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Antimicrobial Activity of Biodegradable Polysaccharide and Protein-Based Films Containing Active Agents

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ABSTRACT

Significant interest has emerged in the introduction of food packaging materials manufactured from biodegradable polymers that have the potential to reduce the environmental impacts associated with conventional packaging materials. Current technologies in active packaging enable effective antimicrobial (AM) packaging films to be prepared from biodegradable materials that have been modified and/or blended with different compatible materials and/or plasticisers. A wide range of AM films prepared from modified biodegradable materials have the potential to be used for packaging of various food products. This review examines biodegradable polymers derived from polysaccharides and protein-based materials for their potential use in packaging systems designed for the protection of food products from microbial contamination. A comprehensive table that systematically analyses and categorizes much of the current literature in this area is included in the review.

Keywords: food packaging, active packaging, antimicrobial agents, biodegradable film

1 Introduction

Films and coatings prepared from biodegradable materials are increasingly being used in the food packaging industry (Rodriguez and others 2006). Biodegradable polymers can be produced from natural, renewable resources (e.g. starch), chemically synthesised from natural sources (e.g. poly(lactic acid)) or made from microbiologically produced materials (e.g. hydroxybutyrate and hydroxyvalerate) (Petersen and others 1999; Cha and Chinnan 2004; Cagri and others 2004; Pommet and others 2005; Perez-Gago and Krochta 2005; Weber and others 2002). These biopolymers can decompose more readily in the environment than their synthetic polymeric counterparts such as polyethylene (PE), polypropylene (PP) and polystyrene (PS) that are derived from crude oils (Cutter 2006; Iovino and others 2008; Tharanathan 2003; Altskär and others 2008; Chick and Ustunol 1998; Guilbert 1986; Dias and others 2010; Lopez-Rubio and others 2006). Consumer demands for preservative-free, high-quality food products, packaged in materials that create less environmental impact have inspired research into the application of biopolymeric materials. In combination with antimicrobial (AM) packaging systems, biopolymer materials with AM properties are emerging as one of the more promising forms of active packaging systems (Hernandez-Izquierdo and others 2008; Krochta and De Mulder-Johnston 1997; Cha and Chinnan 2004). The further development of food packaging materials manufactured from biodegradable polymers such as starch-based materials have the potential to reduce environmental impacts thereby being advantageous over conventional synthetic-based packaging systems (Vlieger 2003).

Active packaging (AP) is a system in which the product, the package and the environment interact in a positive way to extend shelf-life or improve microbial safety or sensory properties while maintaining the quality of food products (Rooney 1995; Suppakul and others

2003; Han 2000; Quintavalla and Vicini 2002; Devlieghere and others 2000; Miltz and others 1995). According to Rooney (1995) and Matche and others (2004), the additional preservation roles rendered by AP systems to the packaged food product differentiates them from traditional packaging systems which offer only protective functions against external influences. A polymeric film mixed with an AM agent can be vital in controlling microbial growth on the surfaces of foods; hence leading to an extension of the shelf-life and/or improved microbial safety of food products (Ojagh and others 2010; Padgett and others 1998). Several researchers have published review articles in the area of bio-based polymers with a detailed discussion of potential food packaging applications (Weber 2000; Krochta and De Mulder-Johnston 1997; Petersen and others 1999; Cagri and others 2004; Tharanathan 2003; Cutter 2006) as well as the general issues affecting AM packaging (Olivas and Barbosa-Canovas 2009). Many of the previous studies focus on key foodborne pathogens such as *Listeria*, *S. aureus*, *E. coli* and *Salmonella* (Ojagh and others 2010; Maizura and others 2008; Shen and others 2010). The reasons for focusing on foodborne pathogens in particular is clear but to food manufacturers the cost/benefit is a major consideration and extending the shelf life of real foods, by diminishing spoilage, is a primary goal. The number of published research studies with AM packages for real foods is however limited. In spite of the importance of the cost/benefit ratio for food manufacturers, a detailed analysis of the cost effectiveness of AM packaging systems developed from bio-polymeric materials is outside the scope of this review.

In the present review, the concept of AM packaging systems with respect to food packaging applications is considered with a focus on biodegradable films, mainly polysaccharides and protein-based materials. This is followed by a detailed discussion of various forms of films incorporated and/or coated with AM agents. Finally, consideration is given to coating and immobilisation of AM agents onto films prepared from biodegradable materials.

2 Polysaccharides and Proteins-Based Materials

Interest has increased recently in the potential uses of films and coatings manufactured from biodegradable polymers particularly polysaccharides and protein-based materials. In the last 15 years or so and especially in recent years the interest in these materials has been primarily for use in food packaging (Krochta and De Mulder-Johnston 1997; Krochta and others 1994; Baldwin and others 1995). Polysaccharides and proteins-based films demonstrate adequate gas barrier properties (Hernandez-Izquierdo and Krochta 2008). Examples of polysaccharide-based polymers that have a potential to be used in AM packaging systems or can be used in conjunction with AM agents include starch, alginate, cellulose, chitosan, carageenan. Examples of proteins-based materials include whey protein, soya protein, corn zein and/or their derivatives (Rodriguez and others 2006; Phan and others 2005; Dawson and others 2002; Brody 2005; Cagri and others 2004; Krochta 2002; 1997). Furthermore, various forms of polysaccharides, protein-based polymers and/or other biodegradable polymers identified by Weber (2002) have the potential to be developed into active packaging materials for food packaging applications. Many bio-based materials such as polysaccharides and protein-based polymers are hydrophilic with a relatively high degree of crystallinity causing processing and performance problems. Therefore, AM packages made from such biodegradable films demonstrate high moisture sensitivity, poor water barrier and poor mechanical properties compared to those made from synthetic polymers (Weber and others 2002).

Packaging materials with suitable physico-mechanical properties can nonetheless be prepared from biopolymers such as starch-based materials when the biodegradable materials are modified by physical, mechanical and/or chemical techniques or by blending them with compatible plasticisers (Arvanitoyannis and others 1998; Davis and Song 2006; Fang and others 2005; Pommet and others 2005; García and others 2000; Tharanathan 2003; 1999). Plasticizers are relatively low molecular weight compounds that can be copolymerized with the polymer or added to the polymer to reduce the intermolecular and intramolecular forces and thereby increase the mobility of the polymeric chains (Sothornvit and Krochta 2005; García and others 2000; Tharanathan 2003). Plasticizers are usually mixed with biopolymers to improve processing, increase film flexibility and lower the glass transition temperature (Avérous and others 2000; Fang and others 2005; Arvanitoyannis and Biliaderis 1999; Brody 2005; López and others 2008; Krochta 2002; Zhang and Liu 2009). Examples of plasticizers that are commonly used with biopolymers include polyols such as glycerol, sorbitol and mannitol, monosaccharides such as fructose, glucose and mannose, and poly(ethylene glycol) (Brody 2005; Kester and Fennema 1986). Water is another important plasticiser for biodegradable films although excess moisture may affect the film properties (Krochta 2002; Van Soest and Essers 1997). Water can be added to a starch-based film in order to break its native granular structure and hydrogen bonding (Yang and Paulson 2000; Mali and others 2002; Myllärinen and others 2002).

When a biopolymer is chemically, mechanically or physically modified, it is able to exhibit thermoplastic properties (Arvanitoyannis and Biliaderis 1999). Modified biodegradable materials such as starch can thus be manufactured into a suitable packaging film using conventional plastic conversion processes like compression moulding, extrusion and thermoforming (Carvalho and others 2005; Jin and Zhang 2008; Kristo and others 2008). Packaging films made from biodegradable polymers such as polysaccharides exhibit low gas permeability, enabling the extension of shelf life of food products without creating anaerobic conditions (Baldwin and others 1995). These biodegradable films or coatings can also be used to prolong the shelf-life of foods such as muscle food products by preventing dehydration, oxidative rancidity and surface browning (Nisperos-Carriedo 1994). Recently, commercially developed starch-based packaging materials like Plantic®, EverCorn™ and Bio-P™ made by Plantic Technologies, Novamont and Bioenvelope respectively, became available (García and others 2009; Robertson 2008). These materials can be used in commercial applications to package food products such as biscuits and snacks. Biodegradable materials have also found successful applications in the pharmaceutical industry as films or coatings to control drug release (Tuovinen and others 2003; Arifin and others 2006; Siepmann and others 2004; Soppimath and others 2001).

3 Preparation of AM Films from Biodegradable Materials

The main processing techniques used for the preparation of biodegradable films are similar to those used in synthetic plastics processing; these include wet and dry processing methods (Brody 2005; Pommet and others 2005). The wet methods comprise solvent casting (which is the most commonly used laboratory-scale technique to prepare AM films from biopolymers) whereas the dry methods usually involve compression moulding or extrusion of the biopolymers that have been modified to become thermoplastic (Liu and others 2006; Pommet and others 2005; Van Soest and Essers 1997; Nam and others 2007; Mehyar and Han 2004; 2006; Thunwall and others 2006; Chaléat and others 2008). The processing techniques may significantly affect the properties of the resultant AM film made from a biodegradable material (Altskär and others 2008). Different factors affect the choice of the processing techniques when preparing an AM packaging film (Han 2005). These include the type and properties of the polymer, the properties of the AM agent (such as polarity and compatibility with the polymer), the heat stability of the latter during processing and the residual AM activity after manufacturing (Han 2000). When a polar AM agent is added to a non-polar polymer to produce an AM film, the incorporated AM agent may affect the physical and mechanical properties of the resultant AM film (Han 2003). However, if the AM agent is compatible with the polymer, a considerable amount of it can be incorporated into the packaging material with minimal physico-mechanical property deterioration (Rupika and others 2008; Suppakul 2004; Han and Floros 1997; Han 2005). Therefore, the polymer and/or the AM agent may require modification prior to film processing in order to increase the compatibility between the two (Cha and Chinnan 2004). During manufacturing of AM films, the temperature and the shearing forces must be carefully considered (Han 2003). High processing temperatures may result in considerable losses of volatile AM agents (Han 2000; Rupika and others 2005; Han and Floros 1997). Moreover, Cooksey (2005) suggested that the AM agent might partly or completely lose its AM activity when incorporated into the film under harsh processing conditions. For example, Nam and others (2007) reported up to 48% recovery of the initial lysozyme activity in an extruded starch-based film upon increasing the extrusion temperature. Therefore, to minimise the loss of AM agent during processing, as low as possible temperatures should be applied as recommended by Han and Floros (1998).

4 Antimicrobial Activity of Biodegradable Films

Numerous studies have identified migratory and non-migratory systems as the two main types of AM packaging systems. A migratory system contains an AM agent that can migrate into the headspace of the package. A non-migratory system contains an AM agent immobilised onto the packaging film. In the latter case, the AM film becomes effective against microbial growth when the food and the packaging material are in direct contact (Brody and others 2001; Vermeiren and others 2002; Davidson and others 2005; Han and Gennadios 2005; Appendini and Hotchkiss 1997; Appendini and Hotchkiss 2002). These forms of AM packaging systems are designed primarily for the purpose of protecting food products from deterioration and spoilage by microorganisms. The following subsections provide a detailed overview of each of the different forms of AM packaging systems by utilising biodegradable films. Table 1 shows that significant progress has been made by effectively integrating AM agents into various biodegradable polymers, particularly polysaccharides such as starch-based and protein-based films. Such AM films have demonstrated inhibitory activity against the growth of various microorganisms. Understandably, the physico-mechanical properties of the films are other important aspects to be considered when designing the film for food packaging applications.

>>>INSERT Table 1

4.1 Antimicrobial Activity of Biodegradable Films Incorporated with AM Agents

Impregnation of an AM agent into a packaging material is a feasible means for achieving optimal AM activity of an AM film (Suppakul and others 2003; Han 2003; Weng and Hotchkiss 1993). This method enables a slow release of the agent onto the food surfaces and the maintaining of an adequate concentration of the agent to effectively inhibit microbial growth throughout the product shelf life (Salleh and others 2007; Cooksey 2005). An AM agent can be incorporated into a packaging material by blending it with a base polymer before manufacturing (extrusion or compression moulding) of the film (Suppakul and others 2006; Rardniyom 2008; Rupika and others 2008; Mistry 2006). This method enables the AM agent to be evenly distributed in the amorphous region of the material (Suppakul 2004).

4.1.1 Antimicrobial Activity of Polysaccharide Films Incorporated with AM agents

Biodegradable polysaccharides can be used for the production of biodegradable films. Polysaccharide-based films demonstrate adequate film-forming properties, although they are sensitive to moisture due to the hydrophilic groups in their structure (Han and Floros 1997; Krochta and others 1994; Baldwin and others 1995). Phan and others (2005) studied the functional properties of agar-based and starch-based films as well as their potential application in food packaging. They reported that films made from agar and cassava starch demonstrated advanced functional properties. However, these films exhibited poor moisture barrier properties compared to low-density polyethylene (LDPE) films because of the inherent hydrophilicity of the polysaccharides. Dias and others (2010) developed biodegradable films based on rice starches that had improved mechanical properties.

Amongst the polysaccharide-based polymers, the starch-based ones are the most abundant and relatively inexpensive renewable materials. Starch is a natural polysaccharide primarily sourced from cereal grains, potatoes, tapioca and arrowroot (Cutter 2006; Baldwin and others 1995; Zhang and Liu 2009). Starch consists of amylose and amylopectin molecules present at different molecular ratios. Amylose is a linear molecule consisting of glucose units connected by 1,4-glucosidic linkages and amylopectin is a highly branched molecule consisting of short 1,4-glucose chains connected by 1,6-glucosidic linkages (Rodriguez and others 2003; Maizura and others 2007; Wu and others 1998; Parker and Ring 2001). Starch is a semicrystalline, very hydrophilic material (Bicerano 2003). The amorphous and crystalline phases affect the physical and chemical properties of starch-based films such as the mechanical and gas barrier properties (Liu 2005; Cha and Chinnan 2004). Films manufactured from starch-based materials have better gas barrier properties than synthetic polymer films but their mechanical properties are poorer. A high amylose starch polymer can be formed into consistent, relatively strong and flexible films that are highly impermeable to oxygen and carbon dioxide. This is in contrast to high amylopectin starch polymers, that can only be formed into non-continuous and brittle films (Gennadios and others 1997; Cha and Chinnan 2004). As expected, starch alone cannot be formed into films with adequate properties for food packaging (Phan and others 2005; Arvanitoyannis and Biliaderis 1998). The intrinsic high level of hydrophilicity, poor mechanical properties and difficulties in processing limit its applications in food packaging unless modified mechanically, physically, chemically or genetically (Arvanitoyannis and others 1998; Davis and Song 2006; 1999; Marron and others 2000; Tharanathan 2003; García and others 2000; Zhang and Liu 2009). Several studies have demonstrated that modified starch-based materials can be used in commercial applications to package dry and other solid food products such as biscuits, snacks, cereals, fresh produce, fruits and vegetables (Cutter 2006; Gennadios and others 1997; Wong and others 1994; Nisperos-Carriedo 1994; Bravin and others 2006; Avérous and others 2001; Debeaufort and others 1998) and/or products with low water activity (Olivas and Barbosa-Canovas 2009).

Table 1 demonstrates that many researchers have made considerable progress by successfully impregnating starch-based films with natural or synthetic AM agents. Such AM starch-based films have shown inhibitory activity to the growth of various microorganisms such as *S. enteritidis*, *L. plantarum*, *B. thermosphaceta B2*, and *L. monocytogenes*, *E. coli O157:H7*, *E. coli*, *S. aureus*, *S. typhimurium*. Durango and others (2006) developed an AM film based on yam starch incorporated with chitosan at different concentrations (1%, 3% and 5% (w/v)) and reported a significant reduction of *S. enteritidis* in liquid culture by each of the films. Nam and others (2007) incorporated 1% (w/w) lysozyme into a pea starch film and demonstrated an AM activity against *B. thermosphaceta B2*. Salleh and others (2007) studied the synergistic effects of wheat starch films incorporated with lauric acid and chitosan and found a significant AM activity of these films against *B. subtilis* but not against *E. coli*. The authors claimed that starch-based films inhibited the growth of all tested microorganisms in liquid culture. The latter observation may be unrealistic in terms of the release of AM agent in the film because the starch-based film presumably dissolves in the liquid culture medium.

Baron and Sumner (1993) showed that starch films impregnated with potassium sorbate and acidified with lactic acid reduced the growth of *S. typhimurium* by 4 log CFU mL^{-1} after 2 h at 37°C. The population count of *E. coli O157: H7* decreased by 2 log CFU mL^{-1} after 3.5 h at 37°C. Furthermore, they found that corn-starch films impregnated with potassium sorbate inhibited the growth of *S. typhimurium* and *E. coli O157 H7* on poultry products stored at 7°C for 12 days. Maizura and others (2008) investigated the antibacterial activity of starch-alginate film incorporated with lemongrass oil. The AM film inhibited the growth of *E. coli O157:H7* and *S. enteritidis* determined by the agar disc diffusion assay but did not show any inhibitory effect on the growth of *S. aureus*. A recent study by Shen and others (2010) showed that sweet potato starch film incorporated with 15% (w/w) potassium sorbate or 5% (w/w) chitosan resulted in a significant reduction of *E. coli* on solid and semi-solid media compared to control film containing no potassium sorbate or chitosan that did not inhibit the growth of *E. coli*. The sweet potato starch film incorporated with 10% (w/w) chitosan suppressed the growth of *S. aureus*. Corrales and others (2009) showed that pea starch films impregnated with grape seed extract inhibited the growth of *B. thermosphaceta B2* on pork loin by 1.3 log CFU mL^{-1} within the first 4 days of storage at 4°C compare to the control film. Pelissari and others (2009) investigated the AM activity of starch-based film incorporated with oregano essential oil (EO). The use of the AM starch-based film effectively inhibited the growth of *E. coli O157:H7*, *B. cereus* and *S. enteritidis* in the agar disc diffusion assay.

Many of the abovementioned studies demonstrated AM activity against various microorganisms using techniques involving agar-based and liquid culture media. Unfortunately, the question of the moisture sensitivity of the starch-based materials and the subsequent usefulness of their films as commercial packaging systems has not been adequately addressed in the literature to date. Therefore, further research is needed to show how to diminish the moisture sensitivity and to enhance the physico-mechanical properties of such starch-based materials so that these can be used for packaging of moist food products. Although, many starch-based materials incorporated with various AM agents demonstrate AM activity, an important aspect to be considered is the effect of increasing the concentration of AM agent on the physico-mechanical properties of the resultant films. Shen and others (2010) reported a deterioration in the physico-mechanical properties of films upon an increase in the potassium sorbate concentration**.** Indeed, such adverse effects could limit the prospects of applying such films in food packaging applications.

In many studies the AM activity of other polysaccharide-based materials such as chitosan incorporated with AM agents has been investigated. Chitosan films have exhibited inhibitory activity on the growth of various microorganisms, when impregnated with AM agents. For example, Ojagh and others (2010) developed chitosan films containing 0.4% to 2% (v/v) of cinnamon EOs and evaluated the AM efficacy of these films against *L. monocytogenes*, *L. plantarum*, *E. coli*, *L. sakei* and *P. fluorescens* in the disc diffusion assay. They reported that chitosan films containing these concentrations of cinnamon EOs inhibited the growth of all the tested bacteria on agar media. Li and others (2006) demonstrated that chitosan films incorporated with 463 international units (IU) of nisin inhibited the growth of *S. aureus*, *L. monocytogenes* and *B. cereus* using the agar diffusion method. However, nisin incorporated into chitosan film had no inhibitory effect against *E. coli*. The later observation is in agreement with the results of Pranoto and others (2005) who studied the AM effect of chitosan films impregnated with nisin at different concentrations against *E. coli*. The impregnated chitosan films were also tested against food pathogens including *S. aureus*, *S. typhimurium*, *L. monocytogenes* and *B. cereus*. In their findings, the AM chitosan film demonstrated inhibitory effects on *L. monocytogenes*, *S. aureus* and *B. cereus*. Increasing the concentration of nisin in the film formulation did not improve the AM activity of the film. Ouattara and others (2000) found that chitosan films containing several organic acids (acetic and propionic) and cinnamaldehyde reduced the growth of *Enterobacteriaceae*, *Serratia liquefaciens* and *Lactobacillus sakei* on the surfaces of vacuum-packed cured meat products (bologna, cooked ham and pastrami) after a storage period of 21 days at 4°C. Duan and others (2008) reported that chitosan films containing lysozyme demonstrated inhibitory activity against *E. coli* and *L. monocytogenes*. A significant release of lysozyme from the films was found. The storage conditions (time and temperature) did not affect the water vapour permeability of the film. Möller and others (2004) studied the AM effectiveness of chitosan-hydroxypropylmethyl cellulose (HPMC) films, chitosan-HPMC films containing stearic and citric acids, and chemically modified chitosan-HPMC films. The chitosan-HPMC films, with and without stearic acid, significantly reduced the growth of *L. monocytogenes*.

Table 1 shows that other studies have evaluated the AM activity of AM agents incorporated into cellulose-based materials such as methylcellulose (MC) films. The cellulose-based materials are some of the naturally occurring polysaccharides with improved film-forming properties. Similarly to the starch-based materials, cellulose-based materials are hydrophilic in nature and have a crystalline structure and so that they are not generally suitable for the packaging of moist food products (Cutter 2002; Baldwin and others 1995).

Many of the cellulose-based materials and/or their derivatives such as MC, HMPC and cellulose acetate are already produced commercially. The latter is widely used in the packaging of baked goods and fresh food products (Weber 2000). Although, there have been a limited number of studies conducted in the past using MC-based materials and/or their derivatives, more recently there has been increased recognition of the potential use of such materials in AM packaging systems for the preservation of food products against microbial contaminations and for the extension of the shelf life of the packaged products. Several researchers have investigated the potential use of cellulose-based materials in AM packaging systems particularly in coating systems as discussed in Section 4.2. For example, Ayana and Nazan (2009) studied the antibacterial effectiveness of olive leaf extract incorporated into MC films against *S. aureus* in an agar disc diffusion test and on surfaces of Kasar cheese. The MC films demonstrated inhibitory activity against *S. aureus* on the agar medium. The films containing 1.5% (w/v) olive leaf extract decreased the population count of *S. aureus* on the surface of Kasar cheese by 1.22 log cycles after 14 days of storage. Santiago-Silva and others (2009) investigated the AM activity of a cellulose-based film incorporated with pediocin. Using the challenge test on sliced ham inoculated with *L. innocua* and *Salmonella spp.* the AM cellulose-based film reduced the growth of *L. innocua* by 2 log cycles after 15 days of storage at 12°C. Similarly, the AM cellulose-based film effectively inhibited the growth of *Salmonella spp* by 0.5 log cycles after 12 days of storage.

Table 1 shows the AM activity of AM agents incorporated into other polysaccharide-based materials such as alginate, poly(lactic acid) (PLA) and pullulan-based films as determined by different researchers. Marcos and others (2007) studied the effect of enterocins incorporated into a series of biodegradable films (alginate, zein and poly(vinyl alcohol)) for the preservation of ready-to-eat food products including sliced ham inoculated with *L.*

monocytogenes. These biodegradable AM films successfully delayed and/or reduced the growth of *L. monocytogenes* during storage at 6°C for 29 days. Recently, Jin and Zhang (2008) investigated a PLA film incorporated with nisin. They found that PLA containing nisin significantly inhibited the growth of *L. monocytogenes* in liquid culture and on liquid egg white. The PLA-nisin film was more active against the growth of *E. coli O157:H7* in orange juice than on liquid culture. Rojas-Grau and others (2006) studied the antibacterial effectiveness of apple puree-based films impregnated with EOs (oregano, cinnamon and lemongrass) against *E. coli O157:H7*. All the evaluated films containing EOs were reported to be effective against *E. coli O157:H7* with the antibacterial activity of oregano oil notably higher than that of lemongrass and cinnamon oils. Kandemir and others (2005) investigated the AM activity of pullulan-based films incorporated with partially purified lysozyme against the growth of *E. coli* and *L. plantarum*. The AM pullulan-based films were found to be effective against *E. coli* but did not show any AM activity against *L. plantarum*. Natrajan and Sheldon (2000) evaluated the antibacterial effectiveness of calcium alginate and agar-based films incorporated with nisin against *S. Typhimurium* on broiler skin. Their results showed that the films containing nisin reduced the population of *S. Typhimurium*.

4.1.2 Antimicrobial Activity of Protein Films Incorporated with AM Agents

Proteins are biopolymeric materials that can be used for the production of biodegradable AM films as they have good film-forming properties. Protein-based polymers have amino acids as their monomer units. Packaging films have been manufactured from different proteins, such as corn zein, wheat gluten, soy protein, whey protein or their derivatives (Hernandez-Izquierdo and others 2008). Packaging films made from protein-based polymers possess adequate physico-mechanical properties (Krochta 2002). Whey protein and corn zein incorporated with natural or synthetic AM agents have been extensively tested *in vitro* and on different food products against the growth of various microorganisms. A summary of the studies investigating the antibacterial effect of AM protein-based films is also presented in Table 1. Although these studies are not directly comparable in term of the AM agents tested or microorganisms tested, the results in general demonstrate that whey protein isolate (WPI) films can be impregnated with AM agents and have the potential to be used as AM food packaging materials. However, no information is readily available in the current literature on the cost/effective benefits of WPI-based films and therefore such information is needed before fabricating AM films from WPI-based materials for commercial applications.

Pintado and others (2010) investigated the inhibitory effects of whey protein films incorporated with nisin, natamycin and malic acid against *P. aeruginosa*, *L. monocytogenes*, *Y. lipolytica*, *P. roqueforti*, *P. commune* using the agar disc diffusion method. They reported that whey protein films incorporated with AM agents demonstrated inhibitory effects against all tested microorganisms. Seydim and Sarikus (2006) tested the AM efficacy of WPI films incorporated with oregano, rosemary and garlic EOs against *E. coli O157:H7*, *S. aureus*, *S. enteriditis*, *L. monocytogenes*, and *L. plantarum*. The AM whey protein films containing oregano EOs at 2% (w/w) level demonstrated a higher inhibitory effect against the tested microorganisms than similar films containing garlic and rosemary extracts. Min and others (2005) investigated the AM effectiveness of whey protein films containing Lactoperoxidase evaluated against *L. monocytogenes* using liquid and agar media as well as on smoked salmon. These films reduced the population of *L. monocytogenes* on smoked salmon by 3 log CFU g^{-1} after 35 days of storage compared with the control film. Gadang and others (2008) evaluated the AM effectiveness of WPI films incorporated with a combination of nisin, malic acid, grape seed extract and EDTA against the growth of *L. monocytogenes*, *E. coli O157:H7*, and *S. typhimurium* inoculated on the surface of a turkey frankfurter. It was found that all the

WPI films incorporated with the combination of AM agents decreased the population of *L. monocytogenes*, *E. coli O157:H7*, and *S. typhimurium* on the surface of the turkey frankfurter by 3.2, 4.2 and 4.6 log CFU g^{-1} after 28 days of storage at 4°C compared to the control film.

Cagri and others (2001) developed WPI films containing 0.5% to 1.5% (w/w) of sorbic acid (SA) or *p*-aminobenzoic acid (PABA) and evaluated the AM efficacy of these AM WPI films against *L. monocytogenes, E. coli* O157:H7 and *S. typhimurium DT104* in a disc diffusion assay. They reported that WPI films containing 1.5% (w/w) PABA or SA inhibited the growth of *L. monocytogenes, E. coli* O157:H7 and *S. typhimurium DT104* in that assay. These results were verified by Cagri and others (2002) who examined the AM effectiveness of WPI films incorporated with 0.5% to 1.0% (w/w) PABA or SA against *L. monocytogenes*, *E. coli O157: H7* and *S. enterica subsp. Enterica serovar typhimurium DT104* inoculated on sliced bologna and summer sausage. Whey protein isolate films containing 1.5% w/w PABA or SA reduced the *L. monocytogenes*, *E. coli* and *S. enterica* population on both products after 21 days at 4 °C. Ko and others (2001) studied the AM activity of WPI, SPI, egg albumin and wheat gluten films incorporated with nisin against *L. monocytogenes*. They found that all these AM protein-based films inhibited *L. monocytogenes*.

Corn zein materials obtained from plant sources are an additional form of proteins that demonstrate good film-forming properties with the potential of being impregnated with AM agents in order to preserve food products from microbial contamination. Previous studies showed that corn zein films containing AM agents demonstrated AM activity against the growth of various microorganisms both *in vitro* and in various food products. A detailed study by Hoffman and others (2001) found that corn zein films incorporated with lauric acid, nisin, EDTA and combinations of these three compounds reduced *L. monocytogenes* in liquid culture, although there was no observed inhibitory effect in films incorporated with EDTA alone. All the films were reported to be bacteriostatic when a 10^4 CFU mL^{-1} *S. enteritidis* initial inoculum was used. Padgett and others (1998) investigated the inhibitory effect of heat-pressed and cast corn zein films containing lysozyme and nisin and reported significant inhibition zones for *Lactobacillus plantarum* by the cast film compared to the heat-pressed films. In another study Padgett and others (2000) found an inhibitory activity of corn zein films incorporated with various levels of lauric acid and nisin on the growth of *L. plantarum* in liquid culture. Gücbilmez and others (2007) developed AM films from corn zein incorporated with lysozyme and albumin proteins. They reported that these films demonstrated AM activity against the growth of *E. coli* and *B. subtilis*.

The AM activity of other types of protein-based films have been studied and reported in the scientific literature by different researchers (see Table 1). Kristo and others (2008) investigated the effectiveness of sodium caseinate (SC) incorporated with nisin, potassium or sodium lactate against *L. monocytogenes*. They found that SC films containing nisin exhibit the highest inhibitory effects on the growth of *L. monocytogenes* followed by films impregnated with potassium sorbate, whereas films containing sodium lactate were only slightly effective. Sivarooban and others (2008) evaluated the AM properties of soy protein isolate (SPI) films containing 1% (w/w) of grape seed extract and nisin (1×10^3 IU g^{-1}). The AM SPI films demonstrated the greatest inhibitory activity against *L. monocytogenes* compared with the other systems that were tested. Oussalah and others (2004) developed a protein-based edible film containing 1% (w/w) oregano and pimento EOs or a mixture of both EOs and evaluated the AM effects of these films on the preservation of whole beef muscle. The results suggested an effectiveness of the AM films against *Pseudomonas spp.* and *E. coli H0157:H7* inoculated on the surface of the beef. Their results also suggested that films containing oregano EO were more effective against the growth of both microorganisms compared to films containing pimento.

4.2 Antimicrobial Activity of Biodegradable Films Coated with AM Agents

In addition to the direct incorporation of AM agents into packaging films discussed above, AM agents can be coated on the surface of packaging materials in order to provide a high concentration of the agent in contact with the surface of food product (Gennadios and others 1997; An and others 2000). The application of an AM agent on a packaging material can be achieved by using various coating techniques including immersion of the substrate or by spraying the substrate with a coating/carrier solution. For this purpose, the AM agent is dissolved in an appropriate solvent such as water, ethanol or isopropanol before applying it to the packaging material (Krochta 2002). Little has been reported on the activity of AM agents coated on biodegradable polymers. Some of the relevant studies are given in Table 1.

Miltz and others (2006) studied the effectiveness of a corn starch-based film coated with the peptide dermaseptin S4 derivative as an AM agent against moulds and aerobic bacteria on cucumbers. They reported that this system was very effective. Coma and others (2001) found that cellulose films coated with nisin inhibited *L. innocua* and *S. aureus* on laboratory media. Chen and others (1996) prepared AM films containing 2% or 4% (w/w) of sodium benzoate and potassium sorbate by casting MC, chitosan and their mixtures. They evaluated the antimycotic activity of the AM films against *Rhodotorula rubra* and *Penicillium notatum* and found that MC and MC/chitosan films containing 2% and 4% (w/w) sodium benzoate and potassium sorbate respectively inhibited the growth of these microorganisms. Ming and others (1997) reported that a cellulose casing coated with pediocin completely inhibited the growth of *L. monocytogenes* on ham, turkey breast and beef products compared to the control film after 12 weeks of storage at 4°C. Janes and others (2002) investigated the AM effect of corn zein films coated with nisin and/or 1% (w/w) calcium propionate against *L. monocytogenes* inoculated on ready-to-eat chicken samples and found that the coated films inhibited the growth of the microorganism. Kim and others (2008) evaluated recently the AM effectiveness of chitosan and WPI coated with lysozyme against the growth of *L. monocytogenes* and *S. enteritidis* inoculated on hard-boiled eggs. The Chitosan-lysozyme system controlled the growth of *S. enteritidis* on hard-boiled shell-on and on peeled eggs. Siragusa and Dickinson (1992; 1993) found that calcium alginate coatings and films containing organic acids effectively reduced the population of *L. monocytogenes, S. typhimurium* and *E. coli O157:H7* on the surface of beef carcass.

4.3 Antimicrobial Activity of Biodegradable Films with Immobilised AM Agents

Effective AM packaging systems can also be achieved by the immobilisation of an AM agent in a polymeric material. According to Steven and Hotchkiss (2003), the AM agents that can be immobilised include peptides, proteins or enzymes. These agents can be synthesised on the surface or extracted separately and then covalently linked to the polymer substrate. An AM agent that is covalently immobilised onto the packaging material is not released but becomes effective in inhibiting microbial growth when in contact with the surface of the packaged food product (Han 2003). Different studies have been conducted focusing on immobilisation of AM agents onto packaging materials. Appendini and Hotchkiss (1997) investigated the efficiency of lysozyme immobilised on polyvinyl alcohol (PVOH) beads, nylon 6,6 pellets and cellulose triacetate (CTA) films. They reported that the viability of *Micrococcus lysodeikticus* was reduced in the presence of immobilised lysozyme on CTA film that was found to show the highest AM activity amongst the studied structures. Cutter and Siragusa (1997) assessed the potential decontamination of raw beef by applying organic acids (lactic or acetic acid) immobilized onto calcium alginate films. They reported a considerable reduction of *L. monocytogenes* growth with the treated films compared to a calcium alginate film without acid treatment. Cutter and Siragusa (1996) studied the AM activity of nisin immobilised onto calcium alginate films against *Brochothrix thermosphacta* on beef surfaces. They found that calcium alginate films treated with nisin suppressed the growth of *B. thermosphacta* by 2.42 log CFU cm^{-2} after 7 days compared to an untreated film. A greater and steady nisin activity was found when the tissues were ground and stored under refrigerated conditions in the AM immobilized film for up to 7 days compared to the use of sprayed nisin only.

5 Summary

Consumer demands and requirements by regulatory agencies to use more environmentally-friendly and less polluting packages have directed researchers to look at packaging materials that are derived from natural or made from renewable resources to replace, at least some, of the synthetic polymers. Biodegradable materials derived from polysaccharides and proteins, when combined with AM agents, have the potential to be manufactured into food packaging films with effective AM properties. Polysaccharide-based materials with AM agents, particularly the starch-based ones, have been studied extensively with some commercial success in the food packaging industry. Many of the studies were carried out in order to obtain a "proof of concept" by measuring the inhibition zones created by the diffusion of the AM agent in solid media. Some modified biodegradable polymers such as starch-based materials can be manufactured into films and used to package dry and/or solid food products such as biscuits, snacks, cereals, fresh produce, fruits and vegetables. Developing commercial biodegradable films with improved physical and mechanical properties is still a challenge due to their hydrophilic nature that limits their application for packaging of food products with a high water activity. The biodegradable and bio-compostable materials are also, many times, more expensive and more difficult to process, a fact that further increases their cost compared to synthetic polymers. However, when considering the cost of a package, the total "cradle to grave" economic approach should be evaluated. Thus, the economic evaluation should include not only the cost of the packaging material and of processing the material into a package but also the cost of disposing of the final package namely, recycling and/or incineration and/or land filling. This is very important especially for the last option, taking into consideration the decreasing number of land filling sites and the diminishing space for garbage disposal in the developed countries. If such considerations are taken into account, the difference between the cost of biodegradable/bio-compostable and synthetic polymers becomes much smaller. Antimicrobial packaging films with improved physical and mechanical properties could be prepared from biodegradable polymers that have been modified and/or blended with other compatible materials incorporated or coated with AM agents. However, additional research and development work is required to reduce the moisture sensitivity of these polymers, enhance their physical properties and improve their process-ability. These goals can be achieved by proper blending with appropriate materials and/or by copolymerization. Biodegradable materials could also be successfully prepared and applied in AM packaging systems by the incorporation of appropriate AM agents. Taking into consideration that the public, as a whole, is already conscious (and becomes even more so as times go by) to the environment, it is conceivable that the future will see more biodegradable and AM biodegradable polymers and/or their devivatives in the packaging of food, agricultural and other products.

Table 1: Antimicrobial activity of AM agents in biodegradable materials

Packaging Material	Antimicrobial Agent	Loading	Applic-ation[a]	Substrate	Microorganism(s)	Observations	References
Polysaccharide Films							
Calcium alginate	Acetic acid	2% (v/v)	C	Lean beef tissue	*L. monocytogenes*	Reduced *L. monocytogenes* growth	Siragusa and Dickson (1992)
Calcium alginate	Acetic acid	2% (v/v)	C	Lean beef tissue	*L. monocytogenes*, *S. typhimurium* and *E. coli O157:H7*	Decreased *L. monocytogenes*, *S. Typhimurium*, *E. coli O157:H7*	Siragusa and Dickson (1993)
Calcium alginate	Lactic acid	1.7% (v/v)	IM	Lean beef tissue	*L. monocytogenes*	Reduced *L. monocytogenes* count	Siragusa and Dickson (1992)
Calcium alginate gel	Nisin	1×102 µg/mL	IM	Lean and adipose beef carcass	*B. thermosphacta*	Reduced 2.84 and 2.91 log of *B. thermosphacta* on lean and adipose respectively	Cutter and Siragusa (1997; 1996)
Cellulose casing	Pediocin	10% (w/v)	C	Fresh poultry, fresh beef, ham	*L. monocytogenes*	Inhibited growth of *L. monocytogenes* in fresh and processed products	Ming and others (1997)
Cellulose	Nisin		IN	Agar medium	*L. innocua* and *S. aureus*	Inhibited growth of *L. innocua* and *S. aureus*	Coma and others (2001)
Cellulose film	Olive leaf extract	0.5-3% (w/v)	IN	Agar method cheese	*S. aureus*	Decrease 1.22 log of *S. aureus* after 14 days	Ayana and Nazan (2009)

Packaging Material	Antimicrobial Agent	Loading	Application[a]	Substrate	Microorganism(s)	Observations	References
Cellulose, chitosan	Potassium sorbate	2-5% (w/v)	C	Agar diffusion	*Rhodotorula rubra* and *Penicillium notatum*	AM activity against *R. rubra* and *P. notatum*	Chen and others (1996)
Cellulose, chitosan	Sodium benzoate	2-5% (w/v)	C	Agar diffusion	*Rhodotorula rubra* and *Penicillium notatum*	AM activity against *R. rubra* and *P. notatum*	Chen and others (1996)
Chitosan	Nisin	4.63-37.04 × 102 IU	IN	Agar diffusion	*S. aureus*, *L. monocytogenes*, *B. cereus*, and *E. coli*	Inhibited growth of *S. aureus*, *L. monocytogenes* and *B. cereus* but not *E. coli*	Li and others (2006)
Chitosan	Acetic acid	1% (w/v)	IN	Ham, bologna, pastrami	*S. liquefaciens*, and *L. sakei*	Reduced growth of *S. liquefaciens* and *L. sakei*	Ouattara and others (2000)
Chitosan	Acetic acid	0.25-1% (w/v)	IN	Ham, bologna, pastrami	*Enterobacteriaceae*, *S. liquefaciens*, *L. sakei*	Growth of *S. liquefaciens* was delayed by film	Ouattara and others (2000)
Chitosan	Cinnamon oil	0.4-2% (v/v)	IN	Agar method	*L. monocytogenes*, *L. plantarum*, *E. coli* ,*L. sakei*, *Ps. fluorescens*	Iinhibited *L. monocytogenes*, *L. plantarum*, *E. coli*, *L. sakei* , *P. fluorescens*	Ojagh and others (2010)

Packaging Material	Antimicrobial Agent	Loading	Application[a]	Substrate	Microorganism(s)	Observations	References
Chitosan	Garlic oil	1-4 × 102 µg/g	IN	Agar method	*E. coli*, *S. aureus*, *S. typhimurium*, *L. monocytogenes* and *B. cereus*	Clear zone of inhibition against *S. aureus*, *L. monocytogenes* and *B. cereus*	Pranoto and others (2005)
Chitosan	Nisin	5.1-204 × 103 IU/g chitosan	IN	Agar method	*E. coli*, *S. aureus*, *S. typhimurium*, *L. monocytogenes* and *B. cereus*	Film inhibited growth of S. *aureus*, *L. monocytogenes* and *B. cereus*	Pranoto and others (2005)
Chitosan	Potassium sorbate	50-200 mg/g	IN	Agar method	*E. coli*, *S. aureus*, *S. typhimurium*, *L. monocytogenes* and *B. cereus*	Demonstrated AM activity against *S. aureus*, *L. monocytogenes* and *B. cereus*	Pranoto and others (2005)
Chitosan	Propionic acid	1% (w/v)	IN	Ham, bologna, pastrami	*S. liquefaciens*, *L. sakei*	All films reduced growth of *S. liquefacien*s for all the storage period.	Ouattara and others (2000)
Chitosan	Lysozyme	60% (w/w)		Agar media	*E. coli* and *L.*	AM activity against E. *coli* and *L. monocytogenes*	Duan and others (2008)

Packaging Material	Antimicrobial Agent	Loading	Application[a]	Substrate	Microorganism(s)	Observations	References
					monocytogenes		
Chitosan-HPMC	Chitosan	0.5-2% (w/v)		Agar method	*L. monocytogenes*	Inhibited *L. monocytogenes*	Möller and others (2004)
PLA	Nisin	0.25 g/mL	IN	Liquid culture, orange juice, egg white	*E. coli O157:H7*, *S. enteritidis*, and *L. monocytogenes*	Films reduced growth of *E. coli O157:H7*, *S. enteritidis*, and *L. monocytogenes*	Jin and Zhang (2008)
Starch-based	Dermaseptin S4	3 mg/L	C	Cucumber	Moulds and aerobic bacteria	Film demonstrated AM activity	Miltz and others (2006)
Starch	Grape seed extract	1-20% (w/v)	IN	Agar media Pork loin	*L. monocytogenes*, *E. coli*, *E. faecalis*, , *E. faecium*, *S. typhimurium*, and *B. thermosphaceta B2*	Reduced growth of *thermosphaceta B2* on pork loin; inhibited Gram-positive bacteria on solid media but not Gram-negative bacteria	Corrales and others (2009)
Starch film	Chitosan	1-9% (w/w)	IN	agar and liquid media	*B. subtilis* and *E. coli*	Inhibited *B. subtilis* and *E. coli*	Salleh and others (2007)
Starch film	Chitosan	5-15% (w/w)	IN	Agar media and semisolid	*E. coli* and *S. aureus*	Inhibited both *E. coli* and *S. aureus*	Shen and others (2010)

Packaging Material	Antimicrobial Agent	Loading	Application[a]	Substrate	Microorganism(s)	Observations	References
Starch film	Chitosan	1-5% (w/v)	IN	Liquid culture	*S. enteritidis*	Inhibitory effect against *S. enteritidis*	Durango and others (2006)
Starch film	Lauric acid	8% (w/w)	IN	Agar and liquid culture media	*B. subtilis* and *E. coli*	Inhibition of *B. subtilis* and *E. coli*	Salleh and others (2007)
Starch film	Lysozyme	1% (w/w)	IN	Agar media	*B. thermosphaceta B2*	Inhibitory effect against *B. thermosphaceta B2*	Nam and others (2007)
Starch film	Potassium sorbate	5-15% (w/w)	IN	Agar media semisolid	*E. coli* and *S. aureus*	Inhibited *E. coli* but not *S. aureus*	Shen and others (2010)
Starch film	Potassium sorbate	20%	IN	Liquid culture, poultry	*S. typhimurium* and *E. coli*	Inhibited *S. typhimurium* and *E. coli O157:H7* by 4 and 2 logs respectively	Baron and Sumner (1993)
Starch-alginate	Lemongrass oil	0.1-0.4% (w/v)	IN	Agar media	*E. coli O157:H7*	Inhibited *E. coli O157:H7* growth	Maizura and others (2008)
Starch-chitosan	Oregano EOs	0.1-1% (w/w)	IN	Agar media	*E. coli O157:H7*, *S. aureus*, *S. enteriditis*, and *B. cereus*	Inhibited *E. coli O157:H7*, *S. aureus*, *S. enteriditis*, *B. cereus*	Pelissari and others (2009)
Starch	Grape seed extract	1-20% (w/v)	IN	Agar media Pork loin	*L. monocytogenes*, *E. coli*, *E. faecalis*, *E. faecium*, *S.*	Reduced 1.3 log CFU mL^{-1} of *B. thermosphaceta B2* on pork loin; inhibited Gram-positive bacteria on solid media but not	Corrales and others (2009)

Packaging Material	Antimicrobial Agent	Loading	Application[a]	Substrate	Microorganism(s)	Observations	References
					typhimurium, and *B. thermosphaceta B2*	Gram-negative bacteria	
Protein Films							
Corn zein	Calcium-propionate	1% (w/w)	C	Ready-to-eat chicken	*L. monocytogenes*	Coated films suppressed *L. monocytogenes* growth	Janes and others (2002)
Corn zein	Lysozyme	479-958 µg/cm2	IN	Agar media	*E. coli* and *B. subtilis*	Effective against *E. coli* and *B. substilis*	Güçbilmez and others (2007)
Corn zein	Nisin	1× 103 IU/g	C	Ready-to-eat chicken	*L. monocytogenes*	Coated films reduced *L. monocytogenes* growth	Janes and others (2002)
Corn zein	Lauric acid	200 mg	IN	Liquid culture	*L. monocytogenes*, and *S. enteriditis*	Significant effect against *L. monocytogenes* but not against *S. enteriditis*	Hoffman and others (2001)
Corn zein	Nisin	0.188 mg	IN	Liquid culture	*L. monocytogenes*, and *S. enteriditis*	Reduced counts of *L. monocytogenes, S. enteriditis*	Hoffman and others (2001)
Proteins-based film	Oregano EOs	1% (w/v)	IN	Beef muscle slices	*Pseudomonas spp.* and *E. coli H0157:H7*	Films containing oregano reduced 0.95 and 1.12 log of *P. spp.* and *E. coli H0157:H7* respectively, after 7 days	Oussalah and others (2004)

Packaging Material	Antimicrobial Agent	Loading	Application[a]	Substrate	Microorganism(s)	Observations	References
Proteins-based film	Pimento EOs	1% (w/v)	IN	Beef muscle slices	*Pseudomonas spp.* and *E. coli H0157:H7*	Films containing pimento EOs were reported to be less effective against *E. coli H0157:H7* and *Pseudomonas*	Oussalah and others (2004)
Sodium caseinate	Nisin	7.5-75 × 10-4 (w/w)	IN	Agar media	*L. monocytogenes*	Effectively reduced *L. monocytogenes*	Kristo and others (2008)
Sodium caseinate	Potassium sorbate	10-25 (w/w)	IN	Agar media	*L. monocytogenes*	Reduced growth of *L. monocytogenes*	Kristo and others (2008)
Sodium caseinate	Sodium lactate	10-40 (w/w)	IN	Agar media	*L. monocytogenes*	Slightly effective against *L. monocytogenes*	Kristo and others (2008)
Soy protein Corn zein	EDTA	15-30m mM	IN	Agar and liquid media	*L. plantarum* and *E. coli*	Inhibited *E. coli* at 30 mM	Padgett and others (1998; 2000)
Soy protein Corn zein	Lauric acid	2.5-133 mg/g	IN	Agar and liquid media	*L. plantarum* and *E. coli*	Inhibited *L. plantarum* but not *E. coli*	Padgett and others (1998; 2000)
Soy protein Corn zein	Lysozyme	2.5-133 mg/g of film	IN	Agar and liquid media	*L. plantarum* and *E. coli*	Inhibited *L. plantarum* and *E. coli*	Padgett and others (1998; 2000)

Packaging Material	Antimicrobial Agent	Loading	Applic-ation[a]	Substrate	Microorganism(s)	Observations	References
Soy protein Corn zein	Nisin	0.01-6 mg/g of film	IN	Agar and liquid media	*L. plantarum* and *E. coli*	Inhibited *L. plantarum* and *E. coli.*	Padgett and others (1998; 2000)
Soy protein isolate	EDTA	0.16% (w/w)	IN	Liquid or solid media	*E. coli O157:H7*, *S. typhimurium*, and *L. monocytogenes*	Enhanced AM activity of nisin and GSE	Sivarooban and others (2008)
Soy protein isolate	Grape seed extract + EDTA	1% (w/w)	IN	Liquid or solid media	*E. coli O157:H7*, *S. typhimurium*, and *L. monocytogenes*	Reduced population of *E. coli O157:H7, S. typhimurium, L. monocytogenes*	Sivarooban and others (2008)
Soy protein isolate	Nisin + EDTA	1 × 103 IU/g	IN	Liquid or solid media	*E. coli O157:H7*, *S. typhimurium*, and *L. monocytogenes*	Reduced population of *E. coli O157:H7, S. typhimurium, L. monocytogenes*	Sivarooban and others (2008)
Soy protein isolate films	Nisin	3-12 × 104 IU/15mL	IN	liquid culture media	*L. monocytogenes*	Inhibition against *L. monocytogenes* was concentration dependent	Ko and others (2001)
Whey protein	Lactoperoxidase	0.01-0.4 (w/v)	IN	Agar and liquid culture	*L. monocytogenes*	Reduced population of *L.*	Min and others (2005)

Packaging Material	Antimicrobial Agent	Loading	Application[a]	Substrate	Microorganism(s)	Observations	References
				media, smoked salmon		*monocytogenes* by 3 log CFU g^{-1} on smoked salmon	
Whey protein	Malic acid	3% (w/v)	IN	Agar media	*L. monocytogenes*, *P. aeruginosa*, *P. commune*, *P. roqueforti* and *Y. lipolytica*	Inhibited *L. monocytogenes* and *P. aeruginosa*	Pintado and others (2010)
Whey protein	Natamycin	2-5×10-3 g/mL	IN	Agar media	*L. monocytogenes*, *P. aeruginosa*, *P. commune*, *P. roqueforti* and *Y. lipolytica*	Inhibited *Y. lipolytica, Penicillium spp.*	Pintado and others (2010)
Whey protein	Nisin	50 IU/mL	IN	Agar media	*L. monocytogenes*, *P. aeruginosa*, *P. commune*, *P. roqueforti* and *Y. lipolytica*	Inhibited *L. monocytogenes*	Pintado and others (2010)
Whey protein isolate	Chitosan-lysozyme	3% (w/w)	C	Hard-boiled egg	*L. monocytogenes* and *S.*	Ineffective against *L. monocytogenes* but reduced	Kim and others (2008)

Packaging Material	Antimicrobial Agent	Loading	Application[a]	Substrate	Microorganism(s)	Observations	References
					enteritidis	growth of *S. enteritidis*	
Whey protein isolate	Garlic oil	1-4% (w/v)	IN	Agar method	*E. coli O157:H7*, *S. aureus*, *S. enteriditis*, *L. monocytogenes*, and *L. plantarum*	Garlic oil inhibit *E. coli O157:H7, S. aureus, S. enteriditis, L. monocytogenes,* and *L. plantarum* at 3-4%	Seydim and Sarikus (2006)
Whey protein isolate	Grape seed extract	1.2-3.6 × 103 ppm	IN	Turkey frankfurter	*L. monocytogenes*, *E. coli O157:H7*, and *S. typhimurium*	Ineffective against *L. monocytogenes*, *E. coli O157:H7* but inhibited growth of *S. typhimurium*	Gadang and others (2008)
Whey protein isolate	Malic acid	1.2-3.6 × 103 ppm	IN	Turkey frankfurter	*L. monocytogenes*, *E. coli O157:H7*, and *S. typhimurium*	Ineffective against *L. monocytogenes*, *E. coli O157:H7* but inhibited growth of *S. typhimurium*	Gadang and others (2008)
Whey protein isolate	Nisin	6-18 × 103 IU/g	IN	Turkey frankfurter	*L. monocytogenes*, *E. coli O157:H7*, and *S. typhimurium*	Ineffective against *L. monocytogenes*, *E. coli O157:H7* but inhibited growth of *S. typhimurium*	Gadang and others (2008)
Whey protein isolate	Oregano	1-4% (w/v)	IN	Agar method	*E. coli O157:H7*, *S.*	Oregano demonstrated Inhibitory effect against *E. coli*	Seydim and Sarikus (2006)

Packaging Material	Antimicrobial Agent	Loading	Application[a]	Substrate	Microorganism(s)	Observations	References
					aureus, *S. enteriditis*, *L. monocytogenes*, and *L. plantarum*	*O157:H7, S. aureus, S. enteriditis, L. monocytogenes*, and *L. plantarum* at 3-4%	
Whey protein isolate	p-aminobenzoic acid	0.5-1.5% (w/v)	IN	Agar media	*L. monocytogenes*, *E. coli O157:H7*, and *S. Typhimurium DT104*	Inhibited *L. monocytogenes*, *E. coli O157:H7*, *S. Typhimurium*	Cagri and others (2001)
Whey protein isolate	p-aminobenzoic acid	0.5-1% (w/v)	IN	Bologna summer sausage	*L. monocytogenes*, *E. coli O157: H7*, and *S. Typhimurium DT104*.	Reduced *L. monocytogenes* by log 1.5-3.4 on bologna slices and increase by log 2.2 under control after 21 days. Population of *E. coli O157: H7* decrease by log 2.7-3.6	Cagri and others (2002)
Whey protein isolate	Rosemary	1-4% (w/v)	IN	Agar method	*E. coli O157:H7*, *S. aureus*, *S. enteriditis*, *L. monocytogenes*, and *L. plantarum*	Ineffective against all the reference microorganisms At all concentrations	Seydim and Sarikus (2006)
Whey protein isolate	Sorbic acid	0.5-1.5% (w/w)	IN	Agar media	*L. monocytogenes*, *E. coli O157:H7*, and *S.*	Inhibited *L. monocytogenes*, *E. coli O157:H7*, *S. Typhimurium DT104*	Cagri and others (2001)

Packaging Material	Antimicrobial Agent	Loading	Application[a]	Substrate	Microorganism(s)	Observations	References
					Typhimurium DT104		
Whey protein isolate	Sorbic acid	0.5-1% (w/v)	IN	Bologna and summer sausage	*L. monocytogenes*, *E. coli O157: H7*, and *S. Typhimurium DT104.*	Decreased population of *L. monocytogenes, E. coli O157: H7, S. Typhimurium*	Cagri and others (2002)
Others							
Apple puree	Cinnamon	0.05-0.5% (w/w)	IN	Liquid culture	*E. coli O157:H7*	Film effective against E. *coli O157:H7*	Rojas-Grau and others (2006)
Apple puree	Lemongrass oil	0.05-0.5% (w/w)	IN	Liquid culture	*E. coli O157:H7*	Inhibited the growth of *E. coli O157:H7*	Rojas-Grau and others (2006)
Apple puree	Oregano oils	0.05-0.1% (w/w)	IN	Agar media/solid media	*E. coli O157:H7*	Highly effective against *E. coli O157:H7*	Rojas-Grau and others (2006)
PVOH, CTA, nylon 6,6	Lysozyme	10-300 mg/g	C	Liquid culture	*Micrococcus lysodeikticus*	All films demonstrated AM activity with nylon 6,6 showing the least effective	Appendini and Hotchkiss (1997)

[a] Application Type: IN = Incorporated: physically/chemically combined; C = coated: incorporated in a coating layer and applied; IM = immobilized: covalently bonded with components of packaging layer.

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