



27 **ABSTRACT**

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

39

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

## 41 **1 Introduction**

42 Films and coatings prepared from biodegradable materials are increasingly being used in the  
43 food packaging industry (Rodriguez and others 2006). Biodegradable polymers can be  
44 produced from natural, renewable resources (e.g. starch), chemically synthesised from natural  
45 sources (e.g. poly(lactic acid)) or made from microbiologically produced materials (e.g.  
46 hydroxybutyrate and hydroxyvalerate) (Petersen and others 1999; Cha and Chinnan 2004;  
47 Cagri and others 2004; Pommet and others 2005; Perez-Gago and Krochta 2005; Weber and  
48 others 2002). These biopolymers can decompose more readily in the environment than their  
49 synthetic polymeric counterparts such as polyethylene (PE), polypropylene (PP) and  
50 polystyrene (PS) that are derived from crude oils (Cutter 2006; Iovino and others 2008;  
51 Tharanathan 2003; Altskär and others 2008; Chick and Ustunol 1998; Guilbert 1986; Dias  
52 and others 2010; Lopez-Rubio and others 2006). Consumer demands for preservative-free,  
53 high-quality food products, packaged in materials that create less environmental impact have  
54 inspired research into the application of biopolymeric materials. In combination with  
55 antimicrobial (AM) packaging systems, biopolymer materials with AM properties are  
56 emerging as one of the more promising forms of active packaging systems (Hernandez-  
57 Izquierdo and others 2008; Krochta and De Mulder-Johnston 1997; Cha and Chinnan 2004).  
58 The further development of food packaging materials manufactured from biodegradable  
59 polymers such as starch-based materials have the potential to reduce environmental impacts  
60 thereby being advantageous over conventional synthetic-based packaging systems (Vlieger  
61 2003).

62  
63 Active packaging (AP) is a system in which the product, the package and the environment  
64 interact in a positive way to extend shelf-life or improve microbial safety or sensory  
65 properties while maintaining the quality of food products (Rooney 1995; Suppakul and others

66 2003; Han 2000; Quintavalla and Vicini 2002; Devlieghere and others 2000; Miltz and others  
67 1995). According to Rooney (1995) and Matche and others (2004), the additional  
68 preservation roles rendered by AP systems to the packaged food product differentiates them  
69 from traditional packaging systems which offer only protective functions against external  
70 influences. A polymeric film mixed with an AM agent can be vital in controlling microbial  
71 growth on the surfaces of foods; hence leading to an extension of the shelf-life and/or  
72 improved microbial safety of food products (Ojagh and others 2010; Padgett and others  
73 1998). Several researchers have published review articles in the area of bio-based polymers  
74 with a detailed discussion of potential food packaging applications (Weber 2000; Krochta and  
75 De Mulder-Johnston 1997; Petersen and others 1999; Cagri and others 2004; Tharanathan  
76 2003; Cutter 2006) as well as the general issues affecting AM packaging (Olivas and  
77 Barbosa-Canovas 2009). Many of the previous studies focus on key foodborne pathogens  
78 such as *Listeria*, *S. aureus*, *E. coli* and *Salmonella* (Ojagh and others 2010; Maizura and  
79 others 2008; Shen and others 2010). The reasons for focusing on foodborne pathogens in  
80 particular is clear but to food manufacturers the cost/benefit is a major consideration and  
81 extending the shelf life of real foods, by diminishing spoilage, is a primary goal. The number  
82 of published research studies with AM packages for real foods is however limited. In spite of  
83 the importance of the cost/benefit ratio for food manufacturers, a detailed analysis of the cost  
84 effectiveness of AM packaging systems developed from bio-polymeric materials is outside  
85 the scope of this review.

86

87 In the present review, the concept of AM packaging systems with respect to food packaging  
88 applications is considered with a focus on biodegradable films, mainly polysaccharides and  
89 protein-based materials. This is followed by a detailed discussion of various forms of films

90 incorporated and/or coated with AM agents. Finally, consideration is given to coating and  
91 immobilisation of AM agents onto films prepared from biodegradable materials.

92

## 93 **2 Polysaccharides and Proteins-Based Materials**

94 Interest has increased recently in the potential uses of films and coatings manufactured from  
95 biodegradable polymers particularly polysaccharides and protein-based materials. In the last  
96 15 years or so and especially in recent years the interest in these materials has been primarily  
97 for use in food packaging (Krochta and De Mulder-Johnston 1997; Krochta and others 1994;  
98 Baldwin and others 1995). Polysaccharides and proteins-based films demonstrate adequate  
99 gas barrier properties (Hernandez-Izquierdo and Krochta 2008). Examples of polysaccharide-  
100 based polymers that have a potential to be used in AM packaging systems or can be used in  
101 conjunction with AM agents include starch, alginate, cellulose, chitosan, carageenan.  
102 Examples of proteins-based materials include whey protein, soya protein, corn zein and/or  
103 their derivatives (Rodriguez and others 2006; Phan and others 2005; Dawson and others 2002;  
104 Brody 2005; Cagri and others 2004; Krochta 2002; 1997). Furthermore, various forms of  
105 polysaccharides, protein-based polymers and/or other biodegradable polymers identified by  
106 Weber (2002) have the potential to be developed into active packaging materials for food  
107 packaging applications. Many bio-based materials such as polysaccharides and protein-based  
108 polymers are hydrophilic with a relatively high degree of crystallinity causing processing and  
109 performance problems. Therefore, AM packages made from such biodegradable films  
110 demonstrate high moisture sensitivity, poor water barrier and poor mechanical properties  
111 compared to those made from synthetic polymers (Weber and others 2002).

112

113 Packaging materials with suitable physico-mechanical properties can nonetheless be prepared  
114 from biopolymers such as starch-based materials when the biodegradable materials are

115 modified by physical, mechanical and/or chemical techniques or by blending them with  
116 compatible plasticisers (Arvanitoyannis and others 1998; Davis and Song 2006; Fang and  
117 others 2005; Pommet and others 2005; García and others 2000; Tharanathan 2003; 1999).  
118 Plasticizers are relatively low molecular weight compounds that can be copolymerized with  
119 the polymer or added to the polymer to reduce the intermolecular and intramolecular forces  
120 and thereby increase the mobility of the polymeric chains (Sothornvit and Krochta 2005;  
121 García and others 2000; Tharanathan 2003). Plasticizers are usually mixed with biopolymers  
122 to improve processing, increase film flexibility and lower the glass transition temperature  
123 (Avérous and others 2000; Fang and others 2005; Arvanitoyannis and Biliaderis 1999; Brody  
124 2005; López and others 2008; Krochta 2002; Zhang and Liu 2009). Examples of plasticizers  
125 that are commonly used with biopolymers include polyols such as glycerol, sorbitol and  
126 mannitol, monosaccharides such as fructose, glucose and mannose, and poly(ethylene glycol)  
127 (Brody 2005; Kester and Fennema 1986). Water is another important plasticiser for  
128 biodegradable films although excess moisture may affect the film properties (Krochta 2002;  
129 Van Soest and Essers 1997). Water can be added to a starch-based film in order to break its  
130 native granular structure and hydrogen bonding (Yang and Paulson 2000; Mali and others  
131 2002; Myllärinen and others 2002).

132

133 When a biopolymer is chemically, mechanically or physically modified, it is able to exhibit  
134 thermoplastic properties (Arvanitoyannis and Biliaderis 1999). Modified biodegradable  
135 materials such as starch can thus be manufactured into a suitable packaging film using  
136 conventional plastic conversion processes like compression moulding, extrusion and  
137 thermoforming (Carvalho and others 2005; Jin and Zhang 2008; Kristo and others 2008).  
138 Packaging films made from biodegradable polymers such as polysaccharides exhibit low gas  
139 permeability, enabling the extension of shelf life of food products without creating anaerobic

140 conditions (Baldwin and others 1995). These biodegradable films or coatings can also be used  
141 to prolong the shelf-life of foods such as muscle food products by preventing dehydration,  
142 oxidative rancidity and surface browning (Nisperos-Carriedo 1994). Recently, commercially  
143 developed starch-based packaging materials like Plantic®, EverCorn™ and Bio-PTM made by  
144 Plantic Technologies, Novamont and Bioenvelope respectively, became available (García and  
145 others 2009; Robertson 2008). These materials can be used in commercial applications to  
146 package food products such as biscuits and snacks. Biodegradable materials have also found  
147 successful applications in the pharmaceutical industry as films or coatings to control drug  
148 release (Tuovinen and others 2003; Arifin and others 2006; Siepmann and others 2004;  
149 Soppimath and others 2001).

150

### 151 **3 Preparation of AM Films from Biodegradable Materials**

152 The main processing techniques used for the preparation of biodegradable films are similar to  
153 those used in synthetic plastics processing; these include wet and dry processing methods  
154 (Brody 2005; Pommet and others 2005). The wet methods comprise solvent casting (which is  
155 the most commonly used laboratory-scale technique to prepare AM films from biopolymers)  
156 whereas the dry methods usually involve compression moulding or extrusion of the  
157 biopolymers that have been modified to become thermoplastic (Liu and others 2006; Pommet  
158 and others 2005; Van Soest and Essers 1997; Nam and others 2007; Mehyar and Han 2004;  
159 2006; Thunwall and others 2006; Chaléat and others 2008). The processing techniques may  
160 significantly affect the properties of the resultant AM film made from a biodegradable  
161 material (Altskär and others 2008). Different factors affect the choice of the processing  
162 techniques when preparing an AM packaging film (Han 2005). These include the type and  
163 properties of the polymer, the properties of the AM agent (such as polarity and compatibility  
164 with the polymer), the heat stability of the latter during processing and the residual AM

165 activity after manufacturing (Han 2000). When a polar AM agent is added to a non-polar  
166 polymer to produce an AM film, the incorporated AM agent may affect the physical and  
167 mechanical properties of the resultant AM film (Han 2003). However, if the AM agent is  
168 compatible with the polymer, a considerable amount of it can be incorporated into the  
169 packaging material with minimal physico-mechanical property deterioration (Rupika and  
170 others 2008; Suppakul 2004; Han and Floros 1997; Han 2005). Therefore, the polymer and/or  
171 the AM agent may require modification prior to film processing in order to increase the  
172 compatibility between the two (Cha and Chinnan 2004). During manufacturing of AM films,  
173 the temperature and the shearing forces must be carefully considered (Han 2003). High  
174 processing temperatures may result in considerable losses of volatile AM agents (Han 2000;  
175 Rupika and others 2005; Han and Floros 1997). Moreover, Cooksey (2005) suggested that the  
176 AM agent might partly or completely lose its AM activity when incorporated into the film  
177 under harsh processing conditions. For example, Nam and others (2007) [reported up to 48%](#)  
178 recovery of the initial lysozyme activity in an extruded starch-based film upon increasing the  
179 extrusion temperature. Therefore, to minimise the loss of AM agent during processing, as low  
180 as possible temperatures should be applied as recommended by Han and Floros (1998).

181

#### 182 **4 Antimicrobial Activity of Biodegradable Films**

183 Numerous studies have identified migratory and non-migratory systems as the two main types  
184 of AM packaging systems. A migratory system contains an AM agent that can migrate into  
185 the headspace of the package. A non-migratory system contains an AM agent immobilised  
186 onto the packaging film. In the latter case, the AM film becomes effective against microbial  
187 growth when the food and the packaging material are in direct contact (Brody and others  
188 2001; Vermeiren and others 2002; Davidson and others 2005; Han and Gennadios 2005;  
189 Appendini and Hotchkiss 1997; Appendini and Hotchkiss 2002). These forms of AM



190 packaging systems are designed primarily for the purpose of protecting food products from  
191 deterioration and spoilage by microorganisms. The following subsections provide a detailed  
192 overview of each of the different forms of AM packaging systems by utilising biodegradable  
193 films. Table 1 shows that significant progress has been made by effectively integrating AM  
194 agents into various biodegradable polymers, particularly polysaccharides such as starch-based  
195 and protein-based films. Such AM films have demonstrated inhibitory activity against the  
196 growth of various microorganisms. [Understandably, the physico-mechanical properties of the](#)  
197 [films are other important aspects to be considered when designing the film for food packaging](#)  
198 [applications.](#)

199

200 >>>INSERT Table 1

201

#### 202 **4.1 Antimicrobial Activity of Biodegradable Films Incorporated with AM Agents**

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

212

#### 213 **4.1.1 Antimicrobial Activity of Polysaccharide Films Incorporated with AM agents**

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

224

225 Amongst the polysaccharide-based polymers, the starch-based ones are the most abundant and  
226 relatively inexpensive renewable materials. Starch is a natural polysaccharide primarily  
227 sourced from cereal grains, potatoes, tapioca and arrowroot (Cutter 2006; Baldwin and others  
228 1995; Zhang and Liu 2009). Starch consists of amylose and amylopectin molecules present at  
229 different molecular ratios. Amylose is a linear molecule consisting of glucose units connected  
230 by 1,4-glucosidic linkages and amylopectin is a highly branched molecule consisting of short  
231 1,4-glucose chains connected by 1,6-glucosidic linkages (Rodriguez and others 2003;  
232 Maizura and others 2007; Wu and others 1998; Parker and Ring 2001). Starch is a  
233 semicrystalline, very hydrophilic material (Bicerano 2003). The amorphous and crystalline  
234 phases affect the physical and chemical properties of starch-based films such as the  
235 mechanical and gas barrier properties (Liu 2005; Cha and Chinnan 2004). Films  
236 manufactured from starch-based materials have better gas barrier properties than synthetic  
237 polymer films but their mechanical properties are poorer. A high amylose starch polymer can

238 be formed into consistent, relatively strong and flexible films that are highly impermeable to  
239 oxygen and carbon dioxide. This is in contrast to high amylopectin starch polymers, that can  
240 only be formed into non-continuous and brittle films (Gennadios and others 1997; Cha and  
241 Chinnan 2004). As expected, starch alone cannot be formed into films with adequate  
242 properties for food packaging (Phan and others 2005; Arvanitoyannis and Biliaderis 1998).  
243 The intrinsic high level of hydrophilicity, poor mechanical properties and difficulties in  
244 processing limit its applications in food packaging unless modified mechanically, physically,  
245 chemically or genetically (Arvanitoyannis and others 1998; Davis and Song 2006; 1999;  
246 Marron and others 2000; Tharanathan 2003; García and others 2000; Zhang and Liu 2009).  
247 Several studies have demonstrated that modified starch-based materials can be used in  
248 commercial applications to package dry and other solid food products such as biscuits,  
249 snacks, cereals, fresh produce, fruits and vegetables (Cutter 2006; Gennadios and others 1997;  
250 Wong and others 1994; Nisperos-Carriedo 1994; Bravin and others 2006; Avérous and others  
251 2001; Debeaufort and others 1998) and/or products with low water activity (Olivas and  
252 Barbosa-Canovas 2009).

253

254 Table 1 demonstrates that many researchers have made considerable progress by successfully  
255 impregnating starch-based films with natural or synthetic AM agents. Such AM starch-based  
256 films have shown inhibitory activity to the growth of various microorganisms such as *S.*  
257 *enteritidis*, *L. plantarum*, *B. thermosphaceta* B2, and *L. monocytogenes*, *E. coli* O157:H7, *E.*  
258 *coli*, *S. aureus*, *S. typhimurium*. Durango and others (2006) developed an AM film based on  
259 yam starch incorporated with chitosan at different concentrations (1%, 3% and 5% (w/v)) and  
260 reported a significant reduction of *S. enteritidis* in liquid culture by each of the films. Nam  
261 and others (2007) incorporated 1% (w/w) lysozyme into a pea starch film and demonstrated  
262 an AM activity against *B. thermosphaceta* B2. Salleh and others (2007) studied the synergistic

263 effects of wheat starch films incorporated with lauric acid and chitosan and found a  
264 significant AM activity of these films against *B. subtilis* but not against *E. coli*. The authors  
265 claimed that starch-based films inhibited the growth of all tested microorganisms in liquid  
266 culture. The latter observation may be unrealistic in terms of the release of AM agent in the  
267 film because the starch-based film presumably dissolves in the liquid culture medium.

268

269 Baron and Sumner (1993) showed that starch films impregnated with potassium sorbate and  
270 acidified with lactic acid reduced the growth of *S. typhimurium* by 4 log CFU mL<sup>-1</sup> after 2 h at  
271 37°C. The population count of *E. coli O157: H7* decreased by 2 log CFU mL<sup>-1</sup> after 3.5 h at  
272 37°C. Furthermore, they found that corn-starch films impregnated with potassium sorbate  
273 inhibited the growth of *S. typhimurium* and *E. coli O157 H7* on poultry products stored at 7°C  
274 for 12 days. Maizura and others (2008) investigated the antibacterial activity of starch-  
275 alginate film incorporated with lemongrass oil. The AM film inhibited the growth of *E. coli*  
276 *O157:H7* and *S. enteritidis* determined by the agar disc diffusion assay but did not show any  
277 inhibitory effect on the growth of *S. aureus*. A recent study by Shen and others (2010)  
278 showed that sweet potato starch film incorporated with 15% (w/w) potassium sorbate or 5%  
279 (w/w) chitosan resulted in a significant reduction of *E. coli* on solid and semi-solid media  
280 compared to control film containing no potassium sorbate or chitosan that did not inhibit the  
281 growth of *E. coli*. The sweet potato starch film incorporated with 10% (w/w) chitosan  
282 suppressed the growth of *S. aureus*. Corrales and others (2009) showed that pea starch films  
283 impregnated with grape seed extract inhibited the growth of *B. thermosphaceta B2* on pork  
284 loin by 1.3 log CFU mL<sup>-1</sup> within the first 4 days of storage at 4°C compare to the control film.  
285 Pelissari and others (2009) investigated the AM activity of starch-based film incorporated  
286 with oregano essential oil (EO). The use of the AM starch-based film effectively inhibited the  
287 growth of *E. coli O157:H7*, *B. cereus* and *S. enteritidis* in the agar disc diffusion assay.

288

289 Many of the abovementioned studies demonstrated AM activity against various  
290 microorganisms using techniques involving agar-based and liquid culture media.  
291 Unfortunately, the question of the moisture sensitivity of the starch-based materials and the  
292 subsequent usefulness of their films as commercial packaging systems has not been  
293 adequately addressed in the literature to date. Therefore, further research is needed to show  
294 how to diminish the moisture sensitivity and to enhance the physico-mechanical properties of  
295 such starch-based materials so that these can be used for packaging of moist food products.  
296 Although, many starch-based materials incorporated with various AM agents demonstrate  
297 AM activity, an important aspect to be considered is the effect of increasing the concentration  
298 of AM agent on the physico-mechanical properties of the resultant films. Shen and others  
299 (2010) reported a deterioration in the physico-mechanical properties of films upon an increase  
300 in the potassium sorbate concentration. Indeed, such adverse effects could limit the prospects  
301 of applying such films in food packaging applications.

302

303 In many studies the AM activity of other polysaccharide-based materials such as chitosan  
304 incorporated with AM agents has been investigated. Chitosan films have exhibited inhibitory  
305 activity on the growth of various microorganisms, when impregnated with AM agents. For  
306 example, Ojagh and others (2010) developed chitosan films containing 0.4% to 2% (v/v) of  
307 cinnamon EOs and evaluated the AM efficacy of these films against *L. monocytogenes*, *L.*  
308 *plantarum*, *E. coli*, *L. sakei* and *P. fluorescens* in the disc diffusion assay. They reported that  
309 chitosan films containing these concentrations of cinnamon EOs inhibited the growth of all  
310 the tested bacteria on agar media. Li and others (2006) demonstrated that chitosan films  
311 incorporated with 463 international units (IU) of nisin inhibited the growth of *S. aureus*, *L.*  
312 *monocytogenes* and *B. cereus* using the agar diffusion method. However, nisin incorporated

313 into chitosan film had no inhibitory effect against *E. coli*. The later observation is in  
314 agreement with the results of Pranoto and others (2005) who studied the AM effect of  
315 chitosan films impregnated with nisin at different concentrations against *E. coli*. The  
316 impregnated chitosan films were also tested against food pathogens including *S. aureus*, *S.*  
317 *typhimurium*, *L. monocytogenes* and *B. cereus*. In their findings, the AM chitosan film  
318 demonstrated inhibitory effects on *L. monocytogenes*, *S. aureus* and *B. cereus*. Increasing the  
319 concentration of nisin in the film formulation did not improve the AM activity of the film.  
320 Ouattara and others (2000) found that chitosan films containing several organic acids (acetic  
321 and propionic) and cinnamaldehyde reduced the growth of *Enterobacteriaceae*, *Serratia*  
322 *liquefaciens* and *Lactobacillus sakei* on the surfaces of vacuum-packed cured meat products  
323 (bologna, cooked ham and pastrami) after a storage period of 21 days at 4°C. Duan and others  
324 (2008) reported that chitosan films containing lysozyme demonstrated inhibitory activity  
325 against *E. coli* and *L. monocytogenes*. A significant release of lysozyme from the films was  
326 found. The storage conditions (time and temperature) did not affect the water vapour  
327 permeability of the film. Möller and others (2004) studied the AM effectiveness of chitosan-  
328 hydroxypropylmethyl cellulose (HPMC) films, chitosan-HPMC films containing stearic and  
329 citric acids, and chemically modified chitosan-HPMC films. The chitosan-HPMC films, with  
330 and without stearic acid, significantly reduced the growth of *L. monocytogenes*.

331

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

338 Many of the cellulose-based materials and/or their derivatives such as MC, HMPC and  
339 cellulose acetate are already produced commercially. The latter is widely used in the  
340 packaging of baked goods and fresh food products (Weber 2000). Although, there have been a  
341 limited number of studies conducted in the past using MC-based materials and/or their  
342 derivatives, more recently there has been increased recognition of the potential use of such  
343 materials in AM packaging systems for the preservation of food products against microbial  
344 contaminations and for the extension of the shelf life of the packaged products. Several  
345 researchers have investigated the potential use of cellulose-based materials in AM packaging  
346 systems particularly in coating systems as discussed in Section 4.2. For example, Ayana and  
347 Nazan (2009) studied the antibacterial effectiveness of olive leaf extract incorporated into MC  
348 films against *S. aureus* in an agar disc diffusion test and on surfaces of Kasar cheese. The MC  
349 films demonstrated inhibitory activity against *S. aureus* on the agar medium. The films  
350 containing 1.5% (w/v) olive leaf extract decreased the population count of *S. aureus* on the  
351 surface of Kasar cheese by 1.22 log cycles after 14 days of storage. Santiago-Silva and others  
352 (2009) investigated the AM activity of a cellulose-based film incorporated with pediocin.  
353 Using the challenge test on sliced ham inoculated with *L. innocua* and *Salmonella spp.* the  
354 AM cellulose-based film reduced the growth of *L. innocua* by 2 log cycles after 15 days of  
355 storage at 12°C. Similarly, the AM cellulose-based film effectively inhibited the growth of  
356 *Salmonella spp* by 0.5 log cycles after 12 days of storage.

357

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

363 *monocytogenes*. These biodegradable AM films successfully delayed and/or reduced the  
364 growth of *L. monocytogenes* during storage at 6°C for 29 days. Recently, Jin and Zhang  
365 (2008) investigated a PLA film incorporated with nisin. They found that PLA containing nisin  
366 significantly inhibited the growth of *L. monocytogenes* in liquid culture and on liquid egg  
367 white. The PLA-nisin film was more active against the growth of *E. coli O157:H7* in orange  
368 juice than on liquid culture. Rojas-Grau and others (2006) studied the antibacterial  
369 effectiveness of apple puree-based films impregnated with EOs (oregano, cinnamon and  
370 lemongrass) against *E. coli O157:H7*. All the evaluated films containing EOs were reported to  
371 be effective against *E. coli O157:H7* with the antibacterial activity of oregano oil notably  
372 higher than that of lemongrass and cinnamon oils. Kandemir and others (2005) investigated  
373 the AM activity of pullulan-based films incorporated with partially purified lysozyme against  
374 the growth of *E. coli* and *L. plantarum*. The AM pullulan-based films were found to be  
375 effective against *E. coli* but did not show any AM activity against *L. plantarum*. Natrajan and  
376 Sheldon (2000) evaluated the antibacterial effectiveness of calcium alginate and agar-based  
377 films incorporated with nisin against *S. Typhimurium* on broiler skin. Their results showed  
378 that the films containing nisin reduced the population of *S. Typhimurium*.  
379

#### 380 **4.1.2 Antimicrobial Activity of Protein Films Incorporated with AM Agents**

381 Proteins are biopolymeric materials that can be used for the production of biodegradable AM  
382 films as they have good film-forming properties. Protein-based polymers have amino acids as  
383 their monomer units. Packaging films have been manufactured from different proteins, such  
384 as corn zein, wheat gluten, soy protein, whey protein or their derivatives (Hernandez-  
385 Izquierdo and others 2008). Packaging films made from protein-based polymers possess  
386 adequate physico-mechanical properties (Krochta 2002). Whey protein and corn zein  
387 incorporated with natural or synthetic AM agents have been extensively tested *in vitro* and on



388 different food products against the growth of various microorganisms. A summary of the  
389 studies investigating the antibacterial effect of AM protein-based films is also presented in  
390 Table 1. Although these studies are not directly comparable in term of the AM agents tested  
391 or microorganisms tested, the results in general demonstrate that whey protein isolate (WPI)  
392 films can be impregnated with AM agents and have the potential to be used as AM food  
393 packaging materials. However, no information is readily available in the current literature on  
394 the cost/effective benefits of WPI-based films and therefore such information is needed before  
395 fabricating AM films from WPI-based materials for commercial applications.

396

397 Pintado and others (2010) investigated the inhibitory effects of whey protein films  
398 incorporated with nisin, natamycin and malic acid against *P. aeruginosa*, *L. monocytogenes*,  
399 *Y. lipolytica*, *P. roqueforti*, *P. commune* using the agar disc diffusion method. They reported  
400 that whey protein films incorporated with AM agents demonstrated inhibitory effects against  
401 all tested microorganisms. Seydim and Sarikus (2006) tested the AM efficacy of WPI films  
402 incorporated with oregano, rosemary and garlic EOs against *E. coli O157:H7*, *S. aureus*, *S.*  
403 *enteritidis*, *L. monocytogenes*, and *L. plantarum*. The AM whey protein films containing  
404 oregano EOs at 2% (w/w) level demonstrated a higher inhibitory effect against the tested  
405 microorganisms than similar films containing garlic and rosemary extracts. Min and others  
406 (2005) investigated the AM effectiveness of whey protein films containing Lactoperoxidase  
407 evaluated against *L. monocytogenes* using liquid and agar media as well as on smoked  
408 salmon. These films reduced the population of *L. monocytogenes* on smoked salmon by 3 log  
409 CFU g<sup>-1</sup> after 35 days of storage compared with the control film. Gadang and others (2008)  
410 evaluated the AM effectiveness of WPI films incorporated with a combination of nisin, malic  
411 acid, grape seed extract and EDTA against the growth of *L. monocytogenes*, *E. coli O157:H7*,  
412 and *S. typhimurium* inoculated on the surface of a turkey frankfurter. It was found that all the

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

416

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

430

431 Corn zein materials obtained from plant sources are an additional form of proteins that  
432 demonstrate good film-forming properties with the potential of being impregnated with AM  
433 agents in order to preserve food products from microbial contamination. Previous studies  
434 showed that corn zein films containing AM agents demonstrated AM activity against the  
435 growth of various microorganisms both *in vitro* and in various food products. A detailed study  
436 by Hoffman and others (2001) found that corn zein films incorporated with lauric acid, nisin,  
437 EDTA and combinations of these three compounds reduced *L. monocytogenes* in liquid

438 culture, although there was no observed inhibitory effect in films incorporated with EDTA  
439 alone. All the films were reported to be bacteriostatic when a  $10^4$  CFU mL<sup>-1</sup> *S. enteritidis*  
440 initial inoculum was used. Padgett and others (1998) investigated the inhibitory effect of heat-  
441 pressed and cast corn zein films containing lysozyme and nisin and reported significant  
442 inhibition zones for *Lactobacillus plantarum* by the cast film compared to the heat-pressed  
443 films. In another study Padgett and others (2000) found an inhibitory activity of corn zein  
444 films incorporated with various levels of lauric acid and nisin on the growth of *L. plantarum*  
445 in liquid culture. Gücbilmez and others (2007) developed AM films from corn zein  
446 incorporated with lysozyme and albumin proteins. They reported that these films  
447 demonstrated AM activity against the growth of *E. coli* and *B. subtilis*.

448

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

463 containing oregano EO were more effective against the growth of both microorganisms  
464 compared to films containing pimento.

465

#### 466 **4.2 Antimicrobial Activity of Biodegradable Films Coated with AM Agents**

467 In addition to the direct incorporation of AM agents into packaging films discussed above,  
468 AM agents can be coated on the surface of packaging materials in order to provide a high  
469 concentration of the agent in contact with the surface of food product (Gennadios and others  
470 1997; An and others 2000). The application of an AM agent on a packaging material can be  
471 achieved by using various coating techniques including immersion of the substrate or by  
472 spraying the substrate with a coating/carrier solution. For this purpose, the AM agent is  
473 dissolved in an appropriate solvent such as water, ethanol or isopropanol before applying it to  
474 the packaging material (Krochta 2002). Little has been reported on the activity of AM agents  
475 coated on biodegradable polymers. Some of the relevant studies are given in Table 1.

476

477 Miltz and others (2006) studied the effectiveness of a corn starch-based film coated with the  
478 peptide dermaseptin S4 derivative as an AM agent against moulds and aerobic bacteria on  
479 cucumbers. They reported that this system was very effective. Coma and others (2001) found  
480 that cellulose films coated with nisin inhibited *L. innocua* and *S. aureus* on laboratory media.  
481 Chen and others (1996) prepared AM films containing 2% or 4% (w/w) of sodium benzoate  
482 and potassium sorbate by casting MC, chitosan and their mixtures. They evaluated the  
483 antimycotic activity of the AM films against *Rhodotorula rubra* and *Penicillium notatum* and  
484 found that MC and MC/chitosan films containing 2% and 4% (w/w) sodium benzoate and  
485 potassium sorbate respectively inhibited the growth of these microorganisms. Ming and  
486 others (1997) reported that a cellulose casing coated with pediocin completely inhibited the  
487 growth of *L. monocytogenes* on ham, turkey breast and beef products [compared to the control](#)

488 film after 12 weeks of storage at 4°C. Janes and others (2002) investigated the AM effect of  
489 corn zein films coated with nisin and/or 1% (w/w) calcium propionate against *L.*  
490 *monocytogenes* inoculated on ready-to-eat chicken samples and found that the coated films  
491 inhibited the growth of the microorganism. Kim and others (2008) evaluated recently the AM  
492 effectiveness of chitosan and WPI coated with lysozyme against the growth of *L.*  
493 *monocytogenes* and *S. enteritidis* inoculated on hard-boiled eggs. The Chitosan-lysozyme  
494 system controlled the growth of *S. enteritidis* on hard-boiled shell-on and on peeled eggs.  
495 Siragusa and Dickinson (1992; 1993) found that calcium alginate coatings and films  
496 containing organic acids effectively reduced the population of *L. monocytogenes*, *S.*  
497 *typhimurium* and *E. coli O157:H7* on the surface of beef carcass.

498

#### 499 **4.3 Antimicrobial Activity of Biodegradable Films with Immobilised AM Agents**

500 Effective AM packaging systems can also be achieved by the immobilisation of an AM agent  
501 in a polymeric material. According to Steven and Hotchkiss (2003), the AM agents that can  
502 be immobilised include peptides, proteins or enzymes. These agents can be synthesised on the  
503 surface or extracted separately and then covalently linked to the polymer substrate. An AM  
504 agent that is covalently immobilised onto the packaging material is not released but becomes  
505 effective in inhibiting microbial growth when in contact with the surface of the packaged food  
506 product (Han 2003). Different studies have been conducted focusing on immobilisation of  
507 AM agents onto packaging materials. Appendini and Hotchkiss (1997) investigated the  
508 efficiency of lysozyme immobilised on polyvinyl alcohol (PVOH) beads, nylon 6,6 pellets  
509 and cellulose triacetate (CTA) films. They reported that the viability of *Micrococcus*  
510 *lysodeikticus* was reduced in the presence of immobilised lysozyme on CTA film that was  
511 found to show the highest AM activity amongst the studied structures. Cutter and Siragusa  
512 (1997) assessed the potential decontamination of raw beef by applying organic acids (lactic or

513 acetic acid) immobilized onto calcium alginate films. They reported a considerable reduction  
514 of *L. monocytogenes* growth with the treated films compared to a calcium alginate film  
515 without acid treatment. Cutter and Siragusa (1996) studied the AM activity of nisin  
516 immobilised onto calcium alginate films against *Brochothrix thermosphacta* on beef surfaces.  
517 They found that calcium alginate films treated with nisin suppressed the growth of *B.*  
518 *thermosphacta* by 2.42 log CFU cm<sup>-2</sup> after 7 days compared to an untreated film. A greater  
519 and steady nisin activity was found when the tissues were ground and stored under  
520 refrigerated conditions in the AM immobilized film for up to 7 days compared to the use of  
521 sprayed nisin only.

522

## 523 **5 Summary**

524 Consumer demands and requirements by regulatory agencies to use more environmentally-  
525 friendly and less polluting packages have directed researchers to look at packaging materials  
526 that are derived from natural or made from renewable resources to replace, at least some, of  
527 the synthetic polymers. Biodegradable materials derived from polysaccharides and proteins,  
528 when combined with AM agents, have the potential to be manufactured into food packaging  
529 films with effective AM properties. Polysaccharide-based materials with AM agents,  
530 particularly the starch-based ones, have been studied extensively with some commercial  
531 success in the food packaging industry. Many of the studies were carried out in order to  
532 obtain a "proof of concept" by measuring the inhibition zones created by the diffusion of the  
533 AM agent in solid media. Some modified biodegradable polymers such as starch-based  
534 materials can be manufactured into films and used to package dry and/or solid food products  
535 such as biscuits, snacks, cereals, fresh produce, fruits and vegetables. Developing commercial  
536 biodegradable films with improved physical and mechanical properties is still a challenge due  
537 to their hydrophilic nature that limits their application for packaging of food products with a

538 high water activity. The biodegradable and bio-compostable materials are also, many times,  
539 more expensive and more difficult to process, a fact that further increases their cost compared  
540 to synthetic polymers. However, when considering the cost of a package, the total "cradle to  
541 grave" economic approach should be evaluated. Thus, the economic evaluation should include  
542 not only the cost of the packaging material and of processing the material into a package but  
543 also the cost of disposing of the final package namely, recycling and/or incineration and/or  
544 land filling. This is very important especially for the last option, taking into consideration the  
545 decreasing number of land filling sites and the diminishing space for garbage disposal in the  
546 developed countries. If such considerations are taken into account, the difference between the  
547 cost of biodegradable/bio-compostable and synthetic polymers becomes much smaller.  
548 Antimicrobial packaging films with improved physical and mechanical properties could be  
549 prepared from biodegradable polymers that have been modified and/or blended with other  
550 compatible materials incorporated or coated with AM agents. However, additional research  
551 and development work is required to reduce the moisture sensitivity of these polymers,  
552 enhance their physical properties and improve their process-ability. These goals can be  
553 achieved by proper blending with appropriate materials and/or by copolymerization.  
554 Biodegradable materials could also be successfully prepared and applied in AM packaging  
555 systems by the incorporation of appropriate AM agents. Taking into consideration that the  
556 public, as a whole, is already conscious (and becomes even more so as times go by) to the  
557 environment, it is conceivable that the future will see more biodegradable and AM  
558 biodegradable polymers and/or their devivatives in the packaging of food, agricultural and  
559 other products.

560

**Table 1: Antimicrobial activity of AM agents in biodegradable materials**

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



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

Packaging Material	Antimicrobial Agent	Loading	Applic- ation <sup>a</sup>	Substrate	Microorganism(s)	Observations	References
Chitosan	Garlic oil	1-4 × 10 <sup>2</sup> µg/g	IN	Agar method	<i>E. coli</i> , <i>S. aureus</i> , <i>S. typhimurium</i> , <i>L. monocytogenes</i> and <i>B. cereus</i>	Clear zone of inhibition against <i>S. aureus</i> , <i>L. monocytogenes</i> and <i>B. cereus</i>	Pranoto and others (2005)
Chitosan	Nisin	5.1-204 × 10 <sup>3</sup> IU/g chitosan	IN	Agar method	<i>E. coli</i> , <i>S. aureus</i> , <i>S. typhimurium</i> , <i>L. monocytogenes</i> and <i>B. cereus</i>	Film inhibited growth of <i>S. aureus</i> , <i>L. monocytogenes</i> and <i>B. cereus</i>	Pranoto and others (2005)
Chitosan	Potassium sorbate	50-200 mg/g	IN	Agar method	<i>E. coli</i> , <i>S. aureus</i> , <i>S. typhimurium</i> , <i>L. monocytogenes</i> and <i>B. cereus</i>	Demonstrated AM activity against <i>S. aureus</i> , <i>L. monocytogenes</i> and <i>B. cereus</i>	Pranoto and others (2005)
Chitosan	Propionic acid	1% (w/v)	IN	Ham, bologna, pastrami	<i>S. liquefaciens</i> , <i>L. sakei</i>	All films reduced growth of <i>S. liquefaciens</i> for all the storage period.	Ouattara and others (2000)
Chitosan	Lysozyme	60% (w/w)		Agar media	<i>E. coli</i> and <i>L. monocytogenes</i>	AM activity against <i>E. coli</i> and <i>L. monocytogenes</i>	Duan and others (2008)

Packaging Material	Antimicrobial Agent	Loading	Applic- ation <sup>a</sup>	Substrate	Microorganism(s)	Observations	References
					<i>monocytogenes</i>		
Chitosan-HPMC	Chitosan	0.5-2% (w/v)		Agar method	<i>L. monocytogenes</i>	Inhibited <i>L. monocytogenes</i>	Möller and others (2004)
PLA	Nisin	0.25 g/mL	IN	Liquid culture, orange juice, egg white	<i>E. coli O157:H7</i> , <i>S. enteritidis</i> , and <i>L. monocytogenes</i>	Films reduced growth of <i>E. coli O157:H7</i> , <i>S. enteritidis</i> , and <i>L. monocytogenes</i>	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	<i>L. monocytogenes</i> , <i>E. coli</i> , <i>E. faecalis</i> , <i>E. faecium</i> , <i>S. typhimurium</i> , and <i>B. thermosphaceta B2</i>	Reduced growth of <i>thermosphaceta B2</i> 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	<i>B. subtilis</i> and <i>E. coli</i>	Inhibited <i>B. subtilis</i> and <i>E. coli</i>	Salleh and others (2007)
Starch film	Chitosan	5-15% (w/w)	IN	Agar media and semisolid	<i>E. coli</i> and <i>S. aureus</i>	Inhibited both <i>E. coli</i> and <i>S. aureus</i>	Shen and others (2010)

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

Packaging Material	Antimicrobial Agent	Loading	Applic- ation <sup>a</sup>	Substrate	Microorganism(s)	Observations	References
					<i>typhimurium</i> , and <i>B. thermosphaceta B2</i>	Gram-negative bacteria	
<b><i>Protein Films</i></b>							
Corn zein	Calcium-propionate	1% (w/w)	C	Ready-to-eat chicken	<i>L. monocytogenes</i>	Coated films suppressed <i>L. monocytogenes</i> growth	Janes and others (2002)
Corn zein	Lysozyme	479-958 µg/cm <sup>2</sup>	IN	Agar media	<i>E. coli</i> and <i>B. subtilis</i>	Effective against <i>E. coli</i> and <i>B. subtilis</i>	Güçbilmez and others (2007)
Corn zein	Nisin	1 × 10 <sup>3</sup> IU/g	C	Ready-to-eat chicken	<i>L. monocytogenes</i>	Coated films reduced <i>L. monocytogenes</i> growth	Janes and others (2002)
Corn zein	Lauric acid	200 mg	IN	Liquid culture	<i>L. monocytogenes</i> , and <i>S. enteriditis</i>	Significant effect against <i>L. monocytogenes</i> but not against <i>S. enteriditis</i>	Hoffman and others (2001)
Corn zein	Nisin	0.188 mg	IN	Liquid culture	<i>L. monocytogenes</i> , and <i>S. enteriditis</i>	Reduced counts of <i>L. monocytogenes</i> , <i>S. enteriditis</i>	Hoffman and others (2001)
Proteins-based film	Oregano EOs	1% (w/v)	IN	Beef muscle slices	<i>Pseudomonas spp.</i> and <i>E. coli H0157:H7</i>	Films containing oregano reduced 0.95 and 1.12 log of <i>P. spp.</i> and <i>E. coli H0157:H7</i> respectively, after 7 days	Oussalah and others (2004)

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

Packaging Material	Antimicrobial Agent	Loading	Applic- ation <sup>a</sup>	Substrate	Microorganism(s)	Observations	References
Soy protein Corn zein	Nisin	0.01-6 mg/g of film	IN	Agar and liquid media	<i>L. plantarum</i> and <i>E. coli</i>	Inhibited <i>L. plantarum</i> and <i>E. coli</i> .	Padgett and others (1998; 2000)
Soy protein isolate	EDTA	0.16% (w/w)	IN	Liquid or solid media	<i>E. coli O157:H7</i> , <i>S. typhimurium</i> , and <i>L. monocytogenes</i>	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	<i>E. coli O157:H7</i> , <i>S. typhimurium</i> , and <i>L. monocytogenes</i>	Reduced population of <i>E. coli O157:H7</i> , <i>S. typhimurium</i> , <i>L. monocytogenes</i>	Sivarooban and others (2008)
Soy protein isolate	Nisin + EDTA	1 × 10 <sup>3</sup> IU/g	IN	Liquid or solid media	<i>E. coli O157:H7</i> , <i>S. typhimurium</i> , and <i>L. monocytogenes</i>	Reduced population of <i>E. coli O157:H7</i> , <i>S. typhimurium</i> , <i>L. monocytogenes</i>	Sivarooban and others (2008)
Soy protein isolate films	Nisin	3-12 × 10 <sup>4</sup> IU/15mL	IN	liquid culture media	<i>L. monocytogenes</i>	Inhibition against <i>L. monocytogenes</i> was concentration dependent	Ko and others (2001)
Whey protein	Lactoperoxidase	0.01-0.4 (w/v)	IN	Agar and liquid culture	<i>L. monocytogenes</i>	Reduced population of <i>L.</i>	Min and others (2005)

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



Packaging Material	Antimicrobial Agent	Loading	Applic- ation <sup>a</sup>	Substrate	Microorganism(s)	Observations	References
					<i>enteritidis</i>	growth of <i>S. enteritidis</i>	
Whey protein isolate	Garlic oil	1-4% (w/v)	IN	Agar method	<i>E. coli O157:H7</i> , <i>S. aureus</i> , <i>S. enteritidis</i> , <i>L. monocytogenes</i> , and <i>L. plantarum</i>	Garlic oil inhibit <i>E. coli O157:H7</i> , <i>S. aureus</i> , <i>S. enteritidis</i> , <i>L. monocytogenes</i> , and <i>L. plantarum</i> at 3-4%	Seydim and Sarikus (2006)
Whey protein isolate	Grape seed extract	1.2-3.6 × 10 <sup>3</sup> ppm	IN	Turkey frankfurter	<i>L. monocytogenes</i> , <i>E. coli O157:H7</i> , and <i>S. typhimurium</i>	Ineffective against <i>L. monocytogenes</i> , <i>E. coli O157:H7</i> but inhibited growth of <i>S. typhimurium</i>	Gadang and others (2008)
Whey protein isolate	Malic acid	1.2-3.6 × 10 <sup>3</sup> ppm	IN	Turkey frankfurter	<i>L. monocytogenes</i> , <i>E. coli O157:H7</i> , and <i>S. typhimurium</i>	Ineffective against <i>L. monocytogenes</i> , <i>E. coli O157:H7</i> but inhibited growth of <i>S. typhimurium</i>	Gadang and others (2008)
Whey protein isolate	Nisin	6-18 × 10 <sup>3</sup> IU/g	IN	Turkey frankfurter	<i>L. monocytogenes</i> , <i>E. coli O157:H7</i> , and <i>S. typhimurium</i>	Ineffective against <i>L. monocytogenes</i> , <i>E. coli O157:H7</i> but inhibited growth of <i>S. typhimurium</i>	Gadang and others (2008)
Whey protein isolate	Oregano	1-4% (w/v)	IN	Agar method	<i>E. coli O157:H7</i> , <i>S.</i>	Oregano demonstrated Inhibitory effect against <i>E. coli</i>	Seydim and Sarikus (2006)

Packaging Material	Antimicrobial Agent	Loading	Applic- ation <sup>a</sup>	Substrate	Microorganism(s)	Observations	References
					<i>aureus</i> , <i>S. enteritidis</i> , <i>L. monocytogenes</i> , and <i>L. plantarum</i>	<i>O157:H7</i> , <i>S. aureus</i> , <i>S. enteritidis</i> , <i>L. monocytogenes</i> , and <i>L. plantarum</i> at 3-4%	
Whey protein isolate	p-aminobenzoic acid	0.5-1.5% (w/v)	IN	Agar media	<i>L. monocytogenes</i> , <i>E. coli O157:H7</i> , and <i>S. Typhimurium DT104</i>	Inhibited <i>L. monocytogenes</i> , <i>E. coli O157:H7</i> , <i>S. Typhimurium</i>	Cagri and others (2001)
Whey protein isolate	p-aminobenzoic acid	0.5-1% (w/v)	IN	Bologna summer sausage	<i>L. monocytogenes</i> , <i>E. coli O157: H7</i> , and <i>S. Typhimurium DT104</i> .	Reduced <i>L. monocytogenes</i> by log 1.5-3.4 on bologna slices and increase by log 2.2 under control after 21 days. Population of <i>E. coli O157: H7</i> decrease by log 2.7-3.6	Cagri and others (2002)
Whey protein isolate	Rosemary	1-4% (w/v)	IN	Agar method	<i>E. coli O157:H7</i> , <i>S. aureus</i> , <i>S. enteritidis</i> , <i>L. monocytogenes</i> , and <i>L. plantarum</i>	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	<i>L. monocytogenes</i> , <i>E. coli O157:H7</i> , and <i>S.</i>	Inhibited <i>L. monocytogenes</i> , <i>E. coli O157:H7</i> , <i>S. Typhimurium DT104</i>	Cagri and others (2001)

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

<sup>a</sup> 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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