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1 **Full Title**

2 Physical Performance of Biodegradable Films Intended for Antimicrobial Food
3 Packaging

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18 **Short version of title:** Performance of biodegradable films (...)

19 **Choice of journal section:** Food Engineering and Physical Properties

Abstract

Antimicrobial films were prepared by including enterocins to alginate, polyvinyl alcohol (PVOH) and zein films. The physical performance of the films was assessed by measuring colour, microstructure (SEM), water vapour permeability (WVP) and tensile properties. All studied biopolymers showed poor WVP and limited tensile properties. PVOH showed the best performance exhibiting the lowest WVP values, higher tensile properties and flexibility among studied biopolymers. SEM of antimicrobial films showed increased presence of voids and pores as a consequence of enterocin addition. However, changes in microstructure did not disturb WVP of films. Moreover, enterocin containing films showed slight improvement compared to control films. Addition of enterocins to PVOH films had a plasticizing effect, by reducing its tensile strength and increasing the strain at break. The presence of enterocins had an important effect on tensile properties of zein films by significantly reducing its brittleness. Addition of enterocins thus proved not to disturb the physical performance of studied biopolymers. Development of new antimicrobial biodegradable packaging materials may contribute to improving food safety while reducing environmental impact derived from packaging waste.

Keywords: biodegradable films, antimicrobial, packaging, enterocins, physical properties.

Practical application: Development of new antimicrobial biodegradable packaging materials may contribute to improving food safety while reducing environmental impact derived from packaging waste.

Introduction

Transformation of dietary habits has resulted in an increasing demand by consumers for ready to eat food products conveniently packed. The resulting environmental impact of the high consumption of plastic materials in the food industry has encouraged special efforts from the packaging industry to develop biodegradable packaging materials. Novamont (Italy), Fkur Kunststoffe Gmbh (Germany), and Nodax (USA), are examples of companies who commercialise packaging materials manufactured with bio-based materials (Liu 2006). Biodegradable packaging materials may be extracted directly from natural sources (e.g. alginate and zein), can be produced by classical chemical synthesis (e.g. polyvinyl alcohol), or can be obtained through microbial action (e.g. polyhydroxyalkanoate).

Alginates are hydrophilic polysaccharides derived from seaweed. Alginates possess good film-forming properties that make them particularly useful in food applications (Nisperos-Carriedo 1994; Cutter 2006; Ashikin and others 2010). Alginates, having negatively charged groups, can form three dimensional networks when react with divalent cations such as calcium (Gennadios and others 1997; Cutter and Sumner 2002).

Zein is a major storage protein of corn. As a result of its low polar amino acid content zein is insoluble in water but soluble in alcohol. Zein films are generally brittle and the use of plasticizers is necessary to obtain flexible films (Soliman and Furuta 2009). Zein has shown great potential for use as edible coatings and packaging material (Buffo and Han 2005). Polyvinyl alcohol (PVOH) is a synthetic water soluble polymer, obtained from the hydrolysis or partial hydrolysis of polyvinyl acetate. PVOH has good film forming properties, which offers good tensile strength, flexibility and barrier properties to oxygen and aroma (DeMerlis and Schoneker 2003). The USDA and EFSA have

reported that the use of PVOH as food coatings does not imply a safety risk (EFSA 2005; FDA 2004)

Antimicrobial packaging is a further potential application of biodegradable materials. Antimicrobial packaging can extend the shelf life and safety of food products by preventing the growth of both pathogenic and spoilage microorganisms (Han 2000; Quintavalla and Vicini 2002). Several studies have reported the antimicrobial effects of biodegradable films with added bacteriocins (Ming and others 1997; Natrajan and Sheldon 2000; Coma and others 2001). Previous studies pointed out that antimicrobial alginate, zein and PVOH films incorporated with enterocins A and B effectively improved the safety of sliced cooked ham (Marcos and others 2007, 2008). The knowledge of the effects of enterocin incorporation on the physical properties of films, though, is of great importance for their subsequent applications to food packaging. Thus, the objective of the present study was to assess the implications of enterocins addition to the physical properties of alginate, zein and PVOH films.

Materials and Methods

Enterocin production

Enterococcus faecium CTC492, isolated from a meat product and producer of enterocins A and B (Aymerich and others 1996; Casaus and others 1997) was grown in modified MRS broth. The composition of standard MRS was modified as follows: reduction of glucose to 0.5%, increase of Tween 80 (Sigma-Aldrich, Saint Louis, MO, USA) to 0.75%, without addition of beef extract. Enterocins were obtained from a 2 litre culture grown for 15 h at 30°C. The cells were removed by centrifugation at 10,000 g for 10 min at 4°C. Ammonium sulphate (300 g/L) was added to the supernatant. The protein precipitate was pelleted by centrifugation at 10,000 g for 30 min and further

dissolved in 50 mM phosphate buffer, pH 6. An additional heat treatment of 10 min at 100°C was applied in order to inactivate any surviving *Enterococcus* cells. The obtained bacteriocins were stored at -80°C.

Antimicrobial activity measurement

The indicator strains, *L. monocytogenes* CTC1010, CTC1011, and CTC1034 were separately grown overnight in Tryptic Soy Broth with 0.6% yeast extract (TSBYE; Merck, Darmstadt, Germany) at 30°C.

Bacteriocin activity was quantified by the agar spot test (Tagg and others 1976). A solid medium composed of 20 g/L beef extract, 20 g/L glucose, and 15 g/L agar, was used to support soft TSBYE (TSBYE with 7.5 g/L agar) seeded with 20 µl of the overnight mixture of *L. monocytogenes*. Enterocin samples were serially diluted two-fold with 50 mM phosphate buffer (pH 6). A 10 µl sample of each dilution was spotted onto soft TSBYE lawn. The plates were incubated overnight at 30°C. An arbitrary unit (AU) was defined as the highest dilution showing growth inhibition of the indicator lawn, and bacteriocin activity was expressed as AU/ml.

Film manufacturing

Film forming solutions were obtained as suggested by Del Nobile and others (2003) and Buonocore and others (2005) with some modifications. Alginate solutions were obtained by stirring for 2 h at 80°C a 5% (w/v) alginic acid (Sigma-Aldrich) solution in distilled water. Glycerol (5% v/v) was added as plasticizer, and the solution was further stirred at ambient temperature for 30 min. Zein solutions, 31% (w/v) zein (Sigma-Aldrich) in ethanol, were dissolved stirring the solution for 2 h at 80 °C. Glycerol (5% v/v) was added, and the solution was further stirred at ambient temperature for 30 min. Polyvinyl alcohol solutions (PVOH), 13% (w/v) polyvinyl alcohol (MW=70,000-

100,000; Sigma-Aldrich) in distilled water, were dissolved by autoclaving the solution for 20 min at 121 °C. After measuring the volume of the film blend, the active solution was obtained by adding the appropriate dilution from the stock solution of enterocins (409,600 AU/ml) to obtain a concentration of 2,000 AU/cm². The solution was stirred at ambient temperature until completely dissolved. The films were manufactured by casting the polymer solutions onto 20×20 cm glass surfaces using a thin-layer chromatography (TLC) plate coater (CAMAG, Muttenz, Switzerland). The gate of the TLC coater was fixed at 1.5 mm to control the thickness of the films. The films were dried at ambient conditions under a laminar flow hood until complete evaporation of the solvent, and peeled off after 48 h. Alginate films were reticulated by immersion in a 2% (w/v) calcium chloride solution. The thickness of the films obtained was measured by means of a Digimatic Micrometer (Mitutoyo, Japan). The value of the film thickness was obtained by averaging 10 measurements.

Colour measurement

Instrumental colour measurement of biodegradable films was performed using a Minolta Chromameter CR200 (Minolta, Japan). C illuminant and 2° standard observer were chosen. L* (lightness), a* (redness), and b* (yellowness) colour values were determined in the 1976 CIELAB system. The colorimeter was calibrated before each series of measurements using a white ceramic plate. The mean of six measurements was recorded for each film. Three independent films from each type were tested.

Scanning electron microscopy

Scanning electron microscopy (SEM) was used to examine the surface and cross-section of the films. Samples were freeze dried and mounted on stubs with double-sided adhesive tape and coated with carbon under vacuum. Samples were observed with a

field emission scanning electron microscope (Hitachi S-4100, Hitachi High-Technologies, Toronto, Canada) with an accelerating voltage of 5 or 10 kV.

Water vapour permeability

A PERMATAN-W® Model 3/33 (Mocon, Minneapolis, USA) was used to measure the water vapour transmission rates through the films according to the standard method ASTM F1249-06. The water vapour transmission rates were determined at 23°C and 98% RH. Water vapour permeability (WVP) was calculated by multiplying the steady state water vapour transmission rate by the average film thickness determined as described above and dividing by the water vapour partial pressure difference across the films:

$$WVP = \frac{WVTR \times t}{P_w}$$

Where WVTR: water vapour transmission rate, t: film thickness, P_w : water vapour partial pressure gradient across the film. Units for WVP were $\text{g} \times \text{mm} \times \text{m}^{-2} \times \text{h}^{-1} \times \text{kPa}^{-1}$. Three independent films from each type were tested.

Tensile properties

A MTS Texture Analyser (MTS Systems Corporation, MN, USA) was used to carry out a Texture Profile Analysis. Film strips (8×3 cm) were placed in the grips of the testing machine, set to an initial separation of 100 mm. Crosshead speed was set at 100 mm/min. Tensile strength (MPa) and strain at break (%) were calculated from the load-deformation curves of tensile measurements. Measurements represent an average of at least 5 replicates. Three independent films from each type were tested.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra of biodegradable films were recorded using a Bomem MB120 spectrophotometer equipped with a Spectra-Tech Analytical Plan microscope. A diamond cell was used as a sample holder. The spectrometer has a KBr beamsplitter and a Glowbar source. The microscope has its own mercury cadmium telluride (MCT) detector refrigerated with liquid nitrogen. The analysis was performed within the spectral region of 4000-720 cm^{-1} with a resolution of 4 cm^{-1} and an accumulation of 100 scans. After attenuation of total reflectance and correction of the baseline, FTIR data was **pre-treated** using the SNV (standard normalised variate). Resulting spectra were compared in order to evaluate the effect of adding enterocins to the films.

Statistical analysis

Data were subjected to analysis of variance using the General Linear Model procedure from the SAS statistical package (SAS © System for Windows, Release 9.1, SAS Institute, Cary, NC, USA). The model included film type, enterocin addition and their interaction as fixed effects. Differences between effects were assessed by the Tukey test ($p < 0.05$).

Results and discussion

Alginate, zein, and PVOH were chosen as carrier polymers for antimicrobial packaging because of their good film-forming properties (Lai and Padua 1997; Hemeda and others 2003; Rhim 2004). Preliminary tests (data not shown) were conducted in order to determine the maximum concentration of enterocins that could be included in the polymer blend without disturbing film formation. Incorporation of enterocins at a concentration of 2,000 AU/ cm^2 to all studied biopolymers proved to form homogenous films. Moreover, these films showed antimicrobial action against *L. monocytogenes*

both *in vitro* and through food packaging (Marcos and others 2007). However, inclusion of antimicrobials could disrupt the physical properties of the studied materials affecting their subsequent application as packaging materials. The physical performance of biodegradable films with added enterocins was assessed.

The interaction between film type and enterocin addition was significant for water vapour permeability, colour and tensile properties, indicating that the inclusion of enterocins influenced the physical properties of the studied films differently depending on the type of polymer. Therefore, the effect of enterocin addition on film properties was studied separately for each film type.

Colour measurements

Colour is an important property of films intended for food packaging as its aspect could influence consumer acceptance of the product. The appearance of control alginate films reticulated with calcium chloride solution was white and opaque. PVOH films were transparent, and zein films were yellow and opaque. The visual examination of the films showed that alginate and PVOH films incorporated with enterocins took a brownish appearance. This appreciation was confirmed by instrumental colour analysis of the films. Table 1 shows a significant decrease of L* values (lightness) and an increase of b* values (yellowness) in enterocin containing alginate and PVOH films, compared with control films. On the other hand, probably due to its naturally stronger coloration, no colour changes ($p>0.05$) were observed in zein films with added enterocins.

Scanning electron microscopy (SEM)

Differences in film morphology by the addition of enterocins were investigated by scanning electron microscopy. The micrographs of film surfaces showed a relatively smooth and continuous surface, and no differences due to the inclusion of enterocins

were detected (data not shown). The SEM images of film cross-sections, though, gave information about the changes on the microstructure of films induced by the incorporation of enterocins. Control alginate films showed a compact structure when observed with SEM (Figure 1a). Inclusion of enterocins to alginate films lead to the formation of void spaces as observed in Fig. 1d. Alginate film structure is obtained through the interaction of COOH groups present in alginate polymer with calcium ions added for reticulation. The observed disruptions in alginate film microstructure suggest that the inclusion of enterocins, which are cationic peptides, could have disturbed the formation of a cross-linked network. Dramatic morphology changes in alginate beads as a consequence of addition of cationic drugs have been previously reported (Stockwell and others 1986; Segi and others 1989; Gombotz and Wee 1998). The authors attributed morphology disruptions to the fact that cationic compounds would Ca^{2+} in the interaction with COOH groups. Figure 1b, cross-sections of control PVOH films, shows a dense and homogenous structure, typical of PVOH films (Chen and others 2008). Addition of enterocins to PVOH films resulted in the formation of some cavities on film cross-sections (Fig. 1e). Finally, the cross-section micrographs of zein films (Fig. 1c) showed a porous structure typical for this type of films (Padgett and others 1998; Mastromatteo and others 2009). Addition of enterocins increased significantly both the number and size of pores of zein films (Fig. 1f). Other authors have reported increased porosity in zein films with added antimicrobials such as thymol and lysozyme (Güçbilmez and others 2007; Del Nobile and others 2008).

The reported images suggest a disruption of the polymeric matrix as a result of the inclusion of enterocins during film formation. Among the polymer films studied, the microstructure of PVOH was least affected by the inclusion of enterocins

237 *Water vapour permeability (WVP)*

238 Figure 2 summarizes water vapour permeability values of studied films. Biopolymer
239 films are generally highly polar polymers, such as proteins and polysaccharides having
240 large degrees of hydrogen bonding and containing hydroxyl groups, which have poor
241 moisture barrier properties (Mendieta-Taboada and others 2008). In fact, all studied
242 films showed WVP higher than $0.42 \text{ g}\times\text{mm}\times\text{m}^{-2}\times\text{h}^{-1}\times\text{kPa}^{-1}$ values that, according to the
243 classification of film properties proposed by Krochta and De-Mulder-Johnston (1997),
244 would provide a poor moisture barrier.

245 PVOH films exhibited the best moisture barrier properties (Fig. 2), with values in the
246 limit of moderate to poor moisture barrier (Krochta and De-Mulder-Johnston 1997).

247 PVOH is a synthetic polymer prepared by partial or complete hydrolysis of polyvinyl
248 acetate. The lower WVP observed for this type of film could be due to the formation of
249 denser 3D networks, what would agree with SEM images, which show a most compact
250 and uniform structure for PVOH films compared to the other film types (Fig. 1b). Zein
251 films presented an intermediate WVP in relation to the others studied. Zein is a natural
252 alcohol-soluble protein that forms films with a relatively low WVP when compared to
253 other biopolymers mainly because of its hydrophobic nature (Ghanbarzadeh and others
254 2006; Tihminlioglu and others 2010). Finally, films prepared with alginate, a natural
255 hydrophilic polysaccharide, showed the highest WVP among all studied films (Fig. 2).

256 The WVP of studied biofilms was not disturbed by enterocin inclusion. Moreover,
257 addition of enterocins induced a reduction of WVP ($p<0.01$), that means an
258 improvement of water barrier properties of films (Fig. 2). The decrease in WVP even if
259 small in number would be important for films like the studied ones with poor barrier
260 properties. Enterocin containing PVOH films indeed presented WVP values in the range
261 considered as moderate water barrier.

Permeability is supposed to be influenced by the hydrophobic or hydrophilic nature of the material, by the presence of voids or cracks, and by steric hindrance and tortuosity in the structure (Wang and Padua 2005). It seems that the already described increase of voids and pores in enterocin containing films would not have altered WVP.

The water vapour transfer generally occurs through the hydrophilic portion of the film and it is believed to be dependent upon the number of available polar (-OH) groups in the polymer (Hambleton and others 2009). Several authors have reported that changing the hydrophilic part of the polymer through chemical cross-linking would alter the permeability of PVOH to water (Krumova and others 2000; Westedt and others 2006). The reported results suggest that enterocins could have reacted with available OH groups in PVOH improving cross-linking and thus reducing WVP.

Tensile properties

Tensile properties are important mechanical characteristics of films that indicate the ability to maintain the film integrity under the stress occurring during food processing, handling, and storage of packaged materials (Rhim and Lee 2004). Tensile strength (TS), expresses the maximum stress developed in a film during tensile testing (Gennadios and others 1994). Strain at break (SB) shows the flexibility and extensibility of films and is important for shaping of films (Krochta and De-Mulder-Johnston 1997). As expected, both tensile parameters showed a significant positive correlation ($r = 0.634$, $p < 0.01$). Measurements of TS and SB of control and antimicrobial films are summarised in Table 2. The range of values observed, indicate that zein and alginate films presented TS and SB below 10 MPa and 10 %, respectively, values that indicate poor mechanical properties according to Krochta and De-Mulder-Johnston (1997) film classification. On the other hand, PVOH films showed moderate mechanical properties.

The use of PVOH films blended with natural biopolymers indeed have been suggested to enhance their physical properties (Park and others 2001; Srinivasa and others 2003). The inclusion of enterocins during film forming affected all film types to some extend (Table 2). Enterocin containing PVOH films exhibited improved tensile properties in relation to control films. The observed reduction in tensile strength and increase in SB parameters due to the presence of enterocins would suggest that enterocins would have behaved as plasticisers. Plasticisers function by weakening the intermolecular forces between adjacent polymer chains, and thereby decreasing TS and increasing film flexibility (Mendieta-Taboada and others 2008). Zein enterocin containing films showed a significant increase of both TS and SB. Zein films are typically very brittle and difficult to manipulate. Therefore it is important to highlight the great improvement registered on flexibility (16.5% increase of SB) registered by the addition of enterocins. Finally, tensile properties of alginate films were the least affected by addition of enterocins (Table 2). The decrease in TS, even if small, was significant ($p<0.05$). The result would confirm, as previously stated, that the presence of enterocins in the alginate blend could have disturbed to some extend the formation of a cross-linked network. Norajit and others (2010) also suggested that reduced TS observed in alginate films containing gingseng extract might have resulted from differences in cross-linking. Moreover, this idea would agree with the presence of voids observed with SEM in enterocin containing alginate films that could contribute to the slight reduction on film toughness.

FTIR spectroscopy

The FTIR spectra of control films and films with added enterocins are shown in Figure 3. Differences among control and enterocin added PVOH and alginate films were observed (Fig. 3). Absorption bands for alginate were observed between 3600 and 3200

cm⁻¹, which are linked to the O-H stretching from inter- and intra-molecular hydrogen bonds (Senna and others 2010). The FTIR spectra of alginate films showed a shift in the absorption band and a variation of peak intensity related to O-H stretching (Fig. 3). This change suggest that inclusion of enterocins to alginate films would result in changes in hydrogen bonding resulting from cross-linking, confirming disturbance of the formation of a cross-linked network as previously hypothesised. The spectra also showed an increase of one of the absorption peaks assigned to free carboxylate (1410 cm⁻¹). As described by Salmieri and Lacroix (2006) a reduction of the peak intensities associated with the free carboxylate groups of alginate (1600 and 1410 cm⁻¹) would be characteristic of interactions between alginate and Ca²⁺. Therefore, the observed increase in enterocin containing films could reflect decreased interactions, due to disturbance of enterocins during cross-linking. PVOH films with added enterocins also showed major changes in the FTIR spectra in the absorption band related to O-H stretching, 3600-3200 cm⁻¹ (Fig. 3). Addition of enterocins resulted in a considerable increase in intensity and change in shift of the O-H peaks from the film. Finally, Figure 3 shows overlapping spectra for zein films with added enterocins and control films, indicating no alteration of the absorbance spectra of zein films due to the inclusion of enterocins.

Conclusions

This study confirms that addition of enterocins to alginate, PVOH and zein was not detrimental to their physical performance. Moreover, water vapour permeability and tensile properties were improved mainly in PVOH and zein films. These results, together with previous studies of the authors showing the effectiveness of those antimicrobial materials against *Listeria monocytogenes* (Marcos and others 2007),

would recommend the use of the studied biopolymers as carrier medium for enterocin application to food.

References

Ashikin WHNS, Wong TW, Law CL. 2010. Plasticity of hot air-dried mannuronate- and guluronate-rich alginate films. Carbohydr Polym: doi: 10.1016/j.carbpol.2010.02.002.

Aymerich T, Holo H, Havarstein LS, Hugas M, Garriga M, Nes IF. 1996. Biochemical and genetic characterization of enterocin A from *Enterococcus faecium*, a new antilisterial bacteriocin in the pediocin family of bacteriocins. Appl Environ Microbiol 62(5):1676-1682.

Buffo RA, Han JH. 2005. Edible films and coatings from plant origin proteins. In: Han JH. Innovations in food packaging. London: Elsevier Academic Press. p 277-300.

Buonocore GG, Conte A, Del Nobile MA. 2005. Use of mathematical model to describe the barrier properties of edible films. J Food Sci 70(2):E142-E147.

Casaus P, Nilsen T, Cintas LM, Nes IF, Hernandez PE, Holo H. 1997. Enterocin B, a new bacteriocin from *Enterococcus faecium* T136 which can act synergistically with enterocin A. Microbiol 143:2287-2294.

Chen Y, Cao X, Chang PR, Huneault MA. 2008. Comparative study on the films of poly(vinyl alcohol)/pea starch nanocrystals and poly(vinyl alcohol)/native pea starch. Carbohydr Polym 73(1):8-17.

357 Coma V, Sebti I, Pardon P, Deschamps A, Pichavant FH. 2001. Antimicrobial edible
 358 packaging based on cellulosic ethers, fatty acids, and nisin incorporation to inhibit
 359 *Listeria innocua* and *Staphylococcus aureus*. J Food Prot 94:470-475.

360 Cutter CN, Sumne SS. 2002. Application of edible coatings on muscle foods. In:
 361 Gennadios A. Protein-based films and coatings. Boca Raton, FL: CRC Press. p 467-
 362 484

363 Cutter CN. 2006. Opportunities for bio-based packaging technologies to improve the
 364 quality and safety of fresh and further processed muscle foods. Meat Sci 74:131-142.

365 Del Nobile MA, Piergiovanni L, Buonocore GG, Fava P, Puglisi ML, Nicolais L. 2003.
 366 Naringinase immobilization in polymeric films intended for food packaging
 367 applications. J Food Sci 68(6):2046-2049.

368 Del Nobile MA, Conte A, Incoronato AL, Panza O. 2008. Antimicrobial efficacy and
 369 release kinetics of thymol from zein films. J Food Eng 89(1):57-63.

370 DeMerlis CC, Schoneker DR. 2003. Review of the oral toxicity of polyvinyl alcohol
 371 (PVA). Food Chem Toxicol 41:319-326.

372 European Food Safety Authority. 2005. Opinion of the scientific panel on food
 373 additives, flavourings, processing aids and materials in contact with food on a request
 374 from the commission related to the use of polyvinyl alcohol as a coating agent for
 375 food supplements. EFSA J 294:1-15.

376 FDA, US Food and Drug Administration. 2004. Agency Response Letter GRAS Notice
 377 No. GRN 000141 [Polyvinyl Alcohol]. Westpoint, PA: CFSAN/ Office of Food
 378 Additive Safety [Last updated: 2009 May 21]. Available from:

379 <http://www.fda.gov/Food/FoodIngredientsPackaging/GenerallyRecognizedasSafeGR>
 380 [AS/GRASListings/ucm153970.htm](http://www.fda.gov/Food/FoodIngredientsPackaging/GenerallyRecognizedasSafeGR).

381 Gennadios A, McHugh TH, Weller CL, Krochta JM. 1994. Edible coatings and films
 382 based on proteins. In: Krochta JM. Edible coatings and films to improve food quality.
 383 Lancaster, USA: Technomic publishing co., inc. p 201-257.

384 Gennadios A, Hanna MA, Kurth LB. 1997. Application of edible coatings on meats,
 385 poultry and seafoods: a review. *Lebensm Wiss Technol* 30:337-350.

386 Ghanbarzadeh B, Oromiehie AR, Musavi M, D-Jomeh ZE, Rad ER, Milani J. 2006.
 387 Effect of plasticizing sugars on rheological and thermal properties of zein resins and
 388 mechanical properties of zein films. *Food Res Int* 39(8):882-890.

389 Gombotz WR, Wee S. 1998. Protein release from alginate matrices. *Adv Drug Delivery*
 390 *Rev* 31(3):267-285.

391 Güçbilmez ÇM, Yemenicioglu A, Arslanoglu A. 2007. Antimicrobial and antioxidant
 392 activity of edible zein films incorporated with lysozyme, albumin proteins and
 393 disodium EDTA. *Food Res Int* 40 (1):80-91.

394 Hambleton A, Debeaufort F, Bonnotte A, Voilley A. 2009. Influence of alginate
 395 emulsion-based films structure on its barrier properties and on the protection of
 396 microencapsulated aroma compound. *Food Hydrocolloids* 23(8):2116-2124.

397 Han JH. 2000. Antimicrobial food packaging. *Food Technol* 54(3):56-65.

398 Hemeda OM, Hemeda DM, Said MZ. 2003. Some physical properties of pure and
 399 doped polyvinyl alcohol under applied stress. *Mech. Time-Depend. Mater* 7:251-268.

400 Krochta JM, De-Mulder-Johnston C. 1997. Edible and biodegradable polymer films:
 401 Challenges and opportunities. Food Technol 51(2):61-74.

402 Krumova M, López D, Benavente R, Mijangos C, Pereña JM. 2000. Effect of
 403 crosslinking on the mechanical and thermal properties of poly(vinyl alcohol). Polym J
 404 41 (26):9265-9272.

405 Lai H, Padua G. 1997. Properties and microstructure of plasticized zein films. Cereal
 406 Chem 74(6):771-775.

407 Liu L. 2006. Bioplastics in food packaging: Innovative technologies for biodegradable
 408 packaging. [Accessed: 2009 Jan 20]. San Jose, CA: San Jose State University.
 409 Available from <http://www.iopp.org/files/public/SanJoseLiuCompetitionFeb06.pdf>.

410 Marcos B, Aymerich T, Monfort JM, Garriga M. 2007. Use of antimicrobial
 411 biodegradable packaging to control *Listeria monocytogenes* during storage of cooked
 412 ham. Int J Food Microbiol 120(1-2):152-158.

413 Marcos B, Aymerich T, Monfort JM, Garriga M. 2008. High-pressure processing and
 414 antimicrobial biodegradable packaging to control *Listeria monocytogenes* during
 415 storage of cooked ham. Food Microbiol 25(1):177-182.

416 Mastromatteo M, Barbuzzi G, Conte A, Del Nobile MA. 2009. Controlled release of
 417 thymol from zein based film. Innovative Food Sci Emerg Technol 10(2):222-227.

418 Mendieta-Taboada O, Sobral PJA, Carvalho RA, Habitante AMBQ. 2008.
 419 Thermomechanical properties of biodegradable films based on blends of gelatin and
 420 poly(vinyl alcohol). Food Hydrocolloids 22(8):1485-1492.

421 Ming X, Weber GH, Ayres JW, Sandine WE. 1997. Bacteriocins applied to food
 422 packaging materials to inhibit *Listeria monocytogenes* on meats. J Food Sci
 423 62(2):413-415.

424 Natrajan N, Sheldon BW. 2000. Inhibition of *Salmonella* on poultry skin using protein-
 425 and polysaccharide-based films containing a nisin formulation. J Food Prot
 426 63(9):1268-1272.

427 Nisperos-Carriedo MO. 1994. Edible coatings and films based on polysaccharides. In: J.
 428 Krochta M. Edible coatings and films to improve food quality. Lancaster, USA:
 429 Technomic publishing co., inc. p 305-337.

430 Norajit K, Kim KM, Ryu GH. 2010. Comparative studies on the characterization and
 431 antioxidant properties of biodegradable alginate films containing ginseng extract. J
 432 Food Eng 98 (3): 377-384.

433 Padgett T, Han IY, Dawson P. 1998. Incorporation of food-grade antimicrobial
 434 compounds into biodegradable packaging films. J Food Prot 61(10):1330-1335.

435 Park SY, Jun ST, Marsh KS. 2001. Physical properties of PVOH/chitosan-blended films
 436 cast from different solvents. Food Hydrocolloids 15:499-502.

437 Quintavalla S, Vicini L. 2002. Antimicrobial food packaging in meat industry. Meat Sci
 438 62(3):373-380.

439 Rhim JW, Lee JH. 2004. Effect of CaCl₂ treatment on mechanical and moisture barrier
 440 properties of sodium alginate and soy protein-based films. Food Sci Biotechnol
 441 13(6):728-732.

442 Rhim JW. 2004. Physical and mechanical properties of water resistant sodium alginate
443 films. . *Lebensm Wiss Technol* 37(3):323-330.

444 Salmieri S, Lacroix M. 2006 Physicochemical properties of alginate/polycaprolactone-
445 based films containing essential oils. *J Agric Food Chem* 54: 10205-10214.

446 Segi N, Yotsuyanagi T, Ikeda K. 1989. Interaction of calcium-induced alginate gel
447 beads with propranolol. *Chem Pharm Bull* 37(11):3092-3095.

448 Senna MM, Salmieri S, El-naggar AW, Safrany A, Lacroix M. 2010. Improving the
449 compatibility of zein/poly(vinyl alcohol) blends by gamma irradiation and graft
450 copolymerization of acrylic acid. *J Agric Food Chem* 58(7): 4470-4476.

451 Soliman EA, Furuta M. 2009. Influence of γ -irradiation on mechanical and water barrier
452 properties of corn protein-based films. *Radiat Phys Chem*, 78 (7-8): 651-654.

453 Srinivasa PC, Ramesh MN, Kuma KR, Tharanathan RN. 2003. Properties and sorption
454 studies of chitosan-polyvinyl alcohol blend films. *Carbohydr Polym* 53(4):431-438.

455 Stockwell AF, Davis SS, Walker SE. 1986. In vitro evaluation of alginate gel systems
456 as sustained release drug delivery systems. *J Controlled Release* 3(1-4):167-175.

457 Tagg JR, Dajani AS, Wannamaker LW. 1976. Bacteriocins of gram-positive bacteria.
458 *Bacteriol Rev*, 40(1):722-756.

459 Tihminlioglu F, Atik ID, Özen B. 2010. Water vapor and oxygen-barrier performance
460 of corn-zein coated polypropylene films. *J Food Eng* 96 (3): 342-347.

461 Westedt U, Wittmar M, Hellwig M, Hanefeld P, Greiner A, Schaper AK, Kissel T.
462 2006. Paclitaxel releasing films consisting of poly(vinyl alcohol)-graft-poly(lactide-

463 co-glycolide) and their potential as biodegradable stent coatings. J Controlled Release
464 111(1-2):235-246.

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473 **Table 1.** Colour parameters of biodegradable films.

Film type	L [*]	a [*]	b [*]
Alginate C	80.25	-0.18	4.16
Alginate E	76.52	-0.30	7.01
SE	0.28	0.03	0.38
p	<0.001	NS	<0.01
PVOH C	83.88	0.08	0.15
PVOH E	80.13	-0.33	4.77
SE	0.51	0.04	0.33
p	<0.01	<0.01	<0.01
Zein C	68.54	5.58	62.12
Zein E	66.43	7.12	60.64
SE	0.82	1.21	0.84
p	NS	NS	NS

474 L*: lightness; a*: redness; b*: yellowness; C: control. E:
 475 enterocin. PVOH: polyvinyl alcohol. SE: standard error. NS:
 476 non-significant (p>0.05).
 477

478 **Table 2.** Tensile properties of biodegradable films.

Film type	Tensile strength (MPa)	Strain at break (%)
Alginate C	2.29	0.58
Alginate E	1.72	0.49
SE	0.14	0.03
p	<0.05	NS
PVOH C	47.28	2.02
PVOH E	32.94	2.74
SE	0.95	0.07
p	<0.001	<0.01
Zein C	4.23	0.61
Zein E	5.92	2.26
SE	0.13	0.13
p	<0.001	<0.01

479 C: control; E: enterocin; SE: standard error. PVOH: polyvinyl
 480 alcohol; NS: non-significant ($p>0.05$).

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Figure 1. SEM micrographs of cross-sections of biodegradable films obtained at different magnifications: control alginate (a), PVOH (b) and zein (c) films; enterocin containing alginate (d), PVOH (e) and zein (f) films. Magnifications of 700× (a), 450× (b), 1100× (c), 1500× (d), 600× (e), and 250× (f).

Figure 2. Water vapour permeability (WVP) of biodegradable films. Values are means of triplicates. Bars indicate standard deviation among replicates. Different letters indicate differences between control and enterocin containing films within a film type.

496 **Figure 3.** FTIR spectra of biodegradable films.

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