J Korean Soc Appl Biol Chem (2012) 55, 651–656
10.1007/s13765-012-2100-y

ORIGINAL ARTICLE

Preparation of a Cotton Seed Meal Protein/Nanoclay Composite Film Containing Carvacrol and Its Effect on the Growth of *Escherichia coli* O157:H7 Inoculated on Bacon during Storage

Wan-Shin Jo · Hye-Yeon Song · Nak-bum Song · Kyung Bin Song

Received: 4 May 2012 / Accepted: 7 August 2012 / Published Online: 31 October 2012

Abstract Cottonseed meal protein (CSP) film containing nanoclay and carvacrol was prepared, and the physical properties of the film were investigated. Nanoclay (Cloisite Na^+) incorporation improved mechanical properties of the CSP film. CSP film containing 3% nanoclay had tensile strength of 4.07 MPa and elongation at break of 26.37%. The optimal condition for the CSP film manufacturing was 3 g cottonseed protein, 3 g fructose, and 0.09 g Cloisite Na^+ in 100 mL of film-forming solution. To inhibit the growth of pathogenic bacteria such as *Escherichia coli* O157:H7, CSP film containing carvacrol was prepared, and microbial growth was examined during bacon packaging and storage using the film. The antimicrobial activity of the CSP film against *E. coli* O157:H7 increased with increasing carvacrol concentration. After 10 d of storage, the population of *E. coli* O157:H7 inoculated on the bacon samples packed with the CSP film containing carvacrol decreased by 1.57 log CFU/g compared to the control. These results suggest that the CSP film containing carvacrol can be used as an antimicrobial packaging film.

Keywords bacon · carvacrol · cottonseed protein · edible film

Introduction

Biodegradable materials are becoming increasingly popular to replace plastic materials in the packaging industry. However, biodegradable materials must demonstrate comparable or better mechanical properties, water barrier properties, and economic efficiency compared to synthetic polymers (Grevellec et al., 2001). Proteins, polysaccharides, and lipids are used as base materials to make edible films, among which protein-based films have the most attractive properties, because these films provide better nutritional value, gas barrier properties, and mechanical properties than polysaccharide- and lipid-based films (Hanani et al., 2012).

Cottonseed is cultivated for use in fibers in more than 70 different countries and is the second most important protein source, following soybeans, in the world (Marquie and Guilbert (2002). The use of cottonseed protein for producing biodegradable packaging material is cost effective and environmentally friendly (Marquié et al., 1997; Marquié, 2001). However, cottonseed protein film requires some improvement in its mechanical properties. Various cross-linking agents, such as formaldehyde, glutaraldehyde, and glyoxal, have been used to improve the physical properties of cottonseed protein-based films (Marquié, 2001). In addition to cross-linking agents, nanoclay can be used to improve the physical properties of the protein films (Bae et al., 2009; Luecha et al., 2010).

It is well known that the addition of small amounts of nanoclay enhances the tensile strength and thermal stability of protein-based films (Lam et al., 2005). Montmorillonite (MMT), which is generally modified through a cationic ion substitution reaction with surface sodium ions, is a type of nanoclay (Park et al., 2002; Jang et al., 2011). Chen and Zhang (2006) reported that the dispersion of fine MMT layers into a soy protein matrix improved the mechanical strength and thermo-stability of the soy protein film.

With the increase in consumption of ready-to-eat food products, edible films containing antimicrobials to enhance food safety against pathogenic bacteria such as *Escherichia coli* O157:H7 have been studied (Du et al., 2008). Incorporating antimicrobials into the edible films extends the shelf life of the food products (Ku

W.-S. Jo · H.-Y. Song · N.-b. Song · K. B. Song (✉)
Department of Food Science and Technology, Chungnam National University, Daejeon 305-764, Republic of Korea
E-mail: kbsong@cnu.ac.kr

et al., 2008). Carvacrol, which is a major component of oregano essential oil and thyme (Lagouri et al., 1993; Arrebola et al., 1994), is generally regarded as safe and has antimicrobial activity against *E. coli* O157:H7 (Burt and Reinders, 2003; Zivanovic et al., 2005). In particular, carvacrol has been applied to potato puree, table grapes, chicken, and sprouts (Burt et al., 2007; Martínes-Romero et al., 2007).

Thus, the aims of the present study were to prepare CSP films from CS oil residue, and to improve the mechanical property and antibacterial activity of CSP film by the addition of nanoclay and carvacrol into film-forming solution for use as edible films and for use in bacon packaging.

Materials and Methods

Materials. Fructose and sorbitol were purchased from Sigma-Aldrich Chemical Co. (USA). Commercial MMT nanoclay (Cloisite Na^+) was obtained from Southern Clay Co. (USA.). Carvacrol was purchased from Fluka (USA). Cotton seed (CS) was obtained from Gyeonggi-do Agricultural Research & Extension Services, Korea, in 2011. Bacon was purchased from a local market (Daejeon, Korea), and bacon slices with a uniform weight (20±1 g) were used for the experiment.

Extraction of cotton seed meal protein. Extraction of CS meal protein was performed according to the method of Liadakis et al. (1993). CS meal was mixed with 0.1 N NaOH at a meal-to-solution ratio of 1:20 (w/v) at 40°C for 30 min with constant stirring. The mixture pH was adjusted to 12 by the addition of 3 N NaOH. During the extraction, pH of the suspension was maintained by adjusting with 0.5 N HCl or 0.5 N NaOH. After 30 min, the product was centrifuged at 2,550 × g for 10 min to remove any insoluble materials, and the supernatant was filtered through cheese cloth. The extract was acidified with 2 N HCl until the isoelectric value was attained. The isoelectric point was determined to be 4.37. After 30 min, the solution was centrifuged at 2,550 × g for 10 min, and the precipitated proteins were washed with distilled water. The protein solutions were subsequently neutralized and lyophilized.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). SDS-PAGE was performed according to the method of Laemmli (1970). Equal amounts of the protein samples were loaded into each lane for comparison, resolved on a 10% separation gel, and stained with Coomassie brilliant blue. The following molecular weight markers were used: myosin (210 kDa), β-galactosidase (117 kDa), bovine serum albumin (97.8 kDa), ovalbumin (55 kDa), carbonic anhydrase (37.5 kDa), soybean trypsin inhibitor (29 kDa), lysozyme (19.7 kDa), and aprotinin (6.9 kDa).

Preparation of the film-forming solution. To prepare the CS meal protein (CSP) film, 3 g of CSP was dissolved in 100 mL of distilled water and mixed with 3 g of fructose as a plasticizer. Cloisite Na^+ was prepared by dispersing various amounts (1, 3, 5, and 7%, w/w) of nanoclay into 100 mL distilled water and then stirring for 24 h. Subsequently, 3 g of CSP and 3 g of fructose were dissolved into a nanoclay solution. The film-forming solution was conditioned in a water bath at 90°C for 30 min and adjusted to 75°C. Varying amounts of carvacrol were added to 100 mL of the film-forming solution to obtain final concentrations of 0.4, 0.6, and 1.0% (w/v) of carvacrol.

Film casting and drying. Film-forming solutions were strained through the cheese cloth and cast onto flat, Teflon-coated glass plates (24×30 cm). A uniform film thickness was maintained by casting a constant quantity of film-forming solution onto each plate. The plates were dried at 25°C for 24 h. Dried films were peeled intact from the casting surfaces, and test specimens were sectioned for water vapor permeability (2×2 cm) and tensile strength (2.54×10 cm) tests.

Measurement of tensile strength and elongation. The tensile strength (TS) of the film and the elongations at break (E) were determined using an Instron Universal Testing Machine (Model 4484, Instron Co., USA) according to ASTM method D638M. The film specimens were conditioned in an environmental chamber at 25°C and 50% relative humidity (RH) for 24 h. An initial grip distance of 5 cm and a cross-head speed of 50 cm/min were used. TS was calculated by dividing the maximum load of a specimen by the initial cross-sectional area, and E was expressed as the percent change from the initial gauge length of a specimen at the point of sample failure. Five replicates of each film were tested.

Determination of film thickness. The film specimens were conditioned in an environmental chamber at 25°C and at 50% RH for 2 days. The film thickness was measured at five random positions using a micrometer (Mitutoyo, Model No. 2046-08, Japan), and the mean value was determined.

Measurement of water vapor permeability. The water vapor permeability (WVP) of the edible films was determined at 25°C and 50% RH using the method of Hong et al. (2009). A polymethylacrylate cup (20 mL) was filled to 1 cm with distilled water and was covered with a film specimen that was conditioned in an environmental chamber at 25°C and 50% RH. The weight loss of the cup was measured over time, and the slope was calculated using linear regression analysis. The WVP (ng m/m^{2}s Pa) was then calculated using the following formula:

$$WVP=(WVTR \times L)/\Delta p,$$

where the water vapor transmission rate (WVTR) is calculated by dividing the slope by the open area of the cup, L is the mean film thickness, and p is the corrected partial vapor pressure difference across the film specimen.

Culture preparation. *E. coli* O157:H7 (NCTC 12079) was cultured at 37°C for 24 h in 50-mL conical tubes containing 25 mL Luria-Bertani (LB) broth (Difco, USA).

Diffusion test for antimicrobial activity. The antimicrobial activity of the film containing carvacrol was determined using agar diffusion method. Tryptic soy agar (Difco) was spread with

E. coli O157:H7 (0.1 mL). Discs (10 mm in diameter) were cut from the films and placed onto the inoculated plates. After allowing the carvacrol to diffuse for 3 h at 4°C, the *E. coli* O157:H7 plates were incubated at 37°C for 24 h. Each microbial count was determined as the mean of three replicates, and the inhibition zone was measured in millimeters using a Digimatic caliper (Model 500-181-20, Mitutoyo Corp., Japan).

Inoculation of pathogens on bacon. *E. coli* O157:H7 was incubated at 37°C in LB until the count reached 10^6 CFU/mL. *E. coli* O157:H7 (1 mL) was spread evenly onto the bacon surface with a sterile glass rod and allowed to rest for 30 min. The initial inoculation levels of *E. coli* O157:H7 in the bacon samples were 6.63 log CFU/g. The bacon was packed in direct contact with the CSP film or the CSP film containing 0.6 g carvacrol/100 mL by wrapping. The samples packed in the polyethylene terephthalate (PET) film were used as a control. All of the samples were stored at 4±1°C.

Microbiological analysis. The inoculated bacon samples (10 g each) were placed in 90 mL of 0.1% peptone water. The samples were homogenized for 3 min in a sterile bag using a Stomacher (MIX 2, AES Laboratoire, France), filtered through sterile cheese cloth, and diluted with 0.1 g of peptone water/100 g water to measure microbial counts. Serial dilutions were performed in triplicate on each selective agar plate. *E. coli* O157:H7 counts were determined by plating appropriately diluted samples onto MacConkey agar (Difco). To quantify the *E. coli* O157:H7 population, each plate was incubated at 37°C for 24 h. Each microbial count was calculated as the mean of three determinations, and the microbial counts were expressed as log CFU/g.

Statistical analysis. Analysis of variance and Duncan's multiple range tests were performed to analyze the data using the SAS program version 8.1 (SAS Institute, Inc., USA). Differences at $p<0.05$ were considered significant. All results are expressed as the mean ± standard deviation.

Results and Discussion

SDS-PAGE. The SDS-PAGE profile of the CSP extracted from CS meal shows that the CSP bands were between 10 and 55 kDa (Fig. 1). The approximate molecular weights of the main bands were 55.5, 48.7, 23.1, and 13.6 kDa, which corresponded to albumins and globulins. Similar to our results, Sadeghi and Shawrang (2007) reported that the major components of CSP were globulin 9S, globulin 5S, and albumin 2S, with molecular weights of 55.2 and 48.8 kDa; 24.1, 23.4, and 21.5 kDa; and 19.8, 15.2, and 13.9 kDa, respectively. Saroso (1989) has also reported that CSP components are primarily globulins (60%) and albumins (30%), with much lower proportions of prolamins (8.6%) and glutelins (0.5%).

Mechanical properties of films. Plasticizers affect the physical properties of films, depending on their concentration, size, and shape (Cho and Rhee, 2002; Wan et al., 2005). Plasticizers disrupt intermolecular interactions between polymer molecules, resulting in a decrease in brittleness and an increase in flexibility (Shaw et al., 2002). Various plasticizers have been used in CSP films (data not shown), and fructose was selected as the best plasticizer, resulting in a TS of 3.17 MPa and an E of 44.25%. Shin et al. (2011) and Jang et al. (2011) also reported that fructose-containing edible films have higher TS and E values than sorbitol-containing films, possibly because fructose has more favorable interactions with the reactive groups of the CSP polymer than other plasticizers (Jang et al., 2011).

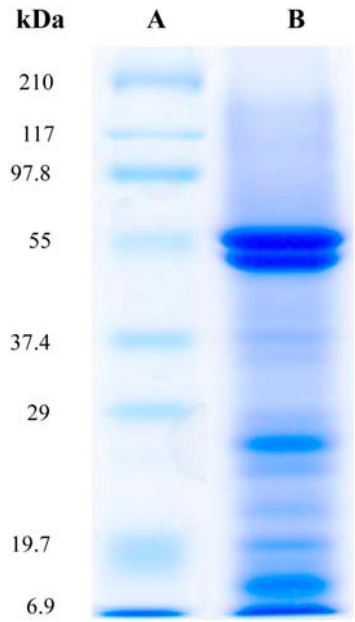

Fig. 1 SDS-PAGE profile of cottonseed protein. (A) molecular weight marker proteins, (B) cottonseed protein

Table 1 Physical properties of the cottonseed protein/Cloisite Na^+ film

Cloisite Na^+ (%)	Thickness (μm)	Tensile strength (MPa)	Elongation (%)	Water vapor permeability (ng m/m^2sPa)
0	68.20±5.75^a	3.17±0.15^c	44.25±2.61^a	2.89±0.16^a
1	53.87±4.72^c	3.81±0.19^b	26.40±5.37^b	2.58±0.08^b
3	59.53±5.71^{bc}	4.07±0.20^{ab}	26.37±7.53^b	2.77±0.07^{ab}
5	61.47±2.96^{ab}	4.12±0.26^a	15.08±7.35^c	2.80±0.07^a

Means ± SD
Values in a column followed by different superscript letters are significantly different ($p<0.05$).

The incorporation of nanoclay into the CSP film enhanced the mechanical properties of the film (Table 1). The TS of nanocomposite films increased significantly ($p<0.05$) with increasing amounts of Cloisite Na^+, with the highest TS value at 5% Cloisite Na^+. Bae et al. (2009) reported that the TS of fish gelatin films were increased by the addition of Cloisite Na^+. The TS of chitosan films was also increased by the addition of Cloisite Na^+ (Xu et al., 2006). The increase in TS by the addition of Cloisite Na^+ can be explained by the formation of an exfoliated state and uniform

Table 2 Mechanical properties of the CSP film containing various concentration of carvacrol

Carvacrol (%)	Thickness (μm)	Tensile strength (MPa)	Elongation (%)	Water vapor permeability (ng m/m^{2}sPa)
0	59.53±5.71^a	4.07±0.20^a	26.37±7.53^a	2.77±0.07^{ab}
0.4	64.40±5.92^b	3.35±0.25^{bc}	29.79±4.32^a	2.73±0.03^b
0.6	64.47±2.39^b	3.51±0.16^b	31.31±3.55^a	2.93±0.05^a
1.0	67.80±4.54^b	3.18±0.09^c	30.55±5.06^a	2.69±0.13^b

Means ± SD
Values in a column followed by different superscript letters are significantly different (p <0.05).

Table 3 Antimicrobial activity of the CSP-carvacrol film against *E. coli* O157:H7

Carvacrol (%)	Diameter of inhibition zone (mm)
	E. coli O157:H7
0.0	0.00±0.00^d
0.4	13.54±0.46^c
0.6	16.18±0.22^b
1.0	19.00±0.35^a

Means ± SD
Values in a column followed by different superscript letters are significantly different (p <0.05).

dispersion of Cloisite Na^+ molecules in the CSP matrix (Jang et al., 2011). In addition, the negative charge in Cloisite Na^+ has high affinity and interactions such as hydrogen bonding with CSP (Chen and Zhang, 2006). However, the E of the composite film decreased significantly (p <0.05) with the addition of Cloisite Na^+. Similar to our results, Lepoittevin et al. (2002) also reported that the E of poly(e-caprolactone) decreased with the addition of Cloisite Na^+. The decline in E values appears to be due to the presence of unexfoliated aggregates (Fornes et al., 2001).

The addition of nanoclay enhanced the water vapor barrier properties of the CSP film. All of the films exhibited lower WVPs than that of the pristine CSP film, due to the barrier properties of the nanoclay. The enhanced water vapor barrier properties of the CSP composite film is primarily attributed to the layered structure of the nanoclay, which retards the transmission of moisture through the CSP matrix (Park et al., 2002). Overall, based on the physical properties of CSP, 3% Cloisite Na^+ was the most desirable content for the composite film.

The CSP films containing various amounts of carvacrol were prepared to provide the antimicrobial activity (Table 2). The film without carvacrol had a TS of 4.07 MPa and an E of 26.37%, whereas the film containing 0.6% carvacrol had a TS of 3.51 MPa and an E of 31.31%. The addition of carvacrol to the CSP film significantly decreased the TS and slightly increased the E. Ravishankar et al. (2009) also reported that the TS of apple-based film decreased and the E increased following the addition of carvacrol. These results are consistent with a previous study by Rojas-Guaü et al. (2007), where the TS decreased and the E increased following the addition of carvacrol. To explain this

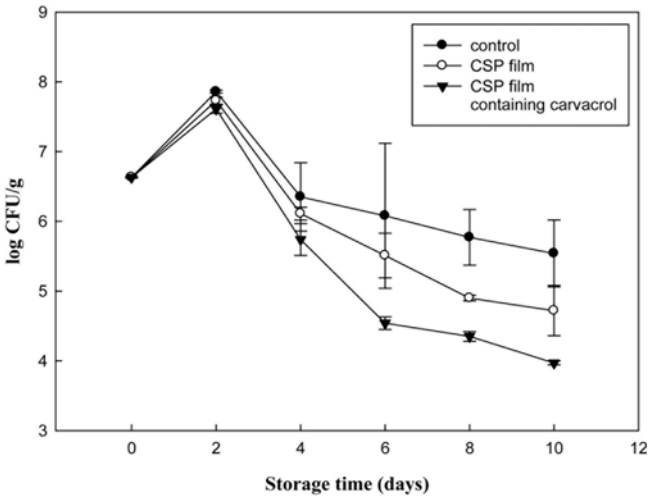

Fig. 2 Change in the populations of *E. coli* O157:H7 in bacons during storage at 4°C.

difference, Benavides et al. (2012) reported that the addition of oregano essential oil to the film affected its ionic interactions, resulting in a decrease in the intermolecular forces along the polymer chains. For the same reason, the flexibility of the film was improved by the addition of carvacrol, resulting in a decrease in the TS and an increase in the E. The WVP of the films increased up to an addition of 0.6% carvacrol. Du et al. (2008) also reported that the addition of carvacrol increased the WVP of tomato films, which is in agreement with the present investigation. This finding is most likely due to the chemical nature of carvacrol, as carvacrol could affect the intermolecular interactions of the structural matrix in the CSP films and increase the vapor transfer across the film (Pranoto et al., 2005). WVP of the CSP film was slightly decreased by the addition of 1% carvacrol, differently from 0.6% carvacrol. Similar to our results, Zivanovic et al. (2005) also reported that WVP of chitosan film was significantly decreased by the addition of 1% oregano oil. The reason for the decrease of WVP by the addition of 1% carvacrol might be due to the decrease of hydrophilic portion by the addition of carvacrol (Benavides et al., 2012).

Antimicrobial activity of films. The antimicrobial activity against *E. coli* O157:H7 in CSP films containing carvacrol is presented in Table 3. The antimicrobial activity of the films was expressed as the size of the inhibition zone. The inhibition zone for *E. coli* O157:H7 increased with increasing concentrations of carvacrol from 0.4 to 1%. One percent carvacrol had the highest antimicrobial activity with a 19.0 mm inhibition zone. Lim et al. (2010) also reported the antimicrobial activity of *Gelidium corneum* (GC) films containing carvacrol. Accordingly, these results show that CSP films containing carvacrol can be used for antimicrobial packaging of foods. Based on the mechanical properties and the antimicrobial activity of the films, the appropriate carvacrol content for bacon packaging is 0.6%.

Microbiological analysis of bacon packed with the CSP film. The microbial growth of a typical pathogenic bacterium, such as *E. coli* O157:H7, inoculated on bacon wrapped with the carvacrol (0.6%)-containing CSP film was monitored during storage at 4°C (Fig. 2). The initial inoculum level of *E. coli* O157:H7 in the bacon samples was 6.63 log CFU/g. The populations of *E. coli* O157:H7 in the bacon increased during the first 2 days after inoculation and then decreased during 10 days of storage. Min and Oh (2009) also reported a decrease in the populations of *E. coil* O157:H7 in beef samples during storage at 4°C. The reason for the decrease during 10 days of storage might be due to low temperature, at which an inhibitory effect on the growth of *E. coli* O157:H7 occurs. After 10 days of storage, the population of *E. coli* O157:H7 for the control was 5.54 log CFU/g, whereas the population of the bacteria for the bacon wrapped with the CSP film was 4.72 log CFU/g, which was lower than the control probably due to low available oxygen content inside the CSP film packaging. In particular, the population of the bacteria for the bacon wrapped with the CSP film containing 0.6% carvacrol was 3.97 log CFU/g, which represents a decrease of 1.57 log CFU/g compared to the control. Oussalah et al. (2004) also reported that the incorporation of oregano essential oils, whose main component is carvacrol, into milk protein-based films decreased the population of *E. coli* O157:H7 in the whole beef muscle by 1.12 log CFU/g. Lim et al. (2010) also reported that the incorporation of carvacrol into GC films enhances antimicrobial activity, which is in agreement with our results. The antimicrobial activity of carvacrol is due to the hydroxyl group of its unique chemical structure, which depletes the ATP pool, resulting in cell death (Ultee et al., 2002).

In summary, edible protein films were prepared using CSP extracted from CS meal. The incorporation of 3% nanoclay (Cloisite Na^+) into the CSP film-forming solution enhanced the TS of the CSP film, and the CSP film containing 0.6% carvacrol exhibited antimicrobial activity against *E. coli* O157:H7 inoculated on bacon. These results suggest that the CSP/nanoclay composite film containing carvacrol can be used in bacon packaging.

References

Arrebola ML, Navarro MC, Jimenez J, and Ocana FA (1994) Yield and composition of the essential oil of thymus serpylloides subsp serpylloides. *Phytochemistry* **36**, 67–72.

Bae HJ, Park HJ, Darby DO, Kimmel RM, and Whiteside WS (2009) Development and characterization of pet/fish gelatin-nanoclay composite/ldpe laminate. *Packag Technol Sci* **22**, 371–83.

Benavides S, Villalobos-Carvajal R, and Reyes JE (2012) Physical, mechanical and antibacterial properties of alginate film: Effect of the crosslinking degree and oregano essential oil concentration. *J Food Eng* **110,** 232–9.

Burt SA, Fledderman MJ, Haagsman HP, F van Knapen, and Veldhuizen EJA (2007) Inhibition of salmonella enterica serotype enteritidis on agar and raw chicken by carvacrol vapour. *Int J Food Microbiol* **119,** 346–50.

Burt SA and Reinders RD (2003) Antibacterial activity of selected plant essential oils against *Escherichia coli* O157:H7. *Lett Appl Microbiol* **36,** 162–7.

Chen P and Zhang L (2006) Interaction and properties of highly exfoliated soy protein/montmorillonite nanocomposites. *Biomacromolecules* **7**, 1700–6.

Cho SY and Rhee C (2002) Sorption characteristics of soy protein films and their relation to mechanical properties. *Lebensm-Wiss Technol* **35**, 151–7.

Du WX, Olsen CW, Avena-Bustillos RJ, McHugh TH, Levin CE, and Friedman M (2008) Antibacterial activity against e-coli o157:H7, physical properties, and storage stability of novel carvacrol-containing edible tomato films. *J Food Sci* **73**, M378–83.

Fornes TD, Yoon PJ, Keskkula H, and Paul DR (2001) Nylon 6 nanocomposites: the effect of matrix molecular weight. *Polymer* **42**, 9929–40.

Grevellec J, Marquie C, Ferry L, Crespy A, and Vialettes V (2001) Processability of cottonseed proteins into biodegradable materials. *Biomacromolecules* **2**, 1104–9.

Hanani ZAN, Roos YH, and Kerry JP (2012) Use of beef, pork and fish gelatin sources in the manufacture of films and assessment of their composition and mechanical properties. *Food Hydrocolloid* **29**, 144–51.

Hong YH, Lim GO, and Song KB (2009) Physical properties of *Gelidium corneum*-gelatin blend films containing grapefruit seed extract or green tea extract and its application in the packaging. *J Food Sci* **74**, C6–10.

Jang SA, Shin YJ, Seo YB, and Song KB (2011) Effects of various plasticizers and nanoclays on the mechanical properties of red algae film. *J Food Sci* **76**, N30–4.

Ku KJ, Hong YH, and Song KB (2008) Mechanical properties of a gelidium corneum edible film containing catechin and its application in sausages. *J Food Sci* **73**, C217–21.

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage t4. *Nature* **227**, 680–5.

Lagouri V, Blekas G, Tsimidou M, Kokkini S, and Boskou D (1993) Composition and antioxidant activity of essential oils from oregano plants grown wild in greece. *Z Lebensmittel-Unters For* **197**, 20–3.

Lam CK, Lau KT, Cheung HY, and Ling HY (2005) Effect of ultrasound sonication in nanoclay clusters of nanoclay/epoxy composites. *Mater Lett* **59**, 1369–72.

Lepoittevin B, Devalckenaere M, Pantoustier N, Alexandre M, Kubies D, Calberg C et al. (2002) Poly(-caprolactone)/clay nanocomposites prepared by melt intercalation: mechanical, thermal and rheological properties. *Polymer* **43**, 4017–23.

Liadakis GN, Floridis A, Tzia C, and Oreopoulou V (1993) Protein isolates with reduced gossypol content from screw-pressed cottonseed meal. *J Agr Food Chem* **41**, 918–2.

Lim GO, Hong YH, and Song KB (2010) Application of gelidium corneum edible films containing carvacrol for ham packages. *J Food Sci* **75**, C90–3.

Luecha J, Sozer N, and Kokini JL (2010) Synthesis and properties of corn zein/montmorillonite nanocomposite films. *J Mater Sci* **45**, 3529–37.

Marquie C (2001) Chemical. Reactions in cottonseed protein cross-linking by formaldehyde, glutaraldehyde, and glyoxal for the formation of protein films with enhanced mechanical properties. *J Agr Food Chem* **49**, 4676–81.

Marquie C and Guilbert S (2002) Formation and properties of cottonseed protein films and coatings. In *Protein-based Films and Coatings.* Gennadios A (1st ed), pp. 139–58, CRC Press, USA.

Marquie C, Tessier AM, Aymard C, and Guilbert S (1997) Hplc determination of the reactive lysine content of cottonseed protein films to monitor the extent of cross-linking by formaldehyde, glutaraldehyde, and glyoxal. *J Agr Food Chem* **45**, 922–6.

Martinez-Romero D, Guillen F, Valverde JM, Bailen G, Zapata P, Serrano M et al. (2007) Influence of carvacrol on survival of botrytis cinerea inoculated in table grapes. *Int J Food Microbiol* **115,** 144–8.

Min BJ and Oh JH (2009) Antimicrobial activity of catfish gelatin coating containing origanum (*Thymus capitatus*) oil against gram-negative pathogenic bacteria. *J Food Sci* **74,** M143–8.

Oussalah M, Caillet S, Salmieri S, Saucier L, and Lacroix M (2004) Antimicrobial and antioxidant effects of milk protein-based film containing essential oils for the preservation of whole beef muscle. *J Agr Food Chem* **52**, 5598–605.

Park HM, Li XC, Jin CZ, Park CY, Cho WJ, and Ha CS (2002) Preparation and properties of biodegradable thermoplastic starch/clay hybrids. *Macromol Mater Eng* **287**, 553–8.

Pranoto Y, Salokhe VM, and Rakshit SK (2005) Physical and antibacterial properties of alginate-based edible film incorporated with garlic oil. *Food Res Int* **38**, 267–72.

Ravishankar S, Zhu LB, Olsen CW, McHugh TH, and Friedman A (2009) Edible apple film wraps containing plant antimicrobials inactivate foodborne pathogens on meat and poultry products. *J Food Sci* **74**, M440–5.

Rojas-Grau MA, Avena-Bustillos RJ, Olsen C, Friedman M, Henika PR, Martin-Belloso O et al. (2007) Effects of plant essential oils and oil compounds on mechanical, barrier and antimicrobial properties of alginate-apple puree edible films. *J Food Eng* **81**, 634–41.

Sadeghi AA and Shawrang P (2007) Effects of microwave irradiation on ruminal protein degradation and intestinal digestibility of cottonseed meal. *Livest Sci* **106**, 176–81.

Saroso B (1989) Chemical properties of protein in cottonseed kernels. *Industr Crops Res J* **1**, 60–5.

Shaw NB, Monahan FJ, O'Riordan ED, and O'Sullivan M (2002) Physical properties of wpi films plasticized with glycerol, xylitol, or sorbitol. *J Food Sci* **67**, 164–7.

Shin YJ, Jang SA, and Song KB (2011) Preparation and mechanical properties of rice bran protein composite films containing gelatin or red algae. *Food Sci Biotechnol* **20**, 703–7.

Ultee A., Bennik MHJ, and Moezelaar R (2002) The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen bacillus cereus. *Appl Environ Microb* **68**, 1561–8.

Wan VCH, Kim MS, and Lee SY (2005) Water vapor permeability and mechanical properties of soy protein isolate edible films composed of different plasticizer combinations. *J Food Sci* **70**, E387–91.

Xu YX, Ren X, and Hanna MA (2006) Chitosan/clay nanocomposite film preparation and characterization. *J Appl Polym Sci* **99**, 1684–91.

Zivanovic S, Chi S, and Draughon AF (2005) Antimicrobial activity of chitosan films enriched with essential oils. *J Food Sci* **70**, M45–51.