Preparation and physical properties of gelatin/CMC/chitosan composite films as affected by drying temperature

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Abstract

This study examined the effects of drying temperatures (25 and 45 °C) on the physical properties of different formulations of gelatin/CMC/chitosan composite films. The physical properties of each formulated film were assessed via Fourier Transform Infra-Red (FTIR) spectroscopy, X-Ray Diffractometry (XRD), Water Vapour Permeability (WVP) and biodegradability. The incorporation of CMC and chitosan significantly influenced film properties. Increased chitosan concentrations reduced the film's amorphous character by increasing its crystalline structure. The blended films also exhibited amino peaks that shifted from 1542 to ~1548 cm^{-1} while NH and/or OH peaks shifted from 3384 to 3288 cm^{-1}. Formulation E had the second lowest WVP for both drying condition and the highest weight loss for biodegradability after burial in soil for 5 days. In conclusion, different temperature did not affect the properties of film produced and formulation E qualified as 'high quality packaging material' with promising potential for the food packaging industry.

Introduction

Biodegradable films fabricated from biodegradable polymers promise to replace the plastic non-degradable films that presently plague the environment. Such polymers are derived from protein sources that are currently of high interest due to their potential application(s) in fields related to environmental protection (Vroman and Tighzert, 2009). Previous studies show that gelatin, a protein sourced material, is widely viewed with much interest by the food-packaging industry for this reason (Fakhreddin *et al*., 2013).

Gelatin, as obtained by the partial degradation of collagen, has received much attention as an edible film because of its abundance, relative low cost, biodegradability and excellent functional properties due to its excellent film forming ability (Arvanitoyannis *et al*., 1997a; Cao *et al*., 2007a). Properties of gelatin films vary depending on the gelatin's source, conditions of preparation and the substances incorporated in a film's formation (Hoque *et al*., 2011). Such films are very transparent and form an excellent gas barrier (oxygen and carbon dioxide). However, their mechanical properties only allow for a moderate water-vapour barrier compared to synthetic films. Hence, several researchers have tried to improve gelatin film properties by developing mixed biopolymer films (Gómez-Estaca *et al*., 2011). Numerous studies have been conducted for this purpose on pig skin derived gelatin films (Andreuccetti *et al*., 2011; Zainal *et al*., 2013); bovine derived gelatin films (Cao *et al*., 2007b; Rivero *et al*., 2009); and fish derived gelatin films (Fakhreddin *et al*., 2013; Rawdkuen *et al*., 2012).

A cellulose derivative, carboxymethyl cellulose (CMC), is mainly used in food processing because of its viscosity and water binding properties as well as its soluble transparency. Among biopolymers, anionic linear polysaccharides derived from cellulose i.e., methylcellulose and carboxymethyl cellulose (CMC) are widely available, easily processed, inexpensive, and have excellent film-making properties. Films prepared with these polymers generally provide a good gas barrier as well as moderate to good mechanical properties but remain exceptionally vulnerable to moisture (Biswal and Singh, 2004).

Chitosan, a polysaccharide derived from chitin, is an interesting cationic polysaccharide biodegradable polymer with excellent film-forming ability compared to other polysaccharides and has several advantages such as biocompatibility, biodegradability and non-toxicity (Fakhreddin *et al*., 2013). Chitosan's film forming ability has been documented by many studies

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for its physical, mechanical and anti-microbial properties (Pereda *et al.*, 2009; Dutta *et al.*, 2009).

The blending of biopolymers emerged to improve properties of films comprising biopolymer material. Many studies have also shown that edible films produced by a select combination of biopolymers have better properties than single component films (Yoo and Krochta, 2011). Previously, biodegradable film blends conducted by researchers include: (i) polysaccharide–polysaccharide or CMC-alginate blended film (Yadav *et al.*, 2014); (ii) protein–protein such as whey protein isolate, i.e., (WPI)-gelatin blended film (Wang *et al.*, 2010); (iii) protein–polysaccharide such as the gelatin-chitosan blended film (Zhong and Xia, 2008); (iv) lipid–protein such as whey protein-wax blended film (Janjarasskul *et al.*, 2014); and (v) polysaccharide–lipid such as konjac glucomannan/CMC/ lipid blended film (Cheng *et al.*, 2008).

Drying is a critical process in the production of edible blended films and most edible films, developed so far, have been dried under ambient conditions (Pérez-Gago and Krochta, 2000; Thakhiew *et al.*, 2010). However, increasing the drying temperature improves mechanical and barrier properties and shortens production time as shown by whey protein isolate (WPI) films, and peanut protein concentrate films (Jangchud and Chinnan, 1999). Higher drying temperatures have also induced methylcellulose crystallinity to produce denser WPI film; and cross-linking in peanut protein concentrate. Hence, the aims of this study are to investigate the effects of different drying temperatures (oven at 45ºC vs. room at 25ºC) on the physical properties of five gelatin/CMC/chitosan composite blended films formulation.

Material and Methods

Materials

The commercial type B bovine skin gelatin with a bloom value of ~225 g; sodium salt, high-viscosity carboxymethyl cellulose (CMC); medium molecular weight chitosan; and glycerol (MW = 92.09) were obtained from Sigma-Aldrich Company Ltd., Poole, Dorset, UK.

Gelatin-based film production

Film solutions were prepared following method by Tongdeesoontorn *et al.* (2009) with slight modification. Gelatin powder was mixed with five different concentrations of CMC and chitosan in distilled water in the following ratios (gelatin/CMC/chitosan): A (80/20/0), B (80/0/20), C (80/10/10), D (60/20/20) and E (60/30/10). Glycerol (30% w/w

total solid) was used to plasticize film formation. All samples were stirred constantly for 24 h in order to solubilize the mix and then cast on a clean Petri dish and divided into two lots for different drying conditions, i.e., room (25°C) and oven (45°C). In order to get uniform thickness, each Petri dish were allocated for film solution by 25 g.

Fourier transform infrared spectroscopy (FTIR) analysis

All infrared spectra of the prepared samples were conducted following Vicentini *et al.* (2005) with some modification, using the Thermo Nicolet 380 FTIR Spectrometer with UATR accessory. Sample scanning frequencies ranged from 4000 to 400 cm^{-1} with a spectra resolution of 4 cm^{-1}. This method determined the effects from interactions between gelatin, chitosan and CMC. All measurements were undertaken at room temperature and repeated three times.

X-ray diffraction (XRD) analysis

XRD patterns for the composite film samples were analyzed with a Rigaku X-Ray Diffractometer with CuKa radiation at 30 mA and 40 kV according to the method described by Rhim *et al.* (2009) with some modifications. Each assay was repeated three times with an angle diffraction range of $2\Theta = 3–80º$ and scanning time of 30 min per run.

Water vapor permeability (WVP)

Water Vapor Permeability (WVP) was measured via method described by Denavi *et al.* (2009) with some modification. Circular aluminum cups (2 x 2 cm) containing 10 g of silica gel (0% RH) were individually sealed for/by each sample. Each cup was then weighed and placed in a desiccator containing distilled water at 30ºC. The cup was then weighed hourly for six hours and water vapor permeability (WVP) was calculated using the following equation:

$$WVP\ (g.mm.h^{-1}.cm^{-2}.pa^{-1}) = \frac{\Delta W\ (g) \times Film\ thickness\ (mm)}{Times\ (h) \times Test\ area\ (cm^2) \times \Delta P\ (Pa)}$$

While ΔW is weight different and ΔP (Pa) is partial pressure

Film biodegradability

Biodegradation testing for the composite films produced was referred to the soil burial method of Karnnet *et al.* (2005), with some modifications. Samples of each film formulation (2 cm^2) were dried in a desiccator until their weights were constant (referred to as 'initial weight'). These samples were

then buried in 100 g of soil and daily weight changes were recorded for five consecutive days. Each sample was then dried until its weight became constant (referred to as 'final weight'). The percent weight loss (W %) was calculated using the following equation:

$$\text{Weight loss (W \%)} = \frac{\text{Initial weight} - \text{final weight}}{\text{Initial weight}} \times 100$$

Statistical analysis

Each statistical analysis was calculated in triplicate. One-way ANOVA variance analysis was performed by Minitab 14.0 software and comparisons of means utilized Tukey's test at a confidence level of $p < 0.05$.

Results and Discussion

Film production

Five (5) composite gelatin/CMC/chitosan films with respective ratios was prepared as follows: A (80/20/0), B (80/0/20), C (80/10/10), D (60/20/20) and E (60/30/10). Each formulation was dried for comparative purposes (room at 25°C vs. oven at 45°C). Films dried at room temperature (25ºC) completely set after 46 days compared to oven dried films (45ºC) which set in 2 days. Drying rates were influenced by temperature and environment and higher temperature correlated with a quicker drying rate due to the rapid evaporation of the solution's aqueous component.

Fourier transform infrared spectroscopic (FTIR) analysis

Fourier transform infrared spectroscopic (FTIR) analysis will provide information of functional properties which correlate functional group and structure of composite blended film. FTIR can identify effects from molecular interactions between gelatin, CMC and chitosan on a film's structure. Figures 1a and 1b show FTIR spectra results for all formulations, which evidenced seven functional group patterns with similar peaks for both drying conditions. These included (i) amide A and free water (3200–3400 cm-1); (ii) the amide I band, which represents C=O or COO coupled with hydrogen bonding (1700–1600 cm⁻¹); (iii) the amide II band, which represents the bending vibrations of N-H groups and stretching vibrations of C-N groups (1500-1600 cm⁻¹); and (iv) the Amide III band's relation to vibrations in plane of C-N and N-H groups of bound amide (1000–1250cm⁻¹). The amide I band is the most sensitive spectral region of the protein's secondary structure. Each peak was noted in the same wavelength position for
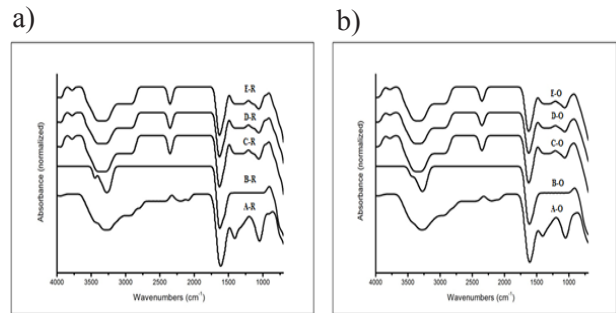

Figure 1. FTIR spectra for gelatin/CMC/chitosan blended films at a) Room dried condition (25 ºC); where (A-R) is formulation A; (B-R) is formulation B; (C-R) is formulation C; (D-R) is formulation D; (E-R) is formulation E.
b) Oven dried condition (45 ºC); where (A-O) is formulation A; (B-O) is formulation B; (C-O) is formulation C; (D-O) is formulation D; (E-O) is formulation E.

both drying conditions but with different intensities (Hanani *et al.*, 2011).

Figures 1a and 1b shows the gelatin based film spectra with the addition of CMC and chitosan, had peaks of O-H stretching that shifted toward lower wavelengths (3384–3288 cm⁻¹). These shifts indicated that hydrogen bonds acting on OH groups in these blends were weaker compared to pure polymer films (Tong *et al.*, 2008). In addition, the amide II peak (Figures 1a and 1b) shifted from 1542 to 1548 cm⁻¹ compared to the amide peak for gelatin films reported by Zhong and Xia (2008). Peaks at 1653–1641 and 1542 cm⁻¹ indicated C=O stretching (amide I) and NH bending (amide II) for pure gelatin films, respectively. By contrast, spectra for blended gelatin/CMC/chitosan films shifted to a lower wavelength for amide I (1641–1632 cm⁻¹), and to a higher wavelength for amide II (1542–1548 cm⁻¹). These differences may be due to alterations in the secondary structure of the gelatin's polypeptide chains due to the addition of chitosan. Amide I's peak for the present study's blended films decreased from 1641 to 1632 cm⁻¹ as shown in Figures 1a and 1b, suggesting that increased chitosan caused conformational changes in the gelatin's polypeptide chains resulting in a decreased presence of single helices, random coils and disordered structures (Fakhreddin *et al.*, 2013).

FTIR spectra for CMC films evidenced peaks at 3296 cm⁻¹ caused by O–H stretching and inter-/intra-molecular hydrogen bonds, and also at 2909 cm⁻¹ due to C–H stretching (Tong *et al.*, 2008). By blending CMC into a gelatin based film, the O-H stretching peak shifted to 3288 cm⁻¹, while pure CMC films exhibited peaks around 1413 cm⁻¹ that have previously been assigned to anti-symmetric and symmetric COO-group vibrations (Tong *et al.*, 2008). Hence, blending CMC with gelatin and chitosan showed a decreased

Table 1. Water Vapour Permeability ($g.mm.h^{-1}.cm^{-2}.Pa^{-1}$) of films prepared from different formulations of gelatin/CMC/chitosan for two drying temperatures

Formulation	Water vapour permeability ($g.mm.h^{-1}.cm^{-2}.Pa^{-1}$)		Biodegradability (gradient, *m*)	
	Room temperature (25 °C)	Oven drying temperature (45 °C)	Room temperature (25 °C)	Oven drying temperature (45 °C)
A	2.208×10^{-7aA}	7.775×10^{-8aA}	3.04	3.03
B	2.491×10^{-7aA}	2.872×10^{-7aA}	3.69	3.05
C	4.070×10^{-7aA}	1.953×10^{-7aA}	3.15	3.56
D	4.377×10^{-7aA}	3.702×10^{-7aA}	3.07	3.28
E	2.250×10^{-7aA}	1.065×10^{-7aA}	4.30	4.20

a – e mean within a row with different letters indicate significant differences ($p < 0.05$) while A-E mean within a colum with different letters indicate significant differences ($p<0.05$).
Formulation A (80/20/0); formulation B (80/0/20); formulation C (80/10/10); formulation D (60/20/20); formulation E (60/30/10).

intensity of the COO- group peak in our analysis, as shown in Figures 1a and 1b.

X-ray diffraction (XRD) analysis

XRD analysis describes the physical properties in terms of crystalline structure and assesses the compatibility of each component material in blended films (Su *et al.*, 2012). Figures 2a and 2b show diffractogram patterns ($2\theta = 20°$) for each composite film formulation (A, B, C, D and E) for both drying conditions (25 and 45ºC), respectively. This peak reflects the distance between amino acid residues along the helix, which is about 0.44 nm (Badii *et al.*, 2013). These diffractogram patterns were all very similar for both drying conditions for film formulations A, B, D and E, while formulation C's pattern (dried at 45ºC) was slightly different with higher intensity in area $2\theta = 20°– 80º$, compared to samples dried at 25°C.

The Gelatin/CMC based composite film, formulation A, had an intermediate characteristic reflection at $2\theta = 20°$. With the addition of chitosan, formulation B showed a stronger reflection (at 20º) of substantially increased intensity, indicating enhancement of the film's crystalline structure. The diffractograms also showed smaller peaks at about $2\theta = 10°$, for films with a chitosan component. Of note is that the smaller peak also increased as the amount of incorporated chitosan increased and was most obvious for formulations B and D. These results echo the work of Bergo and Sobral (2007) who showed a typically wide amorphous peak at $2\theta = 21.8°$ for gelatin film, whereas Cheng *et al.* (2003)

demonstrated two crystal peaks at $2\theta =11.5°$ and 18° for chitosan film.

Film formulations with less chitosan (C, D, E) demonstrated an amorphous phase. Bergo and Sobral (2007) observed a similar structural pattern in gelatin based film, suggesting the amorphous character was due to a lack of re-crystallization during production. This phase may reflect the high stability of the material as well as its increased moisture content, thus, preventing the formation of semi-crystalline regions. The amorphous phase of composite films C, D and E can be attributed to broken hydrogen bonds in chitosan molecules, and implies that hydrogen bonding between gelatin and chitosan favors their compatibility (Cheng *et al.*, 2003).

Generally, observed peak region patterns obtained by XRD were similar for both drying conditions. However, differences in intensity may reflect polymer crystallinity, as shown in Figures 2a and 2b. Effects of temperature on crystallinity as observed in the present study, was in the same agreement with the results obtained by Martin and Bradbury (1952) who showed that a lower casting temperature enhanced crystallinity in gelatin films. These results strongly suggests that films, prepared by drying gels at room (lower temperature), have a predominant collagen-like helical structure, and are referred to as cold-dried film or ''helical gelatin''. On the other hand, films prepared from gelatin solutions dried at higher temperatures (>35 ºC) have a prevailing coil structure and are called ''coiled gelatin'' or hot-dried films (Badii *et al.*, 2013). Films dried at room temperature (25ºC; Figure 2a) demonstrate a higher intensity XRD
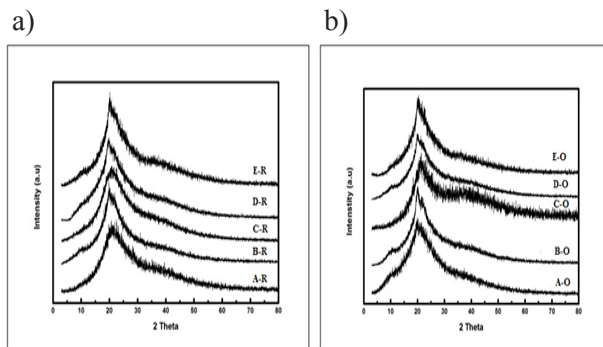
a) b)

Figure 2. XRD spectra of gelatin/CMC/chitosan blended films at a) Room dried condition (25 ºC); where (A-R) is formulation A; (B-R) is formulation B; (C-R) is formulation C; (D-R) is formulation D; (E-R) is formulation E.
b) Oven dried condition (45 ºC); where (A-O) is formulation A; (B-O) is formulation B; (C-O) is formulation C; (D-O) is formulation D; (E-O) is formulation E.

peak at $2\theta = 20°$, compared to oven dried films (45 ºC; Figure 2b). Hence, it appears that casting film at a lower temperature enhances the film's crystallinity while casting at a high temperature (45 ºC) leads to an amorphous structure.

Water vapor permeability (WVP)

Water Vapour Permeability (WVP) results ranged from 7.775×10^{-8} to 4.377×10^{-7} g.mm.h^{-1}.cm^{-2}.Pa^{-1}, suggesting that temperature clearly influences WVP (Table 1). Oven dried films showed no significant difference (p>0.05) of WVP value compared to room dried films. Study by Banker (1966), on synthetic polymer films found that higher temperatures led to increased cohesive strength between polymer chains, thus, creating a more compact structure with less free volume, which achieved lower permeability.

Film composition also did not influenced the WVP of the films in this study as there is no significant difference (p>0.05) of WVP value between each film formulation. The addition of 10 % chitosan increased the WVP value of gelatin/CMC blended film (Formulation A) from 7.775×10^{-8} to 1.953×10^{-7} and 1.065×10^{-7} g.mm.h^{-1}.cm^{-2}.Pa^{-1}, for Formulation C and E, respectively. Followed by chitosan addition up to 20 % in Formulation film B and D, WVP value increased to 2.872×10^{-7} and 3.702×10^{-7} g.mm.h^{-1}.cm^{-2}.Pa^{-1}. This findings was in agreement with study by Bourtoom and Chinnan, (2008) who reported that the biodegradable blend film WVP increased with an increase in the content of chitosan. Furthermore, increasing the chitosan ratio will increase the hydrophilicity groups (NH^{3+}) of the blended film (Bourtoom and Chinnan, 2008). Referring to the XRD test, the addition of chitosan will increase the crystallinity of the film. This crystalline

structure resulting in the increased of water vapour permeability value of the film produced. This finding was supported by Zhong & Xia (2008) which found a decreased in crystallinity of Chitosan/Cassava Starch /Gelatin blended film and resulted in low water vapour permeability value. This results proved that the increased in crystallinity of film may cause the decreased the amorphous properties thus lead to the increase water vapour permeability.

The presence of CMC also did not affect WVP in the present study. Formulations E (30% CMC), A (10% CMC) and C (20% CMC) all showed no significant difference (p>0.05) in WVP results, concurring with Tongdeesoontorn *et al.* (2009) who concluded that the addition of CMC to cassava starch films had no effect on WVP, while the moisture sorption of cassava starch based films increased with increased CMC content.

Film biodegradability

Film biodegradability tests (data not shown) measured daily weight loss (%) for each formulation over five days yielded the average weight loss percentage. Formulation E films, comprised of 60% gelatin, 30% CMC and 10% chitosan, had the best biodegradability indices with gradients (m) of 4.56 and 4.3 for both drying methods (25 and 45 ºC), respectively, and higher average weight loss results. Formulation B films (80/0/20) (gelatin/CMC/Chitosan) had the second highest average weight loss, while recorded losses for formulations A (80/20/0), C (80/10/10) and D (60/20/20) were nearly equal after the 5 days trial burial. Gradients of percent weight loss for oven dried films (45ºC) were 3.03, 3.05, 3.56, 3.28, 3.56; and for room dried films (25ºC) were 3.04, 3.69, 3.15, 3.07, 4.30, respectively, for formulations A, B, C, D and E. Oven dried films showed a lower percentage weight loss compared to room dried films.

Weight loss results for all buried films indicated that all film formulations contained degradable materials capable of undergoing significant changes in chemical structure under specific environmental conditions; i.e., the loss of structural integrity and other properties (Lee *et al.*, 2008). Complete biochemical decomposition of organic molecules by microorganisms is also known as biodegradation, which implies mineralization or the conversion of C, N, S, and P (organic compound content) to inorganic products. Previously, biodegradation was not considered in film production, but due to increased waste management concerns, this characteristic has since become a desirable quality (Robertson, 2013).

Crystallinity also correlated positively with

biodegradability in the present study. The high concentration of chitosan in formulation B (20%) contributed to its crystallinity, thus increasing the film's biodegradability compared to amorphous films. The diffractograms accounted for this crystalline fragility, which easily ruptures at low tensile strength and hastens biodegradability. FTIR spectra of formulation B films also provided direct evidence for the increased presence of OH groups that augmented the degradation rate.

Conclusion

In conclusion, results obtained indicate that different drying temperatures did not affect the physical properties of the gelatin/CMC/chitosan blended films produced and investigated. However, effects and interactions of gelatin, CMC and chitosan on the properties of gelatin-based films which demonstrated that chitosan association substantially influences the physical properties of blended films. A ratio of 60/30/10 (gelatin/CMC/chitosan) for formulation E appears optimal for potential use in food packaging as it had the second lowest water vapour permeability and the highest biodegradability rate.

Acknowledgements

References

Andreuccetti, C., Carvalho, R. A., Galicia-García, T., Martínez-bustos, F. and Grosso, C. R. F. 2011. Effect of surfactants on the functional properties of gelatin-based edible films. Journal of Food Engineering 103(2): 129–136.

Arvanitoyannis, I., Psomiadou, E., Nakayama, S., Aiba, S. and Yamamoto, N. 1997. Edible films made from gelatin, soluble starch and polyols, Part 3. Food Chemistry 60: 593–604.

Badii, F., MacNaughtan, W., Mitchell, J. R. and Farhat, I. A. 2013. The effect of drying temperature on physical properties of thin gelatin films. Drying Technology 32(1): 30–38.

Banker, G. S. 1966. Film coating theory and practice. Journal of Pharmacy and Pharmacology 55: 81–89.

Bergo, P. and Sobral, P. J. A. 2007. Effects of plasticizer on physical properties of pigskin gelatin films. Food Hydrocolloids 21(8): 1285–1289.

Biswal, D. R. and Singh, R. P. 2004. Characterisation of carboxymethyl cellulose and polyacrylamide graft copolymer. Carbohydrate Polymer 57: 379–387.

Bourtoom, T. and Chinnan, M. S. 2008. Preparation and properties of rice starch - chitosan blend biodegradable film. Food science and technology 41: 1633–1641.

Bradbury, E., & Martin, C. 1952. The effect of the temperature of preparation on the mechanical properties and structure of gelatin films. In Proceedings of the Royal Society of London A: Mathematical, Physical and Engineering Sciences (Vol. 214, No. 1117, pp. 183-192). The Royal Society.

Cao, N., Fu, Y. and He, J. 2007a. Preparation and physical properties of soy protein isolate and gelatin composite films. Food Hydrocolloids 21(7): 1153–1162.

Cao, N., Fu, Y. and He, J. 2007b. Mechanical properties of gelatin films cross-linked, respectively, by ferulic acid and tannin acid. Food Hydrocolloids 21: 575–584.

Cheng, M., Deng, J., Yang, F., Gong, Y., Zhao, N. and Zhang, X. 2003. Study on physical properties and nerve cell affinity of composite films from chitosan and gelatin solutions. Biomaterials 24(17): 2871–2880.

Cheng, L. H., Abd Karim, A. and Seow, C. C. 2008. Characterization of composite films made of konjac glucomannan (KGM), carboxymethyl cellulose (CMC) and lipid. Food Chemistry 107: 411 – 418.

Denavi, G. A., Perez-Mateos, M., Anon, M. C., Montero, P., Mauri, A. N. and Gómez-Guillén, M. C. 2009. Structural and functional properties of soy protein isolate and cod gelatin blend films. Food Hydrocolloids 23(8): 2094–2101.

Dutta, P. K., Tripathi, S., Mehrotra, G. K. and Dutta, J. 2009. Perspectives for chitosan based antimicrobial films in food applications. Food Chemistry 114(4): 1173–1182.

Fakhreddin, S., Rezaei, M., Zandi, M. and Farahmand, F. 2013. Preparation and functional properties of fish gelatin – chitosan blend edible films. Food Chemistry 136(3-4): 1490–1495.

Gómez-Estaca, J., Gómez-Guillen, M. C., Fernández-Martín, F. and Montero, P. 2011. Effects of gelatin origin, bovine hide and tuna-skin, on the properties of compound gelatin-chitosan films. Food Hydrocolloids 25(6): 1461–1469.

Hanani, Z. A. N., Roos, Y. H. and Kerry, J. P. 2011. Fourier Transform Infrared (FTIR) Spectroscopic Analysis of Biodegradable Gelatin Films Immersed in Water. In 11 th International Congress on Engineering and Food. Congress conducted at Athens, Greece.

Hoque, M. S., Benjakul, S., Prodpran, T. and Songtipya, P. 2011. Properties of blend film based on cuttlefish *(Sepia pharaonis)* skin gelatin and mungbean protein isolate. International Journal Biological Macromolecules 49(4): 663–673.

Jangchud, A. and Chinnan, M. S. 1999. Peanut protein film as affected by drying temperature and pH of film forming solution. Journal of Food Science 64: 153–157.

Janjarasskul, T., Rauch, D. J., McCarthy, K. L. and Krochta, J. M. 2014. Barrier and tensile properties of

whey protein–candelilla wax film/sheet. LWT - Food Science and Technology 56(2): 377–382.

Karnnet, S., Potiyaraj, P. and Pimpan, V. 2005. Preparation and properties of biodegradable stearic acid-modified gelatin films. Polymer Degradation and Stability 90(1): 106-110.

Lee, D. S., Yam, K. L. and Piergiovanni, L. 2008. Food packaging science and technology. Boca Raton, FL: Taylor & Francis.

Pereda, M., Aranguren, M. I. and Marcovich, N. E. 2009. Water vapor absorption and permeability of films based on chitosan and sodium caseinate. Journal of Applied Polymer Science 111(6): 2777–2784.

Perez-Gago, M. B., & Krochta, J. M. (2000). Drying temperature effect on water vapor permeability and mechanical properties of whey protein-lipid emulsion films. Journal of Agricultural and Food Chemistry 48(7): 2687-2692.

Rawdkuen, S., Suthiluk, P., Kamhangwong, D. and Benjakul, S. 2012. Mechanical, physico-chemical, and antimicrobial properties of gelatin-based film incorporated with catechin-lysozyme. Chemistry Central journal 6(1): 131.

Rhim, J. W., Hong, S. I. and Ha, C. S. 2009. Tensile, water vapor barrier and antimicrobial properties of PLA/ nanoclay composite films. LWT-Food Science and Technology 42(2): 612-617.

Rivero, S., García, M. A. and Pinotti, A. 2009. Composite and bi-layer films based on gelatin and chitosan. Journal of Food Engineering 90: 531–539.

Robertson, G. L. 2013. Food packaging: Principles and Practice. Boca Raton, FL: Taylor & Francis.

Su, J. F., Yuan, X. Y., Huang, Z., Wang, X. Y., Lu, X. Z., Zhang, L. D. and Wang, S. B. 2012. Physicochemical properties of soy protein isolate/carboxymethyl cellulose blend films crosslinked by Maillard reactions: color, transparency and heat-sealing ability. Materials science & engineering C 32(1): 40–6.

Thakhiew, W., Devahastin, S. and Soponronnarit, S. 2010. Effects of drying methods and plasticizer concentration on some physical and mechanical properties of edible chitosan films. Journal of Food Engineering 99: 216-224.

Tong, Q., Xiao, Q. and Lim, L. T. 2008. Preparation and properties of pullulan–alginate–carboxymethyl cellulose blend films. Food Research International 41(10): 1007–1014.

Tongdeesoontorn, W., Mauer, L. J., Wongruong, S. and Rachtanapun, P. 2009. Water vapour permeability and sorption isotherm of cassava starch based films blended with gelatin and carboxymethyl cellulose. Asian Journal Food Agro-Industries 2: 501-514.

Vicentini, N. M., Dupuy, N., Leitzelman, M., Cereda, M. P. and Sobral, P. J. A. 2005. Prediction of cassava starch edible film properties by chemometric analysis of infrared spectra. Spectroscopy Letters 38(6): 749-767.

Vroman, I. and Tighzert, L. 2009. Biodegradable Polymers. Materials 2: 307–344.

Wang, L., Auty, M. A. and Kerry, J. P. 2010. Physical assessment of composite biodegradable films manufactured using whey protein isolate, gelatin and sodium alginate. Journal of Food Engineering 96(2): 199-207.

Yadav, M., Rhee, K. Y. and Park, S. J. 2014. Synthesis and characterization of graphene oxide/carboxymethyl cellulose/alginate composite blend films. Carbohydrate polymers 110: 18–25.

Yoo, S. and Krochta, J. M. 2011. Whey protein–polysaccharide blended edible film formation and barrier, tensile, thermal and transparency properties. Journal of the Science of Food and Agriculture 91(14): 2628-2636.

Zainal, A. N. H., Beatty, E., Roos, Y. H. and Morris, M. A. 2013. Development and characterization of biodegradable composite films based on gelatin derived from beef, pork and fish sources. Foods 2: 1–17.

Zhong, Q. and Xia, W. 2008. physicochemical properties of edible and preservative films from chitosan/cassava starch/gelatin blend plasticized with glycerol. Food Technology Biotechnology 46(3): 262–269.