

Physical, Barrier and Mechanical Properties and Phenolic Content of Pectin/Lotus Seed Flour Composite Films

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Abstract

The lotus seed, which contains high antioxidant value, is of interest amid usage in food systems. It can be produced into flour and utilized as an ingredient. The lotus seed flour can be substituted for pectin up to a maximum of 50% for film-casting. Pectin: lotus seed flour films in the ratio of pectin: lotus seed flour 100:0, 75:25 and 50:50 (in order to obtain a concentration of 2% with 1% glycerol) determined the physical properties and phenolic content. Pectin: lotus seed flour films were brown in color following the color of lotus seed flour. Lotus seed flour content increased the moisture content, a^* , b^* , water vapor permeability and phenolic content of the films. However, tensile strength, solubility and L^* decreased ($p < 0.05$) while elongation was not significantly different ($p > 0.05$). The obtained film can be consumed with food to increase the phenolic content for improved health.

Keywords: Pectin, Lotus seed flour, Film, Physical properties, Phenolic content

1. Introduction

The development of edible films has been extensively studied in the last few decades. Active film alters packaging conditions to extend shelf-life or to improve certain properties of food products, thus denoting a feasible, innovative alternative for food processing and distribution. As a food grade polymer, pectin may serve as a matrix for the production of biodegradable and environmentally friendly edible film. Pectin film formulated to carry antioxidants has been previously developed, for instance pectin-methyl cellulose films with ascorbic acid which developed localized antioxidant activity for preserving the tocopherol content of walnut oil (Perez *et al.*, 2013). Acia-pectin film with thyme essential oil displays antimicrobial activity which protects against *Listeria monocytogenes* (Espitia *et al.*, 2014). were studied by Saha *et al.* (2016). Consequently, methylcellulose/pectin (90:10) gave the best results in terms of mechanical properties. Increasing pomegranate juice in pectin film resulted in enhanced elongation, decreased strength and modulus, increased water vapor permeability and decreased insoluble matter (Azeredo *et al.*, 2016). Pectin film with chitosan nanoparticles exhibits greater mechanical properties when compared with control pectin films (Lorevice *et al.*, 2016).

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The ratio of methylcellulose and pectin film This outcome is similar to the pectin/papaya puree/cinnamaldehyde nanoemulsion edible composite films which were studied by Otini *et al.* (2014) whereby papaya puree reduced films' tensile strength and rigidity while offering increased extensibility and water vapor permeability. Furthermore, cinnamaldehyde nanoemulsions balanced the plasticizing effect of papaya puree by increasing rigidity, yet decreasing extensibility and permeability to water among pectin films. Lotus (*Nelumbo nucifera*) seeds are one source of antioxidants. Thai lotus seeds especially, display 6 times more antioxidants than Chinese lotus seeds. Lotus seeds contain greater levels of proteins, amino acids, non-saturated fatty acids, and ash than lotus stems (Man *et al.*, 2012). Thai lotus seeds contain more crude protein, crude fiber and ash, yet lower crude lipid than Indian lotus seeds (Paiyarach and Punbusayakul, 2009). Pre-gelatinized lotus seed flour exhibits stronger antioxidant activities than raw lotus seed flour (Yen *et al.*, 2005). The antioxidants from lotus seeds may be applied to sausage products which may be effective in retarding lipid oxidation (Qi and Zhou, 2013). Furthermore, the lotus seed flour can be used instead of wheat flour (at up to 10%) in cookies and 5% in noodle products as per the most acceptable formula (Paiyarach and Punbusayakul, 2009; Jirukkakul and Sengkhampan, 2017). This suggests the potential of lotus seed flour as a possible alternative source of functional ingredients. The addition of lotus seeds in pectin film is a new product which shows high phenolic content to protect food products from lipid oxidation.

The aims of the experiment were to determine the physical properties and phenolic content of pectin/lotus seed flour films.

2. Materials and Methods

2.1 Film Preparation

Pectin (poly-D-galacturonic acid methyl ester, 65–70% degree of esterification) was purchased from HIMedia Laboratories Pvt. Ltd. (India), with the lotus (*Nelumbo nucifera*) seeds purchased from a local market in NongKhai, Thailand. The initial moisture content of fresh lotus seeds was 70.61%. The hard seed coat was removed with a sharp knife and dried at 60°C for 8 h. The moisture content of the dried lotus seeds was 8.25%. After that, the pieces were ground in a hammer mill with a mesh size of 80. The protein, fat, fiber, ash and phenolic content of lotus seed flour were 28.18%db, 0.02%db, 4.10%db, 5.80%db and 0.22mg GAE/100mg, respectively. Then, the pectin and lotus seed flour were mixed with distilled water in order to obtain a concentration of 2% (Saha *et al.*, 2016) which consisted of pectin:lotus seed flour in the ratio of 100:0, 75:25 and 50:50, respectively. Lotus seed flour could not be casing standalone film without enough of plasticizer which was more than 2% because the

plasticizing effect in edible films demonstrated by reducing tensile strength and rigidity while increasing elongation of films (Otoni *et al.*, 2014). The lotus seed flour was similar to the methylcellulose which was not possible to cast films constituting of higher proportions of methylcellulose to pectin than 50:50 (w/w) because non-homogeneous film making solutions were obtained due to segregation of the components and excessive viscosity. Moreover, it was irregular thickness and broke easily during peeling from plate (Perez *et al.*, 2013). Glycerol (1%) was added as the plasticizer. The mixed solution was heated at 100°C for 10 min while being stirred. Then, 50 mL film solution was poured onto a plate (15 cm diameter). The plates were then dried at 60°C in a hot air oven for 8 h. Next, the films were peeled off, placed in a plastic bag and stored in a desiccator prior to testing.

2.2 The Physical Characterizations of the Films

2.2.1 The Tensile Strength of the Films

The mechanical properties of the films were evaluated following the ASTM D882 method (ASTM, 1996). The texture analyzer (Micro Stable Systems, Godalming, UK) was set in tensile mode and was used with a 50 N load cell. The films were prepared in 1x12 cm² with initial grip length of 10 cm. The cross head speeds were set at 1 mm/sec. The tensile strength was calculated by dividing the maximum force by the cross-section area. Elongation was calculated in the percentage of the extension length.

2.2.2 The Moisture Content and Water Activity of the Films

The water activity indicates the shelf-life of a film. Generally, films exhibit low moisture content, and are considered as dried material. The water activity of films was examined using AquaLab (series 3, Decagon Devices, USA) and the moisture content of the films was determined by employing AOAC (2000). The films were cut into small pieces of 3 g and dried in a hot air oven 105°C for 24 h. The loss of weight of the film is calculated as a percentage.

2.2.3 The Solubility of the Films

The films were cut into 2x2 cm², weighed (A) and put in 50 mL of distilled water (25°C). Next, the flask was shaken for 24 h at 25°C. Film residue was filtrated from the solution and dried at 104°C for 24 h, then weighed (B). Solubility (%) = $(A-B) \times 100/A$ (Kavoosi *et al.*, 2014).

2.2.4 The Water Vapor Permeability of the Films

The water vapor permeability of the films was measured using ASTM E96 method (ASTM, 2000). The films were cut into 5 cm diameter, and then weighed as the initial weight. The cut film was sealed onto the mouth of the test-cup (5 cm diameter and 3 cm height) which contained 30 ml of distilled water. The test-cups were placed in the control chamber containing silica gel (RH=0%). Chamber RH was 50±5%. Weights were taken every hour at intervals up

to 8 h. A graph was subsequently plotted between the loss of weight and the time taken to obtain the slope of each sample. WVP was calculated as follows: $WVP (g \cdot mm/m^2 \cdot day \cdot kPa) = \text{slope} \times \text{thickness}/(\text{area} \times dp)$; dp = partial pressure between 2 sides of a film.

2.2.5 The Colors of the Films

The films were determined for color via the Hunter Lab Spectrocolorimeter. Films were cut for placing over the 1 cm diameter hole test detector. A white plate calibration was applied for the background. They were then covered with a black test-cup for interference protection. The colors were reported in $L^*a^*b^*$ system for lightness, redness and yellowness.

2.2.6 The Scanning Electron Microscope of the Films

The surface morphology of the paper was examined using scanning electron microscopy (SEM) (JSM-5600, JEOL, Japan). Samples were coated with gold under a vacuum. SEM was carried out to give further insight on the coatings. The examination used an accelerating voltage of 20 kV, and the magnification was 100x for surface of film.

2.3 The Phenolic Content of the Films

Phenolic contents behave as antioxidants due to the reactivity of their phenol moiety. Although there are several mechanisms, the predominant mode of antioxidant activity is believed to be radical scavenging via hydrogen atom donation (Bhat and Sridhar, 2011). The phenolic content of the film was determined according to Bhat and Sridhar (2011) along with some modifications. The 5 g of films was briefly extracted by employing 5 ml of 95% ethanol in a water bath at 95°C for 10 min. Then, 0.5 mL of extraction was mixed with distilled water at the same content and treated with 2 mL of Na_2CO_3 (in 0.1 N NaOH). After 10 min, 0.2 mL of Folin-Ciocalteu's reagent (diluted with water 1:1) was applied to determine the phenolic content expressed as gallic acid equivalents (GAE).

3. Results and Discussion

3.1 The Tensile Strength of the Films

The mechanical properties of films are often presented in terms of tensile strength (TS) and elongation (E). TS expresses the resistance of cohesion forces between chains (Shahidi and Ambigaipalan, 2015). Generally, TS of bioplastic and edible films are weaker than synthetic polymer. Notably, edible film produced from polysaccharide is lower in TS than protein and lipid edible film. Figure 1 shows the value of TS which decreases with lotus seed flour ($p < 0.05$) because of the brittle nature of lotus seed flour which had weak intermolecular interactions between polymeric chain lead to decreased rigidity of the films (Otoni *et al.*, 2014). Moreover, the crystal structure of lotus seed flour was destroyed in the heating process, and it was easy to associate the hydrogen bonding of the water with the hydroxyl of amylose (Zeng

et al., 2015). Similar results were determined by previous studies on pectin films with pomegranate juice and citric acid (Azeredo *et al.*, 2016) shown as 1–10 MPa of TS. E is mechanical properties expressed as the deformation prior to breakage which is required for some food applications. High E values indicate ample flexibility, extensibility and toughness due to the cohesion among polymer chains. The lotus seed flour did not affect E of pectin films ($p>0.05$). It expressed in the range 8.21–12.68% (Figure 1). E value of films in the experiment was greater than the pectin film with chitosan nanoparticles (1–3%) (Lorevice *et al.*, 2016) as well as pectin with acai film (4.9%) (Espitia *et al.*, 2014).

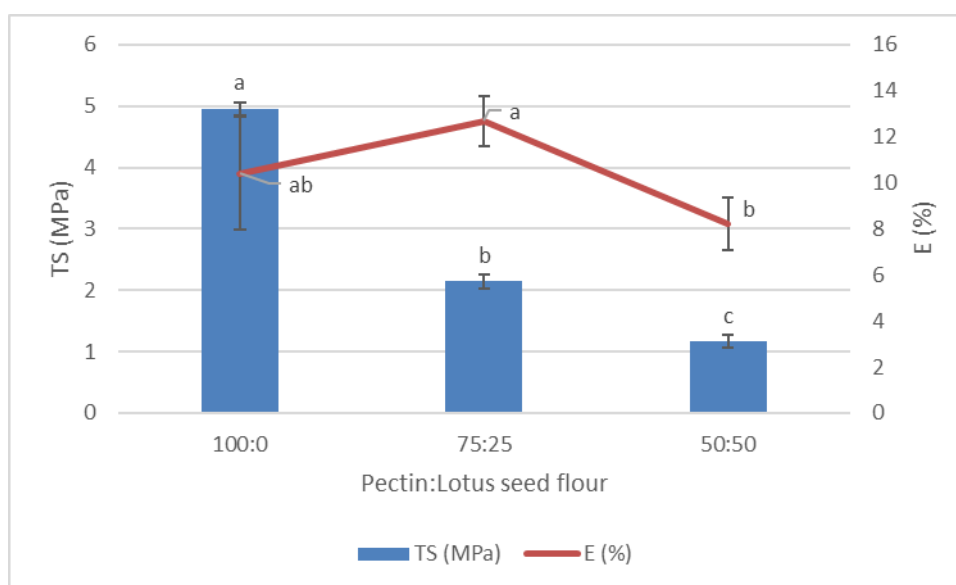


Figure 1 The tensile strength (TS) and elongation (E) of pectin:lotus seed flour (100:0, 75:25 and 50:50) films

3.2 The Moisture Content and Water Activity of the Films

The moisture content of the pectin film with lotus seed flour was high as they were measured at RH 55%. The results were congruent with pectin-methyl cellulose films which were measured at RH 57.5% (Perez *et al.*, 2013). Lotus seed flour affected high moisture content due to the fact that it is a hydrophilic material which absorbs moisture from the environment itself. However, the water activity of all treatments was not significant difference ($p>0.05$). It was in the range 0.36–0.37 which is lower than 0.5 so, the obtained films were considered to dried products.

3.3 The Solubility of the Films

The water solubility percentages of films are important for food packaging as such properties have an influence on applications. Water solubility is an indication of a film's hydrophilicity. The solubility of the films shows a similar trend to moisture content. The lotus seed flour is more water soluble material than pectin. Thus, the solubility of film decreased with lotus seed flour content increase ($p < 0.05$). The ordered rearrangement of amylose and the strong bonding forces, such as the double helix which plays an important role in limiting the starch hydration and solubility (Guo *et al.*, 2015).

Table 1 The moisture content and solubility of pectin:lotus seed flour (100:0, 75:25 and 50:50) films

Pectin:lotus seed flour	Moisture content (%)	Water activity	Solubility (%)
100:0	26.923±0.520 ^a	0.3647±0.013 ^a	57.654±0.120 ^a
75:25	28.281±0.224 ^b	0.3643±0.010 ^a	55.412±1.047 ^b
50:50	28.029±0.291 ^b	0.3657±0.010 ^a	30.240±1.218 ^c

Note: Different letters in the same column indicates the significant difference ($p < 0.05$)

3.4 The Water Vapor Permeability of the Film

Water vapor permeability (WVP) is highly significant for food packaging applications. WVP provides a measure of how easily water vapor can penetrate a material and suggests a material's moisture barrier properties (Otoni *et al.*, 2014). Biodegradable polymers exhibit a lower barrier property than synthetic polymer (Saha *et al.*, 2016). Pectin film WVP incorporated with lotus seed flour is presented in Figure 2. Pectin film WVP was significantly increased by lotus seed flour content due to the pectin dilution effect (Azeredo *et al.*, 2016). The study was congruent with alginate/pectin film (Galus and Lenart, 2013), however it was lesser than pectin films with pomegranate juice and citric acid (Azeredo *et al.*, 2016), and pectin/papaya puree/cinnamaldehyde nanoemulsion edible composite films (Otoni *et al.*, 2014). Such a difference may be caused by dissimilarity in the film preparation and WVP measuring conditions (Galus and Lenart, 2013).

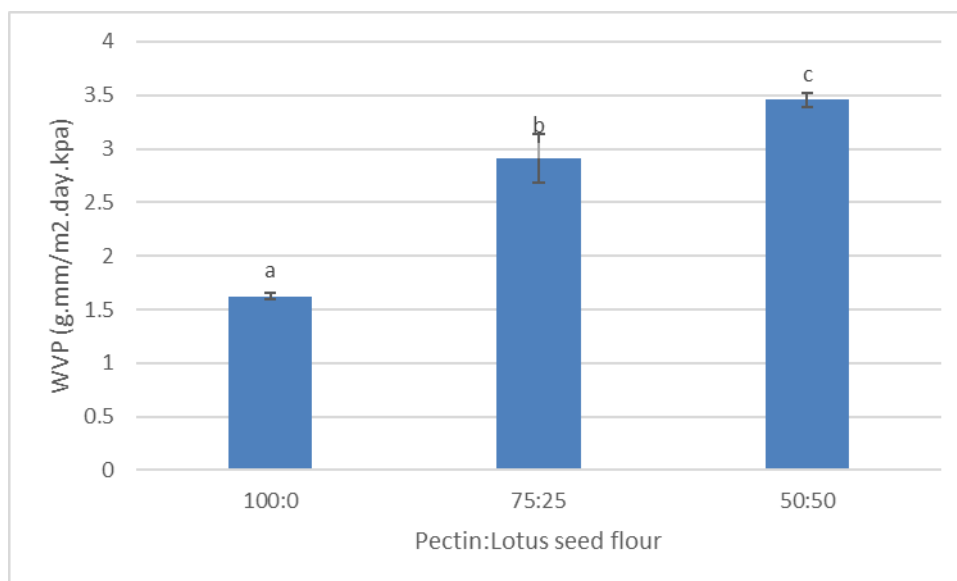


Figure 2 The Water vapor permeability (WVP) of pectin:lotus seed flour (100:0, 75:25 and 50:50) films

3.5 Color of the Films

Food packaging color is a central factor in terms of general appearance and consumer acceptance. The color parameter L^* provides a measurement of lightness. Its dark to light values range from 0 to 100. A positive a^* value is a measure of redness and a negative value of greenness. A positive b^* value is a measure of yellowness and a negative value of blueness (Espitia *et al.*, 2014). The appearance of pectin films without lotus seed flour was transparent, clear, homogeneous and smooth. Whereas the non-homogeneous of pectin:lotus seed flour film making solutions were obtained due to segregation of the components which affected irregular thickness, L^* , a^* and b^* values. The pectin films without lotus seed flour indicated high L^* value and low a^* and b^* values which was comparable to the pectin film from Perez *et al.* (2013) and Galus and Lenart (2013). The L^* of film decreased with lotus seed flour, while the a^* and b^* directly increased (Figure 3). It was noted that the light-brown color of lotus seed flour was correlated with high a^* and b^* values. These results were congruent with Espitia *et al.* (2014) who reported that increasing the amount of apple-skin polyphenols markedly increased the a^* and b^* value with the concomitant decrease in L^* values of pectin film consequent of its antimicrobial activity.



Figure 3 The appearance of pectin:lotus seed flour (100:0, 75:25 and 50:50) films

3.6 The Scanning Electron Microscope of the Films

Scanning electron microscopy observations were carried out to get a better insight in the homogeneity and the microscopic structure of films. The scanning electron microscope (SEM) pictures of the surface of pectin films with lotus seed flour are presented in Figure 4. The scratch of films was due to the clue of mold casing. The pectin film had a compact, smooth, transparent, and homogeneous surface structure due to the ordered-phase and homogeneous network structure (Galus and Lenart, 2013). The addition of the lotus seed flour increased roughness and opaqueness in the film matrix. The roughness and factures might result in weaker mechanical properties and might also lead to lower interactions among polymer chains (Lorevice *et al.*, 2016). The increasing concentration of lotus seed flour caused film brittleness so it could not be added more than 50% in film ingredient. The results were similar to the pure pectin films which presented more homogenous microstructure than the acia-pectin films (Espitia *et al.*, 2014).

3.7 The Phenolic Content of the Films

Antioxidant activity is expressed as phenolic content (Table 2). The major constituent in lotus seed flour is phenolic. In general, the phenolic content of films increases as the amount of lotus seed flour is increased. Phenolic is an antioxidant substance which may be applied to sausage products which could prove effective in retarding lipid oxidation (Qi and Zhou, 2013). Thus, the film, incorporated with lotus seed flour, showed a higher phenolic content than the pectin film ($p < 0.05$). The pectin film, devoid of lotus seed flour, had no phenolic content which was analogous with the results from Ayala-Zavala *et al.* (2013) and Melgarejo-Flores *et al.* (2013) who studied the total phenolic content of pectin with a cinnamon leaf oil-coating.

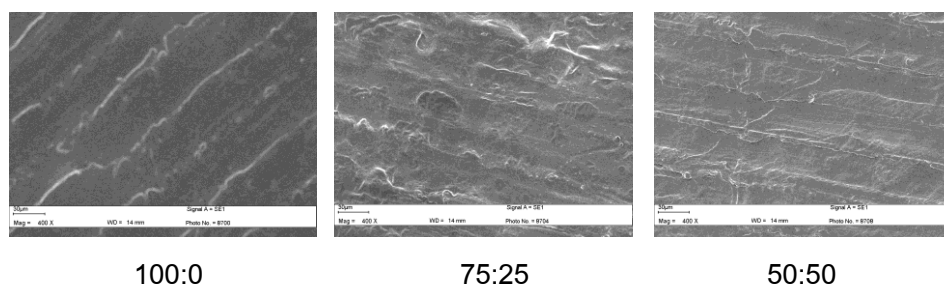


Figure 4 The scanning electron microscope (SEM) of pectin:lotus seed flour (100:0, 75:25 and 50:50) films at 100x magnification

Table 2 The color and phenolic content of films

Pectin: Lotus seed flour	L*	a*	b*	Phenolic (mg/mg film)
100:0	86.78±0.15 ^a	1.16±0.07 ^c	(-)4.42±0.38 ^c	0±0 ^c
75:25	73.04±0.53 ^b	8.41±0.32 ^b	17.84±0.56 ^b	1.71±0.26 ^b
50:50	63.51±0.48 ^c	13.08±0.39 ^a	26.91±1.49 ^a	2.32±0.07 ^a

Note: Different letters in the same column indicates the significant difference ($p < 0.05$)

4. Conclusion

The lotus seed flour content caused moisture content, water vapor permeability a^* and b^* of the film to increase, though it caused tensile strength, solubility and film-lightness to decrease. Meanwhile, elongation changed slightly ($p > 0.05$). In addition, the lotus seed flour impacted on the film's antioxidant activity in terms of phenolic content. Finally, suitable conditions for the physical and antioxidant properties of the film were pectin/lotus seed flour ratio of 50:50.

References

- AOAC. 2000. Official Methods of Analysis. In AOAC International, Gaithersburg, USA.
- ASTM. 1996. Standard test method for tensile properties of thin plastic sheeting D882. In ASTM International, PA, USA.
- ASTM. 2000. Standard test method for water transmission of material E96. In ASTM International, PA, USA.
- Ayala-Zavala, J.F., Silva-Espinoza, B.A., Valenzuela, M.R., Leyva, J.M., Ortega-Ramirez, L.A., Carrasco-Lugo, D.K., Perez-Carlon, J.J., Melgarejo-Flores, B.G., Gonzalez-Aguilar, G.A., and Miranda, M.R.A. 2013. Pectin-cinnamon leaf oil coatings add antioxidant and antibacterial properties of fresh-cut peach. *Flavour and Fragrance Journal*. 28(1):39–45.
- Azeredo, H.M.C., Morrugares-Carmona, R., Wellner, N., Cross, K., Bajka, B., and Waldron, K.W. 2016. Development of pectin films with pomegranate juice and citric acid. *Food Chemistry*. 198:101–106.
- Bhat, R. and Sridhar, K.R. 2011. Influence of ionizing radiation and conventional food processing treatments on the status of free radicals in lotus (*Nelumbo nucifera*) seeds. *Food Chemistry*. 107:174–184.

- Espitia, P.J.P., Avena-Bustillos, R.J., Du, W., Teofilo, R.F., Soares, N.F.F. and McHugh, T.H. 2014. Optimal antimicrobial formulation and physical-mechanical properties of edible films based on acai and pectin for food preservation. *Food Packaging and Shelf Life*. 2:38–49.
- Galus, S. and Lenart, A. 2013. Development and characterization of composite edible films based on sodium alginate and pectin. *Journal of Food Engineering*. 115:459–465.
- Guo, Z., Zeng, S., Lu, X., Zhou, M., Zhenf, M. and Zheng, B. 2015. Structural and physicochemical properties of lotus seed starch treated with ultra-high pressure. *Food Chemistry*. 186:223–230.
- Jirukkakul, N. and Sengkhampan, N. 2017. The physicochemical properties and potential source of the lotus seed flour for noodles. *Songklanakarin Journal of Science and Technology*. Article in press.
- Kavoosi, G. Rahmatollahi, A. and Dadfar, S.M.M. 2014. Effects of essential oil on the water binding capacity, physico-mechanical properties, antioxidant and antibacterial activity of gelatin films. *LWT-Food Science and Technology*. 57:556–561.
- Lorevice, M.V., Otini, C.G., Moura, M.R. and Mattoso, L.H.C. 2016. Chitosan nanoparticles on the improvement of thermal, barrier, and mechanical properties of high- and low-methyl pectin films. *Food Hydrocolloids*. 52:732–740,
- Man, J., Cai, J., Cai, C., Xu, B., Huai, H. and Wei, C. 2012. Comparison of physicochemical properties of starches from seed and rhizome of lotus. *Carbohydrate Polymers*. 88:676–683.
- Melgarejo-Flores, B.G., Ortiga-Ramirez, L.A., Silva-Espinoza, B.A., Gonzalez-Aguilar, G.A., Miranda, M.R.A. and Ayala-Zavala, J.F. 2013. Antifungal protection and antioxidant enhancement of table grapes treated with emulsions, vapors, and coatings of cinnamon leaf oil. *Postharvest Biology and Technology*. 86(1): 321–328.
- Otoni, C.G., Moura, M.R., Aouada, F.A., Camilloto, G.P., Cruz, R.S., Lorevice, M.V., Soares, N.F.F. and Mattoso, L.H.C. 2014. Antimicrobial and physical-mechanical properties of pectin/papaya puree/cinnamaldehyde nanoemulsion edible composite films. *Food Hydrocolloids*. 41:188–194.
- Paiyarach, D. and Punbusayakul, N. 2009. Nutritional quality and a prospected functional food ingredient of Thai lotus (*Nelumbo nucifera*) seed. In *The Thailand Research Fund*, Bangkok, Thailand.
- Perez, C.D., De'Nobili, M.D., Rizzo, S.A., Gerschenson, L.N., Descalzo, A.M. and Rojas, A.M. 2013. High methoxyl pectin-methyl cellulose films with antioxidant activity at a functional food interface. *Journal of Food Engineering*. 116:162–169.

- Qi, S. and Zhou, D. 2013. Lotus seed epicarp extract as potential antioxidant and anti-obesity additive in Chinese Cantonese Sausage. *Meat Science* 93:257–262.
- Saha, N.R., Sarkar, G., Roy, I., Rana, D., Bhattacharyya, A., Adhikari, A., Mukhopadhyay, A. and Chattopadhyay, D. 2016. Studies on methylcellulose/pectin/montmorillonite nanocomposite films and their application possibilities. *Carbohydrate Polymers*. 136:1218–1227.
- Shahidi F. and Ambigaipalan, P. 2015. Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects- A review. *Journal of Functional Foods*. 18: 820–897.
- Yen, G., Duh, P. and Su, H. 2005. Antioxidant properties of lotus seed and its effect on DNA damage in human lymphocytes. *Food Chemistry*. 89: 379–385.
- Zeng, S., Wu, X., Lin, S., Zeng, H., Lu, X., Zhang, Y. and Zheng, B. 2015. Structural characteristics and physicochemical properties of lotus seed resistant starch prepared by different methods. *Food Chemistry*. 186:213-222.