Available online at www.sciencedirect.com
SCIENCE DIRECT
Carbohydrate Polymers 60 (2005) 547–551
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

Short communication

# A combination of gamma irradiation and $CaCl_2$ immersion for a pectin-based biodegradable film

Ho Jin Kang[a,b], Cheorun Jo[b], Na Young Lee[b], Joong Ho Kwon[a], Myung Woo Byun[b,*]

[a]*Department of Food Science and Technology, Graduate School, Kyungpook National University, Daegu 702-701, South Korea*
[b]*Radiation Food Science and Biotechnology Team, Korea Atomic Energy Research Institute, P.O. Box 105, Yuseong, Daejeon 305-600, South Korea*

Received 11 August 2004; revised 16 November 2004; accepted 28 February 2005
Available online 7 April 2005

**Abstract**

Using citrus pectin a biodegradable film was prepared by a combination treatment of gamma irradiation (0, 10, 20, and 30 kGy) and $CaCl_2$ immersion (0, 5, and 10%) cross-linking. The tensile strength of the pectin-based film was the highest in the 5% $CaCl_2$ treatment at 20 kGy of an irradiation dose. The tensile strength of the film with $CaCl_2$ was generally higher than that of the non-$CaCl_2$ treatment. The elongation at break and water vapor permeability were the lowest at a $CaCl_2$ of 5% among the irradiated treatments. The total organic carbon content produced from the *Paenibacillus polymyxa* and *Pseudomonas aeruginosa* showed that the film of the 20 kGy-irradiated film was lower than those of the 0, 10, and 30 kGy-irradiated films. In conclusion, irradiation of the film casting solution at 20 kGy combined with a 5% $CaCl_2$ immersion resulted in film with improved mechanical properties and biodegradability.


*Keywords:* Citrus pectin; Irradiation; $CaCl_2$; Mechanical property; Biodegradable film

## 1. Introduction

Increased use of synthetic packaging films has led to serious ecological problems due to their total non-biodegradability (Tharanathan, 2003). Continuous awareness towards environmental pollution results in the need for a safe, eco-friendly atmosphere and it has led to a paradigm shift on the use of biodegradable materials, especially from renewable agriculture feedstock and marine food processing industry wastes (Tharanathan, 2003). The inert and non-biodegradable plastic materials represent approximately 30% of municipal solid waste, on the other hand biodegradable films can be a source for saving energy and an important issue for environmental protection (Lacroix et al., 2002).

Pectin is one of the largest proportional materials in the citrus by-products. Pectin is a complex of acidic polysaccharides that form an interpenetrating network in the plant cell wall. Plasticized blends of citrus pectin and high amylose starch give strong, flexible films, which are thermally stable up to 180 °C. Potential commercial uses for such films are water-soluble pouches for detergents and insecticides, flushable liners and bags, and medical delivery systems (Tharanathan, 2003). These films are solution cast by air-drying at ambient temperature.

Although pectin is a poor moisture barrier, pectin coatings were reported to retard water loss from food by acting as a sacrificing agent (Krochta & De Mulder-Johnston, 1997). Schultz, Owens, and Maclay (1948) found that $Ca^{2+}$-pectin forms can be used to develop edible film. On the other hand, gamma irradiation, was found to be effective for the improvement of the barrier properties, as well as the mechanical properties (Brault, D'Aprano, & Lacroix, 1997).

The aim of this study was to investigate the physical properties and the biodegradability of a pectin-based film by a combination of gamma irradiation and $CaCl_2$ treatment, which is a major agricultural by-product in citrus processing.

* Corresponding author. Tel.: +82 42 868 8060; fax: +82 42 868 8043.
*E-mail address:* mwbyun@kaeri.re.kr (M.W. Byun).

0144-8617/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2005.02.016

## 2. Materials and methods

### 2.1. Film preparation and irradiation

Pectin (2.5%, w/v, methoxy content was 9.0% dried base from citrus fruits, Sigma-Aldrich Co., St Louis, MO, USA), polyvinyl alcohol (1.25%, w/v, PVA, 98% hydrolyzed, Sigma-Aldrich Co., St Louis, MO, USA), and glycerol (2.5%, w/v, Archer Daniels Midland Company and Jusei Chemical Co., Ltd Tokyo, Japan) were suspended in distilled water (200 mL) at 90 °C after stirring for 30 min. After degassing the solution by sonicating it for 1 h, the solution was put into a brown-colored glass bottle (200 mL) and gamma-irradiated with an absorbed dose of 0, 10, 20, and 30 kGy. Irradiation was performed in the gamma irradiation facility (100 kCi, Co-60, point source, AECL, IR-79, MDS Nordion, ON, Canada) of the Korea Atomic Energy Research Institute, Korea. The temperature of irradiation was $14 \pm 1$ °C and the rate of irradiation was 10 kGy/h. Dosimetry was performed using 5 mm diameter alanine dosimeters (Bruker Instruments, Rheinstetten, Germany). The non-irradiated and irradiated solutions (25 mL) were spread onto a petri dish (10 cm diameter) and air-dried for 48 h at room temperature. The dried films were immersed in a 0, 5, and 10% $CaCl_2$ solution (50 mL) and removed after 5 min. Then the $CaCl_2$-immersed films were air dried for 24 h at room temperature. Before the determination of the mechanical properties and biodegradability, all the films were placed on a static temperature–moisture (25 °C, 50% relative humidity, Model FX 1077, JEIO Tech. Co. Ltd Seoul, Korea) for 24 h.

### 2.2. Film thickness and surface color

Film thickness was measured with the prepared testing film strips ($5 \times 1$ cm) at five different positions (four corners and a center) using a micrometer (Dial Thickness Gauge 7301, Mitutoyo, Tokyo, Japan; 0.01 μm limit) by slowly reducing the micrometer gap until the first indication of a contact and then averaged.

Color of the film surface was measured with the film strip ($5 \times 1$ cm) using the Hunter Colorimeter (Spectrophotometer CM 3500D, Minolta Co., Osaka, Japan) and the Hunter color $L^*$-, $a^*$- and $b^*$-value was reported. The color was measured 10 times for each film at different sites and then averaged.

### 2.3. Water vapor permeability (WVP) and water solubility (WS)

Water vapor permeability (WVP) of the film was measured according to the ASTM E96-95 (1995a) with some modifications using a sample cup made from polymethylacrylate with a 46 mm internal and 87 mm external diameter, and 21 mm depth. Distilled water (16 mL) was added into the cup and the film was tightly covered as a cross-head over the cup without any space remaining. After weighing the initial weight, the weight was measured every 2 h until 12 h. The environment was set at 25 °C and 50% relative humidity. Weight loss of the cups with time was measured and a linear regression analysis was performed to calculate the slope. WVP (ng m/m$^2$ s Pa) was calculated using the following equation

$$\mathrm{WVP} = (\mathrm{WVTR} \times L)/\Delta p$$

where, WVTR indicates the water vapor transmission rate of the sample film (ng/m$^2$ s) calculated by dividing the slope by the open area of the cup, $L$ indicates the average thickness of the sample film (m), and $\Delta p$ indicates the corrected difference of the partial vapor pressure (Pa) across the two phases of the film specimen.

Water solubility (WS) of the sample was determined by the method of Gontard, Guilbert, and Cuq (1992). Three films ($2 \times 2$ cm) were dried at 105 °C for 24 h in a dry oven and then the initial solid content was measured. The samples were then put into a 50 mL beaker with 30 mL distilled water and sealed by parafilm, and placed in a thermostat at 25 °C for 24 h. After drying for 24 h, the films were dried for a further 24 h in a dry oven and then the solid content was measured. The WS (%) of the films was defined as the ratio of the water-soluble solid to the initial solid content.

### 2.4. Tensile strength (TS) and percentage elongation at break (%E)

Tensile strength (TS) and percentage elongation at a break (%$E$) of the sample film ($5 \times 1$ cm) were measured following the American Standard of the Testing Materials (ASTM) (1995b) method using a Texture Analyzer (TA-XT2, Stable Micro System Co. Ltd, Surrey, England). It was set at an initial distance between the grips of 2 cm and a cross-head velocity of 3 mm/s. TS of the film was calculated by dividing the maximum strength by the initial cross-sectional area. The %$E$ was calculated by dividing the initial distance between the grips from the elongated distances until the time that the film breaks.

### 2.5. Biodegradability

*Paenibacillus polymyxa* (KCTC 1663) and *Pseudomonas aeruginosa* (KCTC 1636) were obtained from a Korean collection for type cultures (KCTC, Daejeon, Korea) and used as received. The bacterial cultures were prepared for 24 h in a sterilized nutrient broth medium by inoculating one colony from an agar slant, from which 0.1 mL was transferred to a new broth medium and grown for 18 h. The cultures were centrifuged ($698.75 \times g$ for 20 min at 4 °C) in a refrigerated centrifuge (VS-5500, Vision Scientific, Co., Seoul, Korea). Cultures were washed three times with a sterile saline solution. The pellet was finally suspended in a sterile saline solution to have a cell density of about

$10^7$–$10^8$ CFU/g levels. Ion solutions containing 0.3% $NH_4NO_3$, 0.1% $K_2HPO_4$, and 0.05% $MgSO_4$ were prepared and 1% of the start broth and 0.3% of the film (w/v) were added. The sample solution was incubated at 37 and 30 °C for *P. polymyxa* and *P. aeruginosa*, respectively. After 72 h of incubation the total organic carbon (TOC) was determined using a total organic carbon analyzer (TOC-5000A, Shimadzu Co., Kyoto, Japan). The upper layer of the sample solution (100 μL) after 72 h of incubation was used for the determination.

### 2.6. Scanning electron microscopy (SEM)

Morphological investigation of the surface and cross-section of the films were investigated using a scanning electron microscopy (S-2400, Hitachi Co., Tokyo, Japan). The samples were sputter-coated with gold at a thickness of 14 nm (K-550, Emitech, England) prior to examination to allow for conductivity.

### 2.7. Statistical analysis

The whole experiment was duplicated with three observation numbers adapted for each experiment except for the color measurement. Analysis of the Variance was performed with raw data, and the mean values and the standard errors were calculated by the Statistical Analysis System (SAS, 1989). Differences among the mean values were determined by the Duncan's multiple range test with a significance defined at $p<0.05$.

## 3. Results and discussion

### 3.1. Surface color and mechanical properties

Color of the packaging is an important factor in terms of general appearance and consumer acceptance. The Hunter color $L^*$-value of the pectin-based film increased by an increase of the irradiation dose ($p<0.05$). $CaCl_2$ treatment did not show any difference. Hunter color $a^*$-value was negative ($-2.20$) initially but a $CaCl_2$ of 5, 10% increased the $a^*$-value to $-1.60$–$0.26$, respectively. Irradiation increased the $b^*$-values (Data not shown). The $L^*$-value of the film with cross-linked starch were generally higher than those of the films with native starch (Kim & Lee, 2002). The data of the Hunter color $a^*$- and $b^*$-values were not significantly different among the irradiation dose, but the $a^*$-value of the 5% $CaCl_2$ immersion film was higher than the others. Jo, Kang, Lee, and Byun (2005) reported that the Hunter color $L^*$-, $a^*$-value of the pectin/gelatin-based film decreased with an increase of the irradiation doses, but the Hunter color $b^*$-value increased. The different results between the previous and the present studies might be caused by the protein used in the previous study.

The thickness of the films prepared was $0.17\pm0.01$ μm with no difference among the films. The tensile strength is the most important mechanical property for many applications (Park, Rhim, Jung, & Kang, 1995). The TS of the non-irradiated pectin film increased as the concentration of the $CaCl_2$ immersion increased but the WS, WVP, and $\%E$ were decreased (Table 1). TS of the alginate films prepared by 5% $CaCl_2$ increased, whereas the $\%E$ decreased significantly ($p<0.05$) (Rhim, 2004). Generally, the strength, flexibility of the carbohydrate or protein composite films are negatively

Table 1
Mechanical properties of the pectin-based film treated with a combination of irradiation and $CaCl_2$

| Irradiation dose (kGy) | $CaCl_2$ treatment (%) | Water solubility (%) | Water vapor permeability (ng m/m$^2$ s Pa) | Tensile strength (kPa) | Elongation at break (%) |
|---|---|---|---|---|---|
| 0 | 0 | 63.66a | 0.15a | 153c | 3.45a |
| | 5 | 59.82b | 0.14ab | 193b | 2.60b |
| | 10 | 55.16c | 0.13b | 230a | 1.38c |
| | SEM[a] | 0.137 | 0.004 | 4.4 | 0.033 |
| 10 | 0 | 83.17a | 0.15a | 163c | 2.49a |
| | 5 | 79.86c | 0.10b | 233a | 1.27c |
| | 10 | 82.57b | 0.15a | 193b | 1.86b |
| | SEM | 0.0226 | 0.006 | 4.4 | 0.004 |
| 20 | 0 | 71.23a | 0.11b | 110b | 2.94a |
| | 5 | 52.33c | 0.11b | 269a | 1.02c |
| | 10 | 55.52b | 0.17a | 270a | 2.71b |
| | SEM | 0.018 | 0.004 | 4.0 | 0.019 |
| 30 | 0 | 57.33b | 0.207a | 120b | 2.92a |
| | 5 | 43.64c | 0.110c | 190a | 1.07c |
| | 10 | 83.63a | 0.170b | 93c | 1.60b |
| | SEM | 0.187 | 0.006 | 1.9 | 0.002 |

Different letters (a–c) within the same column differ significantly ($p<0.05$).
[a] Pooled standard error of the mean ($n=9$).

correlated (Lee, Shim, & Lee, 2004). Irradiated pectin film had a different trend from the non-irradiated counterpart. TS of the irradiated pectin film was the highest in 5% of the $CaCl_2$ immersion, whereas the WS, WVP, and *%E* were the lowest at the same level for all the irradiation doses applied. Particularly, TS was the highest and the WS, WVP, and *%E* was the lowest at a 5% $CaCl_2$ immersion with 20 kGy. The WVP values were negatively correlated with the TS. Pavlath, Voisin, and Robertson (1999) reported that the WVP of the pectin film by a 5% $CaCl_2$ immersion was decreased three times more than those of the native pectin films. WVP of the films based on a soya protein isolate was significantly reduced by a gamma irradiation while the TS increased (Lacroix et al., 2002). Ressouany, Vachon, and Lacroix (1998) reported that a gamma irradiation significantly increased the mechanical properties of the films by inducing cross-links between the protein chains. Results of these references indicate that the irradiation increased the cross-linking of the protein or carbohydrate molecules. However, using pectin alone, 30 kGy of irradiation decreased the TS significantly. It suggests that using biomolecules such as carbohydrate in film making, it is not always irradiation dose-dependent. The present pectin film prepared by $CaCl_2$ immersion may have formed $Ca^{2+}$-pectate by cross-linking both the pectin molecular and the calcium ion. Therefore, a successful cross-linked pectin film was obtained by using a 5% $CaCl_2$ immersion and 20 kGy of irradiation with substantial mechanical properties.

### 3.2. Biodegradability and scanning electron microscopy (SEM)

*P. polymyxa* (the strain name changed from *Bacillus polymyxa*) or *Bacillus macerans* produce a strong amylase activity and sometimes they cause a tenderizing of vegetables or foods. They are also very effective in the pectolytic degradation from pectin. *P. aeruginosa* is also a well-known bacterium which can degrade polyvinylalcohol completely (Lacroix et al., 2002). Both *P. polymyxa* and *P. aeruginosa* are found easily in a soil environment.

From the results of the mechanical properties, 5% $CaCl_2$ was selected and further investigated for measuring the biodegradability. In the strain of *P. polymyxa*, the content of the total organic carbon was lower in the pectin-based film with a 20 kGy irradiation (Fig. 1). However, $CaCl_2$ treatment did not affect the biodegradability of the film. With a consideration of the mechanical properties and biodegradability, the film irradiated at 20 kGy was the optimum one from this study.

Biodegradability of *P. aeruginosa* was also shown as the lowest for the 20 kGy-irradiated film both the non- and $CaCl_2$-treated film. There was a difference found between the non- and $CaCl_2$-treated (Fig. 1). It was observed that there were different patterns in the total organic carbon content between the two microbial strains (Jo et al., 2005). Kim and Lee (2000) reported that the biodegradability of starch/PE films was accelerated by an addition of NaOH cross-linked starch to the PE films. The authors indicated that potato starch cross-linked by NaOH treatment was more sensitive than that of the non-treated from the attack of *P. aeruginosa*, resulting in a better biodegradability.

The results of the scanning microscopy confirmed that the irradiation and $CaCl_2$ treatment of the pectin film decreased the interlayer space of the film (Fig. 2). Bigi, Bracci, Cojazzi, Panzavolta, and Roveri (1998) reported that the thickness and interlayer space of the gelatin film decreased dramatically via a cross-linking with glutaraldehyde. Zhai, Yoshii, and Kume (2003) also found that

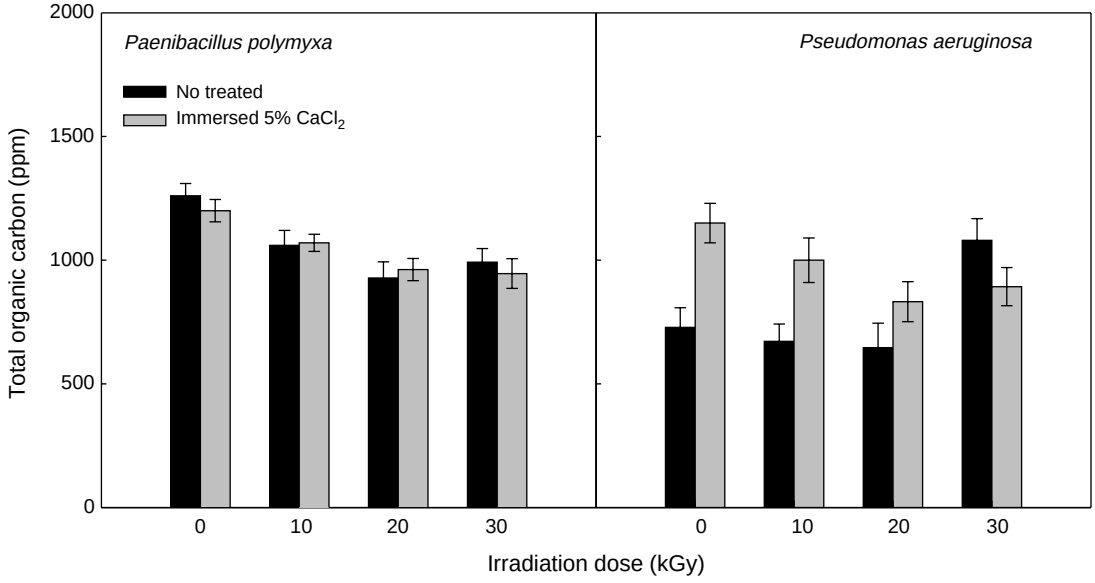


Fig. 1. Content of the total organic carbon (ppm) produced by *Paenibacillus polymyxa* and *Pseudomonas aeruginosa* in the solution containing the pectin-based film as a nutrient source.

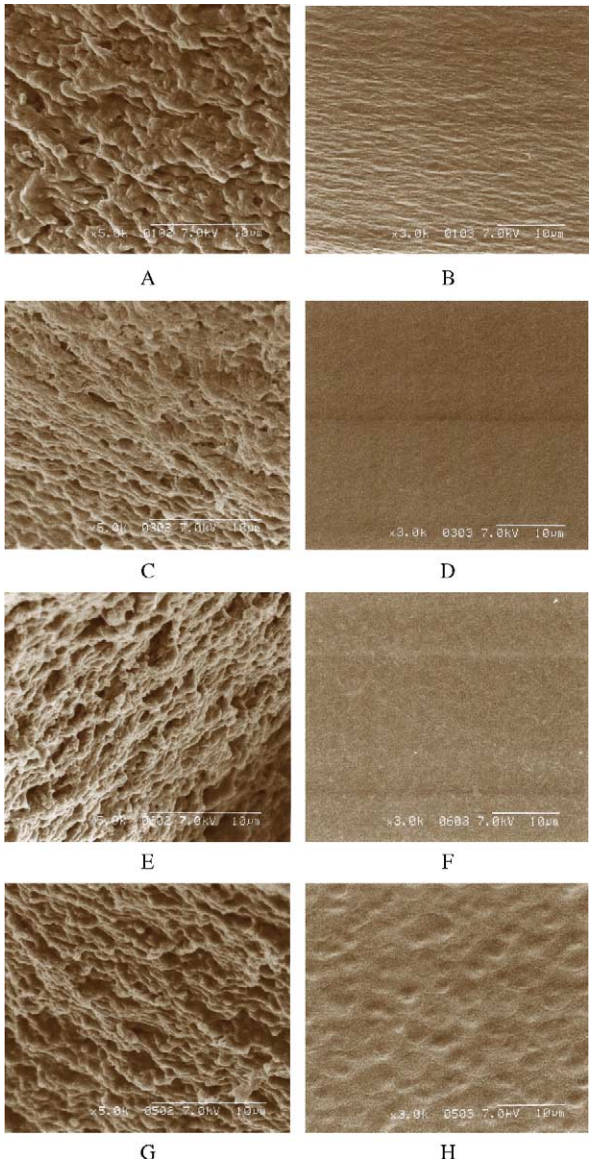


Fig. 2. Scanning electron microscopy (SEM) of the pectin-based film with a combination treatment of $CaCl_2$ (5%) and irradiation. (A) Cross-section (×5000), 0 kGy; (B) surface (×3000), 0 kGy; (C) cross-section (×5000), 0 kGy $CaCl_2$; (D) surface (×3000), 0 kGy $CaCl_2$; (E) cross-section (×5000), 20 kGy; (F) surface (×3000), 20 kGy; (G) cross-section (×5000), 20 kGy $CaCl_2$; (H) surface (×3000), 20 kGy $CaCl_2$.

when a starch-based sheet in a physical gel state was irradiated, an intact, smooth starch-based sheet was formed after drying at the same conditions when compared to the non-irradiation ones.

In conclusion, an irradiation with a combination of $CaCl_2$ treatment results in an improvement of the mechanical properties and biodegradability of the citrus pectin-based film.

## Acknowledgements

This study was supported by Korea Institute of Science and Technology Evaluation and planning (KISTEP) and Ministry of Science & Technology (MOST), Korean government, through its National Nuclear Technology Program.

## References

ASTM (1995a). Standard test methods for water vapor transmission of materials (E96-95). In *Annual book of ASTM standards* (pp. 745–754). Philadelphia, PA: American Society for Testing and Materials.

ASTM (1995b). Standard test methods for water vapor transmission of materials (D882-91). In *Annual book of ASTM standards* (pp. 182–190). Philadelphia, PA: American Society for Testing and Materials.

Bigi, A., Bracci, B., Cojazzi, G., Panzavolta, S., & Roveri, N. (1998). Drawn gelatin films with improved mechanical properties. *Biomaterials*, *19*, 2335–2340.

Brault, D., D'Aprano, G., & Lacroix, M. (1997). Formation of free-standing sterilized edible films from irradiated caseinates. *Journal of Agricultural and Food Chemistry*, *45*, 2964–2969.

Gontard, N., Guilbert, S., & Cuq, J. L. (1992). Edible wheat gluten film: Influence of the main process variables on film properties using response surface methodology. *Journal of Food Science*, *57*, 190–199.

Jo, C., Kang, H. J., Lee, N. Y., & Byun, M. W. (2005). Pectin and gelatin-based film: Effect of gamma irradiation on the mechanical properties and biodegradation. *Radiation Physics and Chemistry*, 72, 745–750.

Kim, M., & Lee, S. J. (2002). Characteristics of crosslinked potato starch and starch-filled linear low-density polyethylene films. *Carbohydrate Polymers*, *50*, 331–337.

Kim, M. R., & Lee, S. J. (2000). Mechanical properties and degradability of degradable polyethylene films containing crosslinked potato starch. *Korean Journal of Food Science and Technology*, *32*(6), 1298–1305.

Krochta, J. M., & De Mulder-Johnston, C. (1997). Edible and biodegradable polymer films: Challenges and opportunity. *Food Technology*, *51*, 61–74.

Lacroix, M., Le, T. C., Ouattara, B., Yu, H., Letendre, M., Sabato, S. F., et al. (2002). Use of γ-irradiation to produce films from whey, casein, and soya proteins: Structure and functional characteristics. *Radiation Physics and Chemistry*, *63*, 827–832.

Lee, K. Y., Shim, J., & Lee, H. G. (2004). Mechanical properties of gellan and gelatin composite films. *Carbohydrate Polymers*, *56*, 251–254.

Park, H. J., Rhim, J. H., Jung, S. T., & Kang, S. G. (1995). Mechanical properties of carrageenan-based biopolymer films. *Journal of KOPAST*, *1*, 38–50.

Pavlath, A. E., Voisin, A., & Robertson, G. H. (1999). Pectin-based biodegradable water insoluble films. *Macromolecular Symposium*, *140*, 107–113.

Ressouany, M., Vachon, C., & Lacroix, M. (1998). Irradiation dose and calcium effect of the mechanical properties of cross-linked caseinate films. *Journal of Agricultural and Food Chemistry*, *46*(4), 1618–1623.

Rhim, J. W. (2004). Physical and mechanical properties of water resistant sodium alginate films. *LWT Food Science and Technology*, *37*, 323–330.

SAS Institute, Inc. (1989). *SAS user's guide*. Cary, NC: SAS Institute, Inc.

Schultz, T. H., Owens, H. S., & Maclay, W. D. (1948). Pectinate films. *Journal of Colloid Science*, *3*, 53–62.

Tharanathan, R. N. (2003). Biodegradable films and composite coatings: Past, present and future. *Trends in Food Science and Technology*, *14*, 71–78.

Zhai, M., Yoshii, F., & Kume, T. (2003). Radiation modification of starch-based plastic sheets. *Carbohydrate Polymers*, *52*, 311–317.