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The Effect of Chitosan-Based Nanocomposite Coating on the Postharvest Life of Papaya (*Carica papaya* L.) Fruits

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Nanochitosan, which was prepared by ionotropic gelation of chitosan and polyphosphate ions, had an average particle size of 112 nm. The nanochitosan was incorporated in formulations for chitosan-based nanocomposite (Ch-NCh) films and coatings. The 80% nanochitosan (v/v) containing film (Ch80NCh + Add) was the most compact and thinnest, followed by the 40% nanochitosan (v/v) containing film (Ch40NCh + Add) and the 1% chitosan-plus-additives film (Ch + Add) which was highly porous and the thickest. The capability of the coating formulations to extend the postharvest life of cv. Sinta papaya fruits, which were stored at 14.6 °C and 79% relative humidity, was evaluated. Control (uncoated) and blank (additives only, Add only) coated fruits reached the limit of marketability on the 7th and 10th days of storage, respectively. The Ch + Add coated fruits reached the limit of marketability on the 14th day of storage. The Ch-NCh coated fruits did not reach the limit of marketability at the end of the 26-d storage period. The Ch-NCh coatings retarded peel color change, minimized disease incidence, shriveling and weight loss, and maintained pulp firmness. Titratable acidity of the Ch80NCh + Add coated fruits peaked later (19th day) than that of the other fruit samples which peaked on the 14th day of storage. Total soluble solids (TSS) content generally increased during storage with the Ch-NCh coated fruits generally having lower TSS readings. Results indicate that chitosan-based nanocomposite coatings retard fruit ripening in papaya cv. Sinta.

Key Words: chitosan, nanochitosan, nanocomposite coating, nanoparticles, papaya, postharvest life

Abbreviations: Add – additives, Ch – chitosan, CI – color index, DD – degree of deacetylation, NCh – nanochitosan, TA – titratable acidity, TSS – total soluble solids

INTRODUCTION

Increasing the shelf life and improving the appearance of many food products through edible coatings have been the treatment of choice in response to the growing demand for good-quality and fresh food products. New and improved technologies, along with suitable applications in the food industry, are emerging in response to these demands and concern for the environment. Nanoscience and nanotechnology are fields of study that have the potential to generate new products and processes in the food industry. The application of nanotechnology in the development of edible coatings and films may help the food industry to produce materials that could alleviate serious global environmental problems of pollution. In addition, the biodegradability of these coatings and films could enhance their potential to extend the shelf life and quality of food products (Tharanathan 2003).

Chitosan is a polymer that has been used as a matrix in edible coatings because it is a nontoxic and biocompatible material (Kean and Thanou 2010). It is a linear co-polymer of β-(1-4) linked 2-acetamido-2-deoxy-β-D-glucopyranose and 2-amino-2-deoxy-β-D-glycopyranose derived from chitin, the second most abundant polysaccharide, next to cellulose (Johney et al. 2014). The material is produced commercially from abundant, renewable resources, primarily from seafood processing waste (Kim et al. 2006).

Several studies have shown the effectiveness of chitosan as a coating in extending the postharvest life of fresh, whole and minimally processed fruits and vegetables. Chitosan has been used to delay ripening and to improve the postharvest quality of guava (Hong et al. 2012), tomato (El-Beltagy et al. 2013), banana (Jafarizadeh Malmiri et al. 2011) and papaya fruits (Barrera et al. 2015). The effectiveness of chitosan as a coating for fruits and vegetables depends on its selective gas permeability

(Wong et al. 1992) and its antimicrobial properties (Romanazzi et al. 2015; Garcia et al. 2014; Lopez-Mata et al. 2013). The United States Food and Drug Administration (USFDA) has approved chitosan coating as a "Generally Recognized as Safe" (GRAS) substance, its application being safe for the consumer and the environment (Romanazzi et al. 2015).

However, as with other naturally occurring polysaccharides when used as a coating or film, chitosan has poor moisture barrier and weak mechanical properties (Elsabee and Abdou 2013; Sorrentino et al. 2007) because of its chemical structure. On the other hand, nanoparticles exhibit unique properties quite distinct from their microsize counterparts, thus the inclusion of nanoparticles into a chitosan matrix could enhance and/or improve its properties.

Nanocomposite films have improved mechanical strength and decreased moisture permeance (Torabi and Nafchi 2013; de Moura et al. 2009). Kerch (2015) found that chitosan films and coatings containing nanoparticles have reduced water vapor permeability and improved mechanical properties. Several studies have demonstrated the potential of nanocomposite coatings to maintain the quality and extend the shelf life of fresh produce (Garcia et al. 2014; Zambrano-Zaragoza et al. 2013). Increased shelf life was also observed in tomatoes and grapes coated with a nanocomposite coating of low molecular weight chitosan with silver nanoparticles (Johney et al. 2014).

Chitosan nanoparticles have been used as fillers for polymer matrices. The incorporation of chitosan-tripolyphosphate nanoparticles significantly improved the mechanical and barrier properties of hydroxypropyl methylcellulose films (De Moura et al. 2009). Chitosan nanoparticles are natural materials that have impressive physicochemical properties, are environmentally friendly and are bioactive (Yang et al. 2009). These nanoparticles have been used in drug (Katas et al. 2013) and gene (Vimal et al. 2013) delivery systems.

Papaya (*Carica papaya* L.), a very popular tropical fruit, has a large export demand (Rivera 2005). It is grown almost throughout the country as a backyard and plantation crop (Lustria et al. 2009 unpublished). It has increasingly contributed to the Philippine economy and has potential to become a major fruit crop like mango, banana and pineapple. The fruit is climacteric, hence, it has a short postharvest life (Ali et al. 2011). Its marketability is affected by increasing incidence of overripe fruit, hence postharvest handling procedures are essential to minimize losses and prolong its shelf life. Chitosan coatings containing low molecular weight chitosan (Dotto et al. 2015) and propolis as an additive (Barrera et al. 2015) have demonstrated effectiveness in extending the shelf life of papaya fruits. These recent findings indicate that edible coatings enhanced with nanofillers can also be an effective way of improving the postharvest life of papaya.

This study aimed (1) to prepare a nanocomposite coating using chitosan as a matrix with chitosan nanoparticles as filler and (2) to evaluate its performance in extending the postharvest life of papaya fruits.

MATERIALS AND METHODS

Preparation of Nanochitosan

Nanochitosan (NCh) was prepared according to the ionotropic gelation procedure of Yang et al. (2009) with some modifications. Sodium polyphosphate was used instead of sodium tripolyphosphate. Dr. Hong Kyoon No of the Catholic University of Daegu, Department of Food Science and Technology, Hayang, South Korea, provided the chitosan samples. Chitosan with a molecular weight (MW) of 223 KDa was directly converted to nanochitosan without treatment with hydrogen peroxide. Five tenths of a gram of chitosan was dissolved in 1 L of 2% acetic acid (Baker Analyzed Reagent, USA) and stirred for 30 min. One hundred mL of the resulting solution was mixed with 40 mL 0.5 gL^{-1} sodium polyphosphate (Merck, Germany) solution and stirred for 2 h at room temperature (RT), thus producing a nanochitosan suspension.

The prepared NCh was subjected to transmission electron microscopy (TEM Hitachi H300, Japan) at the National Institute of Molecular Biology and Biotechnology (BIOTECH), University of the Philippines Los Baños. A drop of the nanochitosan suspension was placed in a 400 mesh copper grid coated with collodion. Excess fluid was removed by blotting with filter paper. The suspension was allowed to set for 1–2 min, and then subjected to negative staining using 1% purified terephthalic acid (PTA) for about 2 min. TEM examination of the specimen was done using 100–120 KV accelerating voltage. Images of the specimen were taken and the film was processed and developed for printing.

Chitosan-Nanochitosan Coating Formulations

Chitosan with a molecular weight of 1100 KDa was used in the preparation of coating formulations. The chitosan was analyzed for its solubility in different solvents (water, 0.1 N CH_3COOH and 0.1 N NaOH) and degree of deacetylation (DD) following the method of Schoch (1964) and Sabnis and Block (1997) as cited by Khan et al. (2002), respectively, with some modifications.

The coating formulations containing chitosan (MW 1100 KD) as the matrix, nanochitosan and additives, were prepared by mixing the components and homogenizing with a Braun MR 350 CA hand blender (Spain) until a clear solution was obtained. The additives included casein as the protein component, ascorbic acid as the acidulant, polysorbate and ethylene glycol as the emulsifier and plasticizer, respectively. Four coating formulations were prepared. The first was made up of additives only (Add only), the second consisted of 1% (w/v) chitosan and additives (Ch + Add), the third contained 1% (w/v) chitosan - 40% (v/v) nanochitosan with additives (Ch40NCh + Add), and the fourth contained 1% (w/v) chitosan - 80% (v/v) nanochitosan with additives (Ch80NCh + Add).

The chitosan containing formulations were cast to form films. The films were subjected to scanning electron microscopy (SEM) using a JEOL 5300 Scanning Electron Microscope at the Physics Department, College of Science of the De La Salle University Taft, Taft Ave., Manila. Film samples were attached to a double-sided carbon tape and coated with gold using a JEOL JFC 1200 Fine coater. Images of the specimen were captured using SEM.

Application of Chitosan-Nanochitosan Coating Formulations

Cv. Sinta papaya fruits at breaker stage [peel color index (CI) 2 – not more than 10% yellow] were harvested from the Eddie Silan farm, Indang, Cavite, Philippines. The fruits were individually wrapped in newspaper, placed in plastic crates and transported by land vehicle to the laboratory. The fruits were washed with detergent and allowed to dry before coating. The coating mixtures were applied on the fruits with an airbrush. The coated fruits were placed in open trays, air-dried at room temperature then stored at 14.6 °C and a relative humidity of 79%. The fruits were observed until each treatment reached the limit of marketability.

Five treatments of the fruit samples were set. Uncoated fruits served as control and the Add only coated fruits served as blank. The other fruits were coated with the other formulations, Ch + Add, Ch40NCh + Add and Ch80NCh + Add.

Separate sets of fruits were used for monitoring the physical attributes and chemical changes of the fruits during storage. Observations of their physical attributes were recorded in triplicate for each treatment while two replicates were taken for each treatment at each sampling time to monitor chemical changes during storage.

Physico-chemical Evaluation of Papaya Fruits during Storage

Physical analyses. Changes in the physical attributes of the papaya fruits were monitored during storage. Fruit color was determined by visual examination using the color index scale developed by the Postharvest Horticulture Training and Research Center, University of the Philippines Los Baños where: 1 = fully green; 2 = breaker, not more than 10% yellow; 3 = more green than yellow; 4 = more yellow than green; 5 = yellow with traces of green; and 6 = fully yellow. Fruit firmness was measured using a fruit penetrometer with a cone-type probe tip (MF10 Imada®, Japan) in kg force unit. The fruits were cut in half, and pulp firmness was estimated from the center of each half. Disease incidence was based on the appearance of the fruit surface by visual examination. Disease severity was rated according to the following disease severity indices: 0 = 0% no disease; 1 = 10% of fruit surface infected; 2 = 25% of fruit surface infected; 3 = 50% of fruit surface infected; and 4 $\geq$ 75% of fruit surface infected.

Shriveling was determined subjectively according to the shriveling index scale given by Quintana and Paull (1993) as cited by Proulx et al. (2005) where 1 = field fresh, no sign of shriveling; 2 = minor signs of shriveling; 3 = shriveling evident but not serious; 4 = moderate shriveling and 5 = extremely wilted and dry. A shriveling rate of 3 was considered the limit of acceptability for sale of the fruit (Proulx et al. 2005). During the course of storage, the weight of the same set of fruits was calculated. Weight loss was reported as the percentage weight loss from the initial weight.

Chemical analyses. Fifty grams of papaya pulp was homogenized with 100 mL distilled water using a blender for 1 min, then filtered and the supernatant was used to determine titratable acidity and total soluble solids.

Titratable acidity (TA) was determined by titrating a 6.00 mL aliquot of the supernatant with standard 0.1N NaOH (AOAC 1995). Percent titratable acidity was expressed as % malic acid.

Total soluble solids (TSS) content was determined by the refractometry method (AOAC 1995). Two to three drops of the supernatant was placed in a hand-held °Brix refractometer (Atago®, Japan) and the refractometer reading was recorded. A dilution factor (DF) was calculated for correction of the TSS reading of the filtrate. The following equations were used:

$$\mathrm{DF} = 1 + Vol\ H_20\ /fresh\ weight\ of\ sample,\ g$$

$$TSS\ ^\circ\mathrm{Brix} = refractometer\ reading\ x\ DF$$

Statistical Analysis

Statistical analysis was conducted using The SAS® System for Windows™ release 6.12. The General Linear Models Procedure employing Analysis of Variance (ANOVA) and Least Significant Difference (LSD) Test at 5% level of significance were applied for the determination of significant differences among treatments.

RESULTS AND DISCUSSION

Characterization of Nanochitosan Particles, Chitosan and Chitosan-Nanochitosan Films

Nanochitosan was prepared by ionotropic gelation with polyphosphate ions instead of tripolyphosphate ions as

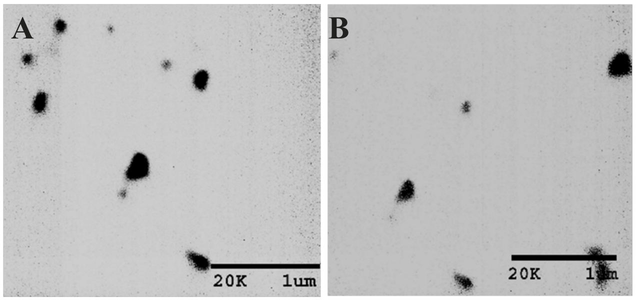

Fig. 1. Transmission electron micrographs of two different fields (A and B) of nanochitosan solution.

Yang et al. (2009) used. The average particle size of the prepared nanochitosan was 112 nm as determined by transmission electron microscopy, TEM (Fig. 1). The nanoparticles appeared spherical except a few that were irregularly shaped. Transmission electron microscopy allows analysis of samples by means of high resolution and high magnification imaging (Ma et al. 2006). The images of a sample are produced when it is illuminated with a focused beam of high-energy electrons and the electrons that are transmitted through the sample are detected. The images produced are significantly of high resolution and hence are commonly used to measure nanoparticle size.

The 1100 KD chitosan had a solubility of 50% in 0.1N CH_3COOH, 6% in water and was insoluble in 0.1 NaOH. Its DD was 57.72 ± 1.4%. This is in agreement with the survey conducted by No and Meyers (1995) which showed that the degree of deacetylation of commercial chitosan ranges from 56% to 97%. Nadres (2007) reported a DD value of 55.50% ± 4.83% for chitosan. Khan et al. (2002), however, stated that chitosan is generally known to have a DD value of 75%. Kim et al. (2006) reported that films prepared from chitosan with a DD of 78.9%, which was considered as low DD, had lower water vapor permeability and higher tensile strength compared to films from chitosan with high DD (92.3%). These findings suggest that the films produced in this study could serve as a barrier to moisture and are relatively strong.

The surface morphologies and thickness of the films cast from the coating formulations were analyzed by SEM. With SEM a beam of high-energy electrons is focused on the surface of the sample producing images from which the morphology and topography of the surface could be analyzed (Ma et al. 2006). Electron micrographs of the films are shown in Figure 2. The Ch + Add film (Fig. 2-A1) exhibited a high degree of porosity with large pore sizes evenly distributed throughout the

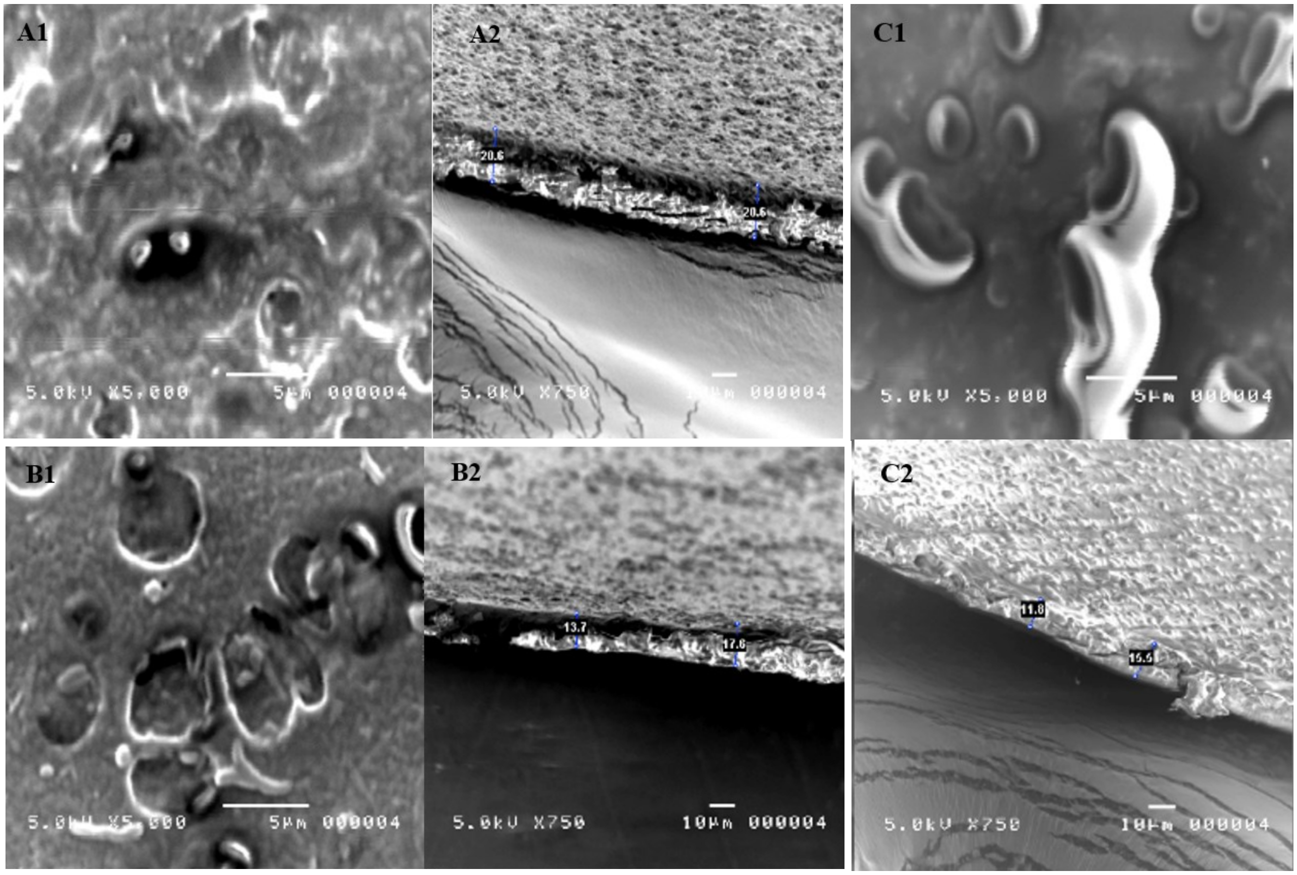

Fig. 2. Scanning electron micrographs of the surface (left) at 5000x magnification and cross-section (right) at 750x magnification of films cast from the coating formulations (A) Ch + Add, (B) Ch40NCh + Add and (C) Ch80NCh + Add.

film while there were fewer and smaller pores in the Ch40NCh + Add film (Fig. 2-B1). Also, the pores of the Ch + Add film were deep while those of the Ch40NCh + Add were shallow. The Ch80NCh + Add film (Fig. 2-C1) was compact compared to the other films. The deep pores observed in the Ch + Add film appeared to have been filled with nanochitosan particles in the Ch80NCh + Add film.

The texture of the films was assessed by comparing the roughness or smoothness of the film surfaces as they appear in the SEM electron micrographs. The SEM micrographs (Fig. 2) show that the presence of a relatively high concentration of nanochitosan improved film texture. The Ch + Add film was rough and coarse compared to the nanochitosan containing films, which were smooth, with the Ch80NCh + Add film being smoother. Also, the Ch80NCh + Add film had a glossy surface while the Ch40NCh + Add film had a dull surface.

These observations agree with those of De Moura et al. (2009) who stated that the large surface area of nanoparticles increases its interaction with the film matrix. Also, the small-sized nanoparticles have the capability to fill in the voids of the porous chitosan matrix. This explains the smoother surface of the films containing nanochitosan. De Moura et al. (2009) observed more compact hydroxypropyl methylcellulose films when nanoparticles were present. Compaction can decrease water permeability and increase tensile strength. These are characteristics of a good coating material.

Among the films, the Ch + Add film was the thickest at 20.6 μm (Fig. 2-A2) followed by the Ch40NCh + Add film with intermediate thickness of 15.7 μm (Fig. 2-B2), then Ch80NCh + Add film being the thinnest with an average of 13.7 μm. The decrease in film thickness with increase in nanochitosan concentration indicates that compaction occurred. Film thickness is an important factor in determining water vapor as well as gas permeability (Donhowe and Fennema 1994). It is possible that the Ch80NCh + Add film had the lowest water vapor and oxygen and carbon dioxide permeabilities among the films because it was the thinnest of the films and it visually exhibited the smallest pore sizes.

Physicochemical Characteristics of Coated Papaya Fruits

To assess the effect of chitosan-based coatings on the postharvest life of papaya fruits, the fruits with a color index 2 (not more than 10% yellow) were used since this stage marks the start of their physiological maturity.

Peel color. Peel color is commonly used as a visual ripening index of climacteric fruits. Change in peel color is important as it depicts loss of chlorophyll, synthesis of carotenoid pigments and unmasking of other pigments formed earlier during fruit development (Basulto et al. 2009). Papaya cv. Sinta fruits were evaluated regularly from start of coating until the end of their marketability. A change in peel color from green to orange-yellow was evident from the first to the last day of storage.

Changes in the peel color of the papaya fruits during storage are given in Table 1. Control fruits exhibited a significant change in peel color from CI 2 to CI 3 on day 3 while the coated fruits remained at CI 2. The additives only and Ch + Add coated fruits showed a significant change in peel color on day 5. The fruits with coatings containing nanochitosan changed color only on day 10. The color index (CI) value of these fruits was significantly lower than that of the other fruits. The control fruits had the highest CI value of 5 (yellow with traces of green) on day 19 while the fruits with nanochitosan had a CI value of 3. On the 26th day of storage, the fruits coated with Ch40NCh + Add and Ch80NCh + Add had a peel color of CI 4. No readings were obtained for the other fruits as these had reached the end of their marketability.

Retardation of color development in papaya fruits coated with formulations containing nanochitosan results from the creation of a modified internal fruit atmosphere (Al Eryani-Raqeeb et al. 2009), which could reduce respiration rate by selective permeability of CO_2 and O_2 gases. The presence of nanochitosan increases the barrier properties of the coatings by interactions between the matrix and nanochitosan particles. Also, the nanochitosan particles fill in the voids in the chitosan matrix, thereby decreasing the permeability of the coating to gases. Oxygen stimulates ethylene synthesis while carbon dioxide is a competitive inhibitor of ethylene action (Burg and Burg 1965). Ethylene is the hormone responsible for chlorophyll breakdown and carotenoid synthesis (Haard and Chism 1996). At low O_2 concentrations and high CO_2 concentrations, ethylene synthesis is retarded as a result of chlorophyll degradation and carotenoid synthesis is inhibited. It may be said that the coatings with nanochitosan can indirectly delay chlorophyll degradation and carotenoid synthesis.

Disease severity. Papaya is susceptible to various postharvest diseases. The two major postharvest diseases of papaya are Anthracnose, caused by the fungus *Colletotrichum gloeosporoides* and black rot, caused by the fungus *Mycosphaerella caricae* (Zhou et al. 2004).

Hot water and fungicidal treatments are procedures carried out prior to storage to prevent pathogenic infection. However, these procedures were not conducted in this study in order to evaluate the effect of the chitosan-nanochitosan coating formulations on the disease severity of papaya fruits.

Disease severity increased with prolonged storage (Table 2). Significant differences among the treatments were observed only on the 10th day of storage. The control fruits were the first to exhibit disease infection, which was on day 7, while the Ch + Add coated fruits showed evidence of disease on the 10th day. Evidences of disease in fruits with the chitosan-nanochitosan coatings were observed only on the last day of storage (day 26). By this time, no fruit samples were left for the other treatments as

Table 1. Color changes in papaya fruits with storage at 14.5 °C and RH 79% as affected by chitosan-based nanocomposite coating formulations.

	Color Index							
	Days of Storage							
	1	3	5	7	10	14	19	26
Control	2.0 ± 0.0^{A}	3.0 ± 0.0^{A}	3.0 ± 0.0^{A}	3.0 ± 0.0^{A}	3.3 ± 0.5^{A}	4.0 ± 0.0^{A}	5.0 ± 0.0^{A}	-
Add only	2.0 ± 0.0^{A}	2.0 ± 0.0^{B}	2.7 ± 0.5^{B}	2.7 ± 0.5^{B}	3.0 ± 0.0^{A}	3.7 ± 0.5^{B}	3.7 ± 0.5^{B}	-
Ch + Add	2.0 ± 0.0^{A}	2.0 ± 0.0^{B}	2.3 ± 0.5^{C}	2.3 ± 0.5^{C}	3.0 ± 0.0^{A}	3.3 ± 0.5^{C}	3.7 ± 0.5^{B}	-
Ch40NCh + Add	2.0 ± 0.0^{A}	2.0 ± 0.0^{B}	2.0 ± 0.0^{D}	2.0 ± 0.0^{D}	2.3 ± 0.5^{B}	3.0 ± 0.0^{D}	3.0 ± 0.0^{C}	4.0 ± 1.1^{A}
Ch80NCh + Add	2.0 ± 0.0^{A}	2.0 ± 0.0^{B}	2.0 ± 0.0^{D}	2.0 ± 0.0^{D}	2.3 ± 0.5^{B}	3.0 ± 0.0^{D}	3.0 ± 0.0^{C}	4.0 ± 0.0^{A}

1 = fully green, 2 = breaker, not more than 10% yellow, 3 = more green than yellow, 4 = more yellow than green, 5 = yellow with traces of green, 6 = fully yellow.

Data are means of three replicates. Means ranks of the same superscript letter in a column are not significantly different at the 5% level by LSD.

Table 2. Disease severity of papaya fruits coated with chitosan-based nanocomposite coating formulations on storage at 14.5 °C and RH 79%.

Treatment	Disease Severity*							
	Days of Storage							
	1	3	5	7	10	14	19	26
Control	0.0	0.0	0.0	1.0^{A}	2.0^{A}	2.7 ± 0.5^{A}	3.7 ± 0.5^{A}	-
Add only	0.0	0.0	0.0	0.1 ± 0.33^{B}	1.0^{B}	2.0^{B}	3.0^{B}	-
Ch + Add	0.0	0.0	0.0	0.0^{B}	0.7 ± 0.5^{C}	1.0 ± 0.8^{C}	2.3 ± 0.5^{C}	-
Ch40NCh + Add	0.0	0.0	0.0	0.0^{B}	0.0^{D}	0.0^{D}	0.0^{D}	1.5 ± 0.6^{A}
Ch80NCh + Add	0.0	0.0	0.0	0.0^{B}	0.0^{D}	0.0^{D}	0.0^{D}	1.5 ± 0.6^{A}

0 = 0% no disease; 1 = 10% of fruit surface infected; 2 = 25% of fruit surface infected; 3 = 50% of fruit surface infected; 4 $\geq$ 75% of fruit surface infected.

Data are means of three replicates. Means ranks of the same superscript letter in a column are not significantly different at the 5% level by LSD.

they had previously reached the end of their marketable life because of infection. On day 26, the chitosan-nanochitosan coated fruits were still marketable while the 1% chitosan (Ch + Add) coated fruits were heavily infected (Fig. 3).

Results indicate that chitosan afforded protection against microbial growth on the papaya fruits, which was enhanced by the addition of nanochitosan to the matrix. The nanochitosan containing coatings provided some protection.

Dotto et al. (2015) reported that papaya fruit coated with chitosan exhibited lower microbial counts compared to uncoated fruits stored at 18–24 °C. The appearance of microbial infection in uncoated fruits was earlier than that in Ch + Add coated fruits and much earlier than that in the chitosan-nanochitosan coated fruits in the present study (Table 2).

Other researchers also reported that chitosan coatings with nanoparticles reduced or prevented microbial infection in coated fruits (Johney et al. 2014; Garcia et al. 2014) and fresh-cut fruits (Pilon et al. 2015). The antifungal and antibacterial activities of chitosan are attributed to its polycationic nature, which allows electrostatic interactions with the anionic components of the cell surface of microorganisms (Kim et al. 2003 and Tsai et al. 2002, as cited by Elsabee and Abdou 2013), thus bringing about changes in their cell surface integrity and permeability (Dotto et al. 2015; Kong et al. 2010).

Shriveling and weight loss. Shriveling of the fruit peel indicates drying or loss of water. Shriveling can be determined subjectively through visual or feel rating. A shriveling rate of 3 (shriveling evident but not serious) is the accepted limit of marketability of papaya fruits (Proulx et al. 2005).

There was a general increase in shriveling of stored papaya fruits (Table 3). Significant differences in shriveling index among all the treatments occurred only on the 10th day of storage. The control, Add only and Ch+Add coated fruits exhibited the first sign of shriveling on the 7th day. The fruits coated with formulations containing nanochitosan exhibited a change in shriveling index only on the 14th day of storage. The control fruits reached their limit of marketability in terms of shriveling on day 10 while that of the fruits coated with Add only on day 14. The fruits coated with Ch40NCh + Add and Ch80NCh + Add did not differ in shriveling until the last day of sampling, day 26. Both sets of fruit samples did not reach the limit of marketability in terms of shriveling index.

Moisture loss in fruits increases during storage, thus resulting in weight loss. The weight loss of the papaya fruits gradually increased with storage (Table 4). However, none of the fruit samples reached a weight loss of about 8%. According to Paull and Chen (1989), as cited by Proulx et al. (2005), a weight loss of approximately 8% in mature green papayas produced rubbery textured, low -glossed, shriveled and unsalable fruits. In our study, the control fruits had the highest percentage of weight loss among all the fruit samples. The Ch-NCh coated fruits generally exhibited significantly lower weight loss values compared to the control and other fruit samples. The Ch-NCh coated fruits did not significantly differ in weight loss during the course of storage. The Ch40NCh + Add coated fruits had the lowest mean weight loss of 1.3% after 19 d of storage.

Results indicate that nanochitosan coatings can prevent shriveling and reduce weight loss in coated fruits. This in turn indicates low water vapor permeability of the

Fig. 3. External appearance of papaya fruits coated with chitosan-based nanocomposite coating formulation after 26 d of storage at 14.5 °C and RH 79%. Papaya fruits coated with: A) Ch40NCh + Add, B) Ch80NCh + Add, and C) Ch + Add.

Table 3. Shriveling indices of chitosan-nanochitosan coated papaya fruits on storage at 14.5 °C and RH 79%.

Treatment	Shriveling Index							
	Storage Time (Days)							
	1	3	5	7	10	14	19	26
Control	1.0^{A}	1.0^{A}	1.0^{A}	2.0^{A}	3.0^{A}	3.7 ± 0.5^{A}	4.0 ± 0.0^{A}	-
Add only	1.0^{A}	1.0^{A}	1.0^{A}	2.0^{A}	2.3 ± 0.5^{C}	3.3 ± 0.5^{A}	4.0 ± 0.0^{A}	-
Ch + Add	1.0^{A}	1.0^{A}	1.0^{A}	1.0^{B}	2.7 ± 0.5^{B}	2.7 ± 0.5^{B}	2.7 ± 0.5^{B}	-
Ch40NCh + Add	1.0^{A}	1.0^{A}	1.0^{A}	1.0^{B}	1.0 ± 0.0^{D}	2.0 ± 0.0^{C}	2.3 ± 0.5^{B}	2.5 ± 0.5^{A}
Ch80NCh + Add	1.0^{A}	1.0^{A}	1.0^{A}	1.0^{B}	1.0 ± 0.0^{D}	2.0 ± 0.0^{C}	2.3 ± 0.5^{B}	2.5 ± 0.5^{A}

1 = field-fresh, no sign of shriveling; 2 = minor signs of shriveling; 3 = shriveling evident but not serious; 4 = moderate shriveling; 5 = extremely wilted and dry.

Data are means of three replicates. Means in a column followed by the same superscript letter are not significantly different at the 5% level of significance using LSD

Table 4. Weight loss of chitosan-nanochitosan coated papaya fruits during storage at 14.5 °C and RH 79%.

Treatment	Weight Loss (%)*					
	Storage Time (Days)					
	3	5	7	10	14	19
Control	0.57 ± 0.14^{A}	0.93 ± 0.65^{A}	1.36 ± 0.08^{A}	1.18 ± 0.59^{B}	1.73 ± 0.51^{A}	4.35 ± 1.78^{A}
Add only	0.63 ± 0.11^{A}	0.64 ± 0.07^{AB}	1.33 ± 0.27^{A}	1.76 ± 0.32^{A}	1.65 ± 0.28^{A}	3.11 ± 0.78^{B}
Ch + Add	0.38 ± 0.06^{BC}	0.49 ± 0.11^{B}	0.80 ± 0.34^{B}	0.96 ± 0.23^{BC}	1.31 ± 0.40^{B}	2.48 ± 1.13^{BC}
Ch40NCh + Add	0.30 ± 0.05^{C}	0.35 ± 0.19^{B}	0.49 ± 0.19^{C}	0.77 ± 0.77^{C}	0.75 ± 0.09^{C}	1.30 ± 0.07^{D}
Ch80NCh + Add	0.41 ± 0.06^{B}	0.44 ± 0.04^{B}	0.66 ± 0.24^{BC}	0.99 ± 0.18^{BC}	1.00 ± 0.16^{BC}	1.61 ± 0.19^{CD}

*Values are expressed as means of three replicates. Means in a column followed by the same superscript letter are not significantly different at the 5% level of significance using LSD.

coatings, which prevented the escape of moisture from the fruit. The presence of the chitosan nanoparticles decreased the water vapor permeability of the chitosan film because of the formation of hydrogen bonds between the nanochitosan and the chitosan matrix, thereby decreasing the diffusion of water (De Moura et al. 2009). The nanochitosan particles, because of their small size, fill in the voids in the porous chitosan film matrix, thus making the film structure more compact and more difficult for water molecules to diffuse through. Water molecules follow a tortuous path as they diffuse through the coating (De Moura et al. 2009).

Pulp firmness. Pulp firmness generally decreased during storage (Table 5). A drastic decrease was observed in the control and Add only coated fruits after 3 d of storage. Similarly, Pereira et al. (2009) noted that untreated 'Tainung-1' papaya fruits softened rapidly with more than 80% softening during the first 3 d of storage at 27 °C. Papaya cv. Golden fruits at a mature green stage of ripeness and stored at 24 °C, however, softened gradually. Similarly, pulp firmness of the cv. Sinta papaya fruits coated with Ch + Add and those coated with the Ch -NCh nanocomposite coatings gradually decreased. The Ch-NCh coated fruits were generally significantly firmer than the control fruits. These results imply that chitosan and the nanochitosan composite coatings retarded processes like hemicellulose and pectin breakdown that cause fruit softening. Such processes occur during fruit ripening by the action of ethylene, which could have been retarded by a modified internal atmosphere in the coated fruits.

Titratable acidity and total soluble solids. Titratable acidity (TA) of the papaya fruits generally increased up to a point and then decreased during storage (Table 6). The control and all the coated fruits except for the Ch80NCh + Add coated fruits had a peak in TA on the 10th day of storage. For the latter, the peak was on the 14th day. At this point, the TA of these papaya fruit samples was significantly higher than that of the other samples, while on the 19th day, their TA was significantly higher than that of the control and the Add only coated fruits. On the 26th day, the chitosan-nanochitosan coated fruits did not significantly differ in TA. Throughout storage, the control and the Add only coated fruits did not significantly differ in TA. Lazan et al. (1989) and Abu-Goukh et al. (2010) also reported a similar increase and then a decrease in TA

Table 5. Pulp firmness index of chitosan-nanochitosan coated papaya fruits during storage at 14.5 °C and RH 79%.

Treatment	Pulp Firmness (kg force)*							
	Storage Time (Days)							
	1	3	5	7	10	14	19	26
Control	2.24 ± 1.30^{AB}	0.040 ± 0.22^{C}	0.12 ± 0.08^{D}	0.06 ± 0.06^{B}	0.06 ± 0.03^{B}	0.11 ± 0.03^{B}	0.03 ± 0.01^{C}	-
Add only	2.73 ± 0.73^{AB}	0.26 ± 0.14^{C}	0.10 ± 0.03^{D}	0.05 ± .03^{B}	0.09 ± 0.01^{B}	0.20 ± 0.05^{B}	0.12 ± 0.03^{B}	-
Ch + Add	1.90 ± 2.00 ^{B}	1.48 ± 0.60^{B}	2.73 ± 0.25^{C}	0.65 ± 0.44^{B}	0.83 ±0.91^{AB}	0.20 ± 0.05^{B}	0.12 ± 0.02^{B}	-
Ch40NCh + Add	3.75 ± 0.78^{A}	2.56 ± 0.34^{A}	3.15 ± 0.14^{B}	1.93 ± 0.92^{A}	1.28 ± 0.92^{AB}	1.15 ± 1.10^{AB}	0.13 ± 0.03^{B}	0.06 ± 0.03^{A}
Ch80NCh + Add	2.50 ± 0.53^{AB}	2.53 ± 0.30^{A}	3.52 ± 0.44^{B}	1.70 ± 0.29^{A}	1.70 ± 1.44^{A}	1.51 ± 1.5^{A}	0.25 ± 0.10^{A}	0.12 ± 0.07^{A}

*Values are expressed as means of two replicates.
Means in a column followed by the same superscript letter are not significantly different at the 5% level of significance using LSD.

Table 6. Changes in titratable acidity and total soluble solids content of chitosan-nanochitosan coated papaya fruits during storage at 4.5 °C and RH 79%.

Treatment	Titratable Acidity (%)*							
	Storage Time (Days)							
	1	3	5	7	10	14	19	26
Control	1.13 ± 0.20^{A}	1.17 ± 0.18^{BC}	1.41 ± 0.24^{A}	1.57 ± 0.25^{A}	1.93 ± 0.53^{A}	1.33 ± 0.25^{B}	1.17 ± 0.24^{B}	-
Add only	0.97 ± 0.30^{A}	1.25 ± 0.18^{AB}	1.45 ± 0.00^{A}	1.61 ± 0.12^{A}	1.77 ± 0.29^{AB}	1.33 ± 0.20^{B}	1.13 ± 0.20^{B}	-
Ch + Add	1.09 ± 0.13^{A}	1.41 ± 0.10^{A}	1.45 ± 0.43^{A}	1.37 ± 0.20^{B}	1.77 ± 0.39^{AB}	1.25 ± 0.10^{B}	1.21 ± 0.22^{AB}	-
Ch40NCh + Add	0.88 ± 0.20^{A}	1.21 ± 0.26^{ABC}	1.37 ± 0.20^{A}	1.29 ± 0.12^{B}	1.65 ± 0.32^{AB}	1.21 ± 0.15^{B}	1.37 ± 0.25^{AB}	0.72 ± 0.26^{A}
Ch80NCh + Add	1.13 ± 0.25^{A}	1.00 ± 0.10^{C}	1.29 ± 0.20^{A}	1.45 ± 0.00^{AB}	1.49 ± 0.10^{B}	1.69 ± 0.26^{A}	1.45 ± 0.26^{A}	0.68 ± 0.10^{A}
	Total Soluble Solids (°Brix)*							
Control	7.61 ± 0.59^{A}	7.17 ± 0.44^{BC}	8.35 ± 0.16^{A}	9.39 ± 0.37^{A}	9.35 ± 1.19^{A}	8.38 ± 0.33^{A}	9.70 ± 1.77^{A}	-
Add only	6.66 ± 0.34^{B}	6.70 ± 0.35^{C}	7.80 ± 0.10^{B}	7.64 ± 0.85^{CD}	8.78 ± 0.78^{A}	8.35 ± 0.78^{A}	10.00 ± 1.44^{A}	-
Ch + Add	6.39 ± 0.30^{B}	7.54 ± 0.24^{AB}	7.30 ± 0.35^{C}	7.98 ± 0.11^{C}	7.91 ± 0.56^{B}	7.87 ± 1.11^{AB}	9.49 ± 1.55^{A}	-
Ch40NCh + Add	7.14 ± 1.19^{AB}	6.63 ± 0.47^{C}	7.10 ± 0.43^{CD}	8.61 ± 0.10^{B}	7.03 ± 0.20^{C}	8.68 ± 0.22^{A}	9.39 ± 1.66^{AB}	9.79 ± 0.99^{A}
Ch80NCh + Add	7.20 ± 0.74^{AB}	7.74 ± 0.72^{A}	6.86 ± 0.13^{D}	7.25 ± 0.15^{D}	7.84 ± 0.27^{B}	7.06 ± 0.88^{B}	7.78 ± 0.11^{A}	8.27 ± 0.22^{B}

*Values are expressed as means of two replicates. Means in a column followed by the same superscript letter are not significantly different at the 5% level of significance using LSD

of papaya fruit during ripening. In contrast, a decrease in titratable acidity during ripening has been reported by Chavez-Sanchez et al. (2013). The increase in TA corresponds to an increase in galacturonic acid as the papaya ripens (Paull and Chen 1999, as cited by Resende et al. 2012). Citric, malic acid, α-ketoglutaric and ascorbic acids are the major acids in papaya (Chan et al. 1971). The utilization of these acids as respiratory substrates for the formation of flavor and aromatic compounds (Ladaniya 2008) may account for the decrease in titratable acidity after the climacteric respiration peak.

The effect of the coating on the ripening of the papaya fruit can be assessed by determining when the peak of titratable acidity occurs. The titratable acidity of papaya fruit steadily decreases during growth and development and reaches a minimum value at physiological maturity, then increases systematically to reach a peak which coincides with the climacteric peak of respiration, and then sharply declines afterwards (Abu-Goukh et al. 2010). Results of TA determination indicate that the Ch80NCh + Add coated fruits attained the climacteric peak later (day 14) than the control and the other coated fruits (day 10). This result implies that ripening was delayed in the former, which could be attributed to the coating of the fruit with the higher concentration of nanochitosan. As mentioned earlier, this coating formulation exhibited compactness of the film which may have decreased gas permeability, thereby resulting in decreased transpiration and respiration rates leading to a delay in fruit ripening.

There was a general increase in total soluble solids (TSS) during storage of the papaya fruits (Table 6). The control fruits generally exhibited the highest TSS readings. Also, there were generally significant differences among the five treatments in all the days of storage. The Ch40NCh + Add and Ch80NCh + Add treatments had generally significantly lower TSS readings than the control from day 3 to 10 and the latter had significantly lower TSS readings on days 14 and 19 compared with the control. On the 26th day, the Ch80NCh + Add coated fruits had TSS values significantly lower than those of the Ch40NCh + Add coated ones. Gomez et al. (2002) and Calegario et al. (1997) reported an increase in soluble sugars content of papaya fruits along with ripening. Papaya fruits do not significantly have high starch content (about 0.1%) when harvested, which could contribute to sweetness due to hydrolysis upon ripening (Gomez et al. 2002). The increase in TSS has been attributed to sucrose synthesis as the fruit ripens with the carbon source coming from the cell wall components. Ali et al. (2011) similarly reported lower soluble solids content in chitosan-coated Esotika II papaya fruits compared with the uncoated control fruits. The lower TSS of the coated fruits may be due to the capability of the coating to retard the ripening of the fruit by decreasing respiration rate and metabolic activity (Ali et al. 2011), thus slowing down sugar synthesis from carbohydrates (Al Eryani-Raqeeb et al. 2009).

CONCLUSION

The incorporation of nanochitosan with a particle size of 112 nm into chitosan-based fruit-coating formulations produced more compact and thinner films with improved texture and smoother and glossy surfaces as seen from SEM electron micrographs. Some physicochemical changes in the properties of coated papaya fruits indicate that the Ch-NCh coatings have the capability to retard ripening and to extend the postharvest life of papaya up to 26 d at 14.5 °C. Color changes and shriveling occurred at a slower rate in the Ch-NCh coated fruits. The Ch-NCh coated fruits did not reach the limit of marketability in terms of shriveling index. The Ch-NCh coated fruits showed signs of disease infection on the 26th day of storage at a very low severity index value. Weight loss, loss of firmness and total soluble solids were generally less in the Ch-NCh coated fruits.

The capability of the Ch-NCh coatings to retard ripening and to extend the postharvest life of papaya fruit compared with the Ch + Add coating could be attributed to the nanochitosan particles that filled in the voids in the coating matrix. This chain of events enhances the interaction of the particles with the chitosan matrix, thereby producing a more compact film coating, which could maintain low respiration and transpiration rates. This interaction could also prevent or reduce shriveling and weight loss, and thus reduce ethylene production as well as enhance the protection provided by chitosan against disease-causing fungi and bacteria.

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REFERENCES CITED

ABU-GOUKH AA, SHATTIR AET, MAHDI EFM. 2010. Physico-chemical changes during growth and development of papaya fruit. I. Chemical changes. Agric Biol J N Am 1: 871–877.

ALI A, MUHAMMAD MTM, SIJAM K, SIDDIQUI Y. 2011. Effect of chitosan coatings on the physicochemical characteristics of Eksotika II papaya (*Carica papaya* L.) fruit during cold storage. Food Chem 124: 620–626.

AL ERYANI-RAQEEB A, MAHMUD TMM, SYED OMAR SR, MOHAMED ZAKI AR, AL ERYANI AR. 2009. Effects of calcium and chitosan treatments on controlling anthracnose and postharvest quality of papaya (*Carica papaya* L.). Int J Agric Res 4: 53–68.

[AOAC] Association of Official Analytical Chemists. 1995. Official Methods of Analysis of AOAC International. 16th ed. Vol. 1. AOAC International, Washington, D.C., USA.

BARRERA E, GIL J, RESTREPO A, MOSQUERA K, DURANGO D. 2015. A coating of chitosan and propolis extract for the postharvest treatment of papaya (*Carica papaya* L. cv. Hawaiiana). Rev Fac Nal Agr 68: 7667–7678.

BASULTO FS, DUCH ES, GIL FE, PLAZA RD, SAAVEDRA AL, SANTAMARIA JM. 2009. Postharvest ripening and maturity indices for maradol papaya. Intercencia 34: 583–588.

BURG SP, BURG EA. 1965. Molecular requirements for the biological activity of ethylene. Plant Physiol 42: 144–152.

CALEGARIO F, PUSCHMANN R, FINGER FL, COSTA AE. 1997. Relationship between peel color and fruit quality of papaya (*Carica papaya* L.) harvested at different maturity stages. Proc Fla State Hort Soc 110: 228–231.

CHAN HT JR, CHANG TSK, TAFFORD AE, BREKKE JE. 1971. Nonvolatile acids in papaya. J Agr Food Chem 19: 263–265.

CHAVEZ-SANCHEZ I, CARILLO-LOPEZ A, VEGA-GARCIA M, YAHIA EM. 2013. The effect of antifungal hot-water treatments on papaya postharvest quality and activity of pectinmethylesterase and polygalacturonase. J Food Sci Tech 50: 101–107.

DE MOURA MR, AOUAD FA, AVENA-BUSTILLOS RJ, MCHUGH TH. 2009. Improved barrier and mechanical properties of novel hydroxypropyl methylcellulose edible films with chitosan/tripolyphosphate nanoparticles. J Food Eng 92: 448–453.

DONHOWE IG, FENNEMA O. 1994. Edible films and coatings: characteristics, formation, definitions and testing methods. In: Krochta JM, Baldwin EA, Nisperos-Carriedo MO, editors. Edible Coatings and Films to Improve Food Quality. Lancaster, PA: Technomic Publishing Company, Inc. p. 1–24.

DOTTO GL, VIEIRA MLG, PINTO LAA. 2015. Use of chitosan solutions for the microbiological shelf life extension of papaya fruits during storage at room temperature. LWT-Food Sci Technol 64: 126–130.

EL-BELTAGY A, AMER EISSA AH, EL-SAYED S. 2013. Development of chitosan-glucose and chitosan-citric complexes edible coating to improve tomatoes post-harvest quality. Agric Eng Int: CIGR J 15(3): 208–216.

ELSABEE MZ, ABDOU ES. 2013. Chitosan based edible films and coatings: a review. Mater Sci Eng 33: 1819–1841.

GARCIA M, CASARIEGO A, DIAZ R, ROBLEJO L. 2014. Effect of edible chitosan/zeolite coating on tomatoes quality during refrigerated storage. Emir J Food Agric 26(3): 238–246.

GOMEZ M, LAJOLO F, CORDENUNSI B. 2002. Evolution of soluble sugars during ripening of papaya fruit and its relation to sweet taste. J Food Sci 67: 442–447.

HAARD NF, CHISM GW. 1996. Characteristics of edible plant tissues. In: Fennema OR, editor. Food Chemistry. 3rd ed. New York: Marcel Dekker, Inc. p. 857–911.

HONG K, XIE J, ZHANG L, SUN D, GONG D. 2012. Effects of chitosan coating on postharvest life and quality of guava (*Psidium guajava* L.) fruit during cold storage. Sci Hortic 144: 172–78.

JAFARIZADEH MALMIRI H, OSMAN A, TAN CP, ABDUL RAHMAN R. 2011. Development of an edible coating based on chitosan-glycerol to delay 'Berangan' banana (*Musa sapientum* cv. Berangan) ripening process. Int Food Res J 18(3): 989–997.

JOHNEY J, RAGUNATHAN R, MOZHI KA. 2014. Development of low molecular chitosan and its silver nanocomposites for the edible fruit coatings to improve the shelf life period. Res J Pharm Biol Chem Sci 5: 40–47.

KATAS H, RAJA MAG, LAM KL. 2013. Development of chitosan nanoparticles as a stable drug delivery system for protein/siRNA. Int J Biomater Vol. 2013, Article ID 146320, 9 pages, 2013. Doi:10/1155/2013/146320. http://www.hindawi.com/journals/ijbm/2013/146320/cta/. Retrieved: March 30, 2015.

KEAN T, THANOU M. 2010. Biodegradation, biodistribution and toxicity of chitosan. Adv Drug Deliv Rev 62: 3–11.

KERCH G. 2015. Chitosan films and coatings prevent losses of fresh fruit nutritional quality: A review. Trends Food Sci Tech 46: 159–166.

KHAN TA, PEH KK, CH'NG HS. 2002. Reporting degree of deacetylation values of chitosan: the influence of analytical methods. J Pharm Pharmaceut Sci 5: 205–212.

KIM KM, SON JH, KIM S-K, WELLER CL, HANNA MA. 2006. Properties of chitosan films as a function of pH and solvent type. J Food Sci 71: E119–E124.

KONG M, CHEN XG, XING K, PARK HJ. 2010. Antimicrobial properties of chitosan and mode of action: a state of the art review. Int J Food Microbiol 144: 51–63.

LADANIYA M. 2008. Citrus Fruit: Biology, Technology and Evaluation. San Diego, CA: Academic Press (Elsevier). 576 p.

LAZAN H, ALI ZM, LIANG KS, YEE KL. 1989. Polygalacturonase activity and variation in ripening of papaya fruit with tissue depth and heat treatment. Physiol Plant 77: 93–98.

LOPEZ-MATA MA, RUIZ-CRUZ S, SILVA-BELTRAN NP, ORNELAS-PZ JDJ, ZAMUDIO-FLORES PB, BURRUEL-IBARRA SE. 2013. Physicochemical, antimicrobial and antioxidant properties of chitosan films incorporated with carvacrol. Molecules 18: 13735–13753.

MA H, SHIEH K-J, QIAO TX. 2006. Study of transmission electron microscopy (TEM) and scanning electron microscopy (SEM). Nature and Science 4: 14–22.

NADRES ET. 2007. Preparation and characterization of a tyrosine -modified chitosan and preliminary evaluation of its use as a chiral stationary phase for high performance liquid chromatography. [M.S. Thesis] College, Laguna, Philippines: University of the Philippines Los Baños. 81 p. (Available at the UPLB Library).

NO HK, MEYERS SP. 1995. Preparation and characterization of chitin and chitosan – A review. J Aquat Food Prod T 42(2): 27–52.

PEREIRA T, DE ALMEIDA PSG, DE AZEVEDO IG, DA CUNHA M, DE OLIVEIRA JC, DE SILVA MC, VARGAS H. 2009. Gas diffusion in 'Golden' papaya fruit at different maturity stages. Postharvest Biol Tech 54: 123–130.

PILON L, SPRICIGO PC, MIRANDA M, DE MOURA MR, ASIS OBG, MATTOSO HC, FERREIRA MC. 2015. Chitosan nanoparticle coatings reduce microbial growh of fresh-cut apples while not affecting quality attributes. Int J Food Sci Tech 50: 440–448.

PROULX M, NUNES CN, EMOND JP, BRECHT JK. 2005. Quality attributes limiting papaya postharvest life at chilling and non-chilling temperatures. Proc Fla State Hort Soc 118: 389–395.

RESENDE ECO, MARTINS PF, DE AZEVEDO RA, JACONMINO AP, BRON IU. 2012. Oxidative processes during 'Golden' papaya fruit ripening. Braz J Plant Physiol 24: 85–94.

RIVERA RA. 2005. A guide to papaya growing and marketing. Retrieved May 20, 2010 from http://hvcc.da.gov.ph/pdf/papaya/pdf.

ROMANAZZI G, FELIZIANI E, BAÑOS SB, SIVAKUMAR D. 2015. Shelf life extension of fresh fruit and vegetables by chitosan treatment. Crit Rev Food Sci Nutr. DOI: 10.1080/10408398.2014.900474.

SCHOCH TJ. 1964. Methods of Carbohydrate Chemistry. Vol. 4. Edited by: Smith RL. New York: Academic Press. p. 106.

SORRENTINO A, GORRASI G, VITTORIA V. 2007. Potential perspectives of bio-nanocomposites for food packaging applications. Trends Food Sci Technol 18: 84–95.

THARANATHAN RN. 2003. Biodegradable films and composite coatings: past, present and future. Trends Food Sci Technol 14: 71–78.

TORABI A, NAFCHI AM. 2013. The effects of SiO_2 nanoparticles on mechanical and physicochemical properties of potato starch films. Journal of Chemical Health Risks (JCHR) 3: 33–42.

VIMAL S, MAJEED SA, TAJU G, NAMBI KSN, RAJ NS, MADAN N, FAROOK MA, RAJKUMA T, GOPINATH D, HAMEED ASS. 2013. Chitosan tripolyphosphate (CS/TPP) nanoparticles: Preparation, characterization and application for gene delivery in shrimp. Acta Trop 128: 486–493.

WONG DWS, GASTINEAU FA, GREGORSKI KS, TILLIN SJ, PAVLATH AE. 1992. Chitosan-lipid films: Microstructure and surface energy. J Agric Food Chem 40: 540–544.

YANG H-C, W-H WANG, K-S HUANG, M-H HON. 2009. Preparation and application of nanochitosan to finishing treatment with anti-microbial and anti-shrinking properties. Carbohyd Polym 79: 176–179.

ZAMBRANO-ZARAGOZA ML, MERCADO-SILVA E, RAMIREZ-ZAMORANO P, CORNEJO-VILLEGAS MA, GUTIERREZ-CORTEZ E, QUINTANAR-GUERRERO D. 2013. Use of solid lipid nanoparticles (SLNs) in edible coatings to increase guava (*Psidium guajava* L.) shelf-life. Food Res Int 51: 946–953.

ZHOU L, PAULL RE, CHEN NJ. 2004. Papaya. In: Gross KC, Wang CY, Salveit M, editors. Agriculture Handbook No. 66: The Commercial Storage of Fruits, Vegetables and Florist and Nursery Stocks. Retrieved June 21, 2010 from http://www.ba.ars.usda.gov/hb66/101papaya.pdf