International Journal of Environment Volume : 05 | Issue : 04 | Oct.-Dec. | 2016
ISSN: 2077-4508 Pages: 43-55

Development of Nano-Chitosan Edible Coating for Peach Fruits Cv. Desert Red

[1]Mohamed M. Gad, [2]Osama A. Zagzog and [3]Osama M. Hemeda

[1]Horticulture Department, Faculty of Agriculture, Zagazig University, Egypt.
[2]Plant Production Department, Faculty of Technology and Development, Zagazig University, Egypt.
[3]Physics Department, Faculty of Science, Tanta University, Egypt.

Received: 10 September 2016 / Accepted: 20 October 2016 / Publication date: 25 October 2016

ABSTRACT

Increasing the storage life and enhancing quality of peach fruits [*Prunus persica* (L.) Bastch.] "cv. Desert Red" was investigated using nano-chitosan. A Chitosan nano particle was prepared by using high energy ball milling. The full mature peach fruits was harvested then coated with one of these concentrations (0.2, 0.4 and 0.8%) and control. The fruits stored at 0±1°C and 90-95% relative humidity for 28 days. The quality parameters were taken in weekly intervals after 7, 14, 21 and 28 days. The results of the two successive seasons 2015 / 16 indicated that the nano-chitosan 0.4% treatment gave the lowest fruit decay percentage (FD %) and TSS/acid ratio compared with other treatments in both seasons. The Highest concentration of nano-chitosan reduced fruit weight losses (FWL) and maintained fruit pulp firmness (FPF). In the advance of cold storage period, FWL, FD% and TSS/acid ratio were gradually increased. While, FPF, TSS and acidity were decreased. The best qualities of peach fruits were obtained from the 0.4% nano-chitosan treatment after 28 days of cold storage, while 0.8% nano-chitosan treatment increased FD%.

Key words: Nano-chitosan, Edible coating, Peach, Desert Red, Storage period and Fruit quality.

Introduction

Peach is an agriculturally important deciduous fruits of global significance. It is widespread in the newly reclaimed areas in Egypt in the last decade (El-Badawy, 2012). Recently, the production and commercialization of stone fruits especially peaches have increased quickly throughout the world because of its high nutrient level and pleasant flavor. Furthermore, peach fruits are rich with ascorbic acid, carotenoids (pro-vitamin A), phenolic compounds and considered a prime source for antioxidants (Byrne, 2001; Tomás-Barberán *et al*., 2001). Peach [*Prunus persica* (L.) *Bastch*.] "cv. Desert Red " is an early ripening cultivar under the Egyptian conditions.

Nowadays, using the nanoparticles of chemical substances has a great scientific interest as they are effectively connect between bulk materials and atomic or molecular structures. A bulk material should have constant physical properties regardless of its size, but at the nanoscale, size-dependent properties are often observed to be changed. In addition, the percentage of atoms at the surface of a material becomes significant as compared to the number of atoms in the bulk of the material (Mavani and Shah, 2013).

Nanoparticles of materials are used for its wide surface area which in role induce high reactivity, effective catalyst of plant metabolism, better penetration into the cell and increase plant activity (Raliya *et al*., 2014).

The consumer demands are increasing day after day not only for high quality but also for a microbiologically safe food, together along with longer product shelf life. Achieving these goals together put the scientists in a challenge to develop new food preservative strategies. One of these strategies is to use the edible films from polysaccharides and lipids that could potentially serve as edible coating materials (Olivas and Barbosa-Cánovas, 2008; Sothornvit *et al*., 2007).

Chitosan is a natural carbohydrate polymer derived by deacetylation of chitin [poly-β-(1→4)-N-acetyl-D-glucosamine], which is a major component of the crustacean shells such as crab, shrimp, and crawfish. Chitosan has three types of relative functional groups such as an amino groups and primary and secondary hydroxyl group at C-2, C-3 and C-6 positions respectively. (Aranaz *et al*., 2009) studied the physic-chemical behavioral and functional properties of chitin and chitosan and also

Corresponding Author: Osama A. Zagzog, 2Plant Production Department, Faculty of Technology and Development, Zagazig University, Egypt. E-mail: zagzog_1000@yahoo.com

studied specific applications in drug delivery, tissue engineering, functional food, food preservative, biocatalyst, immobilization, waste water treatment, molecular imprinting and metal-nano-composites.

Biopolymer thin layer containing chitosan as antimicrobial agent were using. The films were characterized using FTIR spectroscopy. FTIR spectra of the pure components reveal strong absorption bands (Anicuta *et al*., 2010; Dainelli *et al*., 2008). It is a high molecular weight cationic polysaccharide that exhibits the antifungal activity (Ziani *et al*., 2009a) and has film-forming properties (Arvanitoyannis, 2008; Sebti *et al*., 2005). Chitosan-based films have good mechanical properties and selective gas permeability (CO_2 and O_2). However, the high water vapor permeability limits their application (Campos *et al*., 2011). Chitosan is the polysaccharide most frequently used as a base material for forming films and coatings in food packaging due to its intrinsic antimicrobial and edible properties (Chiellini, 2008). Therefore, it was extensively used to protect, improve quality and extend the shelf life of fresh foods (Campos *et al*., 2011). In this respect, chitosan coating was safe and effective method for extending shelf life of strawberry fruits (Vargas *et al*., 2006). In addition, it maintained the total postharvest quality of tomatoes (Mustafa *et al*., 2012). Furthermore chitosan resulted in better control of postharvest rots in apple fruits (de Capdeville *et al*., 2002a) sweet cherries (Romanazzi *et al*., 2003a) citrus fruits (El-Ghaouth *et al*., 2000) papaya fruits (Bautista-Baños *et al*., 2003) and carrots (Molloy *et al*., 2004).

In order to improve chitosan's performance, a mechanical treatment using high-energy ball milling was performed in order to minimize the particle size to enter the range of nanometer. Therefore, the aim of the present study is to investigate the effect of chitosan nanoparticles coating on the storage life and quality of peach fruits.

Materials and Methods

Sample preparation:

Chitosan nano crystallite powder was synthesized by high-energy ball milling. Powder mixture was conducted in a planetary ball mill to 40 h using ball to powder mass ratio of (8:1).

Structural and spectral measurement:

The chitosan samples were examined by X-ray diffraction using a Philips model (PW-1729) diffract meter equipped with Cu Kα radiation source (λ = 1.541178 Å). Infrared spectra (FTIR) for the chitosan samples were carried out at room temperature by using a PERKIN-ELMER-1430 recording infrared spectra in the range 200 to 4000 cm-1 (at Tanta University, Central lab. The microstructure of the sintered samples examined using High Resolution Transmission Electron Microscope (HRTEM) model JOEL EM 2-100).

Plant materials:

Desert red, a peach cultivar (*Prunus persica* L.) was used in this experiment during the both 2015 / 16 seasons. The experiment was carried out at the post-harvest laboratory of Horticulture Department, Faculty of Agriculture, and Zagazig University, Egypt. The fruits of this cultivar were obtained from a private orchard at El-Tahrer north district, El-Bihira Governorate, Egypt. The chosen trees were similar, aged six years old, grafted on nemaguard rootstock, grown in sandy soil at 5×5 meters apart.

Fruits were picked at the first of June using small clippers then packed in carton boxes and directly transferred to the lab. The harvested fruits were mature (at the optimal commercial fresh market flavor development). Chosen fruits were healthy and free of physiological and pathological disorders. Finally, it was washed using tap water then air-dried before treatment. The characteristics of the chosen fruits expressed as average are shown in Table 1.

The experimental design was factorial (4×4) in a complete randomized design, in three replicate and each replicate contained 30 uniform fruits. The main factor was nano-chitosan coating applications (0.0, 0.2, 0.4 and 0.8%) and the sub-main factor was cold storage periods (7, 14, 21 and 28 days).

Table 1: Characteristics of the used fruits storage treatments:

Parameter	1st season	2nd season
Pulp firmness (g/ cm^2)	1443	1317
TSS (Brix°)	10.4	11.2
Acidity (%)	1.07	1.24
TSS/Acidity Ratio	9.72	9.03

Nano-chitosan treated fruits were air-dried, while in the control treatment water were used instead of the nano-chitosan. All treated and untreated fruits were then packed in perforated (0.06% of area) 20 micron thickness low density polyethylene (LDPE) bags then stored at 0±1°C and 90-95% relative humidity for 28 days. The quality parameters were recorded after 7, 14, 21 and 28 days.

Data recorded:

A sample of thirty fruits for each treatment was randomly taken after the tested cold storage periods of each treatment were used for determinations.

Fruit weight losses (FWL) (%):

The fruits were weighed before cold storage to obtain the initial weight, and then weighed after each period of cold storage. FWL % were calculated according to the following equation:

$$FWL \quad \% \quad = \frac{Wi \quad - \quad Ws}{Wi} \times 100 \quad .$$

Where, *Wi* = fruit weight at initial date, *Ws* = fruit weight at sampling date (Hazali *et al*., 2013) (Ibrahim and Gad, 2015).

Fruit decay percentage (FD %): It was determined as percentage of rotted fruits by various fungi.

Fruit pulp firmness (FPF): For determining fruit pulp, firmness five fruits per replicate were used. It was measured using a Push Pull dynamometer (Model FD 101) and the results were expressed as gram (g/cm^2) (Ibrahim and Gad, 2015).

4. Total acidity (TA) (%): It was determined using the titration method (Hazali *et al*., 2013).

Total soluble solids percentage (TSS): It was determined using a hand refractometer and the results were expressed as Brix° (Hazali *et al*., 2013).

TSS / acid ratio: (Ibrahim and Gad, 2015).

Statistical analysis:

Statistical analyses were conducted for all collected data using the Statistic 9 (2008) according to the methodology given by (Snedecor and Cochran, 1989) and the least significant difference test ($P < 0.05$) was used to investigate the significant differences among the treatments for all tested parameters. The figures were drawn using the GraphPad Prism version 6.00 for Windows, (Prism, 2014).

Results

1- Structural and spectral analysis:

FTIR analysis:

The characteristic absorption of the chitosan is the band at 1559.17 cm-1, which is assigned to the stretching vibration of amino group of chitosan and 1333.5 cm-1 assigned to vibration of C-H2. 1650.95 was

due to C=O stretching (amide I). Another band at 3367.1 is due to amine NH symmetric vibration or indicates symmetric stretching vibration of O-H. The peak of 2927.41 cm-1 is typical C-H stretch vibration. The peaks around 896.73 and 1154.19 cm-1 correspond to saccharide structure of chitosan. The broad peak at 1021 and 1080.91 indicates C-O stretching vibration. The broad peak at 1080.91, which indicates C-O stretching vibration in the spectrum of chitosan (Fernandes *et al*., 2011). The sharp peak at 1384 cm-1 was assigned to CH3 in amide group [32]. The absorption bands at 1151 cm-1 was assigned to the anti-symmetric stretching of C-O-C bridge, and 1098 and 1021 cm-1 were assigned to the skeletal vibrations involving the C-O stretching (Silva *et al*., 2012).

Chitosan was characterized in terms of N-acetylation degree and average molecular weight. The degree of N-acetylation (DA) was determined by FT-IR spectroscopy. The amide-I band (ν = 1655 cm−1) was used as the analytical band and the hydroxyl band (ν = 3450 cm−1) as the internal reference band. The DA was calculated according to the method proposed by (Amaral *et al*., 2005; Baxter *et al*., 1992), as follows: DA (%) = (A1655/A3450) × 115

The degree of deacetylation (DD) serves as a diagnostic to classify the biopolymer as chitin or chitosan .In the present study the value of degree of deacetylation (DD) = 65%.

There are three characterization peaks of chitosan at 3424 cm−1 of ν (O-H), 1092 cm−1 of ν(C- O -C) and 1610 cm−1 of ν (N-H2). The spectrum of chitosan after milling (Fig. 2A) is different from that of chitosan. In chitosan nanoparticles the peak of 3424 cm−1 becomes wider, indicating that hydrogen bonding is enhanced and shifted to 3445. In chitosan nanoparticles, the 1610 cm−1 peak of NH2 bending vibration shifts to 1532 cm−1 and a new sharp peak 1640 cm−1 appears.

X-Ray analysis:

Figure shows the X-ray diffraction pattern of pure Chitosan. The XRD analysis was used to study the crystallinity of the chitosan samples. The peaks at 2θ=10° and 20.09° for pure chitosan confirms the semi crystalline nature. The less broad peaks appeared after milling indicates that the sample is going from amorphous to crystalline nature (Martínez-Camacho *et al*., 2010; Monteiro and Airoldi, 1999). It is well known that the rigid crystalline structure of pure Chitosan is stabilized mainly by intra and intermolecular Hydrogen bonds (Vijayalakshmi *et al*., 2014; Wan *et al*., 2004). The distinct crystalline peaks at around 2θ values 100 and 200 are due the presence of plenty of –OH and –NH2 groups in the chitosan structure, which could form stronger inter and intermolecular hydrogen bonds (Wanule *et al*., 2014).

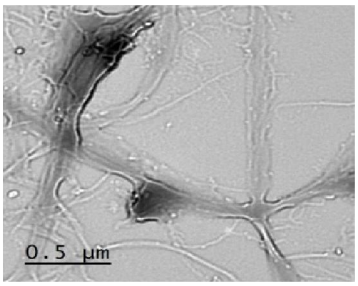
The XRD of the prepared chitosan after milling shows shift in 2θ value also the nature of the peak is different compared with the XRD of pure chitosan before milling, confirms the change in crystallite particles size and its physical properties. The particle size of the chitosan after ball milling was calculated using sheerer equation.

$$t = \frac{0.9\lambda}{B\cos\theta}$$

Where B is the full width at half maximum (FWHM) in radian. The estimated value of particle size was about 50 nm.

TEM analysis:

Transmission electron microscope (TEM) imaging showed a spherical, smooth and almost homogenous structure for nanoparticles. In the present study, TEM images (Photo 1) have shown the morphological properties and surface appearance of nanoparticles, which have nearly spherical shape, smooth surface and size range of about 50-35 nm, which confirm the result of XRD. The size of chitosan nanoparticles, as evident from the TEM images was found to be 50 nm. .TEM analysis of chitosan nanoparticles showed uniform size distribution in nanometer range. The average particle size was found to be 43 nm (Manchanda and Nimesh, 2010). The tem image for chitosan before exposed to milling process, illustrates that the particle size was in the μm range.



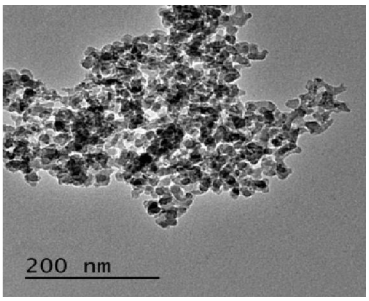


Photo 1: TEM images

2. Effect of treatments on fruits attributes:

Data in Table. 1 indicates that nano-chitosan treatments significantly maintained quality of "Desert Red" peach fruits. Higher concentrations of nano-chitosan reduced FWL and maintained FPF, while increased FD%. The nano-chitosan 0.4% treatment gave the lowest FD% and TSS/acid ratio compared with other treatments in both seasons. The nano-chitosan 0.4 and 0.8% increased TA compared with control and 0.2% in the first season only.

Table 1: Effect of nano chitosan concentrations on fruit weight losses percentage, fruit decay percentage, fruit pulp firmness, total soluble solids, total acidity percentage and TSS/acid ratio in 2015 and 2016 seasons.

Treatments	Fruit weight losses (%)	Fruit decay (%)	Fruit pulp firmness (g/cm^2)	Total soluble solids (Brix°)	Total acidity (%)	TSS/Acid ratio
	1st Season					
Chitosan 0.0 %	1.26 a	32.50 a	279.17 b	10.17 a	0.56 b	20.54 a
Chitosan 0.2 %	0.90 b	12.92 b	276.42 b	9.79 a	0.55 b	20.30 a
Chitosan 0.4 %	0.84 b	4.17 b	332.50 a	10.08 a	0.65 a	16.32 b
Chitosan 0.8 %	0.93 b	27.92 a	353.30 a	10.00 a	0.62 a	17.99 b
2nd Season						
Chitosan 0.0 %	1.18 a	30.83 a	269.86 b	10.54 a	0.58 a	20.34 a
Chitosan 0.2 %	1.01 b	12.08 b	277.78 b	9.96 b	0.54 a	20.35 a
Chitosan 0.4 %	0.78 c	5.83 b	354.72 a	10.25 ab	0.65 a	16.77 b
Chitosan 0.8 %	0.84 c	27.5 a	348.33 a	10.21 ab	0.58 a	20.22 a

3. Effect of cold storage period on fruits attributes:

In order to investigate the effect of cold storage for different periods (7, 14, 21, 28 days), the fruit quality characters were determined (Fig. 1). FWL, FD% and TSS/acid ratio were gradually increased with the advance in cold storage period. The highest values were recorded in fruits stored for 28 days. On the other hand, the highest values for FPF, TSS and acidity were obtained after seven days of cold storage, then decreased gradually afterwards. As shown in Fig 1 B, there was no fruit decay during the first two weeks, while, after 2 weeks the fruit decay started to increase and reached to the highest value at 28 days. At 7 days, the TSS was 10.73 and 10.63 Brix°at the first and second seasons, respectively, which were the highest values then stared to decrease slightly at 14 and 21 days and finally decreased significantly to reach to the lowest values at 28 days.

4. Effect of interaction treatments on fruits attributes:

The effect of the interaction between Nano-chitosan concentrations and cold storage period on fruit quality characteristics are presented in Figs 2 and 3. It is clear from Fig 2 that the lowest value of FWL and FD% was obtained in the fruits coated with 0.4% nano-chitosan after storing for seven days and 28 days, respectively, in both seasons. Although the highest nano-chitosan concentration (0.8%) increased FD% and gave high FPF in the first week then decreased as compared with 0.4% in both seasons. Storing fruits for 28 days gave significantly the lowest TSS, acidity in all treatments, whereas the highest TSS/acid ratio in all

treatments in the two seasons as shown in figs. 2 and 3. Fruits didn't have decay till 14 days with all interaction treatments, and then the fruit decay appeared after 14 days and increased till giving the highest fruit decay at 28 days. As clear from Fig 4 a & b that TSS didn't affected significantly with the cold storage durations interacted with different Nano-chitosan concentrations.

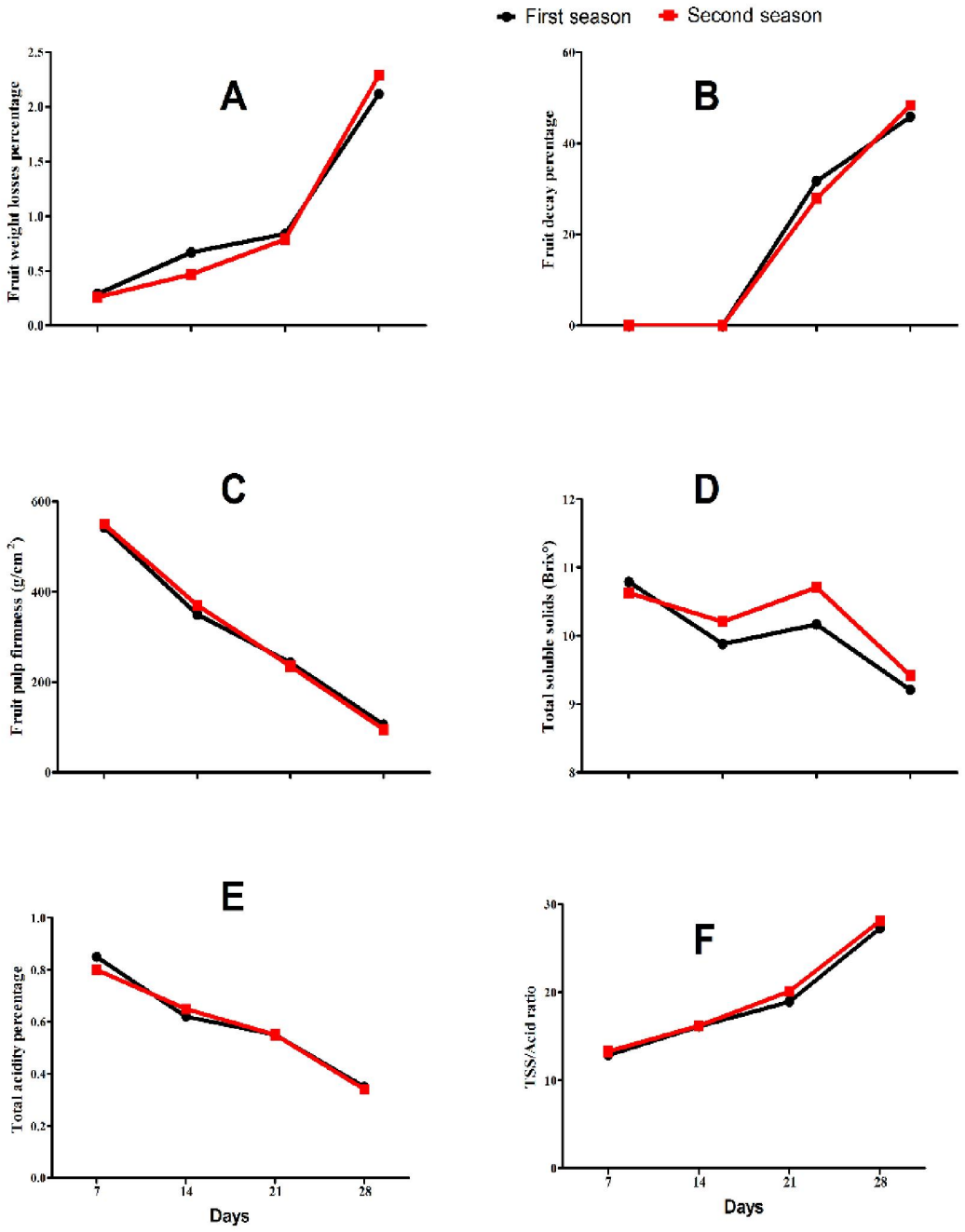


Fig. 1: Effect of cold storage period on fruit weight losses percentage (A), fruit decay percentage (B), fruit pulp firmness (C), total soluble solids (D), total acidity percentage (E) and TSS/acid ratio (F) in 2015 and 2016 seasons.

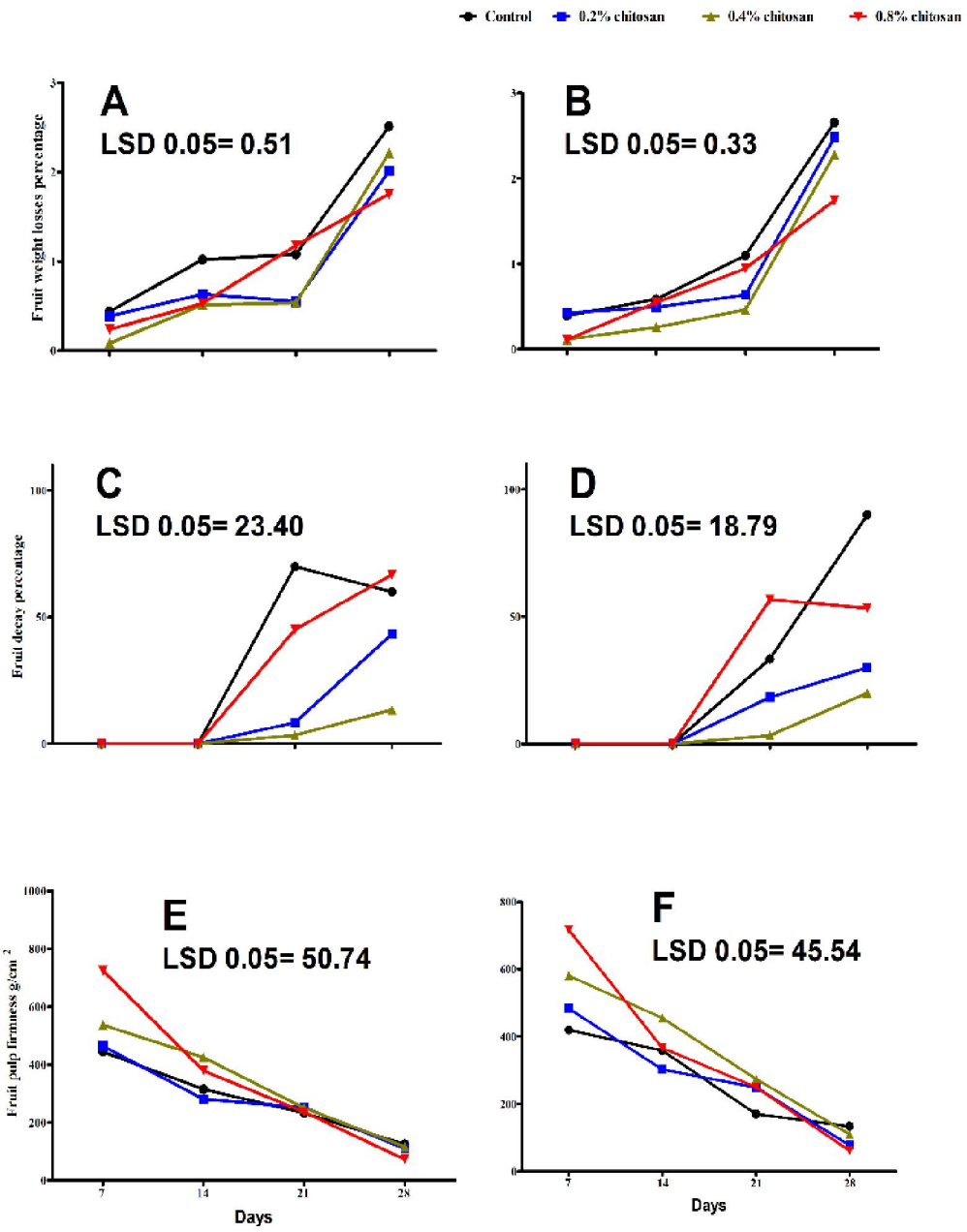


Fig. 2: Effect of the interaction between nano chitosan concentrations and cold storage period on fruit weight losses percentage (A and B), fruit decay percentage (C and D) and fruit pulp firmness (E and F) in 2015 and 2016 seasons, respectively.

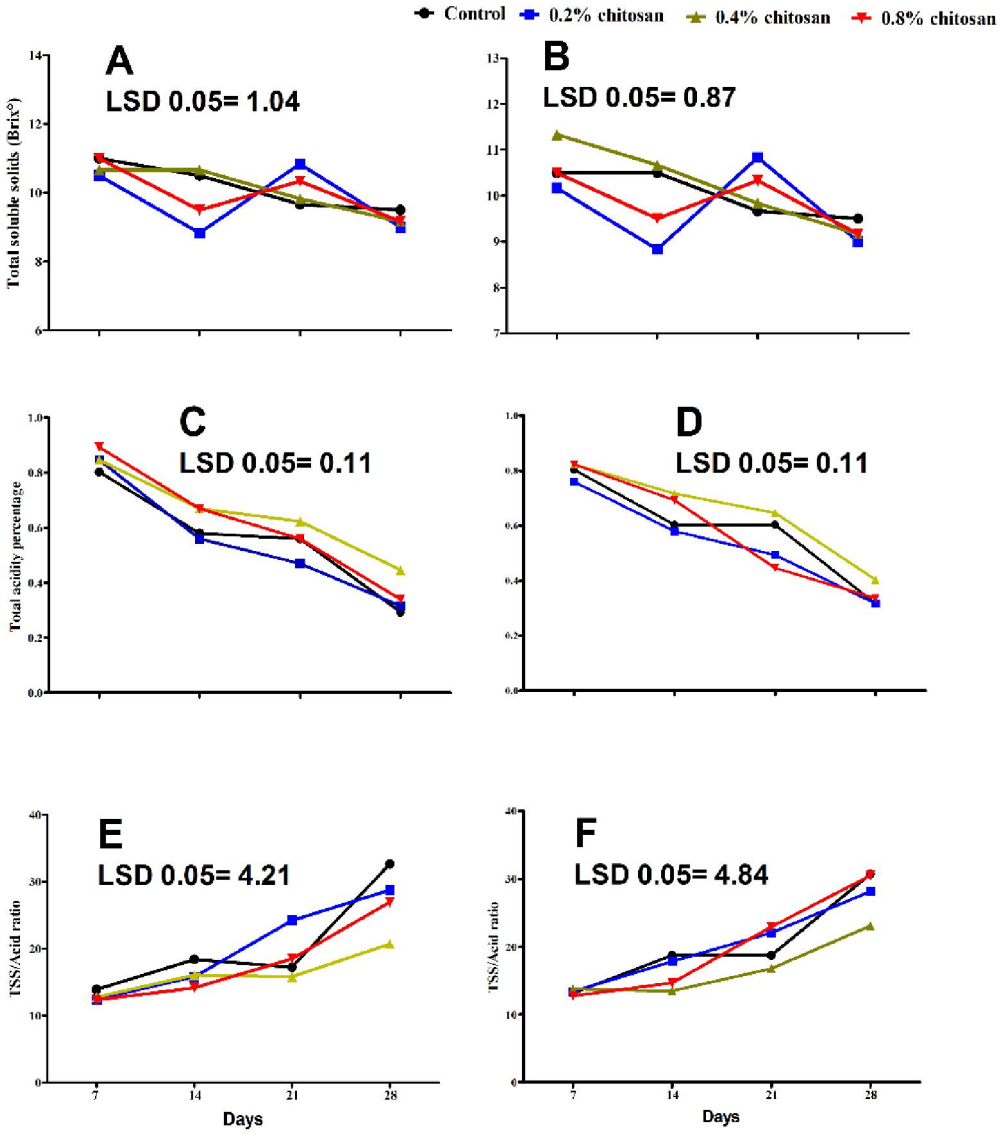


Fig. 3: Effect of the interaction between nano chitosan concentrations and cold storage period on total soluble solids (A and B), total acidity percentage (C and D) and TSS/acid ratio (E and F) in 2015 and 2016 seasons, respectively.

Discussion

Due to the good film-forming capacity of chitosan, it was extensively used to protect, improve quality and extend the shelf life of fresh foods (Fan *et al*., 2009). Chitosan coatings can extend the storage life of fruits and vegetables. It forms a semipermeable film on the fruit surface, which would modify the gas exchange between the outer atmosphere and the internal gas composition (Li and Yu, 2001), and reduces transpiration, water loses and slowed the fruit ripening (Bautista-Baños *et al*., 2006). The water vapor transmission rate of the chitosan-coated films decreased gradually as the concentration of chitosan coating solution increased (Chiellini, 2008). Chitosan in combination with modified atmosphere packaging maintained fruits quality (Simões *et al*., 2009). Water loss can be one of the main causes of deterioration, since it not only resulted in direct quantities losses, but also causes losses in

appearance (due to wilting and shriveling) and nutritional quality (Kader, 1986). The weight loss is a result of water loss from the fruit tissues and partially of the respiration process (Hussein *et al*., 1998).

The reduction of water loss resulted from using chitosan has been reported for numerous horticultural commodities such as tomatoes, longan, apples, mangoes, bananas, bell peppers, Peach, strawberries, etc. (Du *et al*., 1997; Du *et al*., 1998; El-Badawy, 2012; Eshghi *et al*., 2014; Ghaouth *et al*., 1991b; Jiang and Li, 2001; Kittur *et al*., 2001).

The loss of firmness of the chitosan-treated fruits was delayed, suggesting that peach fruit ripening delayed, hence resulting in firmer fruit (Bautista-Baños *et al*., 2003). According to (Hussein *et al*., 1998) rate of insoluble protopectins degradation to simple pectins was increased with the progress of storage period. Also, Pectinesterase activity is expected to increase progressively during storage and as a result decrease hardiness of fruit peel and pulp during storage, as reported by (Ponomarev, 1986) on pear. The loss of firmness is related to the degradation of the cellular wall by poligacturonasis and pectinametilesterasis enzymes and to the loss of water (Kays, 1991).

The reduction in fruit firmness with increasing cold storage period is agreed with the results obtained by (Viskelis *et al*., 2011) on apples. several examples indicated that in strawberries, raspberries, tomatoes, peaches, papayas and others was delayed during the storage period and various reports indicate that the treated fruit was firmer at the end of storage (Bautista-Baños *et al*., 2003; El-Badawy, 2012; El Ghaouth *et al*., 1992a; El Ghaouth *et al*., 1992b; Ghaouth *et al*., 1991a; Li and Yu, 2001).

The acidity was slowly reduced in the chitosan-treated fruits at the end of the storage period, associating this decrease with loss of eating quality (El Ghaouth *et al*., 1992b; Jiang and Li, 2001; Li and Yu, 2001; Srinivasa *et al*., 2002). Decreasing TA during storage period might be due to destruction of organic acids through oxidation and their consumption in respiration processes within fruit tissues. Progress of storage period was found to raise respiration rate of the fresh fruits (Hussein *et al*., 1998).

Chitosan creates a coating film, recorded that chitosan coating often inhibits CO2 production; consequently ethylene production of the commodity. The inhibitory effect of chitosan on decay derives from the combination of its antifungal and eliciting properties. Indeed, chitosan inhibits the in vitro growth of many fungi, including some species causing decay on fruits and vegetables. Also, chitosan coating protecting fruit skin from mechanical injuries and sealing small wounds (Ribeiro *et al*., 2007). Chitosan can reduce disease severity, possibly by increasing the activity of PAL and PPO, lignification resulting from increased biosynthesis of phenolic compounds or induced secondary metabolites and SAR (Apiradee *et al*., 2007).

The antimicrobial activity of chitosan is linked to its positively charged amino group which interacts with negatively charged microbial cell membrane promoting an increase in their permeability and causing disruptions that lead to cell death (Ziani *et al*., 2009b). It was demonstrated that chitosan inhibited the growth of many spoilage and pathogenic bacteria and also yeast and molds (No *et al*., 2007; Roller, 2003). Antimicrobial activity depends on the type of chitosan, degree of acetylation, molecular weight, the target microorganism, the pH of the medium, and presence of other additives or food components (Aider, 2010).

Increasing FD% in 0.8% nano-chitosan appeared as spoilage and darkening in the fruit skin but no rots were observed. So, the high concentrations of nano-particle materials may cause bad effects especially soft fruits as peaches. The inhibitory effect of chitosan on decay was in line with (de Capdeville *et al*., 2002b) on apple; (Romanazzi *et al*., 2003b) on sweet cherries, table grapes and oranges; (Bautista-Baños *et al*., 2003) on papaya and (El-Badawy, 2012) on peach.

Generally, at the end of the storage period, numerous studies reported that TA was increased on the chitosan-treated commodity (strawberries, tomatoes, and peaches), while in other crops such as mangoes and longan, acidity was slowly reduced, associating this decrease with loss of eating quality (El Ghaouth *et al*., 1992b; Jiang and Li, 2001; Li and Yu, 2001; Srinivasa *et al*., 2002). After storage, TSS of chitosan-treated fruits differed according to the commodity. Lower TSS that control fruit were reported in mangoes and bananas coated with chitosan while higher values were reported on treated peaches. However, other studies reported that TSS of chitosan-dipped papayas and zucchinis were the same as the untreated fruit (Bautista-Baños *et al*., 2003; Constantino *et al*., 2001; Du *et al*., 1997; Kittur *et al*., 2001; Srinivasa *et al*., 2002). The content of ascorbic acid was also evaluated in chitosan-treated mango and peaches (Li and Yu, 2001; Srinivasa *et al*., 2002). In those studies, the content of this vitamin in the treated mango gradually decreased during the storage period and was lower than in untreated fruit. However, for peaches, the content of ascorbic acid was higher in chitosan-treated fruit when compared within untreated.

References

Aider, M., 2010. Chitosan application for active bio-based films production and potential in the food industry: Review. LWT Food Science and Technology, 43: 837-842.

Amaral, I., P. Granja and M. Barbosa, 2005. Chemical modification of chitosan by phosphorylation: an XPS, FT-IR and SEM study. Journal of Biomaterials Science, Polymer Edition, 16: 1575-1593.

Anicuta, S.-G., L. Dobre, M. Stroescu and I. Jipa, 2010. Fourier transform infrared (FTIR) spectroscopy for characterization of antimicrobial films containing chitosan. Analele Universităţii din Oradea Fascicula: Ecotoxicologie, Zootehnie şi Tehnologii de Industrie Alimentară, pp: 1234-1240.

Apiradee, U., A.T. Jaime and O. Kullanart, 2007. Chitosan for Improving Orchid Production and Quality. Orchid Science and Biotechnology, 1: 5.

Aranaz, I., M. Mengíbar, R. Harris, I. Paños, B. Miralles, N. Acosta, G. Galed and Á. Heras, 2009. Functional characterization of chitin and chitosan. Current Chemical Biology, 3: 203-230.

Arvanitoyannis, I., 2008. The use of chitin and chitosan for food packaging applications. Environmentally compatible food packaging, pp: 137.

Bautista-Baños, S., A.N. Hernandez-Lauzardo, M.G. Velazquez-Del Valle, M. Hernández-López, E.A. Barka, E. Bosquez-Molina and C. Wilson, 2006. Chitosan as a potential natural compound to control pre and postharvest diseases of horticultural commodities. Crop Protection, 25: 108-118.

Bautista-Baños, S., M. Hernández-López, E. Bosquez-Molina and C. Wilson, 2003. Effects of chitosan and plant extracts on growth of Colletotrichum gloeosporioides, anthracnose levels and quality of papaya fruit. Crop protection, 22: 1087-1092.

Baxter, A., M. Dillon, K.A. Taylor and G.A. Roberts, 1992. Improved method for ir determination of the degree of N-acetylation of chitosan. International Journal of Biological Macromolecules, 14: 166-169.

Byrne, D., 2001. Peach breeding trends: a world wide perspective, V International Peach Symposium, 592: 49-59.

Campos, C.A., L.N. Gerschenson and S.K. Flores, 2011. Development of edible films and coatings with antimicrobial activity. Food and Bioprocess Technology, 4: 849-875.

Chiellini, E., 2008. Environmentally compatible food packaging, First ed. Woodhead publishing limited.

Constantino, G., G. Hernandez, M. Romero, C. Sosa, M. Bosquez and S. Domınguez, 2001. Efecto antimicrobiano de recubrimientos a base de quitosano en calabacita (*Cucurbita pepo* L.) Presented at the IX National Meeting of the Mexican Society for Horticultural Science, Oaxtepec, Morelos Mexico. October.

Dainelli, D., N. Gontard, D. Spyropoulos, E. Zondervan-van den Beuken and P. Tobback, 2008. Active and intelligent food packaging: legal aspects and safety concerns. Trends in Food Science & Technology, 19: S103-S112.

de Capdeville, G., C.L. Wilson, S.V. Beer and J.R. Aist, 2002a. Alternative disease control agents induce resistance to blue mold in harvested'Red Delicious' apple fruit. Phytopathology, 92: 900-908.

de Capdeville, G., C.L. Wilson, S.V. Beer and J.R. Aist, 2002b. Alternative disease control agents induce resistance to blue mold in harvested ‘Red Delicious’ apple fruit. Phytopathology, 92: 900-908.

Du, J., H. Gemma and S. Iwahori, 1997. Effects of chitosan coating on the storage of peach, Japanese pear and kiwifruit. J. Jpn. Hort. Sci., 66: 15-22.

Du, J., H. Gemma and S. Iwahori, 1998. Effects of chitosan coating on the storability and on the ultrastructural changes of Jonagold apple fruit in storage. Food Preservation Science (Japan).

El-Badawy, H., 2012. Effect of Chitosan and Calcium Chloride Spraying on Fruits Quality of Florida Prince Peach Under Cold Storage. Research Journal of Agriculture and Biological Sciences, 8: 272-281.

El-Ghaouth, A., J.L. Smilanick and C.L. Wilson, 2000. Enhancement of the performance of Candidasaitoana by the addition of glycolchitosan for the control of postharvest decay of apple and citrus fruit. Postharvest biology and Technology, 19: 103-110.

El Ghaouth, A., J. Arul and A. Asselin, 1992a. Potential use of chitosan in postharvest preservation of fruits and vegetables. Advances in chitin and chitosan, 1: 440-452.

El Ghaouth, A., R. Ponnampalam, F. Castaigne and J. Arul, 1992b. Chitosan coating to extend the storage life of tomatoes. Hort Science., 27: 1016-1018.

Eshghi, S., M. Hashemi, A. Mohammadi, F. Badii, Z. Mohammadhoseini and K. Ahmadi, 2014. Effect of nanochitosan-based coating with and without copper loaded on physicochemical and bioactive components of fresh strawberry fruit (Fragaria x ananassa Duchesne) during storage. Food and Bioprocess Tech., 7: 2397-2409.

Fan, W., J. Sun, Y. Chen, J. Qiu, Y. Zhang and Y. Chi, 2009. Effects of chitosan coating on quality and shelf life of silver carp during frozen storage. Food Chemi., 115: 4.

Fernandes, L.L., C.X. Resende, D.S. Tavares, G.A. Soares, L.O. Castro and J.M. Granjeiro, 2011. Cytocompatibility of chitosan and collagen-chitosan scaffolds for tissue engineering. Polimeros, 21: 1-6.

Ghaouth, A., J. Arul, R. Ponnampalam and M. Boulet, 1991a. Chitosan coating effect on storability and quality of fresh strawberries. Journal of food science., 56: 1618-1620.

Ghaouth, A., J. Arul, R. Ponnampalam and M. Boulet, 1991b. Use of chitosan coating to reduce water loss and maintain quality of cucumber and bell pepper fruits. Journal of food processing and preservation 15: 359-368.

Hazali, N., A.R.A. Tajudin, N.M. Nor, M. Ibrahim and M.N.A. Yahya, 2013. Physicochemical Characteristics of Belimbing Dayak (*Baccaurea angulata*) Juice Beverages. European International Journal of Science and Technology, 2: 203-210.

Hussein, A.M., M.B. El-Sabrout and A.E. Zaghloul, 1998. Post-harvest physical and biochemical changes of common and late types of seedy guava fruit during storage. Post-harvest physical and biochemical changes of common and late types of seedy guava fruit during storage, 23: 187-204.

Ibrahim, M.M. and M.M. Gad, 2015. The Relationship between Harvest Date and Storage Life of Washington Navel Orange Fruits. Sciences, 5: 1247-1256.

Jiang, Y. and Y. Li, 2001. Effects of chitosan coating on postharvest life and quality of longan fruit. Food Chemistry, 73: 139-143.

Kader, A.A., 1986. Biochemical and physiological basis for effects of controlled and modified atmosphere on fruits and vegetables. Food Technology, pp: 99-102.

Kays, S.J., 1991. Post Harvest Physiology And Handling Of Perishable Plant Products. Van Nostrand Reinhold, New York: AVI Book.

Kittur, F., K. Kumar and R. Tharanathan, 2001. Functional packaging of chitosan films. Z. Tebensmittelunterssuchung undforshung A 206: 44-47.

Li, H. and T. Yu, 2001. Effect of chitosan on incidence of brown rot, quality and physiological attributes of postharvest peach fruit. Journal of the Science of Food and Agriculture, 81: 269-274.

Manchanda, R. and S. Nimesh, 2010. Controlled size chitosan nanoparticles as an efficient, biocompatible oligonucleotides delivery system. Journal of applied polymer science., 118: 2071-2077.

Martínez-Camacho, A., M. Cortez-Rocha, J. Ezquerra-Brauer, A. Graciano-Verdugo, F. Rodriguez-Félix, M. Castillo-Ortega, M. Yépiz-Gómez and M. Plascencia-Jatomea, 2010. Chitosan composite films: Thermal, structural, mechanical and antifungal properties. Carbohydrate Polymers, 82: 305-315.

Mavani, K. and M. Shah, 2013. Synthesis of silver nanoparticles by using sodium borohydride as a reducing agent, International Journal of Engineering Research and Technology. ESRSA Publications.

Molloy, C., L.H. Cheah and J.P. Koolaard, 2004. Induced resistance against Sclerotinia sclerotiorum in carrots treated with enzymatically hydrolysed chitosan. Postharvest Biology and Technology, 33: 61-65.

Monteiro, O.A. and C. Airoldi, 1999. Some studies of crosslinking chitosan–glutaraldehyde interaction in a homogeneous system. International Journal of Biological Macromolecules, 26: 119-128.

Mustafa, M., A. Ali and S. Manickam, 2012. Application of a chitosan based nanoparticle formulation as an edible coating for tomatoes (*Solanum lycoperiscum* L.), VII International Postharvest Symposium., 1012: 445-452.

No, H.K., S.P. Meyres, W. Prinyawiwatkull and Z. Xu, 2007. Applications of chitosan for improvement of quality and shelf life of foods: A review. Journal of Food Science., 72: R87-R100.

Olivas, G.I. and G.V. Barbosa-Cánovas, 2008. Alginate–calcium films: water vapor permeability and mechanical properties as affected by plasticizer and relative humidity. LWT-Food science and technology, 41: 359-366.

Ponomarev, P.F., 1986. Changes in content of pectin and activities of pectolytic enzymes during ripening and storage of pears. Tovarovedenie., 3: 6-10.

Prism, G., 2014. Version 6.00 for windows. GraphPad Software. San Diego (California).

Raliya, R., R. Saran, K. Choudhary and J.C. Tarafdar, 2014. Biosynthesis and characterization of nanoparticles. Journal of Advancement in Medical and Life Science., 1: 4.

Ribeiro, C., A.A. Vicente, J.A. Teixeira and C. Miranda, 2007. Optimization of edible coating composition to retard strawberry fruit senescence. Postharvest Biol. Technol., 44: 7.

Roller, S., 2003. Introduction. In Roller (Ed.), Natural antimicrobials for the minimal processing of foods. Boca Raton: CRC Press.

Romanazzi, G., F. Nigrov and A. Ippolito, 2003a. Short hypobaric treatments potentiate the effect of chitosan in reducing storage decay of sweet cherries. Postharvest Biology and Technology, 29: 73-80.

Romanazzi, G., F. Nigro and A. Ippolito, 2003b. Short hypobaric treatments potentiate the effect of chitosan in reducing storage decay of sweet cherries. Postharvest Biol. Technol., 29: 73-80.

Sebti, I., A. Martial-Gros, A. Carnet-Pantiez, S. Grelier and V. Coma, 2005. Chitosan polymer as bioactive coating and film against Aspergillus niger contamination. Journal of Food Science., 70: M100-M104.

Silva, S.M., C.R. Braga, M.V. Fook, C.M. Raposo, L.H. Carvalho and E.L. Canedo, 2012. Application of infrared spectroscopy to analysis of chitosan/clay nanocomposites. Infrared spectroscopy-materials science, engineering and technology. Croatia: InTech, pp: 43-62.

Simões, A.D.N., J.A. Tudela, A. Allende, R. Puschmann and M.I. Gil, 2009. Edible coatings containing chitosan and moderate modified atmospheres maintain quality and enhance phytochemicals of carrot sticks. Postharvest Biol. Technol., 51: 364-370.

Snedecor, G.W. and W.G. Cochran, 1989. Statistical methods, 8thEdn. Ames: Iowa State Univ. Press Iowa.

Sothornvit, R., C. Olsen, T. McHugh and J. Krochta, 2007. Tensile properties of compression-molded whey protein sheets: determination of molding condition and glycerol-content effects and comparison with solution-cast films. J of Food Eng., 78: 855-860.

Srinivasa, P., R. Baskaran, M. Ramesh, K.H. Prashanth and R. Tharanathan, 2002. Storage studies of mango packed using biodegradable chitosan film. European Food Research and Technology, 215: 504-508.

Tomás-Barberán, F.A., M.I. Gil, P. Cremin, A.L. Waterhouse, B. Hess-Pierce and A.A. Kader, 2001. HPLC-DAD-ESIMS analysis of phenolic compounds in nectarines, peaches, and plums. J of Agric. and Food Chem., 49: 4748-4760.

Vargas, M., A. Albors, A. Chiralt and C. González-Martínez, 2006. Quality of cold-stored strawberries as affected by chitosan–oleic acid edible coatings. Post. Biology and Tech., 41: 164-171.

Vijayalakshmi, K., T. Gomathi and P. Sudha, 2014. Preparation and characterization of nanochitosan/sodium alginate/microcrystalline cellulose beads. Der Pharmacia Lettre, 6: 65-77.

Viskelis, P., M. Rubinskiene, A. Sasnauskas, C. Bobinas and N. Kvikliene, 2011. Changes in apple fruit quality during modified atmosphere storage. J of Fruit and Ornamental Plant Res., 19: 155-165.

Wan, Y., K.A. Creber, B. Peppley and V. Tam Bui, 2004. Ionic conductivity and tensile properties of hydroxyethyl and hydroxypropyl chitosan membranes. J of Polymer Sci. Part B: Polymer Physics, 42: 1379-1397.

Wanule, D., J. Balkhande, P. Ratnakar, A. Kulkarni and C. Bhowate, 2014. Extraction and FTIR Analysis of Chitosan from American cockroach, Periplaneta americana. International Journal of Engineering Science and Innovative Technology (IJESIT) 3.

Ziani, K., I. Fernández-Pan, M. Royo and J.I. Maté, 2009a. Antifungal activity of films and solutions based on chitosan against typical seed fungi. Food Hydrocolloids, 23: 2309-2314.

Ziani, K., I. Fernandez Pan, M. Royo and J. Maté, 2009b. Antifungal activity of films and solutions based on chitosan. Food Hydrocolloids, 23: 2309-2314.