

THE ROLE OF CHITOSAN TO PROLONGE THE FRESH FRUIT QUALITY DURING STORAGE OF GRAPEFRUIT CV. RAY RUBY

WASSEM AHMED^{1*}, RAFIA AZMAT², ABDUL QAYYUM¹, AYAZ MEHMOOD¹, SHAH MASAUD KHAN, ¹M. LIAQUAT¹, SAEED AHMED³ AND SUMEIRA MOIN⁴

¹Department of Agricultural Sciences, University of Haripur, Pakistan

²Department of Chemistry & ⁴Department of Botany, ^{2,4}University of Karachi, Pakistan

³Institute of Horticultural Sciences, University of Agriculture Faisalabad, Pakistan

*Corresponding author's email: waseemuaf12@gmail.com

Abstract

Grapefruit is one of the vital citrus fruit which cultivated in many countries including Pakistan. Conversely, the shelf life of this fruit is rather short under ambient condition. The objective of this study was to extend the fresh fruit quality of grapefruit by using a natural wax coating substance like chitosan. The coating of chitosan in extending the postharvest life of grapefruit was investigated in relation with the phytochemicals like total phenolic compounds (TPC), total antioxidants (TA), total carotenoids (TC), total flavonoids (TF), etc., and physiological changes including chilling injury (CI), weight loss (WL), and gases exchanges. The experiment was intended in a completely randomized design, composed of coating with chitosan at three levels, and stored at 8°C with relative humidity is 95.5%. The results indicated that chitosan application @ 140 mg per fruit maintained the highest fruit quality parameters such as, TPC (172.32 mg GAE/100g), TA (72.09%), TC (17.09 mg/100g), TF contents (52.27 mg CEQ/100g), total limonin contents (15.08 µg/mL) with minimum chilling injuries (1.58 %), and fruit rots (0.66%) were also measured. Overall, fruits coated with chitosan had greater external adequacy than untreated ones. The application of the chitosan @ 140 mg per fruit could be used to reduce deteriorative processes, maintain quality and increase the storage life of grapefruit at 8°C.

Key words: Antioxidants, Chitosan, grapefruit, Phytochemicals, Shelf life.

Introduction

Citrus fruit coated with commercial waxes on the packing line, to enhance gloss, reduce water loss and shrinkage, and lower transpiration process during storage (Petracek *et al.*, 1998). Wax coating may also maintain the fruit quality during the storage periods. There is a trend worldwide to explore new alternative methods to control postharvest diseases and maintaining the fruit quality during storage (Arah *et al.*, 2016). Public concern regarding the environment and human health issues has been increased for the development of natural, biodegradable, edible coatings for maintaining the postharvest quality of fruits and vegetables (Petracek *et al.*, 1998). Fruit quality reflects numerous external and internal attributes for preserving the minimum standards of palatability and commercial acceptability (Davies & Albrigo, 1994). Many degenerative diseases can be prevented through the use of rich phytochemical nutrition diet that provides the essential nutrient needs, and also improves many physiological processes in human body (Petracek *et al.*, 1998). Primary cause of diseases are the losses of essential dietary phytochemical due to excessively use of western diet (Ullah *et al.*, 2017; Nawaz *et al.*, 2008). The use of proper and balanced amount of phytochemical base food can prevent different chronic diseases and ailments of human body (Majhenič *et al.*, 2007; Arah *et al.*, 2016).

Wax coating are commercially used to reduce the moisture loss of fruits and improves fruit quality during storage (Dalal *et al.*, 1971). Different types of wax coating such as wax emulsion, hydrazide, maleic and polysaccharide-based delays, ripening process during storage (Dalal *et al.*, 1971). Films and coatings received much attention in recent years because they improve

quality and extend shelf-life through providing a blockade to mass transference, move nutrition constituents then improve mechanical integrity or handling characteristics of food (Krochta, 1997). Waxing treatments is a regular practice in packing houses, aimed to replace natural waxes on the fruit surface during washing process (Arah *et al.*, 2016). The wax application serves to reduce fruit shrinkage and improve fruit appearance during storage (Majhenič *et al.*, 2007).

Several kind of coating emulsions materials commonly used in fruits and vegetables including lipids, polysaccharides, resins, and proteins (Krochta, 1997). Proteins and polysaccharides are right film-forming materials in fruits (Dalal *et al.*, 1971). However, these films do not show any function as a moisture barriers (Majhenič *et al.*, 2007). Lipids coating on fruit surface shows better moisture barrier, while, present low mechanical integrity after application (Krochta, 1997). To maximize the advantages, many formulations including composite coatings of both groups have been documented (Krochta, 1997; Debeaufort *et al.*, 1998). These composite layers contain chitosan, cellulose derivatives, and acid sucrose fatty ester emulsifiers (Baldwin, 1994). Fruit with wax coating showed more quality parameters as compared to fruit without wax coating (Nawaz *et al.*, 2008; Nisperos-Carriedo *et al.*, 1990; Hagenmaier, 2002).

However, lack of knowledge about the composition of many commercially available coating makes it difficult to predict their performance on fruit quality during storage periods (Perez-Gago *et al.*, 2003). The objective of this study is to investigate the effects of different doses of chitosan coating on grapefruit for their phytochemical, physiological and quality parameters changes during storage periods.

Materials and Methods

Plant material: This experiment was conducted in Orange Research Station Sargodha, Pakistan during the mid-seasons of 2012-2013. Fifteen uniform 12-year-old grapefruit trees grafted onto sour orange root stock with 6 m × 6 m spacing. Each tree was represented as a replicate. The trees were grown under similar cultural practices of irrigation, fertilization, pest management and weeding. The orchard soil texture was sand loamy, pH was 8.2, electrical conductivity (EC) was 1.0 dS^m-1, and CaCO₃ content was 35.0%.

Fruit harvesting, washing, and Fungicides applications: One hundred eight fruits of Ray Ruby were harvested from 18 trees during November and immediately shifted to the laboratory of Department of Post-harvest Centre, Ayub Agricultural Research Institute. Harvested fruits were washed in a plastic tub using sodium hypochloric solution @ 100 ppm before storage. After washing, the fruits were treated with fungicide of Thiabendazole (TBZ) @ 1000 ppm for 5 minutes and then dried at room temperature of 38°C for 5 minutes.

Preparation of wax coating: Chitosan-oleic acid coating was prepared according to a method described by Vargas *et al.* (2004). Chitosan (1-2%) was dispersed in an aqueous solution of glacial acetic acid (1%, v/v) at 40°C. Tween 80 solution at 0.1% (v/v) was added to improve wet ability of desirable solution. After, 8 h of stirring, oleic acid (1-4%) was added to the chitosan solution.

Application of wax coating: Grapefruits were coated using a self-made coating apparatus. The speed of the brush rollers was 160 rpm, and the coating solution was sprayed at 10 mL/min. Each piece of fruit was weighed about 10 seconds before and again 10 seconds after application to determine the wet weight of coating applied (Hagenmaier, 2002). The mean wet weight of the coating was about 0.20 mg per fruit. These fruits were then dried using an electric fan at room temperature (30±2°C) for 30 minutes and placed in cold storage.

Treatments layout

- T₀: Control (without wax applied)
- T₁: Chitosan 120mg per fruit (5 min) dipping
- T₂: Chitosan 130mg per fruit (5 min) dipping
- T₃: Chitosan 140mg per fruit (5 min) dipping

Phytochemicals

Total phenolic contents (mg GAE/100 g): Folin-Ciocalteu reagent method reported by Ainsworth & Gillespie (2007) was used to estimate the total phenolic contents (TPC). The Folin-Ciocalteu reagent (10 mL) was dissolved in distilled water to make the solution 100 mL. In each sample (100 mL), FC-reagent (200 µL) was added and shake thoroughly then 700 mM Na₂CO₃ (800 µL) was added to each sample and incubated at room temperature for 2 h. Sample (200 µL) was transferred to a clear 96-well plate and the absorbance of each well was measured at 765 nm. Amount of TPC was calculated using a calibration curve for Gallic acid. The results were expressed as Gallic acid equivalent.

Total antioxidants (% DPPH inhibition): Amira *et al.* (2012) reported a method for the determination of total antioxidants activities via their scavenging abilities to 2, 2-diphenyl-1-picrylhydrazyl stable radicals. The absorbance was recorded against a blank at 517 nm using micro-plate ELISA reader (BioTek, USA). Inhibition of free radical by DPPH in percent (%) was calculated through following formula:

$$IA \% = (A_{\text{blank}} - A_{\text{sample}} / A_{\text{blank}}) \times 100$$

where A_{blank} is the absorbance of the control reaction mixture excluding fruit sample, and A_{sample} is the absorbance of the test compounds. IC₅₀ values represented the concentration of grapefruit extracts that caused 50% neutralization of DPPH radicals and calculated from the plot of inhibition percentage against concentrations.

Total flavonoids contents (mg CEQ/100 g): Flavonoids were determined by the method of Kim *et al.* (2003). Distilled water (4 ml) was added to 1 ml of fruit juice followed by the addition of 5% sodium nitrite solution (0.3 ml) and 10% aluminum chloride solution (0.3 ml). Test tubes were incubated at ambient temperature for 5 min, and then 2 ml of 1M sodium hydroxide was added to the mixture and the volume of reaction mixture was made up to 10 ml with distilled water. The mixture was thoroughly vortex, and the absorbance of the pink color developed was determined at 510 nm. A calibration curve was prepared with catechin, and the results were expressed as mg catechin equivalents. All the measurements were taken in triplicate, and the mean values were calculated.

Limonin contents: Total Limonin contents were isolated and evaluated for its purity (Brekša *et al.*, 2006). The stock solution 500 µg/ mL was prepared in acetonitrile and stored at 20°C. Juice samples were clarified by centrifugation (16000g, 5 min, 10 °C), and the supernatant was collected and filtered through filter paper (Whatman #1, Whatman Inc., Clifton, NJ) for the estimation of limonin contents. Using these values, the limonin equivalence (µg/mL) of the sample was calculated.

Total carotenoids contents (mg/100g): Total carotenoids contents were estimated according to the method of (Lichtenthaler and Buschmann, 2001). Frozen grapefruit juice (5ml) was extracted with 1mL of pure acetone, and then the mixture was homogenized for 1 min and incubated at 4°C in dark until the cap turned white. The homogenate was centrifuged at 16,000×g for 15min and 200µL of supernatant from each tube were placed in 96-well plates. The absorbance was read at 470 nm in a microplate reader (Power Wave HT, Bio. Tek). The concentration of total carotenoids was calculated as follows:

$$TC (\mu\text{g/mL}) = \frac{(1000 \times A_{470}) / 214, \text{ expressed as mg}}{100 \text{ g fresh weight}}$$

Physiological disorders

Weight loss (%): Ten fruits (n=10) were randomly selected from each treatment unit. These fruits were weighted as fresh and after 30 days interval of the storage period, weight was calculated using the following formula given by (Thakur *et al.*, 2002).

$$WL (\%) = \frac{\text{Original fruit weight} - \text{final fruit weight after storage}}{\text{Average fruit weight}} \times 100$$

Chilling injury (%): Chilling injury (CI) during the storage was calculated by using the following formula:

$$\text{Chilling injury } (\%) = \frac{\text{Number of affected fruits per treatment}}{\text{Total number of fruits per treatment}} \times 100$$

Fruit rot (%): Fruit rot during the storage was estimated by using the following formula:

$$\text{Fruit rot } (\%) = \frac{\text{Number of affected fruits per treatment}}{\text{Total number of fruits per treatment}} \times 100$$

CO₂ and ethylene production: Rates of CO₂ and ethylene production were measured by the static system. Ten fruits per replication were weighed and sealed together in a 3 L container for 2 h. Gas samples of (2, 3, 4, 5) were withdrawn through a rubber septum using a syringe and the percentage of carbon dioxide determined using a Gow-Mac gas chromatograph (Series 580, Bridgewater, N.J.) equipped with a thermal conductivity detector. The respiration rate was calculated using the following formula:

$$\text{Respiration rate (mL CO}_2\text{kg}^{-1}\text{h}^{-1}) = \frac{\% \text{ CO}_2 \text{ volume (mL)}}{\text{Sample weight (kg) X sealed time (h)}} \times 100$$

Ethylene production was measured by injecting a 1 mL gas sample into an HP 5890 gas chromatograph (Hewlett Packard, Avondale, Pa.) equipped with a flame ionization detector. The rate of ethylene production was calculated using the following formula:

$$\mu\text{L C}_2\text{H}_4\text{kg}^{-1}\text{h}^{-1} = \frac{\text{ppm C}_2\text{H}_4 \text{ X void volume (mL)}}{\text{Sample weight (kg) X sealed time (h)}} \times 100$$

Organoleptic evaluation: Organoleptic evaluation of the fruit for sourness, sweetness, taste, and texture was done using the Hedonic scale method of Peryam & Pilgrim (1957).

Statistical analysis: Collected data were statistically analyzed using computer software MSTAT-C. Analysis of variance was used to test the significance of variance. While difference among treatment means were compared using LSD test ($P=0.05$) (Steel *et al.*, 1997). Standard errors (SE) were computed by MS-Excel and data were presented graphically using the same program.

Results

Phytochemical parameters

Total phenolic contents (mg GAE/100 g): Results showed statistically significant differences ($p \leq 0.05$) regarding the effects of wax coating treatments, storage periods and their interaction on total phenolic contents (TPC) in the fruits (Fig. 1). The fruits treated with chitosan @ 140 mg per fruit (T₃) showed higher TPC of 172.23 mg GAE/100 g as compared to the fruits of T₂ (chitosan @ 130 mg per fruit) and T₁ (chitosan @ 120 mg per fruit), and TPC values were 168.00 and 154.56 mg GAE/100 g, respectively. While lower TPC values (133.10 mg GAE/100 g) were recorded in the fruits treated without wax coating (T₀). The fruits were analyzed after 90 days storage showed minimum TPC (143.85 mg GAE/100 g) as compared to 60 and 30 days after storage, and TPC values were 156.59 and 170.47 mg GAE/100 g, respectively. The interaction between wax coating treatments and storage periods found that higher TPC was noted in the fruits of T₃ (180.93 mg GAE/100 g) and T₂ (176.51 mg GAE/100 g) after 30 days storage. While lower TPC (109.17 mg GAE/100 g) were recorded in the fruits treated without wax coating (T₀) after 90 days storage.

Total antioxidants activities (% DPPH inhibition): The Higher amount of total antioxidants activities were recorded in the fruits those were treated with T₃ (72.09%) as compared to the fruits of T₂ and T₁ (Fig. 2). while, lower antioxidants activities (51.92%) noted in the non-treated fruits (T₀). The fruits were analyzed after 30 days storage period showed higher antioxidants activities (73.00%) than the fruits of 60 (62.78%) and 90 (53.35%) days after storage, respectively. The interaction between wax coating treatments and storage periods showed that higher antioxidants activities in the fruits of T₃ (80.40%) after 30 days storage, and lower antioxidants activities (38.69%) were recorded in the untreated fruits (T₀) after 90 days storage.

Total flavonoids contents (mg CEQ/100 g): The results revealed that fruits treated with T₃ showed higher TFC (57.27 mg CEQ/100 g), and these were on equivalence with the fruits of T₂ (56.07 mg CEQ/100 g) followed by T₁ (Fig. 3). While lower TFC of 43.02 mg CEQ/100 g were noted in the fruits without wax coating (T₀). The fruits were analyzed 30 days after storage showed higher TFC (56.60 mg CEQ/100 g) as compared to 60 (51.69 mg CEQ/100 g) and 90 days (45.47 mg CEQ/100 g) after storage, respectively. The interaction between wax coating treatments and storage periods showed that higher TFC (61.14 and 59.92 mg CEQ/100 g) in the fruits of T₃ and T₂ after 30 days storage, respectively, and these were similar to each other. Whereas, lower TFC of 34.47 mg CEQ/100 g were noted after storage of 90 days in the non-treated fruits (T₀).

Total carotenoids contents (mg/100 g): The fruits were treated with T₃ and T₂ showed higher entire carotenoids contents of 17.09 and 16.97 mg/100 g, respectively, and lower amount of total carotenoids were noted in the fruits of T₀ (13.89 mg/100 g) (Fig. 4). The fruits analyzed 30 days after storage showed higher carotenoids contents (17.11 mg/100 g) as compared to 60 and 90 days storage period, which were 16.08 and 14.25 mg/100 g,

respectively. There was a relation established here that TC decreases with the increase of storage period between wax coating treatments and storage periods, where maximum TC were noted in the fruits of T₃ (17.68 and 17.53 mg/100 g) and T₂, analyzed after 30 days storage respectively. Whereas, minimum total carotenoids contents of 10.95 mg/100 g were observed from the fruits treated without wax coating (T₀) after 90 days storage.

Total limonin contents (µg/mL): Lower amounts of total limonin contents (15.07 µg/mL) were noted in the fruits treated with T₃, and higher TLC of 15.08 was found in the untreated fruits (T₀) (Fig. 5). The fruits those were analyzed 30 days after storage showed higher amounts of TLC (14.75 µg/mL) than the fruits those were analyzed 60 and 90 days after storage where TLC values were 13.57 and 12.21 µg/mL, respectively. The interaction between wax coating treatments and storage periods showed higher amounts of TLC (15.35, 15.14 and 15.04 µg/mL) in the fruits of T₀, T₁, and T₂ after 30 and 60 days of storage, respectively (Fig. 5). While lower amounts of TLC of 10.19 µg/mL were observed in the fruits of T₃, analyzed 90 days after storage.

Physiological parameters

Chilling injury (%): Higher chilling injury (CI) index (2.77%) noted in the fruits without wax coating (T₀), and lower chilling injury indexes of 0.111 and 0.222% were found in the fruits of T₃ and T₂, respectively (Fig. 6). The fruits analyzed after a storage period of 90 days showed a higher index of chilling injury (1.58%) than the fruits analyzed 60 and 30 days after storage, where CI were 0.833 and 0.500%, respectively.

Fruit rot (%): The results regarding the fruit rot (%) are given in Fig. 7. Lower fruit rot indexes were observed in T₃ (1.66), and T₂ (2.11%), respectively, and these were on par with each other. While higher fruit rot index (10.33%) was noted in the fruits treated without wax coating (T₀). Fruits were analyzed 90 days after storage showed higher fruit rot index of 6.83% as compared to the fruits of 60 and 30 days after storage, and fruit rot indexes were 4.75 and 2.41%, respectively. The interaction between wax coating treatments and storage periods showed that higher fruit rot index of 14.66% was recorded in the fruits without wax coating (T₀) 90 days after storage. Whereas, fruits of T₃ and T₂ showed lower fruit rot indexes (0.00 and 0.66%) when analyzed 30 days after storage, respectively.

Fruit weight loss (%): Lower weight loss (WL) 2.665% was recorded in the fruits of T₃, and higher weight loss 11.44% was noted in the fruits of T₀ (Fig. 8). The fruits which were analyzed after 90 days storage period showed higher weight loss (8.08%) than the fruits of 60 days (5.83%) and 30 days (3.66%), respectively. The interaction indicated that lower weight losses 1.33 and 2.00% were found from 30 days after storage in the fruits of T₃ and T₂, respectively. However, higher weight loss (15.33%) was recorded in the T₀ fruits (when analyzed 90 days after storage).

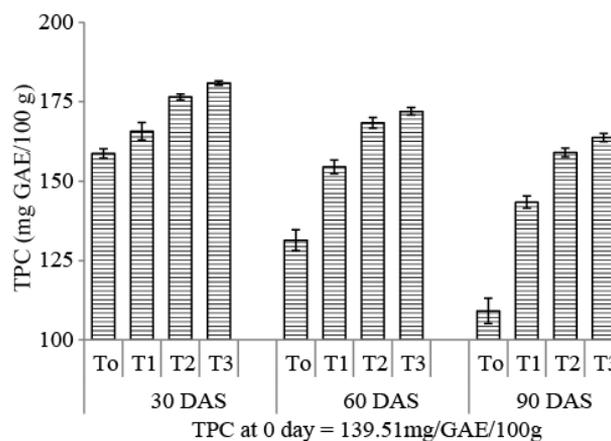


Fig. 1. Effects of wax coating treatments on total phenolic contents (mg GAE/100 g) during storage (8°C) in grapefruit cv. Ray Ruby. T₀ = Without wax coating, T₁ = Chitosan @ 120 mg per fruit for 5 min dipping, T₂ = Chitosan @ 130 mg per fruit for 5 min dipping, T₃ = Chitosan @ 140 mg per fruit for 5 min dipping (DAS = days after storage). Each vertical bar represents mean of three replicates ± S.E.

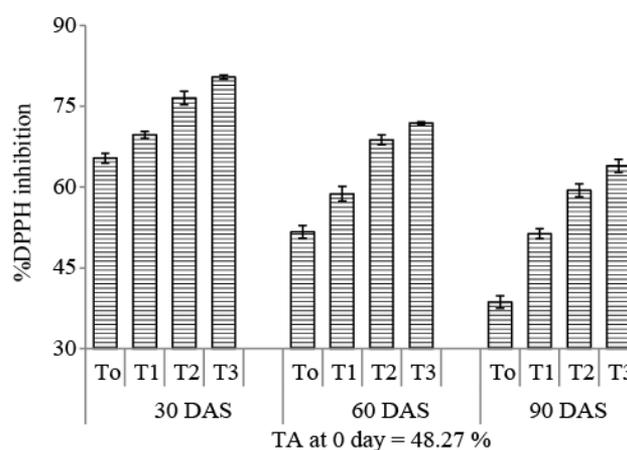


Fig. 2. Effects of wax coating treatments on total antioxidants activities (% DPPH inhibition) during storage (8°C) in grapefruit cv. Ray Ruby. T₀ = without wax coating, T₁ = Chitosan @ 120 mg per fruit for 5 min dipping, T₂ = Chitosan @ 130 mg per fruit for 5 min dipping, T₃ = Chitosan @ 140 mg per fruit for 5 min dipping (DAS = days after storage). Each vertical bar represents mean of three replicates ± S.E.

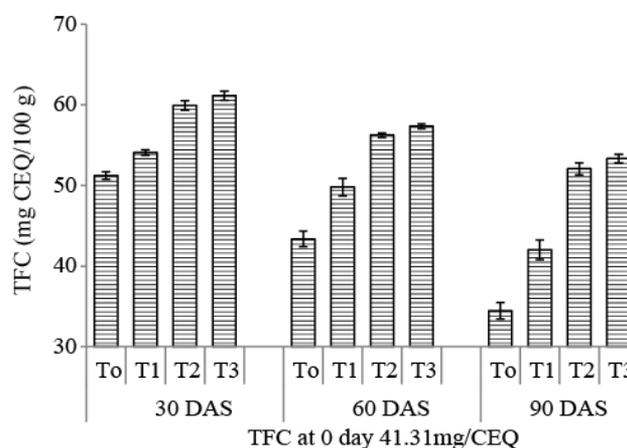


Fig. 3. Effects of wax coating treatments on total flavonoids contents (mg CEQ/100 g) during storage (8°C) in grapefruit cv. Ray Ruby. T₀ = Without wax coating, T₁ = Chitosan @ 120 mg per fruit for 5 min dipping, T₂ = Chitosan @ 130 mg per fruit for 5 min dipping, T₃ = Chitosan @ 140 mg per fruit for 5 min dipping (DAS = days after storage). Each vertical bar represents mean of three replicates ± S.E.

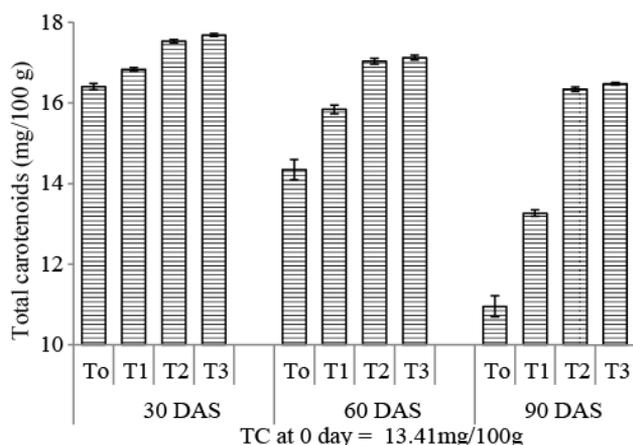


Fig. 4. Effects of wax coating treatments on total carotenoids contents (mg/100 g) during storage (8°C) in grapefruit cv. Ray Ruby. T₀ = Without wax coating, T₁ = Chitosan @ 120 mg per fruit for 5 min dipping, T₂ = Chitosan @ 130 mg per fruit for 5 min dipping, T₃ = Chitosan @ 140 mg per fruit for 5 min dipping (DAS = days after storage). Each vertical bar represents mean of three replicates ± S.E.

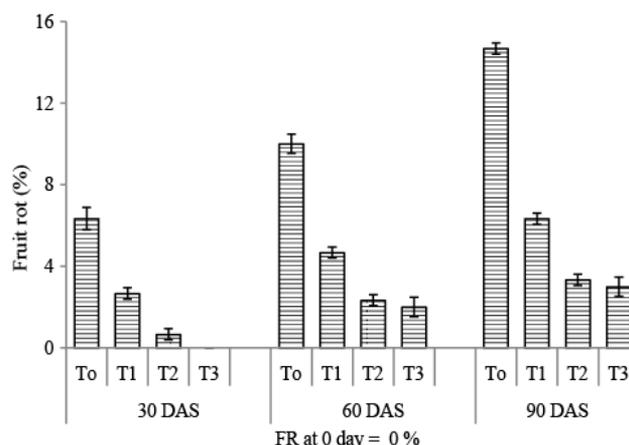


Fig. 7. Effects of wax coating treatments on fruit rot (%) during storage (8°C) in grapefruit cv. Ray Ruby. T₀ = Without wax coating, T₁ = Chitosan @ 120 mg per fruit for 5 min dipping, T₂ = Chitosan @ 130 mg per fruit for 5 min dipping, T₃ = Chitosan @ 140 mg per fruit for 5 min dipping, (DAS = days after storage). Each vertical bar represents mean of three replicates ± S.E.

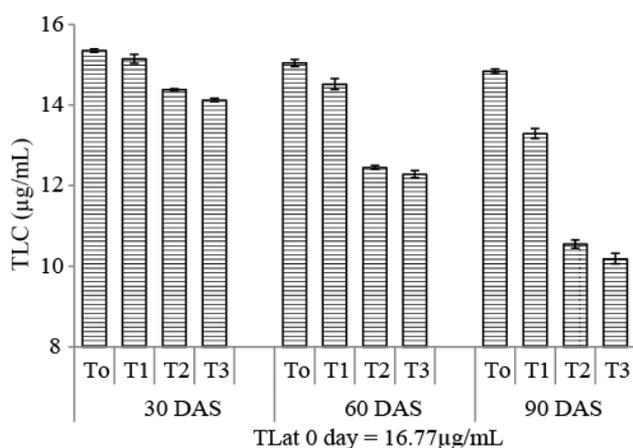


Fig. 5. Effects of wax coating treatments on total limonin contents (µg/mL) during storage (8°C) in grapefruit cv. of Ray Ruby. T₀ = Without wax coating, T₁ = Chitosan @ 120 mg per fruit for 5 min dipping, T₂ = Chitosan @ 130 mg per fruit for 5 min dipping, T₃ = Chitosan @ 140 mg per fruit for 5 min dipping (DAS = days after storage). Each vertical bar represents mean of three replicates ± S.E.

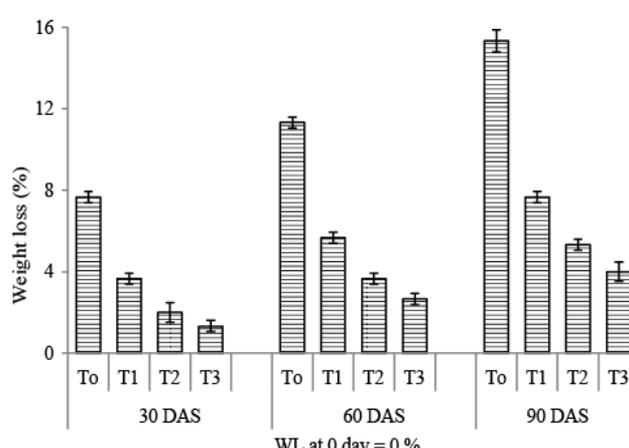


Fig. 8. Effects of wax coating treatments on weight loss (%) during storage (8°C) in grapefruit cv. Ray Ruby. T₀ = Without wax coating, T₁ = Chitosan @ 120 mg per fruit for 5 min dipping, T₂ = Chitosan @ 130 mg per fruit for 5 min dipping, T₃ = Chitosan @ 140 mg per fruit for 5 min dipping, (DAS = days after storage). Each vertical bar represents mean of three replicates ± S.E.

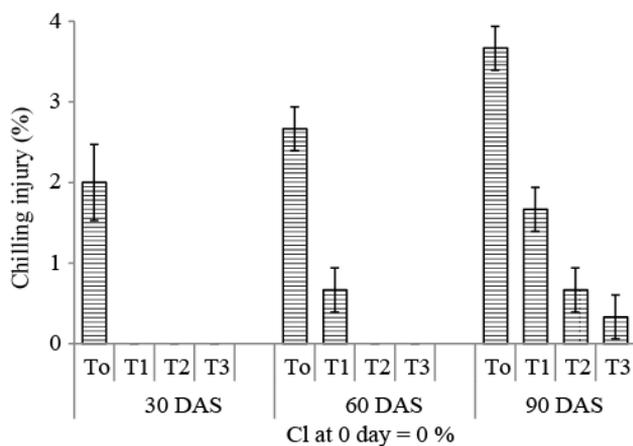


Fig. 6. Effects of wax coating treatments on chilling injury (%) during storage (8°C) in grapefruit cv. of Ray Ruby. T₀ = Without wax coating, T₁ = Chitosan @ 120 mg per fruit for 5 min dipping, T₂ = Chitosan @ 130 mg per fruit for 5 min dipping, T₃ = Chitosan @ 140 mg per fruit for 5 min dipping, (DAS = days after storage). Each vertical bar represents mean of three replicates ± S.E.

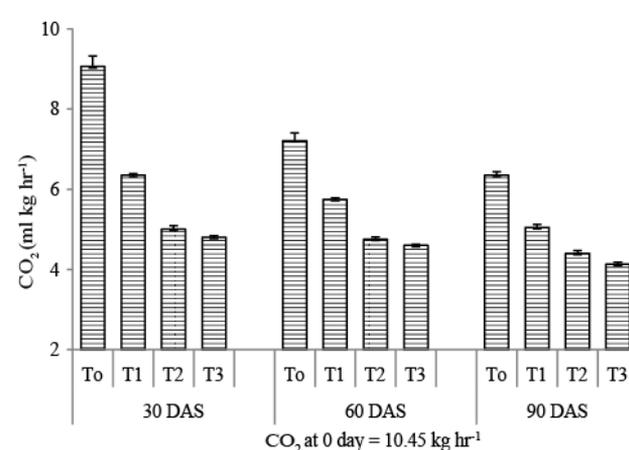


Fig. 9. Effects of wax coating treatments on CO₂ (ml kg hr⁻¹) during storage (8°C) in grapefruit cv. Ray Ruby. T₀ = Without wax coating, T₁ = Chitosan @ 120 mg per fruit for 5 min dipping, T₂ = Chitosan @ 130 mg per fruit for 5 min dipping, T₃ = Chitosan @ 140 mg per fruit for 5 min dipping, (DAS = days after storage). Each vertical bar represents mean of three replicates ± S.E.

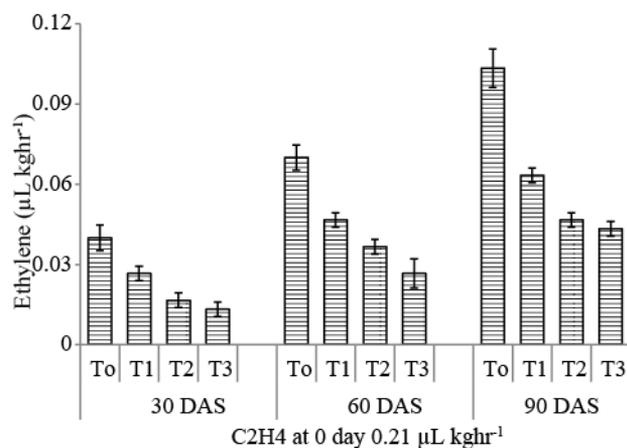


Fig. 10. Effects of wax coating treatments on ethylene ($\mu\text{L kg}^{-1}\text{hr}^{-1}$) during storage (8°C) in grapefruit cv. Ray Ruby.

T₀ = Without wax coating, T₁ = Chitosan @ 120 mg per fruit for 5 min dipping, T₂ = Chitosan @ 130 mg per fruit for 5 min dipping, T₃ = Chitosan @ 140 mg per fruit for 5 min dipping, (DAS = days after storage). Each vertical bar represents mean of three replicates \pm S.E.

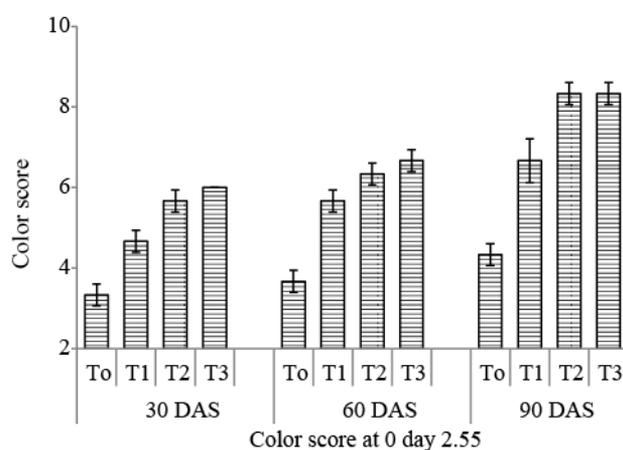


Fig. 11. Effects of wax coating treatments on color score during storage (8°C) in grapefruit cv. Ray Ruby.

T₀ = Without wax coating, T₁ = Chitosan @ 120 mg per fruit for 5 min dipping, T₂ = Chitosan @ 130 mg per fruit for 5 min dipping, T₃ = Chitosan @ 140 mg per fruit for 5 min dipping, (DAS = days after storage). Each vertical bar represents mean of three replicates \pm S.E.

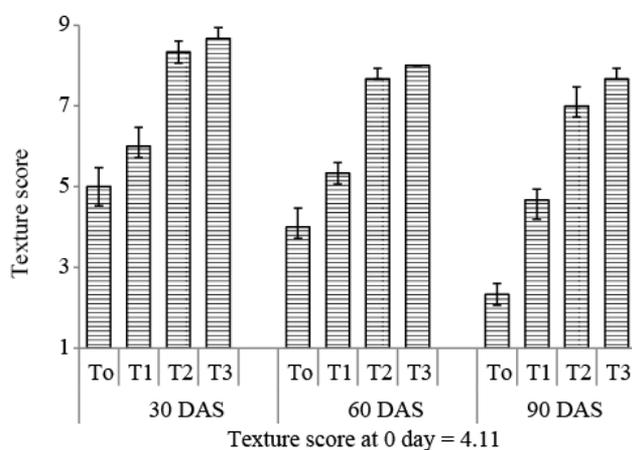


Fig. 12. Effects of wax coating treatments on texture score during storage (8°C) in grapefruit cv. Ray Ruby.

T₀ = Without wax coating, T₁ = Chitosan @ 120 mg per fruit for 5 min dipping, T₂ = Chitosan @ 130 mg per fruit for 5 min dipping, T₃ = Chitosan @ 140 mg per fruit for 5 min dipping, (DAS = days after storage). Each vertical bar represents mean of three replicates \pm S.E.

CO₂ (ml kg⁻¹ hr⁻¹): The effects of wax coating treatments, storage periods and their interaction showed significant differences at $p \leq 0.05$ on CO₂ in the fruits (Fig. 9). Higher CO₂ of 7.54 ml kg⁻¹ was recorded in the fruits of T₀, and lower CO₂ of 4.52 and 4.72 ml kg⁻¹ in the fruits of T₃ and T₂, respectively. The fruits those were analyzed 30 days after storage showed higher CO₂ (6.31 ml kg⁻¹) than to the fruits analysed after 60 and 90 days period of storage, and CO₂ values were 1.58 and 4.98 ml kg⁻¹, respectively. The interaction between wax coating treatments and storage periods showed that lower CO₂ values (4.14 and 4.40 ml kg⁻¹) were noted in the fruits of T₃ and T₂ when analyzed 90 days after storage respectively and these were on par with each other. While higher CO₂ of 9.06 ml kg⁻¹ was recorded from the fruits of T₀ (30 days after storage).

Ethylene ($\mu\text{L kg}^{-1}\text{hr}^{-1}$): Statistically significant differences ($p \leq 0.05$) were found regarding the effects of wax coating treatments, storage periods and their interaction on ethylene production in the fruits (Fig. 10). The fruits of T₃ (Chitosan @ 140 mg per fruit) and T₂ (Chitosan @ 130 mg per fruit) showed lower rates of ethylene production (0.027 and 0.033 $\mu\text{L kg}^{-1}\text{hr}^{-1}$), respectively. While a higher rate of ethylene (0.071 $\mu\text{L kg}^{-1}\text{hr}^{-1}$) was observed in the fruits treated without wax coating (T₀). Fruits were analyzed 90 days after storage showed a higher rate of ethylene (0.064 $\mu\text{L kg}^{-1}\text{hr}^{-1}$) as compared to the fruits of 60 and 30 days after storage, with rates 0.045 and 0.024 $\mu\text{L kg}^{-1}\text{hr}^{-1}$, respectively. The interaction between wax coating treatments and storage periods showed a higher rate of ethylene (0.103 $\mu\text{L kg}^{-1}\text{hr}^{-1}$) in the fruits T₀ (when analyzed 90 days after storage). Although lower rates of ethylene (0.013 and 0.016 $\mu\text{L kg}^{-1}\text{hr}^{-1}$) were observed in the fruits of T₃ and T₂, respectively after 30 days storage. However, these were at par with the fruits of T₃ and T₁ (60 and 30 days after storage), respectively.

Quality related parameters

Colour score: The results regarding the effects of wax coating treatments and storage periods and interaction between them showed significant impact on the color of the fruits as shown in Fig. 11. Higher colour scores of 7.00 and 6.77 were marked by the panelists for the fruits of T₃ and T₂, respectively. While a minimum colour score of 3.77 was marked for fruits of T₀. Higher colour score of 6.91 rated by the panelists for fruits which were analyzed after 90 days storage period than the fruits of 60 and 30 days after storage, where colour scores were 5.58 and 4.91 liked by the panelists, respectively.

Texture score: Texture score showed significant differences at $p \leq 0.05$ regarding the effects of wax coating treatments and storage periods, while their interaction did not show significant differences (Fig. 12). Maximum texture scores were ranked by the panelists for the fruit of T₃ (8.11) and T₂ (7.66), respectively and minimum texture score was rated by the panelists for the fruits of T₀ (3.77). Fruits were analyzed 30 days after storage and found higher texture score (7.00), while lower texture scores of 6.25 and 5.41 were recorded from 30 and 90 days after storage, respectively.

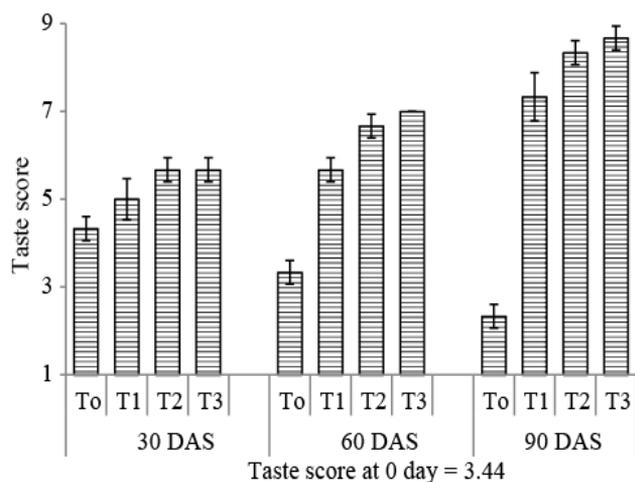


Fig. 13. Effects of wax coating treatments on taste score during storage (8°C) in grapefruit in Ray Ruby. T₀ = Without wax coating, T₁ = Chitosan @ 120 mg per fruit for 5 min dipping, T₂ = Chitosan @ 130 mg per fruit for 5 min dipping, T₃ = Chitosan @ 140 mg per fruit for 5 min dipping, (DAS = days after storage). Each vertical bar represents mean of three replicates ± S.E.

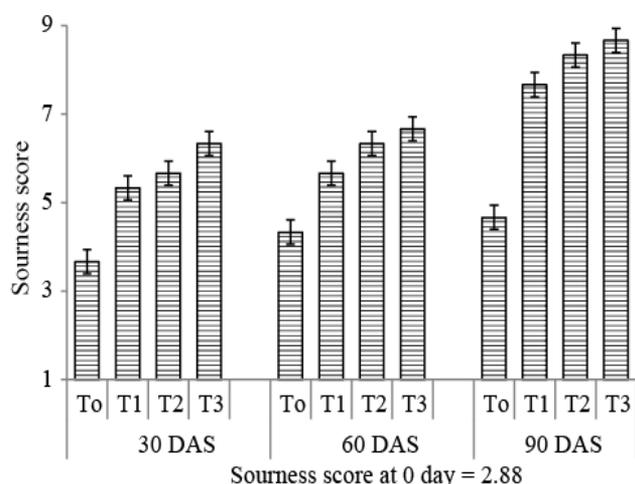


Fig. 14. Effects of wax coating treatments on sourness score during storage (8°C) in grapefruit cv. Ray Ruby. T₀ = Without wax coating, T₁ = Chitosan @ 120 mg per fruit for 5 min dipping, T₂ = Chitosan @ 130 mg per fruit for 5 min dipping, T₃ = Chitosan @ 140 mg per fruit for 5 min dipping, (DAS = days after storage). Each vertical bar represents mean of three replicates ± S.E.

Taste score: The analyzed data presented in Figure 13 showed significant differences ($p \leq 0.05$) regarding the effects of wax coating treatments, storage periods and interaction between them on taste score in the fruits. Maximum taste scores were marked by the panelists for fruits of T₃ (7.11) and T₂ (6.88), respectively. While minimum taste score of 5.16 was found in untreated fruits (T₀). Fruits analyzed 90 days after storage with higher taste score (6.66) than 60 and 90 days after storage with the taste scores of 5.66 and 5.16, respectively. The interaction between wax coating treatments and storage periods showed that higher taste scores were ranked for of T₃ (8.66) and T₂ (8.33) when analyzed 90 days after storage, respectively. Whereas, lower taste scores (2.33 and 3.33) were marked by the panelists in the fruits without wax coating (T₀), when analyzed 90 and 60 days after storage, respectively.

Sourness score: The results regarding the sourness score are presented in the Fig.14. It was found that higher sourness scores were marked by the panelists for fruits of T₃ (7.22) and T₂ (6.77), respectively. While lower sourness was marked by the panelists in the fruits of T₀ (4.22). Fruits were analyzed 90 days after storage showed maximum sourness (7.33), whereas minimum sourness (5.75 and 5.25) were recorded from the fruits of 60 and 30 days after storage, respectively.

Discussion

Films and edible coatings are defined as “a thin application of material that forms a protective covering around an edible commodity and can be consumed along with the coated product” (Guilbert, 1986). Films and coatings have been used traditionally to improve appearance and to conserve food products. Wax coatings for fruits have been applied in China since the 12th century (Dalal *et al.*, 1971). Chitosan is a modified natural carbohydrate polymer derived from chitin and has been found in a wide range of natural sources like crustaceans, fungi, insects and some algae (Tolaimate *et al.*, 2000).

Maximum quantities of TPC, TA, TC, and TF were recorded from 30 and 60 days after storage. Chitosan may inhibit the activity of polyphenol oxidase, which is involved in the process of phenolic compound degradation (Jiang and Li, 2001). It is well known that the bioactivity of chitosan, including antioxidant ability, is mainly attributed to the activity of hydroxyl and amino groups. There are three kinds of hydrogen sources; NH₂ of C₂, OH of C₃, and C₆. It is difficult for 3-OH to take part in the reaction because of steric hindrance (Xie *et al.*, 2001). Fruit treated with chitosan showed maximum TA and TF, TC after 90 days as compared to fruits which kept for 90 days storage. This might be due to chitosan, which has an ability to develop a modified system for the exchange of gases and improves the ability of enzymes activity during storage (Macheix *et al.*, 1990). Untreated fruit and lower concentration of chitosan showed a maximum reduction of these compounds after 90 days due to the breakdown of cell structure and senescence phenomena during storage (Macheix *et al.*, 1990). Fruit treated with chitosan @ 140 mg perfruit (T₃) showed maximum TPC and then TPC after 30 and 60 days of storage and fruits treated with lower concentration or control. Untreated fruits suffered more enzymatic changes than those treated with chitosan. This is temporary due to the advanced stage of oxidation, and molecules gradually lose this property, which causes a drastic reduction in TLC. Several studies have been reported the similar results (Andreasen *et al.*, 2001). Fruit treated with chitosan showed no CI, FR, during the whole storage period, this could be due to antibacterial membranes produced from a mixture of hydrolyzed starch that causes the semipermeable barrier in cell wall which prevents spores entering in the cell wall (Li *et al.*, 2007). Cuticle layer mainly suffers water losses and results in a crack in fruits. These cuticle layers can be damaged easily, and can form micro-cracks that can cause moisture loss continuously (Cohen *et al.*, 1990). Our finding is similar

to that of the previous investigation of Romanazzi *et al.* (2002) & Chien *et al.* (2005). Lower fruit weight losses were observed in the fruits treated with chitosan after 90 days of storage. This was mainly because the chitosan was the derivative of the amino cellulose with the feature of polycation, and it can gather the positive ion on the surface of the negative ion. The interaction between the positive ion and negative ion makes the biological adhesive property, so it can adhere the surrounding molecule to form the colloidal film which was the unique feature of this coating (Ali *et al.*, 2005). T₃ showed a decrease in respiration rate which might be due to reduce oxygen supply on the fruit surface. Yonemoto *et al.* (2002) & Du *et al.* (1997) reported that application of chitosan coating inhibited respiration rates of fruits.

Sensory evaluation showed that the fruits treated with chitosan showed more sweetness and sourness trend which decreases after 90 days of storage. This increase in sweetness might be the result of fast ripening of fruits resulting in increased sugar contents during storage. Sugar level was lower in untreated and control fruit after 90 days of storage. Sourness was reduced by chitosan application, and the increased trend was noted in case of T₀ which may be due to oxidation of citric acid. It has been found previously that sweetness was increased in fruits (Gul *et al.*, 1990; Doreyappa and Huddar, 2001; Srinivasa *et al.*, 2002). Fruit sweetness may be attributed to the presence of organic acids in the fruits which are major contributor of fruit taste (Kays, 1991). Fruit texture is mainly associated to cell wall integrity and stored carbohydrates such as pectin, starch, etc. Chitosan maintained fruit textural score after 90 days storage which may be due to decreased breakdown of insoluble pectin substances (Weichmann, 1987). Coated fruits showed overall good quality than uncoated fruits (T₀). This improvement in quality may be attributed to increase in organic acids during senescence (Baldwin, 1994). Untreated fruit showed lower quality. This poor quality might be due to the change in carbohydrates, proteins, amino acids, lipids and phenolic compounds that can influence the fruits quality (Malundo *et al.*, 2001).

Conclusion

Grapefruit coated by different shrimp chitosan as complexes, to enhanced the fruits quality and shelf life of grapefruits stored at 8±2°C temperature, maintained by the gases exchanges, weight loss and decreasing the quality of untreated fruit during storage periods were measured. The parameters at T₃ showed maximum organoleptic scores for fruit quality and higher phytonutrients like TPC (172.32 mg GAE/100g), TA (72.09 %), TC (17.09 mg/100g), TFC (52.27 mgCEQ/100g) and TLC (15.08 µg/mL). Chitosan coating also protected the grapefruit from diseases and maintained the physiological responses including chilling injury (1.58 and 1.33%), and fruit rot (0.66 and 0.33%) as compared to untreated fruits analyzed 90 days of storage. It is recommended that T₃ (Chitosan 140 mg per fruit) was the best dose and found very effective in improving the overall quality and shelf life of grapefruit during storage period. Maximum quality attributes of grapefruit was maintained after 90 days of storage period. Thus chitosan glucose complex seems to be a new natural preservative, and may be applied as promising applications in the food industry in near future.

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