

Effects of Postharvest Edible Coatings to Maintain Qualitative Properties and to Extend Shelf-life of Pomegranate (*Punica granatum. L*)

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Abstract

Regardless of the low respiration rate in pomegranate fruits, it is a greatly perishable product. An alternative to maintain quality and prolong the shelf life of pomegranate fruits could be edible coatings. In the present study, three different edible coatings including: 1% chitosan, 1000 mg L⁻¹ thymol, and their combined were investigated on the postharvest quality of pomegranate fruits. Changes in weight loss, fruit firmness, total soluble solids (TSS), titratable acidity (TA), juice pH, anthocyanin, total phenolic, and flavonoids content, sensory characteristics and fungal decay incidence of fruits were evaluated during 30, 60, 90 and 120 days storage at 6 °C. The results showed that coated fruits with 1% chitosan + 1000 mg L⁻¹ thymol significantly decreased weight loss and firmness than uncoated fruits. Furthermore, the coated fruits with 1% chitosan + 1000 mg L⁻¹ thymol exhibited significantly higher anthocyanin, total phenolic, flavonoids content, and sensory characteristics than the control treatment. The minimum shelf-life (83.33 days) was observed in uncoated fruits, while the maximum shelf-life (108.33 days) was recorded in fruits coated with 1 % chitosan + 1000 mg L⁻¹ thymol. Duration of storage had a significant effect on weight loss, firmness, juice pH, anthocyanin content, total phenolic content, flavonoids content, sensory characteristics, and decay, whereas there was no significant effect on TSS, TA, and TSS/TA. In conclusion, the combined application of chitosan and thymol can provide a useful alternative for shelf life extension of pomegranate fruits.

Keywords: Anthocyanin, chitosan, flavonoid, phenol, thymol.



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Introduction

Pomegranate (*Punica granatum L.*) is considered native to Iran, and it has been widely cultivated in arid and semiarid regions of this country. Iran, with the total area under pomegranate cultivation of about 70,000 ha and annual pomegranate production of about 800,000 tonnes, ranks second in pomegranate production in the world (Kahramanoğlu and Usanmaz,

2016). The eatable portions of pomegranate fruit (called arils) make up about 50% of the fruit weight, 76–85% juice, and 15–24% seeds (Varasteh et al., 2012). Pomegranate fruits are commonly used fresh and generally for making juice, jelly, and grenadine. It is an excellent source of natural antioxidants, such as anthocyanins, flavonoids, and phenolic acids (Zaouay et al., 2012). In addition,

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pomegranate fruit is rich in vitamins and minerals (Fawole and Opara, 2013).

Regardless of the low respiration rate in pomegranate fruit, it is a greatly perishable product (Barman et al., 2011), because of fruit peel has numerous minute openings that permit free movement of water vapor, and make fruit highly susceptible to water loss (Fawole and Opara, 2013). Furthermore, pomegranate fruit is sensitive to low temperatures, and cold storage at 5 °C or lower will cause chilling injury to the fruits (Elyatem and Kader, 1984). Exposure of pomegranate fruits to low temperatures results in aril browning due to the oxidative damage of membranes, leading to higher activities of certain enzymes such as polyphenol oxidase and peroxidase. Several chemical treatments have been tested for pomegranate fruits to prevent or delay the onset of chilling injury and to extend the maximum storage period without compromising fruit quality (Moradinezhad and Khayyat, 2014; Ranjbari et al., 2016). However, storage at higher temperatures leads to a reduction of shelf life by accelerating physiological and pathological activities (Barman et al., 2011). Therefore, finding a way to maintain quality and extend shelf life of pomegranate fruits is of great importance.

A potential postharvest treatment for quality maintenance and shelf life extension of fruits is surface coatings. Chitosan has significant antimicrobial, ant-oxidation, and biodegradation properties (Delieghere et al., 2004). As a well-known coating material, chitosan has played an essential role in the quality enhancement of fruits (Dotto et al., 2015). In addition, thymol, as one of the most important essential oils, is considerably active against a broad spectrum of microorganisms and can extend the shelf-life of fruits. Addition of essential oils to edible coating can prolong the shelf-life of fruits due to improved and extended antimicrobial activity because essential oil compounds are regularly released overtime on the fruit surface while retaining a suitable

concentration of antimicrobial components during the storage time (Outtara et al., 2000). However, as far as we know, no work has been reported about the effect of thymol essential oil, and the combination of chitosan and thymol essential oil on the physiological and quality attributes of pomegranate fruits. Thus, the objective of the present study was to evaluate the effects of chitosan, thymol essential oil, and their interaction on physicochemical quality enhancement of pomegranate fruit during cold storage. Changes including weight loss, fruit firmness, total soluble solids (TSS), titratable acidity (TA), juice pH, anthocyanin, total phenolic, and flavonoids content, sensory characteristics, and fungal decay incidence of fruits were synchronously examined during storage at 6 °C for 120 days.

Materials and Methods

Plant material

Pomegranate fruits cv. 'Malas Ghermez Siab' were harvested at commercial ripening stage (about 190 days after fruit set) from a commercial orchard located at Poldokhtar, Lorestan province, Iran. Immediately, fruits were transported by a ventilated car to the horticultural laboratory in Arak University, Arak, Iran. Pomegranate fruits were selected for uniformity in size, shape, and color. Injured, sunburn, bruised, and diseased fruits were discarded. The remaining fruits were randomized and divided into four parts of 60 fruits for the following treatments in three replicates (each replicate contained 20 individual fruits).

Treatment and storage conditions

Three types of solution were prepared including 1% chitosan (medium molecular weight, Fluka, Buchs, Switzerland), 1000 mg L⁻¹ thymol [(5-methyl-2-isopropyl-phenol) (Sigma Chemical Co., St. Louis, MO, USA, Minimum 99.5%)] and 1% chitosan combined with 1000 mg L⁻¹ thymol. These concentrations of chitosan and thymol were selected based on the preliminary experiments in the laboratory

condition. Fruits were dipped for 15 min in three prepared solutions. A quarter of the pomegranate fruits were treated in distilled water, which served as control. Fruits dripped in distilled water were used as a control. All samples were air-dried for 60 min before packing in polyethylene terephthalate (gas and water-resistant) and stored at 6 °C and 80 % relative humidity in permanent darkness for 120 days. Five fruits from each replicate were randomly sampled on 30, 60, 90, and 120 days and analyzed for the following parameters:

Weight loss

Weights of individual replicate were recorded following treatment (day 0) and at different intervals (days 30, 60, 90, and 120) during storage. Weight loss percentage was expressed as the percentage of loss of weight concerning the initial weight.

Fruit firmness

The firmness of pomegranate fruits was recorded using a penetrometer (STEP SYSTEM, Germany). A plunger tip with an 8 mm diameter and 21 mm height was used to measure the firmness of the fruits. Readings were expressed as kg cm^{-2} .

TSS, TA, TSS/TA, and pH

Total soluble solids (TSS) concentration was determined in the juice from three individual fruit for each treatment using a refractometer (Atago, PAL-1, Japan) at 20 ± 1 °C and results expressed as the means of % (°Brix). The pH of the juice was recorded using a pH meter (Az 86502, Taiwan). Then titratable acidity (TA) was determined by titration with 0.1 N NaOH up to pH of 8.1, using 1 mL of diluted juice in 25 mL distilled water and results expressed as % citric acid. TSS to TA ratio was calculated by dividing TSS to TA percentages.

Determination of Anthocyanin

The total anthocyanin content of aril juice was determined according to the pH differential method (Kim et al., 2003). Absorbance was measured at 520 and 700

nm and expressed as cyanidin-3-glycoside (molecular weight of 449.2) equivalents per 100 g of fresh weight of fruit.

Total phenolics and flavonoids contents

Total phenolic content in pomegranate juice was determined using the Folin–Ciocalteu method as described by Singleton and Rossi (1965).

Total flavonoids content in the juice was determined by a colorimetric method described by Park et al. (2008).

Sensory evaluation

During the storage, sensory evaluation of the stored pomegranate fruits was achieved by a panel of five experts on a hedonic scale ranging from 0 to 5, where 1 = very bad, 2 = bad, 3 = medium, 4 = good, and 5 = excellent. Color, aroma, appearance, and overall acceptability were done by this method, and the average values were comprised of assessing the acceptability by the consumers. Consequently, fruits that ranked good or excellent, considered consumable.

Fungal decay incidence

Fungal decay incidence of fruits was evaluated as described by Reyes-Avalos et al. (2016). Fungal decay incidence was achieved by counting the number of rotten fruits, divided by the total number of fruits within each package, and was expressed as a percentage (%).

Shelf life of fruits

The shelf life of pomegranate fruit was determined from the day of harvest to the shelf-life expiration date in the storage. The shelf life of fruits was determined by recording the number of days the fruits remained in good condition throughout storage without degeneration. When the degeneration of fruits exceeded 50%, it was considered as the end of shelf life.

Statistical analysis

To evaluate the effect of edible coating (chitosan, thymol essential oil and their

interaction) and duration of storage (30, 60, 90 and 120 days) on the physiological, quality attributes, sensory parameters and shelf life of pomegranate fruits the experiment was conducted as a factorial experiment based on the completely randomized design (CRD). Data were analyzed using GLM procedure SAS software Version 9.1. Significant differences were assessed using Duncan's multiple range test at $P \leq 0.05$. The results were presented as mean values \pm SE.

Results

There was no significant interaction between storage duration and coating treatments on the physiological and quality attributes of pomegranate fruits (data not shown). Therefore, we just mentioned the main effects of storage duration and coating treatments.

Effect of duration of storage

Duration of storage had a significant effect on weight loss ($P < 0.0001$), firmness ($P < 0.0001$), juice pH ($P = 0.0006$), anthocyanin content ($P = 0.0004$), total phenolic content ($P = 0.0007$), flavonoids content ($P < 0.0001$), sensory characteristics ($P = 0.0083$) and decay ($P = 0.0192$) of coated and uncoated fruits, whereas storage time had no significant effect on TSS ($P = 0.0644$), TA ($P = 0.2336$) and TSS/TA ($P = 0.2103$) (Table 1). Weight loss, firmness, juice pH and decay of pomegranate fruits increased considerably with the extended storage time. In contrast, there was a significant decrease in anthocyanin content, total phenolic content, flavonoids content and sensory characteristics of pomegranate fruits with the longer storage duration (Table 1).

Table 1. Effect of duration of storage on physicochemical and qualitative properties of coated and uncoated pomegranate fruits during cold storage

Storage days	Weight loss (%)	Firmness (kg cm ⁻²)	TSS (°Brix)	TA (%)	TSS/TA	Juice pH	Anthocyanin content (mg 100 g ⁻¹)	Phenol content (mg 100 mL ⁻¹)	Flavonoid content (mg 100 mL ⁻¹)	Sensory scores	Decay (%)
30	9.12 \pm 1.92 b	7.06 \pm 1.08 c	15.08 \pm 0.46	0.83 \pm 0.12	18.56 \pm 3.21	3.30 \pm 0.07 c	175.19 \pm 6.45 a	159.09 \pm 7.96 a	94.58 \pm 4.29 a	5.00 \pm 0.00 a	0.00 \pm 0.00 b
60	11.81 \pm 3.07 b	7.63 \pm 0.97 bc	15.40 \pm 0.63	0.78 \pm 0.26	22.07 \pm 9.89	3.53 \pm 0.20 bc	171.41 \pm 8.11 ab	156.01 \pm 9.43 a	89.50 \pm 6.74 a	5.00 \pm 0.00 a	0.00 \pm 0.00 b
90	23.31 \pm 4.89 a	8.43 \pm 1.21 ab	15.55 \pm 0.47	0.74 \pm 0.20	22.23 \pm 6.08	3.70 \pm 0.42 ab	163.88 \pm 14.02 bc	150.59 \pm 11.80 a	83.25 \pm 7.08 b	4.66 \pm 0.49 ab	0.00 \pm 0.00 b
120	25.96 \pm 5.74 a	9.30 \pm 1.15 a	15.82 \pm 0.96	0.67 \pm 0.12	24.13 \pm 4.43	3.88 \pm 0.43 a	154.91 \pm 14.68 c	138.91 \pm 16.08 b	74.13 \pm 9.19 c	4.41 \pm 0.79 b	3.83 \pm 6.93 a
<i>P</i> -value	<0001	<0001	0.0644	0.2336	0.2103	0.0006	0.0004	0.0007	<0001	0.0083	0.0192

Mean values followed by the similar letters within a column are not significantly different from each other at $P \leq 0.05$ (Duncan's multiple range test). Values represent the mean \pm SE.

Effects of edible coatings on the physiological and quality attributes

Fruit weight loss

The weight loss of pomegranate fruits in all treatments increased with the longer storage duration (Table 1). However, the percentage of weight loss was significantly affected by edible coatings, and the higher rate was observed in uncoated fruits (Fig. 1). After 120 days of cold storage, the

maximum weight loss (33.76%) was recorded in uncoated fruits, while this one was 19.33% for 1% chitosan + 1000 mg L⁻¹ thymol, which was the most effective treatment on reducing weight loss. Moreover, there was a significant difference between 1% chitosan + 1000 mg L⁻¹ thymol and the other coated fruits at the end of the storage period (Fig. 1).

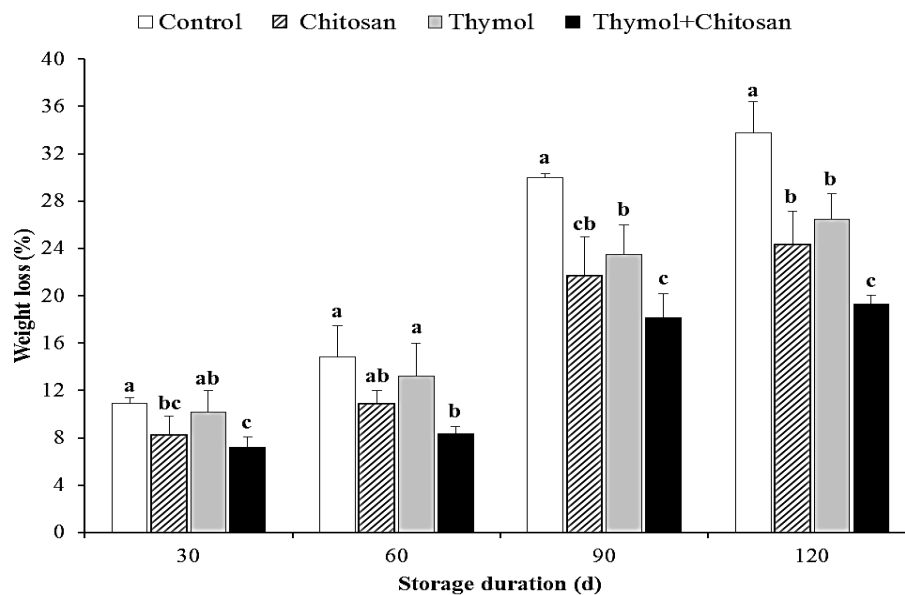


Fig. 1. Effect of edible coatings on weight loss of pomegranate fruits during 120 days of storage at 6 °C. Significant difference between data is expressed by different letters. Significant differences were separately assessed using Duncan's multiple range test at $P \leq 0.05$ on 30, 60, 90 and 120 days. Columns show mean value + standard error.

Fruit firmness

The firmness of pomegranate fruits was considerably affected by edible coatings, and the higher value was found in uncoated fruits (Fig. 2). After 120 days of cold storage, the maximum firmness (10.73 kg

cm^{-2}) was observed in uncoated fruits, while this one was 8.10 kg cm^{-2} for 1 % chitosan + 1000 mg L^{-1} thymol, which was the best treatment on maintaining fruit firmness (Fig. 2).

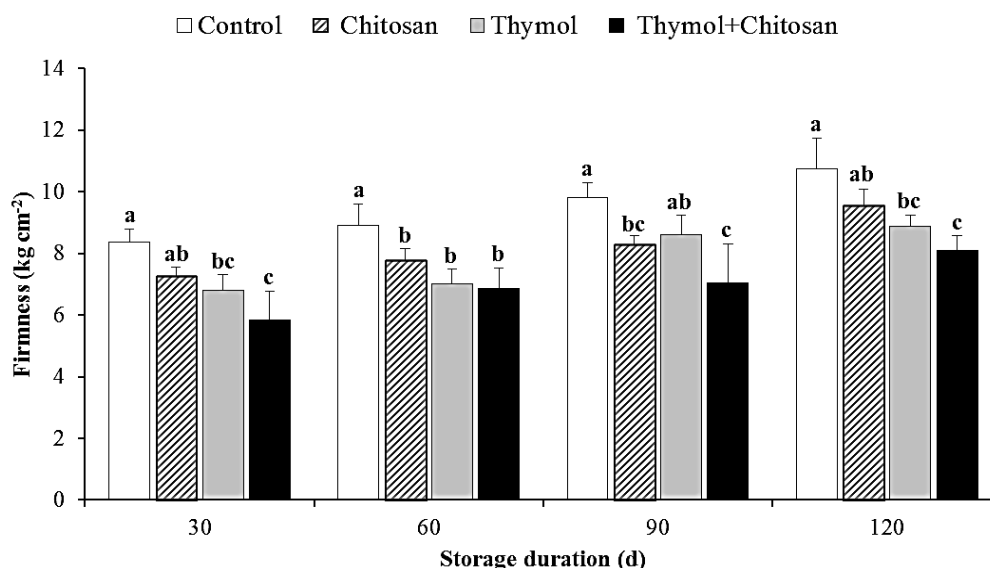


Fig. 2. Effect of edible coatings on the firmness of pomegranate fruits during 120 days of storage at 6 °C. Significant difference between data is expressed by different letters. Significant differences were separately assessed using Duncan's multiple range test at $P \leq 0.05$ on 30, 60, 90 and 120 days. Columns show mean value + standard error.

TSS, TA, TSS/TA, and pH

Figure 3 shows the variation in TSS, TA, and TSS/TA of pomegranate fruits during storage following application of treatments. There was a slight increase in the level of

TSS and a slight decrease in the level of TA during cold storage in the coated and uncoated fruits, although no significant difference was found between control and coating treatments (Fig. 3).

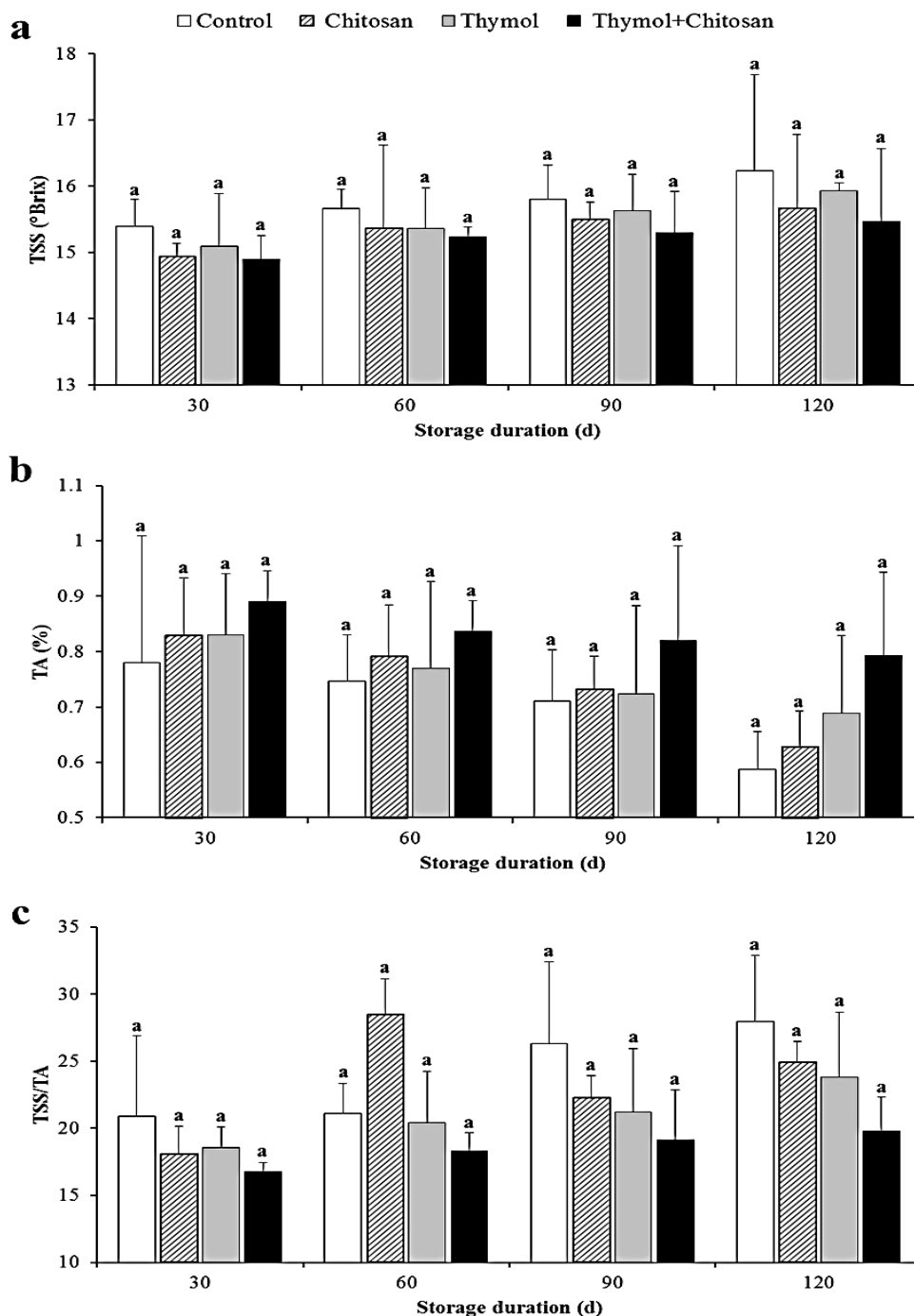


Fig. 3. Effect of edible coatings on (a) the TSS (b) TA, and (c) TSS/TA of pomegranate fruits during 120 days of storage at 6 °C. Significant difference between data is expressed by different letters. Significant differences were separately assessed using Duncan's multiple range test at $P \leq 0.05$ on 30, 60, 90 and 120 days. Columns show mean value + standard error.

The juice pH increased at the end of storage time in the uncoated and coated fruits, reaching maximum values at the end of storage with mean 4.23 in control fruits.

However, no significant difference was found between control and coating treatments (Fig. 4).

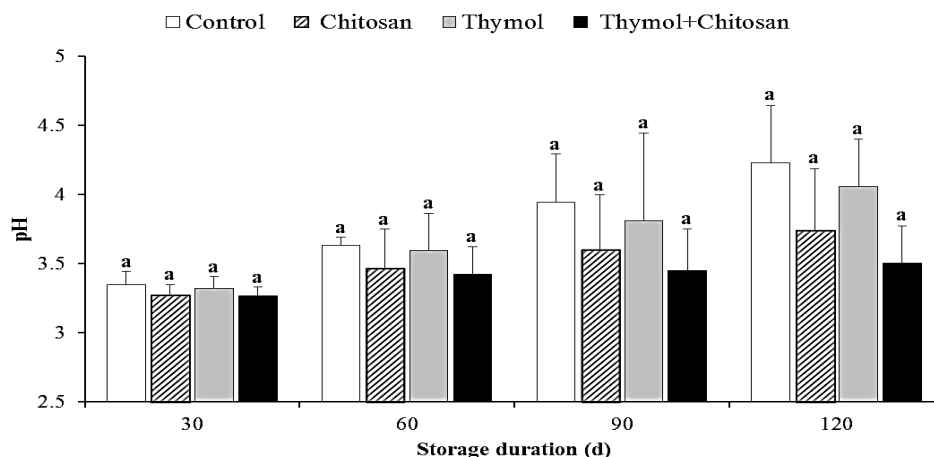


Fig. 4. Effect of edible coatings on the juice pH of pomegranate fruits during 120 days of storage at 6 °C. Significant difference between data is expressed by different letters. Significant differences were separately assessed using Duncan's multiple range test at $P \leq 0.05$ on 30, 60, 90 and 120 days. Columns show mean value + standard error.

Anthocyanin content

The results showed that anthocyanin content reduced in coated and uncoated fruits during cold storage (Table 1). Coated fruits significantly maintained higher anthocyanin content than uncoated fruits during postharvest periods. However, fruits

coated with 1 % chitosan + 1000 mg L⁻¹ thymol showed the maximum anthocyanin content with 170 mg 100 g⁻¹ after 120 days storage at 6 °C, although no significant difference was found between this treatment and 1% chitosan treatment (Fig. 5).

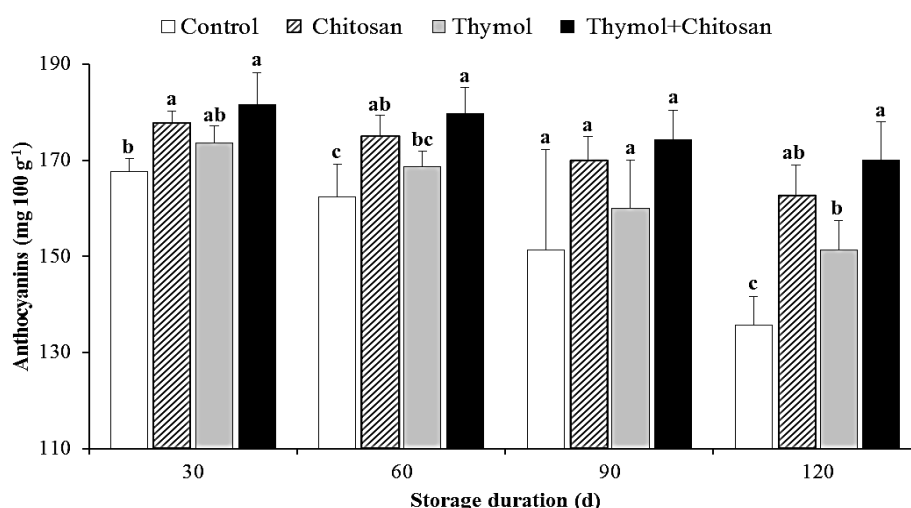


Fig. 5. Effect of edible coatings on the anthocyanin content of pomegranate fruits during 120 days of storage at 6 °C. Significant difference between data is expressed by different letters. Significant differences were separately assessed using Duncan's multiple range test at $P \leq 0.05$ on 30, 60, 90 and 120 days. Columns show mean value + standard error.

Total phenolic content

The results revealed that total phenolic content decreased in coated and uncoated fruits during cold storage (Table 1). After 120 days of cold storage, the minimum

total phenolic content (117.33 mg GAE 100 mL⁻¹) was observed in uncoated fruits, while this one was 158.66 mg GAE 100 mL⁻¹ for 1 % chitosan + 1000 mg L⁻¹ thymol (Fig. 6).

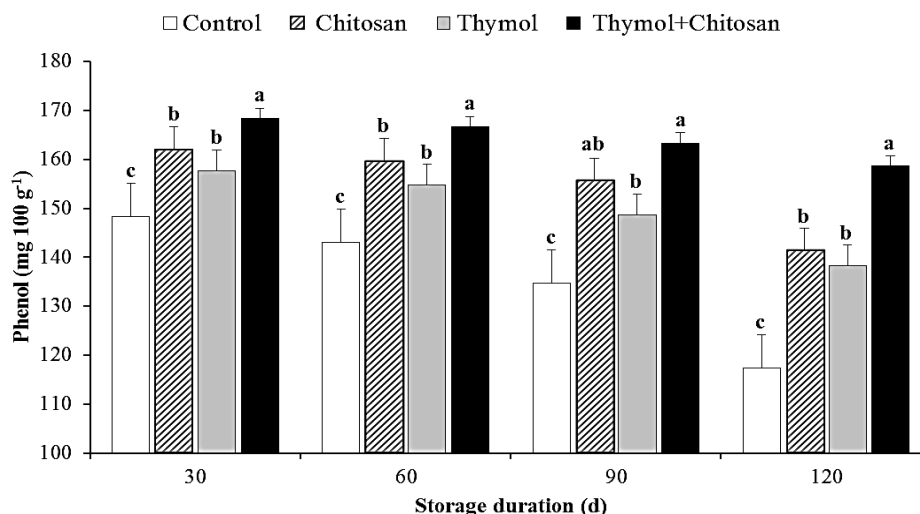


Fig. 6. Effect of edible coatings on the total phenolic content of pomegranate fruits during 120 days of storage at 6 °C. Significant difference between data is expressed by different letters. Significant differences were separately assessed using Duncan's multiple range test at $P \leq 0.05$ on 30, 60, 90 and 120 days. Columns show mean value + standard error.

Flavonoids content

Flavonoids content decreased in coated and uncoated fruits during cold storage (Table 1). After 120 days of cold storage, the

lowest flavonoids value (63.66 mg RE 100 mL⁻¹) was observed in control fruits, while this one was 86.06 mg RE 100 mL⁻¹ for 1% chitosan + 1000 mg L⁻¹ thymol (Fig. 7).

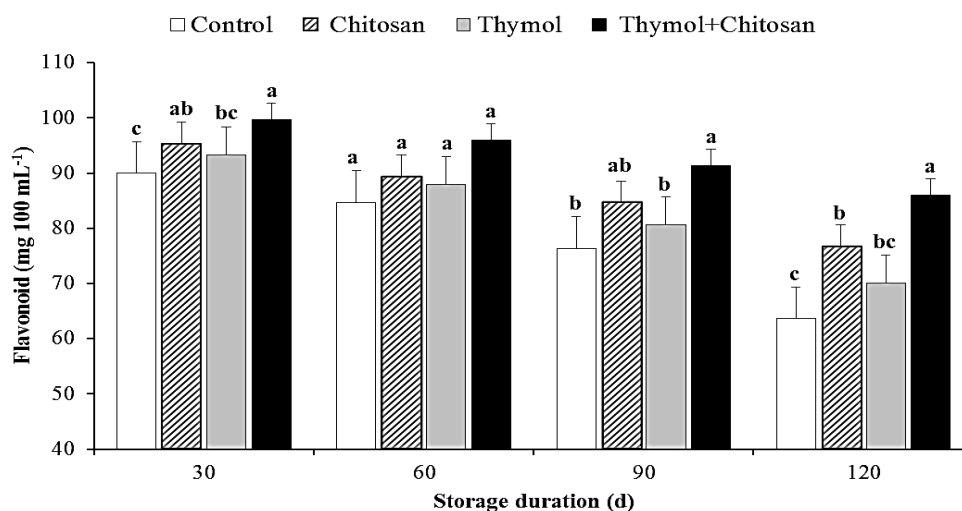


Fig. 7. Effect of edible coatings on the flavonoids content of pomegranate fruits during 120 days of storage at 6 °C. Significant difference between data is expressed by different letters. Significant differences were separately assessed using Duncan's multiple range test at $P \leq 0.05$ on 30, 60, 90 and 120 days. Columns show mean value + standard error.

Sensory evaluation

At the end of the 120 days' storage at 6 °C, all coated fruits were statistically similar but showed higher sensorial scores than

uncoated fruits. Control fruits declined to the critical level of acceptability (3.33) (Fig. 8).

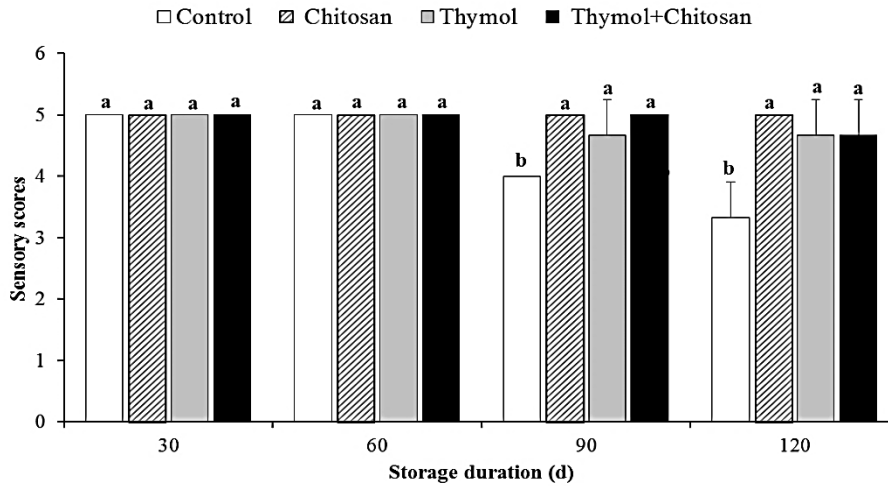


Fig. 8. Effect of edible coatings on the sensory characteristics of pomegranate fruits during 120 days of storage at 6 °C. Significant difference between data is expressed by different letters. Significant differences were separately assessed using Duncan's multiple range test at $P \leq 0.05$ on 30, 60, 90 and 120 days. Columns show mean value + standard error.

Fungal decay incidence

After 120 days of cold storage, the minimum percentage of fungal decay incidence (00.00 %) was recorded in

coated fruits, whereas 15.33% of uncoated fruits were found contaminated with fungal infection (Fig. 9).

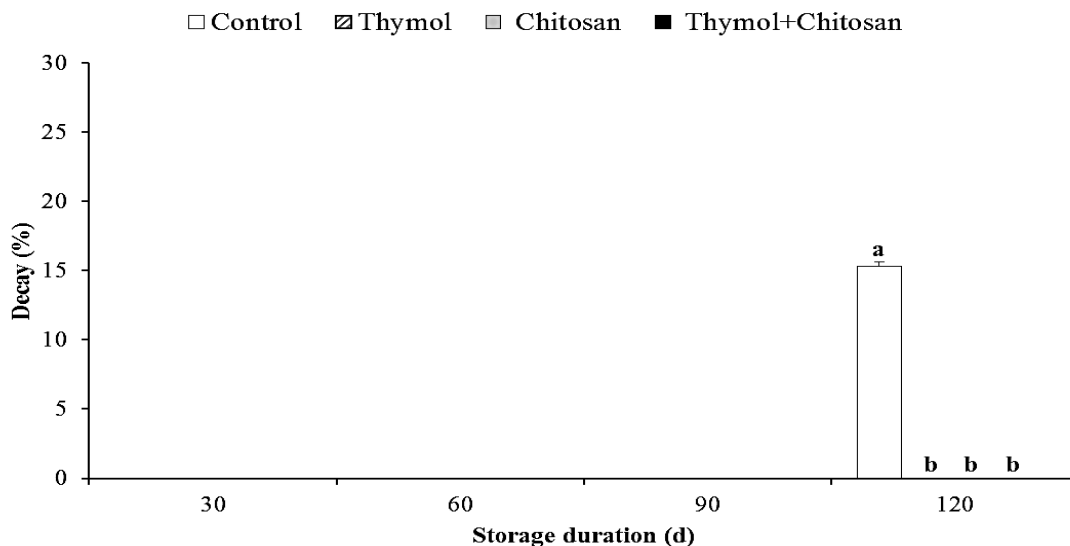


Fig. 9. Effect of edible coatings on the fungal decay incidence of pomegranate fruits during 120 days of storage at 6 °C. Significant difference between data is expressed by different letters. Significant differences were separately assessed using Duncan's multiple range test at $P \leq 0.05$ on 30, 60, 90 and 120 days. Columns show mean value + standard error.

Shelf-life of fruits

Shelf-life of pomegranate fruits was considerably affected by edible coatings (Fig. 10). The minimum shelf-life (83.33 days) was associated with uncoated fruits.

In comparison, the maximum shelf-life (108.33 days) was recorded in coated fruits as no statistical difference was found among fruits coated with chitosan, thymol, or both (Fig. 10).

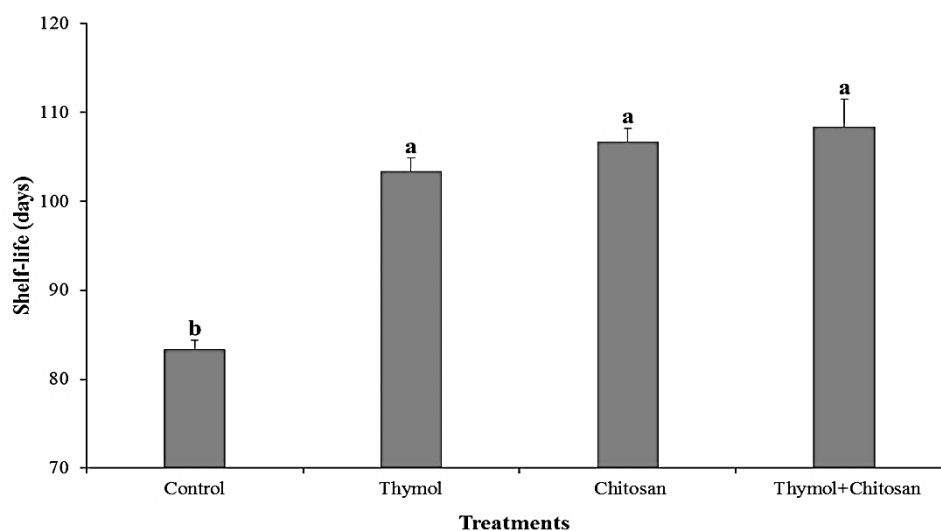


Fig. 10. Effect of edible coatings on the shelf-life of pomegranate fruits stored at 6 °C. Significant difference ($P \leq 0.05$) between data is expressed by different letters. Vertical bars indicate standard error.

Discussion

Edible coatings create a transparent film around the fruit that prevents water loss, limits respiration, and protects against fungal diseases. From the present study, it is revealed that edible coatings, especially 1% chitosan + 1000 mg L⁻¹ thymol, can be used for maintaining the quality of pomegranates during postharvest period. Edible coatings, particularly 1% chitosan + 1000 mg L⁻¹ thymol, considerably reduced weight loss during 120 days storage at 6 °C (Fig. 1). Weight loss in fresh fruit is primarily related to the water loss caused by transpiration and respiration (Reyes-Avalos et al., 2016). Chitosan coatings create a layer on the surface of the fruit and act as a protective barrier that decreases respiration and transpiration across the fruit surface (Meighani et al., 2015). This effect has also been previously reported in (Reyes-Avalos et al., 2016), pomegranate (Meighani et al., 2015), litchi (Lin et al., 2011), and papaya (Ali et al. 2011). In

addition, essential oils have been combined with chitosan to decrease moisture loss in fruit (Sun et al., 2014). Results of the present study suggest that the combination of chitosan and thymol presents much better effect on inhibiting weight loss than their separate use (Fig. 1), which are in agreement with the previous reports (Reyes-Avalos et al., 2016; Dantas Guerra et al., 2016).

Since pomegranate fruits lose water during storage, the texture becomes more compacted and condensed. Therefore, higher force is required to penetrate into the flesh. As a result, chitosan, thymol, and chitosan combined with thymol could positively impact the maintenance of firmness in pomegranates by reducing water loss (Fig. 2). In a similar study, the combination of chitosan with essential oils was also found to delay the loss of orange firmness (Chafer et al., 2012).

The changes in TSS and TA during storage of fruits could be as the result of the

ripening process (Ravanfar et al., 2014; Meighani et al., 2015). Organic acids are the primary respiratory substrates during fruit postharvest storage (Sayyari et al., 2011). Results (Fig. 3) are also in agreement with previous reports in coated sour cherry (Ravanfar et al., 2014) and litchi fruits (Jiang et al., 2005), in which TA reduced during cold storage, and the coating treatment inhibited its loss. In addition, during cold storage, starch, and other nutrients degrade into sugars and other soluble substances, increasing the TSS level (Sabir et al., 2011). However, the minimum level of TSS recorded in 1% chitosan + 1000 mg L⁻¹ thymol coated fruits (Fig. 3) can be related to a decrease in respiration rate by coatings, which is supported by the reports of Barman et al. (2011) and Dantas Guerra et al. (2016). On the other hand, TSS increased with during storage and coincided with the increase in water loss. It is most likely that the increased TSS attributes to the concentration of fruit juice during storage. Pomegranate is a non-climacteric type of fruit and show very low respiration rates (Barman et al., 2011). Therefore, there is low consumption of sugar for respiration during postharvest life in pomegranate fruit.

The juice pH of pomegranate fruits increased at the end of the storage period in the uncoated and coated fruits (Table 1). This result agrees with those reported by Elyatem and Kader (1984), who reported an increase in juice pH of pomegranate during cold storage. The change in juice pH of fruits during storage time has different reasons; it might be due to the effect of treatment on the biochemical condition of the fruit or slower rate of respiration and metabolic activity (Jitareerat et al., 2007).

Our results showed that the edible coatings reduced the degradation of anthocyanin content during the storage time (Fig. 5). It was most possibly because the edible coatings lessen the activity of polyphenol oxidase and peroxidase enzymes in response to changes in the

internal atmosphere of coated fruit (Varasteh et al., 2012). Dong et al. (2004) also reported that the degradation of anthocyanins in litchi fruit is affected by polyphenol oxidase and peroxidase, but application of chitosan coating decreased enzyme activity during storage period.

A continuous decrease in the total phenolic content of the pomegranate fruits was observed during storage period (Table 1); nevertheless, this reduction in coated fruits was smaller than uncoated fruits (Fig. 6). Our findings agree with those reported by Fawole and Opara (2013), who reported a decline in total phenolic content in pomegranate fruits stored under low temperatures for 16 weeks. On the contrary, Sayyari et al. (2011) showed a significant increase in total phenolic content in uncoated and coated pomegranate fruits over 84 days of storage. The changes in total phenolic content during cold storage may be related to fluctuations of phenylalanine ammonia-lyase enzyme activity, the critical enzyme in the first step of the phenylpropanoid pathway directly involved in the biosynthesis of phenolic compounds (Sayyari et al., 2011).

In the present research, there was a steady decline in the flavonoids content of pomegranate fruits during storage period (Table 1). Still, the coating treatments, in particular 1% chitosan + 1000 mg L⁻¹ thymol, reduced the decline in the flavonoids content during storage period (Fig. 7). This result is in agreement with those reported by Meighani et al. (2015). They reported a decline in flavonoids content in pomegranate fruits coated with different coatings stored at 4.5 °C for 120 days. While, this result is in contrast with Ghasemnezhad et al. (2015), which observed a significant increase in flavonoids content in the arils of pomegranate stored at 4 °C for 12 days. The amount of flavonoids is determined by many factors, such as genotype, environmental conditions, production

methods, transportation, handling system, and storage conditions (Ghasemnezhad et al., 2015). The decline could be related to phenols degradation, as previously found in phenolic content (Fig. 6).

In line with the sensory characteristics obtained in the present study (Fig. 8), other studies have reported that the application of chitosan and essential oils alone or in combination as a coating material improved sensory characteristics of fruits during the storage period (Xiao et al., 2010; Xing et al., 2011). This maximum score may be attributed to the minimum water loss from the fruit surface and retention of a better balance between sugars and acids of fruit juice (Jameel Jhalegar et al., 2015).

Numerous studies have reported the efficacy of chitosan in inhibiting the mycelia growth of postharvest pathogenic fungi (Jianglian and Shaoying, 2013). Perdonés et al. (2012) reported that chitosan coatings reduced the percentage of infected strawberries compared to uncoated ones after three days of storage. In addition, Jameel Jhalegar et al. (2015) indicated that essential oils can control fungal decay incidence without causing any injury or harmful effects on Kinnow mandarin, and essential oils can be recommended as a safe method for extending its storage life while maintaining fruit quality. Sánchez-González et al. (2010) reported that chitosan coatings were not effective in inhibiting fungal decay incidence, while the combined application of chitosan and bergamot essential oil as the coating was effective in inhibiting the growth of fungal decay during storage time.

In agreement with the results obtained in the present study (Table 1), changes in physicochemical and qualitative properties of other fruits such as fig (Saki et al. 2019), pomegranate (Meighani et al., 2015), cherries (Goncalves et al., 2007) and strawberry (El Ghaouth et al., 1991) have also been characterized during the storage

period. Results of the present study (Fig. 10) are in agreement with previous studies on mangoes (Taduri et al., 2017), fig (Reyes-Avalos et al., 2016), and pomegranate (Meighani et al., 2015), that showed shelf-life of coated fruits was significantly higher than uncoated fruits under cold storage.

Conclusion

Based on the results, coating treatments significantly affected postharvest physiological responses and all of the assessed quality parameters of pomegranate fruits during cold storage. The combination of chitosan and thymol was more effective than sole chitosan or thymol coatings in maintaining quality parameters and extending the shelf life of pomegranate fruits. Therefore, the combined application of chitosan and thymol can provide a useful alternative for shelf life extension of pomegranate fruits.

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Conflict of interest

The authors indicate no conflict of interest for this work.

References

1. Ali A, Muhammad M.T.M, 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 Chemistry* 124, 620–626.
2. Barman K, Asrey R, Pal R.K. 2011. Putrescine and carnauba wax pretreatments alleviate chilling injury, enhance shelf life and preserve pomegranate fruit quality during cold storage. *Scientia Horticulturae* 130, 795–800.
3. Chafer M, Sanchez-Gonzalez L, Gonzalez-Martínez C. 2012. Fungal decay and shelf life of oranges coated with chitosan and bergamot, thyme, and tea tree essential oils. *Journal of Food Science* 77, 182–187.
4. Dantas Guerra I.C, de Oliveira P.D.L, Fernandes Santos M.M, Carneiro Lúcio A.S.S,

- Tavares J.F, Barbosa-Filho J.M, Madruga M.S, de Souza E.L. 2016. The effects of composite coatings containing chitosan and *Mentha (piperita L. or x villosa Huds)* essential oil on postharvest mold occurrence and quality of table grape cv. Isabella. *Innovative Food Science and Emerging Technologies* 34, 112–121.
5. Delieghere F, Vermeulen A, Debevere J. 2004. Chitosan: antimicrobial activity, interactions with food components and applicability as a coating on fruit and vegetables. *Food Microbiology* 21, 703–714.
 6. Dong H., Cheng L., Tan J., Zheng K, Jiang Y. 2004. Effects of chitosan coating on quality and shelf life of peeled litchi fruit. *Journal of Food Engineering* 64, 355–358.
 7. Dotto G.L, Pinto L.A.A, Hachicha M.A, Knani S. 2015. New physicochemical interpretations for the adsorption of food dyes on chitosan films using statistical physics treatment. *Food Chemistry* 171, 1–7.
 8. El Ghaouth A., Ponnampalam R., Boulet M. 1991. Chitosan coating effect on storability and quality of fresh strawberries. *Journal of Food Science* 56, 1618–1621.
 9. Elyatem S.M, Kader A.A. 1984. Postharvest physiology and storage behavior of pomegranate fruits. *Scientia Horticulturae* 24, 287–298.
 10. Fawole O.A, Opara U.L. 2013. Effects of storage temperature and duration on physiological responses of pomegranate fruit. *Industrial Crops and Products* 47, 300–309.
 11. Ghasemnezhad, M., Zareh, S., Shiri, M.A. and Javdani, Z., 2015. The arils characterization of five different pomegranate (*Punica granatum*) genotypes stored after minimal processing technology. *Journal of food science and technology* 52(4), 2023–2032.
 12. Goncalves B, Silva A.P, Moutinho-Pereira J, Bacelar E, Rosa E, Meyer, A.S. 2007. Effect of ripeness and postharvest storage on the evolution of colour and anthocyanins in cherries (*Prunus avium L.*). *Food Chemistry* 103, 976–984.
 13. Jameel Jhalegar M.D, Sharma R.R, Singh D. 2015. *In vitro* and *In vivo* activity of essential oils against major postharvest pathogens of Kinnow (*Citrus nobilis* × *C. deliciosa*) mandarin. *Journal of Food Science and Technology* 52(4), 2229–2237.
 14. Jiang T, Feng L, Li J. 2012. Changes in microbial and postharvest quality of shiitake mushroom (*Lentinus edodes*) treated with chitosan-glucose complex coating under cold storage. *Food Chemistry* 131, 780–786.
 15. Jiang Y, Li J, Jiang W. 2005. Effects of chitosan coating on shelf life of cold-stored litchi fruit at ambient temperature. *LWT - Food Science and Technology* 38, 757–761.
 16. Jianglian D, Shaoying Z. 2013. Application of Chitosan Based Coating in Fruit and Vegetable Preservation: A Review. *Journal of Food Processing & Technology* 4(5).
 17. Jitareerat P, Paumchai S, Kanlayanarat S. 2007. Effect of chitosan on ripening enzymatic activity, and disease development in mango (*Mangifera indica L.*) fruit. *New Zealand Journal of Crop and Horticultural Science* 35, 211–218.
 18. Kahramanoğlu I, Usanmaz S. 2016. *Pomegranate Production and Marketing*. CRC Press. pp 148.
 19. Kim D, Jeong S.W, Lee C.Y. 2003. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. *Food Chemistry* 81, 321–326.
 20. Li T.T, Li J.R, Hu W.Z, Zhang X.G, Li X.P, Zhao J. 2013. Shelf-life extension of crucian carp (*Carassius auratus*) using natural preservatives during chilled storage. *Food Chemistry* 135, 140–145.
 21. Lin B.F, Du Y.M, Liang X.Q, Wang X.Y, Wang X.H, Yang, J.H. 2011. Effect of chitosan coating on respiratory behavior and quality of stored litchi under ambient temperature. *Journal of Food Engineering* 102, 94–99.
 22. Meighani H, Ghasemnezhad M, Bakhshi D. 2015. Effect of different coatings on post-harvest quality and bioactive compounds of pomegranate (*Punica granatum L.*) fruits. *Journal of Food Science and Technology* 52(7), 4507–4514.
 23. Moradinezhad F, Khayyat M. 2014. Effects of Intermittent Warming and Prestorage Treatments (Hot Water, Salicylic Acid, Calcium Chloride) on Postharvest Life of Pomegranate Fruit cv. ‘Shishe-Kab’ during Long-Term Cold Storage. *International Journal of Horticultural Science and Technology* 1(1), 43–51.
 24. Outtara B, Simard R.E, Piette G, Begin A, Holley R.A. 2000. Diffusion of acetic and propionic acids from chitosan-based antimicrobial packaging films. *Journal of Food Science* 65, 768–773.
 25. Park Y.S, Jung S.T, Kang S.G, Heo B.G, Arancibia-Avila P., Toledo F., Drzewiecki J.,

- Namiesnik J., Gorinstein, S. 2008. Antioxidants and proteins in ethylene-treated kiwifruits. *Food Chemistry* 107, 640–648.
26. Perdones A, Sanchez-Gonzalez L, Chiralt A, Vargas M. 2012. Effect of chitosan–lemon essential oil coatings on storage-keeping quality of strawberry. *Postharvest Biology and Technology* 70, 32–41.
27. Pezo D, Salafranca J, Nerin C. 2006. Design of a method for generation of gas-phase hydroxyl radicals, and use of HPLC with fluorescence detection to assess the antioxidant capacity of natural essential oils. *Analytical and Bioanalytical Chemistry* 385(7), 1241–1246.
28. Qiu X.J, Chen S.J, Liu G.M, Yang Q.M. 2014. Quality enhancement in the Japanese sea bass (*Lateolabrax japonicus*) fillets stored at 4 degrees C by chitosan coating incorporated with citric acid or licorice extract. *Food Chemistry* 162, 156–160.
29. Ranjbari F, Moradinezhad F, Khayyat M. 2016. Effect of nitric oxide on biochemical and antioxidant properties of pomegranate fruit cv. Shishe-kab during cold storage. *International Journal of Horticultural Science and Technology* 3(2), 211–219.
30. Ravanfar R, Niakousari M, Maftoonazad N. 2014. Postharvest sour cherry quality and safety maintenance by exposure to Hot- water or treatment with fresh *Aloe vera* gel. *Journal of Food Science and Technology* 51(10), 2872–2876.
31. Reyes-Avalos M.C, Aguilar-González C.N, Femenia A., Minjares-Fuentes R., Esparza-Rivera J.R, Contreras-Esquivel J.C, Meza-Velázquez J.A. 2016. Improvement of the quality and the shelf life of figs (*Ficus carica*) using an alginate–chitosan edible film. *Food and Bioprocess Technology* 9, 2114–2124.
32. Sabir A, Sabir F.K, Kara Z. 2011. Effects of modified atmosphere packing and honey dip treatments on quality maintenance of minimally processed grape cv. Razaki (*V. vinifera* L.) during cold storage. *Journal of Food Science and Technology* 48(3), 312–318.
33. Sánchez-González L, Chafer M, Chiralt A, González-Martínez C. 2010. Physical properties of edible chitosan films containing bergamot essential oil and their inhibitory action on *Penicillium italicum*. *Carbohydrate Polymers* 82, 277–283.
34. Sayyari M, Babalar M, Kalantari S, Martínez-Romero D, Guillén F, Serrano M., Valero D. 2011. Vapour treatments with methyl salicylate or methyl jasmonate alleviated chilling injury and enhanced antioxidant potential during postharvest storage of pomegranates. *Food Chemistry* 124, 964–970.
35. Singleton V.L, Rosi J.A. 1965. Colorimetric of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture* 16, 144–158.
36. Sun X, Narciso J, Wang Z, Ference C, Bai J, Zhou K. 2014. Effects of chitosan-essential oil coatings on safety and quality of fresh blueberries. *Journal of Food Science* 79, 955–960.
37. Taduri M, Reddy N.N, Lakshmi J, Joshi V. 2017. Effect of pre harvest treatments on shelf life and quality of mango CV. Amrapali. *The Pharma Innovation Journal* 6(7), 54–59.
38. ValizadehKaji B, Ershadi A, Tohidfar M. 2013. In vitro propagation of two Iranian commercial pomegranates (*Punica granatum* L.) cvs. ‘Malas Saveh’ and ‘Yusef Khani’. *Physiology and Molecular Biology of Plants* 19(4), 597–603.
39. Varasteh F, Arzani K, Barzegar B, Zamani Z. 2012. Changes in anthocyanins in arils of chitosan-coated pomegranate (*Punica granatum* L. cv. Rabbab-e-Neyriz) fruit during cold storage. *Food Chemistry* 130, 267–272. <https://doi.org/10.1016/j.foodchem.2011.07.031>.
40. Xiao C, Zhu L, Luo W, Song X, Deng Y. 2010. Combined action of pure oxygen pretreatment and chitosan coating incorporated with rosemary extracts on the quality of fresh pears. *Food Chemistry* 121, 1003–1009.
41. Xing Y, Li X, Xu Q, Yun J, Lu Y, Tang Y. 2011. Effects of chitosan enriched with cinnamon oil on qualitative properties of sweet pepper (*Capsicum annuum* L.). *Food Chemistry* 124, 1443–1450.
42. Zaouay F, Mena P, Garcia-Viguera C, Mars, M. 2012. Antioxidant activity and physico-chemical properties of Tunisian grown pomegranate (*Punica granatum* L.) cultivars. *Industrial Crops and Products* 40, 81–89.