Pomegranate (Punica granatum L.) Fruit Storability Improvement Using Pre-storage Chitosan Coating Technique

F. Varasteh¹, K. Arzani²*, M. Barzegar³, and Z. Zamani⁴

ABSTRACT

Application of edible coatings to enhance storage life of fresh fruit has recently been under considerable attention. This experiment was conducted in order to explore the effect of chitosan coating and storage temperature on the postharvest life of pomegranate (Punica granatum L.) fruit. Therefore, pomegranate fruits ‘Rabbab-e-Neyriz’ were dipped in 0, 1 and 2% aqueous chitosan solutions, air dried and then stored at 2 and 5°C with 90% relative humidity for up to 135 days. The application of chitosan coating retarded the respiration rate and weight loss of the fruit regardless of temperature during storage period; however, the retention was higher at 2 than 5°C. After 135 days of storage, the highest (18.19%) and lowest (9.33%) weight losses were recorded in the control fruit stored at 5°C and 2% chitosan-treated fruit stored at 2°C, respectively. The results revealed that postharvest weight losses in pomegranate fruit mainly related to weight losses in the fruit peel and 4-8% reduction in peel weight percentage (of whole fruit) was recorded in the treated fruit. A slight decline in Soluble Solids Content (SSC) and Titratable Acidity (TA) was found during storage in all treatments, while pH and maturity index increased slowly. Scanning electron microscopy of the treated fruit demonstrated that chitosan covered whole pericarp surface and pores of the coated fruit, and revealed more shrivelling symptoms on the peel of the non-coated fruit during storage.

Keywords: Maturity index, Postharvest storage, Scanning electron microscopy, Storage period, Weight loss.

INTRODUCTION

Pomegranate (Punica granatum L.) as a non-climacteric fruit should be harvested fully ripe to ensure the optimal qualitative attributes. In addition, the abundant content of water in the fruit and numerous natural pores on the peel is subject to the weight loss (Kader et al., 1984). One of the main ways to extend the commercial life of pomegranate is optimizing the environmental condition such as temperature, relative humidity and atmosphere composition, to reduce water loss as well as respiration rate and preserve commercial quality and minimize the physiological and fungal decay losses (Ben-

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Arie et al., 1984). It has been demonstrated that temperature is the most important factor to control the respiratory activity of pomegranate (Elyatem and Kader, 1984; Artes and Tomas-Barberan, 2000). Recommended temperatures to store pomegranate have varied from 0 to 10°C with a storage life ranging from 2 weeks to 7 months depending on the cultivar (Nanda et al., 2001; Gil et al., 1996; Kader et al., 1984; Elyatem and Kader, 1984).

The use of edible films and coating to extend shelf life and improve the quality of fresh, frozen, and fabricated foods has been investigated due to their biodegradable and ecofriendly nature (Shahidi et al., 1999). Application of semi-permeable coating has been shown to enhance the storability of perishable crops (Jiang and Li, 2001). Edible coatings have the potential to decrease moisture loss, restrict oxygen uptake, reduce respiration, decrease ethylene production, seal in flavor volatiles and carry additional functional ingredients (such as antioxidants and antimicrobial agents) that retard discoloration and microbial growth (Baldwin et al., 1995).

Chitosan, a high molecular-weight cationic polysaccharide produced by deacetylation of chitin, is applied widely in postharvest treatments because of its excellent film forming and bio chemical properties (Lin et al., 2008; Jianglian and Shaoying, 2013; Shiekh et al., 2013). Chitosan films are tough, long lasting, flexible and very difficult to tear (Shahidi et al., 1999). Chitosan coating, due to form an ideal semi-permeable film on fruit surface, modify the fruit internal atmosphere, regulate gas exchange, reduce transpiration losses, delay the ripening and maintain the quality of harvested fruit (Li and Yu, 2000; Jiang and Li, 2001; Bautista-Banos et al., 2006; Kaya et al., 2016).

'Rabbab-e-Neyriz' is one of the commercial and popular pomegranate cultivars grown in Iran and, due to its good taste, thick peel, beautiful red color of peel and aril, is suitable more for long time cold storage and export compared to other Iranian cultivars (Varasteh et al., 2009, 2012). However, it also is prone to lose a lot of moisture through the pores existing in the peel. The aim of the present research was to assess the potential of chitosan coating in extending postharvest life and maintaining quality of this commercial pomegranate cultivar ('Rabbab-e-Neyriz').

**MATERIALS AND METHODS**

**Plant Materials and Treatments**

Commercially mature pomegranate (*Punica granatum* L.) fruit ‘Rabbab-e-Neyriz’ were harvested in October, 2008, from a commercial orchard in Neyriz, Fars Province, Iran. On the same day, fruit were transported by a ventilated car to the laboratory of Department of Horticultural Science at Tarbiat Modares University (TMU), where pomegranates were selected for uniformity, shape, color and size (300-350 g), and any blemished or diseased fruit were discarded. For each treatment, three replicates were used. In addition, 45 fruits were used as the control for assessments in Day 0 at three replications. Note that 15 fruits were used in each replication, so the total of 810 fruit was used in 54 groups (treatments/replications). Medium molecular weight chitosan (viscosity: ~200 mPs, mol wt ~400,000, Fluka, Buchs, Switzerland) was purchased and aqueous solutions of 1 and 2% chitosan (W/V) in 1% acetic acid (V/V) were prepared according to the method of Jiang and Li (2001) with some modification. Pomegranate fruit were dipped in 1 or 2% aqueous chitosan solutions and the control fruit were dipped in distilled water with 1% acetic acid without chitosan. Fruit were allowed to dry for 12 hours at room temperature (20±1°C). Then, fruit were placed in the baskets (15 fruits per basket) and stored at 2±0.5 and 5±0.5°C at 90±5% relative humidity. The samples of each treatment were assessed at 45 days intervals (0, 45, 90 and 135) after three days.
in shelf life conditions (20°C and 40±5% RH).

Weight Loss

Weights of individual replicates were recorded following treatment (day 0) and at the different sampling dates. Weight losses were calculated as percentage loss of initial fruit weight.

Respiration Rate

To measure respiration rate, the method described by Mirdehghan et al. (2007) was used with some modification. For this purpose, 2 fruit of each replicate individually were placed in a 2-L container and sealed for 1 hour. One millilitre of the head-space gas sample was withdrawn with a gas sampling valve and CO₂ content quantified using an Agilent 6890 N gas chromatograph (USA) equipped with a Flame Ionization Detector (FID) and the Nickel catalyst reactor G2747A. The gas sample was separated on an Agilent HP Plot Q column (L: 60 m, OD: 0.53 mm, FT: 5 µm) and passed over the hot catalyst in the presence of hydrogen, which converted the CO₂ peak to CH₄. Injector and detector temperatures were 180 and 250°C, respectively. The oven temperature was increased to 180°C with a linear ramp of 7°C min⁻¹. Helium was used as carrier gas at a flow rate of 8 mL min⁻¹. Respiration rate was expressed as mg CO₂ kg⁻¹ h⁻¹. The fruit fresh weight was determined using an analytical balance (Sartorius BP 160P, Germany).

Peel, Aril and Juice Percentage

For quantitative and qualitative measurements, the peels were manually separated from the arils and their weight percentage were recorded. Then, arils juice was extracted by pressing the arils using a Garlic press instrument and the juice percentage was recorded.

Soluble Solids Content (SSC), Titratable Acidity (TA), pH, Maturity Index (MI)

The juices (50 mL) were centrifuged (15 minutes at 4,000 rpm at 4°C) and then the supernatant was divided into small vials for the following analyses (Alighourchi et al., 2008). Soluble Solids Content (SSC) of juice was measured by a temperature compensated refractometer (Atago N1, Tokyo, Japan) at 20°C. Titratable Acidity (TA) was expressed as % citric acid in juice. Citric acid was reported to be the predominant organic acid in pomegranates (Elyatem and Kader, 1984; Poyrazoglu et al., 2002). TA was determined potentiometrically using 0.1M NaOH to the end point of pH 8.1 and expressed as grams of citric acid per litre (Alighourchi et al., 2008). The pH measurements were performed using a digital pH meter (Metrohm model 827, Switzerland) at 20°C. Maturity Index (MI) was calculated as the SSC/TA ratio.

Scanning Electron Microscopy (SEM) Evaluation

For SEM evaluation, dried peel segments of each treatment were shifted onto transparent double-sided tape on the disc surface of polished aluminium stubs. The sample on each stub was sputter-coated with a gold layer of 200 °A thick (Arzani et al., 2005, Varasteh and Arzani, 2009). Fruit peels were observed in a Stereo scan 360 Scanning electron microscope (Philips XL30, Netherlands), operating at 20 KV, and photographed at 100X-5000X.

Statistical Analysis

The experiment was arranged based on completely randomized design in three
replicates with split plot arrangement. The obtained results of analytical determinations were subjected to Analysis of Variance (ANOVA). Mean comparisons were performed according to Fisher’s least significant difference test; differences were considered statistically significant at \( P \leq 0.05 \). The results were expressed as means±SE. The analyses were performed using SAS software.

RESULTS

Weight Loss and Respiration Rate

Weight loss of pomegranate fruit increased with advancing storage duration (Figure 1). Chitosan coating of pomegranate fruit considerably reduced the weight loss during storage at different temperatures. Weight loss on stored fruit at 2°C was lower than those kept at 5°C. In addition, increasing the concentration of chitosan from 1 to 2% resulted in significantly greater weight retention.

After 135 days of storage, among all the treatments, the weight loss of the control (non chitosan-coated pomegranate fruit) stored at 5°C and of the 2% chitosan-coated pomegranate fruit stored at 2°C, was the highest (18.19%) and the lowest (9.33%), respectively.

According to the obtained results after 90 days, pomegranate fruit had low respiration rate (Table 1). Furthermore, the treatments significantly affected respiration rate of samples. Therefore, pomegranate fruit coated with chitosan had less respiration rate and the fruit coated with 2% chitosan exhibited lower respiration rate. In addition, respiration rate was lower at 2 than 5°C of storage temperature.

Soluble Solids Content, Titratable Acidity, pH and Maturity Index

Chitosan coating and temperature during storage for up to 135 days had little effect on Soluble Solid Content (SSC) (Figure 2-A), Titratable Acidity (TA) (Figure 2-B), Maturity Index (MI) (Figure 2-C) and pH (Figure 2-D) of the pomegranate fruit. A slight and non significant decrease in SSC and TA was found after storage in all storage duration (Days)

![Figure 1](image.png)

Figure 1. Effect of chitosan (Chit.) coating and storage temperature (Temp.) (2 and 5°C) on the weight loss (%) of 'Rabbab-e-Neyriz' pomegranate fruit during 135 days storage. The results are expressed as means±SE. Each mean is an average of 45 fruits.
Table 1. Effect of chitosan coating and storage temperature (2 and 5˚C) on the respiration rate (mg CO$_2$ kg$^{-1}$ h$^{-1}$) of ‘Rabbab-e-Neyriz’ pomegranate fruit after 90 days.$^a$

<table>
<thead>
<tr>
<th>Storage duration (Days)</th>
<th>Storage temperature (˚C)</th>
<th>Treatment</th>
<th>Respiration rate (mg CO$_2$ kg$^{-1}$ h$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td>Control</td>
<td>7.80±0.12</td>
</tr>
<tr>
<td>90</td>
<td></td>
<td>Control</td>
<td>8.03±0.12</td>
</tr>
<tr>
<td>90</td>
<td>5</td>
<td>1% Chitosan</td>
<td>5.57±0.09</td>
</tr>
<tr>
<td>90</td>
<td>5</td>
<td>2% Chitosan</td>
<td>4.93±0.09</td>
</tr>
<tr>
<td>90</td>
<td>2</td>
<td>Control</td>
<td>6.17±0.17</td>
</tr>
<tr>
<td>90</td>
<td>2</td>
<td>1% Chitosan</td>
<td>4.50±0.23</td>
</tr>
<tr>
<td>90</td>
<td>2</td>
<td>2% Chitosan</td>
<td>3.53±0.12</td>
</tr>
</tbody>
</table>

$^a$ The results expressed as means±SE. Each mean is an average of 6 fruit. Means in the column with the same letter are not significantly (P≤0.05) different.

Figure 2. Effect of chitosan (Chit.) coating and storage temperature (Temp.) (2 and 5˚C) on soluble solid content (A), titratable acidity (B), maturity index (C) and pH (D) pomegranate fruit during 135 days storage with 3 days at 20˚C. The results are expressed as means±SE. Each mean is an average of 15 fruits.

...treatments. However, an increase was recorded in soluble solids content in the applied treatments during 45 days after storage. After 45 days until the end of storage period, the decrease in SSC and TA was lower in the coated than non-coated fruit. This might be due to higher rates of respiration in the non-coated fruit as compared to a reduced rate in the coated fruit. In addition, SSC and TA reductions were higher in pomegranates stored at 5 than 2˚C. The pH and Maturity Index (MI) were enhanced...
slowly during storage in all treatments, however, no significant differences were observed in the applied treatments (Figure 2.).

**Peel, Aril and Juice Percentage**

During storage, there was a reduction in the peel weight percentage, whereas the percentages of aril and juice (of whole fruit) increased with progress in the storage period [Figure 3 (A, B, and C)].

Coating of pomegranate and low temperature of storage reduced peel moisture loss. Therefore, the fruit coated with 2% chitosan concentration had higher peel percentage. Also, storage temperature had significant effect on peel moisture loss, therefore, a higher peel percentage was recorded on the fruit stored at 2 than 5°C.

**Scanning Electron Microscopy**

The SEM of the peel of the treated pomegranate fruit showed that chitosan films covered pericarp surface and natural porosity on the peel and, therefore, inhibited water loss [Figure 4 (A, B, and C)]. In addition, after 3 months of storage at 2°C with 90% relative humidity, some shriveling symptoms and micro cracks appeared on the peel of the non-coated fruit, while the peel of the coated fruit preserved their integrity, though a few cracks were observed.

**DISCUSSION**

In order to avoid the influence of fruit size on fruit quality attributes (Jafari et al., 2014), in the present research, pomegranate fruit were selected for uniformity, shape, color, and size (300-350 g). In general, the reduction in fruit weight, total soluble solids, and titratable acidity demonstrated senescence and quality deterioration of the fruit. In the last few years, researchers have used chitosan coating to prolong storage life of numerous horticultural commodities such as cucumber and bell pepper (El Ghaouth et al., 1991a), strawberry (El Ghaouth et al., 1991b),...
Figure 4. Scanning Electron Micrographs (SEMs) of the pericarp surface of the peel in 'Rabbab-e-Neyriz' pomegranate stored at 2˚C after 90 days, photographed at 250X. (A, B, C): the pericarp surface of 0, 1 and 2% chitosan coated fruit, respectively. The arrows show the pores and microcracks in the control.

1991b), tomato (El Ghaouth et al., 1992), peach, Japanese pear and kiwifruit (Du et al., 1997), apple (Du et al., 1998), longan (Jiang and Li, 2001), mango (Abbasi et al., 2009), citrus (Chien et al., 2007) and red kiwifruit (Kaya et al., 2016). Application of chitosan as an edible film causes the formation of a barrier on fruit surface, and decreases the respiration, transpiration, and water loss, resulting in controlled enzymatic browning in the stored fruit (Shahidi et al., 1999; Jianglian and Shaoying, 2013). Weight loss is one of the most important factors to limit pomegranate storability (Kader et al., 1984; Ben-Arie et al., 1984). Although pomegranate peel seems to be thick, it has numerous minute openings that enable free movement of water vapor and make the fruit highly prone to water loss (Kader et al., 1984). ‘Rabbab-e-Neyriz’ is a late maturing pomegranate cultivar whose fruit consist of about 48 and 52% of peel and aril, respectively. The aril juice is approximately 40.5% of a mature pomegranate fruit (Varasteh et al., 2009). The results of the present study were similar to our previous findings. According to the present research results, weight loss in pomegranate fruit increased during storage period, and the application of chitosan coating delayed the change in eating quality and retarded weight loss of pomegranate fruit regardless of storage temperature. The weight loss was reduced when the coated fruit were stored at 2˚C. In addition, 2% chitosan concentration showed better results in term of the prevention of fruit weight loss in the storage. Our results were consistent with those reported in other fruit. Weight loss increased gradually throughout storage in the control, although the chitosan coated reduced weight loss of various fruit such as Longan (Jiang and Li, 2001), pear (Lin et al., 2008), apricot (Ghasemnezhad et al., 2010), plum (Bal, 2013), sapota (Ahlawat et al., 2015) and red kiwi fruit (Kaya et al., 2016). The chitosan film formed on the surface of the fruit can delay the migration of moisture from the fruit into the environment, thus reducing weight loss during storage (Ghasemnezhad et al., 2013).
The obtained results showed that pomegranate fruit had a relatively low respiration rate and the result agreed with previous reports about respiration rate of pomegranate fruit (Elyatem and Kader, 1984; Ben-Arie et al., 1984; Gross et al., 2016). It has been indicated that respiration rate of pomegranate fruit declined with storage time extension and decrease in storage temperature (Elyatem and Kader, 1984; Gross et al., 2016) have reported respiration rate of 4 to 8 mg CO$_2$ kg$^{-1}$ (fruit weight) h$^{-1}$ at 5˚C, for pomegranate fruit, which is in agreement with the results of the present research. Reduction of respiration rate as a result of chitosan coating with a greater effect at higher concentration has also been reported for strawberry (El Ghaouth, 1991b), peach, pear and kiwifruit (Du et al., 1997), apple (Du et al., 1998) and longan (Jiang and Li, 2001). Thus, coating with chitosan has the ability to modify internal atmosphere of the fruit and slow down the respiration rate of fresh fruit. In another study, less respiration rate was recorded in shrink wrapped pomegranate fruit stored at different temperature condition in contrast with non shrink wrapped fruit (Nanda et al., 2001).

Our findings showed that soluble solids content and titratable acidity decreased slightly during storage period. The reduction of SSC during storage at 1˚C in non-treated Asian pear fruit has also been reported (Arzani et al., 2011). In the present research, it was found that both concentrations of chitosan retarded the reduction of SSC and TA in fruit as compared to the control, with a slightly greater effect at higher concentration. Furthermore, higher SSC and TA were recorded at lower storage temperature condition. It is supposed that chitosan coating on the pomegranate fruit, by modifying internal atmosphere, caused decrease in usage of sugars and organic acids due to reduction of respiration and metabolism. In addition, low storage temperature could reduce respiration and metabolism. However, because of low respiration rate of pomegranate fruit, difference among treatments was not significant.

Reports from numerous studies also revealed the less SSC and TA reductions of chitosan treated fruit compared with values of the control fruit after storage. This is generally attributed to the modification of the endogenous levels of O$_2$ and CO$_2$ and the inhibition of respiratory activities and the reduction of ethylene biosynthesis (El Ghaouth, 1991b; Du et al., 1997, Li and Yu, 2000; Jiang and Li, 2001; Jiang et al., 2005; Lin et al., 2008, Shiekh et al., 2013). Hence, chitosan coating of fruit had a beneficial effect on the delay of the senescence.

The influence of storage atmosphere, i.e. O$_2$ and CO$_2$ concentrations, on the eating quality of pomegranate fruit during storage period is previously demonstrated by other studies (Artes et al., 1996, 2000; Eris and Turk, 2000; Hess-Pierce and Kader, 2003). It has been determined that SSC and TA declined throughout the storage period in ‘Wonderful’ pomegranate stored at controlled atmosphere condition, whereas pH slightly increased (Hess-Pierce and Kader, 2003). In addition, with modified atmosphere packaging, the highest weight loss was observed in the control fruit, SSC and TA showed a reduction, and an increase in pH was recorded (Artes et al., 2000; Eris and Turk, 2000). However, quality parameters of ‘Mollar’ pomegranate such as SSC, pH, and TA did not change during cold storage at 5˚C and 90-95% RH (Gil et al., 1996). Chitosan coating resulted in the retainment of a higher content of titratable acid, pH, as well as firmness in plum, however, total soluble solids and ascorbic acid contents were not significantly affected by the coating (Bal, 2013).

Maturity Index (MI) is considered as a good indicator for maturity and important quality parameter for pomegranate cultivars (Artes et al., 1996). In our study, the changes of MI were reduced by chitosan coating. Furthermore, a lower level of MI was recorded with reducing storage temperature. In general, since pomegranate is a non-climacteric fruit, it is presumed that
these quality parameters are slightly changed during cold storage. We also concluded that, due to decrease in weight loss and preservation of fruit eating quality, 2°C is an appropriate temperature to store ‘Rabbab-e-Neyriz’ cultivar for a long time storage period. Eris and Turk (2000) determined that 2-3°C is the suitable storage temperature for ‘Hicaz’ pomegranate cultivar, which is in agreement with our finding about suitable storage temperature for ‘Rabbab-e-Neyriz’ pomegranate. In another study a gradual decrease in TSS/TA during storage of uncoated pomegranate arils has been reported, but arils coated with chitosan showed a significantly increased MI during 12 days of storage (Ghasemnezhad et al., 2013).

We have found that the postharvest weight loss in pomegranate fruit is mainly related to weight loss in the peel. The peel weight percentage of the whole fruit decreased at 2°C storage temperature and 1% chitosan concentration treatment. Therefore, reduction of the peel weight percentage of 4-8% was recorded depending on the treatment. Elyatem and Kader (1984) have also indicated that due to natural porousness of the peel of ‘Wonderful’ pomegranate, water loss mostly happens from the peel. Nanda et al. (2001) reported similar results when pomegranate fruit had been stored for 3 months.

The results obtained from the photographs taken by the SEM revealed that the shriveling symptoms of the peel and micro cracks on the chitosan coated fruit were less than the non-coated fruit. It seems that the concentration of the applied chitosan for coating affects the amount of the moisture transmission and respiration rate. Therefore, in the present research, due to reduction of weight loss and respiration, fruit coated with 2% chitosan had less shrivel symptoms and better visual quality after 90 days storage compared to fruit coated with 1% chitosan. Our findings agreed with the report on 1 and 1.2% chitosan coated apples which showed much less deep cracks than uncoated fruit (Du et al., 1998). The thickness of chitosan layer on the surface of the coated fruit depends on the molecular weight and concentration of the utilized chitosan for coating (Du et al., 1998). Low molecular weight chitosan coating improved firmness, titratable acidity, ascorbic acid and the water content for citrus stored at 15°C for 56 days, therefore, the fruit quality was maintained longer compared to the fruit coated with high molecular weight chitosan (Chien et al., 2007).

In conclusion, it was determined that chitosan has potential to be used as a coating technique for better pomegranate fruit storage. Chitosan as a preservative coating material for fruit may be used as a surface coating on pomegranate fruit to modify the internal fruit atmosphere, reduce the supply of oxygen to decrease the respiration rate, and might be an alternative to modified atmosphere packaging. Furthermore, postharvest storage temperature is a key factor to prolong shelf life of fresh pomegranate fruit. The coatings allow preserving pomegranate fruit at 2°C which can reduce weight losses, maintain their quality, and extend storage life of the fruit. The results of this study revealed that pomegranate would be stored for more than 3 months using chitosan coatings. Further research using pomegranate fruit of different cultivars with different fruit size will warrant using chitosan in the storage as a coating agent at the commercial scale.

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Pomegranate Storability Improvement Using Chitosan


بهنود قابلیت تغییر نمای میوه انار 

(Punica granatum L.) با روش پوشش کیتوزان

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چکیده

این مطالعه تأثیر پوشش کیتوزان (Punica granatum L.) به میوه انار، میوه در دمای 15 و 20 درجه سانتی‌گراد و فرآیند کشت به ترتیب بر روی نرخ تغییر نمای میوه، نرخ گشادگی پوست میوه، نرخ شیب گل، نرخ رشد ریشه، نرخ رشد بدن، نرخ تغییر نمای سیالی و نرخ تغییر نمای پوشش را بررسی کرد. نتایج نشان داد که در دمای 20 درجه سانتی‌گراد، پوشش کیتوزان به کاهش نرخ تغییر نمای میوه و کاهش نرخ گشادگی پوست میوه و تغییر نمای سیالی برای انار میوه موجب شد. این نتایج نشان داد که پوشش کیتوزان به کاهش نرخ تغییر نمای میوه انار و کاهش نرخ گشادگی پوست میوه و تغییر نمای سیالی برای انار میوه موجب شد.