Extending Shelf Life of Mandarin Fruit using Pomegranate Peel Extract

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ABSTRACT
Currently, the use of chemicals is reduced due to environmental problems and their negative impacts on human health. Besides, their use has been prohibited in some cases. Postharvest decay caused by fungal pathogens is one of the most important challenges facing the fresh citrus industry. So, the use of pomegranate peel extract (PPE) at the concentrations of 0, 33, 50, and 100% were used to reduce decay and maintain the postharvest qualitative characteristics of mandarin fruit. After treatment with PPE, the fruits were stored for 20 days. Traits such as weight loss, decay percentage, total soluble solids, total acidity, fruit firmness, color index, and sensory evaluation were investigated every five days. The lowest weight-loss percentage (14.71%) was obtained for the fruits treated with 100% PPE as compared with the untreated fruits (42.28%). The fruits treated with PPE maintained firmness compared with the untreated fruits. The fruits treated with PPE showed the lowest acidity reduction at the end of the storage period. At the end of the storage period, the fruits treated with 100% PPE and the untreated fruits showed the lowest and highest fruit decay (16.3% and 39.6%), respectively. The results showed that the application of PPE increased the overall acceptance of panelists from the treated samples. By increasing the concentrations of PPE to 100%, all sensory parameters were improved and could gain a better acceptance by panelists. The highest level of utility and final acceptance in pomegranate peel-treated fruits were obtained at 100%. The untreated fruits showed a lower overall acceptance rate. The findings showed that the postharvest application of PPE improved the qualitative characteristics and sensory properties, so it is recommended to use 100% PPE to reduce the decay and extend the postharvest storage of mandarin.

Introduction*
Citrus fruits are physiologically non-climacteric and have a very low rate of postharvest respiration and ethylene production (Kharchoufi et al., 2018). Due to its watery skin, they are prone to be damaged by physical, physiological, and pathological factors. Physical injuries including wounding and abrasion during harvesting, handling,
packing, and storing, lead to the penetration of microbial agents into the tissue and microbial contamination of the fruit during storage. The green mold infection caused by the fungal pathogen *Penicillium digitatum* is the main postharvest disease in citrus fruits (Palou et al., 2015).

The use of waxes to coat citrus fruits has a long history, but most of the used waxes are based on the chemical synthetics, such as oxidized polyethylene, ammonia, or morpholine, and they sometimes contain fungicidal compounds that are dangerous to humans (Khorram et al., 2017). Therefore, ecofriendly alternative strategies have been developed to preserve fruits and are considered as human safety requirements, followed by the recent increasing consumer tendencies toward the consumption of more natural and healthier food products (Sandhya et al., 2018). Pomegranate peel extract (PPE) clearly has more capacity to inhibit hydroxyl and peroxyl radicals. A considerable amounts of bioactive compounds are also present in its non-edible portions such as peel (Ismail et al., 2012). The ellagitannin and phenolic acids in the pomegranate peel have antibacterial and antifungal activity (Sandhya et al., 2018). Numerous research studies have been investigated the screening of plant extracts for new antifungal compounds, which can be applied to control postharvest diseases (Gatto et al., 2011). The edible coating with PPE has been applied to inhibit the contamination and to keep safe some fruits such as guava (Nair et al., 2018) and citrus (Kharchoufi et al., 2018; Taherpour et al., 2020). The antifungal effect of PPE on the control of *Penicillium digitatum* was investigated by Tayel et al. (2009). The results showed that the use of PPE controlled the green rot caused by *Penicillium digitatum* in citrus. In another study on concentrated PPE, high concentrations of phenols have been reported and its use has been suggested against fungal plant pathogens (Romeo et al., 2015).

The present research aimed to develop safer and eco-friendly alternative strategies for replacing synthetic additives with natural substances in postharvest fruits. Therefore, the research was conducted to develop PPE formulations on the quality and shelf life of mandarin fruits during cold storage.

**Material and Methods**

*Fruit sample preparation*

The mandarin fruits (*Citrus reticulate* var. Kharo) used in present study were purchased from a commercial garden located at Choram, Kohgiluyeh, and Boyer-Ahmad province, Iran, and were immediately transported to the postharvest laboratory of Islamic Azad University, Yasooj Branch. In the laboratory, the fruits were categorized in terms of size and uniformity. Then, the damaged and non-uniform fruits were removed and the healthy fruits with the same size and color were retained for the treatments. After washing with tap water, the fruits were rinsed and superficially dried. The mandarin fruits were, then, immersed in the PPE formulations in three replicates. The formulations included control (C), 33% pomegranate peel extract (33% PPE), 50% pomegranate peel extract (50% PPE), and 100% pomegranate peel extract (100% PPE).

*Preparation of pomegranate peel extract (PPE)*

The pomegranate fruits (*Punica granatum* L.) were purchased from a local market and were washed with distilled water, peeled, and their edible sections were carefully separated. Then, they were air-dried in an oven at 40 °C and 32 ± 4% RH for 48 h until they reached a constant weight (the moisture content of the dried peel was 13.3%). The dried peels were stored at -70 °C until usage. Before treatments, they were milled into a fine powder by an electric blender (Philips, HR 2815, Japan) and passed through a sieve with an aperture size of
470 µm. The dried powder (100 g) was suspended in 800 mL of double distilled water at 40 °C and shaken at room temperature for 24 h as described by Taherpour et al. (2020). The PPE extract was filtered through a Whatman No. 2 filter paper for the removal of peel particles.

**Fruit decay**
Shafiee et al. (2010) method was used to determine the fruit decay. The observations were categorized on a scale of 0–4, where 0 = normal (no decay), 1 = trace (up to 5% affected surface), 2 = slight (5–20% affected surface), 3 = moderate (20–50% affected surface), and 4 = severe (>50% affected surface) decay.

**Fruit firmness**
The fruit firmness was determined by measuring the maximum force (N) with a texture analyzer (CT-3, Brookfield, USA). The length of compression and the speed of the probe were 3 mm and 1 mm s⁻¹, respectively. The amount of force (N) was measured at two points on the surface (Sogvar et al., 2016).

**Weight loss (WL)**
The fruits of each treatment were weighed at the beginning of the experiment and also at the certain intervals during the storage, and the percentage of weight loss was calculated based on Eq. 1 (Hosseinifaraih et al., 2020) in which W₁ is the weight at day 0 and W₂ is the weight at sampling times.

\[
\text{Weight loss} (%) = \frac{W_1 - W_2}{W_1} \times 100 \quad (1)
\]

**Titratable acidity (TA) and total soluble solids (TSS)**
A mortar and pestle was used to juice the fruits in order to measure the TA, TSS and pH content. A hand-held refractometer (ATAGO, Japan) was used to determine the TSS (Hadadinejad et al, 2018). TA was determined by titration using 0.1 M NaOH as the citric acid percent. The TA results were expressed as grams of citric acid per 100 ml of mandarin juice. A pH-meter (Metrohm Switzerland) was employed to determine pH (Radi et al., 2017)

**Color measurement**
The fruit peel color parameters $L^*$, $a^*$ and $b^*$ were measured using a Konika-Minolta Chroma-meter (CR400, Japan) to assess the external color: $L^*$ for the lightness from black (0) to white (100), $a^*$ from green (−) to red (+), and $b^*$ from blue (−) to yellow (+). The color analysis was conducted at four points of the mandarin surface, and the mean values were recorded (Hosseinifaraih et al., 2020).

**Sensory evaluation**
The sensory evaluation of the fruits was performed using a test panel composed of 10 trained panelists. The panelists evaluated the quality parameters, such as taste, color, aroma, and overall acceptance (Martínez-Romero et al., 2008) based on a 5-point scale (5 to 1) in which 5 = extremely liked (very characteristic of the fruit), 4 = moderately liked, 3 = neither liked nor disliked, 2 = moderately disliked, and 1 = extremely disliked.

**Statistical analysis**
The experiment was conducted based on a complete randomized design (CRD). Then, two-way variance analysis was applied in SAS (ver. 9.2) to examine the quality parameters. The difference in the means was determined using the LSD test at P = 0.01.

**Results**

**Decay and Firmness**
Decay (%) was significantly (P < 0.005) increased during storage in all treatments and decay in control fruits was more than the treated fruits. At the end of the experiment, the fruits treated with 100% PPE showed the lowest percentage of fruit decay by 16.3% and the untreated fruits showed the highest
increase in fruit rot (39.6% compared to the beginning of the experiment) (Fig 1A). Overall, fruit firmness of all treatments was significantly reduced during the storage time (p < 0.005) (Fig 1B). Figure 1B displays the firmness of the treated samples as compared with the untreated ones. The statistical analysis revealed a significant difference between PPE applications in maintaining firmness during storage. At the end of the storage period, the fruits treated with 100% PPE had the lowest firmness (25.5% compared to the beginning of experiment) and the untreated fruits showed the highest reduction in firmness (52.7%).

**Fig. 1.** Changes in fruit decay (A) and fruit firmness (B) of mandarin fruits treated with pomegranate peel extract (PPE) during storage.

**Fruit quality (WL, TSS, TA, and pH)**

The weight loss is shown in Figure 2A. At the beginning of the experiment, all samples had a similar weight loss percentage and did not have a significant difference (P < 0.05). Weight loss was decreased in the PPE-treated fruits in comparison with the control during storage. After 5 days of storage, 100% PPE treatment maintained fruit weight better than the other treatments. The fruits treated with 100% PPE exhibited the lowest weight loss percentage of 14.71% and the untreated fruits exhibited the highest weight loss percentage of 42.28% on day 20. The pattern of changes in total soluble solids, acidity, and pH for all treatments are shown in Figure 2B,C,D. Titratable acidity did not show an obvious difference within the first 5 days of storage in both treated and untreated fruits, but it declined in all of them as approaching the end of the storage period (Fig. 2B). The fruits treated with PPE resulted in greater effectiveness in maintaining TA during storage. PPE treatment resulted in more TA compared with the control fruit. The highest acidity was observed in the fruits treated with 100% PPE and the lowest in the untreated fruits on day 20.
In the early stages of storage, no significant difference was observed in the TSS content, but it then started to increase although the TSS content of the fruits treated with PPE was decreased more quickly than the control (Fig. 2C). The application of PPE reduced the increasing trend of soluble solids. On day 20, the highest percentage of soluble solids (15.33%) was observed in the untreated fruits and the lowest (13.27%) was detected in the fruits treated with 100% PPE. pH was significantly increased during storage in all treated and control fruits (Fig. 2D). With increasing fruit storage time, the pH of the fruit juice was increased, but this amount was lower in the fruits treated with PPE. The application of PPE reduced the pH of the fruit juice compared to the untreated fruits. After 20 days of storage, the highest pH was recorded in the untreated fruits and the lowest in the fruits treated with PPE.

**Color**
The color indices of mandarin fruits, which are important quality parameters, are shown in Figure 3. The results for the $L^*$ index are shown in Table 1. The color index $L^*$, which indicates the intensity of light, increased during storage. This increase was greater in the fruits treated with PPE than the untreated fruits. At the end of the storage period, the highest light intensity was observed in the fruits treated with 100% PPE and the lowest in the untreated fruits. Color index $a^*$, which indicates green to red color, showed a trend similar to index $L^*$ so that the highest amount...
of $a^*$ was observed in the fruits treated with PPE and the lowest in the untreated fruits (Table 1). Color index $b^*$ was also increased during the storage period so that the highest amount of $b^*$ was observed in the untreated fruits and the lowest in the fruits treated with PPE (Table 1).

![Fig. 3. The effect of pomegranate peel extract (PPE) on organoleptic properties of the mandarin fruits at the end of the storage period](image)

**Sensory evaluation**

Figure 3 depicts the effects of PPE treatments on the organoleptic sensory and acceptance parameters of mandarin fruits. PPE maintained the fruit color, taste, and aroma at an acceptable level until day 20 of storage. The untreated fruits had low sensory values for organoleptic sensory. By increasing the concentration of PPE, all sensory indicators were improved and gained better acceptance by the panelists. The highest desirability and final acceptance were obtained in the fruits treated with 100% PPE. In addition, PPE did not have undesirable effect on the mandarin fruit.

**Table 1.** Effect of different concentrations of pomegranate peel extract (PPE) on the skin color indices ($L^*$, $a^*$ and $b^*$) of mandarin during the ordinary storage period

<table>
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<tr>
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<tr>
<td>$a^*$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.08± 0.0a</td>
<td>3.41± 0.091c</td>
<td>6.30± 0.0c</td>
<td>9.50± 2.31c</td>
<td>12.60± 0.02d</td>
</tr>
<tr>
<td>PPE 33%</td>
<td>0.08± 0.0a</td>
<td>4.52± 0.01b</td>
<td>8.11± 0.0b</td>
<td>11.02± 0.1b</td>
<td>14.20± 0.67c</td>
</tr>
<tr>
<td>PPE 50%</td>
<td>0.08± 0.0a</td>
<td>6.8± 0.14a</td>
<td>9.58± 0.08a</td>
<td>13.58± 0.0a</td>
<td>16.92± 0.0b</td>
</tr>
<tr>
<td>PPE 100%</td>
<td>0.08± 0.0a</td>
<td>6.92± 0.37a</td>
<td>9.50± 0.12a</td>
<td>13.92± 0.08a</td>
<td>17.23± 0.14a</td>
</tr>
<tr>
<td>$b^*$</td>
<td></td>
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<tr>
<td>Control</td>
<td>13.11± 0.0a</td>
<td>20.01± 0.6a</td>
<td>29.11± 0.02a</td>
<td>37.10± 0.0a</td>
<td>46.18± 0.0a</td>
</tr>
<tr>
<td>PPE 33%</td>
<td>13.11± 0.0a</td>
<td>15.24± 0.0b</td>
<td>22.5± 0.37b</td>
<td>32.41± 0.11b</td>
<td>38.01± 0.0b</td>
</tr>
<tr>
<td>PPE 50%</td>
<td>13.11± 0.0a</td>
<td>13.99± 0.18c</td>
<td>18.37± 0.51c</td>
<td>22.08± 0.19c</td>
<td>32.62± 0.1c</td>
</tr>
<tr>
<td>PPE 100%</td>
<td>13.11± 0.0a</td>
<td>13.51± 0.01c</td>
<td>16.28± 0.29d</td>
<td>20.31± 0.3d</td>
<td>27.11± 0.90d</td>
</tr>
<tr>
<td>$L^*$</td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td>34.12± 0.0a</td>
<td>36.18± 0.28b</td>
<td>37.11± 0.01c</td>
<td>37.14± 0.02c</td>
<td>40.02± 0.0d</td>
</tr>
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Discussion
The treated samples showed a significant low amount of decay compared to the control sample. These findings are in agreement with the findings obtained in previous studies (Nicosia et al., 2016; Osorio et al., 2010; Taherpour et al., 2020). Salahvarzi et al. (2011) and Tehranifar et al. (2011) demonstrated the antimicrobial effect of PPE with regard to phenolic compounds in pomegranates. Fruits lose their weight due to dehydration or biological changes. The low weight loss of the treated fruits could be due to the effect of PPE as an efficient form of water-vapor barrier and O₂ uptake restrictor. It is suggested that the water vapor permeability of fruits and vegetables decreases due to the hydrophobic property of the PPE (Nair et al., 2018). Our results for weight loss are in agreement with studies on citrus (Taherpour et al., 2020) and guava (Nair et al., 2018). The results of the treatment of grapes with grapefruit seed extract also showed that the treated fruits experienced lower weight loss than the control. Essential oils act as a barrier between the fruit and the environment, thus reducing external exchanges and ultimately preventing the loss of fruit juices (Xu et al., 2009).

The untreated fruits showed the highest weight loss during storage, so their soluble solids increased at the end of the experiment. But, the fruits treated with PPE showed the lowest amount of soluble solids because the coating prevented weight loss. During ripening, an increase in soluble solids increases with decreasing acidity due to acid metabolism and the breakdown of starch into simple sugars during storage. Similar results have been reported as to the reduction of the increasing trend of soluble solids in guava fruits coated with chitosan enriched with pomegranate peel extract (Nair et al., 2018). One of the reasons for the increase in soluble solids during storage is the weight loss and water loss from the fresh produce (van Meeteren and Aliniaiefard, 2016) and the concentration of fruit juice during storage, which have led to an increase in the concentration of soluble solids (Sayari et al., 2009; Vieira et al., 2016). Also, the increase in soluble solids can be due to the further hydrolysis of fruit starch to hexose sugars (Larrigaudiere et al., 2002).

Also, the acidity decreased with increasing storage time, but this decreasing trend in fruits treated with PPE was less than the untreated fruits. In fact, the use of PPE slowed down the process of reducing acidity. The TA usually decreases during storage due to the consumption of organic acids in the respiration process (Gol et al., 2015). Acidity increased at a slower pace in the untreated fruits during storage which could be related to the increase in respiration in the untreated fruits due to high caries and increased ethylene production, both of which caused the consumption of fruit organic acids and reduced acidity. The decomposition of organic acids during fruit ripening depends on the rate of respiration because these acids are used in the enzymatic activity of respiration (Nafussi et al., 2001).

Skin color is a more important attribute in determining fruit quality (Nair et al., 2018). According to our results, PPE formulations showed significant differences in lightness (L*) among them, but 100% PPE showed significantly higher lightness during storage.

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<tbody>
<tr>
<td>PPE 33%</td>
<td>34.12±0.0a</td>
<td>36.92±0.14b</td>
<td>38.02±0.06b</td>
<td>8.22±0.12b</td>
<td>41.39±0.0c</td>
</tr>
<tr>
<td>PPE 50%</td>
<td>34.12±0.0a</td>
<td>37.27±0.33a</td>
<td>39.0±0.0a</td>
<td>39.08±0.14a</td>
<td>43.04±0.61b</td>
</tr>
<tr>
<td>PPE 100%</td>
<td>34.12±0.0a</td>
<td>37.62±0.01a</td>
<td>39.18±0.0a</td>
<td>39.31±0.67a</td>
<td>44.09±0.37a</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation Different letters within the same column in each treatment indicate a significant difference (p <0.05).
compared to the untreated fruits. A similar result was reported by Taherpour et al. (2020) in sweet lemon fruits coated with PPE with sodium alginate. The use of PPE increased the color index L*, which indicates the intensity of light, and the color index a*, that indicates the intensity of green to red. However, the use of PPE prevented the increase in color index b*, which indicates blue-yellow (Yagoobi-Soureh et al., 2013). In fact, PPE delayed fruit ripening and reduced chlorophyll degradation to yellow, thereby increasing shelf life. Similar results have been reported in studies on guava (Nair et al., 2018) and lemons (Hosseini Farahi and Haghanifard. 2017).

**Conclusion:**
In conclusion, the findings of present study confirm that the application of pomegranate peel extract (PPE) inhibited fruit decay and maintained the mandarin quality in storage. These treatments could retard weight loss, maintain fruit firmness, delay changes in titratable acidity (TA), total soluble solids (TSS), and improved sensory quality. In general, the postharvest use of PPE for quality improvements of mandarin during storage is recommended. Therefore, postharvest use of treatments with PPE could be a promising strategy to maintain the quality of mandarin fruit during ordinary storage and can provide a particular perspective for further biological analysis.

**Conflicts of Interest**
The authors declare that they have no conflicts of interest regarding the publication of this paper.

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van Meeteren U, Aliniaelfard S. 2016. Stomata and

