Effect of chitosan and thymol essential oil on quality maintenance and shelf life extension of peach fruits cv. ‘Zaferani’

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Purpose: Peach is a climacteric fruit which have rapid ripening. An alternative to maintain quality and extend the shelf life of this fruit could be the use of edible coatings. Research Method: In the present study, the effect of three different coatings; 0.5% chitosan, 200 mg L⁻¹ thymol essential oil and their combined use on postharvest quality of peach fruits cv. ‘Zaferani’ were investigated. Dipping fruits in distilled water was used as a control. Changes in weight loss, fruit firmness, total soluble solids (TSS), sensory attributes, decay incidence, anthocyanin and carotenoid content of fruits were evaluated after 10, 15, 20, 25 and 30 days of storage at 6 °C. Findings: The results showed that the combination treatment of chitosan and thymol presented more effective preservative effect than chitosan or thymol coatings alone. The coated fruits with 0.5% chitosan + 200 mg L⁻¹ thymol showed significantly lower weight loss, fungal decay and TSS than control treatment. Furthermore, the coated fruits with 0.5% chitosan + 200 mg L⁻¹ thymol exhibited significantly higher firmness, anthocyanin and carotenoid content and sensory characteristics than untreated control. In addition, the highest shelf life (28.33 days) was recorded in combination treatment of chitosan and thymol. Research limitations: No limitations were founded. Originality/Value: The coating composed of chitosan and thymol essential oil can provide an efficient alternative for quality maintenance and shelf life extension of peach fruits.
INTRODUCTION

Peach (*Prunus persica* L.) is a climacteric fruit which have rapid ripening. This ripening is responsible for its short shelf life and produce serious problems for its handling and transportation. After harvesting quick softening and regular microbial infections lead to losses in the market chain. So, the fruits are harvested at a pre climacteric stage in order to have better handling process (Tareen et al., 2012). Chilling injuries like, external and internal browning, flesh degradation, discoloration, loss of ability to ripen and increased decay incidence due to low temperature during storage of peach fruit (Lurie, 1993). Peach fruit quality is highly affected by several pre and post-harvest factors. After harvesting peach fruit passes through different critical situations and being highly perishable and deterioration rate is high. The magnitude of post-harvest losses of fresh fruits and vegetables are higher as compared to pre-harvest losses (Serrano et al., 2004). The post-harvest quality of any produce cannot be improved; however quality losses can be minimized (Rahman et al., 2016). Thus, studies on quality conservation and shelf life enhancement of peach fruits are a high importance.

Use of the edible coatings is a potential postharvest treatment for quality conservation and shelf life enhancement of fruits. An accepted and harmless cationic polysaccharide is chitosan that has extensive antimicrobial property, anti-oxidation and biodegradation (Delieghere et al., 2004). Chitosan has played a significant role in quality maintenance of fruits, vegetables, and some aquatic products (Dotto et al., 2015). Use of chitosan was effective in extending the fruit shelf life in strawberries, sweet cherries and papaya (Bill et al., 2014). Essential oils are volatile oily liquids achieved from different parts of plants and usually used as food flavors. Thymol, one of the most significant essential oils, is significantly active against a broad range of microorganisms. In spite of having been long known for their antibacterial, antifungal, antiviral, insecticidal and antioxidant features (Pezo et al., 2006), the recent attention in other natural materials has led to a new scientific awareness of these materials. Alternatively, it has been shown by previous studies that the effect of only chitosan coating treatment on food quality improvement is inferior to that of composite treatments (Saki et al., 2019). To overcome this limitation, several additives have been introduced as the composite material of chitosan, such as glucose (Jiang et al., 2012), tea polyphenols (Li et al., 2013), and citric acid (Qiu et al., 2014). Saki et al. (2019) showed that the coating compound of chitosan and thymol can be an effective alternative for quality conservation and shelf life enhancement of fresh fig fruits. Nevertheless, to the extent that we know, no study has done regarding the effect of thymol essential oil and the combination of chitosan and thymol essential oil on the physiological and quality traits of peach fruits. Therefore, the aim of present study was to assess the effects of chitosan, thymol essential oil and their combined usage on quality conservation and shelf life enhancement of peach.

MATERIALS AND METHODS

**Plant material**

Peach fruits cv. ‘Zaferani’ were harvested at commercial mature stage from a commercial orchard located at Khondab, Markazi province, Iran. Harvested fruit were transported by a ventilated car to the Horticultural Laboratory in Arak University, Iran. 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 lots of fruits for
following treatments in three replicates (each replicate contained at least 10 individual fruits for each sampling time).

**Treatment and storage conditions**
Three types of solutions were prepared including 0.5% chitosan (medium molecular weight, Fluka, Buchs, Switzerland), 200 mg L\(^{-1}\) thymol \([5\text{–methyl-2-}\text{isopropylphenol}]\) (Sigma Chemical Co., St. Louis, MO, USA, Minimum 99.5%) and 0.5% chitosan combined with 200 mg L\(^{-1}\) thymol. These concentrations of chitosan and thymol were selected based on preliminary experiments done in the laboratory. Fruits were dipped for 15 min in three prepared solutions and fruits dipped in distilled water were served as control. All samples were air dried for 60 min before packaging made of polyethylene terephthalate (gas and water resistant) and stored at 6 °C and 80% relative humidity in permanent darkness for 30 days. Fruits from each replicate were randomly sampled on days of 10, 15, 20, 25 and 30 of storage and analyzed for all parameters.

**Weight loss**
Weights of individual replicate were recorded following treatment (day 0) and at different intervals (days 10, 15, 20, 25 and 30) during storage. Weight loss percentage was reported as the percentage of loss of weight with respect to the initial weight.

**Total soluble solids content (TSS)**
Total soluble solids (TSS) concentration was determined in the juice from two individual fruits from each treatment with a refractometer (Atago, PAL-1, Japan) at 20±1 °C and results expressed as the means of % (°Brix).

**Fruit firmness**
Firmness of fruits was recorded using a penetrometer (STEP SYSTEM, Germany) with 8 mm diameter plunger. After removing the epidermis at two equatorial sites a 5 mm plunger tip was used to measure the firmness. Readings were expressed as kg cm\(^{-2}\).

**Anthocyanin content**
Total anthocyanin content 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. The results were calculated as follows (1):

\[
A_{sp} = (A_{520} - A_{700})_{pH\,1.0} - (A_{520} - A_{700})_{pH\,4.5}
\]  

(1)

The content of total anthocyanins (TA) was calculated as follows (2):

\[
TA = \frac{(A_{sp} \times M \times DF \times 1000)}{(\varepsilon \times \lambda \times m)},
\]

(2)

where DF is the dilution factor, \(\lambda\) is the cuvette optical pathlength (1 cm), and m is the weight of the sample (g).

**Carotenoid content**
Carotenoid content was extracted according to the methodology proposed by Lichtenthaler (1987), and was expressed in mg g\(^{-1}\) pulp. Two grams of each sample were immersed in 18
mL of acetone 80%, stirred, and centrifuged at 3000 rpm for 10 minutes. The quantification was done using a spectrophotometer.

**Sensory evaluation**
During the storage, sensory evaluation of stored peach fruits was done by a panel of five experts on 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 included for assessing the acceptability by the consumers.

**Fungal decay incidence**
Fungal decay incidence was determined by counting the number of rotten fruits, divided by the total number of fruits within each package, and was expressed as percentage (%).

**Shelf life**
The shelf life of fruit was accounted from the date of harvesting to the last edible stage. The shelf-life of fruits was determined by recording the number of days the fruits remained in good condition during storage without spoilage. When the spoilage of fruits exceeded 50% it was considered as the end of shelf-life (Taduri et al., 2017).

**Statistical analysis**
The experiment was conducted as completely randomized design (CRD) with 3 replications. 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 ± SD.

**RESULTS AND DISCUSSION**

**Fruit weight loss**
With the prolonged storage time, the weight loss of fruits in all treatments increased (Fig. 1). Nevertheless, coating treatments significantly affected the percentage of weight loss ($P \leq 0.05$) and the highest rate was observed in control fruits (Fig. 1). After 30 days of storage at 6 °C, maximum weight loss (14.59 %) was observed in control fruits, whereas this one was 11.25% for 0.5% chitosan + 200 mg L⁻¹ thymol, although no significant difference was detected between this treatment and other coating treatments. In fresh fruits, weight loss is mainly related with the water loss originated from transpiration and respiration (Reyes-Avalos et al., 2016). Chitosan coatings make a layer on the surface of the fruit and operate as a protective barrier that decrease respiration and transpiration through the fruit surface (Meighani et al., 2015). This effect has also been characterized in fig (Reyes-Avalos et al., 2016), pomegranate (Meighani et al., 2015), litchi (Lin et al., 2011), and papaya (Ali et al., 2011). Essential oils, due to have hydrophobic traits, have been combined with chitosan coatings to decrease moisture loss in fruits (Sun et al., 2014). Our results indicate that the compound of chitosan and thymol presents much better effect on avoiding weight loss than only chitosan or thymol coating, which is in agreement with the earlier reports (Reyes-Avalos et al., 2016; Dantas Guerra et al., 2016; Saki et al., 2019). Application of edible coatings on fresh fruit can deliver an alternative method to changed atmospheric storage by decreasing quality changes and slowing down quantity losses through alteration and control of the internal atmosphere of the individual fruits (Turhan, 2009). Previous works have revealed that the combination of essential oils and chitosan can significantly increase their
antimicrobial activities and several of their physicochemical traits, such as biodegradability and the capability to form films and water vapor barriers, thus prolonging the shelf life of fruit (Dantas Guerra et al., 2016).

**Fruit firmness**

Coating treatments significantly affected firmness of peach fruits and the higher rate was observed in coating treatments (Fig. 2). After 30 days of storage at 6 °C, minimum firmness rate (4.63 kg cm\(^{-2}\)) was detected in control fruits, whereas this one was 6.45 kg cm\(^{-2}\) for 0.5% chitosan + 200 mg L\(^{-1}\) thymol, although no significant difference was observed between this treatment and other coating treatments (Fig. 2).

![Fig. 1](image1.png)

**Fig. 1.** Effect of different coatings on the weight loss of peach fruits during storage at 6 °C. Significant difference (P ≤ 0.05) between data is expressed by different letters. Vertical bars indicate standard deviation.

![Fig. 2](image2.png)

**Fig. 2.** Effect of different coatings on the firmness of peach fruits during storage at 6 °C. Significant difference (P ≤ 0.05) between data is expressed by different letters. Vertical bars indicate standard deviation.
Fig. 3. Effect of different coatings on total soluble solids (TSS) of peach fruits during storage at 6 °C. Significant difference ($P \leq 0.05$) between data is expressed by different letters. Vertical bars indicate standard deviation.

Fig. 4. Effect of different coatings on the anthocyanin content of peach fruits during storage at 6 °C. Significant difference ($P \leq 0.05$) between data is expressed by different letters. Vertical bars indicate standard deviation.
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Fig. 5. Effect of different coatings on the carotenoid content of peach fruits during storage at 6 °C. Significant difference ($P \leq 0.05$) between data is expressed by different letters. Vertical bars indicate standard deviation.

Fruit texture is an important quality trait for customer acceptability of fresh fruit and vegetables. Fruit texture is affected by cell turgidity and the construction and composition of the cell wall polysaccharides (Koh & Melton, 2002). Flesh softening of fruits is related to the action of cell wall degrading enzymes, which hydrolyze starch to soluble sugars and protopectin to water-soluble pectin (Ali et al., 2011). Furthermore, microbial contamination plays a significant role in the loss of fruit firmness (Sun et al., 2014). Therefore, chitosan, thymol and chitosan combined with thymol could positively influence the conservation of firmness in peach fruits by decreasing water loss and fruit senescence and reducing cell wall degradation through the inhibition of microbial propagation. The useful effect of the edible coatings on fruit firmness has also been stated for tomato, peach, Japanese pear, kiwifruit and ‘Murcott’ tangor (Bill et al., 2014).

Total soluble solids (TSS)
Figure 3 presents the variation in TSS of peach fruits for the duration of storage under the four treatments. There was very slight increase in level of TSS throughout cold storage in coated and uncoated fruits (Fig. 3). After 30 days of storage, the highest (12.23 °Brix) and the least (10.86 °Brix) level of TSS was observed in uncoated and 0.5% chitosan + 200 mg L$^{-1}$ thymol coated fruits, respectively (Fig. 3).

In fruits, the changes of TSS during storage might be the result from ripening process (Ravanfar et al., 2014; Meighani et al., 2015). During cold storage, starch and other nutrients reduce into sugars and other soluble materials, resulted in an increase in level of TSS (Sabir et al., 2011). Nevertheless, the lowest rate of TSS (13.83 °Brix) recorded in 0.5% chitosan + 200 mg L$^{-1}$ thymol coated fruits can be related to a reduction in respiration rate by coatings, which is confirmed by the reports of Barman et al. (2011) and Dantas Guerra et al. (2016).
Anthocyanin content
The results indicated that anthocyanin concentration decreased in coated and uncoated fruits during the cold storage. Fruit coating treatments could significantly retain higher anthocyanin concentration than control during postharvest periods. Nevertheless, fruits coated with 0.5% chitosan + 200 mg L\(^{-1}\) thymol exhibited maximum anthocyanin content with 1.65 mg 100 g\(^{-1}\) after 30 days storage at 6 °C (Fig. 4).

Our results indicated that coating treatments reduced degradation of anthocyanins over storage period. It was most possibly due to that coating treatment decreases the activity of polyphenoloxidase and peroxidase enzymes in response to modifications in the internal atmosphere of coated fruit (Varasteh et al., 2012). Dong et al. (2004) also stated that degradation of anthocyanin in litchi fruit caused by polyphenoloxidase and peroxidase, but application chitosan coating treatment reduced enzyme activity over storage period.

Carotenoid content
The results indicated that carotenoid content decreased in coated and control fruits during cold storage (Fig. 5). After 30 days of cold storage, the lowest carotenoid content (9.66 mg g\(^{-1}\)) was detected in control fruits, whereas this one was 21.03 mg g\(^{-1}\) for 0.5% chitosan + 200 mg L\(^{-1}\) thymol (Fig. 5).

Our findings are in agreement with those reported by Flores-López et al. (2016), who stated a decline in carotenoid content in fruits and vegetables coated with different coatings.

Sensory evaluation
At the end of the 30 day storage at 6 °C, the highest score was given to peach fruits treated with 0.5% chitosan + 200 mg L\(^{-1}\) thymol (4.33) followed those treated with 0.5% chitosan (3.00) and 200 mg L\(^{-1}\) thymol (2.66), whereas control fruits (0.00) dropped to the critical level of acceptability (Fig. 6 and 7).

It could be concluded that the coating treatments had a positive effect on consumers mind as the application of this treatment affected general appearance of the fruits. This maximum score can be related to the minimum water loss from the fruit surface and maintenance of better balance between sugars and acids of fruit juice (Jameel Jhalegar et al., 2015).

Decay incidence
Decay incidence was only visible in peach fruits after 25 days of storage. Coating treatments did not significantly affected decay incidence of peach fruits (Fig. 8). Nevertheless, after 30 days of cold storage, the highest decay incidence (7.63%) was observed in uncoated fruits, while it was 0% in 0.5% chitosan + 200 mg L\(^{-1}\) thymol treatment (Fig. 8).

Several works have described the efficiency of chitosan in the inhibition of the mycelial growth of postharvest pathogenic fungi (Jianglian & Shaoying, 2013). Perdones et al. (2012) stated that chitosan coatings reduced the percentage of infected strawberries as compared to control ones after 3 days of storage. Furthermore, Jameel Jhalegar et al. (2015) showed that essential oils have the potential to control fungal decay incidence without causing any damage or destructive effects on Kinnow mandarin, and essential oils can be suggested as a harmless technique for prolonging its storage life while keeping fruit quality. Our result shows that the combination of chitosan and thymol has much better effect on preventing decay incidence than only chitosan or thymol coating, which is in agreement with the earlier reports (Mohammadi et al., 2015). In another study, Hu et al. (2003) displayed that thymol in mixture with chitosan significantly enhanced the antimicrobial activity. Our results also confirm Wang et al. (2011) study that reported the synergistic interaction of cinnamon essential oil with chitosan.
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Fig. 6. Effect of different coatings on the sensory characteristics of peach fruits during storage at 6 °C. Significant difference ($P \leq 0.05$) between data is expressed by different letters. Vertical bars indicate standard deviation.

Fig. 7. Peach fruits appearance after 30 days of storage at 6 °C. (A) the coated fruits with 0.5 % chitosan + 200 mg L$^{-1}$ thymol, (B) the coated fruits with 0.5 % chitosan, (C) the coated fruits with 200 mg L$^{-1}$ thymol, (D) uncoated fruits.
Fig. 8. Effect of different coatings on the decay incidence of peach fruits during storage at 6 °C. Significant difference ($P \leq 0.05$) between data is expressed by different letters. Vertical bars indicate standard deviation.

Shelf life of fruits
Coating treatments significantly affected shelf-life of peach fruits (Fig. 9). The minimum shelf life (16.67 days) was observed in control fruits, while the maximum shelf life (28.33 days) was found in fruits coated with 0.5% chitosan + 200 mg L$^{-1}$ thymol. Nevertheless, no
significant difference was observed between this treatment and other coating treatments (Fig. 9). Our results 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 indicated shelf life of coated fruits was considerably greater than control under cold storage.

CONCLUSION

Our results indicated that coating treatments affected all of the measured quality traits of peach fruits during cold storage. The results revealed that a combination of chitosan and thymol offered more effective protective effect than chitosan or thymol coating alone. Hence, the coating compound of chitosan and thymol can have an effective alternative for quality preservation and shelf life enhancement of peach fruits. This treatment has several benefits including natural, edible and efficient. In spite of substantial results of the present study, further studies are required to be done to determine more effective combination treatments to maintain the quality of peach fruits during cold storage. Moreover, composed treatments must be studied in the future in naturally infected fruit in order to provide an efficient decay control measure to the organic peach fruit industry.

CONFLICT OF INTEREST

The authors have no conflict of interest to report.

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