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Impact of Menthol and Thymol in Combination with Modified Atmosphere Packaging for the Safety and Quality Retention of Apricot Fruits during Cold Storage

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ABSTRACT

The efficiencies of menthol and thymol fumigation in combination with modified atmosphere packaging were studied to preserve apricot fruit quality. For this purpose, 0 (control), 20, and 30 µl of menthol or thymol were placed on a sterile gauze inside a package and sealed with low-density polyethylene (LDPE) film. Following the treatments, all packages were stored at 1.5±1 °C and 85±5% relative humidity for 30 days. They were analyzed for quality parameters during cold storage. The application of menthol 30 µl, thymol 20 µl, and 30 µl reduced the microbial population of the packed fruits to at least 53, 57, and 69%, respectively. Other parameters related to fruit quality such as weight loss, softening, color changes, and malondialdehyde content, which were delayed in treated fruits, compared to the control. The treated fruits exhibited higher bioactive compounds in terms of b-carotene, ascorbic acid, and titratable acidity, respectively, compared to the control at the end of the storage period. The combination of MAP with the vapor phase of menthol and thymol at appropriate concentrations can maintain apricot fruit quality for consumers and growers to increase the possibility of extending apricot shelf life.

Introduction

Apricot is a popular temperate zone fruit of high commercial value and is rich in minerals. The fruit contains a high concentration of bioactive phytochemicals such as carotenoids, flavonoids, phenolic compounds, and antioxidants and is regarded as a functional food (Alajil et al., 2021). Apricot is an early-season fruit and has excellent quality. The perishable nature of apricot fruit was reportedly related to high respiration rate, rapid ripening, softening, decay (Infant et al., 2008), and sensitivity to ethylene, as the main characteristics of climacteric fruits, while being extremely susceptibility to mechanical injuries (Hajilou and Fakhimrezaei, 2013). Brown rot, caused by *Monilinia fructicola* (G. Wint.), and gray mold, caused by *Botrytis cinerea*, are the most important postharvest diseases of stone fruit, such as apricot (Palou et al., 2003).

Modified atmosphere packaging (MAP) in combination with cold storage and edible coating (Ziaolhagh and Kanani, 2021) have been recommended to preserve fruit quality and delay changes in the ripening of stone fruits (Serrano et al., 2005). In addition, pre-storage treatment of fresh apricot fruit in an atmosphere with high O_2

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(40%) and CO_2 (20%) concentrations had a positive effect on the post-harvest quality attributes and shelf life of apricot cultivar 'Shahroudi (Dorostkar et al., 2022). However, the texture and quality of fresh fruit packed by the MAP technique can be enhanced by treating the fruits with essential oils (EOs) through antimicrobial properties (Chu et al., 1999). Fresh sweet cherry fruits were reportedly treated with antifungal EOs, such as eugenol, thymol, and menthol, thereby imparting certain benefits to several quality parameters (Serrano et al., 2005). While the applications of fungicides such as benomyl, iprodione, and triforine are usually limited to postharvest treatments of stone fruits to control brown rot, several alternatives to the chemical control of postharvest decay of stone fruit have emerged. Liu et al. (2002) demonstrated that fumigation of apricot with 2 mg L^{-1} of thymol vapor reduced the germination of *M. fructicola* to 2%, compared to 98% observed in untreated fruits. Another benefit of EOs is their bioactivity in the vapor phase, a characteristic that makes them useful as possible fumigants for stored commodity protection. In addition, it was revealed that *T. vulgaris* and *E. caryophyllata* oils could serve as natural alternatives to fungicides in controlling *M. fructicola* and *B. cinerea* infections in apricot fruits (Hassani et al., 2011).

The potential of using EOs followed by the use of MAP to control postharvest decay has been examined in the case of some stone fruits. Chu et al. (1999) showed that fumigation of sweet cherry with thymol at 30 mg L^{-1}, followed by MAP and storage at 0 oC, reduced the incidence of gray mold rot from 35% in untreated fruits to 0.5%, while considering that *B. cinerea* is an ethylene producing factor *in vitro*.

Apricot is a climacteric fruit that undergoes rapid ripening, thereby inhibiting the ethylene emission of packed fruits which may show other benefits in terms of maintenance of organoleptic properties (Hajilou and Fakhimrezaei, 2013). The fumigation of apricots with thymol resulted in firmer fruits and higher surface browning, but total soluble solids as well as titratable acidity were not affected. In plums, this resulted in higher total soluble solids, whereas firmness and titratable acidity were not affected (Liu et al., 2002). Similarly, Aminifard and Mohammadi (2013) concluded that black caraway, fennel, and peppermint extract reduced the respiration rate in plums and had a positive influence on controlling the weight loss of stored fruits. Thus, the objective of this study was to evaluate the beneficial effects of adding thymol and menthol at low concentrations (20 and 30 µl inside the packages) to control microbial activity, quality, safety, and functional properties of apricot fruits during cold storage.

Materials and Methods

Plant materials and treatments

Apricot (*Prunus armeniaca* L. cv. Gheisi) was picked in June from a commercial orchard near Rafsanjan city (Davaran), Kerman province. The fruits were harvested at commercial maturity when they were still firm, with a light green color background and yellow spots (Dorostkar et al., 2022). The fruits were transferred to the laboratory immediately. Uniform fruits, free of visual defects, were selected, randomized, and divided into 15 lots of 40 fruits per treatment in 4 replicates. Each experimental unit contained 10 individual fruits. Fruit samples were packed in plastic trays (60×165×245 mm) and treatments were performed by placing 20 or 30 µl of menthol or thymol (99.5% purity and purchased from Sigma) on sterile gauze inside the package, and hermetically sealed with a low-density polyethylene (LDPE) film. It was then stored at 1.5±1 °C in permanent darkness and 85±5% RH. Different qualitative properties were measured at 0 (before storage), 20, and 30 days of cold storage.

Measurement of weight loss and microbial analysis

Weight loss was determined by weighing the fruit packages before transferring them to cold storage at the beginning of the experiment and at various intervals during storage. Then, the cumulative weight loss was calculated and expressed in percentages (Hajilou and Fakhimrezaei, 2013).

$$WL\ (\%) = \frac{(prestorage\ FW - afterstorage\ FW)}{prestorage\ FW} \times 100$$

To determine microbial activity, 10 g of fruit tissue was weighed under sterilized conditions and transferred into sterile BagFilter (InterScience, France) containing 90 ml 1% sterilized peptone water before being homogenized using a stomacher (Bag Mixer 400, InterScience, France). A dilution series of each sample was prepared from 10^{-1} to 10^{-6} and 1 ml of diluted solution was added to the agar petri dish (1%) in duplicates for mold counts. After incubation for 3 days at 25 ^{o}C, the number of colonies was counted in the petri dish corresponding to dilutions between 30 and 300 (Serrano et al., 2005). The results were expressed as log CFU g^{-1}.

Determination of β-Carotene, malondialdehyde (MDA) and firmness

β-carotene was assayed using the procedure

described by Nagata and Yamashita (1992). Frozen samples (1 g) from the mesocarp of fruits were homogenized in 10 ml of acetone–hexane (4:6) solution using a pestle and mortar. The solution was shaken well in a test-tube and automatically separated into two phases. An aliquot was taken from the upper solution to measure the optical density at 663, 645, 505, and 453 nm in a spectrophotometer (T80+ UV/VIS, PG Instruments Ltd, England). β-carotene contents were calculated according to the following equations:

$$\beta carotene\ (mg\ 100\ ml^{-1}\ of\ extract) = (0.216 \times A_{663}) - (1.22 \times A_{645}) - (0.304 \times A_{505}) + (0.452 \times A_{453})$$

MDA content was determined according to a method used by Zhao et al. (1994). Accordingly, 0.25 g of flesh tissue was homogenized in 5 ml of trichloroacetic acid 0.1% (w/v). The homogenate was centrifuged at 10,000 g for 10 min. One ml aliquot of the supernatant was added to 4 ml of 20% (w/v) trichloroacetic acid, containing 0.5% (w/v) thiobarbituric acid. The mixture was heated at 95 °C for 15 min and cooled immediately. The absorbance of the supernatant was measured in a UV–Vis spectrophotometer (T80+ UV/VIS, PG Instruments Ltd, England) at 450, 532 and 600 nm, respectively. The concentrations of MDA were expressed as μmol g^{-1} FW.

$$MDA\ content\ (\mu mol\ g^{-1}\ FW) = 6.45\ (OD_{532} - OD_{600}) - 0.56\ OD_{450}$$

Apricots firmness was recorded in 5 fruits per replicate, using a digital pressure tester (model Lutron FG5020, Taiwan) fitted with an 8 mm tip in diameter which was pressed gradually on the surface of the fruit on its equatorial zone, with nearly a constant speed to break the fruit skin. Ultimately, the data were expressed as KgF.

Color measurements

The fruit skin color was evaluated in terms of several parameters, *L**, *a**, and *b**, which were measured on the fruit surface using a Minolta Chromameter CR-400 (Osaka, Japan). Color measurements were performed using the Hunter Lab System. The hue angle [H^o= arctangent (b^*/a^*)] parameter was calculated for each sample during a period of cold storage. Color values were obtained for 5 fruits per replicate. Three measurements were taken from the equatorial region of each fruit.

Titratable acidity (TA), Total soluble solids (TSS), ascorbic acid and pH determination

Juice was extracted from fresh apricot samples of each experimental unit and used for analysis. The TA was expressed as a percentage of malic acid, obtained by titrating 5 ml of apricot juice with 0.1 N NaOH against 1 ml of phenolphthalein (1%) as an indicator of an endpoint with a slight pink color (Hajilou and Fakhimrezaei, 2013).

TSS was measured using a digital refractometer (Atago Co, Ltd, Tokyo, Japan) with a range of 0–32 ^{o}Brix by placing 1–2 drops of clear fruit juice, expressed in percentages.

Ascorbic acid content was determined by titrating a known weight of a sample against 2,6-dichlorophenol-indophenol dye using 3% metaphosphoric acid as a medium for extraction. L-ascorbic acid was used as a standard and the results were expressed as mg 100 g^{-1} FW.

Statistical analysis

Data from measured parameters were subjected to analysis of variance (ANOVA). Mean comparisons were performed using Tukey's test to examine the differences between the treatments and storage time ($p<0.05$). All graphs were drawn by Sigmaplot 11.0 in Microsoft Windows.

Results

Weight loss and microbial activity

Weight loss in the stored apricots increased during storage and was higher in the control group, compared to the fruits treated with 20 and 30 μl menthol and thymol. However, the differences among treatments were not significant after 20 days of the storage period. At the end of the storage time, the control fruits exhibited a significantly higher weight loss (~ 25%) than the packages integrated with menthol or thymol (Fig. 1).

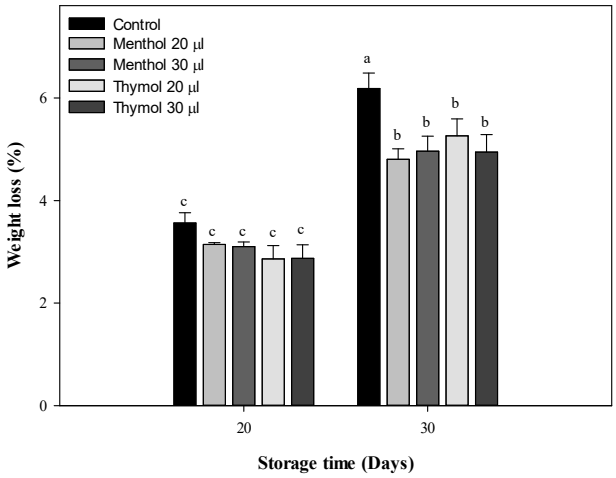

Fig. 1. Influence of menthol and thymol on weight loss of apricot fruit (cv. Gheisi) during storage at 1.5±1 °C (mean value ± SE).

Adding menthol and thymol inside the package led to a reduction in microbial loads of the packed fruits, which became significantly lower as the concentration increased. The microbial count was not significantly different in menthol (20 µl), compared to the control. The addition of menthol 30 µl, thymol 20, and 30 µl reduced the microbial population of packed fruits to at least 53, 57, and 69% respectively (Fig. 2).

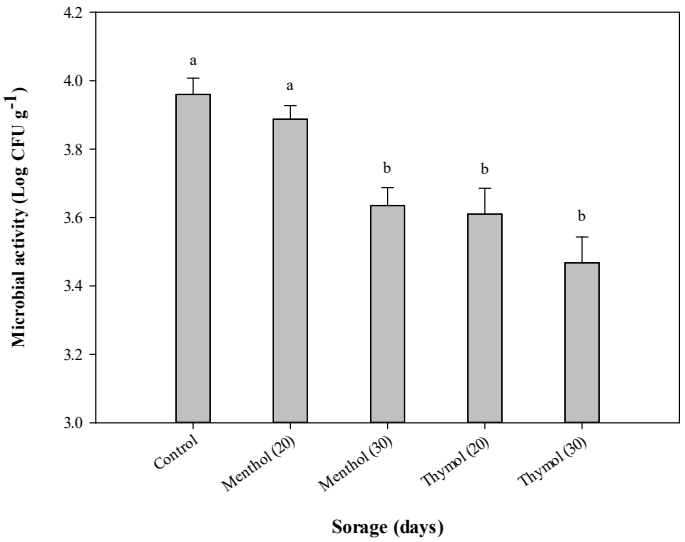

Fig. 2. Influence of menthol and thymol on microbial activity of apricot fruits (cv. Gheisi) during storage at 1.5±1 °C (mean values ± SE).

β-Carotene, MDA and firmness

As shown in Fig. 3A, the β-carotene content decreased toward senescence during storage and finally reached less than 2 mg 100 g^{-1} FW (about 80% reduction in comparison to initial content) in the control group during 30 days of storage. However, the level was higher in all EO-integrated fruits. The highest level of β-carotene was observed in thymol 30 µl after 20 and 30 days of storage.

The MDA content was also quantified in apricot

during storage and was influenced by EOs treatments. The level of this membrane phospholipid peroxidation index was increased over the 30-days of storage and reached the value of 20 nmol g^{-1} FW in control at the end of storage, while the lowest level of 12 nmol g^{-1} FW was observed in thymol 30 µl. After 20 days of storage, the malondialdehyde level was lower in EOs treatments, whereas the differences between the control and treated apricot were higher at the end of storage.

The firmness values of the control and treated apricots and their changes during storage are shown in Fig. 3C. Firmness values decreased throughout the storage in agreement with the progress of the ripening process. Fruit samples treated with thymol and menthol showed significantly higher firmness ($p<0.05$) at 20 and 30 days of storage, which indicates that the EOs possibly affected the dissociation of the fruit cell wall.

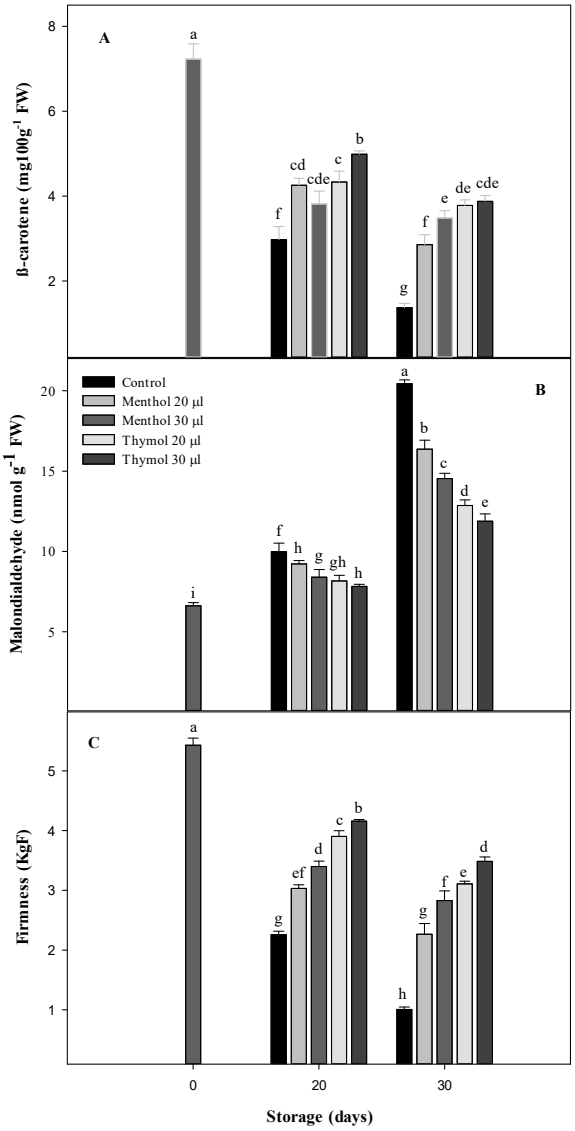

Fig. 3. Influence of menthol and thymol on β-carotene (A), malondialdehyde (B) and firmness (C) of apricot fruit (cv. Gheisi) during storage at 1.5±1 °C (mean values ± SE).

Color parameters

The results of chromatic coordinate changes in apricot fruits (Fig. 4) during cold storage at 1.5±1 oC. The a* level increased during storage in the control and treated fruits, but the value was higher in the control, compared to other treatments (Fig. 4A). The initial level of fruit skin b* index was about 50 at 0 day of storage period. The b* level started to decline in the control at 20 and 30 days of storage (Fig. 4B), while the value was nearly constant in all treated apricots. As shown in Fig. 4C, the hue angle value decreased throughout the storage time but the level was lower in the control, compared to the EO-integrated fruit after 20 and 30 days of storage. The decrease in the hue angle and L* value, as well as an increase in the a* value, represented the darkening of apricot fruit and color changes from greenish-yellow to reddish yellow, respectively. These changes were significantly suppressed by thymol and menthol applications.

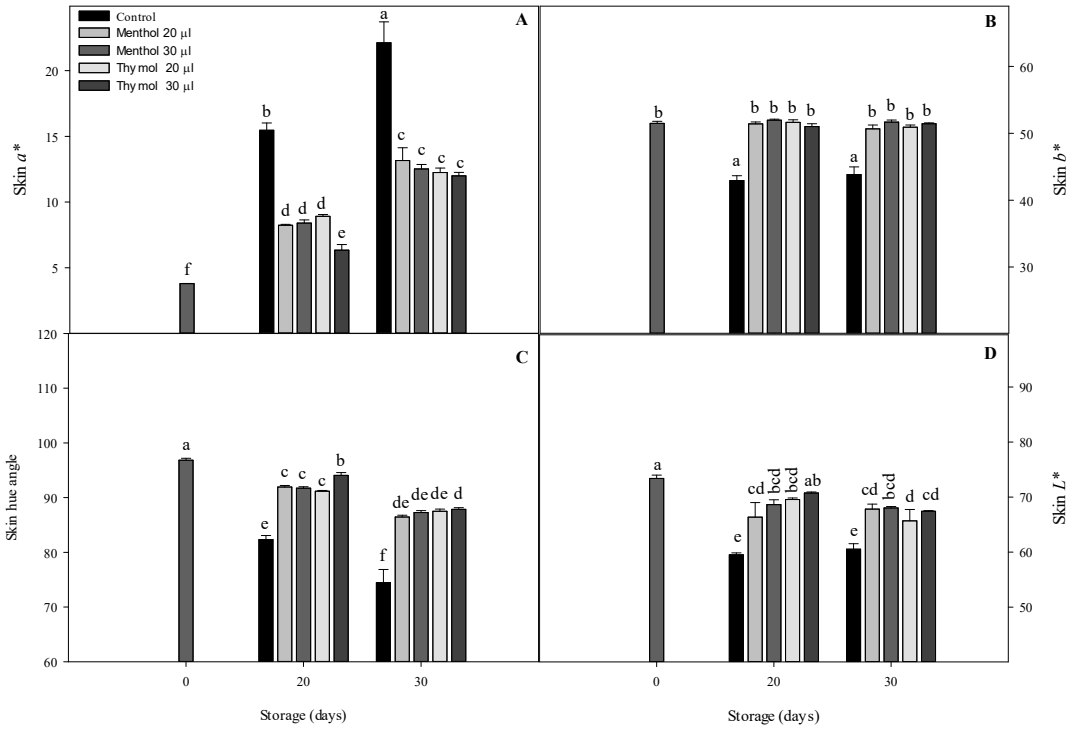

Fig. 4. Influence of menthol and thymol on skin a* (A), b* (B), hue angle (C) and L* (D) of apricot fruits (cv. Gheisi) during storage at 1.5±1 °C (mean values ± SE).

TSS, pH, ascorbic acid, and titratable acidity (TA)

Fruit juice analysis was significantly affected by thymol and menthol integration into MAP. The pH of apricot fruits increased significantly through the storage period, but the values remained lower in all fruits combined with EOs in the package (Fig. 5A). While the value was decreased as the storage enhanced, the levels were higher in all apricots treated with EOs (Fig. 5B). The TSS of apricot increased from harvest to the end of storage, regardless of the applied treatments (Fig. 5C). After 30 days of storage, the control treatment showed the highest TSS of 13.75%, whereas the TSS of the treated apricots was lower than that of the control in all sampling dates during storage.

The total acid content decreased in the control fruit until the twentieth day of storage and then slightly increased thereafter. Treated fruits showed significantly higher contents of titratable acidity than the control fruit ($p<0.05$). However, the levels of total acids in EO-treated fruits varied, depending on menthol and/or thymol (Fig. 5D).

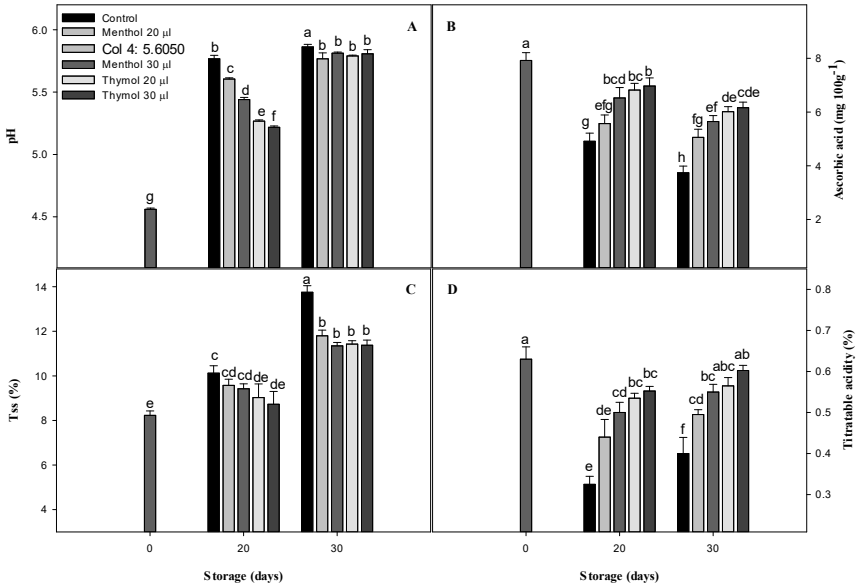

Fig. 5. Influence of menthol and thymol on pH (A), ascorbic acid (B), TSS (C) and titratable acidity (D) of apricot fruits (cv. Gheisi) during storage at 1.5±1 °C (mean values ± SE).

Discussion

Several reports have indicated that EO vapor can delay the loss of water and microbial loads in table grapes, cv. 'Crimson seedless' (Valverde et al., 2005), sweet cherry (Serrano et al., 2005), peach (Montero-Prado et al., 2011) and apricot (Hassani et al., 2011). Although weight loss was controlled by modified atmosphere packaging, adding EOs was effective in the respiration process, intervening with enzymatic reactions of the mitochondrial membranes such as the respiratory electron transport, proton transport, and phosphorylation mechanism (Knobloch et al., 1989), which finally led to a decrease in respiration rate.

The application of EOs in combination with MAP reportedly affected fruit quality by reducing *Botrytis cinerea* and *Rhizopus stolonifer* infections on nectarine fruits (Rashid and Abdel-Rahman, 2019). Jahani et al. (2020) suggested that using EOs, especially thymus and clove oils on pomegranate fruit, may be a useful alternative to the use of synthetic fungicides. Furthermore, among EOs, thyme EO (800 µl L^{-1}) led to the highest firmness and the lowest weight loss. The growth of *A. niger* was completely inhibited by the application of clove oil at concentrations of 200, 400, 600, and 800 µl L^{-1} on the first and tenth day, as well as thyme application at concentrations of 800 µl L^{-1} (Jahani et al., 2020). The EOs of *Mentha piperita* and *Thymus vulgaris* in the vapor phase were inhibitors against bacterial growth (Tyagi and Malik, 2011). The inhibitory impact of EOs against the microbial activity was often related to their direct effects on mycelial growth, spore germination, and their cellular metabolism (Juglal et al., 2002). Furthermore, it was reported that EOs application could change the permeability and integrity of the pathogen cell membrane and ion gradient (Ultee et al., 1999).

The integration of MAP and EOs delayed the MDA increase during storage. Some studies have shown the induction of ROS accumulation in plant cells under low temperature conditions can cause lipid peroxidation (Larkindale and Huang, 2004). The integration of MAP with EOs could maintain membrane integrity by preventing lipid peroxidation. It has been reported that the application of chitosan with EOs in sweet peppers and jujube (Xing et al., 2015) decreased MDA accumulation. Clove oil reduced the MDA content in citrus by enhancing the activity of defense enzymes and increased resistance against fruit decay (Chen et al., 2019). The lower level of MDA in EOs-treated fruits may be related to stronger defense enzymes, as induced by EOs. Thus, according to our results and previous studies, using EOs could reduce decay in apricot or other crops by controlling membrane lipid peroxidation.

Apricot fruits are considered a rich source of carotenoids, especially β-carotene, which accounts for more than 50% of the total carotenoid content (Sass-Kiss et al., 2005). Similar to our results, when thyme and savory EO were applied on peaches and nectarines, the carotenoid content was maintained during the storage of fruits (Santoro et al., 2018). The application of two EOs on tomato led to the highest value of lycopene and b-carotene, while the lowest content was observed in the control samples (Aminifard and Mohammadi, 2012).

A decrease in tissue firmness of fruits happens during postharvest storage and leads to quality loss and a decrease in shelf life (Valverde et al., 2005). The combination of antimicrobial substances such as EOs along with MAP caused delay in reducing texture firmness of cherries (Serrano et al., 2005). Lemongrass and thyme integration into MAP caused a decrease in anthracnose in avocado and maintained the fruit firmness (Mpho et al., 2013). It could be concluded that the presence of thymol vapor in MAP helped reduce the microbial growth and activity of cell wall degradation enzyme, thereby maintaining apricot firmness. The process of firmness loss in apricots largely depends on pectin methyl esterase and glycosidases activities (Cardarelli et al., 2002).

Fruit color changes are usually attributed to the ripening process and affect the quality of apricots while marketing. Changes in fruit color were related to the degradation of chlorophyll and the accumulation of carotenoids and anthocyanin content. The integration of lemongrass into MAP reportedly delayed the ripening process of avocado fruits (Mpho et al., 2013). Higher hue angle and L^* values were observed in grapes, when the fruits were treated with eugenol, thymol, or menthol, along with MAP, compared to the control group (Valverde et al., 2005). The results indicated that thymol and menthol integration into MAP delayed fruit softening, ripening process and color changes during storage at cold temperature. This conclusion was further supported by another study in which the application of EOs in peach and nectarine fruits caused higher levels of L^* and hue angle, respectively, compared to the control (Santoro et al., 2018).

Sugars and organic acids are the major components that determine the taste and flavor of fruits (Espitia et al., 2012). An increase in respiration rate usually leads to higher biochemical activity while polymeric carbohydrates are converted to monosaccharides such as glucose, fructose, and sucrose. Grapes treated with basil, fennel, summer savory, and thyme EOs have shown lower TSS than control fruits at the end of storage (Abdolahi et al., 2010). In addition, the application of EOs in apricot and grapes reduced TSS levels at the end of the storage period (Hassani et al., 2011; Valverde et al., 2005).

The pH of fruit juice increased during storage due to the consumption of organic acids through the respiration process and their conversion to sugars (Chen et al., 2019). Similar to our results, pH levels increased during the storage period in papaya fruit because of the ripening process (Espitia et al., 2012). Tzortzakis et al. (2007) reported that the application of eucalyptus and cinnamon EO increased strawberry pH during storage.

The best-known performance of ascorbic acid is its contribution to the detoxification of active oxygen species in chloroplasts, cytosol, and peroxisomes by oxidation through ascorbate peroxidase (Talano et al., 2008). Ascorbic acid content was reportedly decreased by fungal infection which degraded the fruit cell wall through time in storage. This observation was likewise documented in the case of the control and EO-treated apricots in the current research. However, the most effective treatments for apricot were observed with the application of thymol at 20 and 30 µl. Similar to our results, ascorbic acid became higher in strawberries treated with myrtle during storage (Ulukanli and Oz, 2015).

The TA content of the EO-treated apricots was higher than that of the control group. The application of eugenol in combination with MAP increased the TA levels in table grapes (Valverde et al., 2005). Rathore et al. (2007) found that the TA value decreased during storage time in mango. They concluded that a decrease in TA might be caused by the destruction of citric acid, which could be related to the higher activity of citric acid glyoxylase during ripening. Thus, EO application might delay the ripening and senescence process of apricots during the storage period, thereby maintaining higher levels of ascorbic acid and TA, but lower levels of pH and TSS.

Conclusion

MAP is one of the most important methods of preserving fruit quality. The current results showed that the combination of MAP with the vapor phase of menthol and thymol at appropriate concentrations had beneficial effects on apricots by reducing microbial activity and preserving fruit quality during storage and marketing. These benefits can contribute to the quality of fresh produce for consumers and

growers, while increasing shelf life.

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Conflict of Interest
The authors indicate no conflict of interest for this work.

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