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Combined effects of oxalic acid treatment and modified atmosphere packaging on postharvest quality of loquats during storage

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Abstract: Effectiveness of two different (3 mM and 6 mM) concentrations of oxalic acid (OA) application in combination with modified atmosphere packaging (MAP) on skin browning, fruit skin color (L^*, a^*, b^*) values, fruit flesh firmness, total soluble solids (TSS), titratable acidity (TA), ratio of TSS/TA, pH total phenolic content (TPC), total flavonoid content (TFC), organic acids and soluble sugars, and percentage of O_2/CO_2 change in Hafif Çukurgöbek loquats stored at 5 °C for 30 days were evaluated. Findings indicated that skin browning was reduced by pre-storage OA application in loquats. Furthermore, OA treatment produced the promising effects of inhibiting increases in fruit firmness and maintaining higher levels of organic acids, TPC, and TFC. The results showed that higher (6 mM) concentrations of OA significantly limited both development of skin browning and increasing firmness, and it maintained quality and extended the life of loquat fruit postharvest.

Key words: Browning, flavonoid, loquat, organic acids, oxalic acid, phenol

1. Introduction

Turkey has a great potential to produce (12,900 t) loquats in the Mediterranean region, and Mersin and Antalya are the largest loquat producers in the country (www.tuik.gov.tr). Postharvest life and marketing of loquats (*Eriobotra japonica* Lindl.) are limited due to sensitivity to mechanical damage, skin browning, and the development of an off-flavor as well as moisture loss, decay, and chilling-sensitivity during storage (Çandir et al., 2011; Pareek et al., 2014). In order to extend storage life, many different postharvest treatments have been applied to loquats, such as low-temperature storage, modified atmosphere storage (Ding et al., 2002; Çandır et al., 2011), controlled atmosphere storage (Ding et al., 2006), and some postharvest treatments such as 1-methylcyclopropene (Cai et al., 2006; Oz and Ulukanli, 2011) or methyl jasmonate (Cao et al., 2009), polyamine (Zheng et al., 2000a), calcium chloride (Akhtar et al., 2010), sulfur dioxide (Zheng et al., 2000a), heat treatment (Rui et al., 2009; Shao and Tu, 2014), and chitosan coating (Ghasemnezhad et al., 2011; Petriccione et al., 2015). Heat treatment is a safe physical treatment that can decrease sensitivity to chilling injury and delay ripening (Shao and Tu, 2014). Exposure to high temperature can overcome fruit stress conditions (Lara et al., 2009). Cold storage is effective in prolonging loquat shelf life, but it can increase the incidence of flesh leatheriness, which is the major problem in postharvest loquats. In addition, little is known about the real sensitivity of loquats to browning. Therefore, the degree of browning susceptibility and minimum safe temperature for each cultivar should be determined, and strategies to take the highest quality fruits to market should be pursued. In addition, consumers now pay more attention to fresh fruit safety (Cordenunsi et al., 2003). On the other hand, the use of appropriate protective packaging significantly reduces bruising, and the associated browning, of loquats. Oxalic acid (OA) is an organic acid that is naturally present in plants and increases resistance to environmental stress (Liang et al., 2009). It has been noted that OA delays ripening of banana, prevents browning of litchi fruit and banana, enhances resistance to chilling injury, and extends postharvest quality (Yoruk et al., 2002; Zheng and Tian, 2006; Zheng et al., 2007; Huang et al., 2013a). Moreover, surface treatment of fresh fruit not only delays physiological disorders but also positively affects fruit quality (Akhtar et al., 2010). Modified atmosphere packaging (MAP) storage successfully maintains postharvest quality, extending the storage life of fresh commodities by decreasing oxygen (O_2) levels in packaging and the respiration rate of fresh commodities (Erkan and Eski, 2012). Various postharvest

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treatments have been applied for extending the quality of loquats. To the best of our knowledge, effects of oxalic acid treatment (OAT) on the postharvest life of loquats have not been reported. Thus, the aim of the present study was to monitor the combined effects of oxalic acid treatment (OAT) and MAP on some quality attributes.

2. Materials and methods

2.1. Fruit material treatments and packaging

The variety Hafif Çukurgöbek is one of the main national loquat varieties and has a long storage life compared to many other varieties. Hafif Çukurgöbek loquats were harvested from an orchard in Erdemli district, Mersin, in the Mediterranean region of Turkey. Fruits were hand-harvested at the commercially ripe stage (end of April) according to fruit skin color (fully yellow-orange) in 2014. They were transferred to the laboratory within 3 h of harvest and graded according to visual defects and uniformity of shape, size, and color. Then fruits were divided into three groups; I: untreated fruits were packed in MAP bags and used as the control group, II: fruits were dipped into a solution containing 3 mM oxalic acid (OAT-I) for 15 min, and III: fruits were dipped into solution containing 6 mM oxalic acid (OAT-II) for 15 min at room temperature (20 °C). The OA treatment was chosen according to previous reports in several harvested fruits (Zheng and Tian, 2006; Deng et al., 2015). The solution was mixed until dissolved. Then loquats were transferred onto sterilized and sieved trays, and the excess solution was allowed to drip off with air circulation at room temperature (20 °C). Next, three replicates of each treatment (4 kg of fruit in each replicate) were sealed in MAP (20 µm thick; LifePack). After treatment, all loquats (treated and control) were stored for 30 days at 5 °C with 90% RH. Samples were analyzed from each treatment after 0, 4, 8, 16, 24, and 30 days including chemical analysis and textural and fruit skin color measurements.

2.2. Fruit quality evaluation during storage

TSS of loquat juice was measured with a digital refractometer (Krüss, Hamburg, Germany). The results were expressed as Brix at 20 °C. TA was determined by titration of fruit juice (Titrette GmBD + Co KG, Wertheim, Germany) with 0.1 N NaOH to pH 8.1, and the results were expressed as % malic acid. TSS and TA were measured and expressed as TSS/TA ratios. The pH value of the juice was measured using a ThermoScientific Orion 2 Star CG 710 pH meter. Loquat skin color was measured by two readings from two different symmetrical faces of the fruit in each replicate. Fruit skin color was recorded as CIE (L^*, a^*, b^*) value using a Chroma Meter (CR-400; Konica Minolta, Japan). Fifty fruits were selected per replicate, and browning index was measured by measuring browning area, as described by Akhtar et al. (2010), and expressed as a percentage. The following scale was used: 0: no browning, 1: less than 1/4 browning, 2: 1/4 to 1/2 browning, 3: 1/2 to 3/4 browning, and 4: more than 3/4 browning. The browning index was calculated as follows: $[(1 \times N_1 + 2 \times N_2 + 3 \times N_3 + 4 \times N_4)/(4 \times N)] \times 100$, where N is total number of fruits. Firmness of fruit was determined by peeling of two equatorial sides of the fruits by Firmness Tester (model FT) and measured using a texture analyzer (CT3 model; Brookfield Engineering Labs Inc., Middleboro, MA, USA). Loquats were put onto a metal plate on the TA-BT-Kit Fixture Base Table, and the General Probe Kit (TA44 Cylinder, 4 mm D) was used. Maximum force was measured and firmness expressed in newtons (N). To monitor headspace of MAP packages, O_2 and CO_2 gas composition percentages were measured with a checkpoint O_2/CO_2 gas analyzer (PBI-Dansensor, Ringsted, Denmark) during storage.

2.3. Total phenolic content and total flavonoid content

TPC analyses of loquats were performed according to the Folin–Ciocalteu method, with slight modifications as described by Meda et al. (2005). Fresh tissue was centrifuged at 10,000 × g at 4 °C for 20 min. A supernatant was prepared from fruit juice samples for analysis of phenolic content. The absorbance of the resulting solution was measured at 765 nm using a spectrophotometer. The results were expressed as mg of gallic acid kg^{-1} of loquat. The determination of TFC was performed as described by Meda et al. (2005). The total flavonoids standard was quercetin. Results were expressed as mg of quercetin kg^{-1} of loquat. Measurements were done at 415 nm using a spectrophotometer (Shimadzu Spectrophotometer UV-1800, Kyoto, Japan).

2.4. Sugars and organic acids

Loquat samples were analyzed for sugar percentage (glucose, fructose, and sorbitol) separately using the procedure of Miron and Schaffer (1991). The liquid chromatography apparatus (Hewlett Packard Agilent 1100 Series, Germany) with a refractive index detector (RID) and Shim-Pack HRC NH_2 (300 × 7.8 mm, 5 µm) column was used at room temperature. Loquat juice samples were analyzed for organic acids (malic acid and succinic acid) using the methods of Bozan et al. (1997) with a liquid chromatography apparatus (Hewlett Packard Agilent 1100 Series, Germany), a UV detector, and HPX 87H (300 × 7.8 mm, 5 µm) column.

2.5. Statistical analysis

Experiments were performed using a completely randomized design with 3 replicates per treatment. The obtained data were analyzed by using IBM SPSS Statistics 19 (IBM, NY, USA). The results were obtained using GLM univariate procedures, which provide analysis of variance by one or more factor variables. Means were compared by the least significant difference test at significance levels of $P < 0.05$.

3. Results and discussion

3.1. In-package gas (CO_2/O_2) gas concentration

The effects of oxalic acid treatment on Hafif Çukurgöbek in package gas composition (percentage of O_2/CO_2 production) of loquats are shown in Figures 1A and 1B. Initially, in-package gas concentrations were 20.4%–20.8% O_2 and 0.4%–0.7% CO_2 in MAP. Then % CO_2 increased while % O_2 decreased during storage time, as reported by Ding et al. (2002). The oxygen level was lower in the control (16.6%) than in OAT-I (17.9%), and the highest level was found in OAT-II atmosphere (18.8%) (Figure 1A). Consumption of O_2 made the level of O_2 decrease continuously during respiration in control fruits, while there were some fluctuations in O_2 level in oxalic acid treated groups. The CO_2 percentage was between 0.35% and 0.7% at the beginning; then it gradually increased during storage (Figure 1B). Control fruits had the highest value with 2.3% CO_2, while the OAT-I and the OAT-II oxalic acid treated fruits both had lower CO_2 percentage (1.7%). The respiration rate of oxalic acid treated fruit was lower than in control fruit. The higher oxalic acid treatment had the lowest respiration rate among loquat fruit. Zheng et al. (2007) reported that oxalic acid treatment in Bayuecui peach kept the fruit respiration rate low (5 mM oxalic acid) compared to 1 mM oxalic acid in control groups.

3.2. Fruit skin color

Unripe loquats are green, and most change to orange or yellow in association with ripening (Pareek et al., 2014). Hafif Çukurgöbek (L^*, a^*, b^*) color changes were not significantly different by treatment or storage days. There were fluctuations in L^* value during storage, while there were only small changes in L^* color of loquat at the end of storage compared to initial values. L^* color value was 68.9 in the control, 72.6 in OAT-I, and 71.9 in OAT-II at the beginning of storage (Figure 2A). L^* value in the control and OAT-I fruit was 70 while it was 72.3 in OAT-II fruit. The initial a^* value was about 11.4 in the control treatment, 10.73 in OAT-I, and 10.9 in OAT-II (Figure 2B). During storage a^* value increased slightly, and at the end of storage the control fruit a^* value was 14.4, OAT-I was 12.9, and OAT-II was lowest at 12.6. The control fruit appeared more orange than OA treatment fruits. While b^* value was 47.4 in the control, it was 49.3 in OAT-I and 45.5 in OAT-II (Figure 2C). Huang et al. (2013b) reported that oxalic-acid–treated banana fruit skin color had delayed chlorophyll degradation and kept the skin color.

3.3. Browning index

Flesh and skin browning is a serious problem for postharvest storage and processing of loquats; the fruit is very sensitive to mechanical injury as well. Browning is mainly caused by enzymatic oxidation of endogenous polyphenols into quinones (Paerek et al., 2014). The loquat browning index increased slightly at each storage assessment time; it was at 4.5% in control, 1.4% in OAT-I, and 0.7% in OAT-II after 8 days of storage. By the end of storage, the highest value was in control fruits (10.3%) and 5.8% in OAT-I and 5.3% in OAT-II (Figure 2D). The browning index was significantly lower in the oxalic acid treated fruit than in control group. As reported by Zheng and Tian (2006), higher concentrations of OA inhibited oxidation of phenolic compounds. Another researcher (Huang et al., 2013b) showed that OA treatment of banana fruit peel reduced browning; moreover, previous research in litchi, peach, and banana slices (Yoruk et al., 2004; Zheng and Tian, 2006; Zheng et al., 2007) supported our research results. Oxalic acid, which is an inhibitor of PPO, has very effective anti-browning activity (Huang et al., 2013b).

3.4. Firmness

Loquat firmness is a controversial subject and differs among varieties and according to storage conditions (Abbasi et al., 2013). There was a significant difference among treatments. The firmness of Hafif Çukurgöbek loquat was between 3.2 and 3.4 N after harvest, but it increased gradually during storage (Figure 3). Fruit firmness in control fruits increased to 5.3 N, while fruit firmness in OAT-I and OAT-II treatments was 4.8 N and 4.5 N at end of storage, respectively (Figure 3A). Increase in flesh firmness during 30 days of storage was also reported by Abbasi et al. (2013). In general, a higher firmness level in fruit would indicate greater freshness, but this is not

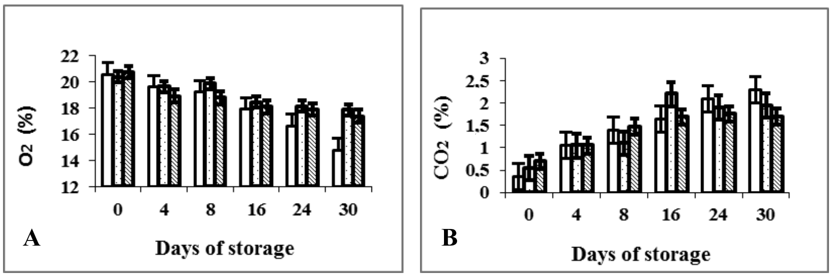

Figure 1. In-package O_2 (%) and CO_2 (%) levels in control (□), 3 mM oxalic acid (▫), and in 6 mM oxalic acid (Ø) treated fruit during 30 day storage at 5 °C. The mean difference is significant at the 5% level. Error bars indicate the standard error.

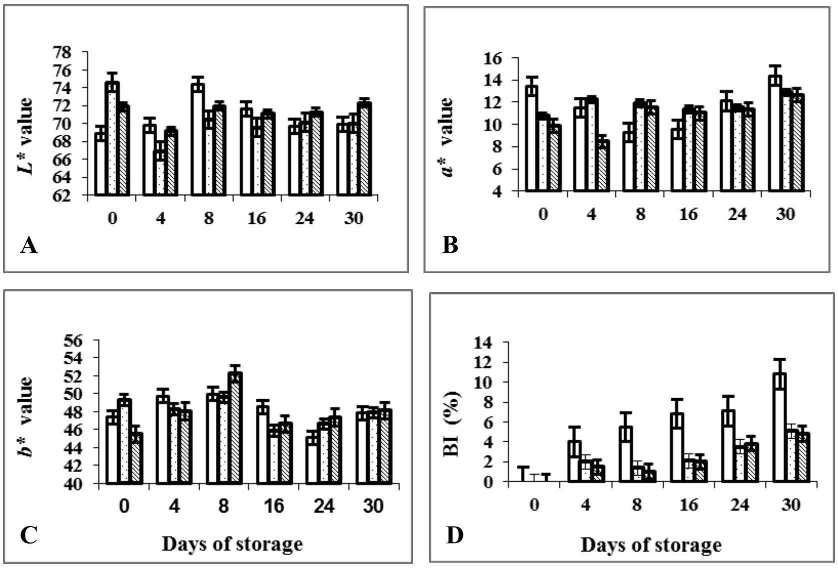

Figure 2. L (lightness), a* (+red, -green), and b* (+yellow, -blue) color values and browning index (%) in control (□), 3 mM oxalic acid (▫), and 6 mM oxalic acid (Ø) treated loquat fruit during 30 day storage at 5 °C. The mean difference is significant at the 5% level. Error bars indicate the standard error.

true in loquats. As Pareek et al. (2014) Cai et al. (2006), and Cao et al. (2010) reported, loquat firmness is different, because it increases according to storage period, and this is positively related with an increase in fruit lignin.

3.5. TSS, TA, TSS/TA, and pH

TSS has a profound effect on the assessment of fruit quality. There were significant differences in TSS content; the value for OAT-I was 10, while the control and OAT-II were 11.2 (Figure 4A). In general, TSS level of the control group was higher compared to OA treated groups according to storage period; however, all treatments were similar at the end of storage (Figure 4A). TA value shows an estimate of organic acids, and for loquat one main acid is malic acid (Petriccione et al., 2015). TA of loquat was about 1.2% at the beginning of storage, and then it started to decrease until the end of storage (Figure 4B). The lowest TA value was about 0.77% in the control fruit, and the highest value was 0.90% in OAT-II fruit (Figure 4B) at the end of storage. The ratio of TSS/TA is important for fruit flavor. A lower ratio of TSS/TA makes fruit more favorable (Ding et al., 2006; Petriccione et al., 2015). TSS/TA increased more in the control fruit and less in oxalic acid treated fruit (Figure 4C). OA treatment may limit the senescence of loquats. The pH value of loquat was about 3.6, and pH increased slightly during storage (Figure 4D). There were slight differences between treatments. The highest pH value was 3.81 in the control fruit followed by 3.78 in OAT-I, and the lowest pH value was 3.77 in OAT-II (Figure 4D). In a previous study (Ding et al., 1998) both TSS and TA values decreased in loquats during cold storage, which caused loquat flavor degradation. The lowest TA values were in the control and highest in OAT-II. Similarly, Ghasemnezhad et al. (2011) reported that a decrease in TA value was the result of using organic acids in the respiration of fruit during storage.

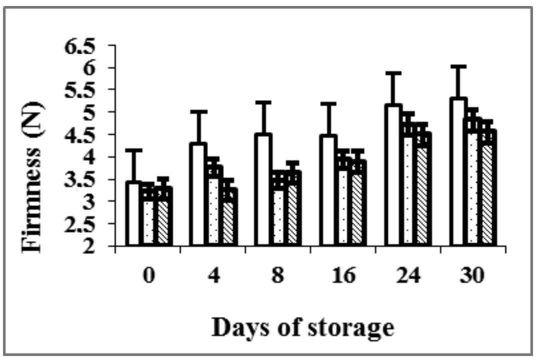

Figure 3. Fruit firmness (N) value changes in control (□), 3 mM oxalic acid (▫), and 6 mM oxalic acid (Ø) treated loquat fruit during 30 day storage at 5 °C. The mean difference is significant at the 5% level. Error bars indicate the standard error.

3.6. Total phenolic and flavonoid content

The loquat is rich in natural antioxidants such as polyphenols and flavonoids, and these profiles in loquats vary among cultivars (Petriccione et al., 2015). Total phenolic content of Hafif Çukurgöbek loquats was 413 mg kg^{-1} in the control, 384 mg kg^{-1} in OAT-I, and 385 mg kg^{-1} in OAT-II (Figure 5A) at the time of initial storage. In general, phenolic content of loquats tended to decrease during storage even though there were slight fluctuations

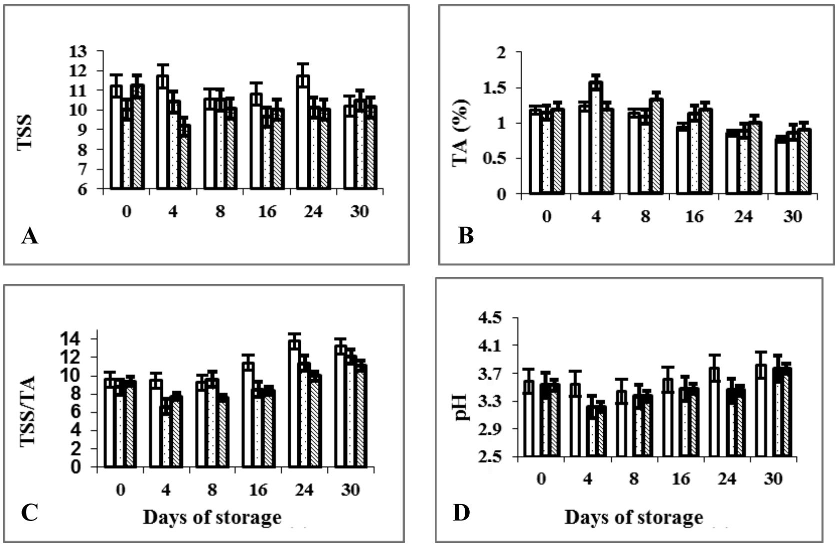

Figure 4. TSS (%), TSS/TA, TA (%), and pH changes in control (□), 3 mM oxalic acid (▫), and 6 mM oxalic acid (Ø) treated loquat fruit during 30 day storage at 5 °C. The mean difference is significant at the 5% level. Error bars indicate the standard error.

in phenolic content. After 30 days in storage, the lowest phenolic content was found in the control treatment with 179.5 mg kg^{-1}. OA-I had the highest value with 350.9 mg kg^{-1}, and OAT-II was 288 mg kg^{-1} (Figure 5A). Phenolic content of loquats increased with oxalic acid concentration compared to control fruits. Phenolic compounds play an important role and positively affect color and flavor in fruits and vegetables (Spanos and Wrolstad, 1992). The present results showed an important correlation between reduction in total phenolic content and increase in browning index percentage. Moreover, Cai et al. (2006), Toor and Savage (2006), and Huang et al. (2013) supported our results, showing that there were important correlations between reduction in total phenolic content and an increase in the browning index, which caused deterioration of cellular structure, or senescence. Flavonoid content was initially between about 56 and 64 mg kg^{-1} (Figure 5B). However, flavonoid content fluctuated during storage. In control fruits, flavonoid content increased from about 62 to 75 mg kg^{-1}, and OAT-I and OAT-II flavonoids were about 83 and 72 mg kg^{-1} at the end of storage, respectively (Figure 5B). Flavonoid content of loquats increased in all treatments at the end of storage.

3.7. Sugar content

Loquats are popular all over the world owing to their mild, subacid, and sweet taste and fructose, glucose, and sucrose are important to fruit taste and quality (Shao et al., 2013). Ripe loquats have a higher amount of glucose, fructose, and sucrose but only a small amount of sorbitol (Pareek et al., 2014). Glucose was between 4.4% and 5.4% at the beginning of storage in Hafif Çukurgöbek loquats (Figure 6A). Glucose content of control fruit decreased from 5.4% to 4.7% by the end of storage; however, OAT-I stayed almost constant (Figure 6A). Glucose concentration was highest in Hafif Çukurgöbek loquats; however, Shao et al. (2013) showed that fructose was the predominant sugar

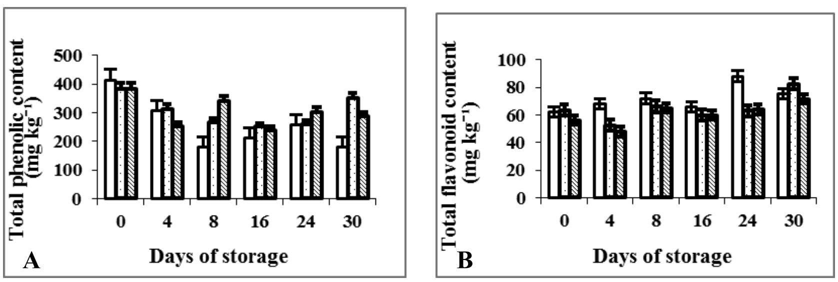

Figure 5. Total phenolic content (mg kg^{-1}) and total flavonoid content (mg kg^{-1}) changes in control (□), 3 mM oxalic acid (▫), and 6 mM oxalic acid (Ø) treated loquat fruit during 30 day storage at 5 °C. The mean difference is significant at the 5% level. Error bars indicate the standard error.

in loquats. Hafif Çukurgöbek loquat fructose content was between 2% and 3.2% at the beginning of storage (Figure 6B). Fructose content was higher in the control in the first 4 days of storage; however, in OAT-II the fruit fructose content increased between days 4 and 8 of storage. Fructose content decreased both in the control and in OAT-II fruit; however, in OAT-I the fruit fructose content increased slightly at the end of 30 days of storage (Figure 6B). Sorbitol is a sugar alcohol (Hamauzu, 1997), and it generally increases by senescence. It was highest in the control (0.50%), 0.44% in OAT-I treated fruit, and lowest in OAT-II fruit (0.40%) (Figure 6C) at the end of storage. Even if there was fluctuation in sorbitol content of loquats between days 8 and 16 of storage, OA treatment kept fruit sorbitol content lower compared to control for the rest of storage. Wang et al. (2016) reported that OA treatment retained the sorbitol content of apricot fruit, and these findings supported our results.

3.8. Organic acids content

The predominant organic acid in unripe loquats is malic acid, which accounts for 90% of all acids, and there are small amounts of citric, succinic, and fumaric acids (Pareek et al., 2014). Organic acid content in fruits decreases during storage due to its conversion to sugars during fruit respiration (Abbasi et al., 2013). Succinic acid was 0.084% in control, 0.069% in OAT-I, and 0.72 in OAT-II fruits at the initial measurement (Figure 7A). Succinic acid decreased in both the control and OAT-II fruits, while it increased in OAT-I treatment (Figure 7A). Malic acid was highest among other organic acids in the Hafif Çukurgöbek loquat variety (Figure 7B). However, malic acid fluctuated in all treatments during storage. Decreasing sugars and organic acids and an increase in the sugar/acid ratio led to a loss in fruit flavor (Ding et al., 2006). Organic acid consumption, mainly malic acid, caused the decline of TA (He et al., 2005). Results showed that increases in fruit

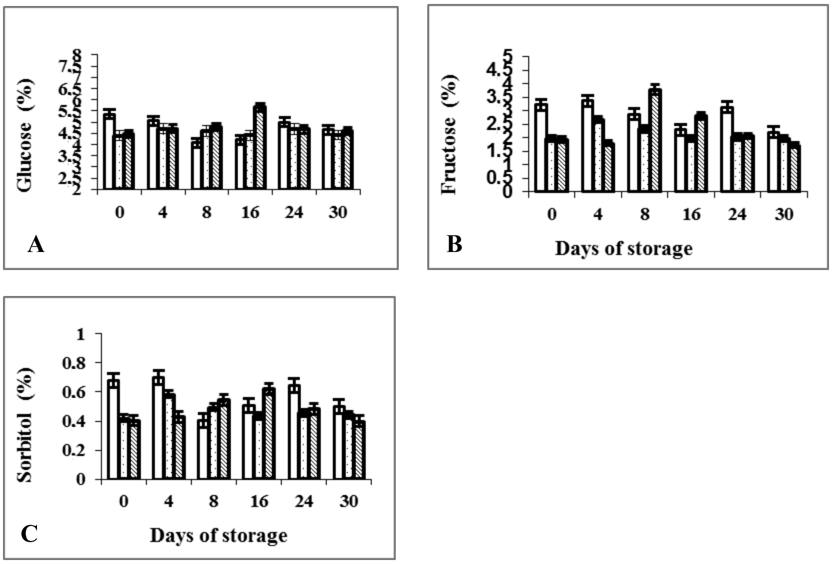

Figure 6. Glucose, fructose, and sorbitol (%) content changes in control (□), 3 mM oxalic acid (▣), and 6 mM oxalic acid (Ø) treated loquat fruit during storage at 5 °C. The mean difference is significant at the 5% level. Error bars indicate the standard error.

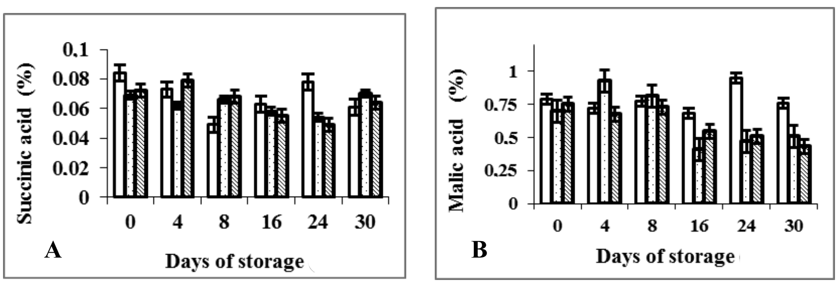

Figure 7. Succinic acid (%) and malic acid content changes in control (□), 3 mM oxalic acid (▣), and 6 mM oxalic acid (Ø) treated loquat fruit during storage at 5 °C. The mean difference is significant at the 5% level. Error bars indicate the standard error.

firmness, browning index percentage, sorbitol, and CO_2 levels in control fruit indicated more senescence and skin browning and a higher respiration rate during storage. However, decreases in total phenol content and O_2 levels in the control group, compared to OA treated fruit, indicated more browning and respiration in control fruit. The *a** value of control fruit was higher than the value of treated fruit, indicating more change to an orange color. Fructose and glucose levels dropped in the control fruit, compared to the initial measurement time, while sorbitol was higher which indicated senescence according to storage period. Results showed that oxalic acid treatment combined with MAP showed promising results for maintaining loquat quality and limiting skin browning, extending storage life at 5 °C for 30 days.

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