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Evaluating the Effects of Citric Acid Application on Reducing Decay, Maintaining Edibility and Shelf Life of Peach Fruits in Cold Storage

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ABSTRACT

Peach fruit (*Prunus persica* L.) was harvested at the stage of commercial harvest and was then treated with 0, 1, 2, 3 mM citric acid (CiA). A factorial model was planned on a completely randomized block design with three replications. Two factors were used in the experimental design, i.e. 4 storage durations × 4 concentrations of CiA. The impact of CiA was evaluated on postharvest quality parameters, decay incidence (DI), vitamin C, total phenolic compounds, and antioxidant capacity, as the peach fruits were maintained in cold storage at 0 ± 0.5 °C and 85-90% relative humidity (RH) for 40 days. By the end of the storage time, peach fruits that were treated with 3 mM CiA showed statistical significance and resulted in the highest values of fruit firmness (FF) (1.75 N), titratable acidity (TA) (0.24%), vitamin C (VC) (3.58 mg.100g^{-1} FW), total phenolic compounds (TPC) (58.49 mg GAE.100g-1 WF) and antioxidant capacity (AC) (52.96%). CiA treatments significantly controlled the DI by about 30.17 % and remarkably extended the shelf life by about 11.66 days, compared to the control samples during the cold storage. Our findings suggested that using CiA, especially at 3 mM, could be a promising treatment in helping to maintain edibility and to inhibit decay in peach fruits. While these can play an important role in the marketing and export of peach fruits, other benefits include a low cost of the chemical inputs and more safety for human consumption.

Abbreviations

Citric acid (CiA), Decay incidence (DI), Vitamin C (VC), Total phenolic compounds (TPC), Antioxidant capacity (AC), Fruit Weight loss (FWL), Fruit firmness (FF), Titratable acidity (TA), Total soluble solid (TSS), Relative humidity (RH)

Introduction

As an important stone fruit, peach (*Prunus persica* L.) is cultivated widely across the world. Its worldwide cultivation covers an area of 1527052 ha, with an annual production of 16.854 million tons (FAOSTAT, 2021). It is a climacteric fruit that shows significant changes in quality characteristics after harvest, resulting in rapid softening, weight loss, dehydration, rotting, and browning (Lurie and Crisosto, 2005). In peach, like other fruits and vegetables, about 80-90% of the fruit weight is water which decreases significantly during the postharvest stage and leads to a short shelf life (Dhall, 2013).

Peach fruit is highly perishable in the post-harvest stage, and its shelf life usually lasts for 5-7 days at room temperature. About 30-40% of the

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total production of peach fruit is usually wasted due to post-harvest decay (Hodges et al., 2011). Storage at low temperatures is a general way to extend the postharvest life of this fruit and to preserve its quality (Nascimento Nunes, 2009). Meanwhile, the current challenges in cold storage research are focused on ways to find alternative methods that can control postharvest loss, prioritize health, evade the negative effects of pesticides, and curb the use of chemicals.

Citric acid is mainly considered safe and is one of the most important organic acids, often as a food additive (Sommers et al., 2003). Organic acids, such as citric acid, can have antiseptic roles in food products and, thus, can attract consumer attention (De Oliveira et al., 2017). Citric acid is an important natural antioxidant, an anti-stress agent that plays a remarkable role as a signaling molecule in some plant physiological processes and defense mechanisms (EL Kobisy D.S. et al., 2005). Recent studies showed that the application of citric acid improved table grape quality (Kok and Bal, 2019), controlled fruit weight loss, maintained fruit firmness, upheld membrane integrity and extended the storage life of fruits.

It has been reported that CiA effectively prolonged shelf life and preserved the quality of fresh-cut CWC (Chinese water chestnut) slices during storage at low temperatures (Jiang et al., 2004). The combination of chitosan with citric acid remarkably increased the activity of antioxidant enzymes, but reduced polyphenol oxidase (PPO) activity and free radical content, thereby limiting oxidative stress (Liu et al., 2016). In another research, it was illustrated that the application of citric acid on apples maintained fruit quality, nutraceutical properties, and the color of 'Red Spur' apple (Allahveran et al., 2018). It was recently determined that combining chitosan with citric acid , as a coating, preserved quality parameters to some extent during storage and maintained sensorial characteristics in fresh-cut guava (Nascimento et al., 2020). Citric acid prolonged storage time in mushrooms, which naturally have a short shelf life of 1-3 days at ambient temperature, while preserving fruit firmness, delaying the weight loss of fruits, and limiting color changes (Ventura-Aguilar et al., 2017).

The aim of this study was to estimate the effects of citric acid on preventing decay and maintaining the edibility of peach fruits during cold storage at 0 ± 0.5 °C and 85-90% relative humidity for 40 days. The emphasis was on developing a new strategy for extending shelf life and maintaining good-quality peach fruits.

Materials and Methods

Fruit material

Peach fruits (*Prunus persica* L.) cv. 'Alberta early ripening' were hand-harvested at the commercial harvest stage (22 July 2020) when more than half of the external surface of the fruits changed from green to yellow (Kader, 1999). After harvest, the fruits were transported to the laboratory within an hour. The fruits were selected for uniform size and color and were free of mechanical damage. The selected fruits were randomly divided into four treatment groups (0, 1, 2, 3 Mm CiA), and each group had 200 fruits.

Citric acid treatment

The postharvest treatment involved dipping the fruits in either 0 (control), 1, 2 or 3 mM citric acid solution for 5 min at 20 °C. Then, the fruits were removed from the citric acid solution and were allowed to be air-dried at room temperature for 2 hours. The fruits were stored at 0 ± 0.5 °C and 85-90% relative humidity for 40 days. In the current study, the data were recorded every 10 days with regard to several physical and chemical properties, decay incidence and shelf life of the peach fruits.

Determination of quality parameters

Fruit weight loss (FWL) was measured every 10 days. Fifteen fruits of each treatment were individually assorted and then divided into three replicates. They were weighed every 10 days and then put back in their storage place. The rate of weight loss was calculated using the following formula (Alhaj Alali et al., 2020):

$$fruit\ weight\ loss(\%) = \frac{(Initial\ weight - final\ weight)}{Initial\ weight} \times 100$$

Fruit firmness (FF) was measured by removing the peel from two positions of the fruit surface (Alhaj Alali et al., 2020) and by using a handheld penetrometer (PFT327, New Zealand) equipped with a 5 mm conical plunger.

Total soluble solids (TSS) were determined using a drop of the extracted juice from each fruit on a hand-held refractometer (Atago N-32, Tokyo) at laboratory temperature. The results were expressed as °Brix (Chéour et al., 1991).

Titratable acidity (TA) was determined by the titration of 5 mL filtered juice with 0.1 N NaOH, until a pH value of 8.1-8.3 was obtained by the pH meter (3520 Bench pH Meters, UK). The results were expressed as a percentage of malic acid (Shokri Heydari et al., 2020).

Determination shelf life of fruits

In postharvest studies, shelf life is known as the amount of time from harvest to the last day when the crop is still edible. The shelf life of peach fruit was calculated by recording the number of days that the fruits maintained optimal marketing quality, edibility and appearance during storage, without substantial signs of degradation on the fruits. When fruit degradation exceeded 50%, the fruits were considered to have reached the end of shelf life (Malekshahi and ValizadehKaji, 2021).

Determination of decay incidence (DI)

Decay incidence was determined according to a method used by Yang et al., (2019). Thirty fruits were divided into three biological replicates and were screened for DI per treatment. The DI was calculated according to the following formula:

$$DI(\%) = \left(\frac{Number\ of\ decayed\ fruit}{Total\ number\ of\ fruit}\right) \times 100$$

Determination of vitamin C (VC)

VC was measured by titration with iodine and potassium iodide. First, 16 g of potassium iodide was dissolved in 1 liter of distilled water, and then 1.27 g of iodine crystals were added. The solution was stirred on a heater. Ultimately, VC was measured by adding filtered fruit juice (5 ml) to distilled water (20 ml) and starch solution (2 ml, 1%). This mixture was then titrated with potassium iodide as an iodine solution until a dark blue color appeared. The amount of VC was calculated using the following formula:

$$VC(mg/100\ \text{g}-^{1}\ \text{juice}) = \frac{(0.88 \times V)}{5} \times 100$$

Where V is the volume of potassium iodide that was consumed (ml) (Majedi, 1994).

Determination of total phenolic compounds (TPC)

Determination of total phenolic compounds in the fruits was carried out according to Ough and Amerine (1988). Accordingly, 0.5 g of fresh fruit was completely crushed in 4 ml ethanol (80%) until the solution was completely homogenized. The homogenate was centrifuged for 20 minutes at 9500 rpm and filtered. Then, the absorbance was recorded with a spectrophotometer at 760 nm (Perkin, Elmer, Lambda EZ 201, USA). Ultimately, the results were expressed as mg of gallic acid equivalent (GAE) per 100 g of fresh weight.

Determination of antioxidant capacity (AC)

The antioxidant capacity was measured using the DPPH method, adopted from Tadolini et al. (2000). In this method, 3.4 ml of 2,2-diphenyl-1-picrylhydrazyl (DPPH) (0.1 mM) was added to 1 ml of the clear methanol extract. The mixture was maintained at room temperature and in the dark for one hour. AC was measured via absorbance at 517 nm using a UV-V spectrophotometer (Perkin, Elmer, Lambda EZ 201, USA). The absorbance of the blank solution of DPPH (2 ml) at 517 nm was used as a control. The decline in DPPH was calculated according to the following equation (%):

$$\%\ inhibition\ of\ DPPH = \frac{(Abs\ control - Abs\ sample)}{Abs\ control} \times 100$$

Statistical analysis

The study was carried out in a completely randomized block design with three replications. All statistical analyses of variance were calculated on two factors during storage, i.e. citric acid treatment (0, 1, 2, 3 mM) and time (0, 10, 20, 30, 40 d). Calculations were performed via GenStat statistical software 17th ed. A two-way analysis of variance (ANOVA) test was used and the differences between mean values were assessed by Duncan's multiple range test ($P < 0.05$).

Results

Qualitative parameters

FWL significantly increased ($P<0.05$) in response to all treatments during storage time. By the end of the storage period, the control group statistically showed the highest value of FWL (4.71%), whereas peach fruits in the 3 mM CiA treatment group showed the lowest value of FWL (2.87%) (Fig. 1). The results showed a remarkable difference between samples of the control and those that were treated with citric acid ($P < 0.05$). No significant difference occurred in weight loss when comparing peach fruits in response to the 2 mM and 3 mM CiA treatments.

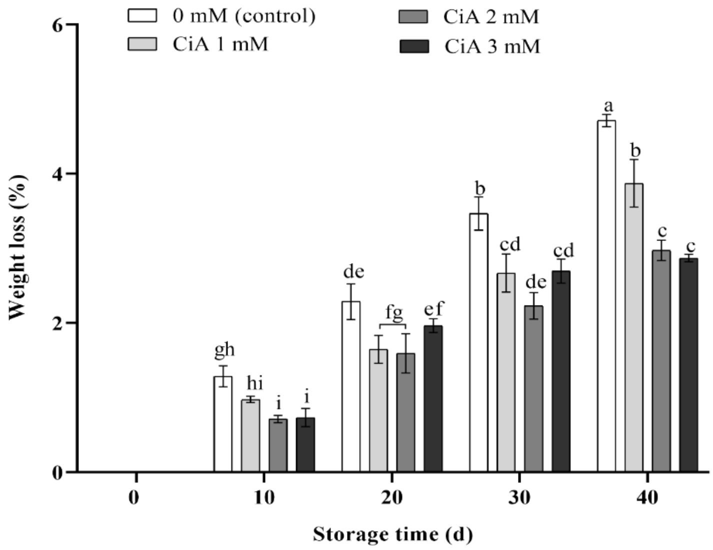

Fig. 1. Effect of postharvest treatments of citric acid (CiA) on weight loss of peach fruit during storage at 0 ± 0.5 °C for 40 d. Data represent mean values of n = 3 and the error bars represent standard errors of the means. Duncan's multiple range test at p ≤ 0.05 level.

The results illustrated a significant decrease in FF in all treatments during the cold storage period. The firmness of the control decreased rapidly, while the firmness of the treated fruits decreased at a significantly slower rate than that of the control (Fig. 2) ($P < 0.05$).

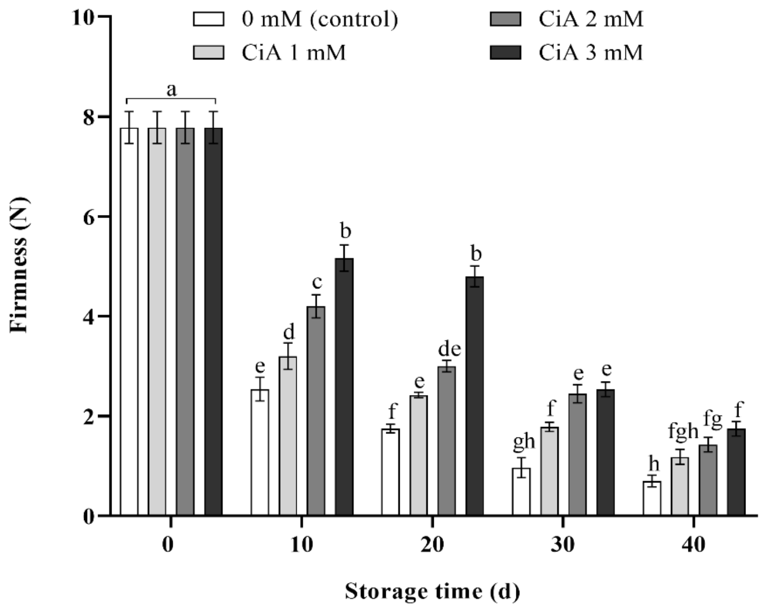

Fig. 2. Effect of postharvest treatments of citric acid (CiA) on firmness of peach fruit during storage at 0 ± 0.5 °C for 40 d. Data represent mean values of n = 3 and the error bars represent standard errors of the means. Duncan's multiple range test at p ≤ 0.05 level.

The data showed that the TSS increased sharply during the first 20 days after storage in the control, but then gradually decreased to the lowest level (7.33 ˚Brix) at the end of the storage period (from 22 July to 30 August 2020) (Fig. 3). However, there was no significant difference between the TSS of fruits in the control and those of the 1 mM CiA treatment. Furthermore, the application of 2 and 3 mM CiA significantly limited the increase in TSS, compared to that of the control, particularly when considering the timespan between 10 and 20 days of storage ($P < 0.05$). A higher loss of moisture was noted in the control, resulting in quicker dehydration and an increase in the TSS of fruits. The results showed that the TA content significantly decreased in response to all treatments throughout the storage period, but higher levels of TA were observed in response to 2 and 3 mM CiA treatments, compared with 1 mM CiA and the control (Fig. 4) ($P < 0.05$).

Shelf life of fruits

CiA treatments significantly prolonged the shelf life of peach fruits. The results showed that the 3 mM CiA treatment extended the shelf life to 37.33 days, which was more than the result of any other treatment, while the fruits of the control group had a remarkably short shelf life (25.67 days) (Fig. 5).

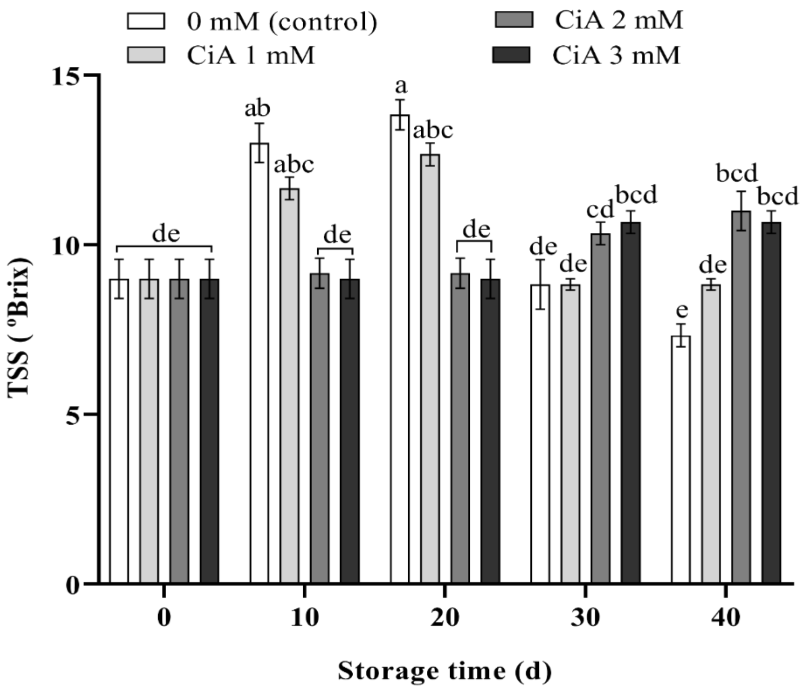

Fig. 3. Effect of postharvest treatments of citric acid (CiA) on TSS of peach fruit during storage at 0 ± 0.5 ˚C for 40 d. Data represent mean values of n = 3 and the error bars represent standard errors of the means. Duncan's multiple range test at $p \leq 0.05$ level.

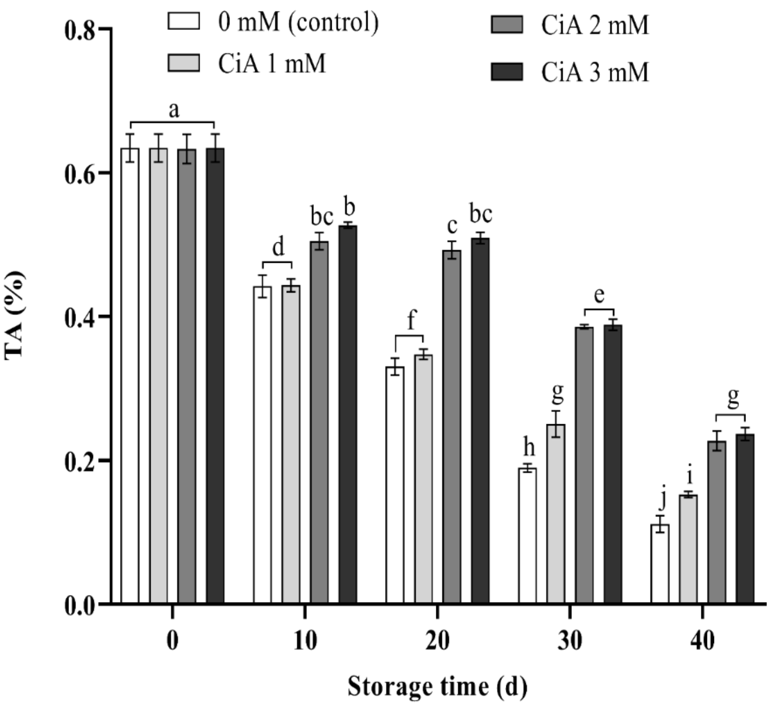

Fig. 4. Effect of postharvest treatments of citric acid (CiA) on TA of peach fruit during storage at 0 ± 0.5 °C for 40 d. Data represent mean values of n = 3 and the error bars represent standard errors of the means. Duncan's multiple range test at $p \leq 0.05$ level.

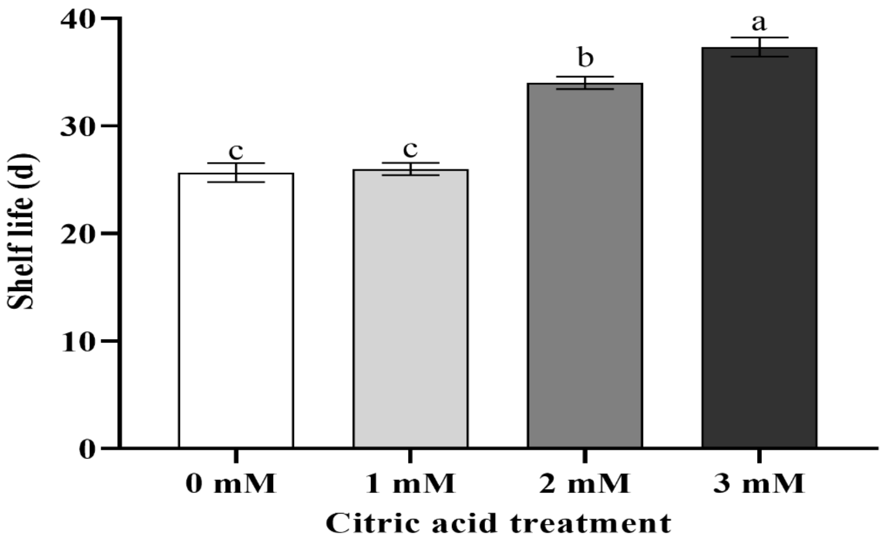

Fig. 5. Effect of postharvest treatments of citric acid (CiA) on the shelf life of peach fruit during storage at 0 ± 0.5 °C for 40 d. Data represent mean values of n = 3 and the error bars represent standard errors of the means. Duncan's multiple range test at $p \leq 0.05$ level.

Decay incidence

In the first 10 days of storage, the decay incidence of the control group was 15.61%, while no sign of decay occurred in the fruits of the CiA treatments during the first 10 days (Fig. 6). In the second 10 days of storage, the decay incidence was controlled at a level of 11.72% in response to the 1mM CiA treatment. As the storage time was prolonged, the decay rate significantly increased, regardless of the control group or the CiA treatments. At the end of storage time, the control group statistically showed the highest level of decay (49.83%), while fruits treated with 3 mM CiA exhibited the lowest level of decay (19.66%) ($P < 0.01$).

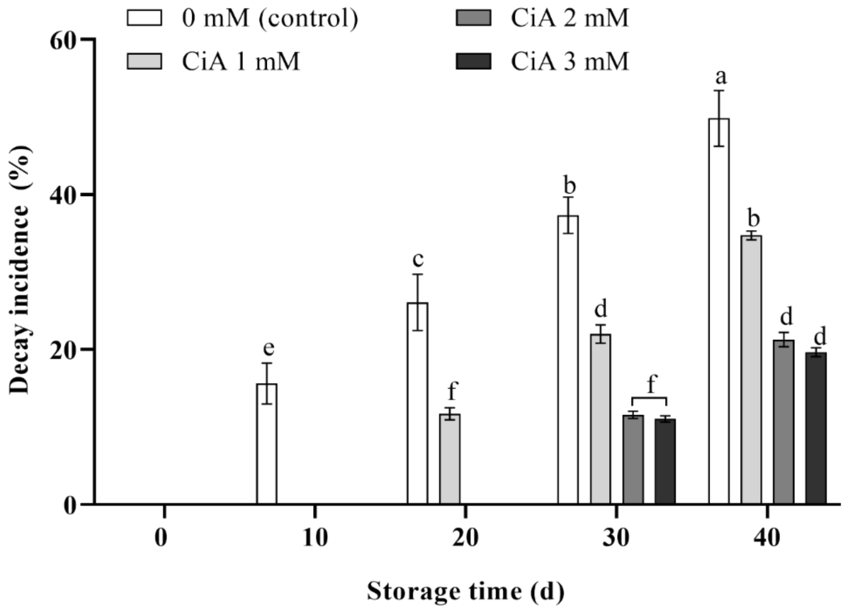

Fig. 6. Effect of postharvest treatments of citric acid (CiA) on decay incidence of peach fruit during storage at 0 ± 0.5 °C for 40 d. Data represent mean values of n = 3 and the error bars represent standard errors of the means. Duncan's multiple range test at $p \leq 0.05$ level.

Vitamin C (VC)

CiA treatments were successful in preventing VC loss during storage time, especially as a result of the 2 and 3 mM CiA treatments, which optimally maintained VC values at 7.128 and 7.509, respectively, compared to that of the control at the end of the storage time. Statistically, no significant difference occurred between the VC values of samples treated with 2 and 3 mM CiA (Table 1).

Total phenolic compounds (TPC)

Regardless of the control or CiA treatments, the total phenolic content in peach fruits increased in the first 20 days, but then decreased thereafter until the end of storage, even as the temperature was maintained at 0 ± 0.5 °C and as the relative humidity remained at 85-90%. In contrast, peach fruits that were treated with citric acid showed the highest TPC (58.49 mg GAE. $100g^{-1}$FW) in response to 3 mM CiA. This had a significant difference ($P < 0.05$) compared to other samples in a similar duration of storage, temperature and humidity (Table.1).

Antioxidant capacity (AC)

The antioxidant capacity gradually increased during the first 20 days of storage, but then showed a declining trend thereafter until the end of storage. When the storage period ended, the results illustrated that 2 and 3 mM CiA treatments caused the highest levels of AC (61.11 and 63.80) in the fruits, respectively ($P < 0.05$), compared to fruits that were treated with 1 mM CiA and the control (Table 1).

Discussion

Peach fruits are easily perishable and usually undergo significant changes in their edibility, resulting in rapid softening, rotting and browning after harvest. Thus, they have a limited market life

of about 2-3 days at room temperature and about 15 days in cold storage (Adel A. Kader., 2001).
FWL is an essential feature of fruits during storage time (Lin et al., 2008), and a previous research showed that CiA played a prominent role in preventing the weight loss of fruits (Liu et al., 2016). Abd El-Aziz (2020) reported that citric acid effectively controlled weight loss in guava fruits, compared to the control. The present study showed that the CiA treatment remarkably reduced the FWL, compared to that of the control. Citric acid has an important role as a major osmotic compound (Hussain et al., 2017). Due to the accumulation of organic acids, citric acid specifically counters the decrease in osmotic potential and prevents a drop in cell turgor pressure in plant tissues (Hummel et al., 2010). Consequently, citric acid can close the stomata, reduce the transpiration rate and limit the weight loss of fruits.

Table 1. Effect of postharvest treatments of citric acid (CiA) on AC, TPC and VC of peach fruit during storage at 0 ± 0.5 °C for 40 d.

Time (d)	CiA treatment (mM)	Antioxidant capacity (AC) (%)	Total phenolic content (TPC) (mg GAE. $100g^{-1}$ FW)	Vitamin C (VC) (mg.$100g^{-1}$ FW)
0	Initial	50.92 ± 3.81 ef	45.74 ± 2.25 fg	8.272 ± 0.304 a
10	0 (control)	54.38 ± 3.95 bcde	51.24 ± 1.67 ef	5.104 ± 0.102 ef
	1	55.72 ± 4.27 bcde	52.81 ± 2.82 de	6.981 ± 0.059 bc
	2	59.42 ± 1.87 abcd	55.05 ± 1.93 cde	7.128 ± 0.134 bc
	3	60.10 ± 1.57 abc	56.75 ± 1.07 bcde	7.509 ± 0.155 ab
20	0 (control)	56.59 ± 4.07 abcde	54.79 ± 3.02 cde	3.931 ± 0.256 gh
	1	57.63 ± 2.26 abcde	58.34 ± 1.36 abcd	6.395 ± 0.211 cd
	2	61.11 ± 1.67 ab	60.94 ± 2.19 abc	6.805 ± 0.155 bc
	3	63.80 ± 1.97 a	64.41 ± 1.47 a	6.891 ± 0.587 bc
30	0 (control)	50.86 ± 3.71 efg	43.88 ± 3.14 gh	2.933 ± 0.310 ij
	1	51.99 ± 2.24 de	50.71 ± 0.82 ef	4.693 ± 0.587 fg
	2	57.21 ± 2.32 abcde	60.15 ± 2.50 abc	5.749 ± 0.469 de
	3	59.03 ± 1.42 abcd	61.85 ± 0.94 ab	5.808 ± 0.619 de
40	0 (control)	41.39 ± 2.02 h	39.10 ± 2.75 h	1.819 ± 0.059 k
	1	44.01 ± 2.49 fh	45.95 ± 1.60 fg	2.171 ± 0.256 jk
	2	52.11 ± 1.52 de	51.88 ± 1.16 ef	3.227 ± 0.293 hi
	3	52.96 ± 1.65 cde	58.49 ± 0.35 abcd	3.579 ± 0.256 hi
significant	df			
treatment	3	**	**	**
time	4	**	**	**
T×T	12	ns	**	**
CV		5.8	2.2	2.6

Data are represented mean ± standard error of three replications. Different letters indicate significance at P ≤ 0.05.*, ** Significance at P = 0.05 and 0.01 level, respectively.

Changes in firmness, TSS, and TA are closely associated with ethylene production (Moya-León et al., 2006). Preventing the biosynthesis of endogenous ethylene and reducing the severity of fruit respiration can lead to prolonged fruit storage. In fact, CiA can participate in the tricarboxylic acid (TCA) cycle throughout the process of fruit ripening and senescence. When exogenous CiA enters the tricarboxylic acid (TCA) cycle, it directly causes feedback inhibition of citrate synthase and accelerates the progress of the TCA, thereby reducing the rate of ethylene production in fruits and delaying the peak of ethylene release (Hussain et al., 2017). The results in the current research confirmed similar findings by Abd El-Aziz (2020) in that the combination of citric acid and hydro-cooling can effectively maintain the firmness of fruits during cold storage for 28 days.
The loss o fruit firmness was related to the breakdownof the cell wall in fruits, resulting from enzymatic activities that involved pectin-esterase, polygalacturonase and pectate lyases which facilitate the destruction of pectin (White, 2002). On the other hand, slowing down the rate of fruit softening during the storage period in treated fruits could be due to the important role of organic acids in alleviating the chilling injury of peach fruits (Wang et al., 2006).
Irrespective of CiA treatments, the TSS of the

peach fruits increased during the storage period. The CiA treatment (2 and 3 mM) significantly prevented the increase in TSS, compared to the control group and the 1 mM citric acid treatment, particularly from day 10 to 20 after harvest ($P < 0.05$). The increase in TSS during the storage period could be due to weight loss, which ultimately means an increase in fruit juice concentration (Moreno et al., 2008). Similar results were observed in cherimoya (Liu et al., 2016).

Regardless of the control or citric acid treatments, a decreasing trend was observed in TA at the end of storage. Maximum TA was maintained in response to the 3 mM CiA treatment. This may be due to a slower degradation of citric acid, probably through the weakened activity of citric acid glyoxylase. In turn, this lowered the respiration rate and ultimately decelerated the metabolic processes in fruits (Abbasi et al., 2009). In the available literature, the use of citric acid effectively prevented H2O2 production and increased the susceptibility of both *Penicillium digitatum* and *P. expansum* in peel disks of citrus (Macarisin et al., 2007). This suggested that the CiA treatment effectively inhibited decay in fruits by enhancing their resistance to microbiological diseases. Vardar et al. (2012) showed that the CiA treatment remarkably reduced decay in strawberry fruits. Liu et al. (2016) indicated that CiA reduced the rate of decay in cherimoya, compared to the control. Perkins et al. (2017) reported that CiA effectively controlled postharvest decay in red bayberry. Our results in the present study showed that CiA significantly prevented decay, compared to the control. The mechanism by which CiA enhanced the biocontrol of decay may have resulted from the effects of CiA on pathogens. On the other hand, the acidification of fruit tissues with some postharvest treatments like organic acids may offer protection against postharvest decay, especially where alkalizing fungi are involved (Perkins et al., 2017).

Vitamin C is a primary bioactive compound that is susceptible to processing and storage. Our findings in the current study showed that CiA treatment effectively preserved VC during the storage period, compared to fruits of the control. Maximum VC values were retained by the effect of 3 mM CiA on fruits, probably because of less oxidative deterioration in fruits, and led to a smaller loss of VC. Similar results were obtained by Ansorena et al. (2014) when studying the effects of CiA on VC in broccoli. Pushkala et al., (2012) reported that carrots maintained more VC in response to chitosan coating when used in combination with the CiA pretreatment.

The increase of phenolic compounds in fruits may be due to the remarkable role of the CiA treatment which acts as a natural antioxidant in decreasing the respiration rate (Elad, 1992). On the other hand, CiA reportedly enhanced the activity of phenylalanine ammonialyase (PAL) which is the key enzyme responsible for phenolic biosynthesis in plants (Winkel-Shirley, 2001). Our results are in line with previous studies that showed how the CiA treatment enhanced total phenolic content and total flavonoids in apple fruits (Allahveran et al., 2018).

As an antioxidant, citric acid has anti-stress effects that protect and maintain antioxidant properties in plants and in their organs (Fayed, 2010). In the present research, an increase in antioxidant capacity by exogenous application of citric acid may be due to the remarkable role of CiA which acts as a natural antioxidant in increasing the antioxidant capacity of fruits, while enhancing vitamin C and phenolic compounds. Previous studies indicated a significant, positive correlation between antioxidant activity, vitamin C and phenol contents (Barzegar et al., 2018; Tulipani et al., 2008). Also, Allahveran et al. (2018) reported that foliar application of CiA significantly increased total antioxidant activity in apple fruits. Meanwhile, the results of the current study suggested that the CiA could be a potential postharvest treatment for improving the health benefits of peach fruits by increasing the antioxidant capacity.

Conclusion

In conclusion, the postharvest application of CiA could reduce decay and diminish WFL in peach fruits. Moreover, CiA treatment remarkably preserved the postharvest edibility of peach by maintaining good firmness and high TSS, TA, and VC levels. This research showed that the 3 mM CiA was the most effective treatment in alleviating disorders and in maintaining the nutritional quality of peach fruits during the postharvest period. Thus, CiA could be a promising treatment for the maintenance of postharvest edibility and for the extension of shelf life in peach fruits. Citric acid could be an alternative way to control postharvest loss and to avoid the negative effects of pesticides on human health, thereby allowing a decrease in the use of chemical agents.

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Conflict of interest

The authors indicate no conflict of interest for this work.

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