Effect of Pre-Harvest Salicylic Acid and Iron Treatments on Postharvest Quality of Peach Fruits

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Abstract
Peach is a highly corrosive fruit with a short shelf life (less than 7 days at room temperature) susceptible to diseases, pathogens and physical damage. The storage or marketable life of horticultural crops can be extended by various treatments applied to them after and/or before harvesting. Fruits are usually treated with a range of materials [(e.g. salicylic acid (SA)] to improve their appearance or delay deterioration. In the present study, effects of pre-harvest treatment of SA (1, 2, and 4 mM) and iron sequestrine (Fe) (5 and 10 mg L^{-1}) on antioxidant capacity, ion leakage, ethylene production, ascorbic acid and carotenoids content of peach fruit (*Prunus persica* L. Batsch cv. Za'ferani) were examined. The results showed that Fe and SA treatments are effective methods for alleviating ion leakage, weight loss and ethylene production in peach fruit during cold storage. Application of 4.0 mM SA and 10.0 mg L^{-1} Fe were the most effective treatments to maintain fruits quality parameters. SA and Fe treatments maintained peach fruits quality parameters until 40 days. These results suggest that the SA and Fe treatment are useful materials with potential postharvest application for reducing ethylene production, maintain quality, and improve the health benefits of peach fruit by increasing its antioxidant capacity.

Keywords: Postharvest, Antioxidant activity, Ethylene production, Ion leakage, Ascorbic acid, Peach.

Introduction

Peach fruit [*Prunus persica* (L.) Batsch] is considered as one of the most popular and common fruit in the world (Sansavini et al., 2006). Its origin is warm region of China. Peach delicious taste and pleasant flavor with high nutrition level have popularized it across the world (Layne and Bassi, 2008). It is one of the most important stone fruit of Iran with total cultivated area of 21989 ha with annual production of 422365 tones that has 7th rank in the world (FAO, 2017). It is a typical climacteric fruit that shows a dramatic increase in ethylene production and respiration rate during ripening (Serek et al., 1994). Peaches are highly perishable climacteric fruit and, would suffer rapid ripening and deterioration after postharvest, and thus have a limited postharvest life at room temperature. During storage time, peach fruit may undergo softening and rotting,

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which largely loss its quality. Storage at low temperature is the most common way to extend postharvest life and maintain quality of peach fruits (Nunes, 2008).

Today, the global challenges is how to find alternative ways to control postharvest losses, to prioritize healthy methods and avoid the negative effects of pesticides on human health, resistance to fungicides and reducing the use of chemicals. Salicylic acid (SA) or ortho-hydroxyl benzoic acid is an endogenous plant growth regulator of phenolic nature classified as a growth promoter. SA has been widely applied either at the preharvest or postharvest stages. SA is a safe chemical, used to control postharvest quantity or quality losses of perishable crops (Hayat et al., 2013). As shown by recent research reports, SA can improve physical properties of fruits such as size (Marzouk and Kassem, 2011), weight (Elwan and El-Hamahmy, 2009) and firmness (Srivastava and Dwivedi 2000; Zhang et al., 2003; Shafiee et al., 2010). In addition, SA was found to hasten maturity of both climacteric and non-climacteric fruits like strawberry (Karlidag et al., 2009) and tomato (Mady, 2009; Yildirim and Dursun, 2008). Furthermore, SA has positive effects on reducing fruit respiration, ethylene biosynthesis (Srivastava and Dwivedi, 2000), weight loss, and the softening rate (Babalar et al., 2007; Shafiee et al., 2010, Moradinezhad and Jahani, 2016) during storage. Exogenous application of SA was found to be effective in modeling of plant metabolic and physiological processes that may increase resistance to water deficit. SA application at various concentrations through roots, seed soaking and foliar spraying in a concentration-dependent manner alleviated the negative effect of water deficit on tissue water status, stomatal conductance, chlorophyll content, membrane properties and plants physiological activities (Horvath et al. 2007; Hayat et al. 2010). SA treatment increased phenylalanine ammonia lyase (PAL) activity and the concentration of

antioxidant compounds at 7 days post-treatment (Falcioni et al., 2014). Exogenous application of SA increases the antioxidant enzyme and plant resistance to abiotic stresses (Srivastava and Dwivedi, 2000). Exogenous application of SA enhances defense mechanisms and the production of antioxidants in fruits leading to the decrease in lipid peroxidation of cell membrane (Huang et al. 2008; Mo et al. 2008; Wei et al. 2011).

Iron (Fe) deficiency affects several physiological processes and therefore retards plant growth and plant yield. Control of Fe deficiency is often not effective enough and therefore lots of effort has been made to screen plants for Fe efficiency. The symptoms of Fe deficiency may occur at different Fe levels in plants, and this deficiency is highly dependent on soil, plant, nutritional, and climatic factors. The earlier symptom of Fe deficiency is interveinal chlorosis of young leaves (Kabata-Pendias and Pendius, 1999). Fe is a redox element for electron transfer of many complexes (e.g. cytochromes, peroxidases, and catalases). It is also required for chlorophyll synthesis (Marschener, 1986). Iron also plays an important role in the synthesis of rRNA and mRNA in chloroplast with a further effect on the synthesis of chlorophyll (Noort and Wallace, 1966). As an example of improving effects of Fe on horticultural products, it has been reported that total soluble solids (TSS) of onion bulbs will be improved by the use of Fe. This effect of Fe in treated bulbs may be attributed to enhance metabolic processes involved in biosynthesis of TSS such as carbohydrates, organic acid, amino acids and other inorganic constituents (Trivedi and Dhumal, 2013).

Due to susceptibility of peach fruits to losses and its storability problems, the aim of this study was to investigate the effect of pre-harvest treatment of Fe and SA on some quality characteristics of peach at postharvest conditions.

Materials and methods

Plant material and experimental design

Six-year-old peach trees (*Prunus persica* L. Batsch cv. Za'ferani) with the same size were selected from Imam Khomeini Institution's Educational Gardens (35.77 N, 50.94 E) in Karaj, Iran. Different concentrations of SA (0 as control, 1, 2 and 4 mM; Sigma Aldrich Co., USA) were prepared by dissolving the white crystalline powder in hot distilled water and 20 mL of ethanol for better solubility and bringing it to a final volume of 20 L with water and Tween 20® (Sigma Aldrich Co., USA) at the rate of 0.1% as a surfactant. Iron Sequestrine 138 from Artal Co., Spain (0 as control, 5 and 10 mg L^{-1}) were dissolved in distilled water and bringing to the final volume of 20 L with water. The prepared solutions were sprayed directly to the selected trees (5 L per tree) at 40 and 80 days after full bloom.

Peach fruits were harvested manually at commercial maturity and immediately transported to the postharvest laboratory of the College of Agriculture & Natural Resources of University of Tehran. Then, homogeneous fruits were selected from three replicates of five fruits and were used to determine the characteristics at the time of harvest. All fruits were stored at 1°C temperature and relative humidity of 85-90%. The sampling schedule was as follows: every 20 days 3 samples of five fruits from each treatment were taken at random and then the analytical determinations were performed. Fruit firmness, total soluble solids, total acidity, ethylene production and skin color were assayed individually in each fruit. Most of the analytical parameters were determined in all sampling dates. For ethylene production, color, ion leakage, antioxidant capacity and carotenoids data at day 0 and 60 were collected.

Determination of Quality parameters

Fruit flesh firmness was determined using a penetrometer with a 8 mm diameter plunger (PFT327, New Zealand) at two opposite points on the fruit's equator and the results were expressed as kg cm^2. TSS was measured using a refractometer (Brix TE-RM50B, Victoria, Australia). Titratable acidity (TA) was assessed by titration of 5 mL fruit juice from a sample made up of 3 fruits with 0.1 N NaOH until the organic acids were neutralized at pH 8.1-8.3 with a pH meter (3520 Bench pH Meters, UK). Results were expressed as a percentage of citric acid.

Determination of ethylene released from fruit

Ethylene production of peach fruits was determined on three replicates from the two fruits placed in a flask. It was determined following the method of Srivastava and Dwivedi (2000) with some modifications. Fruits were placed in a 1 L sealed jar, capped with a rubber stopper for 24 h and then one mL gas samples were taken from the headspace of the containers by syringe and ethylene concentrations measured by flame ionization gas chromatography using a Shimadzu gas chromatograph of model GC-14A (Shimadzu, Kyoto, Japan).

Determination of antioxidant capacity, ascorbic acid and carotenoids contents

The antioxidant capacity was measured using the DPPH method adapted from Brand-Williams et al. (1995). Briefly, 100 μL of the methanolic extract was added to 3.4 mL of fresh DPPH radical solution (98.9 μM in methanol) and centrifuged for 15 min at 9500 ×rpm in the dark at room temperature. The absorbance of the samples was measured at 515 nm after 1 h. Fruits antioxidant activity was calculated as:

$$\% AA = 1 - \frac{A517\ (Sample)}{A517\ (Control)} \times 100$$

For determination of carotenoids content, a known weight of the leaf tissue was extracted with 80% acetone and the absorbance recorded with a spectrophotometer (Lambda EZ 201, USA) at 510 and 480 nm according to the method of Arnon (1949).

Ascorbic acid of peach fruits was measured by titration with Iodine and potassium iodide (Majedi, 1994). Fruits vitamin C value was calculated as:

$$\frac{mg\ vitamin\ C}{100g\ expressed\ juice}$$

$$= \frac{used\ iodine\ solution\ \times 0.88\ \times 100}{5}$$

Weight loss and ion leakage

Separate samples of fruits in 3 replications of each treatment were kept for the evaluation of fruit weight loss (%) at the end of the experiment. Physiological loss in weight (PLW) was recorded by subtracting final weight from the initial weight of the fruits and then expressed as percent weight loss with reference to the initial weight.

The rate of ion leakage was determined as described by Mirdehghan et al. (2007b), in duplicate for each sample, using 6 discs (10 mm) of peel tissue (1.50 ±0.02 g) that were cut with a cork borer. Conductivity was measured after 4 h of incubation in 25 mL of 0.4 M mannitol under constant shaking, using a Crison conductivity meter (Met Rohm, 664). After readings were taken, the vials were autoclaved at 121 °C for 20 min, held for 24 h and conductivity was measured again for total electrolytes. The rate of electrolyte leakage was expressed as percentage of total:

$$Ion\ leakage = \frac{initial}{Total} \times 100$$

Statistical analysis

All statistical analyses were performed using SAS software package. The data were analyzed by one-way analysis of variance (ANOVA). Mean separations were performed by Duncan's multiple range tests and significant differences are given at $P < 0.05$.

Results

Peach quality parameters and weight loss

The results showed that fruit flesh firmness decreased significantly (Table 1) during storage at 1°C in all of treatments levels (Fe and SA applications). Fruits treatment with SA (Specially 1 mM concentration) was effective in slowing the decline in firmness and Pre-harvest treatments improved the retention of fruit firmness compared to the control (Fig. 1). Fruits treatment with Fe had no significant effect on firmness.

The results showed that fruits treated with all SA treatments had lower TSS than the control fruits. At storage period, regardless SA and Fe treatments, TSS significantly increased during time of storage from 0 to 60 days. There is no significant difference between 1.0 mM and 2.0 mM treatments of SA on fruit TSS contents (Fig. 1). However, iron treatment increased TSS of fruits. Fruit TSS was increased with increasing the Fe concentrations compared to the controls.

Titratable acidity (TA) is related to the organic acids content in fruits. Generally, TA and total organic acid content decline throughout ripening process. TA increased in the day 40 and then reduced until the day 60 (Fig. 1).

Table 1. ANOVA for dependent variables for salicylic acid (SA) and Fe treatments, storage time and their interactions for peach cv. Za'ferani fruits

	Storage time	Salicylic acid	Fe	SA × Time	Fe × Time
Fruit firmness	121[**]	1.58[ns]	1.77[ns]	0.74[ns]	0.88[ns]
Total soluble solids	391.54[**]	10.28[**]	22.35[**]	7.484[**]	3.132[ns]
Titratable acidity	0.2935[**]	0.1309[**]	0.1797[**]	0.0227[*]	0.0073[ns]
Weight loss	8456.9[**]	35.95[ns]	106.4[**]	19.4[ns]	16.26[ns]
Ethylene production	75.57[**]	44.55[**]	7.6[*]	0.12[ns]	5.68[*]
Antioxidant capacity	3015.47[**]	361.65[**]	339.97[**]	14.92[ns]	2.26[ns]
Ascorbic acid	170.9[**]	40.66[**]	22.32[**]	1.78[ns]	4.97[ns]
Carotenoids content	0.2[**]	0.0048[*]	0.028[**]	0.0114[**]	0.0186[**]
Electrolyte leakage	14701[**]	118.04[*]	547.53[**]	82.44[ns]	401.69[**]

[a] **, and * represent significance at the 0.01, and 0.05 levels, respectively, and NS represents non-significance at $P < 0.05$.
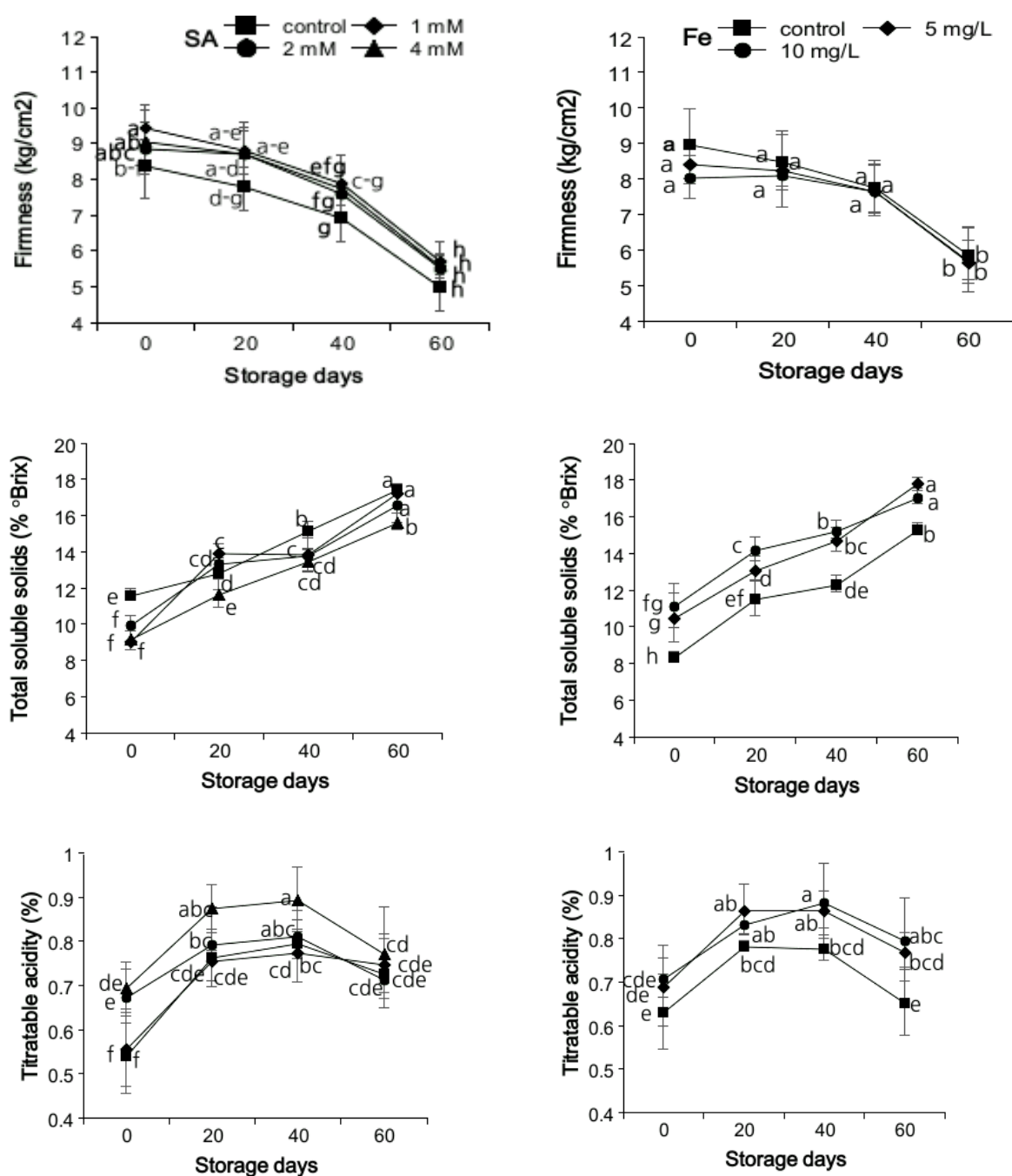
Fig. 1. Effects of different concentrations of salicylic acid (SA, left) and Fe (right) on firmness, soluble solids content and Titratable acidity of peach fruit (cv. Za'ferani) during 60 days of storage at 0 ± 1 °C. Vertical bars indicate ±SE of the mean values.

Percentage of weight loss was increased in all the treatments during the storage period. However, least weight losses were observed in 4.0 mM and then 1.0 mM SA treated fruits with respect to other SA concentrations during 60 days of storage (Fig. 2). However, Fe treatment had no significant effect on fruits water loss during cold storage with respect to control. Fruits treated with 10 mg L^{-1} of iron had lower effects than 5 mg L^{-1} treatment and the control but this reduction was not significant (Fig. 2).
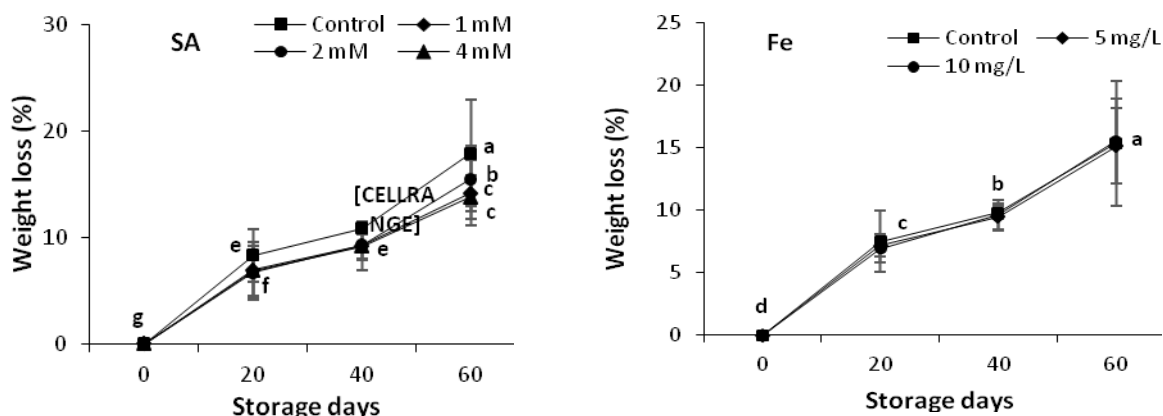
Fig. 2. Effects of different concentrations of salicylic acid (SA, left) and Fe (right) on weight loss of peach fruit (cv. Za'ferani) during 60 days of storage at 0 ± 1 °C. Vertical bars indicate ±SE of the mean values.

Ethylene production

Salicylic acid significantly affected fruit postharvest ethylene production (Table 1). The most effective SA concentration was 4.0 mM, which led to more than 50% reduction in ethylene production of peach fruits in comparison with the control fruits. Other SA concentrations (1.0 and 2.0 mM) were less effective than the 4.0 mM concentration (Fig. 3). Peach fruits ethylene production significantly increased with prolonging time of storage from 0 to 60 days.

Fe fertilization had no significant effect on ethylene production in peach fruits at the end of storage time (Fig. 3).
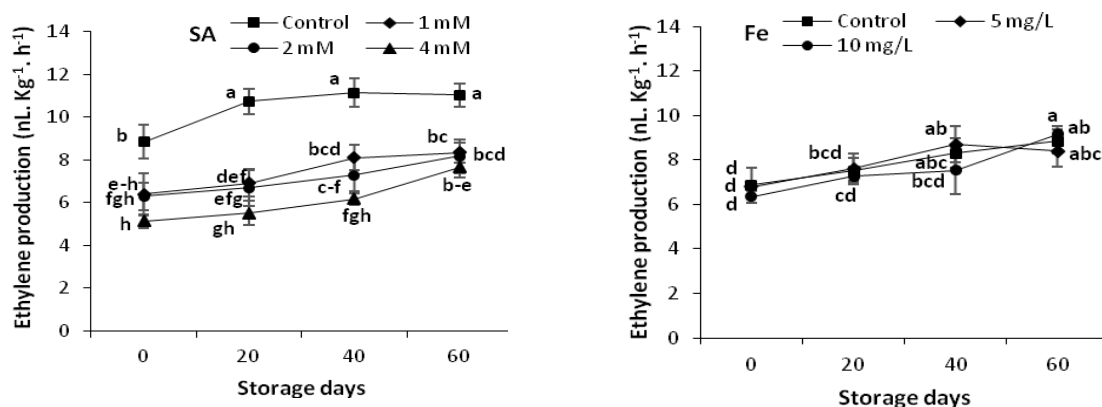

Fig. 3. Effects of different concentrations of salicylic acid (SA, left) and Fe (right) on Ethylene production of peach fruit (cv. Za'ferani) 60 days of storage at 0 ± 1 °C. Vertical bars indicate ±SE of the mean values.

Antioxidant capacity, ascorbic acid and carotenoids contents

The antioxidant activity decreased significantly during storage time. Antioxidant activity showed significant differences among the treatments (Table 1). For both SA and Fe treatments, at the initial of the storage period antioxidant activity was higher than their activity on the end of the storage period. Peach fruits treated with 4.0 mM SA and 10.0 mg L^{-1} Fe concentration were recorded with the highest antioxidant activity levels when compared to the controls (Fig. 4). However, lowest antioxidant activity was found in all of the control fruits during storage period.

Peach fruits ascorbic acid content decreased significantly during the storage period (Table 1). Among the applied SA treatments, higher concentration (4.0 mM) was found to be more effective in maintaining ascorbic acid content than the lower concentrations (1.0 mM and control).

After 60 days of storage, highest ascorbic acid content (12.71 mg/100 g FW) was recorded for the interaction between SA 4.0 mM and Fe 10 mg/L (Fig. 4).

The result showed that regardless of the treatments, carotenoids content of fruit increased during the storage period. SA treatments delayed the formation of carotenoid pigments compared to the control (Fig. 4). Control fruit showed rapid increase in total carotenoids content from initial day of storage. At the end of the experiment, total carotenoids content of SA-treated fruits was not significantly different from that of control fruits. However, at the end of the experiment, no significant difference was detected between treated and control fruits. This indicates that SA treatment did not hamper the synthesis of carotenoid pigments. In contrast, Fe treatments caused increase in the peach carotenoids content, especially at 40 and 60 days after storage (Fig. 4).
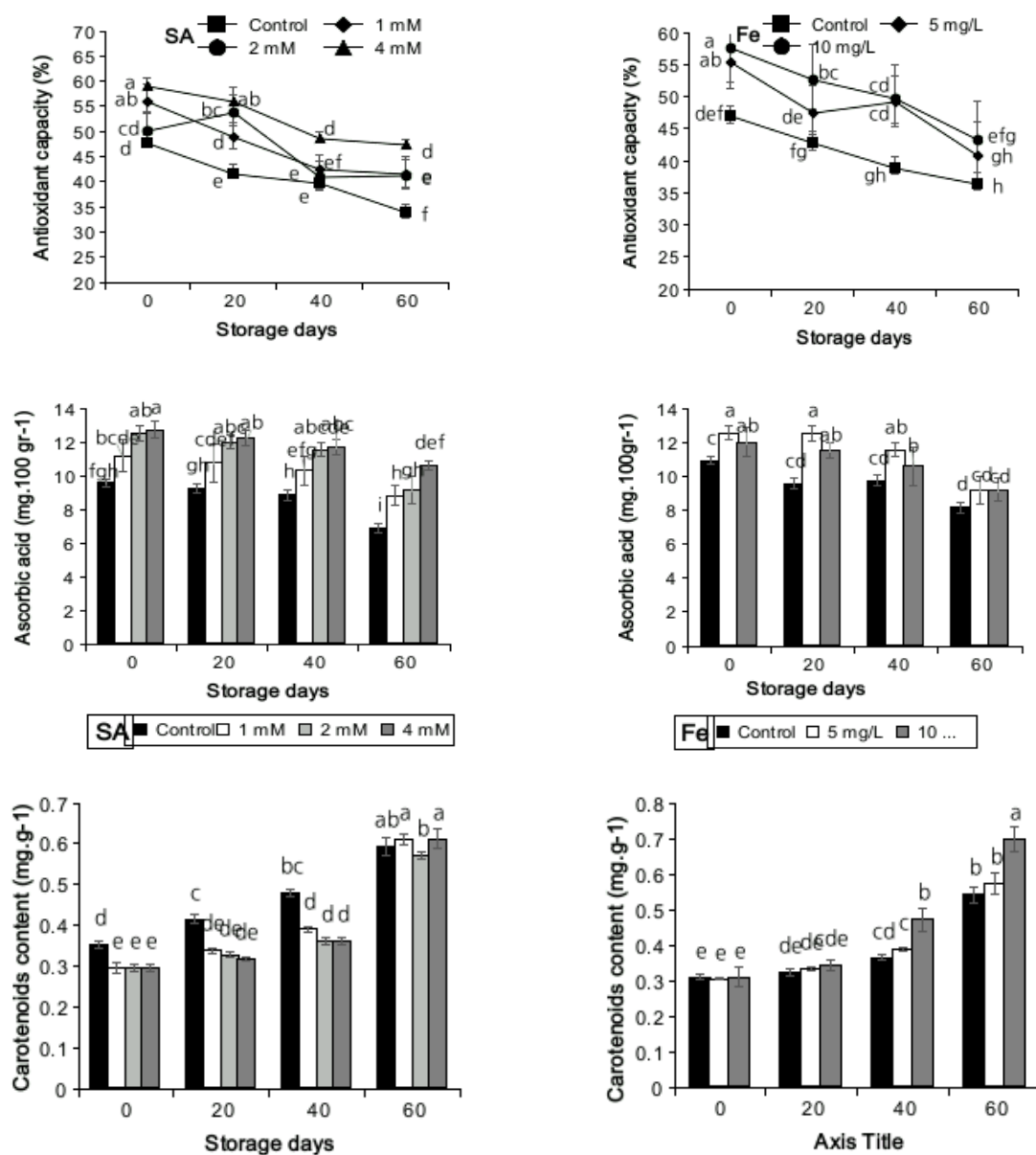

Fig. 4. Effects of different concentrations of salicylic acid (SA, left) and Fe (right) on antioxidant capacity, ascorbic acid and carotenoids contents of peach fruit (cv. Za'ferani) during 60 days of storage at 0 ± 1 °C. Vertical bars indicate ±SE of the mean values.

Fruits ion leakage

Membrane damage can be measured by the ion leakage. Fruits ion leakage in controls was significantly higher than in treated fruits. Ion leakage in peach fruits increased slightly during the initial 40 days of cold storage but increased sharply thereafter. Fruit treated with SA showed a similar trend, but SA treatment inhibited the leakage increase in respect of the control on day 60 (Fig. 5).

The most effective Fe concentration was 10.0 mg L^{-1}, which led to a reduction in ion leakage of peach fruits in comparison with the control fruit during the storage period except 20 days after onset of storage (Fig. 5).
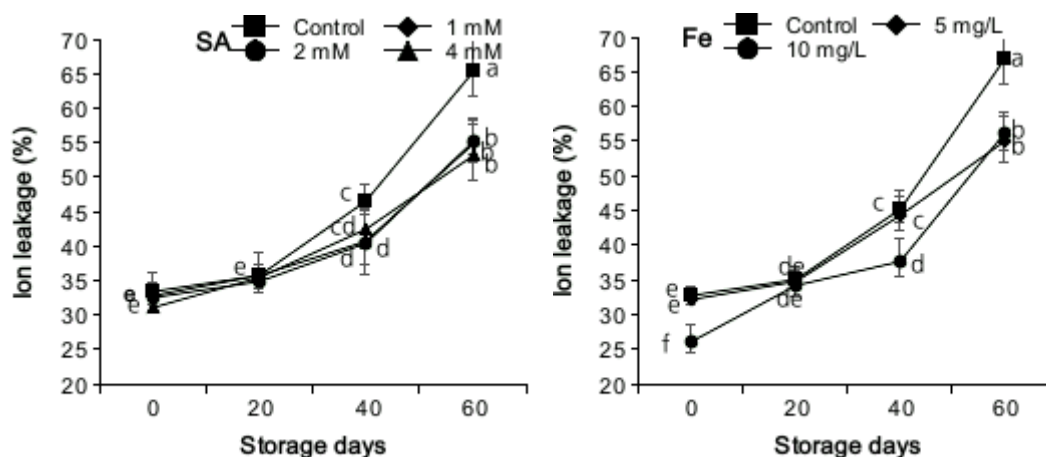

Fig. 5. Effects of different concentrations of salicylic acid (SA, left) and Fe (right) on ion leakage of peach fruit (cv. Za'ferani) 60 days of storage at 0 ± 1 °C. Vertical bars indicate ±SE of the mean values.

Discussion

SA preharvest treatments (especially at 1 mM concentration) were significantly effective in inhibiting the fruit firmness loss during the storage. Changes in cell wall components and its deterioration because of the tissue softening during fruits senescence is relate to the climacteric enhancement in ethylene production (Supapvanich and Tucker, 2011). As an ethylene inhibitor and antioxidant activator, SA delays the fruit ripening and prevents fruit softening by decreasing the activity of cell walls degrading enzymes and membrane lipid peroxidation (Srivastava and Dwivedi, 2000). Moradinejad and Jahani (2016) reported lower firmness of apricots as a result of fruit treatment with SA (0.5 mM) or $CaCl_2$ (2%, 19, and 26.3%).

In this study, TSS of control fruits was more than the treated fruits during storage. Generally, SA as an ethylene inhibitor could delay decline in total sugar content and maintains TSS in fruits during storage (Asghari and Aghdam 2010). SA inhibits the invertase activity and decrease in the level of reducing sugars and also delayed starch breakdown in banana fruits during ripening (Srivastava and Dwivedi, 2000). It has been reported that foliar Fe spray maintains the quality of March-harvested fruits in *Citrus* sp. through increases in fruits size and change in citric acid concentration, whereas fruits TSS increased significantly in tangerine (Pestana et al., 2000). Treatment of kiwifruit with SA maintains higher TA than the control fruit during storage (Kazemi et al., 2011a, b). Similarly, it has been reported that both pre- and postharvest SA treatments maintain higher TA of winter pineapple fruit than the control fruits (Lu et al., 2011). Fruits treatments with Fe improve juice contents, TSS, TA, color index, and other fruits quality parameters (Bañuls et al., 2003).

Fruit weight loss decreased during storage period, but the highest weight loss

was observed in the control fruits. SA (1 and 4 mM) considerably prevented fruits weight loss. Salicylic acid prevents normal respiration as a result decreases the weight loss (Shafiee et al. 2010). Therewith, fruit treatment with SA can reduce respiration rate by inhibition of ethylene action or biosynthesis (Srivastava and Dwivedi 2000). SA also can close the stomata and leads to decreased transpiration and fruits weight loss will be decreased.

SA in 4 mM concentration was better than the other concentrations and control, which led to lower ethylene production than control fruit. SA prevents elevation of ACC synthase transcripts and inhibits ethylene synthesis (Li et al. 1992). Similarly, it has been reported that SA delays the ripening of banana fruits, probably by inhibition of ethylene biosynthesis or its action (Srivastava and Dwivedi 2000).

The fruits antioxidant activity significantly reduced during storage period, which is in agreement with the findings of Habibi (2017) on four grape cultivars. They reported that fruit treatments with SA and Fe maintain antioxidant activity during storage. SA is one of the most important phenolic compounds that robust fruit defense system by boosting antioxidant enzymes biosynthesis (Huang et al., 2008). The amount of free radicals in peach plant that are faced with iron-deficiency are more than those that are grown in optimum conditions, therefore the amount of antioxidant content due to reacting and reducing these free-radicals decrease in plant tissues (Molassiotis et al., 2006).

The ascorbic acid content of peach fruits decreased significantly during the storage, further, 4.0 mM SA treatments was more effective than other treatments. SA treatment in 2 mM concentration was more effective than control in reducing ascorbic acid loss in the pomegranate husks based on the study of Sayyari et al. (2009). Similar results have been reported in the pulp of navel orange (*Citrus sinensis* L. Osbeck.),

that pretreatment with SA may result from an acceleration of biosynthetic pathways or a decrease in catabolism, through an accumulation of dehydroascorbate can leads to high ascorbic acid contents (Huang et al., 2008). Pila et al. (2010) demonstrated that application of SA (0.1 mM) to tomato fruits was beneficial for retarding degradation of ascorbic acid and maintaining the chlorophylls, which is in agreement with Turkyylmaz et al. (2005) who reported that foliar application of SA increased chlorophyll a and b contents in bean (*Phaseolus vulgaris*). Alvarez-Fernandez (2003a) shown that Fe chelate fertilization increases the total organic acids, which may be the reason for increased vitamin C concentration in peach fruits.

The lower initial value of carotenoids in SA-treated fruits might be due to delay in the ripening process by suppressing the ethylene evolution and delaying the ethylene climacteric peak (Barman and Asrey, 2014). Results from the present study are in agreement with reports on mango (Barman and Asrey, 2014) and sweet cherry (Giménez et al., 2014; Valero et al., 2011).

Ion leakage in treated fruits was significantly lower than control fruits, further, SA and Fe treatments were also effective in reducing ion leakage during storage. Retardation of ion leakage by SA treatment has also been reported by Sayyari et al. (2009) for pomegranates. One of the main causes of chilling injury and increase in the ion leakage is the production of activated oxygen species (Kang et al., 2003). It has been reported that SA reduces cell membrane damage by decreasing the activity of cell membrane oxidizing enzymes through increasing the integrity and decreasing the permeability of the cell membrane. To alleviate or prevent stress injury, plants have evolved mechanisms to scavenge these toxic and reactive species by antioxidant compounds and by enzymatic antioxidant systems, such as Superoxide dismutase (SOD),

catalases (CAT), peroxidases (POX) and ascorbate peroxidase (APX) (Wise, 1995). Some of these enzymes contain Fe, either in heme (CAT, POX) or non-heme (Fe-SODs) forms. Fe fertilization in optimum range can also increase photosynthesis and fruit quality.

In climacteric fruits, SA can slow down the ripening process during storage by suppressing and delaying ethylene production and maintaining the related parameters such as TSS, TA, and firmness (Srivastava and Dwivedi, 2000) in some fruits such as sugar apples (Mo et al., 2008). In kiwifruit, acetylsalicylic acid (ASA) pre-treatment before storage, either at 20 °C or 0 °C slows down the softening rate, which was positively associated with the free SA content since ASA applied to fruit will be immediately convert to SA (Zhang et al., 2003).

Conclusion

In conclusion, the data presented here showed that SA reduces weight loss and electrolyte leakage, delays postharvest ripening processes and enhances antioxidant potential by increasing or maintaining antioxidant compounds such as carotenoids and ascorbic acid in peach fruit. The SA and Fe concentrations of 4.0 mM and 10.0 mg L^{-1} were the most effective treatments for keeping fruits quality parameters such as ascorbic acid, antioxidant activity and carotenoids contents. SA and Fe treatments maintained peach fruits quality parameters until 40 days. However, quality and marketability of peaches at 60 days after storage was severely reduced.

Conflict of interest

The authors declare no conflict of interest for this study.

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