

Salicylic acid treatment of peach trees maintains nutritional quality of fruits during cold storage

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All relevant data are within the paper and its Supporting Information files.

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Abstract: Peach trees were treated with salicylic acid at 0 (control) and 1.5 mM at 15 days before harvest to study the impacts of salicylic acid on nutritional quality of peach fruits at harvest and during storage at 1°C for 28 days. Total phenols, flavonoids, and ascorbic acid contents were significantly higher in salicylic acid treated peach fruits after cold storage, leading to fruits with higher DPPH^{*} and FRAP radicals scavenging capacity. In addition, peach fruits treated with salicylic acid exhibited higher antioxidant enzymes catalase (CAT), ascorbate peroxidase (APX) and superoxide dismutase (SOD) activity after storage at 1°C for 2-4 week, leading to fruits with higher firmness and lower weight loss. Thus, salicylic acid treatment of peach trees could increase nutritional quality of peach fruits consumption, due to its effect on increasing antioxidant molecules, with additional effect on delaying the fruit postharvest senescence by increasing the ROS scavenging enzymes activity.

1. Introduction

Peach (*Prunus persica* L.) is highly perishable climacteric stone fruit and is a rich source of ascorbic acid, carotenoids, and phenolics that are good sources of antioxidants (Tomas-Barberan *et al.*, 2001). However, the rapid softening of fruit during storage at ambient temperature results in a short shelf-life of the commodity and reduced commercial fruit quality and consumer acceptance (Nunes, 2008). Due to its economic impact and also human health, great efforts have been done by researchers for delaying postharvest senescence of peach fruits during cold storage leading to fruits with higher sensory and nutritional quality by applying postharvest treatment such as modified and controlled atmosphere storage, heat treatment, glycine betaine, nitric oxide, brassinolide; 1-methylcyclopropene, methyl jasmonate, oxalic acid and salicylic acid (Cao *et al.*,

2010; Liu *et al.*, 2015; Kang *et al.*, 2016; Gao *et al.*, 2016; Razavi and Hajilou, 2016; Shan *et al.*, 2016; Yu *et al.*, 2016).

Fruit ripening with oxidative fact is associated with reactive oxygen species (ROS) such as superoxide radical (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH^\cdot) accumulation leading to membrane deterioration, lipid peroxidation and DNA mutation and ultimately economical quality and quantity losses of fruits results from deterioration of their cellular metabolism (Halliwell and Gutteridge, 1989). For overcome to oxidative stress during ripening which is crucial for delaying fruits deterioration and maintaining fruits sensory and nutritional quality, fruits cells employed an antioxidant system, superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) as enzymatic and ascorbate and glutathione, tocopherols, phenolics, flavonoids, alkaloids and carotenoids as non-enzymatic antioxidants (Apel and Hirt, 2004). SOD vocalizes the first line of ROS scavenging and catalyzes the dismutation of O_2^- to H_2O_2 . Then, H_2O_2 is eliminated by the actions of APX and CAT. APX dismutates H_2O_2 by conversion of AA to DHA (Foyer and Noctor, 2005). Due to extra ROS production and or incapable antioxidant system activity, fruits may encounter oxidative damage during ripening leading to quality losses. Thus, delaying fruits ripening and maintaining fruits quality, due to its economic impact and also human health, can be results from effective antioxidant system activity in fruits during ripening, which is achievable by using of environmentally friendly technologies such as salicylic acid (SA) as safe signaling molecule (Hodges *et al.*, 2004; Asghari and Aghdam, 2010; Kumar *et al.*, 2014), which have potential in delaying ripening, enhancing quality and attenuating biotic and abiotic stress of fruits (Asghari and Aghdam, 2010).

According to capability of SA in using as preharvest treatment, Gimenez *et al.* (2014) reported that sweet cherry fruits treated at preharvest with SA at 0.5 mM and ASA at 1 mM exhibited higher total phenolics and total anthocyanins, as well as higher hydrophilic and lipophilic antioxidant activity at commercial harvest (Gimenez *et al.*, 2014). Also, Gimenez *et al.* (2017) reported that sweet cherry fruits treated at preharvest with SA at 0.5 mM and ASA at 1 mM exhibited higher total phenolics and total anthocyanins, as well as higher hydrophilic antioxidant activity during storage at 2°C for 28 days. Also, cherry fruits treated at preharvest with SA and ASA exhibited higher antioxidant enzymes catalase (CAT), ascor-

bate peroxidase (APX) and superoxide dismutase (SOD) during storage at 2°C for 28 days. Valverde *et al.* (2015) reported that the sweet cherry fruits treated with preharvest 1 mM methyl salicylate exhibited higher total phenolics and anthocyanins content at harvest and during storage at 2°C for 28 days, leading to fruit with higher hydrophilic TAA (H-AA). Also, sweet cherry fruits treated with preharvest 1 mM methyl salicylate exhibited higher antioxidant enzymes CAT, APX and SOD activities during storage at 2°C for 28 days. Thus, salicylates treatment of sweet cherry trees enhances health boosting attributes of cherry fruits consumption, by increasing antioxidant molecules, with supernumerary impacts on delaying the sweet cherry fruits postharvest senescence by enhancing ROS scavenging enzymes activities (Gimenez *et al.*, 2017). It has been suggested that the preharvest salicylates treatments would have commercial fondness with low earnings costs and with considerable profits in fruit nutritional quality (Gimenez *et al.*, 2017). Moreover, postharvest treatment of peach fruit with SA at 2 mM enhanced higher levels of POD, CAT and SOD activities during cold storage as compared with control peaches, which were accompanied by lower polyphenol oxidase (PPO) activity. In addition, SA treated fruits exhibited higher firmness and radical scavenging activity (Tareen *et al.*, 2012).

Then, the aim of this research was to evaluate for the first time the impacts of preharvest SA treatment of peach trees on bioactive molecules and the antioxidant enzymes SOD, CAT, and APX activities at harvest and during cold storage.

2. Materials and Methods

Fruits and treatments

The experiment was carried out on 5-year-old peach [*Prunus persica* (L.) Batsch 'Anjiry maleki'] trees grafted on GF 677 rootstock, in a commercial orchard located in the north-west Iran. The trees were spaced at 6×5 m, receiving identical cultural practices and trained to an open vase system. Twelve trees were selected for uniform size and fruit load and sprayed with SA at concentrations of 1.5 mM on whole tree and control trees receiving only water. A surfactant (Tween-20) was added to each solution as a wetting agent for maximum SA absorption, and sprays were applied at 15-day before commercial harvest. Fruit from control and SA treated trees were

harvested at commercial maturity and immediately transported to the laboratory. The fruits were selected for uniform size, color and absence of mechanical damage, and then one group was analyzed 24 h after harvest and another groups stored at $1\pm 0.5^{\circ}\text{C}$ and 90% RH for 28 d. At 7-day intervals, 5 fruits from each of three replications were selected, and left for a further 24 h at 20°C (shelf-life), and subjected to physicochemical analysis.

Flesh firmness and weight loss

At each sampling date, flesh firmness (N) was measured on the opposite sides of the fruit after peel removal using an Effegi penetrometer (Model FT 011) equipped with an 8 mm diameter probe. To determine the weight loss, five fruits for each replicates were weighed at harvest and at 7 day intervals during cold storage. Results were expressed as percentage of weight loss relative to the initial fruit weight.

Antioxidant enzymes activity assays

Crude extract for APX enzymes was performed by homogenizing 1 g of frozen fruits tissue with 5 mL of phosphate buffer 100 mM, pH= 7.8 containing 1% (w/v) PVP, 1 mM EDTA and 5 mM ascorbic acid. The homogenate was centrifuged at 18,000 g for 10 min at 4°C and the supernatant used for enzyme assay. APX activity was determined by the method of Nakano and Asada (1987) with some modification. The reaction mixture consisted of 3 mL of 50 mM potassium phosphate, pH 7.0, 0.2 mM ascorbic acid, 0.2 mM EDTA and 0.5 mL of crude extract, and the reaction was allowed to start by adding 0.5 mL of 0.5 mM H_2O_2 . The decrease in absorbance at 290 nm was recorded spectrophotometrically for 3 min and APX activity expressed as U mg protein⁻¹.

Crude extract for CAT and SOD enzymes was performed by homogenizing 1 g of frozen fruits tissue with 3 mL of phosphate buffer 50 mM, pH= 7.8 containing 2% (w/v) PVP, 1 mM EDTA. The homogenate was centrifuged at 14,000 g for 20 min at 4°C and the resulting supernatant was used for enzyme assay. CAT activity was quantified following the method described by Zhang *et al.* (2013). The reaction mixture consisted of 50 mM phosphate buffer (pH 7), 15 mM H_2O_2 and 0.1 mL of crude extract in a final volume of 3 mL. Decreases in absorbance at 240 nm at intervals of 30 s were recorded spectrophotometrically. CAT activity expressed as U mg protein⁻¹. SOD activity was assayed according to the method described by Zhang *et al.* (2013). One unit of SOD activity was defined as the amount of enzyme that

causes a 50% inhibition of nitro blue tetrazolium reduction under assay conditions and the results were expressed as U mg protein⁻¹. Total protein content in the enzyme extract was assayed according to the method described by Bradford (1976).

Total phenolics, flavonoids and ascorbic acid contents

The amount of total phenolics in extracts was determined according to the Folin-Ciocalteu reagent method (Singleton and Rossi, 1965). Gallic acid was used as a reference standard, and the total phenolic contents of extract were expressed as mg gallic acid equivalent 100 g⁻¹ fresh weight (FW). Total flavonoids content was determined in accordance with a protocol described by Kaijv *et al.* (2006). A calibration curve was obtained using quercetin as a standard, and the results were expressed as μmol quercetin equivalent (QE) 100 g⁻¹ FW. Ascorbic acid content in the fruits was measured by 2,6-dichlorophenol indophenol dye method (AOAC, 1984). For each sample, 10 g fresh fruits pulp was homogenized with 3% metaphosphoric acid solution and the mixture was made up to 100 mL. An aliquot of 10 ml was then titrated against the dye (2,6-dichlorophenol indophenol) till the pink color appeared. Ascorbic acid content was estimated from a calibration curve of L-ascorbic acid and results expressed as mg ascorbic acid equivalents 100 g⁻¹ FW.

Total antioxidant activity

The FRAP assay was carried out using TPTZ (2,4,6-tripyridyl-Striazine) solution according to the procedure described by Benzie and Strain (1999). FRAP reagent was prepared freshly by mixing 2.5 ml of solutions TPTZ (10 mM, dissolved in 40 mM HCl) and FeCl_3 (20 mM) in 25 ml of acetate buffer (300 mM concentration and 3.6 pH). A 50 μL of the diluted sample was added to 1.5 mL of FRAP reagent. The absorbance of the mixture was measured at 593 nm after 4 min. incubation at 37°C using a UV-visible spectrophotometer (T-60, PG Instrument UK). A calibration curve was built using a standard solution of FeSO_4 and the FRAP values of extract were expressed as mmol Fe(II)/g fresh weight.

The method of Dehghan and Khoshkam (2012) was used for measuring the DPPH radical scavenging ability of peach extracts. The amount of 50 μL of peach extract was allowed to react with 1.95 mL of DPPH radical solution (0.1 mM in methanol) for 30 min. The decrease in absorbance from the resulting solution (AS) was monitored at 517 nm in a UV-visible spectrophotometer (T-60, PG Instrument UK). Absorbance of the blank solution of DPPH (2 ml) was

used as an experimental control (AC). The radical scavenging activity (RSA %) of the peach fruits extracts was calculated according to the following formula:

$$\text{RSA \%} = \frac{100 (\text{Ac-As})}{\text{Ac}}$$

Statistical analysis

The experiment was performed using a factorial design with SA treatment and storage time as the two factors. Differences among means of data were analyzed by Duncan's test at $p \leq 0.05$ ($n=3$). All statistical analyses were performed with SPSS version 20.0.

3. Results and Discussion

As shown in figure 1, fruits weight loss increased and fruits firmness decreased during cold storage in control and treated fruits, but fruits weight loss was significantly lower ($P < 0.05$) and fruits firmness were significantly higher ($P < 0.05$) after storage at 1°C for 2-4 weeks in peach fruits coming from SA treated trees than in controls (Fig. 2). Cell wall degradation by cell wall hydrolases such as polygalactosidase (PG), pectin methyl esterase (PME), β -galactosidase (β -Gal) and xylanase along with cell membrane deterioration led to fruits softening that are associated with climacteric rise in ethylene production (Srivastava and Dwivedi, 2000). Zhang *et al.* (2003) reported that kiwifruit treated with acetyl salicylic acid exhibited lower ethylene biosynthesis during fruit ripening, results from lower ACC oxidase (ACO) and ACC synthase (ACS) activity. Kiwifruits during postharvest softening of at 20°C exhibited lower endogenous SA content, which was concurrent with higher LOX activity and higher ethylene production.

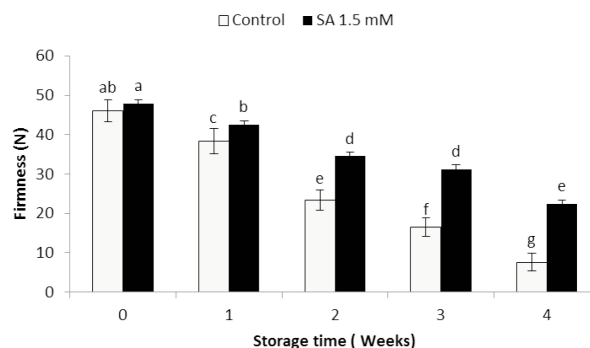


Fig. 2 - Firmness of peach fruits treated with preharvest SA at 1.5 mM stored at $1 \pm 0.5^\circ\text{C}$ for up to 28 days. Data shown are mean \pm standard deviation of three replicate ($n = 3$).

LOX by generation of O_2^- have a regulatory role in ethylene biosynthesis via a contribution in ACC conversion to ethylene (Xu *et al.*, 2000). Kiwifruit treated with acetyl salicylic acid exhibited higher endogenous SA accumulation associated with lower LOX activity, O_2^- accumulation, and ACS and ACO activities and eventually delayed ethylene biosynthesis. Zhang *et al.* (2003) suggested that the higher fruits endogenous SA accumulation leads to lower ethylene biosynthesis and higher firmness. Also, Srivastava and Dwivedi (2000) reported that SA treatment delayed banana fruits ripening, results from lower ethylene biosynthesis due to lower ACS and ACO enzymes activity. They also reported that SA treatment maintains fruits firmness, results from lower PG, xylanase and cellulase activity. Maintaining firmness in peach fruits treated with SA may be result of directly inhibition of cell wall degradation enzymes activity, indirectly decreasing ethylene production, and also higher firmness in peach fruit treated with SA could be attributed to endogenous SA accumulation which lead to lower LOX activity and ROS accumulation. Loss of weight in stored peach is mainly due to evaporation of water from the fruits and becomes apparent as shriveling. The lower weight loss in peach fruits treated with SA could be attributed to stabilization of cell membrane as well as cell wall integrity and the permeability of tissues.

Maintaining ascorbic acid content in fruits during postharvest ripening is crucial for human health, due to antioxidant function of ascorbic acid and also for human disability for ascorbic acid synthesis (Davey *et al.*, 2000; Hassanpour *et al.*, 2011). As shown in figure 3, ascorbic acid content decreased in control and treated fruits during storage at 1°C for 28 days, but ascorbic acid content was significantly higher during cold storage in SA treated peach fruits than in controls ($P < 0.05$) (Fig. 3). Huang *et al.* (2008) reported

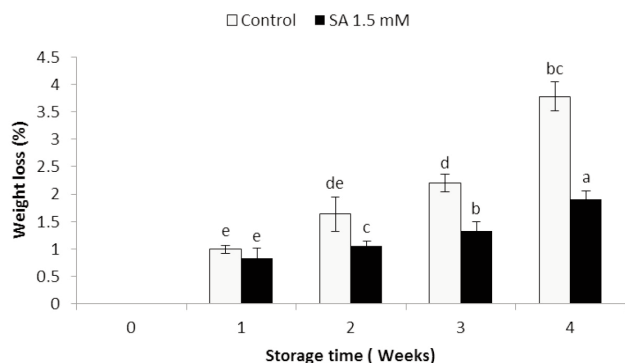


Fig. 1 - Weight loss of peach fruits treated with preharvest SA at 1.5 mM stored at $1 \pm 0.5^\circ\text{C}$ for up to 28 days. Data shown are mean \pm standard deviation of three replicate ($n = 3$).

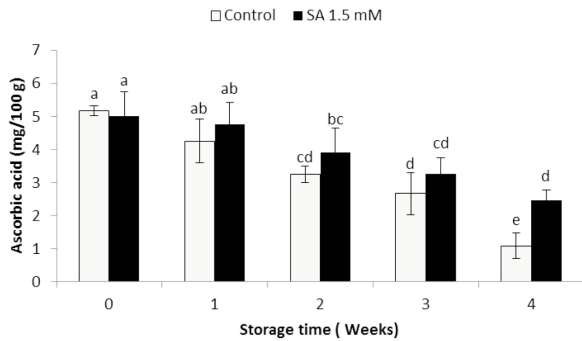


Fig. 3 - Ascorbic acid content of peach fruits treated with preharvest SA at 1.5 mM stored at $1\pm 0.5^\circ\text{C}$ for up to 28 days. Data shown are mean \pm standard deviation of three replicate (n = 3).

that navel orange fruit treated with SA exhibited higher ascorbic acid content, results from increasing cytosolic Ca^{+2} , enhancing GR enzyme activity, which in turn could increase GR/APX system activity leading to higher ascorbate/dehydroascorbate (AA/DHA) and glutathione/glutathione disulfide (GSH/GSSG) ratios. Also, Rao *et al.* (2011) reported that the sweet pepper treated with SA and CaCl_2 exhibited higher ascorbic acid content, which results from lower ascorbic acid oxidase (AAO) enzyme activity. Higher AA content in peach fruits treated with SA may be attributed to higher GR/APX system activity due to increase of cytosolic Ca^{+2} concentrations and/or lower AAO enzyme activity.

Enhancing phenols accumulation in fruits during postharvest ripening is crucial not only due to their contribution in nutritional quality attributes of fruits such as color, astringency, bitterness and flavor, but also phenols are superior antioxidants and display ROS scavenging activity (Hassanpour *et al.*, 2011). Due to phenols ROS scavenging capacity and their function in decreasing low-density lipoproteins (LDL), consumption of fruits with higher phenols would be associated with lowered risk of heart disease (Vinson *et al.*, 2001). As shown in figure 4 and 5, total phenols and flavonoids contents were significantly higher after storage at 1°C for 2-4 week in SA treated peach fruits than in controls ($P < 0.05$).

It has been reported that sweet cherry fruits treated at preharvest with SA at 0.5 mM and ASA at 1 mM exhibited higher total phenolics and total anthocyanins, as well as higher total antioxidant activity at commercial harvest (Gimenez *et al.*, 2014) and during storage at 2°C for 28 days (Gimenez *et al.*, 2017). Also, Valverde *et al.* (2015) reported that the total phenolics and anthocyanins content were significantly higher in methyl salicylate treated sweet cherry

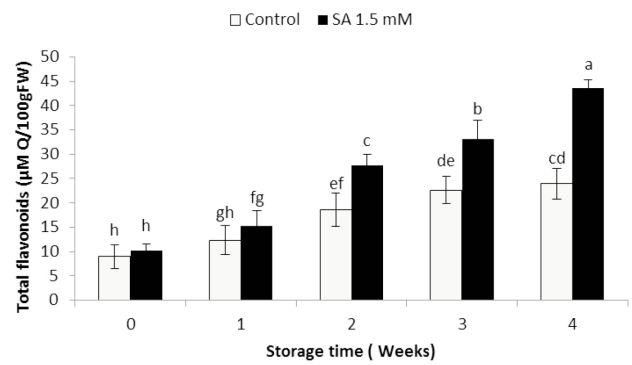


Fig. 4 - Total flavonoids content of peach fruits treated with preharvest SA at 1.5 mM stored at $1\pm 0.5^\circ\text{C}$ for up to 28 days. Data shown are mean \pm standard deviation of three replicate (n = 3).

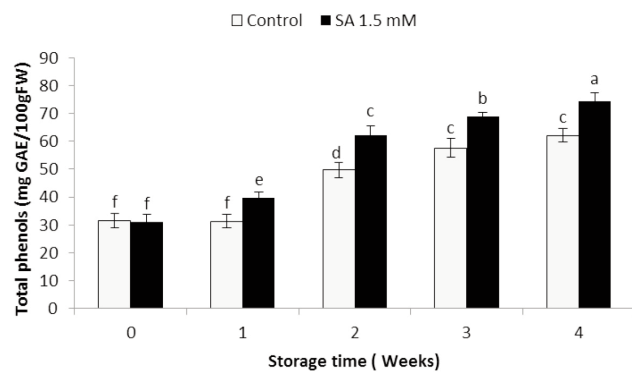


Fig. 5 - Total phenolics content of peach fruits treated with preharvest SA at 1.5 mM stored at $1\pm 0.5^\circ\text{C}$ for up to 28 days. Data shown are mean \pm standard deviation of three replicate (n = 3).

fruits at harvest and during storage at 2°C for 28 days, leading to fruits with higher hydrophilic TAA (H-AA). Due to higher PAL enzyme activity in cornelian cherry fruits treated with SA and CaCl_2 , which was associated with higher total phenols, flavonoids and anthocyanins accumulation (Aghdam *et al.*, 2013; Dokhanieh *et al.*, 2013), it can be postulated that the higher total phenols and flavonoids contents in SA treated peach fruits may attributed to higher PAL activity. Wang *et al.* (2015) reported that the apricot fruits treated with SA exhibited higher total phenols and flavonoids accumulation and higher hydrophilic antioxidant capacity. Higher hydrophilic antioxidant capacity in apricot fruits treated with SA was associated with higher PAL enzyme activity. Also, apricot fruits treated with SA exhibited higher SOD enzyme activity and lower CAT and APX enzymes activity, which leads to lower O_2^- and higher H_2O_2 accumulation. H_2O_2 as second messenger can activate PAL enzyme activity, as a key enzyme in phenylpropanoids pathway, and ultimately higher total phe-

nols and flavonoids accumulation (Wang *et al.*, 2015). Since peach fruits treated with SA exhibited higher CAT and APX enzymes activity, higher total phenols and flavonoids contents cannot be attributed to higher H₂O₂ accumulation.

As shown in figure 6 and 7, DPPH and FRAP scavenging capacity of the peach fruits treated with SA were significantly enhanced during storage at 1°C for 28 days ($P < 0.05$), showing that SA treatment stimulated the scavenging capacity of the peach fruits on DPPH and FRAP radicals, which may be results from higher total phenols and flavonoids accumulation (Razavi and Hajilou, 2016). Dokhanieh *et al.* (2013) and Aghdam *et al.* (2013) reported that the cornelian cherry fruits treated with SA and CaCl₂ exhibited higher total phenols, flavonoids, and anthocyanins accumulation results from higher PAL enzyme activity as key enzyme in phenylpropanoid pathway which is responsible for antioxidant molecules biosynthesis. We proposed that SA treatment may stimulate the accumulation of phenol, and flavonoid in the peach fruits by activating phenylpropanoid pathway. Higher

DPPH and FRAP scavenging capacity of the peach fruits treated with SA may be results from higher total phenols and flavonoids accumulation due to higher PAL enzyme activity concurrent with higher ascorbic acid accumulation due to higher cytosolic Ca²⁺ and or lower AAO enzyme activity.

As shown in figure 8, 9 and 10, antioxidant enzymes CAT, SOD and APX activity increased during storage at 1°C for 28 days in control and treated fruits, and SOD and APX activities were significantly higher during all the storage period at 1°C for 28 days in SA treated peach fruits than in controls ($P < 0.05$), while concerning CAT, higher activity was observed only after 3-4 weeks of storage. Valverde *et al.* (2015) reported that sweet cherry fruits treated at preharvest with methyl salicylate exhibited higher antioxidant enzymes CAT, APX and SOD during storage at 2°C for 28 days. Gimenez *et al.* (2017) reported that sweet cherry fruits treated at preharvest with SA at 0.5 mM and ASA at 1 mM exhibited higher antioxidant enzymes CAT, APX and SOD during storage at 2°C for 28 days. Thus, salicylates treatment of peach trees enhances health boosting attributes of peach fruits consumption, by increasing antioxidant

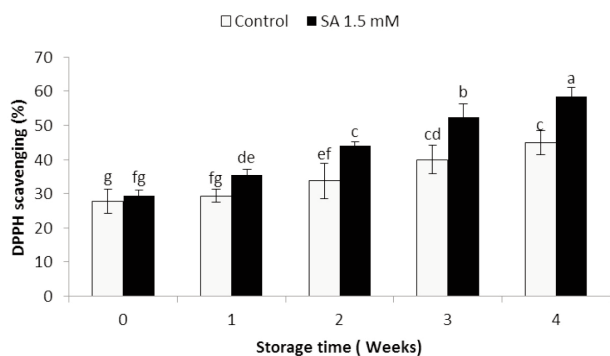


Fig. 6 - DPPH scavenging capacity of peach fruits treated with preharvest SA at 1.5 mM stored at 1 ± 0.5°C for up to 28 days. Data shown are mean ± standard deviation of three replicate (n = 3).

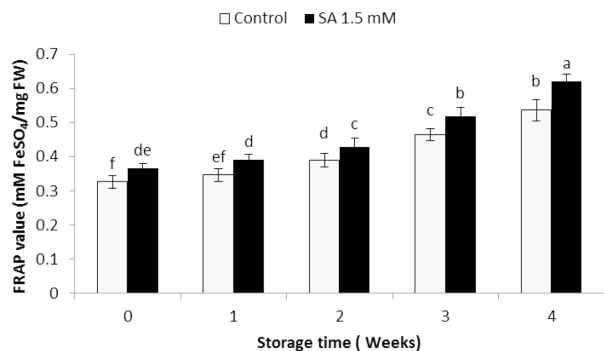


Fig. 7 - FRAP scavenging capacity of peach fruits treated with preharvest SA at 1.5 mM stored at 1 ± 0.5°C for up to 28 days. Data shown are mean ± standard deviation of three replicate (n = 3).

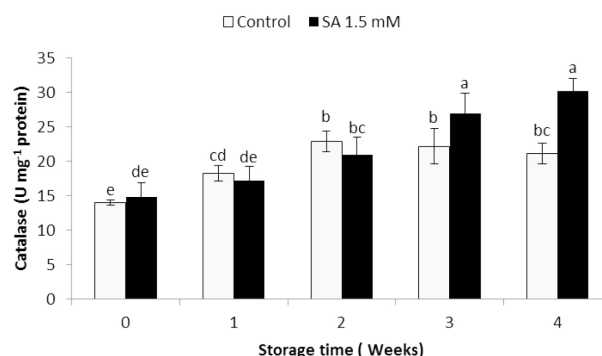


Fig. 8 - CAT activity of peach fruits treated with preharvest SA at 1.5 mM stored at 1 ± 0.5°C for up to 28 days. Data shown are mean ± standard deviation of three replicate (n = 3).

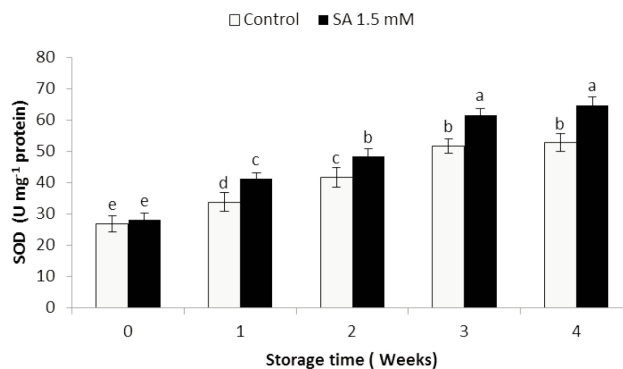


Fig. 9 - SOD activity of peach fruits treated with preharvest SA at 1.5 mM stored at 1 ± 0.5°C for up to 28 days. Data shown are mean ± standard deviation of three replicate (n = 3).

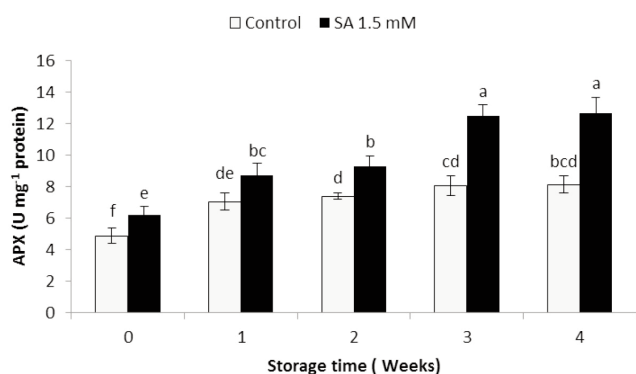


Fig. 10 - APX activity of peach fruits treated with preharvest SA at 1.5 mM stored at $\pm 0.5^{\circ}\text{C}$ for up to 28 days. Data shown are mean \pm standard deviation of three replicate ($n = 3$).

molecules, with supernumerary impacts on delaying the peach fruits postharvest senescence by enhancing ROS scavenging enzymes activities. Higher antioxidant enzymes activity, together with higher antioxidants molecules accumulation, in peach fruits during storage, as a results of preharvest SA treatment, could contribute to ROS scavenging during the postharvest ripening, which in turn, leads to delaying peach fruits postharvest ripening and senescence. SA enhance antioxidant systems activity by avoiding and/or scavenging ROS, which led to decrease oxidative stress during peach fruits ripening and ultimately maintain postharvest quality by prevention of adverse effects of ROS on fruits quality.

4. Conclusions

SA, as safe signaling molecule, could enhance nutritional quality and improve health promoting attributes of peach fruits consumption. In addition, the increase in antioxidant enzymes by SA preharvest treatment may result in a high ROS scavenging potential, and in turn in delaying senescence process leading to the preservation of fruits quality attributes.

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