JOURNAL OF HORTICULTURE AND POSTHARVEST RESEARCH
2022, VOL. 5(1), 79-92

Journal of Horticulture and Postharvest Research

Journal homepage: www.jhpr.birjand.ac.ir

University of Birjand

Exogenous salicylic acid preserves the quality and antioxidant metabolism of avocado 'Quintal' cultivar

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ARTICLE INFO

Original Article

Article history:
Received 28 October 2021
Revised 12 January 2022
Accepted 28 January 2022
Available online 12 February 2022

Keywords:
Antioxidant metabolism
Avocado
Physicochemical
Postharvest
Salicylic acid

DOI: 10.22077/jhpr.2022.4820.1249
P-ISSN: 2588-4883
E-ISSN: 2588-6169

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ABSTRACT

Purpose: This work aimed to evaluate whether salicylic acid (4.0 mM) is able to preserve the quality and antioxidant metabolism of avocado fruit 'Quintal' cultivar. **Research method:** 'Quintal' avocados harvested at physiological maturity were immersed in salicylic acid (SA) solution (0 and 4.0 mM) for 15 minutes at 25 ºC and stored at 15 ºC and 85 % RH for 16 days. The physical-chemical and biochemical quality parameters of the fruits were evaluated every four days. **Findings:** The treatment with SA (4.0 mM) reduced/delayed respiratory activity by up to two days compared to untreated fruits (control). There was also less loss of fresh mass, firmness, sugar synthesis (soluble solids), and degradation of organic acids (titratable acidity). In antioxidant metabolism, SA preserved the activity of phenylalanine ammonia lyase resulting in higher phenolic content, antioxidant activity, and lower polyphenoloxidase activity at the end of 16 days. **Research limitations:** One of the main limitations of this research is that ethylene production (a key player for climacteric fruit) was not measured in Avocado fruits during storage. **Originality/Value:** In addition to being the first report on the application of SA in postharvest avocados, our results demonstrate that SA (4.0 mM) is an effective and low-cost alternative to preserve the quality and antioxidant potential of avocados 'Quintal'.

INTRODUCTION

Avocado (*Persea americana* L.) is a tropical fruit originating from the American continent, with Mexico and Guatemala as centers of diversity (Fischer et al., 2011) and presents in its composition important vitamins, minerals, proteins, fibers, high content of fatty acids and bioactive compounds (Duarte et al., 2016; Piazza et al., 2018; Kassim & Workneh, 2020).

Avocado is mainly marketed in its fresh form, however, as it is a climacteric fruit, its high respiratory rate and ethylene production after harvesting give it high perishability under environmental conditions (Russo et al., 2013; Defilippi et al., 2018). Thus, controlling the changes related to ripening is essential to maintain the quality and antioxidant potential of the fruit, which is reflected in the extension of the shelf life, especially when the objective is to reach more distant markets (Woolf et al., 2020; Munhuweyi et al., 2020).

Among the various existing technologies to preserve fruit quality during storage, salicylic acid (SA) is an effective and low-cost method whose postharvest benefits have already been mentioned in) several horticultural crops (Aghdam et al., 2016).

This SA conservation potential is due to its signaling role capable of reducing the activity of ACC oxidase, a precursor enzyme for ethylene synthesis (Taiz et al., 2017), ie, the hormone responsible for ripening/senescence in fruits. Other benefits include maintenance of pulp firmness through inhibition of enzymes responsible for cell wall degradation and activation of antioxidant metabolism through its involvement in aquiring systemic resistance (Ding & Ding, 2020).

Despite the benefits associated with salicylic acid in the scientific community, there are no reports of its application in avocados. Thus, this work aims to investigate whether exogenous application of SA can preserve the quality and antioxidant metabolism of 'Quintal' avocados during refrigerated storage.

MATERIALS AND METHODS

Plant material

Fruits of avocado 'Quintal' cultivar were harvested at physiological maturity, with an average weight of 650g and transported in plastic boxes to the Product Technology Laboratory of the Federal University of Pará, Campus Altamira, Pará, Brazil. In the laboratory, the fruits were selected for the absence of physiological defects, mechanical injuries, or pests or diseases, washed in running water, sanitized in 5 $mg.L^{-1}$ sodium hypochlorite solution for five minutes, and then dried under benches at room temperature.

Salicylic acid treatment and storage

Prior to the installation of the experiment, preliminary tests were carried out with concentrations of salicylic acid - SA (Sigma-Aldrich, Brazil) ranging from 1.0 to 4.0 mM. The 4.0 mM concentration was chosen due to the best results regarding the physicochemical quality of the fruits (less loss of fresh mass, greater firmness and soluble solids content) during the storage period. The fruits were submerged in an aqueous solution of SA (4.0 mM) and 0.5 mL L^{-1} of Tween-20 as a surfactant for 10 minutes at 25 ± 2 ºC. Untreated fruits (control) were submerged in distilled water for an equal period. After the immersion time, all fruits were allowed to air dry at room temperature and then stored in a cold chamber (15 ± 1 ºC) and 85 ± 1% relative humidity (RH) for 16 days. The physicochemical and biochemical analyzes were performed at intervals of every four days.

Physicochemical and biochemical analyzes

Respiratory activity
It was calculated from a curve obtained by daily fruit evaluation. The determination of respiration rate was performed and quantified by respirometer by the measurement of released CO_2, according to methodology adapted from Bleinroth et al. (1976).

Fresh weight loss (FWL)
FWL was calculated based on the change in fruit mass after different storage periods by weighing the mangoes on a semi-analytical balance with an accuracy of 0.01 g (Mars, model AS 2000, São Paulo, Brazil). The results are expressed as percentages (%).

Firmness
Mesocarp (pulp) firmness was evaluated after removing two regions of epicarp on opposite sides of five replicate fruit, using a texturometer (Effegi Fruit Tester, Italy) equipped with an 8 mm tip. The results are expressed in $g.F^{-1}$ as described by Watkins and Harman (1981).

Soluble solids content (SSC)
It was quantitated using liquid samples obtained by pressing 10 g of pulp with a digital refractometer (Alpha, Atago Co., Ltd, Japan). Results were expressed as percentages (%) (AOAC, 2016).

Titratable acidity (TA)
TA was determined by titrating 10 g of pulp with 0.1 N NaOH, using 0.1 % phenolphthalein as an indicator. The results are expressed as gram equivalents of citric acid per kilogram of pulp (g 100 g^{-1} citric acid) (AOAC, 2016).

pH The pH of the fruit pulp was determined using a pH meter (Orion 3 Star, Thermo Scientific, USA) (AOAC, 2016).

Sample preparation
Pulp (50 g) were homogenized, frozen in liquid nitrogen, and stored at -20 °C, and ground in a ceramic mortar and pestle to obtain a fine powder for analysis of antioxidant metabolism. All analyzes were performed in triplicate.

Total phenolic compounds
It was determined by the Folin-Ciocalteau spectrophotometric method described by Singleton et al. (1999), using gallic acid as a standard. The extract was obtained using 1 g of pulp. In test tubes, an aliquot of 0.5 ml of the extract was added to 2.5 mL of Folin Ciocalteau reagent and 2 mL of 4% sodium carbonate and left to rest for 2 hours, protected from light. Absorbance was measured in a spectrophotometer at 740 nm. A blank sample was conducted under the same conditions. Results were expressed in µg GAE 100 g^{-1} fresh weight.

Antioxidant activity
It was evaluated by the DPPH method (1,1-diphenyl-2-picrylidrazil) according to Mensor et al. (2001) with modifications. The pulp (5 g) was extracted with 5 ml of hexane after 2 minutes of vortexing (VX – 38, Warmnest, Brazil). The obtained extract (0.5 ml) was homogenized with 3 ml of 99 % ethanol and 0.3 ml of DPPH. The blank was composed of 3 ml 99 % ethanol, 0.3 ml 80 % ethanol, and 0.3 ml DPPH. After 45 minutes of rest in the dark,

the reading was performed in a spectrophotometer (Shimadzu, UV-1280, Japan) at 517 nm. Results were expressed in % Trolox equivalents, based on a Trolox calibration curve with concentrations ranging from 0 to 200 µM.

Phenylalanine ammonia-lyase assay

The extraction was performed according to the method proposed by Mori (2001). In an ice bath, the pulp (2.0 g) was homogenized with 4 mL of 10 mM Tris-HCl buffer (pH 8.4), containing EDTA and polyvinylpyrrolidone (PVP), followed by centrifugation (Thermo Scientific, ST16-R, USA) at 10,000 x g for 10 minutes at 4°C. The reaction mixture was composed of 5 µl of βmercaptoethanol, 25 µl of extract, 145 µl of 100 mM Tris-HCl buffer (pH 8.4), and 50 µl of 40 mM L-phenylalanine. After 1 hour of incubation in a water bath at 30 °C, the reaction was stopped by the addition of 25 µL of 6 M HCl. The absorbance was determined at 290 nm and a standard curve was constructed based on the amount of trans-cinnamic acid formed and the results were expressed in µmol ac.transcin.h^{-1}.mg^{-1}P.

Polyphenoloxidase assay

The extraction and determination of polyphenol oxidase (PPO) were performed according to the method of Cano et al. (1997) with adaptations. The extract was obtained by weighing 5 g of pulp and homogenizing with 10 ml of 100 mM sodium acetate buffer followed by centrifugation (Thermo Scientific, ST16-R, USA) for 20 minutes at 15,000 xg at 4 °C. In test tubes, 0.3 mL of extract and 1.85 mL of catechol (0.1 M) were added. The blank was prepared with 1.85 ml of catechol (0.1 M), 0.8 ml of perchloric acid, and 0.3 ml of the extract. After 30 minutes in a water bath at 30 ºC, the reaction was stopped with the addition of 0.8 mL of perchloric acid, and the reading was performed in a spectrophotometer (Shimadzu, UV-1280, Japan) at 395nm. The results were expressed as EAU.min^{-1}.mg^{-1}P of fresh weight.

The protein content was determined using the Bradford (1976) method with bovine serum albumin as the standard.

Experimental design and statistical analysis

The experimental design adopted was completely randomized in a 2x5 factorial arrangement, with two treatments (with and without salicylic acid) and five storage times (0, 4, 8, 12, and 16 days) with five replications and the experimental plot consisting of three fruits. Data were subjected to analysis of variance and means were compared by the Tukey test ($p<0.05$) of probability using the R software (R Core Team, 2020).

RESULTS AND DISCUSSION

Treatment with salicylic acid (SA) delayed the respiratory activity of fruits by two days compared to the control. In the control fruits, the peak of respiratory activity occurred on the fifth day of storage (0.45 mL of CO^2 Kg^{-1} h^{-1}), while the fruits treated with SA were on the seventh day (0.42 mL of CO^2 Kg^{-1} h^{-1}) (Fig. 1).

The delay in the climacteric peak of fruits treated with SA may be related to the inhibition of ethylene synthesis in these fruits. According to Shi et al. (2021), the application of exogenous SA affects the expression of genes related to ethylene biosynthesis, such as 1-aminocyclopropane-1-carboxylic acid (ACC) synthase (ACS) and ACC oxidase (ACO) resulting in delayed maturation and senescence of pears.

The fresh weight loss gradually increased with storage time, being significantly higher ($p<0.05$) in fruits of the control treatment (10.09 %) compared to those treated with SA (8.56 %) after 16 days (Fig. 2).

Postharvest loss of fresh mass is mainly caused by water deficit through processes such as transpiration and respiration that compromise product quality (Lufu et al., 2020). Studies indicate that SA influences the permeability of the plasma membrane ensuring less water vapor loss to the environment (Rosarolla et al., 2012), justifying the lower mass loss observed in fruits treated with SA throughout the period of 16 days.

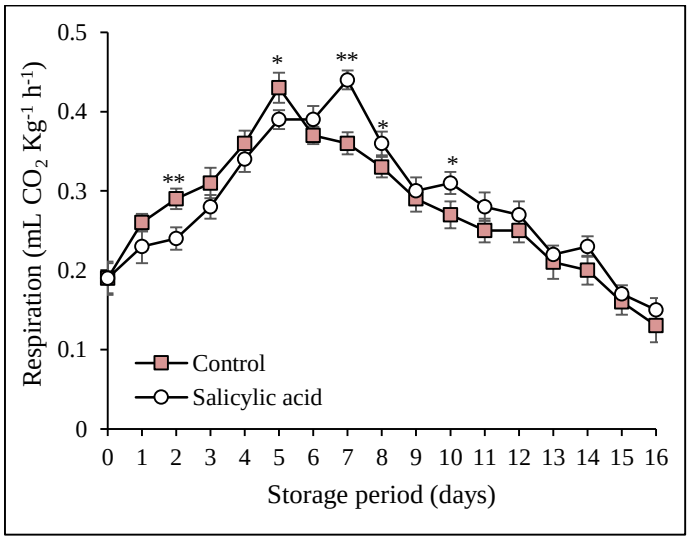

Fig. 1. Respiratory activity of 'Quintal' avocados treated with salicylic acid (4.0 mM) and stored under refrigeration (15 °C) for 16 days. Significant difference at P<0.05 (**) and P<0.01 (*) for treatments within each storage period. The bars represent the standard deviation of 5 repetitions.

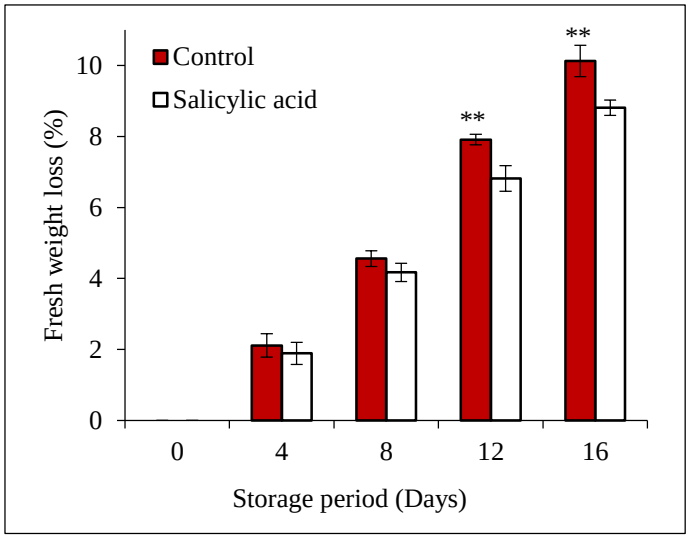

Fig. 2. Fresh weight loss in 'Quintal' avocados treated with salicylic acid (4.0 mM) and stored under refrigeration (15 °C) for 16 days. Significant difference at P<0.05 (**) and P<0.01 (*) for treatments within each storage period. The bars represent the standard deviation of 5 repetitions.

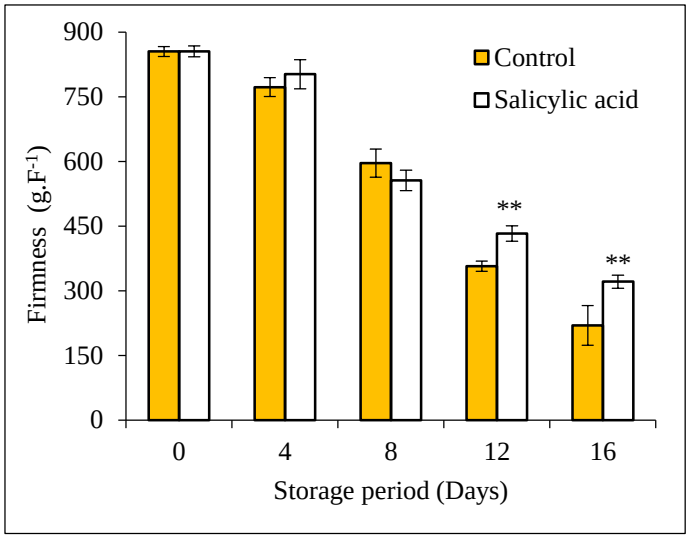

Fig. 3. Firmness in 'Quintal' avocados treated with salicylic acid (4.0 mM) and stored under refrigeration (15 °C) for 16 days. Significant difference at P<0.05 (**) and P<0.01 (*) for treatments within each storage period. The bars represent the standard deviation of 5 repetitions.

Similar effects of SA were observed on jabuticaba (Sanches et al., 2015) and 'Kampai' peach (Santos et al., 2016) where concentrations of 4.0 and 3.0 mM resulted in less fruit mass loss in relation to control, respectively.

Avocado firmness was reduced with storage time, however fruits treated with SA were firmer (321.09 g.F^{-1}) when compared to control fruits (219.71 g.F^{-1}) ($p < 0.05$) after 16 days of storage (Fig. 3).

In general, the greater firmness observed in fruits treated with SA compared to control is due to their inhibitory action of cell wall degrading's enzymes such as polygalacturonase, lipoxygenase, cellulase and pectinamethylesterase.Which leading to a reduction in the process. softening of fruits (Aghdam et al., 2016) and consequently greater firmness. This positive effect of SA on firmness preservation has been reported in fruits such as apple (Hadian-Deljou et al., 2017).

Soluble solids (SS) content increased up to 12 days of storage (20.21 ºBrix) ($p < 0.05$) followed by a decrease on 16th day, despite this reduction the SS content was significantly higher ($p<0.05$).) in fruits treated with SA (16.88 ºBrix) in relation to fruits of control treatment (11.95 ºBrix) (Fig. 4). This increase in SS content is associated with fruit ripening that promoted sugar synthesis mainly due to starch degradation. The decrease is an indication that the SS began to serve as an energy source for respiratory metabolism characterized by a decrease in its content. In this sense, fruits treated with SA presented a delay in ripening / senescence due to lower SS consumption after 16 days and which may be associated with delayed climateric peak (Fig. 1). For Einhardt et al. (2017) this effect of SA on ripening is mainly associated with its action on respiration and the control of ethylene synthesis during storage.

Treatment with SA (4.0 mM) delayed the synthesis of sugars (SS) and consequently the ripening of jabuticabas stored at 25 ºC for eight days (Sanches et al., 2015).

Reduction in titratable acidity (TA) occurred in both treatments with storage time, however, fruits immersed in SA solution presented significantly higher content ($p < 0.05$) of 0.29 g citric acid. 100g^{-1} relative to 0.18 g citric acid. 100g^{-1} pulp control treatment after 16 days of storage (Fig. 5).

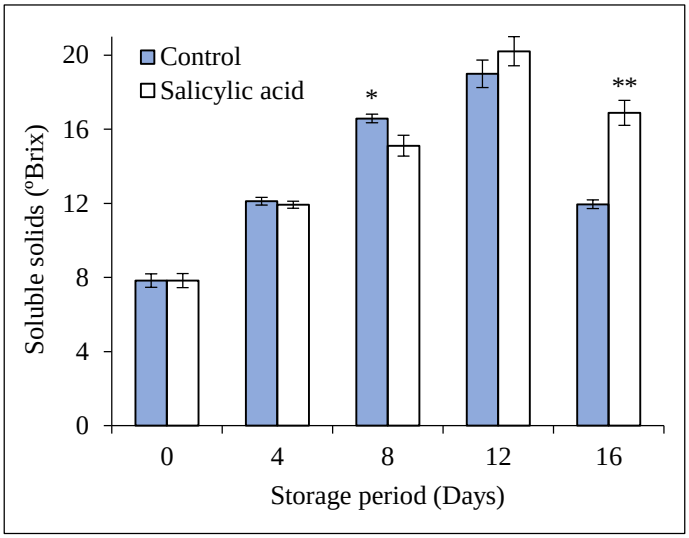

Fig. 4. Content of soluble solids in 'Quintal' avocados treated with salicylic acid (4.0 mM) and stored under refrigeration (15 ° C) for 16 days. Significant difference at P<0.05 (**) and P<0.01 (*) for treatments within each storage period. The bars represent the standard deviation of 5 repetitions.

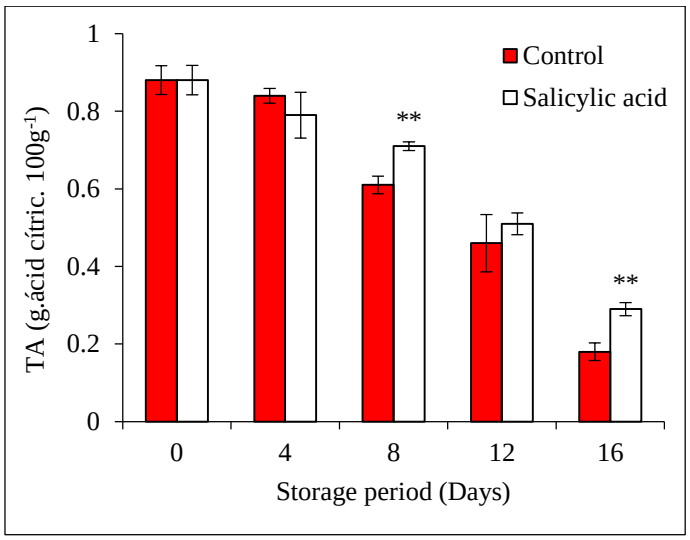

Fig. 5. Titratable acidity (TA) of 'Quintal' avocados treated with salicylic acid (4.0 mM) and stored under refrigeration (15 ° C) for 16 days. Significant difference at P<0.05 (**) and P<0.01 (*) for treatments within each storage period. The bars represent the standard deviation of 5 repetitions.

According to Baswal et al. (2020), the reduction in TA contents with storage time is due to the solubilization of acids to sugars so that respiration is used as an energy substrate, so preserving the acid content is essential for maintaining shelf life after harvest. Thus, it can be inferred that immersion of avocados in a solution at 4.0 mM SA by delaying the climateric peak (Fig. 1) delayed fruit ripening through lower consumption of organic acids (Fig. 5) and higher content SS (Fig. 4) at the end of storage.

There was no significant difference between treatments for the pH variable (p> 0.05). Overall, there was an increase in fruit pH from 5.53 on day zero to 6.75 on day 16 of storage (p<0.05) (Fig. 6).

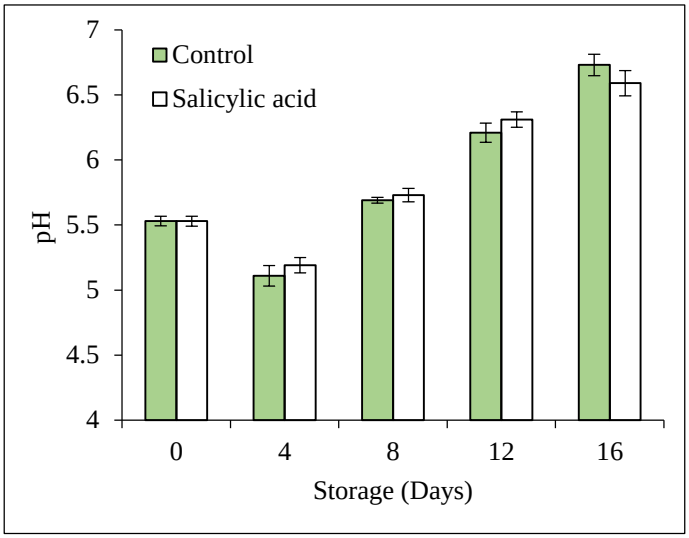

Fig. 6. pH of 'Quintal' avocados treated with salicylic acid (4.0 mM) and stored under refrigeration (15 ° C) for 16 days. Significant difference at P<0.05 (**) and P<0.01 (*) for treatments within each storage period. The bars represent the standard deviation of 5 repetitions.

The increase in pH is a response to the reduction of organic acids (Fig. 5) present in the fruit pulp that is being used for respiration. In jabuticabas that were treated with 4.0 mM SA, low pH values were observed during 8 days of storage at 25 ºC and this was correlated with the preservation of fruit quality (Sanches et al., 2015).

Antioxidant metabolism was influenced by SA treatment, overall antioxidant activity (Fig. 7A) and phenolic content (Fig. 7B) decreased with storage time but remained significantly higher ($p < 0.05$) in fruits treated with SA when compared to control fruits after 16 days.

As an elicitor, SA acts by activating plant defense mechanisms leading to the induction of acquired systemic resistance (ASR) through the synthesis of phenolic compounds, for example (Aghdam et al., 2016; Taiz et al., 2017). In this study, SA acted by delaying the phenolic degradation (29.87 µg.GAE.100g^{-1}) preserving the antioxidant activity of the fruits (29.26 DPPH%) when compared to the control (21.17 µg.GAE.100g^{-1} and 21.09 DPPH%), respectively on the 16 days of storage.

Red delicious apples stored for 193 days at 0 °C had higher phenolic content when treated with SA (4.0 mM) than untreated fruits (control) (Hadian-Deljou et al., 2017). Ezzat et al. (2017) found that SA (2.0 mM) stimulated and preserved antioxidant capacity against degradation in apricot cultivars when compared to control treatment over 28 days of storage at 1 ºC. This positive effect of SA on the induction/preservation of phenolic compounds has also been reported in strawberries (Geransayeh et al., 2015) and tomato (Kumar et al., 2018).

Phenylalanine ammonia lyase (FAL) is the major biosynthesis enzyme of phenolic compounds in the phenylpropanoid pathway (Taiz et al., 2017). According to Figure 8, there was a decrease in FAL activity with storage time, but SA treated avocados had significantly higher activity ($p < 0.05$) (1.22 µmol ac.transcin.h^{-1}.mg^{-1} P) compared to control fruits (0.76 µmol ac.transcin.h^{-1}.mg^{-1} P) on the 16th day of storage.

This result justifies the higher levels of antioxidant activity (Fig. 7A) and phenolic compounds (Fig. 7B) in the treated fruits at the end of the experiment, demonstrating that SA stimulated FAL activity and phenolic synthesis. Salicylic acid also stimulated FAL synthesis during the storage of blackberry (Borsatti et al., 2015).

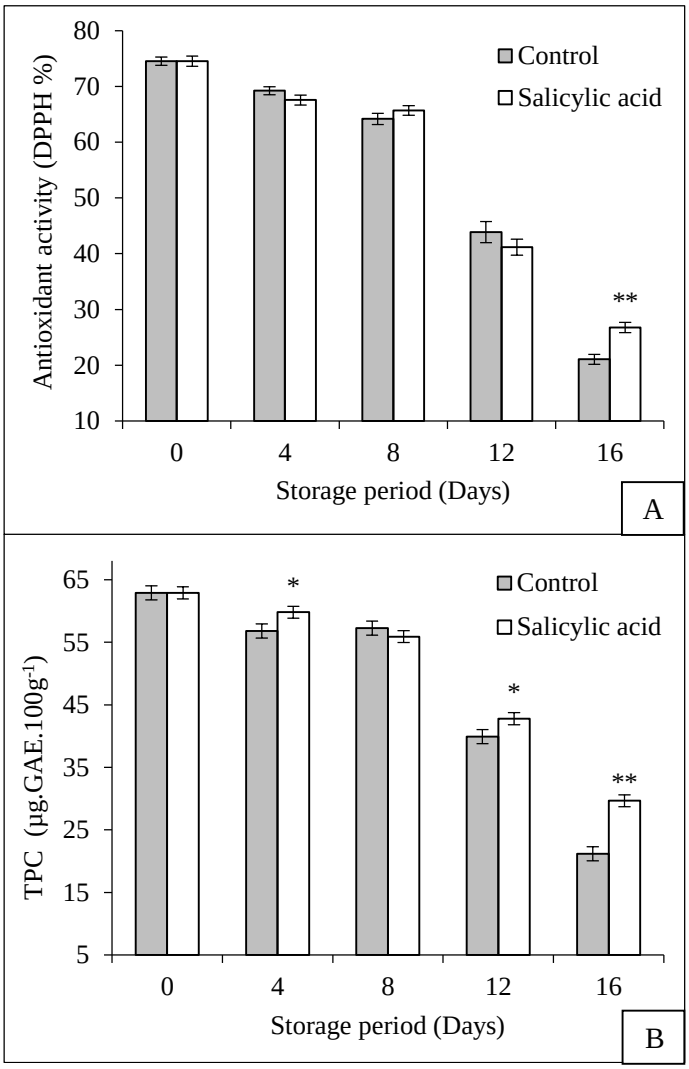

Fig. 7. Antioxidant activity (A) and total phenolic compounds – TPC (B) in 'Quintal' avocados treated with salicylic acid (4.0 mM) and stored under refrigeration (15 ° C) for 16 days. Significant difference at P<0.05 (**) and P<0.01 (*) for treatments within each storage period. The bars represent the standard deviation of 5 repetitions.

Polyphenoloxidase (PPO) is an oxidoreductase enzyme that promotes chemical degradation/oxidation of phenolic compounds (Yan et al., 2013). In this study, PPO activity increased with storage time from 0.39 UA.min^{-1}.mg^{-1} P on day zero to 2.36 UA.min^{-1}.mg^{-1} P on day 16, but this activity is significantly lower ($p<0.05$) in fruits treated with SA compared to control, especially after 8 days of storage (Fig. 9).

This lower degradation rate associated with higher synthesis (Fig. 8) explains the statistically higher phenolic content and antioxidant capacity (Fig. 7A and 7B) of fruits treated with SA. In pineapple, PPO activity was reduced after 20 days of storage at 10 ºC when fruits were treated with 5.0 mM SA compared to control (Lu et al., 2015). Furthermore, SA can acidify the pH and inhibit the catalytic activity of PPO, which is active in the neutral pH range, leading to the loss of its structure and ability to bind with its substrate, the phenolic compounds (Liao et al., 2021).

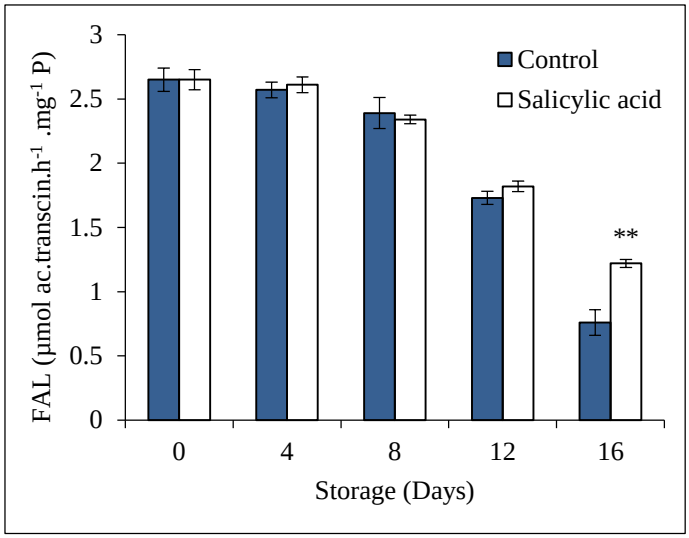

Fig. 8. Phenylalanine ammonia-lyase (FAL) activity in 'Quintal' avocados treated with salicylic acid (4.0 mM) and stored under refrigeration (15 °C) for 16 days. Significant difference at P<0.05 (**) and P<0.01 (*) for treatments within each storage period. The bars represent the standard deviation of 5 repetitions.

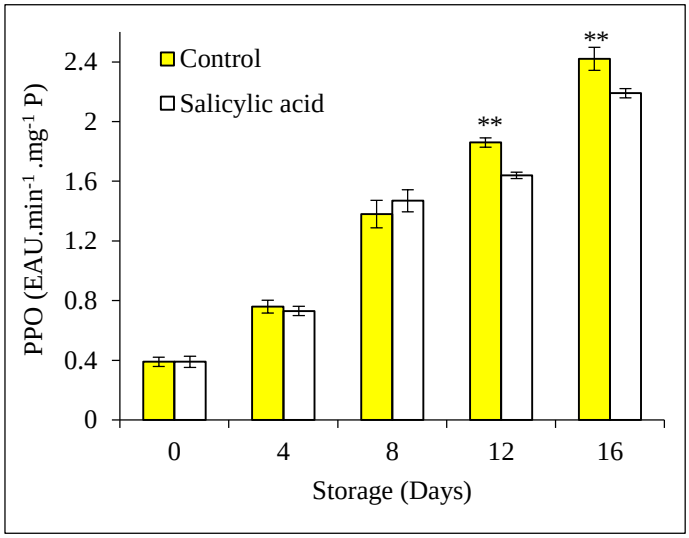

Fig. 9. Polyphenoloxidase (PPO) activity in 'Quintal' avocados treated with salicylic acid (4.0 mM) and stored under refrigeration (15 °C) for 16 days. Significant difference at P<0.05 (**) and P<0.01 (*) for treatments within each storage period. The bars represent the standard deviation of 5 repetitions.

CONCLUSION

Treatment with salicylic acid (SA - 4.0 mM) reduces/delays the respiratory activity of fruits and preserves the physicochemical quality during storage. SA activated the antioxidant metabolism resulting in the preservation of phenolic compounds and antioxidant activity. Regarding quality parameters, the role of SA in this study was associated with regulatory action on ethylene biosynthesis, which delayed the physiological processes related to fruit ripening. In antioxidant metabolism, SA may have acted as an osmoprotector and activated signaling pathways for the synthesis of phenolic compounds.

Conflict of interest

The authors declare that there is no conflict of interest.

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