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EFFECT OF POSTHARVEST SALICYLIC ACID TREATMENTS ON THE INDUCTION OF DISEASE RESISTANCE AND QUALITY PRESERVING OF APRICOT DURING COLD STORAGE

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Abstract Apricots are stone fruits, climacteric, that are highly perishable and have a limited storage life after harvest**.** Effects of salicylic acid (SA) postharvest treatment combined with cold storage on the quality features, fruit decay and antioxidant system of apricot fruits were investigated. Three concentrations of SA (0.5, 1 and 2 mM/L) were applied and apricots were stored at 1°C and 90 % RH for 20 days. Weight loss, acoustic firmness index, soluble solids content, titratable acidity, total phenolics, vitamin C, total carotenoids, antioxidant capacity, fruit decay and antioxidant enzymes were evaluated. Our results showed that treatment with SA at 2 mM/L concentration strongly reduces weight loss and the occurrence of fungal decay, and maintain an high content of acid ascorbic, phenolics and carotenoids in fruits after 20 days of cold storage. Also, results showed that maximum antioxidant activity and activities of peroxidase and superoxide dismutase enzymes were found in the fruit treated with 2mM/L SA. The postharvest application of SA in 0.5mM/L concentration had no positive effects on the quality and antioxidant capacity of the apricots during cold storage.

Key words: *antioxidant capacity, fruit decay, firmness, soluble solids*

INTRODUCTION

Apricots are a significant source of carbohydrate, antioxidants, dietary fiber, vitamins and minerals, but are highly perishable and have a limited storage life after harvest (Alajil et al., 2021). They remain fresh for only two weeks when stored at 1°C, depending on the cultivar, and can be stored for only up to 5 days at room temperature (Aubert & Chanforan, 2007). Postharvest losses are mainly due to fruit stage maturity, harvesting techniques, storage conditions and improper handling, which can lead to deterioration of fruit quality, the appearance of lesions on the surface that increase susceptibility to pathogens and lead to considerable losses.

Consumer demands for higher quality, microbiologically safe fruits with a longer storage period are increasing day by day. These goals have challenged scientists to develop new research focused on the use of various postharvest treatments to maintain fruit quality, disease resistance and extend the storage period. Recently, the preservation of fruit quality and extension of their storage period are maintained by using different natural compounds (chitosan, chitin, salicylic acid, essential oils, plant extracts) that act as barriers to oxygen and moisture,

modify the atmosphere around the fruit and can delay some physiological processes, such as respiration and transpiration, and are natural biocides (Riva et al., 2020; Chen et al., 2023).

In recent years, salicylic acid (SA) has been widely used to maintain postharvest quality, delay fruit ripening and increase nutritional value of different fruit during storage like strawberries (Babalar et al., 2007), sweet cherries (Gimenez et al., 2016), apples (Delijou et al., 2017), peaches (Tareen et al, 2012; Khademi & Erashadi, 2013), apricots (Wang et al., 2015; Ezzat et al., 2020), mandarins (Haider et al., 2020). Being a natural hormone, salicylic acid is considered safe compound as a postharvest treatment for fruits (Aghdam et al., 2016; Riva et al., 2020).

Previous researches suggest that exogenous application of salicylic acid can decrease metabolic rate, delay ethylene production, and improve the quality of stored apricot fruits (Wang et al., 2015; Ezzat et al., 2017; Ezzat et al., 2020). Also, it was reported that salicylic acid treatment could effectively suppress postharvest disease and enhance resistance to pathogens of different fruits including apricot (Ezzat et al., 2020), citrus (Moosa et al., 2019), apple (da Rocha Neto et al, 2016), plum (Zhang et al., 2024), pear (Tian et al, 2006), sweet cherry (Yao & Tian, 2005).

Thus, in the present study we evaluated and compared the changes in apricots quality and antioxidant compounds as well as decay incidence during apricots storage at low temperature in response to treatment with different concentrations of salicylic acid.

MATERIALS AND METHODS

Apricots of the "Favorit" cultivar (created at Research Station for Fruit Tree Growing Băneasa, Bucharest) were obtained from the experimental field of Research Station and immediately used for the storage experiments. The experiments were carried out following a completely randomized design with three treated variants and one untreated control with three replicates each and 20 fruits in each replicate.

Before each analisys, apricots showing no visible wounds were carefully hand-selected and washed with tap water, surface-sterilized by immersion for 2 min in 0.2% sodium hypochlorite, rinsed with sterile distilled water, and then air-dried at room temperature. Fruits for analisys were dipped in salicylic acid solutions of three concentration 0.5, 1 and 2mM/L for 10 minutes, and control fruits were dipped into distilled water and then all fruits was stored at 1°C and 90% RH. The analytical determinations were done at 5, 10, 15 and 20 days after cold storage.

Weight loss was determined by the difference between initial and final weight of each fruit during storage and expressed as percent. Soluble solids content (SSC) was measured with a digital refractometer (Atago, Tokyo, Japan) at 20°C from juice extracted from the fruits and expressed in °Brix (AOAC, 1990). Titratable acidity (TA) was quantified in fruit juice by titration with 0.1N NaOH up to pH 8.2. Titratable acidity was expressed as percent of malic acid, which is the predominant acid in this fruit species (mg ac. malic /100g juice).

Acoustic firmness index of fruits was measured at the equator of the unpeeled fruit in two repetitions using an AWETA Acoustic Firmness Sensor. In this device, an acoustic signal is generated by means of gentle impact on the equator of the fruit. The signal is processed and transformed to obtain a peak of natural frequency, which was used to calculate the stiffness factor as $f^2 \times m^{2/3}$, where f represents the frequency and m is the fruit mass.

The total phenolics were determined by using Folin-Ciocalteu method (Singleton & Rossi, 1965). Fruit tissue (1g) was extracted with ethanol 70 %. An amount of 250 µL extract was dissolved in 250 µL distilled water and then samples were mixed with 2.5 mL of 10-fold-

diluted Folin Ciocalteu reagent and 2 mL 7.5 % sodium carbonate. The mixture was shaken for 1.5 hours before the absorbance was measured by a UV-Visible spectrophotometer (model 2010, Cecil Instr. Ltd., Cambridge, UK) at 765 nm. The results were expressed as mg gallic acid equivalents in 100 g fresh weight (mg GAE/100 g f.w.).

Assessment of vitamin C (ascorbic acid) content was achieved by quantitative reduction of 2,6 diclorphenol-indophenol and the excess of dye was spectrophotometrically determined at 500 nm (AOAC, 1990). The results were expressed as mg vitamin C/100g f.w.

Carotenoids content was determined from whole fruit by measuring the absorbance of acetone extract at 450 nm with UV-VIS spectrophotometer using the method of Rodriguez-Amaya (2001) and expressed as mg β-carotene/100g f.w.

Total antioxidant capacity of fruits was determined by the DPPH (1, 1-diphenyl-2-picrylhydrazyl) method described by Brand-Williams et al. (1995). Briefly, 1 g of fruit tissue was extracted with 10 mL methanol (85 %). A quantity of 1 mL extract was mixed with 2mL DPPH (0.15mM/L) in methanol. The mixture was shaken vigorously and left to stand for 30 minutes under dark condition. The control was prepared by adding 2 mL DPPH to 1mL methanol. Absorbance of the extract was measured at 517 nm by a UV-Visible spectrophotometer (model 2010, Cecil UK). The results were expressed in mg trolox equivalents /100g fresh weight (mgTE/100g f.w.).

The fruit decay incidence was determined by examining the fruits regularly following each storage period by following method described earlier by Bal and Torcuk (2020). It was assessed as symptoms of superficial decayed on the fruit surface, expressed as percentage.

Superoxide dismutase (SOD) activity was carried out by method of Du & Bramlage (1994) using nitroblue tetrazolium chloride. The absorbance was measured at 560 nm. The enzyme activity was expressed as Units min^{-1} g^{-1} f.w. The peroxidase (POX) activity was evaluated according to the method of Hammerschmidt et al. (1982) using guaiacol as the substrate, measured at 470 nm and expressed Units min^{-1} g^{-1} f.w.

The experiment was arranged as a completely randomized design with three replicates. Analysis of Variance (ANOVA) was used for analyzing data using the Microsoft Excel 2003 statistical software. The results were expressed as mean ±SD of three replicates in each experiment.

RESULTS AND DISCUSSIONS

Fruits weight loss of the "Favorit" apricot cultivar increased during cold storage in all three variants treated with SA (Table 1). The fruits weight loss in the control variant was significantly greater after 20 days of storage (17.9 %) compared to samples treated with SA solutions. SA treatments with concentrations of 2 and 1 mM/L reduced fruit weight loss to similar values (between 4.5 and 4.8%) under cold storage conditions (Table 1). Among the treated samples, the highest weight loss (14.1%) was observed in fruits where the concentration of 0.5mM/l was applied while the lowest weight loss (4%) was observed in fruits from the variant treated with a 2mM/L SA solution. Our results are consistent with those obtained by Ezzat et al. (2017).

Fruit weight loss is mainly associated with high respiration intensity and water evaporation through the peel. The thin peel of apricot fruits makes them susceptible to rapid water loss leading to shriveling and spoilage (Aubert & Chanforan, 2007). The lower weight loss in apricot samples treated with salicylic acid is likely due to the maintenance of cellular integrity and tissue permeability by these treatments.

The acoustic firmness index of the fruits decreased progressively throughout the storage period in all SA treated variants as well as in the control (Table 1). It was observed that the acoustic firmness index of the fruits remained at an increased value especially in the variant treated with SA 2mM (3.9) followed by 1mM (3.8) compared to the untreated fruits (2.8) after 20 days of cold storage. Treatment with 0.5 mM/L salicylic acid did not provide any significant benefit in preserving apricots firmness and performed similarly to the control treatment, where fruit firmness declined sharply during 20 days of cold storage, reaching low level (3.0). SA is known as an inhibitor of ethylene biosynthesis, thus reducing fruit softening and delaying fruit overripening in addition to improving the water vapor permeability (Chen et al., 2023).

The effect of storage time on the soluble solids content of apricots treated with the three SA concentrations and untreated fruits was significant. SSC decreased slightly in the first 10 days of cold storage in all variants analyzed (Table 1). However, the decrease of SSC in samples treated with SA in concentrations of 2 and 1mM/L was lower than that in the control sample and the one treated with 0.5mM. Treatment with salicylic acid at low concentrations (0.5 mM/L) did not show beneficial effects on the maintenance of SSC content, its values being very close to the untreated control sample (Table 1). Between 10 and 20 days of storage, soluble solids content began to increase slightly for all three treated variants as well as for the untreated variant.

The decrease in soluble solids content is possible due to the use of sugars during respiration (Ezzat et al., 2017). The results obtained in this study sustain the experiments conducted by Adhikary et al. (2021) which indicate that salicylic acid at low concentrations effectively delayed the respiration rate while maintaining adequate SSC content. However, the authors argue that a higher concentration of salicylic acid could induce the aggregation of toxic substances which can accelerate the deterioration of fruit quality.

The titratable acidity of the fruit decreased slightly in the first 5 days of storage, after which it remained relatively constant in the next 5 days and then increased slightly up to 20 days of storage (Table 1). In this regard, a beneficial effect of the two concentrations of SA (2 and 1mM) was observed up to 15 days of storage, after which the ratio between SSC and TA changes decreased below 12, thus leading to inadequate fruit quality. Similar results, were reported by Batool et al. (2022) and Wang et al. (2015) for apricots.

Table 1. The effect of treatment with salicylic acid (SA) on the qualitativeparameters of "Favorit" apricots during cold storage

Cultivar	Storage time (days) / Treatment		Weight loss (g %)	Acoustic Firmness index	Soluble solids (°Brix)	Titratable acidity (mg ac. malic/100 mL juice)
Favorit	Harvest		0	4.4±0.07	14.6±0.35	1.2±0.03
	5 days	M (untreated)	8±0.04	3.8±0.02	13±1.02	1.1±0.02
		SA (0.5mM/L)	4.2±0.12	3.9±0.05	13.3±1.00	1±0.10
		SA (1mM/L)	2.7±0.09	4.2±1.10	14.4±1.54	0.95±0.11
		SA (2mM/L)	2.8±0.01	4.3±0.12	14.5±0.11	0.97±0.21
	10 days	M	10±0.05	3.5±0.04	12±1.07	1.2±0.04
		SA (0.5mM/L)	7.9±0.11	3.8±0.09	13.1±1.05	0.8±0.11
		SA (1mM/L)	2.75±0.10	4.1±1.20	14.1±1.45	1.2±0.16
		SA (2mM/L)	2.8±0.04	4.2±0.15	14.4±0.19	1.1±0.18
	15 days	M	14±0.09	3.2±0.04	12.9±0.60	1.3±0.09
		SA (0.5mM/L)	13.6±0.18	3.5±0.08	13.5±1.19	1.3±0.13
		SA (1mM/L)	4.6±0.14	3.9±1.18	14.6±1.16	1.1±0.24

		SA (2mM/L)	4.4±0.06	4.0±0.10	14.7±0.21	1.2±0.21
	20 days	M	17.9±1.01	2.8±0.09	13.5±0.88	1.4±0.04
		SA (0.5mM/L)	14.1±0.14	3.0±0.09	14.7±1.21	1.3±0.03
		SA (1mM/L)	4.8±0.07	3.8±1.22	15.2±1.00	1.5±0.30
		SA (2mM/L)	4.5±0.09	3.9±0.16	14.9±0.26	1.5±0.20

Plants have developed a defense mechanism based on their antioxidant system that can scavenge reactive oxygen species (ROS) and thus its activation can protect cells from oxidative damage. This system includes both antioxidant compounds and enzymes that have various beneficial biological functions that play a role in defense against abiotic or biotic factors (Zhang et al., 2024).

In our study all applied treatments were effective in reducing the loss of vitamin C compared to the untreated control during the cold storage period of apricots. The vitamin C content of apricots decreased gradually throughout the fruit storage period up to 20 days. The most pronounced decrease was observed in untreated fruits, in which vitamin C levels declined to 5.0 mg /100g f.w. by the end of the storage period (Table 2).

After 20 days of cold storage, the highest values of ascorbic acid were recorded in fruits treated with 2mM/L SA followed by 1mM/L which support the fact that the treatment with the two concentrations of SA delays fruit ripening and maintains an increased vitamin C content (Table 2). However, the fruits treated with SA 0.5mM/L showed a significant decrease in vitamin C content compared to the other two treatments, with values very close to the untreated control. This suggests that treatment with this concentration of SA is less beneficial for maintaining fruit quality and extending storage period.

Table 2. Effect of treatments with salicylic acid (SA) on fruit vitamin C content of 'Favorit' apricots during cold storage

Vitamin C (mg /100g f.w.)	harvest	5 days	10 days	15 days	20 days
Control	8.5±0.02	7.9±0.08	7.5±0.06	6.1±0.05	5.0±0.09
SA 0.5mM/L		8.1 ±0.15	7.7±0.04	7.0±0.20	5.5 ±0.21
SA 1mM/L		8.0±1.14	7.9±1.10	7.7±1.12	7.5 ±1.19
SA 2mM/L		8.3±0.18	8.2±0.20	8.0±0.15	8.0±0.18

Apricots contain an adequate amount of carotenoid pigments that give them their yellow-orange color, thus contributing to the visual appearance of the fruits and also to antioxidant activity. The carotenoid content increases intensely in the untreated control variant compared to the variants treated with SA up to 20 days of storage. SA concentrations of 1 and 2mM increased the carotenoid content but this happened much more slowly up to 20 days of storage (Table 3).

Thus, we can explain this by the fact that in the untreated fruits a faster synthesis of carotenoid pigments took place, while, the SA treatments in variants treated with 1 and 2mM/L concentrations delayed the synthesis of carotenoids and therefore the intensification of the fruit color and thus ripening. Variant treated with 0.5mM/L salicylic acid had a similar trend to the untreated control, the fruits had concentrations very close to it (Table 3).

Table 3. Effect of treatments with salicylic acid (SA) on fruit total carotenoids content of 'Favorit' apricots during cold storag**e**

Carotenoids (mg β-carotene /100g f.w.)	harvest	5days	10 days	15 days	20 days
Control	8.1±0.02	9.5±0.06	10.3±0.06	11.5±0.11	12.1±0.16
SA 0.5mM/L		9.4 ±1.08	10±1.04	10.7±1.20	11.8 ±1.17
SA 1mM/L		8.9±0.14	9.3±0.11	9.8±0.09	10.4 ±0.20
SA 2mM/L		8.5±2.18	9±2.20	9.50±2.25	10.2±2.30

Total phenolics and ascorbic acid are important antioxidant components, closely associated with tissue damage and fruit aging. Thus, the role of SA in controlling fungal decay may also be due to the activation of antioxidant defense response (Wang et al., 2006) in fruits sustained through phenols and ascorbic acid content or due to its direct antifungal effect. The results obtained in this study showed that the phenolic content of apricots treated with SA was significantly higher compared to untreated fruits, indicating that SA is effective in mitigating oxidative stress induced by reactive oxygen species (Table 4).

The phenolic content of the treated apricots remains at a similar level, around 190 mg gallic acid/100g f.w. for both variants treated with SA, 2 and 1mM/L, and was more pronounced than for the untreated control throughout the storage period (Table 4). The results of our study are consistent with the results obtained by Ezzat et al. (2017) on three apricot varieties, which showed that treatments with SA in different concentrations maintained the amount of total phenolics at a high level up to 21 days of cold storage.

The results of Batool et al. (2022) support that treatment with SA maintains increased values of total phenolic content in apricots of different varieties, by exogenous application of the 4mM/L SA solution. However, the study of Tian et al. (2006) sustained that total phenolics content in "Hosui" pears showed decreasing trend during postharvest storage and this trend was significantly attenuated by SA treatment.

Table 4. Effect of treatments with salicylic acid (SA) on fruit total phenols of 'Favorit' apricots cultivar during cold storage

Total phenols (mg GAE/100g f.w.)	harvest	5 days	10 days	15 days	20 days
Control	252±0.12	200.2±0.18	185.1±0.27	163.1±0.20	100.1±0.31
SA 0.5mM/L		210.6 ±1.05	187.3±2.04	175.8±2.04	163.3±2.02
SA 1mM/L		226.4±1.34	204.6±1.56	200.7±1.10	190.1 ±1.36
SA 2mM/L		231.4.1±0.32	218.2±0.56	207.4±1.37	196.1±1.86

The antioxidant capacity of all treated fruits increased gradually up to 10 days of storage after which it began to decrease slightly (Figure1). In untreated fruits, the antioxidant capacity decreased slowly until the end of the storage period. However, fruits treated with SA 0.5mM/L showed a similar antioxidant capacity throughout the storage period but slightly increased compared to untreated fruits at the end of storage (Figure 1). This increase up to the 10th day of storage was probably due to the content of antioxidant compounds already present in the apricots, which, in turn, is attributed to their hydrogen donor capacity. At the end of storage, the highest antioxidant activity (76.3 mg TE/100g f.w.) was observed in fruits treated with SA 2mM/L and the lowest (60.90 mg TE/100g f.w.) in untreated fruits .

The increase of antioxidant capacity was closely related to the intensification of antioxidant enzyme activity. The results of the present study are in accordance to many previous studies that reported the protective effects of salicylic acid treatment by activating defense

systems and reducing the occurrence of disease symptoms in strawberries (Babalar et al., 2007), plums (Zhang et al., 2024), peaches (Tareen et al., 2012) and cherries (Bal & Torcuk, 2020).

Fungal decay incidence of "Favorit" apricot fruits increased as storage time progressed (Figure 2). Decay was first observed after 5 days of storage for untreated fruit, while in SA treated fruits was observed after 15 days of cold storage. The protective action of SA treatments was notable in apricot fruits during cold storage. After 20 days of storage, the incidence of decay in the control and treated fruits with two concentration (2 and 1 mM/L) of SA was 26.5, 7.1 and 10.2%, respectively, showing that SA treatment reduced the occurrence of rot in apricot fruits during cold storage. This can be explained either by indirect effect related to the induction of resistance or by the direct antimicrobial action of SA.

Incidence of fungal decay was more pronounced (22.1%) after 20 days of storage at treated apricots with 0.5mM/L SA, very close to the fruit untreated variant (26.5%) (Figure 2). Fruit decay was affected by SA treatments compared to control and was correlated with enhancing antioxidant power in treated apricots. Fruit decay incidence decreased with increasing SA concentration. Our results suggest that 2mM/L SA treatment can effectively inhibit the appearance of disease symptoms of apricots during cold storage. The study further revealed that SA in a concentration of 2mM/L was much effective in reducing the decay percentage compared to SA in 0.5mM/L concentration and control (Figure 2).

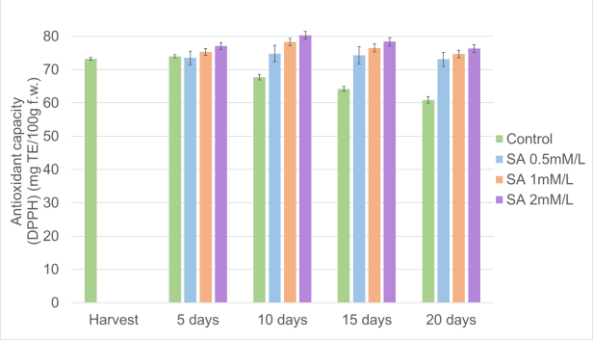

Figure 1. Effect of salicylic acid (SA) treatments on antioxidant capacity in "Favorit" cultivar apricots during cold storage.

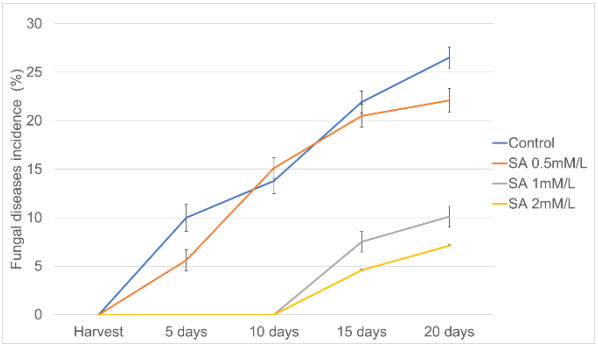

Figure 2. Effect of salicylic acid (SA) treatments on fungal decay incidence in "Favorit" cultivar apricots during cold storage

The beneficial effects of SA treatment in reducing decay incidence in apricots of the "Favorit" variety are in line with previous report conducted on peaches (Wang et al., 2006). The work carried out by Ezzat et al. (2020) showed that postharvest treatments with salicylic acid of 2 and 5 mM/L concentrations significantly reduced the incidence of fungal diseases and lesions diameter in apricot fruits during storage at room temperature for a period of 8 days. Thus, they indicated that the increased level of phenolic content and antioxidant capacity, induced by salicylic acid, was correlated with a delay in the appearance of brown rot symptoms on apricots.

In another previous study realized by Ezzat et al. (2017), SA treatments decreased significantly fruit decay values in three cultivars analyzed compared to water-treated fruits during 28 days of cold storage. The results of Luo et al. (2011) showed that resistance of "Qingnai" plums to pathogens during cold storage was greatly improved by postharvest treatment with SA, the appearance of fungi being observed in treated fruits only after 30 days of storage at 1°C.

Our results showed a general increasing trend in SOD activity until 15 days storage, after that decrease slightly both in treated and untreated fruits (Figure 3). However, there are some differences between the untreated and SA treated apricots. Thus, SOD activity was approximately 2 times higher in 1 and 2mM/L SA treated fruits compared to control. Application of 0.5mM/L SA shows an SOD activity similar to that determined in untreated apricots after 20 days storage, suggesting that this concentration of SA does not induce an increase in antioxidant activity. Our results are in line with those of Wang et al. (2015) who supported that SOD activity increases in apricots of the "Dahuang" variety treated with 2mM/L SA, reaching a maximum value at 15 days storage, being higher than in untreated fruits.

Recently, experiments conducted by Adhikary et al. (2021) found that exogenous treatment with SA in 2 and 3mM/L concentrations clearly reduced the incidence of fungal pathogens and significantly stimulated the activity of superoxide dismutase enzyme during pear "Patharnakh" cultivar cold storage, so that on the 60th day of storage, treated pears showed a lower incidence of rotting compared to untreated fruits which was consistent with our results.

The activity of peroxidase leads to the rigidity of the cell wall by increasing the content of polysaccharide and glycoprotein molecules. Thus, this enzyme plays an essential role in strengthening the cell wall through lignin synthesis, providing a physical barrier of protection against infection by fungal pathogens (Moosa et al., 2019).

Peroxidase activity of apricots showed a gradual increase and reached a maximum at the end of the storage period and enzyme activity was enhanced by SA treatment (Figure 4).

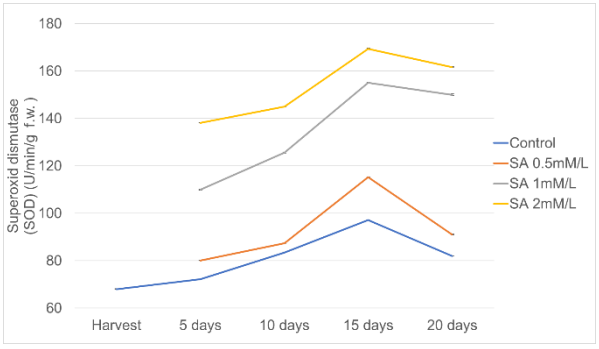

Figure 3. Effect of salicylic acid (SA) treatments on superoxide dismutase activity in "Favorit" cultivar apricots during cold storage.

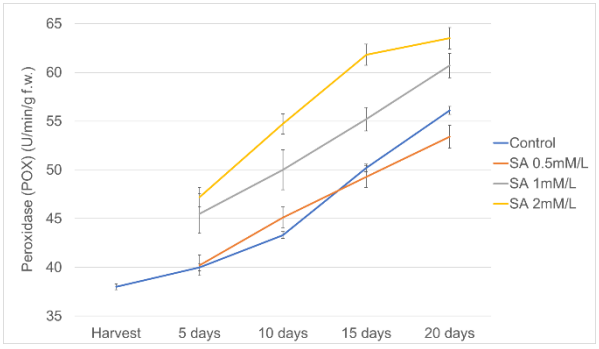

Figure 4. Effect of salicylic acid (SA) treatments on peroxidase activity in "Favorit" cultivar apricots during cold storage

The maximum effect (63.5 U/min/g f.w.) was observed in fruits treated with 2 mM/L followed by fruits treated with 1 mM/L (60.7 U/min/g f.w.). At the end of storage, POX activity in fruits treated with salicylic acid 1 and 2 mM/L was 7 and 11% higher, respectively, than in untreated fruits (Figure 4). Similar findings were reported by Wang et al. (2015) in "Dhuang" apricots cultivar. Thus, our results regarding the reduction of fungal decay incidence during cold storage of the "Favorit" apricots cultivar treated with SA solutions can be explained by indirect mechanisms of resistance induction supported by the increased content in polyphenols, carotenoids and antioxidant enzyme activity (such as SOD and POX) and also the maintenance of an adequate amount of vitamin C.

CONCLUSIONS

The results in our study support that the postharvest treatment of apricots with concentrations of 1 and 2mM/L of salicylic acid in conjunction with low temperatures extend the fruit storage period to 20 days.

Fruit treated with 2mM/L SA was also able to maintain the content of antioxidant compounds (total phenolics, vitamin C and carotenoids), firmness and taste and antioxidant activity at high level until the end of the storage period.

The postharvest application of SA in 0.5mM/L concentration had no positive effects on the quality and antioxidant capacity of the apricots during cold storage, so this concentration has no practical value.

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