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Postharvest application of salicylic acid enhanced antioxidant responses of peach cv. 'Elberta' fruit during storage

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ABSTRACT

Cold storage has been widely used to prolong postharvest life of horticultural crops. However, some fruit are sensitive to low temperature and develop physiological disorders with extended cold storage period. Salicylic acid (SA) enhanced plant tolerance to low temperature during growth. Moreover, SA treatment has been applied to reduce chilling induced disorders of postharvest horticultural crops. This study investigates the effect of salicylic acid (SA) on chilling injury (CI) and antioxidant responses in Elberta peach fruit during cold storage. The effect of salicylic acid on physiological responses of Elberta peach (*Prunus persica* L.) fruit was investigated. The treatments used in this experiment are the control, 1 and 2 mM concentrations of salicylic acid. Then, the fruits were stored at 0 degrees Celsius and relative humidity of 95% for 25 days. After 0, 5, 10, 15, 20 and 25 days, fruits were removed from cold storage, transferred at 20 °C and then analyzed. The experimental design was implemented in the form of a completely randomized design. Statistical analyses were performed using SPSS, with data analyzed by one-way ANOVA and mean separations by Duncan's new multiple-range test. According to the results of this research, SA at the concentrations of 1and 2 mM effectively reduced chilling injury of Elberta peach fruit during 25 days storage at 0 °C, while SA at 2 mM showed the best effect. SA treatment also reduced the lipid peroxidation and hydrogen peroxide content of peach fruit for storage. Results of physiological response in peach fruit showed that SA induced the activity of antioxidant enzymes including catalase and peroxidase. These results indicate that the elicitation of an antioxidant response in peach fruit by SA may be associated with chilling injury alleviation. Moreover, application of SA maintained the peach quality by decrease of lipid peroxidation and hydrogen peroxide content. So, the present study is the evidence that SA enhance peach fruit tolerance to cold stress and therefore results in improving the fruit quality.

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1. Introduction

Fruits quality and health have been considered the subjects of ongoing research with practical concerns. Various health benefits have been associated with the consumption of fruits and vegetables. Postharvest handling and storage conditions of agricultural produce are important determinants of the marketability and shelf-life of fruits (Batool *et al*., 2022). Peach is a climacteric fruit which undergoes rapid ripening. Peaches rapidly soften after commercial maturity, and are highly sensitive to chilling injuries and can be easily damaged during shelf life (Lurie and Crisosto, 2005). This is responsible for its short shelf-life and represents a serious constraint for its efficient handling and transportation (Akbudak and Eris. 2004; Johnson, 2008). It is therefore very important to optimize the postharvest care of fruits to obtain satisfactory quality. Fruit quality losses after harvest because of environmental stress and pathogen infection (Layne and Bassi, 2008; Kluge and Jacomino, 2002). Sensory quality, in the broadest sense, e.g. flavour, taste, texture etc., is one of the primary reasons consumers purchase peach fruit, and the development of knowledge to evaluate eating quality has become an important focus for postharvest biologists and technologists (Allegra *et al*., 2015a; Sortino *et al*., 2017). Visual quality is one of the most important quality parameters, according to the consumers, and this is, often, altered by fruit browning, dehydration and decay. When fruit comes to browning, the enzyme polyphenol oxidase (PPO) (Li-Qin *et al*., 2009) and the antioxidant content are important aspects to take into accounts (Oms-Oliu *et al*., 2010). The development of new technologies in controlling the fruit ripening process allows us to prolong its shelf life, reducing distribution loss and supplying high-quality fruit to the market.

Several promising methods have been developed to alleviate increasing the shelf life of peach fruit. These include postharvest temperature conditioning (Chang *et al*., 2000; Crisosto *et al*.,2004; Girard *et al*., 2005), modified atmosphere packaging (Garner *et al*., 2001; Gorny *et al*., 1999; Zhou *et al*., 2000a), and chemical treatments with plant growth regulators (Abbasi *et al*.,2010).

Salicylic acid (SA), as an endogenous signaling molecule, plays a pivotal role in plants growth and development as well as in responses to environmental stressful conditions (Horvath *et al*., 2007; Gharibiyan *et al*., 2023; Zebro and Heo., 2024). SA may alleviate postharvest chilling injury(CI) in fruits and vegetables via different mechanisms such as enhanced alternative antioxidant changes such as ascorbate peroxidase (APX) and glutathione reductase (GR) activity and other antioxidant enzymes (Cai and Chen, 2006). SA is considered as a plant hormone (Raskin,1992), inhibiting ethylene biosynthesis and delaying the fruit senescence (Zhang *et al*, 2003).The involvement of SA in systemic acquired resistance, associated with the production of pathogenesis-related proteins, has been extensively reported (Amani *et al*., 2023; Asgharia and Aghdam, 2010 Hazarika, and Marak, 2022). Moreover, dietary salicylates from fruit and vegetables are described as bioactive compounds with heath care potential (Hayat, and Ahmad, 2007; Song *et al*., 2023), and considered as generally considered as safe. There are several studies indicating beneficial influences of SA treatment on storability of fruits storage (Wang *et al*., 2022). During kiwifruit ripening, the pattern of decrease in endogenous SA levels was related to accelerated softening, while the application of acetylsalicylic acid (a derivative of SA) slowed down the softening rate of kiwifruit by inhibiting ethylene production and maintaining higher endogenous SA levels (Zhang *et al*, 2003). SA also prevented softening of tomato and kiwifruit during ripening (Ding *et al*., 2002; Zhang *et al*, 2003). It has been reported that SA application either pre-harvest or postharvest reduced fungal decay in sweet cherry (Fung *et al*., 2006), Grape (Hou *et al*., 2022; Nia *et al*., 2022), strawberry (Babalar *et al*, 2007; Liu *et al*., 2022) and peach fruits (Kaur *et al*., 2022; Wang *et al*, 2006) through induction of the defense resistance system and stimulation of antioxidant enzymes. With attention to the risk of improper use of chemicals in postharvest technology and consumer's demand for healthy products, a study on application of postharvest treatments along with cold storage is necessary. The application of postharvest treatments may alleviate postharvest CI in fruits and vegetables via different mechanisms such as enhanced alternative antioxidant changes. We aimed to investigate the impact of SA application on the occurrence of oxidative stress, activation of the antioxidant defense system, and increase in storage life of peach fruits. By monitoring H_2O_2 and MDA content as an oxidative index in the cell membrane of peach fruits, The objectives of this study were to evaluate the effects of SA on chilling injury, lipid peroxidation content, hydrogen peroxide content, and the induction of antioxidant enzymes, such as catalase (CAT) and peroxidase (POD), in peach cv. Elberta fruit during storage at 0 °C.

2. Materials and Methods

2.1. Elberta peach fruit and treatment

This study was conducted during the summer season in 2023, on Elberta harvested from 15 trees, grafted on GF 677 (*Prunus persica* × *Prunus amygdalus*) rootstock, grown in a commercial orchard located in Mahan city in Kerman province (Iran). Elberta peach fruits were picked at ripe stage (RP). Elberta fruits were selected for uniformity of size and absence of defects. The experiment was carried out in the laboratory of Horticultural Science Department, Faculty of Agriculture, Shahid Bahonar University, Kerman, Iran. Peach fruits without wounds or rot were selected based on uniformity of size and absence of physical injury or disease. The harvested fruits were disinfected with 1% sodium hypochlorite (v/v) for 2 min, washed, and dried in air. Subsequently, they were randomly divided into 3 groups. Two of the groups were immersed in aqueous solution containing, respectively, 1 and 2 mM of SA for 5 min, based on our preliminary experiments, and the third group was immersed in distilled water for 5 min and served as a control. All fruits were enclosed in plastic boxes with polyethylene film bags to maintain the relative humidity at about 95% and stored at 0°C. Treated fruits were stored in a cold room at 0°C and 95% relative humidity for 25 days, and the temperature and humidity were monitored with a thermometer and hygrometer every day. 3 repetitions were considered for each treatment and 10 peach fruits were considered in each repetition. The experimental design was implemented in the form of a completely randomized design.

2.2. Evaluation of chilling injury (CI)

The symptoms of CI include surface pitting and browning. The CI index was determined according to the method described by Zhang and Tian (2010). Grade levels were classified as follows: grade 0, the orange fruits are unaffected; grade 1, less than 25% of the fruit area shows CI symptoms in the peel; grade 2, 25–50% of the fruit area shows CI symptoms; and grade 3, more than 50% of the fruit area shows CI symptoms.

The CI index is calculated using the following formula: CI index (%) = (CI Grade ×number of fruit at this level)/(highest level × total fruit number) × 100. Three replicates for each treatment were performed, and each replicate contained 25fruits.

2.3. Measurement of lipid peroxidation content

Lipid peroxidation content was determined and expressed as malondialdehyde (MDA) equivalents, according to the method of Rajinder *et al*. (1981), with slight modifications. Fruit tissue (4.0 g) from peach fruit were homogenized with 20 mL of 10% trichloroacetic acid and then centrifuged for 10 min at 5000 × g. One milliliter of the supernatant was mixed with 3 mL of 0.5% thiobarbituric acid (TBA) dissolved previously in 10% trichloroacetic acid. The reaction mixture solution was heat-treated for 20 min at 95 ◦C, quickly cooled, and then centrifuged for 10 min at 10,000 × g to clarify precipitation. Absorbance at 532 nm was measured and subtracted from the nonspecific absorbance at 600 nm. The amount of MDA was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1} and expressed as mM g^{-1} of fresh weight (FW).

2.4. Assay of antioxidant enzyme activity

Samples were obtained from peach fruits at each time point during the storage at 0 °C. Fresh tissue samples (4 g) with 0.1 g polyvinylpolypoyrrolidone (PVPP) were homogenized in 10 mL of ice-cold PBS (25 mM) containing 1 mM EDTA. The homogenate was centrifuged at 12,000 rpm for 20 min at 4 °C, and the resulting supernatant was collected for the enzyme assay (Egley *et al*., 1983).

Catalase (CAT) and peroxidase (POD) activity was analyzed according to Xing *et al*. (2011), with a slight modification.

The reaction mixture consisted of 2 mL sodium phosphate buffer (50 mM, pH 7.0), 0.5 mL H2O2 (40 mM) and 0.5 mL enzyme extract. The decomposition of H2O2 was measured by the decline in absorbance (A) at 240 nm. CAT specific activity was expressed as U kg^{-1} of FW, where U = Δ-A at 240 nm per s. For POD determination, 0.5 mL enzyme extract were incubated in 2 mL buffered substrate (100 mM sodium phosphate, pH 6.4 and 8 mM guaiacol) for 5 min at 30 ◦C and the increasing absorbance measured at 460 nm every 30 s for 120 s after adding 1 mL of H2O2 (24 mM). POD and CAT activity was expressed as U.mg $protein^{-1}$, where U = ΔA at 470 nm per s.

2.5. H_2O_2 assay

The assay for H_2O_2 content was carried out by the procedure described by Prassad (1996). Fresh tissues (2g) were homogenized with 10 ml of acetone at 0°C. After centrifugation for 15 min at 6000g at 4°C, the supernatant phase was collected. The supernatant (1m1) was mixed with 0.1 ml of 5% titanium sulphate and 0.2 ml ammonia, and then centrifuged for 10 min at 6000g and 4°C. The pellets were dissolved in 3 ml of 10% (v/v) H_2SO_4 and centrifuged for 10 min at 5000 rpm. Absorbance of the supernatant phase was measured at 410 nm. H_2O_2 content was calculated using H_2O_2 as a standard and then expressed as μg g^{-1} on fresh weight basis (Prassad,1996).

2.6. Data analysis

The experimental design was implemented in the form of a completely randomized design. All statistical analyses were performed with SPSS version 13.0 (SPSS Inc., Chicago, IL, USA). Data at each time point were analyzed by one-way ANOVA, and mean separations were performed by Duncan's new multiple range test. Differences at $P < 0.05$ were considered significant. Each treatment consisted of three replicates (10 peach fruits were considered in each repetition) and the experiment was repeated six times.

3. Results

3.1. Effect of SA on CI of peach fruit

The effect of SA on CI of peach fruit during cold storage is shown in Fig. 1. In the untreated control peach fruit, CI symptoms (surface pitting and browning as shown in Fig. 1) occurred at 5 day after storage, and the CI index was as high as 48.35% at 25 days in control peaches (Fig. 1). In peach fruits treated with SA, CI symptoms occurred at 10 days after storage (DAS).

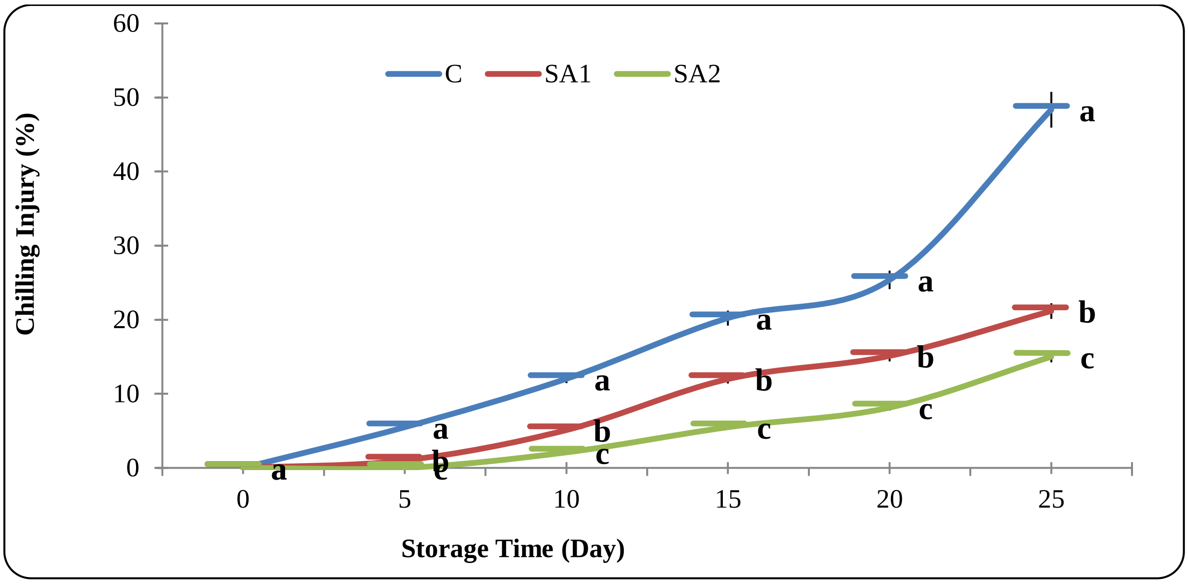


Figure 1. Effect of salicylic acid (SA) on chilling injury of Elberta peach (*Prunus persica* L.) fruit during storage under low temperature. (C: control fruit; SA1: salicylic acid 1mM; SA2: salicylic acid 2mM). Means followed by same letter are not significantly different at P<0.05 according to Duncan's multiple range tests.

Moreover, the CI index in SA-treated fruit was significantly lower compared to control fruit. Among all the treated fruit, SA at 2mM was the most effective in alleviating CI.

3.2. Lipid peroxidation and H_2O_2 content

Lipid peroxidation content has been used as direct indicators of membrane injury, which is often associated with CI. As shown in Fig. 2, a continuous increase in lipid peroxidation content was observed, both in control and in the treated peach fruits stored at 0°C, although the application of SA to peaches significantly delayed the increase of lipid peroxidation during storage. At the end of the storage period (Day 25), the lipid peroxidation content of samples treated with SA at 2mM was significantly lower compared to the lipid peroxidation of control samples. This pattern observed in peach fruits in Fig. 2.

Changes in H_2O_2 content of peach fruit are presented in Fig. 3. The initial H_2O_2 content was low (10.1 – 10.12 µg.g FW^{-1}) in peach fruits. In general, H_2O_2 content increased as storage time increased. At the end of the storage period (25 DAS), the H_2O_2 content of SA-treated samples and control samples was (15.2 and 21.11µg.g FW^{-1}, and 34.27 µg.g FW^{-1} in tissue) respectively, (Fig.3). The increase of H_2O_2 content in the control groups was much higher than that in the SA-treated oranges. H_2O_2 content, is as an indicator of membrane integrity (Gualanduzzi *et al*., 2009; Huang and Guo, 2005; Xu *et al*., 2009), and our results show that membrane integrity was maintained as a result of SA treatment.

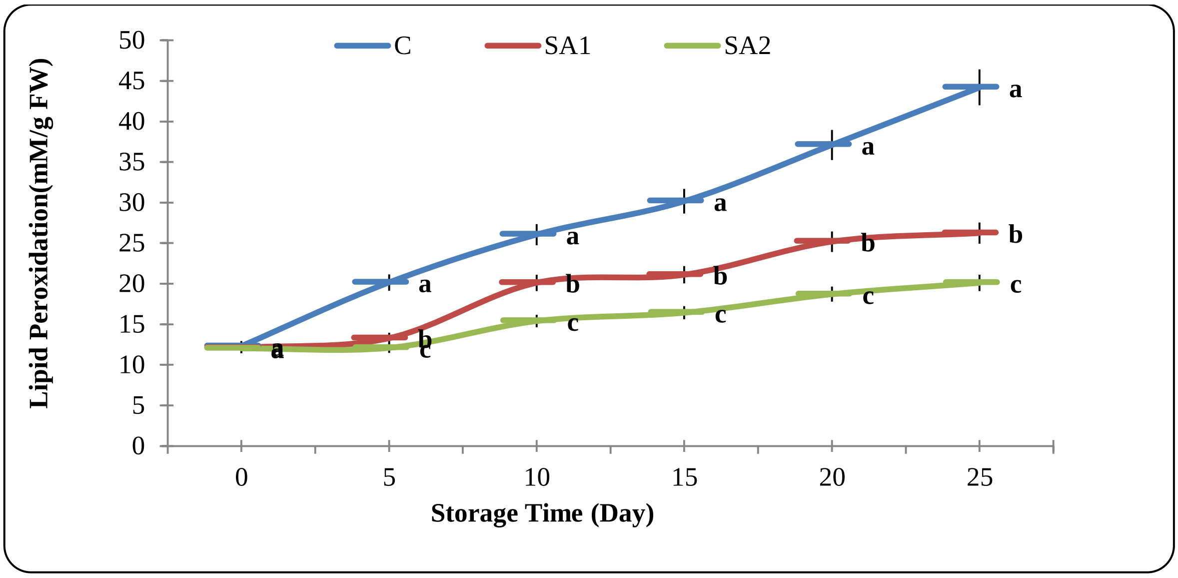


Figure 2. Effect of salicylic acid (SA) on lipid peroxidation of Elberta peach (*Prunus persica* L.) fruit during storage under low temperature. (C: control fruit; SA1: salicylic acid 1mM; SA2: salicylic acid 2mM). Means followed by same letter are not significantly different at P<0.05 according to Duncan's multiple range tests.

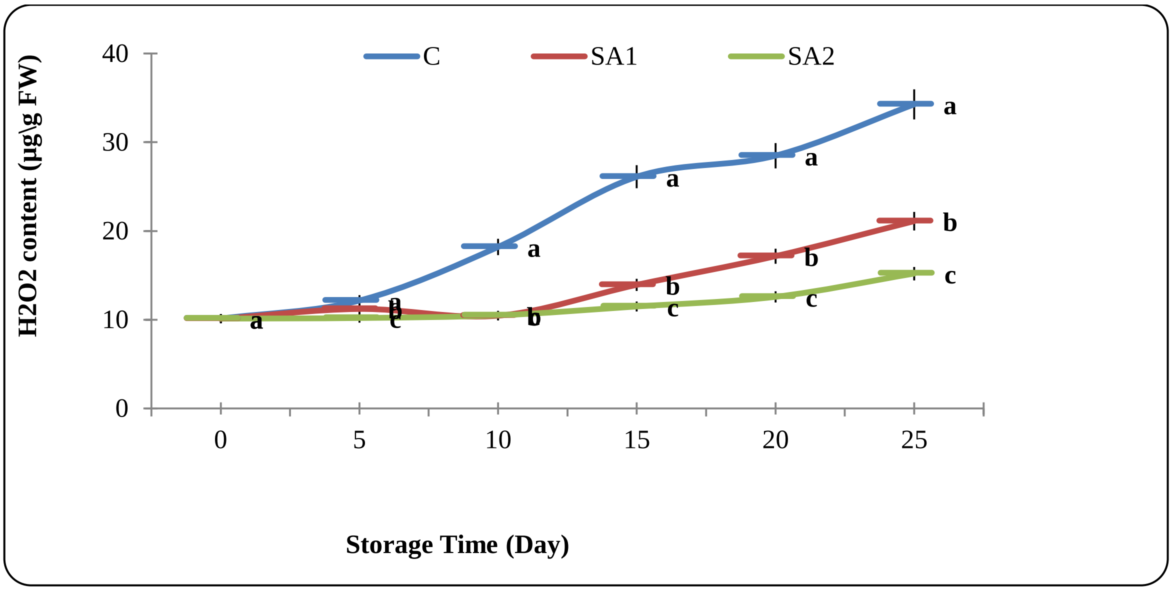


Figure 3. Effect of salicylic acid (SA) on H_2O_2 content of Elberta peach (*Prunus persica* L.) fruit during storage under low temperature. (C: control fruit; SA1: salicylic acid 1mM; SA2: salicylic acid 2mM). Means followed by same letter are not significantly different at P<0.05 according to Duncan's multiple range tests.

3.3. Effect of SA on induction of antioxidant enzymes

As illustrated in Fig. 4 and 5, SA treatments at 1 and 2 mM (especially 2mM) induced the activity of CAT and POD, enzymes in peach fruit stored at 0 °C. CAT and POD activity showed a similar pattern, during storage (Fig. 4 and 5). In control and SA treatments, activity of CAT and POD, enzymes peaked at the end of the storage period (25Day). Both enzymes in peaches treated with SA at 2 mM showed higher levels compared to controls during the entire storage period. All SA treatments induced a higher CAT and POD activity than that in control fruits (Fig. 4 and 5).

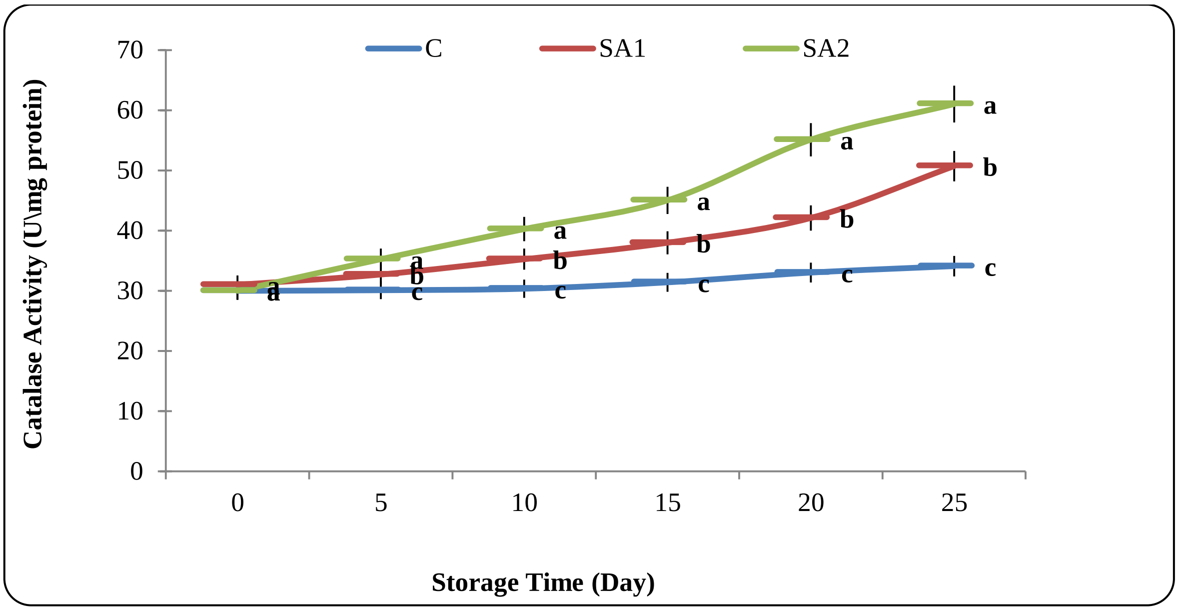


Figure 4. Effect of salicylic acid (SA) on catalase activity of Elberta peach (*Prunus persica* L.) fruit during storage under low temperature. (C: control fruit; SA1: salicylic acid 1mM; SA2: salicylic acid 2mM). Means followed by same letter are not significantly different at P<0.05 according to Duncan's multiple range tests.

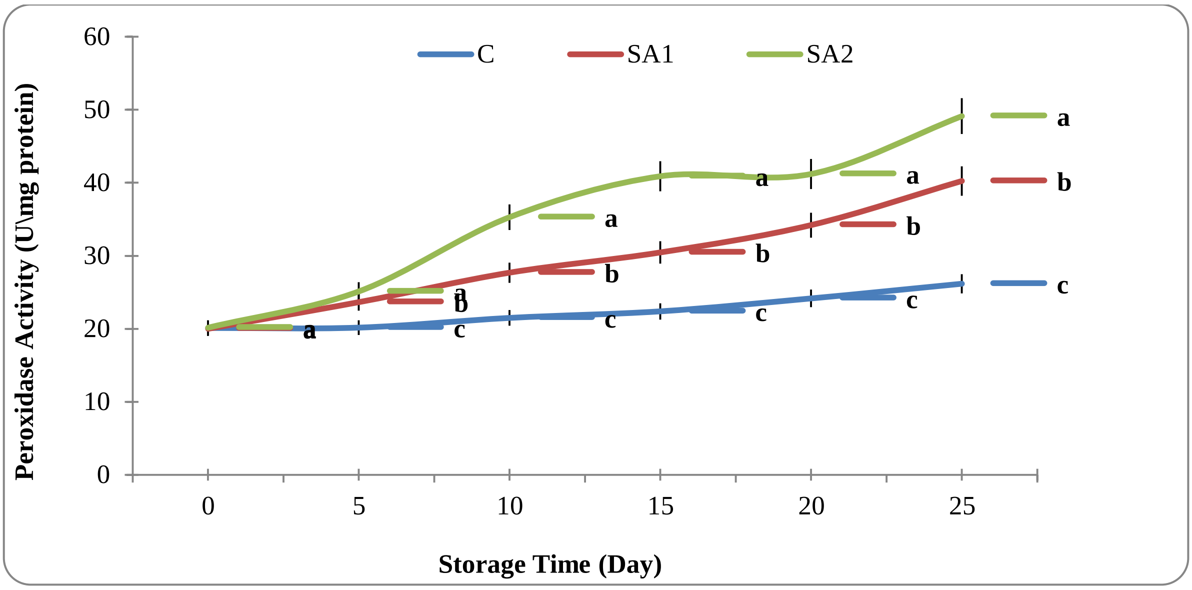


Figure 5. Effect of salicylic acid (SA) on peroxidase activity of Elberta peach (*Prunus persica* L.) fruit during storage under low temperature. (C: control fruit; SA1: salicylic acid 1mM; SA2: salicylic acid 2mM). Means followed by same letter are not significantly different at P<0.05 according to Duncan's multiple range tests.

3.4. Correlation analysis

The correlation plot (Fig. 6) shows the correlations between antioxidant responses in peach under different postharvest time and different salicylic acid concentration. The results showed that there was a positive and significant relationship between antioxidant enzyme activity, chilling injury, peroxidation content and H_2O_2 content (Figure 6). It has been shown that increasing the activity of antioxidant enzymes in peach fruit tissues reduces chilling injury, lipid peroxidation, and hydrogen peroxide, which are the causes of product deterioration during storage.

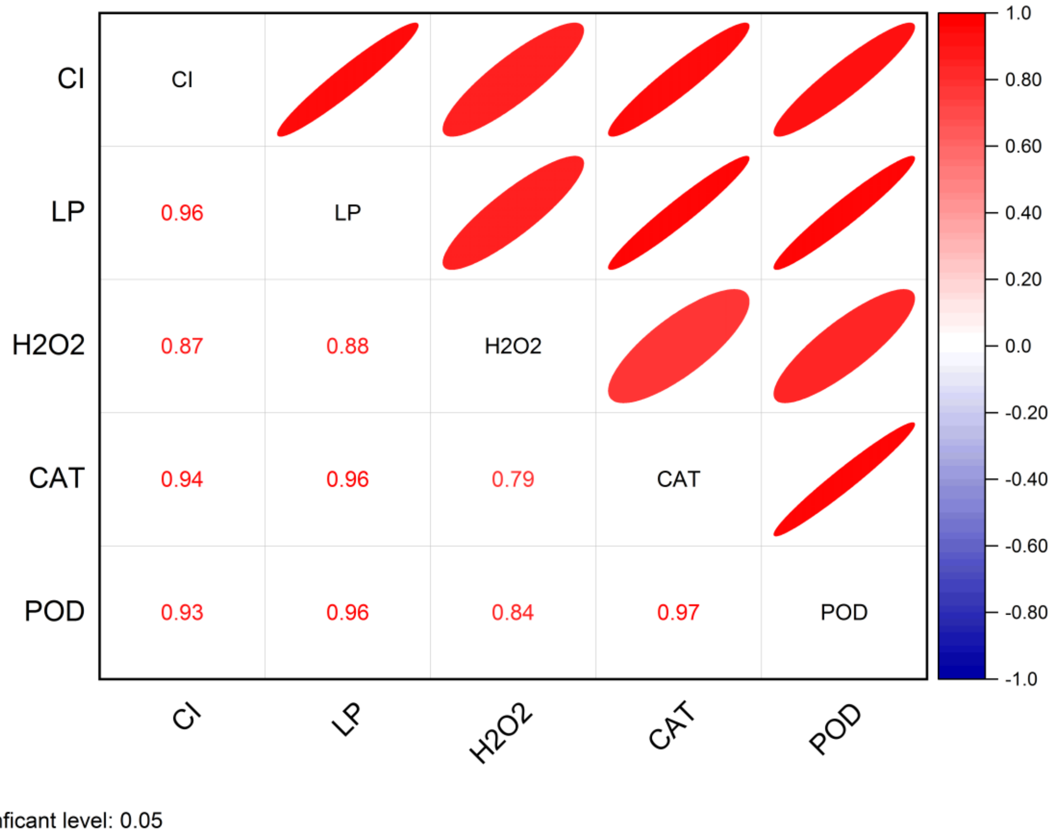
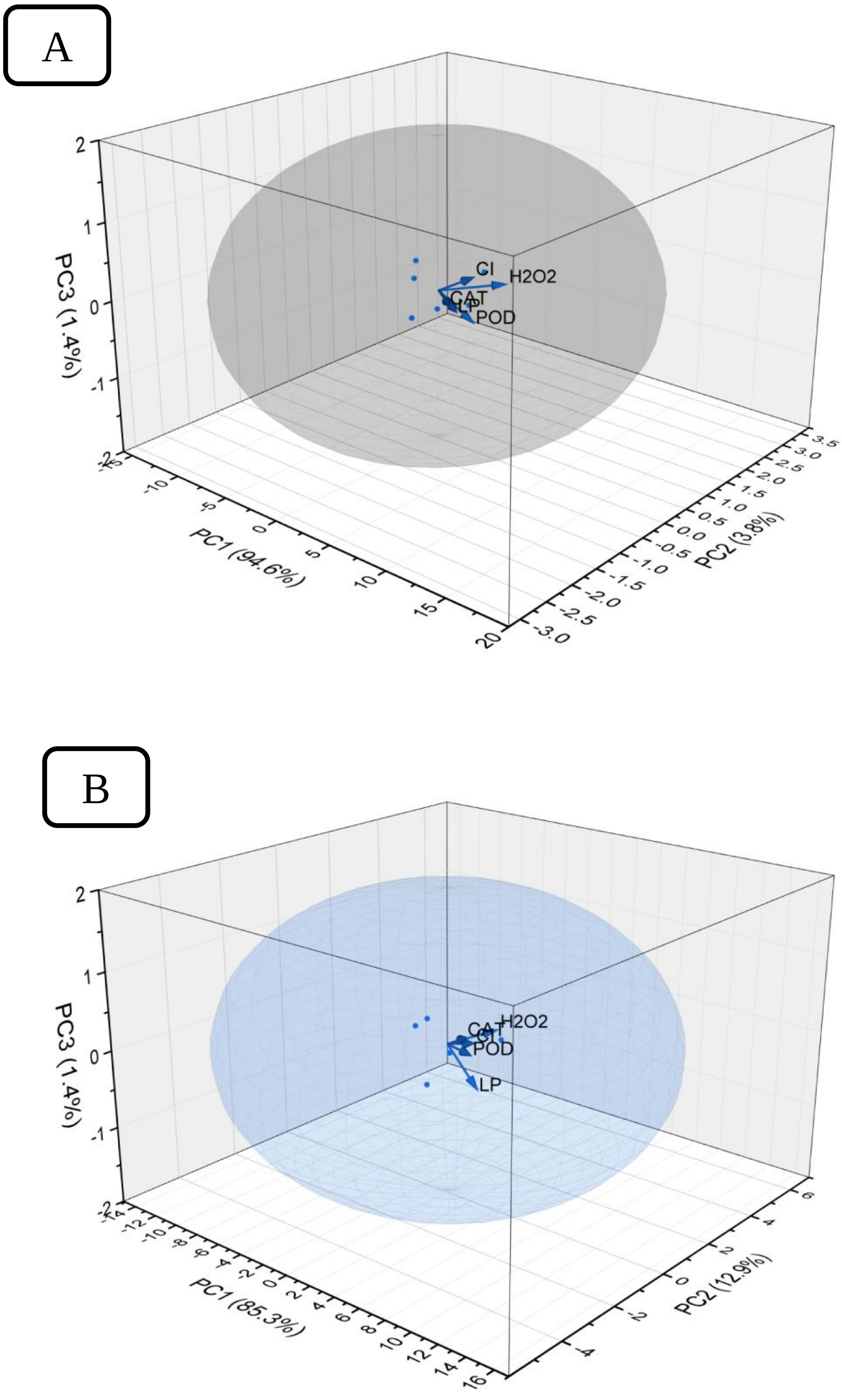


Figure 6. Correlation plot between antioxidant capacity of peach in postharvest. The size and color intensity of circles are proportional to Pearson's correlation coefficient at P<0.01. Red circles indicate positive correlations, while blue are negative correlations. In the correlogram scale from−1 to+1, Pearson's correlation coefficient for variables is on the vertical and horizontal axis. *Indicates values that are statistically different at P<0.05. A correlation plot was drawn with Origin Pro software version 2024.

3.5. Principal component analysis

After standardizing the data to achieve a zero mean and unit variance, principal component analysis (PCA) was conducted to examine changes in five parameters across three salicylic acid concentration and different postharvest times of peach. The magnitude of each vector in the PCA plot reflects the influence of the corresponding parameter, while its direction is determined by PCA1 and PCA2 values. The PCA analysis visualized changes five parameters across three salicylic acid concentration and different postharvest times of peach in the following conditions: A (Control), B (1 mM SA) and (2 mM SA). Control, 1 mM SA and 2 mM SA the percentages of explained variance were 85.6%, 95.3%, and 97.3%, respectively (Fig. 7A, 7B and 7C).


A
PC3 (1.4%)
PC1 (94.6%)
PC2 (3.8%)
CI
H2O2
CAT
LP
POD
B
PC3 (1.4%)
PC1 (85.3%)
PC2 (12.9%)
CAT
H2O2
CI
POD
LP

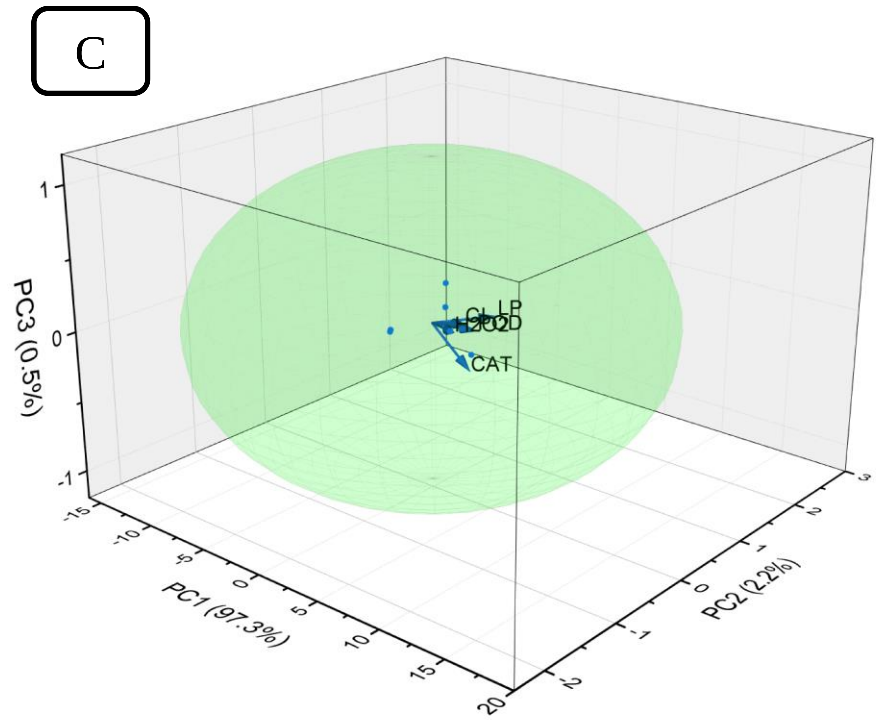


Figure 7. Vector graphs show the relative "contribution" of each input variable to the formation of the principal components. Principle component biplot based on variations of three levels of salicylic acid concentration, A (Control), B (1 mM SA) and (2 mM SA) under different time of postharvest of peach. Principal component analysis and biplots were performed using Origin pro software version 2024.

4. Discussion

Chilling injury (CI) is a major factor in reducing the quality and limiting the storage time of fruits, including peach. We observed that SA treated fruits showed a decrease in MDA and H_2O_2 content, which led to an improvement in fruit quality. To prevent CI development and extend shelf life, several strategies, including physical and chemical treatments, have been evaluated (Abbasi *et al*., 2010; Chang *et al*., 2000; Crisosto *et al*.,2004; Garner *et al*., 2001; Girard *et al*., 2005 Gorny *et al*., 1999; Wagstaff *et al*., 2010; Zhou *et al*., 2000a). In the present study, the plant hormone SA was applied and the results indicated that SA significantly reduces CI of peach fruits during storage at 0 °C (Fig. 1). Our finding was consistent with previous reports that exogenous application of SA is effective in protecting loquat (*Eriobotrya japonica*)(Cai *et al*., 2006), tomato and mango (Ding *et al*., 2001; Ding *et al*., 2007), Orange (Hou *et al*., 2022), grape(Hazarika and Marak, 2022; Zebro and Heo. 2024), pineapple (Lu *et al*., 2010), pomegranate(Sayyari *et al*., 2009) and peaches (Wang *et al*., 2006) against cold stress. However, to the best of our knowledge, this is the first report that SA has been shown to have beneficial effects against CI and antioxidant responses of postharvest peach fruits. CI occurrence is often accompanied by oxidative damage, which can be followed through lipid peroxidaion content. It is a final product of lipid peroxidation (Asghari and Aghdam, 2010; Gualanduzzi *et al*., 2009; Senaratаа *et al*., 2004). In this study, there was a continuous increase in lipid peroxidaion content in all fruits, but the application of SA significantly delayed the increase of lipid peroxidaion (Fig. 2). Moreover, the change in membrane permeability (revealed by H_2O_2 content) showed trends like to lipid peroxidation content; that is, fruit tissue H_2O_2 content increased with storage duration, but SA markedly delayed the increase (Fig. 3). SA has been considered to be involved in a network of interacting signal transduction pathways, which regulate defense responses to abiotic stress (Cao *et al*., 2010; Ding *et al*., 2002; Fung *et al*., 2006; Horvath *et al*., 2007; Liu *et al*., 2022; Kaur *et al*., 2022; Gharibiyan *et al*., 2023; Shakirova *et al*., 2003). Dat *et al*. (2002) demonstrated that SA-mediated stress tolerance in tobacco shows interactions with abscisic acid, ethylene and salicylic acid pathways. In our study, the high activity of antioxidant enzymes such as CAt and POD along with the increased shelf life of peach in the SA-supplemented fruits, can be attributed to the higher efficiency of SA in enhancing the antioxidant system compared to non-treated fruits. The mechanism by which SA induced cold resistance in peach was investigated in this study. When horticultural crops are exposed to severe abiotic stresses, including cold stress, large amounts of intracellular ROS are generated, Recent studies have also shown that Se improves the ability of many plants to scavenge ROS through the enzymatic system by SA (Amani *et al*., 2023; Eraslan *et al*., 2008; Song *et al*., 2023; Wang *et al*., 2007; Wang *et al*., 2022). Overall, our research findings are in agreement with previous studies that have reported the beneficial effects of SA treatment in activating the antioxidant system and improving fruit quality. The detoxification of ROS is dependent on antioxidant enzymes such as CAT and POD (Nia *et al*., 2022; Singh *et al*., 2012; Ding *et al*., 2007). The increase in these enzymes' activity contributes to the adaptation of plants to cold stress and ameliorates oxidative damage such as lipid peroxidation (lipid peroxidation increase as indicator) and H_2O_2 content (Shakirova *et al*., 2003; Eraslan *et al*., 2007). In the present study, we found that the activity of the two enzymes in peach was induced by SA treatment during the storage at 0 °C (Fig. 4 and 5). Similar results have been reported in previous studies. Dat *et al* (2000) demonstrated that exogenous SA was effective in promoting growth of oxidative stressed tobacco plants by elicitation of CAT, POD, and APX(ascorbate peroxidase). Khodary (2004) reported that SA enhanced the activity of CAT, APX, and SOD (superoxide dismutase) in maize leaves, and apoplastic H_2O_2 in SA-induced antioxidant defense. We suggest that the SA induction of antioxidant enzyme activity in peach fruits may be a key factor in lowering oxidative damage caused by cold stress, thus improving the cold tolerance and alleviating CI of peach stored at 0 °C. The application of SA reduced CI of peaches stored at 0 °C and maintained peaches quality as well. The chilling injury, lipid peroxidation, H_2O_2 were significantly reduced by salicylic acid treatment especially at 2 mM SA treatment induced cold resistance may be due to stimulation of antioxidant enzymes and protection against membrane oxidative damage, decreased lipid peroxidation and H_2O_2 content in peach fruits. These results may have implications for the use of SA in managing postharvest CI of other temperate fruits stored at low temperatures.

Conclusion

Based on our results, the treatment of fruits with SA lead to maintaining peach quality-related features and, consequently, the storage life of treated fruits. Both the application of the concentration of SA resulted in a significant slowdown of the chilling injury processes of peach fruits and could be effective for maintain quality. Indeed, fruit treated with SA and had values of activity of enzymes such as catalase and peroxidase higher after 25 days of cold storage, which is an excellent result for commercial purposes. However, treated and untreated fruit also differed in chilling injury. Nevertheless, after 25 days of cold storage, the effects of SA on evaluated parameters such as activity of enzymes, was greater than control. Treatment of fruits with SA is as a protective barrier that decreases chilling injury and the accumulation of hydrogen peroxide. Finally, this study indicates that SA treatment significantly decrease the chilling injury in peach fruits during storage. This opens perspectives of potential diffusion and future research activity.

Author Contributions: Conceptualization: Z.P, V.R.S.; Methodology: Z.P, V.R.S.; Software: Z.P., Validation: Z.P, V.R.S.; Technical Investigation: Z.P, V.R.S.; Writing H.M., Writing Z.P, V.R.S.; Supervision; Z.P, V.R.S.

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Conflicts of Interest: The authors declare no conflict of interest.

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