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The effect of exogenous salicylic acid on antioxidant activity, bioactive compounds and antioxidant system in apricot fruit

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ABSTRACT

To improve the postharvest nutritional and functional qualities of apricot fruit, the fruits were treated with salicylic acid (SA) by vacuum infiltration. The effects of SA treatment were investigated via analyzing of the quality attributes especially related to antioxidant activity, active oxygen metabolism and antioxidant system during the storage of apricot fruit at 2 °C for 25 days. Our results showed that significant higher hydrophilic total antioxidant activity (H-TAA) and content of phenolic acids, flavonoids were found in fruits treated with SA. SA treatment reduced the lipophilic total antioxidant activity in apricot fruit. During storage, application of SA significantly enhanced activity of phenylalanine ammonia-lyase (PAL) and content of hydrogen peroxide. Treated fruits showed significantly lower activity of catalase and ascorbate peroxidase but higher activity of superoxide dismutase and peroxidase than those in control fruits. Moreover, SA treatment retarded the ripening progress and quality loss. Thus, SA treatment might enhance the activity of PAL, H-TAA and content of phenolic acids via regulating the metabolism of hydrogen peroxide in apricot fruit.

1. Introduction

Apricots contain different level of nutritional compounds such as various vitamins, organic acids, sugars, minerals, polyphenols (Sartaj et al., 2011). Apricots are popular around the world because of their nutrition value, pleasant and delightful aroma (Solis-Solis et al., 2007). In addition, various phenolic compounds including caffeic acid, ferulic acid, catechin, epicatechin, *p*-coumaric acid have been identified in apricot fruit (Dragovic-Uzelac et al., 2005; Sochor et al., 2010). Flavonols and carotenoids have also been identified in apricot fruit (Dragovic-Uzelac et al., 2007).

As one kind of climacteric fruits, apricot fruit would suffer rapid ripening and deterioration after postharvest, and thus have a limited postharvest-life at room temperature. During the storage time, apricot fruit undergo the softening and rotting which largely attribute to the loss of quality. Nowadays, low temperature is the mostly applied mean to extend the postharvest-life and maintain the quality of apricots.

Salicylic acid, as a natural phenolic acid, used to be applied to enhance the local and systemic resistance in fruits against pathogens (Chan et al., 2007). In addition, SA treatment has shown its effects on inhibiting the production of ethylene, respiration and senescence (Chan et al., 2007), promoting the quality of fruits (Tareen et al., 2012).

Recently, the effects of postharvest treatment on bioactive compounds and antioxidant capacities of fruit and vegetable have attracted the interest of numerous scientists. Treatment with SA could maintain higher total antioxidant activity, contents of phenolic acids of various kinds of fruits (Tareen et al., 2012; Dokhanieh et al., 2013). However, most work just focus on the positive relation between SA treatment and total phenolic acid and total antioxidant activity of fruits. As far as we know, just one report focused on the effects of SA postharvest treatment on H-TAA and L-TAA of sweet cherry (Valero et al., 2011).

Phenylalanine-ammonia lyase (PAL) is a key enzyme for regulating the biosynthesis of phenolic compounds, could be regulated by SA (Dokhanieh et al., 2013). Latest research suggested that reactive oxygen species (ROS) can be associated with gene expression of PAL, activity of PAL, and accumulation of phenolic compounds (Jacobo-Velázquez et al., 2011).

Major ROS-scavenging enzymes include superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidise (APX), which could be regulated by postharvest SA treatments (Huang et al., 2008). SOD can remove superoxide radical ($O_2\bullet^-$) by converting $O_2\bullet^-$ to hydrogen peroxide (H_2O_2), which is then decomposed by APX and CAT

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(Rao et al., 1997). POD is stimulated by H_2O_2 to enhance the defense of cell (Xu and Tian, 2008).

The balance of the activity of these antioxidant enzyme is crucial for determining the rate of ($O_2\bullet^-$) production and content of H_2O_2 (Xu and Tian, 2008). Postharvest SA treatments can modulate the metabolism of ROS via regulating the activity of antioxidant enzymes.

To the best of our knowledge, there is no literature about the physiological basis for the postharvest SA treatment induced phenolic accumulation of fruits. The aim of this research was to determine the effect of SA on phenylalanine ammonia-lyase (PAL), antioxidant enzymes (SOD, APX, CAT, POD) and ROS to provide physiological evidence for the positive relation between SA treatment and content of phenolic, antioxidant activity in fruits.

2. Materials and methods

2.1. Materials and treatments

Apricot fruit (*Prunus armeniaca* L. cv. Dahuang) of commercial maturity were harvested on 7th, July, 2013 in Beijing, China. Apricot fruit used in the experiment were sorted by uniform size, without physical damage. Apricot fruit were vacuum-infiltrated (0.02 MPa, 2 min) with different concentrations of SA solution at room temperature. The concentrations of SA solution were 0 (control), 1.0 and 2.0 mM, respectively. Control fruits were treated with distilled water. The fruit were then air-dried at room temperature. Fourty apricot fruit from each replicate were put into plastic boxes (600 × 100 × 70 mm). All fruit were then stored at 2 °C, and 85–90% relative humidity (RH). Each treatment contained 240 apricot fruit. For each treatment, apricot fruit were randomly divided into triplicate (80 apricots each replicate).

2.2. Determination of hydrophilic total antioxidant activity (H-TAA) and lipophilic total antioxidant activity (L-TAA)

Total antioxidant activity (TAA) of both hydrophilic (H-TAA) and lipophilic (L-TAA) compounds was determined in the same extraction according to a previous described method (Valero et al., 2011). Briefly, for each sample, three grams of tissue were homogenized ice on with 5 mL phosphate buffer (50 mM, pH 7.8) and 3 mL of ethyl acetate, and then centrifuged at 12000 × *g* for 20 min at 4 °C. The upper fraction was used for L-TAA and the lower for H-TAA. The lower fraction was diluted with phosphate buffer (50 mM, pH 7.8) to a final volume of 20 ml. For both cases, TAA was determined using an enzymatic system. The absorbance of $ABTS^+$ radicals are monitored at 730 nm. The calibration curve was performed with Trolox from Sigma. The results of H-TAA and L-TAA are expressed as mg Trolox equivalent (TE)/g FW and μg Trolox equivalent (TE)/g FW, repectively.

2.3. Extraction and measurement of total phenols, total flavonoids

Bioactive compounds were extracted by the procedure reported previously with some modification (Meyers et al., 2003). Two grams of frozen flesh from each replicate were homogenized at 4 °C with 16 ml of ethanol, and then centrifuged at 12,000 × *g* for 30 min. After centrifugation, the supernatant was collected and then were diluted to 20 ml with 80% ethanol. The extracts were used for the measurement of total polyphenol, flavonoids.

The content of total phenols was determined using the modified Folin–Ciocalteu method (Meyers et al., 2003). The content of total phenols was expressed as mg catechin equivalents (CAE)/g FW, based on a standard curve.

The content of flavonoids was quantified using the method reported previously with slight modification. A volume of 1 ml extracted sample was diluted with 4 ml deionized water, followed by addition of 0.3 ml 5% $NaNO_2$, 0.3 ml 10% $AlCl_3{\cdot}6H_2O$. Absorbance was monitored at 510 nm. Concentration of Total flavonoids was calculated as mg rutin equivalents/g FW.

2.4. Measurement of content of hydrogen Dioxide (H_2O_2) and rate of superoxide radical ($O_2\bullet^-$) production

The content of H_2O_2 was assayed using the method previously described with slight modification (Velikova et al., 2000). Approximately 3 g of flesh was extracted with 3 mL 0.2% (w/v) TCA on ice. The homogenate was then transferred into a tube and centrifuged at 12,000 × g for 30 min at 4 °C. To determine the concentration of H_2O_2, 1.5 mL of the supernatant was added to 1.5 mL potassium phosphate buffer (10 mM, pH 7.0) and 3 mL KI (1 M). The absorbance of supernatant was read at 390 nm. The content of H_2O_2 was assayed by a standard curve and expressed as μmol/g FW.

The rate of $O_2\bullet^-$ production was determined according to a method previously described (Huang et al., 2008). The assay was based on oxidation of hydroxylamine with $O_2\bullet^-$, and nitriteconcentration was determined by a color reaction with 4-aminobenzenesulfonic acid and a-naphthylamine. Absorbance of the color was determined at 530 nm. The rate of $O_2\bullet^-$ production was expressed as nmol/g/min FW.

2.5. Determination of activity of SOD, APX, CAT, POD and PAL

For the assay of relative activity of SOD, APX, CAT, POD and PAL activity, flesh (4 g) from slices was homogenized on ice with 10 ml of 0.1 M phosphate buffer (pH 8.0), containing 2 mM ethylene diamine tetraacetic acid (EDTA) and 8 g/L crosslinking polyvingypyrrolidone (PVPP), and then centrifuged at 12,000 × *g* for 30 min at low temperature (4 °C). The supernatant was collected as the source of crude enzyme extract. The content of protein was determined according to the method described previously (Bradford, 1976). The assay of all the enzyme activity was expressed as U/mg protein.

Superoxide dismutase (SOD) activity was assayed according to the method described previously with little modification (Huang et al., 2008). The reaction mixture was incubated for 10 min under a fluorescent light. Absorbance was determined at 560 nm. One unit of SOD activity was defined as the amount of enzyme required to inhibit 50% of photochemical reduction of NBT.

Ascorbate peroxidase (APX) activity was assayed according to the method described previously with little modification (Huang et al., 2008). The decrease in the absorbance was read at 340 nm. One unit of enzyme was defined as the decrease of 0.1 per minute.

CAT activity was assayed using the procedure described previously with little modification (Huang et al., 2008). The absorbance of the reaction solution was recorded at 240 nm. One unit of enzyme was defined as the decrease of 0.1 per minute.

The activity of POD activity was assayed according to the method described previously with some modification (Saba et al., 2012). The change of absorbance was recorded at 410 nm over a 3 min period. One units of POD activity was defined as increase of 0.1 per minute.

PAL activity was calculated using the procedure described previously with little modification (Saba et al., 2012). The absorbance of reaction mixture was recorded at initial (0 min) and final time (1 h). One unit of PAL activity was defined as the increase of 1 per hour in absorbance at 290 nm.

2.6. Analysis of ethylene production and the rate of respiration

Three fruits were sealed in 1 L flasks for 1 h at 25 °C. The assay was done according to the method previously described (Valero et al., 2011). One mL gas of the headspace from chamber was