Scientia Horticulturae 200 (2016) 95–101

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Short communication

Enhancement of postharvest nutritional quality and antioxidant capacity of peach fruits by preharvest oxalic acid treatment

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ARTICLE INFO

Article history:
Received 14 September 2015
Received in revised form 7 January 2016
Accepted 8 January 2016
Available online 19 January 2016

Keywords:
Antioxidant potential
Flavonoid
Phenol
Prunus persica L.
Postharvest storage

ABSTRACT

To improve the postharvest nutritional quality and antioxidant potential of peach fruit, the fruits were treated with oxalic acid (OA) by preharvest spray at 15 day before harvest. Fruit nutritional quality was analyzed in weekly intervals during the storage of peach fruits at 1 °C for 28 days. The results showed that the application of OA significantly enhanced antioxidant enzymes catalase, peroxidase and superoxide dismutase activities in peach fruits during cold storage. In addition, the increases in total flavonoids, phenolics, and antioxidant activity were higher in treated than in control ones, leading to fruit with high bioactive compounds and antioxidant potential assayed by 1, 1-diphenyl-2-picrylhydrazyl and ferric reducing antioxidant power methods. At the end of storage period, the highest value of total flavonoids among the applied treatment was observed in OA 5 mM. During storage the softening rate was higher in non-treated fruit. Thus, preharvest treatments with OA could be promising strategy to maintain fruit quality and antioxidant capacity as well as maintain a high flesh firmness following post-harvest storage and export.

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1. Introduction

Peach fruit [*Prunus persica* (L.) Batsch] is considered one of the most popular and common fruit in the world (Sansavini et al., 2006). Peaches are highly perishable climacteric fruit and, would suffer rapid ripening and deterioration after postharvest, and thus have a limited postharvest life at room temperature. During storage time, peach fruit may undergo softening and rotting, which largely cause loss of quality. Nowadays, low temperature is the mostly applied mean to extend postharvest life and maintain quality of peach fruits (Nunes, 2008).

During postharvest life, due to internal and external factors, chemical and physical changes occur in fruits and vegetables, which result to losses in nutritional and sensory quality. To prevent these adverse effects caused by postharvest factors, use of environmentally friendly technologies such as oxalic acid (OA) treatment was recommended. OA is an organic acid widely distributed in plants and is generally recognized as safe (GRAS) compound. Recent studies showed that OA might play important roles in stress response, systemic resistance, programmed cell death and redox homeostasis in plant (Zheng et al., 2007a; Liang et al., 2009; Wang et al., 2009; Kim et al., 2008). Recently, the effects of pre- and postharvest treatments on bioactive molecules and antioxidant potential of fruit and vegetable have attracted the interest of scientists. Both pre- and postharvest OA treatments for extending shelf-life and maintaining quality of postharvest fruits and vegetables have been investigated and developed for commercial use. Valero et al. (2011) treated sweet cherries with OA at 1 mM and stored them for 20 days under cold temperature, and reported that OA treatments delayed the postharvest ripening process, manifested by lower acidity, color changes and firmness losses, and maintained quality attributes for longer periods than controls. In addition, total phenolics, anthocyanins and antioxidant activity increased in untreated fruit during the first 10 days of storage and then decreased, while in fruits of OA treatments, these parameters increased continuously during storage. Martinez-Espla et al. (2014) treated sweet cherries with OA at 0.5, 1.0, and 2.0 mM at 98, 112, and 126 days after full blossom and reported that OA treatments increased fruit size at harvest, manifested by higher fruit volume and weight in cherries from treated trees than from controls, the higher effect being found with 2.0 mM OA. Also, Martinez-Espla et al. (2014) reported that the increases in total anthocyanins, total phenolics, and antioxidant activity associated with the ripening process were higher in treated than in control cherries, leading to fruit with high bioactive compounds and antioxidant potential at commercial harvest. Furthermore, it was reported that pre-harvest application of OA on kiwifruit plants

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http://dx.doi.org/10.1016/j.scienta.2016.01.011
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increased the postharvest quality and disease resistance of the fruit, especially inhibited the blue mold rot and patulin accumulation by *Penicillium expansum* (Zhu et al., 2016).

The losing of quality in fruits and vegetables under low temperature storage is due to imbalance between the production and elimination of reactive oxygen species (ROS) (Tsantili et al., 2010). The ROS are highly reactive and toxic and affect cellular functions by damaging DNA, oxidizing proteins, and causing lipid peroxidation. Antioxidant systems in fruits and vegetables, enzymatic and non-enzymatic, protect cells from oxidative damage by scavenging of ROS (Gill and Tuteja, 2010). Pre- and postharvest treatments such as OA may maintain nutritional quality of fruits and vegetables by enhancing antioxidant system activity (Zheng et al., 2007; Ding et al., 2007; Sayyari et al., 2010). Thus, the aim of this research was to determine the effect of OA on antioxidant enzymes [superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT)] and relation between OA treatment and nutritional quality [total phenols and flavonoids and total antioxidant activity assayed by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) methods] of peach fruits.

2. Materials and methods

2.1. Fruits and treatments

The study was conducted in a commercial orchard at Azarbayzan (Iran). Five-year-old peach [*Prunus persica* (L.) Batsch 'Anjiry maleki'] trees were selected for field sprays. Different OA concentrations (0, 1, 3 and 5 mmol L^{-1}) were sprayed on peach trees by using a hand-sprayer until fruit were wet to run off. Tween-20 was added to each solution as a wetting agent and, sprays were applied at 15-day before harvest. Fruit from control and OA-treated trees were harvested at commercial maturity and immediately transported to the laboratory. Then, fruit were divided into twelve groups of 40 fruit each and stored at 1 °C and 90% RH for 28 days. 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. Firmness was measured on opposite sides of each fruit with an Effegi penetrometer (FT 011, Fujihira Industry Co., Ltd., Tokyo, Japan) fitted with an 8 mm diameter probe. The mean of the two measurements was expressed in newtons (N).

2.2. Antioxidant enzymes activity assays

Crude extract for SOD, CAT and POD enzymes was performed by homogenizing 5 g of frozen samples with 10 mL of phosphate buffer 50 mM, pH = 7.0 containing 1% (w/v) PVP and 1 mM EDTA. The homogenate was centrifuged at 15,000 × *g* for 30 min at 4 °C and the supernatant used for enzyme assay. For CAT activities, the protocol described by Zhang et al. (2013) was used. In brief, 100 mL of the crude extract were added to 2.9 mL of reaction mixture containing 15 mM H_2O_2 and 50 mM phosphate buffer pH = 7.0. The degradation of H_2O_2 was measured by the decrease of absorbance at 240 nm during 3 min. One enzymatic unit (U) was defined as 0.01 absorbance decrease per minute, and CAT activity expressed as U mg protein^{-1}. POD activity was assayed according to the method of Ghamsari et al. (2007). The assay mixture contained 3 ml of 0.1 M citrate–phosphate–borate buffer system (pH 7.0), 50 microliters (μL) of 480 mM guaiacol, 50 μL of 96 mM H_2O_2 and 50 μL of extract. The increase in absorbance at 470 nm was recorded spectrophotometrically for 6 min and POD activity expressed as U mg protein^{-1}.

SOD activity was determined photochemically as described in Zhang et al. (2013). The reaction solution contained 50 mM phosphate buffer, pH 7.8, 5 mM methionine, 100 mM EDTA and 65 mM nitro-blue-tetrazolium (NBT). To 2.9 mL of this solution were added 25 μL of enzyme extract and 40 μL of 0.15 mM riboflavin. The tubes were then placed in a fluorescent light incubator (40 W, 10 min) and the formation of blue formazan was monitored by recording the absorbance at 560 nm. One unit (U) of SOD activity is defined as enzyme that causes a 50% inhibition of NBT reduction under assay conditions. The results are reported as U mg protein^{-1}. For all antioxidant enzyme activities results are the mean ± standard deviation (SD) of three replications. Total protein content in the enzyme extract was quantified according to Bradford (1976).

2.3. Total phenols and flavonoids content

Total phenolics were determined by the spectrophotometric method (Singleton and Rossi, 1965). Briefly, 0.1 mL of extract was mixed with 2 ml of 2% Na_2CO_3 and allowed to stand for 2 min at room temperature. For each sample 0.1 mL of 50% (v/v) Folin–Ciocalteu reagent was added with mixing and allowed to stand for 30 min, and the absorbance was measured at 720 nm. Total phenolic contents of sample were expressed as mg gallic acid equivalent 100 g^{-1} fresh weights.

Total flavonoids were determined using a colorimetric assay developed by Kaijv et al. (2006). 75 μL of aqueous $NaNO_2$ (5%) was added to 0.25 ml of peach extracts. After 5 min, 0.15 mL of 10% aqueous $AlCl_3$ was added and was vortexed. The mixture was allowed to stand for 6 min at room temperature. Then 0.5 mL of 1 mol L^{-1} NaOH was added to this mixture. The final volume was adjusted to 2.5 ml with deionized water. The absorbance was measured against the blank at 507 nm. Total flavonoids of samples were expressed on fresh weight basis as μmol quercetin equivalent (QE) 100 g^{-1}.

2.4. Total antioxidant activity

The FRAP assay was done according to the method 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), the light blue reagent contains Fe^{3+}-TPTZ that changes to dark blue after interaction with antioxidants, which is explained by the presence of Fe^{2+}-TPTZ in the reagent. A 50 μL aliquot of extract 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. FeSO4 (0–1 mM) was used as a reference standard and the results were expressed as mmol Fe(II)/g fresh weight.

The DPPH assay of peach extracts was carried out according to the procedure described by Dehghan and Khoshkam (2012) with some modifications. Peach fruit extracts (50 μL) were allowed to react with 1.95 mL of DPPH radical solution (0.1 mM in methanol) for 30 min in the dark covered with aluminum foil. The decrease in absorbance from the resulting solution (AS) was monitored at 517 nm in a UV–vis 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 extracts was expressed as the percentage of inhibition of the DPPH radical:

$$\text{RSA\%} = \frac{100(\text{AC} - \text{AS})}{\text{AC}}$$

2.5. Statistical analysis

The experiment was designed as a completely randomized design. All statistical analyses of variance were calculated over two factors: treatment and time in storage with SPSS 20.0. Differences among means of data were compared by Tukey's HSP test. Differences at $P \leq 0.05$ were considered significant.

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