



# Sodium nitroprusside (SNP) spray to maintain fruit quality and alleviate postharvest chilling injury of peach fruit



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## ABSTRACT

Peaches, cultivar 'G.H. Hill', from trees that were sprayed with 0, 25, 50 and 100  $\mu\text{mol L}^{-1}$  sodium nitroprusside (SNP) 14 days before harvest were stored at 4 °C for 4 weeks. Chilling injury (CI), ethylene production, weight loss, firmness, malondialdehyde, hydrogen peroxide and vitamin C concentrations, antioxidant capacity (AC), and superoxide dismutase, peroxidase and polyphenol oxidase activities in the fruit were measured during storage. SNP treatments reduced ethylene production and maintained firmness, AC and vitamin C of the fruit. Treatment with 25 and 50  $\mu\text{mol L}^{-1}$  SNP reduced CI but 100  $\mu\text{mol L}^{-1}$  SNP increased it. SNP at 100  $\mu\text{mol L}^{-1}$  promoted the accumulation of reactive oxygen species, decreased the activity of antioxidant enzymes and accelerated peroxidation in fruit during storage. These results indicated that preharvest SNP (25 and 50  $\mu\text{mol L}^{-1}$ ) treatment increased chilling tolerance of peach fruit through suppressing ethylene production, maintaining firmness, AC and vitamin C and enhancing anti-oxidative enzyme activity.

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## 1. Introduction

Low storage temperatures close to 0 °C during storage of harvested fruit usually reduce the rate of many metabolic processes that lead to fruit senescence, deterioration, and loss of quality. However, sub-tropical and tropical fruits are often susceptible to chilling injury (CI) at these low temperatures and represent a major factor limiting their storage life. Peaches can develop CI symptoms, such as internal browning, mealiness and loss of flavour when they are stored at 0–5 °C (Lurie and Crisosto, 2005). As mealy fruits lack juice and are unacceptable to consumers, this can lead to serious economic losses by the peach fruit industry (Zhu et al., 2009) and limits the storage potential of the fruit (Koushesh Saba et al., 2012).

Plant injury induced by chilling is thought to involve an imbalance between the production and consumption of reactive oxygen species (ROS) (Wise, 1995). The production of ROS such as hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) and superoxide is considered an important event in fruit during storage (Vicente et al., 2006). Although ROS can act as signals and secondary messengers for the activation of stress and defense pathways, their accumulation can cause oxidative damage to biomolecules, e.g. lipid peroxidation, and eventually lead to cell death (Hariyadi and Parkin, 1991). Malondialdehyde (MDA) is a

useful biomarker for lipid peroxidation (Hariyadi and Parkin, 1991). Various enzymes, including superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX), are involved in the process of scavenging ROS in plants. SOD catalyses the dismutation of superoxide to  $\text{H}_2\text{O}_2$  and oxygen, and  $\text{H}_2\text{O}_2$  is further detoxified by CAT and/or POX to water and oxygen (Mittler, 2002).

Different treatments that have potential to alleviate CI and prolong fruit storage; these include plant growth regulators such as jasmonic acid (Zhu et al., 2004), salicylic acid (Jin et al., 2009), polyamines (PAs) (Koushesh Saba et al., 2012) and nitric oxide (NO) (Cao et al., 2010). NO acts as a multifunctional signaling molecule in plants, and it is involved in many physiological processes including ripening of non-climacteric and climacteric fruit (Leshem and Pinchasov, 2000; Singh et al., 2009). There is increasing evidence that NO can prolong postharvest life of fruit, vegetables and fresh-cut flowers (Wills et al., 2007; Zhu et al., 2006). Postharvest exogenous application of NO has been reported to delay fruit ripening in a range of climacteric or non-climacteric fruit (Manjunatha et al., 2010; Zaharah and Singh, 2011). Softening of peaches was inhibited by 10  $\mu\text{L L}^{-1}$  NO gas during storage at 5 °C and 25 °C (Zhu et al., 2006). Recently postharvest NO fumigation has been reported to alleviate CI in cold stored Japanese plum (Singh et al., 2009). Postharvest exogenous application of NO has been reported to have mechanisms such as suppressed respiration rate, reduced ethylene biosynthesis and CI, delayed development of browning disorders, disease incidence and skin colour changes, flesh soft-

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ening and reduced activity of softening enzymes (Ku et al., 2000; Manjunatha et al., 2010).

NO could be involved in storage protocols as a senescence retarding agent (Leshem et al., 2000). Moreover, Ku et al. (2000) have found that NO is capable of reducing the water loss of a range of commodities susceptible to wilting or shriveling during postharvest storage. Peach fruit is a perishable product with a relatively high ethylene production rate that needs temperature management procedures, alone or in combination with other technological supplements (e.g. controlled atmospheres, 1-methylcyclopropene), to extend its shelf life and avoid storage disorders. SNP as a nitric oxide (NO) donor was used to suppress ethylene production and affect fruit quality (Flores et al., 2008; Zhu et al., 2009), but there are no report of the effects of preharvest SNP on CI in peach and associated enzyme activities such as polyphenoloxidase (PPO) activity. SNP was discovered in 1850 and found to be useful in medicine in 1928 (Friederich and Butterworth, 1995). It has been used to lower blood pressure and considered by the world health organization as an essential medicine (World Health Organization, 2013). The objective of the current study was to investigate if SNP application to peach before harvest would prolong the fruit storage period at 4 °C, and what effects these treatments had on CI, changes in ethylene production, antioxidant capacity (AC) and activities of antioxidant enzymes in the fruit.

## 2. Materials and methods

### 2.1. Plant materials and treatments

Mature 9-year-old 'G. H. Hill' peach (*Prunus persica* L. Batsch) trees planted in north-south row at the spacing of 4 m within row and 8 m between rows were used. Cultural practices of orchard management were carried out according to regular commercial production. Trees with same growth potential were randomly selected in a trial based on randomized complete block design for the field treatments of sprays with 25, 50 or 100  $\mu\text{mol L}^{-1}$  SNP (Sigma–Aldrich, USA) or distilled water as control with 3 replications of 3 trees. 0.1% Tween-20 (v/v) was added to the solutions as a surfactant. Four similar branches on each side of tree were selected and labeled before treatments. All treatment applications were applied 14 days (d) before commercial harvest, with a hand sprayer and sprayed to run off. Fruit were hand harvested according commercial harvest and fruit color change, immediately transferred to postharvest laboratory and fruit which were free of mechanical damage and infection were selected. Fruit of each replication of each treatment divided randomly into 3 sets of 26 fruit and stored at 4 °C (80–90% relative humidity). After 0, 14, and 28 d, one set of fruit for each replicate was transferred to 20 °C for another 1 or 4 d prior to measurements and analysis, to simulate marketing periods.

### 2.2. Ethylene production

At each sampling time, three fruit per replicate were placed in 2 L glass jar hermetically sealed for 2 h. One mL of headspace gas was withdrawn with a gas syringe, and the ethylene was quantified using a GC (Shimadzu, Model C-R4A, Japan) equipped with a flame ionization detector and an Alumina F-1 stainless steel column. The carrier gas (nitrogen) flow rate was 0.5 mL s<sup>-1</sup>. The column temperature was 70 °C, and injector and detector temperatures were 120 °C. Ethylene production rate was expressed as pmole kg<sup>-1</sup> s<sup>-1</sup>.

### 2.3. CI evaluation in the fruit

CI was evaluated using 10 fruit per each replicate. Symptoms of CI, including gel breakdown, flesh woolliness, and flesh browning (Lurie and Crisosto, 2005), were visually assessed, and the

incidence was recorded. A severity index was determined as follows: [(number of fruit with no disorder × 0) + (number of fruit with slight disorder (<25%) × 1) + (number of fruit with medium disorder (25–50%) × 2) + (number of fruit with severe disorder (>50%) × 4)] / (4 × total number of fruit in the treatment).

### 2.4. Fruit quality measurements

Fruit flesh firmness was measured using a texture analyzer (Santam, STM- 1, Iran), fitted with an 11.1 mm probe with constant speed of 20 mm min<sup>-1</sup>. Two different measurements were carried out on two opposite side of central zone of 10 fruit of each replicate. Values were expressed as newton (N). A wedge-shaped slice of flesh taken from each fruit was pooled and juiced for vitamin C, SSC and TA measurements. SSC was measured using an Atago Digital Refractometer (Brix 0–32%, Atago, Japan). TA was measured by titrating 10 mL of juice with 0.1 N NaOH to pH 8.1 and expressed as percentage of malic acid. The fruit weight of each replicate was recorded on the day of harvest and at each sampling time. Weight loss (WL) was expressed as percentage loss of the original fresh weight.

### 2.5. Determination of MDA content

After CI evaluation, two wedged-shaped slice of flesh tissue from two opposite sides of each fruit was removed, immediately frozen in liquid nitrogen, and stored at –80 °C until used for extraction and analysis of MDA, H<sub>2</sub>O<sub>2</sub>, AC content and enzyme activity.

One gram of frozen fruit tissue was homogenized in 10 mL of 10% trichloroacetic acid (TCA) and centrifuged for 10 min at 10,000g and 4 °C. Then, MDA content was determined according Heath and Packer, (1968) as described previously (Zhu et al., 2008).

### 2.6. Determination of H<sub>2</sub>O<sub>2</sub> content

Frozen tissue from each replicate (1 g) was homogenized in 2 mL of 0.05 mol L<sup>-1</sup> phosphate buffer (pH 7) and centrifuged at 4 °C for 15 min at 14,000g. The supernatant (0.5 mL) was then mixed with 1.5 mL of ferrous oxidation/xylenol orange reagent in a 5 mL micro centrifuge vial, vortexed and incubated for 2 h at room temperature, at which time colour development was virtually complete. The absorbance at 560 nm was read after removal of any flocculated materials by centrifugation. The H<sub>2</sub>O<sub>2</sub> standard curve were prepared as described by Wolff (1994). The content of H<sub>2</sub>O<sub>2</sub> was expressed as mol kg<sup>-1</sup> fresh weight (Zhu et al., 2008).

### 2.7. Vitamin C and AC assay

Aliquots of 2 mL fresh fruit juice (Bulk sample) was used for vitamin C assay immediately after juicing. Vitamin C content was determined by titration with 2, 6-dichlorophenol indophenol (AOAC, 2000), using different AA concentrations for the standard curve, and expressed as mg kg<sup>-1</sup> of vitamin by fresh weight.

Antioxidant was extracted by homogenizing 0.7 g of frozen tissue from each replicate with 2 mL of ice cold 1% HCl-methanol solution using mortar and pestle and then centrifuged at 4 °C for 10 min at 12000g. The supernatant was collected and used for antioxidant determination. AC was determined by the 2,2-diphenyl-1-picryl-hydrazil (DPPH) radical-scavenging method according to Sánchez Moreno et al. (1998). The absorbance was measured at 517 nm, using a spectrophotometer (UV-S2100). AC was expressed as the percentage inhibition of the DPPH radical and was determined by following equation:

$$AC = \frac{ABS \text{ Sample} - ABS \text{ Control}}{ABS \text{ Control}} \times 100$$

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