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Oxalic acid and 1-methylcyclopropene alleviate chilling injury of 'Youhou' sweet persimmon during cold storage

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ABSTRACT

Sweet persimmon softens rapidly during storage at room temperature. While low temperature can slow ripening, fruit are sensitive to chilling injury (CI) at low storage temperatures. In this study, we investigated the effects of oxalic acid (OA), 1-methylcyclopropene (1-MCP), and combined OA and 1-MCP treatments on the CI of 'Youhou' sweet persimmons during storage at 1 °C. Chilling-induced membrane damage was inhibited by all treatments as indicated by lower electrolyte leakage and malondialdehyde concentrations than found in control fruit. The treatments suppressed phenolics oxidation associated with polyphenol oxidase and peroxidase activities, resulting in lower skin and flesh browning. The treatments enhanced pectin solubilization and maintained higher juice yield compared with the control. The combined treatment of OA and 1-MCP was more effective than either one alone resulting in the lowest CI incidence in fruit kept at 1 °C.

1. Introduction

The 'Youhou' sweet persimmon is a non-astringent cultivar, which is extremely admired due to its excellent taste and rich cosmetic beauty (Zhang et al., 2010). Sweet persimmon fruit after harvest soften easily when stored at room temperature. While low temperature can delay softening, the fruit are sensitive to chilling injury (CI) at 0–4 °C (Collins and Tisdell, 1995; Woolf et al., 1997).

CI commonly refers to the physiological breakdown of susceptible plant tissues when they are exposed to temperatures above freezing; CI symptoms often appear only when fruit are moved from cold storage to room temperature (Macrae, 1987). CI symptoms in sweet persimmon are expressed as flesh and skin browning, flesh gelling, and a loss of juiciness (Besada et al., 2015; Kim et al., 2002). The mechanisms with which low-temperature stress lead to CI have not been thoroughly described, but alterations in membrane conformation and protein dysfunction have been considered as earlier events of CI which consequently affect the permeability of cell membrane (Lyons, 1973; Raison and Orr, 1990). These alterations result in the enhancement of electrolyte leakage, loss of structural integrity of plasma membrane, changes in lipid composition (Sevillano et al., 2009) and many other physiological events (Cantre et al., 2017). Moreover, CI can impair pectin solubilization as a result of abnormal polygalacturonase (PG) and pectin methylesterase functions (Khademi et al., 2014), resulting in retention of water in gel-form (Ben-Arie and Lavee, 1971).

Oxalic acid (OA) is a common ingredient of plants and has a strong chelating ability with multivalent cations (Kayashima and Katayama, 2002). OA treatment can inhibit CI development in peach (Jin et al., 2014b), mango (Li et al., 2015), and tomato (Li et al., 2016). Liang et al. (2017) reported that OA decreased the malondialdehyde (MDA) concentration, while inhibiting ethylene production in kiwifruit. However, effects of OA treatment on CI of persimmon fruit have not been investigated. It has been reported that the effects of 1-methylcyclopropene (1-MCP) treatment greatly induced CI inhibition due to its interference at the receptor level, thus controlling ethylene responses over extended periods of time (Sisler and Serek, 1999). Prominent fruit examples are Asian pear (Cheng et al., 2015), persimmon (Khademi et al., 2014), and sweet persimmon (Zhang et al., 2010). Previous studies have also found that treatment combinations are more beneficial for maintaining fruit or vegetable quality after harvest. For example, Jin et al. (2014a) reported that loquats treated with hot water (HW) in combination with methyl jasmonate (MeJA) had a lower level of CI than both control and single treatment of either HW or MeJA due to inhibition of the activities of peroxidase (POD) and polyphenol oxidase (PPO). Maximal levels of PG activity and water-soluble pectin content, as well as minimal level of protopectin content were also observed in response to combined treatment in loquat fruit. However, combined treatment with OA and 1-MCP on the fruit of the sweet persimmon has not been reported to date.

The objective of the present study was to investigate the effects of

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treatments with OA, 1-MCP, and a combination of OA with 1-MCP on sweet persimmon during cold storage at 1 °C.

2. Materials and methods

2.1. Plant materials and treatments

Fruit (3600) of the sweet persimmon (*Diospyros kaki* L. cv. Youhou) were harvested from a commercial orchard in Fuping, Shaanxi Province, China. Fruit were chosen on the basis of uniform shape and size, and free of disease, insect pests, or mechanical injury.

2.2. Chemical treatments

Fruit were randomly divided into four homogenous groups (treatment) viz: control, OA, 1-MCP, and OA + 1-MCP treatments. Each treatment consisted of three replicates (300 fruit per replicate). Fruit were exposed to air during the control treatment. For the OA treatment, sweet persimmon fruit were dipped into 5 mM OA solution (Sigma-Aldrich, Madrid, Spain) for 10 min. For the 1-MCP treatment, fruit were fumigated in 1.00 μL L^{-1} of 1-MCP (Rohm and Hass, Philadelphia, PA, USA) for 24 h in a 980 L sealed plastic container at room temperature. After both treatments, fruit were exposed to atmospheric conditions for 0.5 h at room temperature. For the combined treatment of OA + 1-MCP, OA treatment was applied first.

According to previous studies and an initial experiment by our group, the utilized concentrations and processing times of experimental reagents were confirmed as optimal. All the treated fruit were stored at 1 ± 0.5 °C with a relative humidity of 90–95%. Fifteen fruit were randomly selected every 7 d from each replicate of each treatment to assess firmness, juice yield, and electrolyte leakage. For this, fruit were peeled, pulp tissue samples were collected, and cut into small cubes, frozen in liquid nitrogen and stored at −80 °C for further analysis of enzyme activities, pectin and MDA concentrations. In addition, 30 fruit per treatment were removed from cold storage every 7 d and subjected to 25 °C for 5 d to assess the CI index.

2.3. Determination of CI index

The CI index was evaluated via the method described by Zhang et al. (2010) with some modifications. Fruit (30) were randomly picked from each treatment to assay CI symptoms based on the percentage of flesh and skin browning as well as flesh gelling. The degree of CI was rated on a scale of 0 to 4: 0 (no symptoms), 1 (≪25%), 2 (25–50%), 3 (50–75%), and 4(≫ > 75%) and the CI index was calculated according to the following equation: CI index = [Σ (CI scale) × (number of fruit at the CI level)/(total number of fruit in the treatment) × 5].

2.4. Determination of fruit firmness and ethylene production

Fruit firmness was determined via pressure tester (FT 327; Effegi, Alfonsine, Italy) at two points of the peeled fruit at the equatorial region, using an 11-mm diameter, and expressed as Newton (N).

For the evaluation of ethylene production, five fruit per replicate were enclosed in 9.8 L airtight glass containers for 1 h at 1 °C. A syringe was used to collect 2 mL gas sample and injected into a gas chromatograph (GC-14A; Daojin, Japan), and the results were expressed in μg kg^{-1} s^{-1}.

2.5. Determination of MDA concentration and electrolyte leakage

Electrolyte leakage was measured following the method of Cai et al. (2006). The equatorial flesh of three fruit per replication were cut into 1-cm round cubes with a hole punch. These cubes were cut into slices of 2 mm thickness and each fruit provided 15 slices. All slices were place into 30 mL of 0.8 mM mannitol solution and incubated at 20 °C for 2 h. The conductivity of the solution (EC_0) was measured with a DDS-307 conductivity meter (Shanghai, China). The slices were then boiled for 5 min and incubated at 20 °C for 30 min. The conductivity was re-adjusted to a volume of 30 mL to obtain the EC_T and the electrolyte leakage was calculated as (EC_0/EC_T) × 100%.

The MDA concentration was measured with the method described by Xi et al. (2017). Frozen tissue samples (1 g) of each replicate were ground on ice in 5.0 mL of 5% (w/v) trichloroacetic acid and centrifuged at 10,000 × *g* for 20 min at 4 °C. Then, 2 mL of the supernatant were mixed with 0.67% TBA at a ratio of 1:1 (v/v). The mixture was heated to 100 °C for 20 min and then immediately cooled on ice. After centrifugation at 10,000 × *g* for 10 min, the absorbance of the supernatant was measured at 450, 532, and 600 nm using a UV-2600 spectrophotometry. The MDA concentration was calculated via the formula: $6.45 \times (A_{532} - A_{600}) - 0.56 \times A_{450}$.

2.6. Enzyme extraction and activity assay

PG activity was determined according to the method described by Salvador et al. (2007) with some modifications. Samples of frozen tissue (1 g) to each replicate were ground on ice and homogenized with 10 mL 0.1 M ice-cold sodium citrate buffer (pH 4.5), containing NaCl (200 mM), EDTA (13 mM), β-mercaptoethanol (10 mM), and 1% (w/v) polyvinylpyrrolidone (PVP). The homogenate was stirred for 1 h and then centrifuged at 12,000 × *g* for 20 min. The supernatant was collected and filled to 25 mL with the same buffer, using the final supernatant for enzyme extraction. The reaction mixture included 0.5 mL enzyme and 0.5 mL of 0.2% (w/v) polygalacturonic acid and 1 mL sodium acetate buffer (100 mM, pH 4.5); the mixture was then incubated for 1 h at 37 °C. Reactions were terminated by adding 0.1 M borate buffer (pH 9.0). The reactions were conducted immersed in boiling water for 10 min with 1 mL of 1% (w/v) 2-cyanoacetamide and the absorbance was obtained at 276 nm. The amount that caused a change of 0.01 absorbance per hour at 276 nm was defined as one unit (U) of PG activity. PG activity was expressed as U g^{-1} protein. Protein levels were determined with the method of Coomassie Brilliant Blue and using bovine serum albumin as the standard. Each determination was in triplicate.

PPO activity was assayed according to the method described by Jiang (1999) with some modifications. Frozen tissue samples (1 g) of each replicate were ground and homogenized in 4 mL of 0.1 M sodium phosphate buffer (at pH 7.2), and centrifuged at 12,000 × *g* for 30 min at 4 °C. The supernatant was collected as the enzyme extracting solution. 4 mL of 50 mM sodium phosphate buffer (pH 7.2), 1 mL of 50 mM catechol, and 200 μL of enzyme extract were combined as the reaction mixture. The absorbance at 420 nm was automatically recorded for 3 min. The amount that caused a change of 0.01 absorbance per minute at 420 nm was defined as one unit (U) of PPO activity. PPO activity was expressed as U g^{-1} protein. The protein determination was in the same method as above.

POD activity was analyzed with a previously published method described by Lee (1973) with some modifications. Frozen tissue samples (1 g) were extracted with 4 mL of 0.1 M sodium phosphate buffer (pH 6.8), followed by centrifugation at 12,000 × *g* for 30 min at 4 °C. The supernatant was collected as the enzyme extract. The reaction mixture consisted of 3 mL of 25 mM guaiacol, 200 μL of 50 mM H_2O_2, and 1 mL enzyme extract. The amount that caused a change of 0.01 absorbance per minute at 470 nm was defined as one unit (U) of POD activity. POD activity was expressed as U g^{-1} protein. The protein determination was in the same method as above.

2.7. Determination of pectin and juice yield

The contents of protopectin and water-soluble pectin were determined using a previously published method described by Bu et al. (2013). Frozen tissue samples (1 g) of each replicate were ground on ice