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Oxalic acid alleviates chilling injury in peach fruit by regulating energy metabolism and fatty acid contents

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ABSTRACT

The effects of postharvest oxalic acid (OA) treatment on chilling injury, energy metabolism and membrane fatty acid content in 'Baifeng' peach fruit stored at 0 °C were investigated. Internal browning was significantly reduced by OA treatment in peaches. OA treatment markedly inhibited the increase of ion leakage and the accumulation of malondialdehyde. Meanwhile, OA significantly increased the contents of adenosine triphosphate and energy charge in peach fruit. Enzyme activities of energy metabolism including H^+-adenosine triphosphatase, Ca^{2+}-adenosine triphosphatase, succinic dehydrogenase and cytochrome C oxidase were markedly enhanced by OA treatment. The ratio of unsaturated/saturated fatty acid in OA-treated fruit was significantly higher than that in control fruit. These results suggest that the alleviation in chilling injury by OA may be due to enhanced enzyme activities related to energy metabolism and higher levels of energy status and unsaturated/saturated fatty acid ratio.

1. Introduction

Peach fruit (*Prunus persica* Batsch.) is very perishable due to rapid ripening and softening after harvest, which confers sensitivity to mechanical injury and pathogen infection. Refrigeration is widely used to delay ripening and control fruit decay. However, peaches are susceptible to chilling injury (CI), which limits the advantage for maintaining fruit quality during long period storage at low temperature (Wang, 1990). The typical symptoms of CI in peaches include internal browning (IB), lack of juiciness (woolliness or leatheriness), poor flavour and failure to ripen, which greatly reduce commercial quality (Lurie & Crisosto, 2005).

Energy supply in cellular is an important factor in controlling fruit ripening and senescence after harvest. Physiological disorder and browning in postharvest fruits may be related to inadequate supplies and reduced efficiency of cellular energy generation (Jiang et al., 2007). Membranes are thought to be the primary sites for development of CI. A higher ratio of unsaturated to saturated fatty acids provided higher tolerance to chilling temperature in various kinds of fruits such as loquat, banana and mango (Cao, Zheng, Wang, Jin, & Rui, 2009; Li, Zheng, Liu, & Zhu, 2014; Promyou, Kesta, & Van Doorn, 2008). It has been suggested that the damage of cell membranes was associated with lack of energy status, and ATP played important roles in synthesis of fatty acid and repair of membranes (Rawyler, Pavelic, Gianinazzi, Oberson, & Braendle, 1999).

Oxalic acid (OA) is a natural organic acid present in plants. It has been reported that OA may play a role in response to environmental stress, systemic resistance and programmed cell death in plants (Liang, Strelkov, & Kav, 2009). In recent years, treatment of OA in postharvest fruits has received much mention. It has been noted that OA delayed ripening of banana, inhibited decay of mango and prevented browning of litchi fruit (Huang et al., 2013; Zheng & Tian, 2006; Zheng, Ye, Jiang, Jing, & Li, 2011). Moreover, application of OA could increase resistance to chilling injury and maintain postharvest quality of peach, plum and pomegranate fruit (Sayyari et al., 2010; Wu et al., 2011; Zheng, Tian, Meng, & Li, 2007). However, to the best of our knowledge, little information on the effect of OA treatment on energy metabolism and fatty acid changes associated with chilling injury in peach fruit has been available. The objective of this study was to evaluate the effect of OA treatment on energy status content, enzymes activities of energy metabolism, and fatty acid composition in peach fruit stored at low temperature.

2. Materials and methods

2.1. Fruit material and treatment

Peach fruit (*P. persica* Batsch cv 'Baifeng') were hand-harvested at 30–32 N firmness and 10–11% total soluble solids (TSSs) from a

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commercial orchard in Nanjing, China. After harvest the fruits were immediately transported to the laboratory within 2 h and selected for uniform size, colour, and absence of defects. Then peach fruit were randomly divided into 2 groups, each with 360 fruits for 3 replicates. Based on our previous experiments, the first group was immersed in 5 mM OA solution for 10 min, whereas the second group of fruit was soaked in sterile deionised water for 10 min and served as control. All fruit were then air dried for approximately 30 min. After treatment, fruit were transferred to 0 °C for 35 days. Samples were collected from 10 fruit on the 5th, 15th, 25th and 35th day for biochemical analysis. Samples were mixed and frozen immediately in liquid nitrogen, then stored at −80 °C. Another sample of 10 fruit were removed after the 5th, 15th, 25th and 35th day of storage at 0 °C, and held at 20 °C for 3 d to simulate shelf condition for CI index, fruit firmness and extractable juice evaluation. Each treatment was replicated three times and the experiment was conducted twice with similar results.

2.2. Internal browning index measurement

As the main symptom of CI in 'Baifeng' peach was internal browning (IB), IB index was assessed visually according to the internal browning of peach flesh. The severity of IB was evaluated 3 d after transfer of peaches from 0 to 20 °C by rating on a scale of 0–4 with 0 = none; 1 = slight; 2 = moderate; 3 = moderately severe; and 4 = severe. Results were expressed as IB calculated using the following formula: IB = Σ(IB scale) × (number of fruit at that IB)/(4 × total number of fruit in each treatment) × 100%.

2.3. Ion leakage and malondialdehyde content measurements

Ion leakage was determined using 20 flesh disks (5 mm thickness × 10 mm diameter) removed with a cork borer from flesh tissues of 10 fruit at the equatorial region. Disks were immersed into 25 mL double distilled water in glass vials for 1 h, and an initial conductivity of the sample solution was determined with a conductivity metre (DDS-11A, Shanghai, China). Then the disk solution was boiled for 30 min, cooled to room temperature, re-adjusted to a volume of 25 mL and a final conductivity was measured. Ion leakage was expressed as relative conductivity (the initial conductivity of tissue solution/the final conductivity).

To analysis malondialdehyde (MDA) content, 2 g of flesh tissue was homogenized with 5 mL of 0.5% (w/v) trichloroacetic acid (TCA) and then centrifuged at 10,000*g* for 10 min at 4 °C. MDA levels were determined following the method of Hodges, Delong, Forney, and Prange (1999). MDA content was expressed as nmol g^{-1} fresh weight (FW).

2.4. Energy metabolism enzymes measurements

Crude mitochondria were extracted from peach fruit according to the method of Jin et al. (2013). H^+-ATPase and Ca^{2+}-ATPase activities were determined by measuring the release of phosphorus. The reaction was initiated by the addition of 100 μl of 0.03 mol L^{-1} ATP-Tris (pH 8.0), and stopped with 5% (w/v) TCA after 20 min of incubation at 37 °C. One unit of H^+-ATPase and Ca^{2+}-ATPase activities was defined as the release of 1 μmol of phosphorus in absorbance per minute at 660 nm under the assay conditions (Jin et al., 2013).

Succinic dehydrogenase (SDH) activity was determined according to the method of Ackrell, Keamery, and Singer (1978). The assay medium contained 0.3 ml of crude mitochondria extract, 3 ml of 0.2 mmol L^{-1} potassium phosphate buffer (pH 7.4), 1 ml of 0.2 mmol L^{-1} sodium succinate, 0.1 ml of 1 mmol L^{-1} di-p-chlorophenylmethyl carbinol, and 0.1 ml of 0.33% (w/v) methyl sulfenyl phenazine. One unit of SDH activity was defined as an increase of 0.01 in absorbance per minute at 600 nm under the assay conditions.

Cytochrome C oxidase (CCO) activity was assayed by the method of Chen et al. (2012). The assay medium contained 0.2 ml of crude mitochondria extract, 0.2 ml of 0.04% (w/v) cytochrome C solution, and 0.5 ml 0.4% (w/v) dimethyl phenylene diamine. One unit of CCO activity was defined as an increase of 0.1 in absorbance per minute at 510 nm under the assay conditions.

Protein content in the enzyme extracts was determined according to Bradford (1976) method, using bovine serum albumin as a standard. Specific activity of all the enzymes was expressed as units per milligram protein.

2.5. ATP, ADP and AMP content and energy charge measurements

Extract and measurement of ATP, ADP and AMP content were conducted by the method of Yi et al. (2008). Flesh tissue (2 g) of peaches was ground with 5 ml of 0.6 mol L^{-1} perchloric acid. The homogenate was then centrifuged at 20,000*g* for 15 min at 4 °C. A volume of 3 ml of supernatant was taken and quickly adjusted to pH 6.5–6.8 using KOH solution, diluted to 4 mL and passed through a 0.45-μm filter (Millipore Corp., Bedford, MA). ATP, ADP and AMP measurements were performed by a Agilent 1100 high-performance liquid chromatography (HPLC, Agilent Corp., Santa Clara, CA) using a reserved-phase Nova-Pak C18 column (5 μm, 5 × 250 mm, Agilent Corp.) and an ultraviolet detector at 254 nm. Mobile phase A consisted of 0.06 mol L^{-1} dipotassium hydrogen phosphate and 0.04 mol L^{-1} potassium dihydrogen phosphate dissolved in deionised water and adjusted to pH 7.0 with 0.1 mol L^{-1} KOH. Mobile phase B was pure acetonitrile. Elution was conducted by a linear gradient program with 75–100% A and 0–25% B for 7 min. The flow rate was 1.2 mL min^{-1}. Sample of 10 μL was injected into the HPLC system for analysis of ATP, ADP and AMP concentrations according to the external standard program. Energy charge was calculated by [ATP + 1/2 ADP]/[ATP + ADP + AMP].

2.6. Fatty acid quantification

Total lipids of flesh tissue were extracted according to Cao et al. (2009). 20 g of tissue was homogenised in 10 mL of chloroform: methanol: 0.1 mol L^{-1} HCl:water (200:100:1) and then 10 mL of 0.1 mol L^{-1} HCl were added before centrifugation at 4000*g* for 10 min. The organic phase was collected and taken to dryness. Methylation of fatty acids was carried out by adding 1 mL of 140 mL L^{-1} boron trifluoride in methanol at boiling temperature for 10 min. Methylated fatty acids were extracted with hexane, taken to dryness and redissolved in 200 μL of chloroform before injection. Fatty acids were separated and quantified according to Mirdehghan et al. (2007) by gas chromatography (Hewlett Packard Co, Palo Alto, CA, USA) equipped with a flame ionization detector. Authentic methylated fatty acid (Sigma–Aldrich 47801) was used as external standard to identify and quantify peaks; corrections were made at this stage for losses using the C17:0 internal standard. The unsaturated/saturated fatty acid ratio was calculated by the formula: (18:1 + 18:2 + 18:3)/(16:0 + 18:0), where 16:0 is palmitic acid; 18:0 is stearic acid; 18:1 is oleic acid; 18:2 is linoleic acid; and 18:3 is linolenic acid.

2.7. Statistical analysis

Experiments were performed using a completely randomized design. All statistical analyses were performed with SPPS Version 14.0 (SPSS Inc., Chicago, IL, USA). Data were analysed by one-way analysis of variance (ANOVA). The main effects and the interactions were analysed and the means were compared by Duncan's multiple range tests at a significance level of 0.05.