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1-Methylcyclopropene alleviates chilling injury by regulating energy metabolism and fatty acid content in 'Nanguo' pears

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ABSTRACT

This study investigated the effects of 1-methylcyclopropene (1-MCP) treatment on chilling injury (CI), energy metabolism, and membrane fatty acid content in harvested 'Nanguo' pears during the shelf life after cold storage at 0 °C. 1-MCP treated fruit showed slower CI injury development, lower ion leakage and malondialdehyde accumulation, increased adenosine triphosphate content and energy charge during a 25 day shelf life period at 20 °C. Activities of enzymes associated with energy metabolism, including H^+-adenosine triphosphatase, Ca^{2+}-adenosine triphosphatase, succinic dehydrogenase, and cytochrome C oxidase increased after 1-MCP treatment. The ratio of unsaturated to saturated fatty acids in 1-MCP-treated fruit was higher than that in control fruit. These results suggest that alleviation of CI after 1-MCP treatment might be due to enhanced energy metabolism-related enzyme activities and higher levels of energy and unsaturated to saturated fatty acid ratio.

1. Introduction

'Nanguo' pear (*Pyrus ussuriensis*) is grown in the northeastern cold regions of China. This pear cultivar is preferred by consumers because of its good taste, fragrant flavor, and pleasant aroma. Pears are climacteric fruit that show increased ethylene production and respiration rates during ripening. Hence, they remain firm for a limited time after harvest due to ripening. Rapid ripening and softening makes them sensitive to mechanical injury and pathogen infection (Li et al., 2014a,b; Paul et al., 2012). While storage at low temperature delays fruit senescence and extends postharvest life of the fruit, pears are susceptible to development of chilling injury (CI)-associated disorders, including peel and core browning and decay (Wang, 1990).

Ensuring sufficient cellular energy is an important factor in controlling fruit ripening and senescence after harvest. Physiological disorders and browning in harvested fruit might be related to inadequate supplies and reduced efficiency of cellular energy generation (Jiang et al., 2007). Cell membranes are thought to be the primary sites for the development of CI. A higher ratio of unsaturated/saturated fatty acids has been shown to improve tolerance to chilling temperature in various kinds of fruit such as loquat (Cao et al., 2009), banana (Jiang et al., 2004), and mango (Li et al., 2014a,b). Cell membrane damage is thought to be associated with the lack of energy, and ATP is known to play an important role in fatty acid synthesis and membrane repair (Rawyler et al., 1999).

1-Methylcyclopropene (1-MCP), an inhibitor of ethylene perception, is thought to interact with ethylene receptors and thereby prevent ethylene-dependent responses (Watkins, 2006). 1-MCP has been shown to inhibit CI incidence in some horticultural products such as apple (Apollo Arquiza et al., 2005), avocado (Pesis et al., 2002), pineapple (Fan et al., 1999), persimmon (Salvador et al., 2004), 'Fallgold' tangerine and grapefruit (Dou et al., 2005), 'Nova' and 'Ortanique' mandarins (Salvador et al., 2006), plum (Candan et al., 2011), and loquat (Cao et al., 2010). On the other hand, CI symptoms were induced in banana (Jiang et al., 2004), 'Shamouti' orange (Porat et al., 1999), and peach (Fan et al., 2002) by 1-MCP.

The effects of 1-MCP on energy metabolism and fatty acid-related changes associated with CI in 'Nanguo' pear fruit have not been investigated. Thus, this study aimed to evaluate the effect of 1-MCP treatment on energy status, energy metabolism-related enzyme activities, and fatty acid composition in 'Nanguo' pear fruit during the shelf life after storage at low temperatures.

2. Materials and methods

2.1. Fruit material and treatment

Hard, mature (green skin and light cream color) 'Nanguo' pears were harvested from a commercial orchard located at Anshan City

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(longitude, 122.97E; latitude, 41.07N), Northeast China. The fruit were packed in fiberboard cartons and transferred to the laboratory at the Shenyang Agriculture University within 3 h. Fruit with uniform shape and size and free from damage and fungal infection were selected.

The fruit were divided into two groups (150 fruit per group). Three replicates were used for treatment (50 fruit per replicate). Each replicate of treated fruit was exposed to 0.5 $\mu L L^{-1}$ 1-MCP (EthylBloc, Floralife Inc., USA) for 20 h at 20 °C in an airtight 1 m^3 plastic container. 1-MCP was released in the dissolved form in 1 mL distilled water in a glass dish to ensure the release of 1-MCP. For the control treatment, 150 fruit were placed in an identical plastic container without 1-MCP. Following treatment, the samples were placed in polyethylene bags (0.02 mm), secured with rubber bands, and stored under 80–90% relative humidity (RH) and at 0 °C. After 90 days, fruit from both the groups were transferred from cold storage to ambient temperature (20 °C) with 60–65% RH, and 10 fruit from each group were used for analysis at 5-day intervals during the remaining shelf life.

2.2. CI index

Ten fruit were cut equatorially to document the severity of CI. The CI index was determined using a rating scale ranging from 0 to 4 on the basis of common visual symptoms of water-soaked appearance of the flesh cross-section and the affected core area or flesh tissue. The following scale was used: 0 = no damage; 1 = very slight damage; 2 = slight damage; 3 = moderate damage, and 4 = severe damage. The CI index was calculated using the following formula: $[\sum(A \times B)/5C]$, where A is the injury score of individual fruit, B is the number of fruit affected, 5 is the total number of scores (0–4) used, and C is the total number of fruit recorded (Zaharah and Singh, 2011).

2.3. Electrolyte leakage and malondialdehyde content measurement

Discs were excised from the fruit by using a 3-mm diameter cork borer. The discs were briefly rinsed with deionized water and blotted dry on a slightly moistened Whatman filter paper. Twelve discs were then incubated in 30 mL of 300 mM mannitol for 3 h in a 50-mL capped polypropylene centrifuge tube. The conductivity of the bathing solution was measured after 4 h of incubation at 25 °C by using a DDS-307 conductivity meter (Shanghai Precise Science Instrument Co., Shanghai, China). Total electrolyte conductivity was determined after the discs and bathing solution were heated in a boiling water bath for 30 min. The discs and bathing solution were then cooled to room temperature, and the conductivity was measured once again. Electrolyte leakage was expressed as the percentage of the conductivity of total tissue electrolyte. Three replicates were used for each treatment (Luo et al., 2011).

The content of malondialdehyde (MDA) was measured using the thiobarbituric acid (TBA) reaction method (Zhang et al., 2009), with some modifications. Tissue samples (1.0 g) were homogenized in 4.0 mL of 5% (w/v) trichloroacetic acid and centrifuged at 10,000 × g for 20 min. The supernatant was mixed with 2.0 mL of 0.67% TBA, heated at 100 °C for 30 min, and then immediately cooled on ice. After the supernatant was centrifuged at 5000 × g for 10 min, its absorbance was measured using a spectrophotometer (TU-1810 DSPC; Beijing Puxi Instrument Co., Beijing, China) at 450, 532, and 600 nm. The MDA concentration was calculated according to the formula: $6.45 \times (A_{532} - A_{600}) - 0.56 \times A_{450}$. Three replicates were used for each treatment.

2.4. Measurement of activities of enzymes related to energy metabolism

Mitochondria were isolated from pear fruit as described by Qin et al. (2009) with some modifications. In brief, approximately 60 g peeled pear tissue from 10 fruit was gently cut and homogenized in 300 mL ice-cold extraction buffer containing 250 mM sucrose, 1 mM ethylenediaminetetraacetic acid (EDTA), 0.5% w/v polyvinylpyrrolidone (PVP), 0.1% w/v bovine serum albumin (BSA), 10 mM β-mercaptoethanol, and 50 mM Tris-HCl (pH 7.5). The homogenate was filtered through four layers of sterile cheesecloth, followed by centrifugation at 1200 × g for 15 min. The supernatant was decanted and centrifuged at 16,000 × g for 20 min. The pellets were re-suspended in washing buffer (250 mM sucrose, 0.1% w/v BSA, and 50 mM Tris-HCl, pH 7.5) by using a soft brush. The crude mitochondria were purified using density-gradient centrifugation with 28% (v/v) Percoll. The extraction buffer consisted of 250 mM sucrose, 1.0 mM EDTA, 0.5% PVP, 0.1% (w/v) BSA, 10 mM β-mercaptoethanol, and 50 mM Tris-HCl, pH 7.5. The washing buffer consisted of 10 mM Tris, 0.3 M mannitol, 1.0 mM EDTA, and 0.1% (w/v) BSA, pH 7.2.

The concentration of proteins was determined spectrophotometrically at 595 nm, as described by Bradford (1976) by using BSA as the standard.

Respiratory activity was determined as described previously (Mazorra et al., 2013). Briefly, 1 mg of protein was used to determine the respiratory activity of mitochondria by using an oxygen electrode (Hansatech, Respire 1, UK) in 1.0 mL reaction medium [0.35 M mannitol, 10 mM phosphate buffer, 10 mM KCl, 5 mM $MgCl_2$, 0.5% (w/v) BSA, pH 7.2] at 25 °C. The entire assay was performed in the presence of 200 μM glutamate as the substrate for respiration. The addition of 100 nM ADP to the reaction medium induced the transition from phosphorylation (State 3) to resting (State 4) respiration, indicating the phosphorylation capacity of the mitochondria. The mitochondrial respiratory control rate was calculated as follows: (State 3)/(State 4).

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 0.03 M ATP-Tris (pH 8.0), and stopped with 5% (w/v) trichloroacetic acid 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 μM of phosphorus per second at 660 nm under the assay conditions (Jin et al., 2013).

Succinic dehydrogenase (SDH) activity was determined according to the method of Ackrell et al. (1978). The assay medium contained 0.3 mL of the crude mitochondria extract, 3 mL 0.2 mM potassium phosphate buffer (pH 7.4), 1 mL 0.2 mM sodium succinate, 0.1 mL 1 mM dichlorophenylmethyl cardinal, and 0.1 mL 0.33% (w/v) methyl sulfenyl phenazine. One unit of SDH activity was defined as an increase of 0.01 in absorbance per second at 600 nm under the assay conditions.

Cytochrome c oxidase (CCO) activity was assayed according to the method of Zhu et al. (2012). The assay medium contained 0.2 mL of the crude mitochondria extract, 0.2 mL 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 second at 510 nm under the assay conditions. Specific activity of all the enzymes was expressed as units per kilogram protein.

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

ATP, ADP, and AMP contents were extracted and measured using the method of Yi et al. (2008). Flesh tissue (2 g) of pears was ground in 5 mL 0.6 M perchloric acid. The homogenate was then centrifuged at 20,000 × g for 15 min at 4 °C. Next, 3 mL of the