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Effect of tea seed oil treatment on browning of litchi fruit in relation to energy status and metabolism

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ABSTRACT

'Dadingxiang' litchi fruit were treated with 0.1% tea seed oil (TSO) and then stored at 25 °C for up to 8 days to investigate the effects of TSO and possible mechanisms on pericarp browning. The results showed that TSO effectively delayed the development of pericarp browning and the loss of red color in litchi fruit. TSO treatment markedly maintained membrane integrity as indicated by a lower relative electrical conductivity, which might contribute to delayed fruit senescence. In addition, TSO treatment enhanced the ATP level, energy charge and activities of H^+-ATPase and Ca^{2+}-ATPase and regulated the expression of four genes related to energy metabolism, including *LcAtpB*, *LcAAC1*, *LcAOX1 and LcSnRK2*. These results indicate that the process of browning and senescence in litchi fruit may be closely associated with energy status via the regulation of energy metabolism-related enzymes and genes. We suggest that TSO treatment is a convenient and safe approach for reducing browning of harvested litchi fruit.

1. Introduction

Litchi (*Litchi chinensis* Sonn.) is a non-climacteric, subtropical and tropical fruit belonging to *Sapindaceae* family that is extensively cultivated in Southeast Asia (Holcroft and Mitcham, 1996). Owing to its bright red pericarp surrounding translucent fleshy aril, exotic aroma, delicious taste and extraordinary nutritional properties, litchi has a high commercial value in the international market (Jiang et al., 2004). However, harvested litchi is perishable and prone to pericarp browning, leading to deteriorated quality and reduced market value (Jiang et al., 2004). Traditional technique of sulfur dioxide (SO_2) fumigation can effectively block pericarp browning and maintain the red color of harvested litchi fruit (Jiang et al., 2003). However, alternative strategies for browning inhibition have been sought due to public concerns for health and food safety (Jiang et al., 2003).

To date, various physical, chemical and biological techniques as alternatives to SO_2 fumigation have been tested to control litchi browning (Zhang et al., 2015). The major mechanisms behind the observed protection involve inhibition of enzymatic and/or non-enzymatic browning and maintenance of cell redox homeostasis in litchi fruit (Jiang et al., 2004; Neog and Saikia, 2010). It has been recently recognized that the browning and senescence of harvested litchi fruit may also be ascribed to reduced cellular energy level and restricted utilization of energy (Jiang et al., 2007). Some postharvest approaches, including exogenous application of ATP and ethylene inhibitors, and high oxygen and short-term anaerobic exposure have been confirmed to enhance the ATP level and energy status, contributing to the delay of browning in harvested litchi fruit (Yi et al., 2008; Qu et al., 2006; Liu et al., 2015).

ATP is mostly produced via mitochondrial aerobic respiration. ATP level and energy status are tightly associated with the synthesis, transportation and dissipation of nucleotides, which are regulated by specific proteins (Wang et al., 2013). Among these regulatory proteins, ATP synthase located in the mitochondrial membrane is a key enzyme that catalyzes ATP formation from ADP and inorganic phosphate (Pedersen et al., 2000). The subunit β (Atp B) of ATP synthase is involved in the synthesis and degradation of ATP and is considered a novel cell death regulator (Chivasa et al., 2011). The ADP/ATP carrier (AAC) is located in the mitochondrial inner membrane, as the core protein of the mitochondrial adenosine nucleotide transportation system in higher living organisms, is responsible for regulating the mitochondrial ATP concentration by transporting ATP from the

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synthesis site to the utilization site (Spetea et al., 2012). Alternative oxidase (AOX) is one of the main energy dissipation systems in plant mitochondria and plays a pivotal role in increasing heat dissipation with reduced ATP synthesis, suppressing reactive oxygen species (ROS) generation, enhancing resistance to stress and maintaining the functions of mitochondria (Vanlerberghe, 2013). In addition, sucrose non-fermenting-1-related protein kinase (SnRK), an energy regulator, participates in several signal-transduction pathways and regulates energy metabolism in plant cells under different stress conditions (Baena-González et al., 2007). However, the underlying molecular mechanisms involved in energy regulation are far from being clarified in harvested litchi and other fruits.

Tea seed oil (TSO) extracted from *Camellia oleifera* Abel (Theaceae family) is one of the most important edible oils, with an attractive light color, pleasant aroma and perfect cooking quality, making it comparable to olive oil (Lee and Yen, 2006; Fazel et al., 2008). TSO comprises rich unsaturated fatty acids, especially oleic acid (≈70%) and linoleic acid (≈10%), and contains high levels of antioxidants and vitamins, such as phenolics, flavonoids, β-carotene, sterols, squalene and tocopherols (Fazel et al., 2008; Ma et al., 2011). Undoubtedly, tea seed oil as a cooking oil has positive benefits to promote overall human health. TSO has been refined into medicines that display remarkable pharmacological activities in remedying cardiovascular disease, stomachache, empyrosis, inflammation and boosting the immune system (Wu et al., 2005; Li et al., 2011). In addition, TSO has been employed as a natural antioxidant to increase the stability of fish oil (Fazel et al., 2008) and storability of sunflower and olive oils (Sahari et al., 2004), indicating that it could be a valuable raw material and functional product to be applied in the food industry. To the best of our knowledge, there is no available information regarding the efficacy of TSO as a preservative in extending the shelf-life of harvested fruit and its possible physiological and molecular mechanisms related to energy metabolism. The objective of this study was to investigate the effects of TSO on postharvest browning, pericarp color, membrane permeability, energy status, and the expression levels of the energy metabolism-related genes in harvested litchi fruit.

2. Materials and methods

2.1. Plant material and treatments

Litchi (*L. chinensis* Sonn. cv. Dadingxiang) fruit were hand-harvested at commercial maturity from an orchard in Yongxing town of Haikou city in the Hainan province of China. Fruit were packed in plastic box covered with one layer of polyethylene film and transported to postharvest facilities within 2 h in an air-conditioned cargo van at 25 °C. Fruit with uniform size, shape, color, and free from disease and mechanical injury were selected for the following experiments.

Litchi fruit were sterilized with Sportak® fungicide solution (0.1%, v/v) for 3 min, rinsed with tap water, and assigned randomly to 2 treatment groups, with 20 kg in each group. The first group of fruit was immersed in 0.1% (v/v) TSO (100% purity, Guangzhou Daily Chemical Products Co., Ltd., Guangzhou, China) solution containing 0.05% (v/v) Tween 80 at 25 ± 1 °C for 5 min. The second group of fruit (control) was immersed in distilled water containing 0.05% (v/v) Tween 80 at 25 ± 1 °C for 5 min. The optimal concentration of TSO solution (0.1%) was chosen based on preliminary experiments at 0.05, 0.1, 0.25, 0.5 and 1% (data not shown). After treatments, fruit were air-dried and then packed into polyethylene bags (200 × 150 mm, 0.03 mm thickness, each side of bag had nine holes with 10 mm diameter and 15 fruit per bag) and stored at ambient conditions of 25 °C and 85–90% relative humidity. The browning index, color and membrane permeability were evaluated at 0, 2, 4, 6 and 8 d of storage. To assay energy level, enzyme activity and gene expression levels, the pericarp tissue samples were collected at 2-d interval as described above, rapidly frozen in liquid nitrogen, pounded into small pieces with a rolling pin, and then held at −80 °C until analysis. There were three replicates for each treatment at each sampling time, the browning index was investigated in 30 fruit per replicate, and the other parameters were analyzed in 15 fruit per replicate.

2.2. Browning index

Browning index of the litchi pericarp was assessed using a rating scale of 0 to 4 based on browned area on affected fruit surface (Zhang et al., 2015). The scale was implemented as follows: 0 = no browning; 1 = slight (< 5% browning); 2 = moderate (< 25% browning); 3 = severe (25–50% browning); 4 = extremely severe (> 50% browning). The browning index was calculated as follows: Σ (browning scale × number of fruit in each scale)/4 × total number of fruit in each treatment. The experiment was performed twice with similar results.

2.3. Pericarp color measurement

Pericarp color was assessed by determining CIE L^* a^* b^* color system using a colorimeter (Model: Minolta CR-400; Konica Minolta Sensing, Inc., Osaka, Japan). L^* values represent the lightness and darkness. The a^* and b^* values going from negative to positive indicate green to red color and blue to yellow color, respectively. Two measurements for each fruit were implemented at 2 symmetry points on the middle position of fruit pericarp.

2.4. Membrane permeability

Membrane permeability was expressed by the relative electrical conductivity, which was measured according to Zhang et al. (2015) with minor modification. Thirty pericarp discs were derived from 15 fruit (3 replicates per treatment) with a cork borer (8 mm in diameter), washed twice and incubated in 30 mL of de-ionized (di) water at 25 °C for 30 min. The initial electrolyte value of the bathing solution was determined using a conductivity meter (Model: DDSJ-308A; INESA Analytical Instrument Co., Ltd, Shanghai, China). Afterwards, the solution with discs was boiled for 20 min, quickly cooled, and replenished with di-water to 30 mL; then the total electrolyte value of the solution was measured again. Relative conductivity rate was expressed as the percentage (%) of the electrolyte value obtained following the 30-min incubation at 25 °C relative to the total electrolyte value after boiling.

2.5. HPLC analysis of ATP, ADP and AMP

Adenosine triphosphate (ATP), adenosine diphosphate (ADP) and adenosine monophosphate (AMP) contents were determined with high-performance liquid chromatography (HPLC). Three grams of thawed pericarp tissues were ground and homogenized with 6 mL of pre-cooled perchloric acid solution (0.6 M) in an ice bath. The homogenates were centrifuged at 8000 × *g* for 10 min at 4 °C. Three milliliters of supernatant were neutralized to pH 6.8 with 1.0 M KOH and then placed in an ice bath for 30 min to precipitate $KClO_4$. The solution was centrifuged at 8000 × *g* for 5 min at 4 °C, and the supernatant was diluted to 5 mL with di-water and passed through a 0.45 μm-Millipore membrane. Aliquots (20 μL) were injected into an HPLC (Model: Agilent 1260; Agilent Technologies Inc., Palo Alto, CA, USA) equipped with an ultraviolet (UV) detector and an Agilent ZORBAX Extend-C18 (4.6 × 250 mm, 5 μm) column to determine the levels of ATP, ADP and AMP. The protocol was as follows: Mobile phase: 100% phosphate buffer (20 mM KH_2PO_4 and 20 mM K_2HPO_4 dissolved in di-water, adjusted to pH 7.0 with 0.1 M KOH); flow rate: 1.2 mL min^{-1}; elution time: 20 min. ATP, ADP and AMP were identified and quantified by referring to the retention time and peak area obtained from pure compounds (HPLC purity, Sigma Aldrich, St Louis, MO, USA). The contents of ATP, ADP and AMP were expressed as mg kg^{-1} fresh weight (FW). Energy charge (EC) was calculated as ([ATP]

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