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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]

+ 0.5 × [ADP])/([ATP] + [ADP] + [AMP]).

2.6. Determination of H^+-ATPase and Ca^{2+}-ATPase activities

H^+-ATPase and Ca^{2+}-ATPase activities in litchi pericarp were measured with an enzyme-linked immunosorbent assay (ELISA) kit (Bangyi Biotechnology Co. Ltd., Shanghai, China) according to the instructions and information provided by manufacturer. H^+-ATPase and Ca^{2+}-ATPase activities were calculated based on a standard curve and expressed as in U L^{-1}. One unit (U) was defined as the amount of enzyme required to catalyze the release of 1 μmol of phosphorus per minute.

2.7. Gene expression analysis

Expression level of genes related to energy metabolism was determined by real-time quantitative PCR (RT-qPCR). Total RNA was extracted from 1.5 g of pericarp tissues using an RNAprep Pure Plant Kit (DP441; Tiangen Biotech Co., Ltd., Beijing, China) according to the manufacturer's instructions. Total RNA was treated with DNase I (TaKaRa Biotechnology Co., Ltd., Dalian, China) and then purified following the manufacturer's instructions. Purified RNA was used as the template for synthesis of first-strand cDNA by reverse transcription PCR (RT-PCR) using a FastQuant RT Kit (KR106-02; Tiangen Biotech Co., Ltd., Beijing, China) according to the manufacturer's instruction. RT-qPCR was carried out using an Real-Time Detection system (Model: ABI7500; Advanced Biotechnology, Inc., Eldersburg, MD, USA) with 10 μL of SYBR® Premix Ex Taq™ II (2 ×) (TaKaRa Biotechnology Co., Ltd., Dalian, China), 0.8 μL of forward primer (10 μM), 0.8 μL of reverse primer (10 μM), 0.4 μL of ROX Reference Dye II (50 ×) (TaKaRa Biotechnology Co., Ltd., Dalian, China), 2.0 μL of cDNA, and 6 μL of ddH_2O. The RT-qPCR protocol is composed of an initial denaturation step at 95 °C for 15 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 32 s. In the present study, the expression levels of four energy metabolism-related genes, *LcAtpB* (GenBank Accession No. JQ349005), *LcAOX1* (JQ349006), *LcAAC1* (JQ349007) and *LcSnRK2* (JQ349008), were analyzed. *LcGAPDH* (JF759907.2) was used as a reference gene for quantitative normalization. The specific primers for these genes are shown in Table 1. Relative expression of the gene was calculated using the $2^{-\triangle\triangle CT}$ method (Livak and Schmitten, 2001), where $\triangle\triangle CT = (CT_{Target} - CT_{GAPDH})\ Time_x - (CT_{Target} - CT_{GAPDH})\ Time_0$. CT_{Target} and CT_{GAPDH} represent the CT values of the target gene and *LcGAPDH*, respectively. $Time_x$ represented any time point, and $Time_0$ denoted the one-time expression of the target gene normalized to *LcGAPDH*. Litchi pericarp tissues at 0 d were used as the calibrator sample and assigned a nominal value of 1.

2.8. Statistical analysis

Experiments were conducted using a completely randomized design with two treatments and three replications for each treatment. The data were evaluated by one-way analysis of variance (ANOVA) using SPSS statistical analysis software (version 16.0; SPSS Inc., Chicago, IL, USA). All data were expressed as the mean values ± standard error (SE). Asterisks were used to indicate significant differences between control and TSO-treated fruit at the same time point ($^*P < 0.05$, $^{**}P < 0.01$).

3. Results and discussion

3.1. Pericarp browning index and color

As shown in Fig. 1A, browning index steadily increased in control fruit as storage time prolonged. Browning development was effectively suppressed by TSO treatment, in which browning index in TSO-treated fruit was averagely 49.8% lower than that in control fruit through 2 to 8 d of storage (Fig. 1A). In agreement with inhibition of browning, TSO treatment greatly maintained the original color of the fruit pericarp, and this directly reflected in the results from the measurement of pericarp color based on chromaticity L^*, a^* and b^* system. In general, chromaticity L^*, a^* and b^* values in both control and TSO-treated fruit continuously declined throughout storage (Fig. 1B–D). The decline in chromaticity a^* value throughout storage was strongly suppressed in TSO-treated fruit (Fig. 1C), indicating that the red color of litchi pericarp could be retained well by TSO treatment. In comparison, the effect of TSO treatment on chromaticity L^* and b^* was less pronounced than that for chromaticity a^*, with the values of chromaticity L^* at 4 d and chromaticity b^* within the initial 4 d not differing significantly in control and TSO-treated fruit (Fig. 1B, C and D).

3.2. Membrane permeability

Membrane permeability was expressed as relative electrical conductivity. Relative electrical conductivity in control fruit increased steadily from an initial value of 12.4 ± 0.08% to 39.8 ± 1.81% at the end of storage (Fig. 2). In contrast, relative electrical conductivity was remarkably suppressed by TSO treatment, with the value averaging 24.8% less than that in control fruit from 2 to 8 d of storage (Fig. 2). Membrane permeability is an important indicator measuring membrane integrity, the loss of which may cause de-compartmentalization of enzymes and substrates, leading to pericarp browning of litchi fruit (Zhang et al., 2015). The present result suggests that TSO may reduce the damage to membrane integrity, contributing to the delay of browning and senescence in litchi fruit.

3.3. ATP level and energy charge

It is known that senescence and browning in harvested horticultural crops are closely associated with deficiencies in cell energy levels (Liu et al., 2015). In our study, HPLC analysis revealed that ATP content in 'Dadingxiang' litchi fruit at harvest day (0 d) was 25.6 ± 0.4 mg kg^{-1} (Fig. 3A), approximately 25% lower than level reported for 'Huaizhi' litchi fruit, a cultivar cultivated in Guangdong Province, China (Wang et al., 2013). ATP content in control fruit gradually decreased through 8 d of storage along with the progression of browning (Figs. 3A and 1A). This decreasing tendency in ATP levels in non-climacteric litchi fruit after harvest is different from the trend observed in climacteric fruit such as kiwifruit, in which ATP is gradually synthesized and peaks along with respiratory climacteric before decreasing due to fruit senescence (Huang et al., 2014). After treatment with TSO, ATP content sharply increased from an initial value of 25.6 ± 0.4 to

Table 1
Primers used for real time quantitative PCR analysis.

Gene	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)
LcGAPDH	CGGATTTGGAAGGATTGG	GGACCGTGAACACTGTCGTA
LcAtpB	TTAACCGTAGCAAGAGCCC	CCTTCGCAGTAACTTCATCG
LcAAC1	GGCAGGCTGTCTGAACCC	GAAGTAACGGATGACATTAGCAGTA
LcAOX1	ATGCTTACTTCCTGGGCTATCT	GTTCTCAATGTTGCCTTTGTCTA
LcSnRK2	CATTTCCAGATTATGTCCGC	GTTATTTACATCGTTGCTTTGC

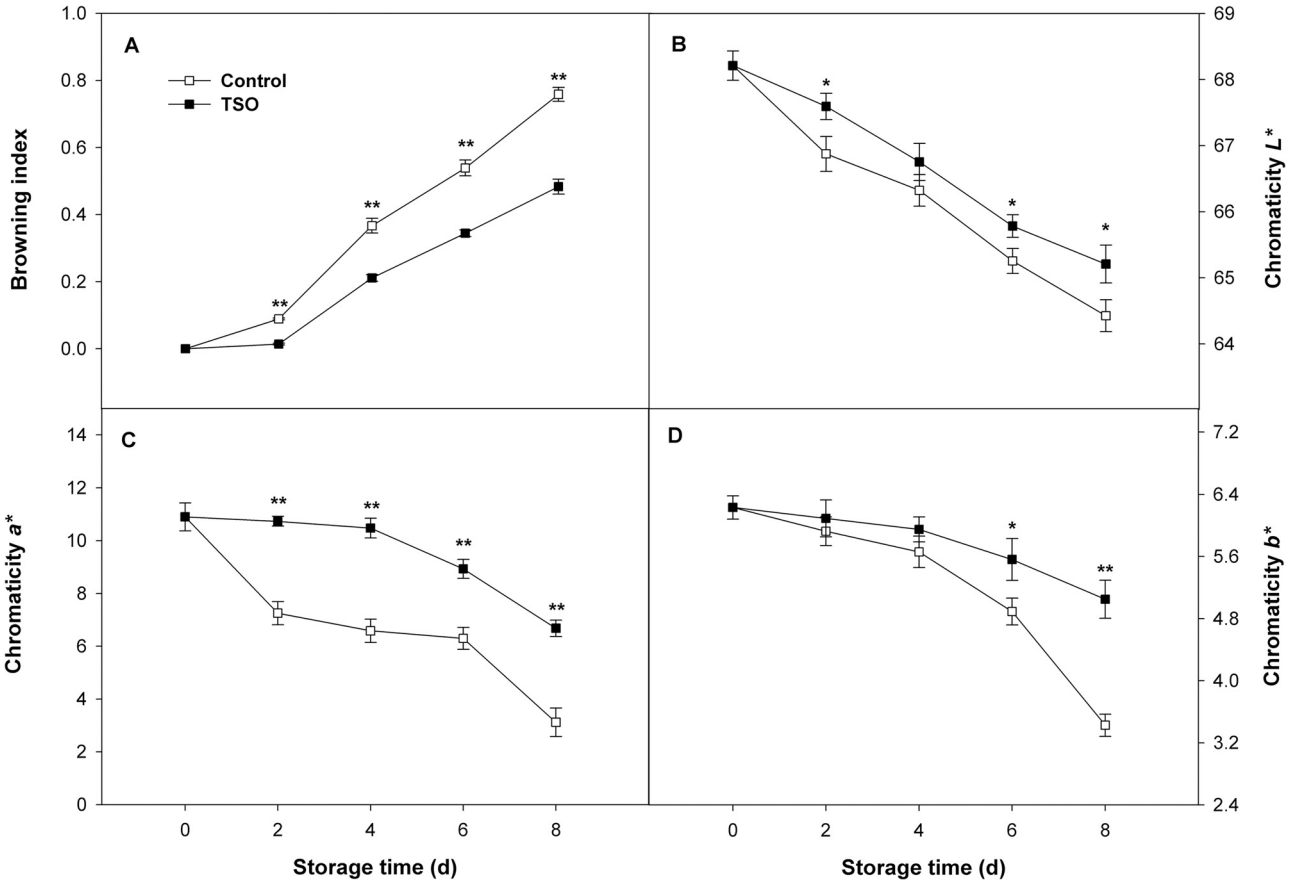

Fig. 1. Browning index (A) and chromaticity L^* (B), a^* (C) and b^* (D) of pericarp in 'Dadingxiang' litchi fruit during storage at 25 °C after treatment with 0.1% TSO or water (control). Vertical bars represent standard error of the means of triplicate assays. Asterisks represent that values are significantly different between control and TSO-treated fruit at the same time point (* $P < 0.05$, ** $P < 0.01$). (□) Control; (■) TSO.

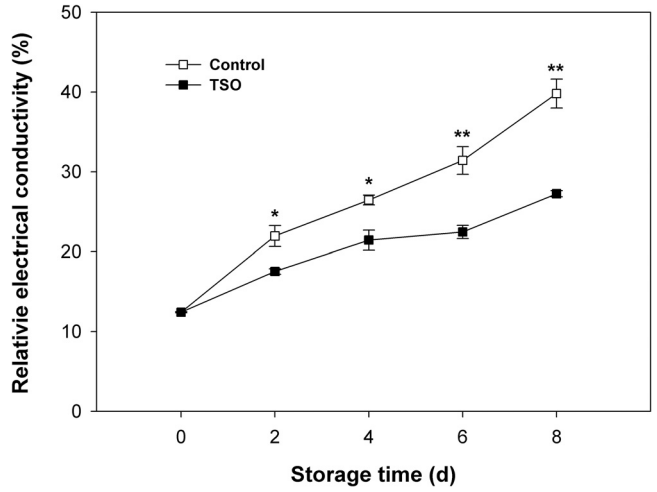

Fig. 2. Relative electrical conductivity in 'Dadingxiang' litchi fruit during storage at 25 °C after treatment with 0.1% TSO or water (control). Vertical bars represent standard error of the means of triplicate assays. Asterisks represent that values are significantly different between control and TSO-treated fruit at the same time point (* $P < 0.05$, ** $P < 0.01$). (□) Control; (■) TSO.

29.8 ± 0.5 mg kg^{-1} within 2 days of storage, thereafter continuously decreased (Fig. 3A). Trajectory analysis showed that pericarp browning index had a high negative correlation with ATP content in untreated (r = −0.98) and TSO-treated (r = −0.75) fruit throughout storage, indicating that the browning and deterioration of litchi fruit could be attributed to an energy deficit, which is in agreement with previous investigations in number of harvested fruits such as apple (Saquet et al., 2000), pear (Saquet et al., 2000; Veltman et al., 2003), litchi (Yi et al., 2010) and longan (Chen et al., 2014). The mechanisms might be that ATP is involved in reinforcement of antioxidant systems and synthesis of membrane unsaturated fatty acids, which would help to maintain membrane integrity and cellular compartmentalization and therefore prevent the oxidation of phenolic compounds (Yi et al., 2010; Chen et al., 2014). In the present study, TSO induced a remarkable accumulation of ATP, which might contribute to the delay of browning in harvested litchi fruit. Interestingly, TSO-induced pattern of ATP changes was not fully consistent with previous reports, in which some exogenous treatments, including ethylene inhibitor dipping, high oxygen and short-term anaerobic exposure, caused a delayed decline rather than inducing the accumulation of ATP in litchi fruit during storage at ambient temperature (Qu et al., 2006; Liu et al., 2015).

As shown in Fig. 3B, energy charge in control fruit increased slightly within the first 2 d of storage and then remained at a steady level of 0.75 (Fig. 3B). Energy charge in TSO-treated fruit increased from 0.74 ± 0.004 to 0.78 ± 0.003 within 2 d and then remained stable level during the remainder of storage (Fig. 3B). Induced increase in energy charge by TSO treatment indicates a surge in anabolic metabolism, which could be beneficial in retarding browning and senescence in litchi fruit. However, no negative correlation between energy charge and browning index was observed in both control (r = 0.56) and TSO-treated (r = 0.26) litchi fruit during storage, suggesting that the contribution of energy charge to regulation of the senescence process in litchi fruit could be less than that of ATP level, which was in line with findings from Liu et al. (2015) for 'Huaizhi' litchi fruit and was also supported by a report from Meyer and Wagner (1986) who observed a higher energy charge in senescent tobacco leaves and proposed the impossibility of senescence triggered by energy charge.

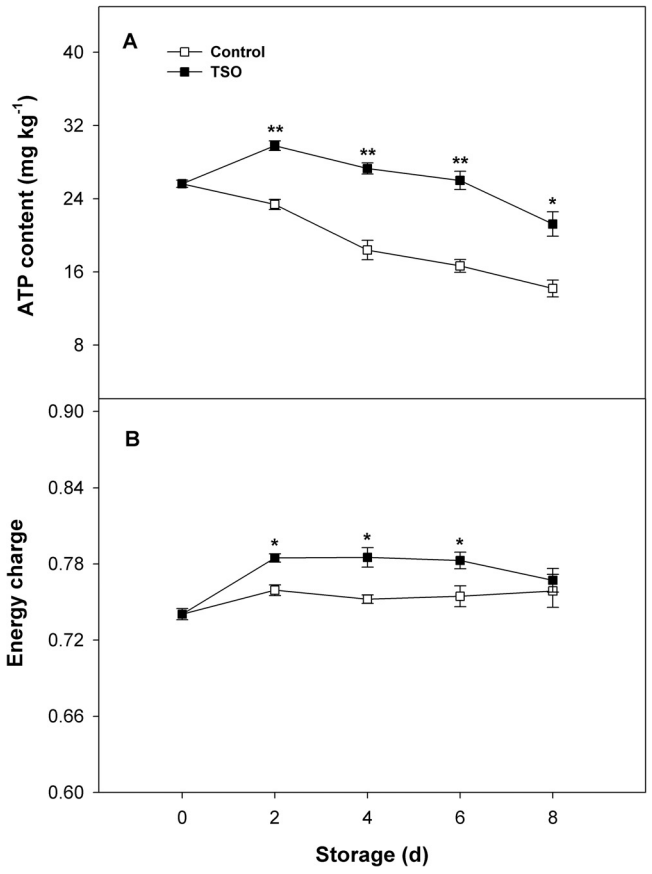

Fig. 3. ATP content (A) and energy charge (B) in 'Dadingxiang' litchi fruit during storage at 25 °C after treatment with 0.1% TSO or water (control). Vertical bars represent standard error of the means of triplicate assays. Asterisks represent that values are significantly different between control and TSO-treated fruit at the same time point (* $P < 0.05$, ** $P < 0.01$). (□) Control; (■) TSO.

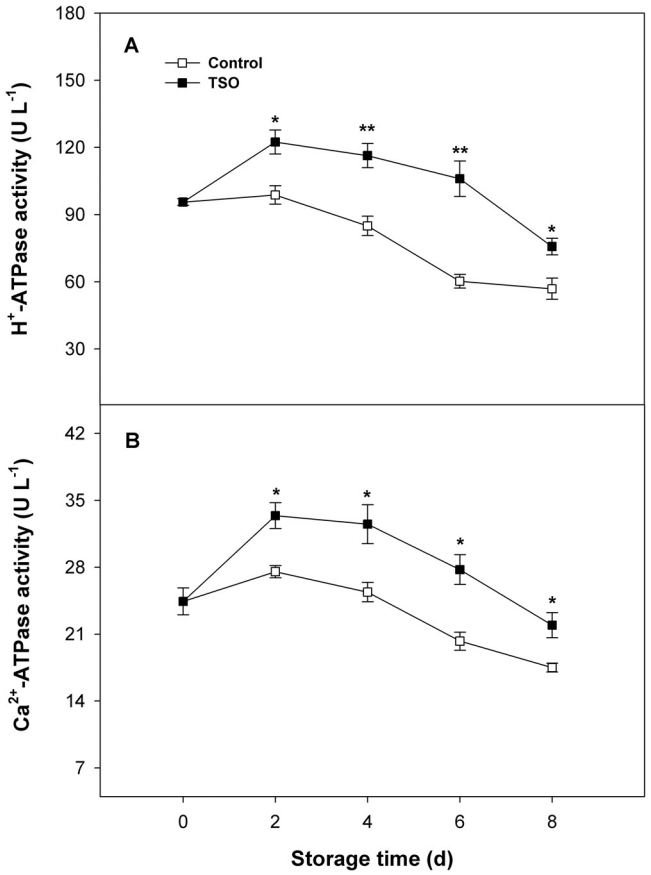

Fig. 4. H^+-ATPase (A) and Ca^{2+}-ATPase (B) activities in 'Dadingxiang' litchi fruit during storage at 25 °C after treatment with 0.1% TSO or water (control). Vertical bars represent standard error of the means of triplicate assays. Asterisks represent that values are significantly different between control and TSO-treated fruit at the same time point (* $P < 0.05$, ** $P < 0.01$). (□) Control; (■) TSO.

3.4. H^+-ATPase and Ca^{2+}-ATPase activities

H^+-ATPase activity in control litchi fruit did not alter within the initial 2 d of storage, but thereafter rapidly declined until the end of storage (Fig. 4A). Ca^{2+}-ATPase activity in control fruit increased slightly within the first 2 d of storage and then decreased steadily until the end of storage (Fig. 4B). Compared to control fruit, treatment with TSO resulted in approximately 1.3- and 1.4-fold increases in H^+-ATPase and Ca^{2+}-ATPase activities in litchi fruit within 2 d of storage, respectively (Fig. 4A and B). Subsequently, activity of both enzymes in TSO-treated fruit exhibited a decreasing trend throughout the remainder of storage (Fig. 4A and B).

H^+-ATPase is a P-type proton pump that resides in the plant plasma membrane and is responsible for coupling ATP hydrolysis and transport of H^+ across the membrane, and thereby generating electrochemical gradients to force the transmembrane transport of nutrients (Sondergaard et al., 2004). Ca^{2+}-ATPase, located in the plasma membrane, endoplasmic reticulum and tonoplast in plants, is a primary regulator of Ca^{2+} efflux from cytosol to extracellular matrix (Guan et al., 2006). High levels of cytosolic Ca^{2+} may disorder intracellular metabolism, while Ca^{2+}-ATPase can regulate Ca^{2+} homeostasis to maintain normal cell function (Jin et al., 2014; Wang et al., 2015). Both H+-ATPase and Ca^{2+}-ATPase are involved in modulating resistance to senescence and chilling injury stress in a variety of harvested fruits (Li et al., 2016; Liu et al., 2016; Pan et al., 2017). Azevedo et al. (2008) noted that loss of ionic and pH homeostasis could be driven by a rapid decline in H^+-ATPase activity during post-climacteric stage in papaya fruit, contributing to a remarkable effect on fruit senescence process. It was reported that the trends in Ca^{2+}-ATPase activity changes roughly paralleled the pattern of respiratory climacteric in peach fruit during storage at ambient temperature, and further treatments with Ca^{2+}-ATPase inhibitors (sodium orthovanadate and erythrosin B) resulted in aggravated senescence in peach fruit, indicating a close relationship between Ca^{2+}-ATPase and senescence (Guan et al., 2006). Using the non-climacteric strawberry as a model system, Fan et al. (2003) observed that Ca^{2+} accumulation resulting from decreased Ca^{2+}-ATPase and the destruction of redox equilibrium during storage together promoted the senescence of fruit. The present results demonstrate that the higher activities of H^+-ATPase and Ca^{2+}-ATPase in TSO-treated litchi fruit was linked with suppressed browning, indicating an important role of both enzymes in regulating browning and senescence in litchi fruit.

3.5. Gene expression

Energy metabolism-related genes expression is shown in Fig. 5. *LcAtpB* expression in control fruit rapidly increased to a peak within 4 d of storage, and the peak value was 3.5-fold higher than the initial level (Fig. 5A). TSO treatment strongly suppressed the expression of *LcAtpB*; the peak for this gene was delayed and appeared at 6 d of storage, with the value being 57% lower than that of control fruit at 2 d (Fig. 5A). After expression peaked, *LcAtpB* expression in both control and TSO-treated fruit decreased drastically as fruit senesced (Fig. 5A). *LcAAC1* expression in both control and TSO-treated fruit peaked at 4 d of storage (Fig. 5B). *LcAAC1* expression in TSO-treated fruit was higher than that in control fruit throughout storage (Fig. 5B). *LcAOX1* expression in control fruit increased continuously during storage, showing a 7.6-fold increase at 8 d relative to the initial value at 0 d (Fig. 5C). TSO treatment suppressed the expression of *LcAOX1*, with expression values

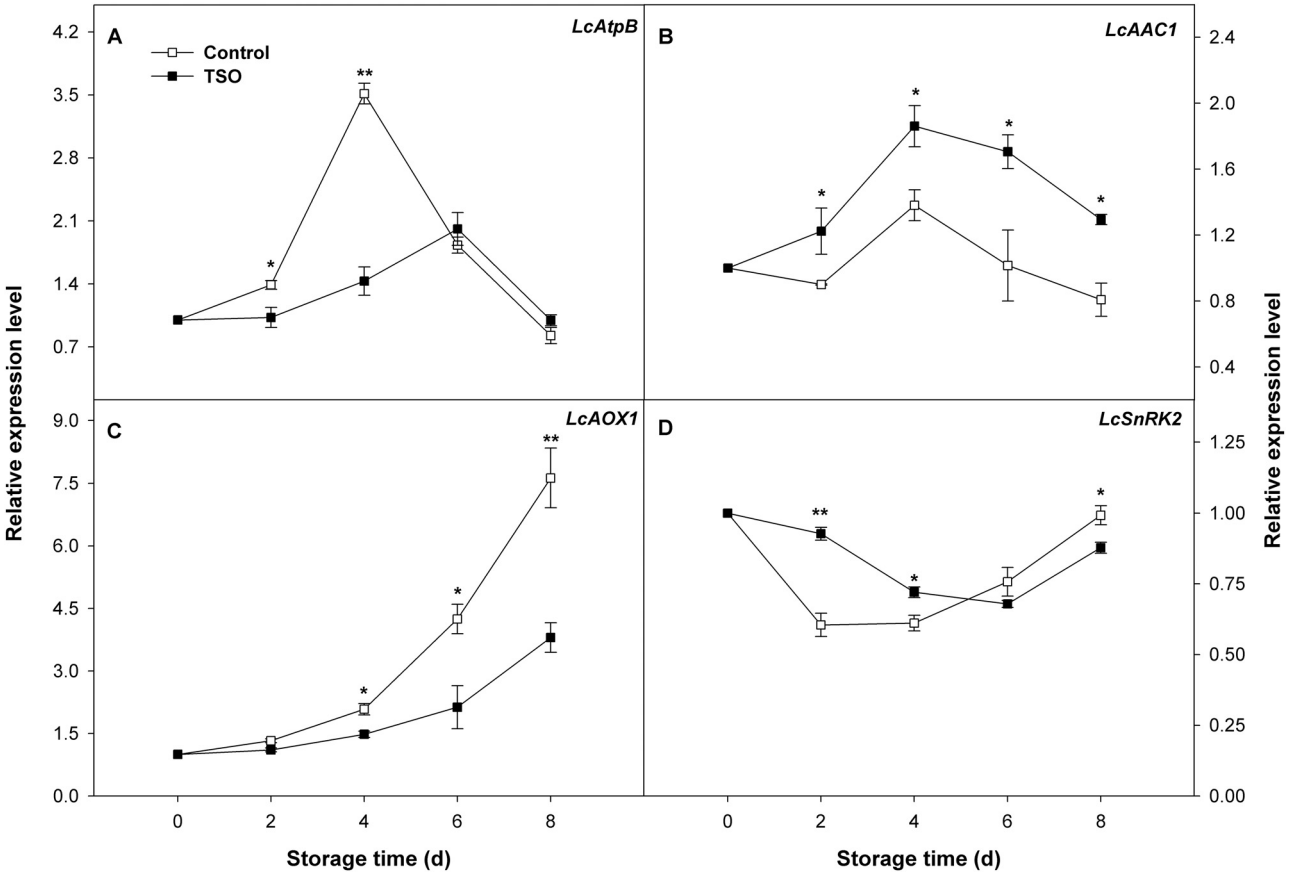

Fig. 5. Relative expression of *LcAtpB* (A), *LcAAC1* (B), *LcAOX1* (C) *and LcSnRK2* (D) in 'Dadingxiang' litchi fruit during storage at 25 °C after treatment with 0.1% TSO or water (control). Vertical bars represent standard error of the means of triplicate assays. Asterisks represent that values are significantly different between control and TSO-treated fruit at the same time point (* $P < 0.05$, ** $P < 0.01$). (□) Control; (■) TSO.

averaging 43% lower than those of control fruit from 4 to 8 d (Fig. 5C). *LcSnRK2* expression in control fruit decreased sharply from the initial value of 1 ± 0.00 to 0.6 ± 0.04 at 2 d of storage and then maintained unchanged over 2–4 d before rapidly increasing and reaching a level comparable to the initial value on the last day of storage (Fig. 5D). *LcSnRK2* expression in TSO-treated fruit continuously declined through the first 6 d of storage, thereafter sharply increasing until the end of storage (Fig. 5D).

AtpB is a pivotal constituent in the catalytic domain of ATP synthase, and mutation in this subunit may directly impact the mechanochemical coupling of ATP synthase (Mnatsakanyan et al., 2011). Lapaille et al. (2010) reported that down-regulation of *AtpB* by RNAi technology severely impaired the coupling of ATP synthesis and respiration, resulting in mitochondrial deformities of in green alga *Chlamydomonas reinhardtii.* In addition, AtpB has been proposed as a novel programmed cell death protein that regulates cell death in plants (Chivasa et al., 2011). In the present study, the rapid increase in *LcAtpB* expression in control fruit in middle period of storage indicates that this gene may trigger the onset of senescence in litchi fruit, which is in accordance with reports in harvested 'Huaizhi' litchi (Wang et al., 2013; Liu et al., 2015) and kiwifruit (Huang et al., 2014). Dramatic decline in *LcAtpB* expression during the late stage of storage might reflect a feedback inhibition of *LcAtpB* transcription resulting from senescence and dysfunction of the cell. Delayed browning and senescence in TSO treated litchi fruit might be partially attributed to its suppression of expression of *LcAtpB*. A similar slowing of browning and senescence in relation to inhibited *LcAtpB* expression was reported in 'Huaizhi' litchi fruit with exposure to short-term anaerobic, pure oxygen and low temperature conditions (Liu et al., 2015).

AAC is the most abundant carrier proteins in mitochondrial membrane and is regarded as one of the key factors in programmed cell death; it is mainly responsible for mediating the export of high-concentrated ATP towards cytoplasm while importing an equal number of ADP into mitochondria, thereby regulating the balance of ATP concentration in mitochondria (Spetea et al., 2012). Any deficiency or dysfunction in AAC may lead to abnormal cell metabolism and thus cause morphologic variations (Palmieri, 2013). For example, knockout of AAC may inhibit respiratory metabolism and slow the growth of roots in *Arabidopsis* (Klingenberg, 2008). In the present study, TSO treatment up-regulated the expression of *LcAAC1*, which might increase cellular ATP supply and maintain cell viability, consequently contributing to delay of browning and senescence in litchi fruit.

AOX is a terminal oxidase of cyanide-resistant respiration pathway and is existed in mitochondrial inner membrane of the higher plants. It may directly accept electrons from the ubiquinone pool and transfer them to O_2, which bypasses energy conservation sites (complexes III and IV), thus decreasing ATP synthesis and increasing heat production (Vanlerberghe, 2013). AOX pathway may also dampen the generation of superoxide radical, which will prevent its conversion to other ROS, thereby reducing damage to membrane and maintaining mitochondrial function of under environmental stresses (Vanlerberghe, 2013). It has been verified that *AOX*-silenced tomato fruit down-regulates ripening-related genes and fails to ripen as indicated by no typical respiratory climacteric and ethylene production, while in the contrast, accelerated accumulation of lycopene was observed in *AOX*-overexpressed tomato fruit, indicating that AOX is involved in ethylene-regulated ripening process in tomato fruit (Xu et al., 2012). Furthermore, Oliveira et al. (2015) reported that *AOX* transcript was prior to ethylene burst, and transcript abundance was maintained at a high level throughout ripening stages in papaya fruit. The authors suggest that AOX expression

could be a vital event during papaya ripening as a preparation for fruit senescence, which was suggested to be independent of ethylene and responsive to multiple and complex signals of respiratory status (Oliveira et al., 2015). In the present study, control litchi fruit exhibited a remarkable up-regulation of *LcAOX1* expression that was inhibited by TSO treatment during storage, which was associated with ATP levels. The results indicate that AOX may be a key factor regulating litchi senescence, which is consistent with previous studies on 'Huaizhi' litchi fruit (Wang et al., 2013; Liu et al., 2015).

SnRK, a homologue of sucrose non-fermenting 1 (SNF1) in yeast and AMP-activated protein kinase (AMPK), is extensively distributed in plants (Nietzsche et al., 2014). SnRK is a cellular energy sensor that can perceive ATP deficiency and stimulate downstream energy-synthesizing reactions while impairing energy-consuming biochemical processes, and therefore it plays an important role in regulating cellular energy homeostasis, especially under stress conditions (Nietzsche et al., 2014). A notable increase in *LcSnRK2* expression was observed in control litchi fruit during late storage, suggesting that up-regulation of *LcSnRK2* might be triggered by decreased ATP levels. Interestingly, we noted that the expression pattern of *LcSnRK2* in control fruit during the early stage of storage exhibited a dramatic decline, which might suggest a hysteretic perception of *LcSnRK2* transcription with regard to ATP deficiency. Compared with control fruit, the milder change in *LcSnRK2* expression in TSO-treated fruit might indicate a smaller need to compensate for energy synthesis due to not serious ATP deficiency. The results are consistent with reports for 'Huaizhi' litchi fruit treated with exogenous ATP (Wang et al., 2013).

4. Conclusion

The results presented in this study demonstrate that 0.1% TSO effectively delayed pericarp browning and retaining its red color in harvested litchi fruit. TSO treatment markedly reduced membrane permeability, which might maintain membrane integrity and contribute to the delay of fruit senescence. Furthermore, TSO treatment enhanced the ATP level, energy charge, H^+-ATPase and Ca^{2+}-ATPase activities, and regulated the expression of four genes including *LcAtpB*, *LcAAC1*, *LcAOX1 and LcSnRK2*, indicating that the browning and senescence process may be closely associated with energy status via the regulation of related enzymes and genes. Because TSO is a natural product with low-dose usage, we propose that the application of TSO could be a convenient, safe and low-cost approach to control pericarp browning and extend the shelf life of harvested litchi fruit.

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