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Melatonin alleviates pericarp browning in litchi fruit by regulating membrane lipid and energy metabolisms

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ABSTRACT

Melatonin functions as a pivotal bio-active molecule that is involved in diverse physiological processes in plants. This study investigated the effects of exogenous melatonin on browning, membrane phospholipids, membrane fatty acids, energy status, and activities of enzymes associated with lipid and energy metabolisms in harvested litchi (*Litchi chinensis* Sonn. cv. A4Wuhe) fruit during storage at 25 °C. The results exhibited that 0.4 mM melatonin treatment to litchi fruit retarded the development of browning and reduced the changes of cellular membrane permeability during storage. Melatonin treatment suppressed the increment of phospholipase D (PLD), lipase, and lipoxygenase (LOX) activities while prevented the hydrolysis of phosphatidylcholine (PC) into phosphatidic acid (PA). Compared to untreated fruit, higher contents of unsaturated fatty acids (USFA) (oleic acid, linoleic acid and linolenic acid), lower contents of saturated fatty acids (SFA) (palmitic acid and stearic acid) and a higher ratio of USFA to SFA were also found in litchi fruit receiving melatonin. In addition, melatonin treatment maintained the higher energy status, as indicated by the enhanced adenosine triphosphate (ATP) content and energy charge, which could be attributed to the enhanced activities of energy metabolism-related enzymes including H^+-ATPase, Ca^{2+}-ATPase, succinate dehydrogenase (SDH), and cytochrome C oxidase (CCO). These results suggest that melatonin could display a better performance to retard browning and senescence in litchi fruit by modulating membrane lipid and energy metabolisms.

1. Introduction

Litchi (*Litchi chinensis* Sonn.) is a well-known subtropical crop with a remarkable economic benefit in the international market for its appealing red pericarp, translucent aril, favorable taste and rich health-promoting phytonutrients (Pareek, 2016). China is the largest producer and one of the major exporters of litchi fruit (Pareek, 2016). Nevertheless, litchi fruit is perishable after harvest and displays high susceptibility to pericarp browning when stored under ambient conditions, leading to rapid quality deterioration and extremely restricts the trade of products (Li et al., 2019c).

The integrity of the biological membrane system (cytoplasmic and intracellular membranes) is closely associated with the development of browning and senescence in harvested fruit (Wang et al., 2018a). Impairment of the membrane structure and function may initiate decompartmentalization of the substrates and enzymes in fruit cells, resulting in enzymatic oxidation of phenols to *o*-quinones via catalysis by peroxidase and polyphenol oxidase (Zhang et al., 2015). The destructive process of membrane system in postharvest fruit may be ascribed to the hydrolysis of membrane phospholipids into free fatty acids and peroxidation of unsaturated fatty acids (USFA), which involves the coordinated actions of a series of lipid metabolizing enzymes (Wang et al., 2018a, b). In addition, excessive reactive oxygen species (ROS) bursts due to cellular redox imbalance under environmental stress may also trigger membrane lipid peroxidation, promoting the occurrence of browning in harvested fruit (Zhang et al., 2018b).

Accumulating evidence indicates that energy in cells is essential for the biosynthesis of phospholipids and the repair of membranes. Moreover, a low energy status may aggravate ROS accumulation and profoundly affect membrane function in postharvest fruit during storage (Saquet et al., 2003). Cellular energy status is generally measured by energy charge (EC), which represents the relationship among adenosine triphosphate (ATP), adenosine diphosphate (ADP) and adenosine monophosphate (AMP) (Aghdam et al., 2018). Mitochondrial

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ATPases, succinic dehydrogenase (SDH) and cytochrome *c* oxidase (CCO) are critical enzymes associated with energy metabolism, participating in the synthesis, transportation and utilization of ATP (Pan et al., 2019). Some postharvest strategies have been examined to increase energy metabolism-related enzymes activity and energy status, which could account for alleviated quality deterioration, as demonstrated in a variety of harvested crops (Aghdam et al., 2018).

Melatonin (N-acetyl-5-methoxytryptamine) is a naturally generated indoleamine that ubiquitously exists in organisms (Arnao and Hernández-Ruiz, 2015). In the plant kingdom, melatonin is considered as an important endogenous multi-functional molecule that modulates a diversity of physiological processes, including seed germination, flowering, circadian rhythm, photosynthesis productivity, leaf senescence, fruit maturation, and protection against various biotic and abiotic stresses (Arnao and Hernández-Ruiz, 2018). More recently, a melatonin receptor, CAND2/PMTR1, has been identified in *Arabidopsis thaliana*, suggesting the possibility of melatonin being a new member of plant hormones, which will help to deepen the understanding of melatonin signal transduction (Wei et al., 2018). Apart from the above-mentioned phytohormonic functions, melatonin has been confirmed to be an amphiphilic molecule that can easily cross over membranes and enter all subcellular compartments, such as vacuoles, nuclei and mitochondria, to act as a robust ROS scavenger and stimulator of the antioxidant system, contributing to the maintenance of intracellular redox homeostasis (Allegra et al., 2003; Zhao et al., 2012). Owing to its natural properties, melatonin has been developed as medicines and supplements that show the extraordinary pharmacological activities of anti-inflammation, anti-senescence, improvement of sleep, anti-tumor and modulation of physiological dysfunction (Arnao and Hernández-Ruiz, 2015).

The role of melatonin as a novel preservative agent on postharvest crops has recently gained much attention. It has been demonstrated that exogenous melatonin application may remarkably delay senescence in peaches (Gao et al., 2016), suppress decay and maintain quality in strawberries (Aghdam and Fard, 2017; Liu et al., 2018), ameliorate chilling injury (CI) symptoms in peaches (Cao et al., 2016, 2018; Gao et al., 2018) and tomatoes (Aghdam et al., 2019; Jannatizadeh et al., 2019), retard fruit ripening and softening in bananas (Hu et al., 2017) and pears (Zhai et al., 2018; Liu et al., 2019b), and induce disease resistance in tomatoes (Li et al., 2019b; Liu et al., 2019a). In a previous study, we observed that 0.4 mM melatonin dipping treatment effectively mitigated the symptoms of pericarp browning and senescence in 'Ziniangxi' litchi fruit stored under ambient temperature, which might be associated with inhibited phenolic oxidation, enhanced antioxidant processess and improved repair capacity of protein oxidative damage (Zhang et al., 2018b). These results suggest that melatonin application could be favorable procedure for inhibiting quality deterioration and prolonging the shelf life of harvested litchi fruit. However, whether the prevention of litchi browning and senescence in response to melatonin could be involved in the regulation of lipid and energy metabolisms have not been investigated. Our objective was to therefore elucidate the influences of exogenous melatonin treatment on the changes in membrane phospholipids, composition of membrane fatty acids, energy status, and the activities of enzymes associated with lipid and energy metabolisms in litchi fruit during storage under ambient conditions.

2. Materials and methods

2.1. Fruit material and treatment

Litchi fruit (*L. chinensis* Sonn. cv. 'A4Wuhe') were picked in the morning from a local orchard in Haikou, China. Fruit maturity at harvest reached to a commercial grade, with a fruit weight of 28 ± 1.4 g, pericarp hue angle of 32.1 ± 1.7 and pulp soluble solids content of 17.4 ± 0.4 % (n = 15). Fruit were transported to the laboratory within 2 h via a van with air-conditioning. Fruit with uniform appearance were selected and then subjected to disinfection with sodium hypochlorite solution (0.5 %, v/v) for 10 s. After rinsing with tap water and air-drying, the fruit were divided into 2 treatment groups, with 500 fruit per group. The first group was immersed in a solution of 0.4 mM melatonin (Macklin Biochemical Co., Ltd., Shanghai, China) dissolved in 20 L diH_2O for 5 min, while the second group (control) was dipped in diH_2O for 5 min. The melatonin concentration of 0.4 mM was selected based on our previous research (Zhang et al., 2018b) and a preliminary experiment using the 'A4Wuhe' litchi cultivar. After air-drying, the fruit were packed and stored at 25 ± 1 °C and 85 ± 5 % RH for 8 d according to our previous procedure (Zhang et al., 2018b). The pericarp browning index and membrane permeability were assessed every two days during storage. At the same intervals, fruit pericarp samples were taken, frozen in liquid N_2, processed into pieces and then immediately preserved at −80 °C until use. Frozen pericarps were pulverized into powders prior to analyzation of each biochemical index. Each treatment was implemented in three replications, in which the browning index was evaluated using 30 fruit per replicate while the other indices were measured with 15 fruit for each replicate.

2.2. Pericarp browning index

The pericarp browning of litchi fruit was visually classified into the scale of 0–4 based on the browned area covering the fruit surface (Zhang et al., 2015), where 0, no browning; 1, slight (≤5 % of browning); 2, moderate (6–25 % of browning); 3, severe (26–50 % of browning); 4, extremely severe (> 50 % of browning). The browning index was calculated with a formula: Σ (browning class × number of fruit in each class)/ 4 × total number of fruit in each treatment (Zhang et al., 2015).

2.3. Measurement of membrane permeability

Membrane permeability of fruit was reflected in the relative electrolyte leakage that was determined according to our previously published method (Zhang et al., 2017), with minor modifications. Specifically, sixty pericarp discs were derived from 15 fruit of each replicate using an 8-mm diameter cork borer, washed with di H_2O and incubated in 50 mL di water at 25 °C for 30 min. The initial electrolyte value (E_0) of the solution with discs was recorded by a FE 30 conductivity meter (Mettler-Toledo Instruments Co., Ltd., Shanghai, China). Thereafter, the solution with discs was boiled for 20 min, quickly cooled in an ice bath, and supplemented di-water to an equal weight as that before heating, then the total electrolyte value (E_t) of the solution was read. Relative electrolyte leakage (%) was calculated as: $E_0/E_t \times 100$ %.

2.4. Determination of lipid metabolizing enzymes activity

One gram of pericarp powder was used for determining the phospholipase D (PLD), lipase and lipoxygenase (LOX) activities, following the methods described in previous reports (Zhang et al., 2018a). One unit (U) of PLD and lipase activities was defined as a change in absorbance of 0.1 at 520 nm per h and per min, respectively. One U of LOX was defined as the enzyme amount leading to an increase in absorbance of 0.1 at 234 nm per minute. Specific PLD, lipase and LOX activities were expressed on the fresh weight (FW) as units per kilogram (U kg^{-1}).

2.5. Measurement of of phosphatidylcholine (PC) and phosphatidic acid (PA) contents

PC content was measured with high-performance liquid chromatography (HPLC) according to the method of Zhang et al. (2018a). Two hundred milligrams of pericarp powders were mixed with 1 mL of chloroform/methanol solution (1:1, v/v) for extraction at 4 °C for 12 h.

The mixture was centrifuged at 8000 × *g* for 10 min at 4 °C. The supernatant was collected and diluted to 5 mL with same chloroform/methanol solution (1:1, v/v). The mixture was then filtered through a 0.45-μm membrane filter. Aliquots (10 μL) were injected into a Rigol L3000 HPLC system (RIGOL Technology Co., Ltd. Beijing, China) equipped with a Kromasil C18 reversed-phase column (4.6 × 250 mm, 5 μm) (Eka Chemicals, AB, Bohus, Sweden) and an ultraviolet detector. The chromatographic conditions included a mobile phase of methanol/acetonitrile (1:1, v/v) at a flow rate of 0.8 mL min^{-1}, column temperature of 30 °C, wavelength of 206 nm and run time of 30 min. The PC content was calculated according to standard curve obtained from an authentic compound and expressed as g kg^{-1} FW.

For the PA assay, 0.1 g of powdered pericarp was homogenized in 1 mL of prechilled phosphate-buffered saline (pH 7.4). The homogenate was centrifuged at 5000 × *g* for 30 min at 4 °C. The PA content in the supernatant was measured using a PA enzyme-linked immunosorbent assay (ELISA) kit (Jianglai industrial Limited by Share Ltd, Shanghai, China) according to the manufacturer's instructions. The PA content was calculated based on a standard curve and expressed as g kg^{-1} FW.

2.6. Membrane fatty acids (FA) assay

One gram of the powdered pericarp tissue was used for extracting and analyzing the membrane FA composition, including SFA (palmitic acid, C16:0 and stearic acid, C18:0) and USFA (oleic acid, C18:1, linoleic acid, C18:2 and linolenic acid, C18:3). Amounts of SFA and USFA compositions were determined using a gas chromatography-mass spectrometry (model: Agilent 7890A-5975C Series GC/MSD, Agilent Technologies Inc., Wilmington, DE, USA) according to the published method of Yi et al. (2009). The relative content of the individual FA component accounting for the total FA and the ratio of USFA/SFA were calculated following the method of Zhang et al. (2018a).

2.7. Assay of energy status

One gram of pericarp powders was utilized to measure the contents of adenosine triphosphate (ATP), adenosine diphosphate (ADP) and adenosine monophosphate (AMP) according to our previously reported HPLC method (Zhang et al., 2017). The contents of ATP, ADP and AMP were expressed as mg kg^{-1} FW. Energy charge was calculated by the following formula: energy charge = ([ATP] + 1/2 × [ADP])/([ATP] + [ADP] + AMP]).

2.8. Measurement of energy metabolism-related enzymes activity

H^+-ATPase, Ca^{2+}-ATPase, succinate dehydrogenase (SDH) and cytochrome C oxidase (CCO) activities in mitochondria were measured using 2 g of powdered pericarp tissues following the published method (Jin et al., 2015). Definition of one U for H^+-ATPase and Ca^{2+}-ATPase activity was as the enzyme amount that catalyzes the production of 1 μmol phosphorus per min at 660 nm. One U of SDH and CCO was defined as an increment of 0.01 in absorbance per min at 660 and 510 nm, respectively. The specific activity of these four enzymes was expressed as U kg^{-1} FW.

2.9. Statistical analysis

The data were analyzed using SPSS statistical software version 22.0. An independent-samples *t*-test was performed to compare the means. Asterisks on the same day represent significant differences between the control and melatonin treatment (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

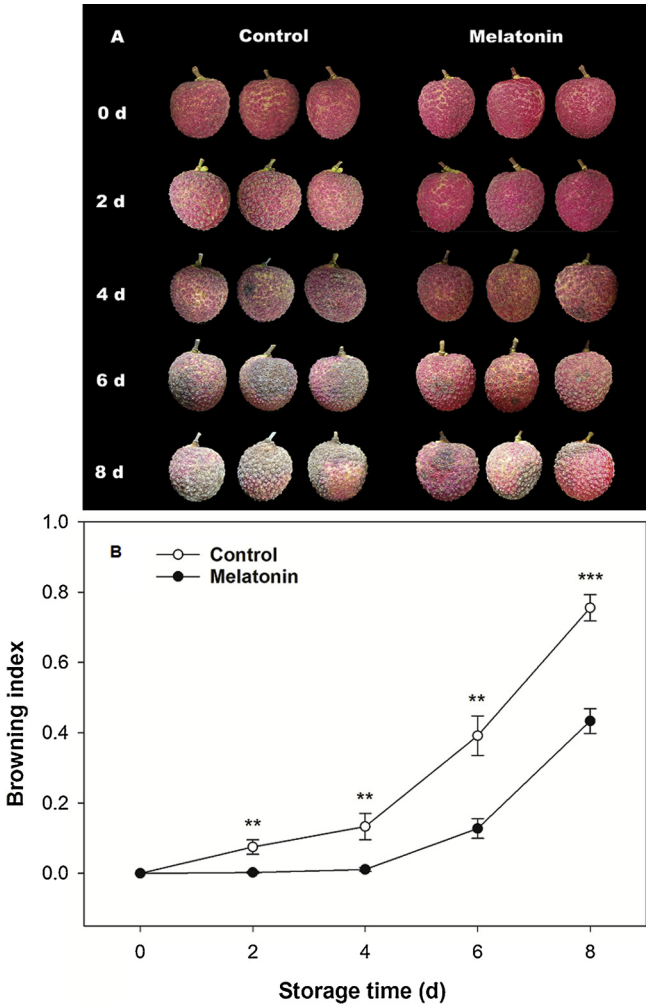

Fig. 1. Visual appearance (A) and pericarp browning index (B) in 'A4Wuhe' litchi fruit during storage at 25 °C after treatment with 0.4 mM melatonin or water (control). Vertical bars represent the standard error of the mean (n = 3). Asterisks at each time point represent significant differences between the treatment and control (* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$).

3. Results

3.1. Browning development

Control 'A4 Wuhe' litchi fruit began to emerge with slight browning symptoms after 2 d of storage and exhibited a sharp increase in browning severity after 4 d of storage (Fig. 1A and B), with a browning index reaching 0.76 ± 0.04 on day 8 of storage (Fig. 1B). Melatonin-treated fruit did not appear the browning within the initial 4 d of storage, followed by continuous augmentation over the rest of storage (Fig. 1 A and B). Comparatively, browning indices for fruit receiving melatonin were lower than those in untreated fruit during the whole storage (Fig. 1B).

3.2. Relative electrolyte leakage

Relative electrolyte leakage in control fruit steadily augmented from an initial value of 24.9 ± 1.2 to a maximum of 42.9 ± 1.7 % after 8 d of storage (Fig. 2). The trend for relative electrolyte leakage in melatonin-treated fruit was parallel with that of the browning index, in which melatonin-treated fruit had significantly ($P < 0.05$) lower relative electrolyte leakages compared to the untreated fruit throughout storage (Fig. 2).

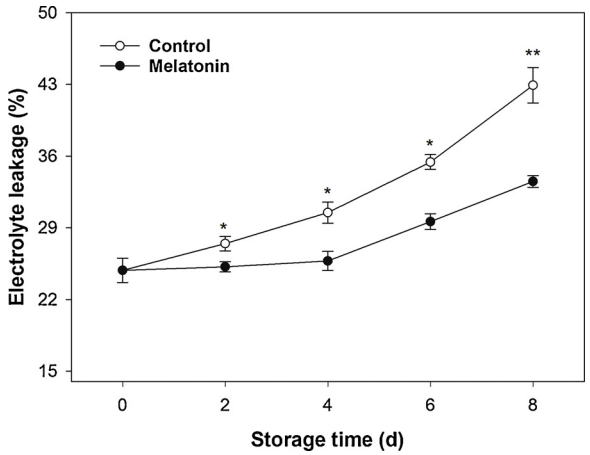

Fig. 2. Relative electrolyte leakage in 'A4Wuhe' litchi fruit during storage at 25 °C after treatment with 0.4 mM melatonin or water (control). Vertical bars represent the standard error of the mean (n = 3). Asterisks at each time point represent significant differences between the treatment and control (* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$).

3.3. PLD, lipase and LOX activities

Control fruit showed increased overall tendency in PLD, lipase and LOX activities during storage (Fig. 3A–C). Melatonin treatment inhibited the increments in PLD, lipase and LOX activities, in which the activities of these three enzymes in melatonin-treated fruit were lower than those of control fruit, with the exception of 2 d of storage (Fig. 3A–C).

3.4. PC and PA contents

PC content in control fruit continuously declined from the initial value of 0.57 ± 0.03 to 0.38 ± 0.01 g kg^{-1} at the last day of storage (Fig. 4A). A slower decline in PC content occurred in litchi fruit treated with melatonin, in which the PC contents in melatonin-treated fruit at 4 through 8 d were significantly higher than those in untreated fruit (Fig. 4A).

PA content in control fruit showed an increase of 17 % during storage (Fig. 4B). Melatonin treatment evidently inhibited the accumulation of PA during the middle and late stages of storage, in which the PA content in melatonin-treated fruit at 4, 6 and 8 d of storage was 3.6, 6.6 and 7.0 % lower than that in control fruit, respectively (Fig. 4B).

3.5. Fatty acids (FA) contents

As depicted in Fig. 5A and B, the initial relative contents of two SFA (palmitic acid, C16:0 and stearic acid, C18:0) in litchi fruit at harvest were 26. 1 ± 0.4 and 10.4 ± 0.3 %, respectively. Control fruit showed constant increments in relative contents of both SFA, with respectively attaining 37.3 ± 1.1 and 19.3 ± 0.4 % at the end of storage (Fig. 5A and B). Melatonin treatment lowered relative contents of palmitic acid and stearic acid, with significant difference between melatonin treatment and control being observed at 4, 6 and 8 d of storage (Fig. 5A and B). As shown in Fig. 5C–F, the initial contents of the three tested USFA on harvest day were ranked as: linoleic acid, C18:2 (49.2 ± 0.2 %) > linolenic acid, C18:3 (10.3 ± 0.3 %) > oleic acid, C18:1 (4.0 ± 0.1 %). Contrary to the change in SFA, the relative contents of these tested USFA in control fruit exhibited varying degrees of decline as storage time progressed (Fig. 5C–E). Melatonin treatment retarded the declines in contents of these three USFA, but more profound effect of melatonin was noted in content of linoleic acid when compared to oleic acid and linolenic acid (Fig. 5C–E). The ratio of USFA to SFA in both control and melatonin-treated fruit steadily dropped as storage progressed, but the ratios of melatonin-treated fruit were significantly higher than those of control fruit during the whole storage (Fig. 5F).

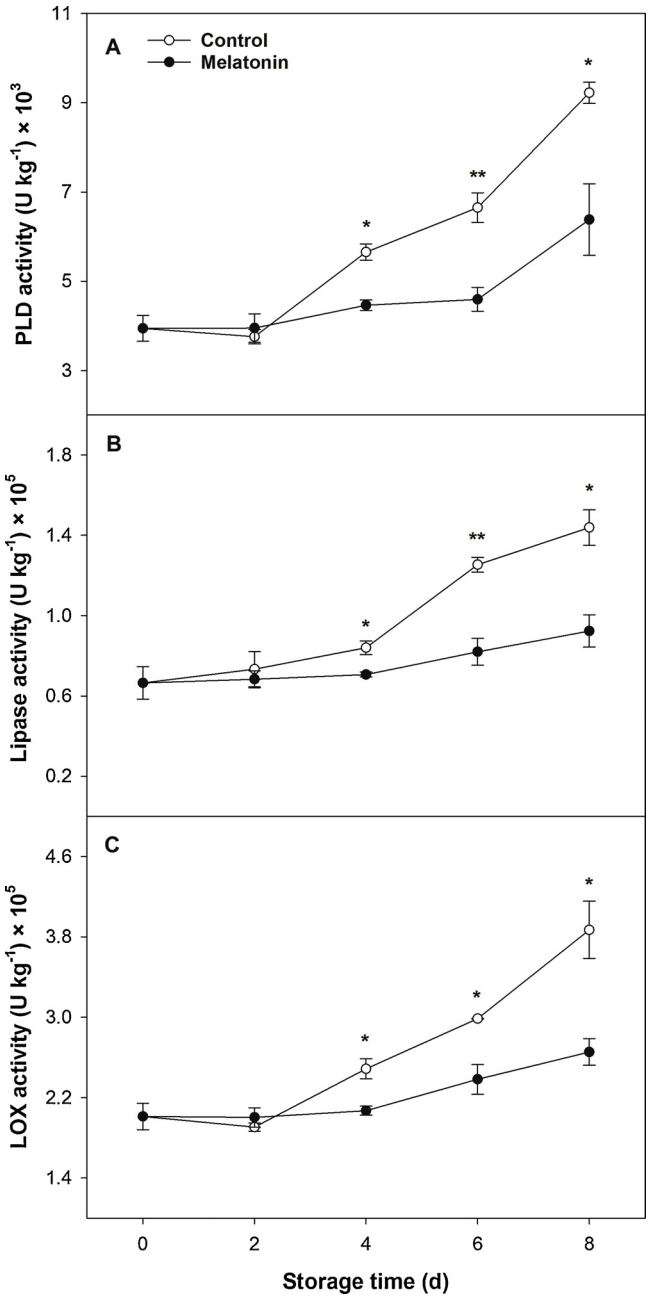

Fig. 3. Activities of phospholipase D (PLD) (A), lipase (B), and lipoxygenase (LOX) (C) in 'A4Wuhe' litchi fruit during storage at 25 °C after treatment with 0.4 mM melatonin or water (control). Vertical bars represent the standard error of the mean (n = 3). Asterisks at each time point represent significant differences between the treatment and control (* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$).

3.6. ATP, ADP and AMP contents and EC

Control fruit showed decreases of 38 and 60 % in ATP and ADP contents after 8 d of storage, respectively (Fig. 6A and B). Compared to control fruit, the higher levels of ATP at 6 and 8 d as well as ADP from 4 to 8 d were maintained in fruit receiving melatonin (Fig. 6A and B). AMP content in control fruit slightly declined within 2 d, followed by a steady rise over the reminder of storage (Fig. 6C). Melatonin treatment

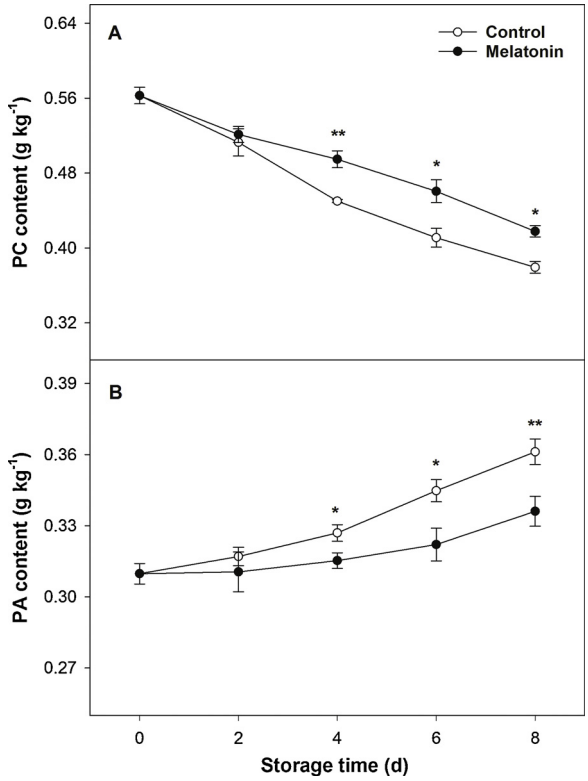

Fig. 4. Contents of phosphatidylcholine (PC) (A) and phosphatidic acid (PA) (B) in 'A4Wuhe' litchi fruit during storage at 25 °C after treatment with 0.4 mM melatonin or water (control). Vertical bars represent the standard error of the mean (n = 3). Asterisks at each time point represent significant differences between the treatment and control (* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$).

significantly lowered AMP contents from 4 to 8 d of storage (Fig. 6C). EC in control fruit decreased from an initial value of 0.79 ± 0.01 to 0.75 ± 0.005 after 8 d of storage (Fig. 6D). EC in melatonin-treated fruit did not diverge from that of control fruit within the initial 4 d, subsequently maintained a higher level of EC than that of control fruit (Fig. 6D).

3.7. H^+-ATPase, Ca^{2+}-ATPase, SDH and CCO activities

H^+-ATPase, SDH and CCO activities in both control and melatonin-treated fruit exhibited transient upward trends within 2 d of storage and thereafter dropped with an approximately parallel mode over until end of storage (Fig. 7A, C and D). Compared to untreated fruit, higher activities of H^+-ATPase, SDH and CCO were noticed in melatonin-treated fruit during most of storage period, except for CCO activity at 2 d (Fig. 7A, C and D). Ca^{2+}-ATPase activity in control and melatonin-treated fruit constantly dropped during storage, but higher Ca^{2+}-ATPase activity occurred in fruit receiving melatonin, except day 2 (Fig. 7B).

4. Discussion

4.1. Effect of melatonin on litchi pericarp browning in relation to membrane integrity

Pericarp browning, as a typical senescence trait, is a major bottleneck that restricts the development of litchi postharvest industry (Zhang et al., 2017). Up to the present, varieties of safe strategies have been attempted to control postharvest browning of litchi fruit, which include heat treatment (Jacobi et al., 1993), controlled atmosphere storage (Mahajan and Goswami, 2004), modified atmosphere packing (Tian et al., 2005), chitosan coatings (Joas et al., 2005; Jiang et al., 2018), and treatments with natural compounds (Zhang et al., 2015, 2017; Shah et al., 2017; Su et al., 2019). In the current study, exogenous melatonin application by means of dipping was shown to be effective in suppressing the development of pericarp browning in 'A4Wuhe' litchi fruit during storage at 25 °C. In agreement with the present results, a delay in browning or other forms of quality deterioration owing to exogenous melatonin exposure was previously observed in stored 'Ziniangxi' litchi fruit (Zhang et al., 2018b) and other harvested crops, including peaches (Gao et al., 2016; Cao et al., 2018), cassavas (Ma et al., 2016), tomatoes (Jannatizadeh et al., 2019), strawberries (Liu et al., 2018), pears (Zhai et al., 2018), Chinese cabbages (Tan et al., 2019) and bamboo shoots (Li et al., 2019a).

The integral cellular membrane system is a basic guarantee for the normal physiological activities of organisms. The integrity of the membrane is reflected in membrane permeability, which can be measured by changes in relative electrolyte leakage (Zhang et al., 2017). In the present work, lower relative electrolyte leakage accompanied by delayed browning was noticed in melatonin-treated litchi fruit compared to that of control fruit during storage at ambient temperature. The results indicate that melatonin treatment might maintain the integrity of membrane structure and limit the contact of enzymes and substrates, consequently leading to reduced occurrence of enzymatic browning of litchi pericarp... Similar inhibition of relative electrolyte leakage in relation to reduced browning or other forms of quality deteriorations was also observed in melatonin-treated peaches (Gao et al., 2016), strawberries (Liu et al., 2018) and tomatoes (Aghdam et al., 2019).

4.2. Effect of melatonin on litchi pericarp browning in relation to membrane lipid metabolism

Components of cell membrane mainly comprise lipids, proteins and sugars, of which the lipids account for the majority (Meyer and Terry, 2010). Phospholipids, mainly presented as phosphoglycerides (e.g. PC and phosphatidylinositol), constitute the most important part of membrane lipids in plants (Lin et al., 2017). PLD, lipase and LOX are crucial enzymes that participate in lipid metabolism (Lin et al., 2017). Among these enzymes, PLD is a membrane-binding enzyme and pertains to the phosphohydrolase family, which is uniquely responsible for hydrolyzing the ester bond of constructional phospholipids (e.g. PC), generating PA (Wang et al., 2018a). In one sense, the degree of phospholipid hydrolysis can be directly manifested by the PLD activity (Sirikesorn et al., 2013). PA can be further decomposed into free fatty acids under the catalysis of lipase (Paliyath and Droillard, 1992). LOX can catalyze the oxidation of double bonds in polyunsaturated free fatty acids, such as linoleic acid, linolenic acid and arachidonic acid, promoting the formation of hydroperoxides and free radicals that can further attack the membrane system; therefore, LOX is deemed a crucial factor in free fatty acid metabolism and lipid peroxidation (Lin et al., 2017). A number of studies indicate that augmented activities of PLD, lipase and LOX in parallel with augmented membrane permeability are programmed processes in senescing tissues of harvested crops (Wang et al., 2018a, b). Therefore, suppression of membrane lipid-metabolizing enzymes activity would be favorable for maintaining cellular membrane integrity and improving quality of postharvest fruit. Sun et al. (2011) reported that n-butanol pretreatment specifically inhibited the PLD activity in litchi fruit during storage, which contributed to diminished degradation of membrane phospholipids and alleviated enzymatic browning. Lin et al. (2017) noted that exogenous treatment with propyl gallate, an antioxidant widely used in food industry, remarkably lowered the activities of PLD, lipase and LOX and maintained the ratio of USFA to SFA in longan fruit, consequently contributing to the retention of pericarp color and the improved storability of products. In our study, the repressed activities of PLD, lipase and LOX, along with

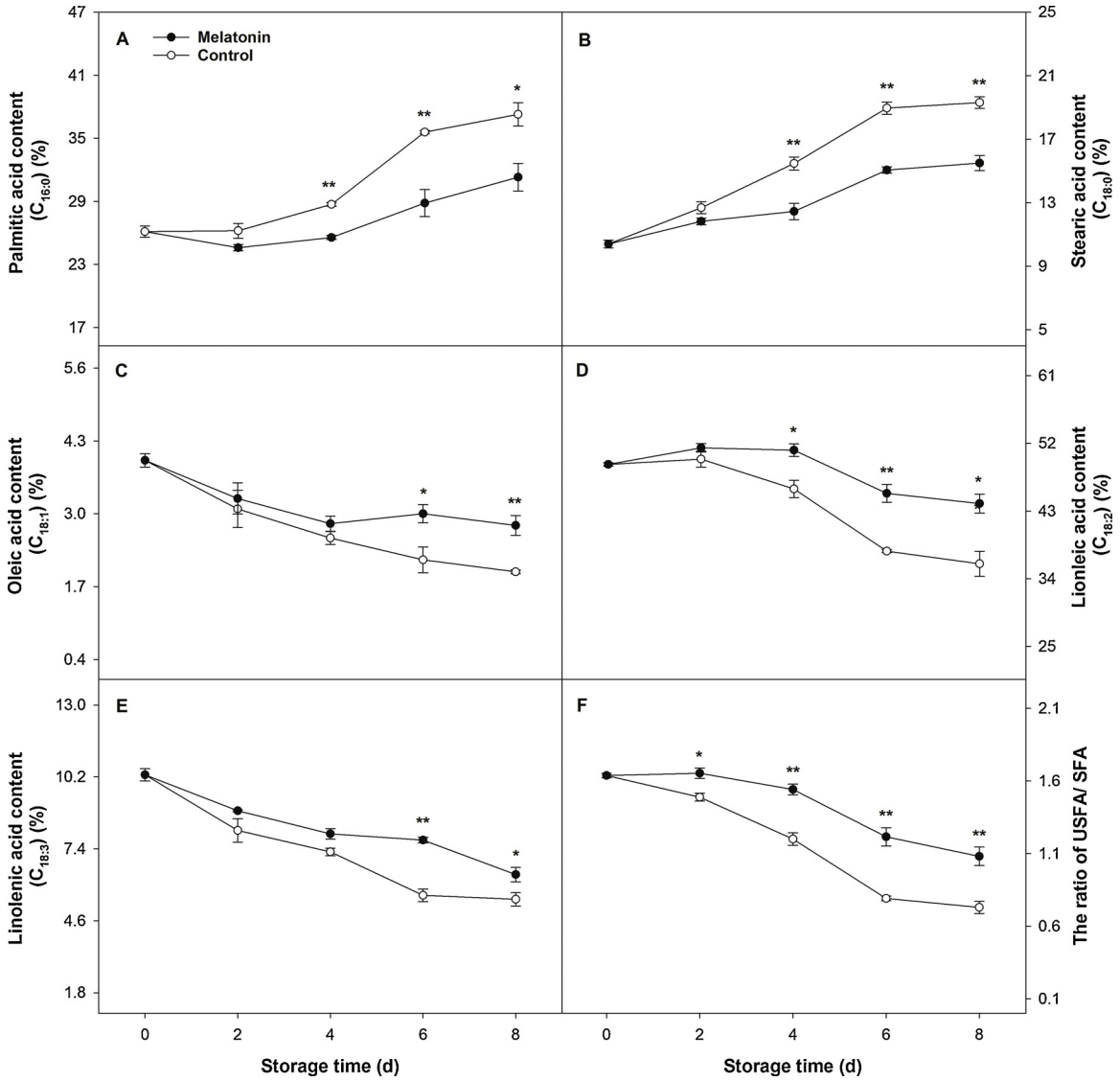

Fig. 5. Relative contents of palmitic acid (A), stearic acid (B), oleic acid (C), linoleic acid (D), and linolenic acid (E), and the ratio of unsaturated fatty acids (USFA) to saturated fatty acids (SFA) (F) in 'A4Wuhe' litchi fruit during storage at 25 °C after treatment with 0.4 mM melatonin or water (control). Vertical bars represent the standard error of the mean (n = 3). Asterisks at each time point represent significant differences between the treatment and control (* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$).

higher PC but lower PA contents, were found in melatonin-treated litchi fruit compared to those of untreated fruit during storage, indicating that delay of litchi pericarp browning in response to melatonin might be associated with inhibition of lipid metabolism. Recent studies have also shown that melatonin treatment effectively alleviated the symptoms of CI in tomatoes and pomegranates during cold storage, which could be ascribed to the suppressed activities of PLD and LOX and the expression of genes encoding these enzymes (Jannatizadeh, 2019; Jannatizadeh et al., 2019). An earlier study testified that melatonin can easily penetrate the hydrophobic lipid phase, suggesting that the biological action of melatonin could possibly occur at membrane level (Costa et al., 1995), which supports the previous and present results obtained for above-mentioned fruits. However, it should also be emphasized that the period of interference with lipid-metabolizing enzymes activity is crucial since enzymatic inhibition will have a weak influence once membrane deterioration has been initiated (Bhushan et al., 2015).

It is well known that the fatty acid composition of membrane lipid profoundly affects cell membrane fluidity that provides the stability and functionality of membranes (Lin et al., 2017). A shorter fatty acid chain with higher degree of unsaturation is conductive to maintaining the fluidity of membranes, contributing to preservation of postharvest quality in fruit (Jin et al., 2014). The view could be supported by Cao et al. (2011) who noted that less susceptibility to core browning occurred in cold-stored 'Qizhong' loquat fruit relative to the 'Fuyang' cultivar, which could be attributed to higher membrane lipid unsaturation in the former. In general, during natural senescence or under environmental stresses, the composition and unsaturated level of fatty acids may be subjected to significant alteration due to catalysis of LOX and attack by ROS, displaying a decrease in USFA levels but an enhancement in SFA levels (Zhang et al., 2018a). The present data exhibited that melatonin treatment delayed the declines in USFA contents, including oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3), while suppressing increases in SFA contents, including palmitic acid (C16:0) and stearic acid (C18:0). Moreover, a higher ratio of USFA to SFA was well maintained in melatonin-treated litchi fruit during storage. Our results were in agreement with the research on melatonin-pretreated tomato fruit during refrigeration (Jannatizadeh et al., 2019). Taken together, these findings imply that melatonin might retard senescence, quality deterioration and stress-induced injury in postharvest fruit by maintaining the unsaturation of membrane fatty

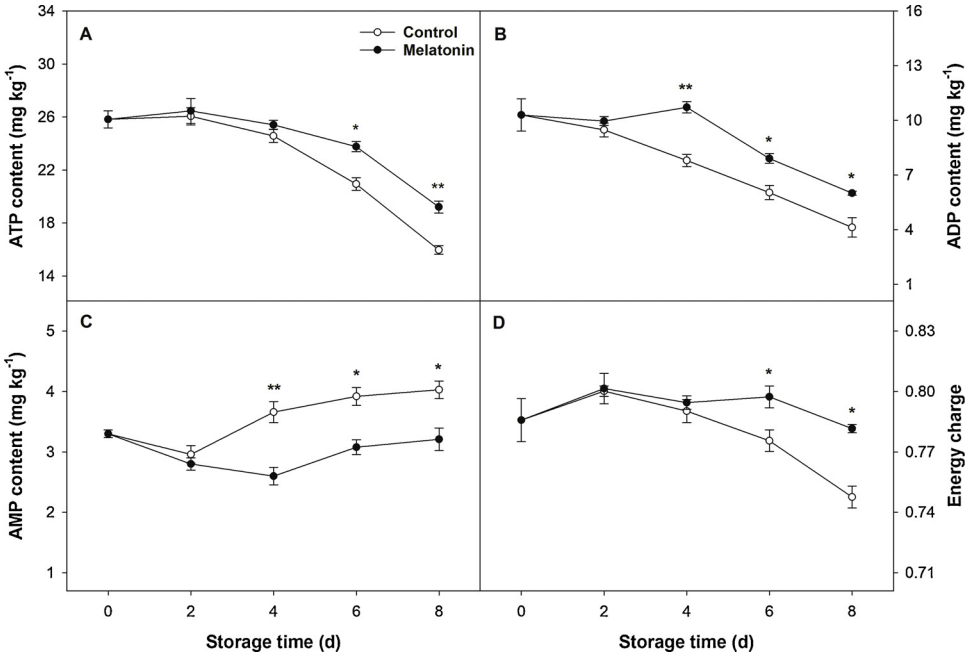

Fig. 6. Contents of adenosine triphosphate (ATP) (A), adenosine diphosphate (ADP) (B) and adenosine monophosphate (AMP) (C), and energy charge (D) in 'A4Wuhe' litchi fruit during storage at 25 °C after treatment with 0.4 mM melatonin or water (control). Vertical bars represent the standard error of the mean (n = 3). Asterisks at each time point represent significant differences between the treatment and control (* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$).

acids.

4.3. Effect of melatonin on litchi pericarp browning in relation to energy status and energy metabolism

Evidence indicates that membrane integrity is intimately associated with cellular energy status, by which ATP depletion can severely restrict lipid synthesis by diminishing desaturation of the fatty acyl chain (Rawyler et al., 1999). Liu et al. (2011) noticed accelerating pericarp browning in 'Huaizhi' litchi fruit within a short period of time under ambient conditions after being transferred from cold storage, which was accompanied by decreased energy levels and enhanced activities of PLD and LOX. In contrast, it was reported that the application of exogenous ATP was able to maintain the ratio of USFA to SFA in 'Huaizhi' litchi fruit, conferring the resistance of fruit against senescence and pathogen infection (Yi et al., 2009). In this work, higher energy status, as signified by higher ATP levels and EC, was observed in melatonin-treated litchi fruit, which might contribute to the maintenance of membrane integrity and delayed browning. Similarly, higher intracellular ATP levels in relation to the preservation of membrane fluidity and conferred cold tolerance were observed in melatonin-pretreated tomato fruit under chilling stress (Jannatizadeh et al., 2019).

Cellular energy status in plant tissues can be regulated by energy metabolism-related enzymes, including ATPases, SDH and CCO (Bhushan et al., 2015). ATPases are a category of enzymes that can hydrolyze ATP into ADP and phosphate, resulting in the discharge of energy (Liu et al., 2011). Suppression of H^+-ATPase and Ca^{2+}-ATPase activities could stimulate a decrease in membrane energization, accounting for destruction of membrane integrity and cell collapse (Liu et al., 2016). SDH acts as a key enzyme in the tricarboxylic acid cycle,

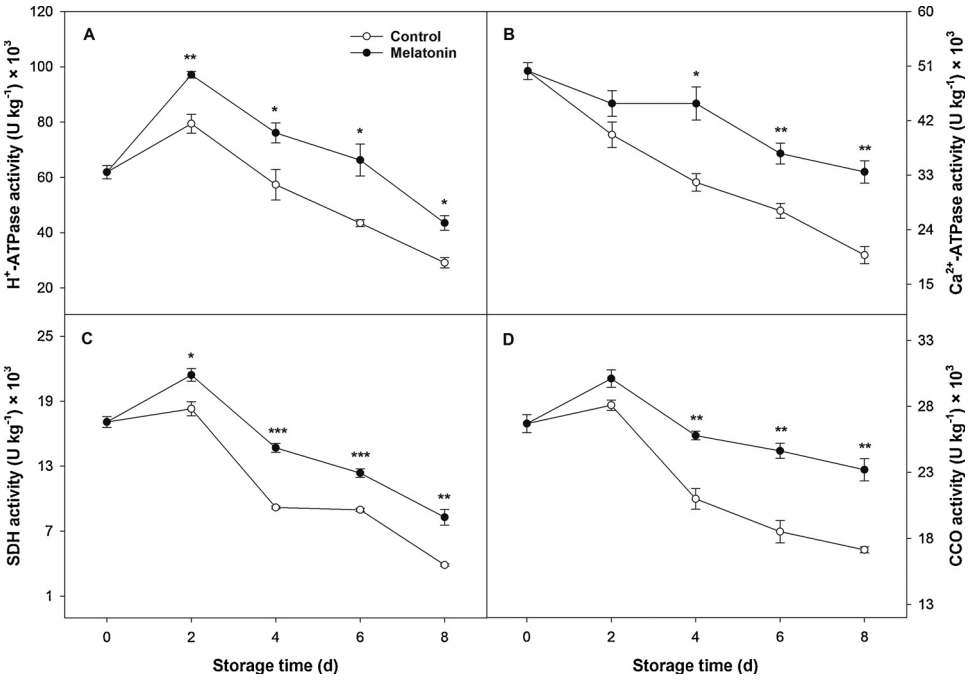

Fig. 7. Activities of H^+-ATPase (A), Ca^{2+}-ATPase (B), succinate dehydrogenase (SDH) (C), and cytochrome C oxidase (CCO) (D) in 'A4Wuhe' litchi fruit during storage at 25 °C after treatment with 0.4 mM melatonin or water (control). Vertical bars represent the standard error of the mean of triplicate assays. Asterisks at each time point represent significant differences between the treatment and control (* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$).

catalyzing the conversion of succinate into fumarate while transferring electrons to the respiratory chain, which contributes to the production of ATP (Jin et al., 2014). CCO is the terminal oxidase of the respiratory electron transport chain and is accountable for the acceptance and transfer of electrons, producing energy through oxidative phosphorylation in mitochondria (Pan et al., 2019). Inactivation of SDH and CCO may cause mitochondrial dysfunction and a deficit of ATP supply, eventually leading to cellular senescence and death. Augmentation of energy metabolism-related enzymes activity in association with improved quality, delayed senescence and attenuated stress has been demonstrated in different varieties of harvested crops, as summarized in a literature review by Aghdam et al. (2018). The present data showed higher activities of H^+-ATPase, Ca^{2+}-ATPase, SDH and CCO in accordance with delayed pericarp browning in melatonin-treated litchi fruit during storage, suggesting that melatonin-promoted anti-senescence could be involved in the improvement of energy status under the regulation of energy metabolism-related enzymes. Similarly, Jannatizadeh et al. (2019) reported that melatonin application to tomatoes induced the enhancements of H^+-ATPase, Ca^{2+}-ATPase, SDH and CCO activities, which was critical for raising energy status and ameliorating fruit CI during cold storage.

5. Conclusion

In summary, the occurrence of browning in 'A4Wuhe' litchi fruit during storage at 25 °C was effectively alleviated by melatonin treatment. The delay in browning and senescence by melatonin could be involved in regulation of membrane lipid and energy metabolisms. Nevertheless, the sophisticated molecular mechanism of melatonin-afforded antibrowning in harvested litchi fruit needs further study. The present study may also open a new perspective aiming to develop melatonin as a preservative agent in the postharvest industry.

Declaration of Competing Interest

The authors declare that there is no conflict of interests.

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