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Short-term anaerobic, pure oxygen and refrigerated storage conditions affect the energy status and selective gene expression in litchi fruit☆

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ABSTRACT

Harvested litchi fruit were stored under cold temperature, short-term anaerobic and pure oxygen conditions to explore the energy characteristics and the genes that are related to pericarp browning. Fruits in short-term anaerobic and pure oxygen environments were stored at 25 °C. Additionally, fruits were stored at 1 °C for 1, 2 or 3 weeks and were then subjected to a post cold storage at 25 °C for up to 48 h. Short-term anaerobic and pure oxygen storage decreased the browning index and relative abundance of the ATP synthase β subunit (*LcAtpB*) and increased respiration intensity, ATP content, the contribution of an alternate oxidation pathway to the total respiration ($\rho V_{AP}/Vt$) and the transcription of plant uncoupling mitochondria protein 1 (*LcUCP1*), with short-term anaerobic treatment being more significant than pure oxygen. Cold storage decreased the ATP levels but increased the browning index, respiration intensity, $\rho V_{AP}/Vt$ and the transcription of alternative oxidase 1 (*LcAOX1*), *LcUCP1*, ADP/ATP carrier 1 (*LcAAC1*) and sucrose non-fermenting-1-related kinase 2 (*LcSnRK2*) during the post storage shelf-life at 25 °C. The data suggest that the energy level is closely related to the respiratory activity and is involved in the browning control of harvested litchi fruit. Transcripts of the 5 genes were found to be involved in the energy regulation and quality control of postharvest litchi fruit.

1. Introduction

Litchi (*Litchi chinensis* Sonn.) is a subtropical fruit with a high market value. The commodity rate and transportation of this fruit are greatly restricted by rapid fruit deterioration and pericarp browning during room storage and post-cold storage under ambient conditions (Liu et al., 2011; Reichel, Carle, Sruamsiri, & Neidhart, 2010). Therefore, the mechanisms of litchi browning and quality deterioration have been extensively studied (Jiang et al., 2006; Kumar et al., 2012; Martínez-Castellanos et al., 2011; Sivakumar & Korsten, 2010). Recent studies have shown that the senescence or browning of harvested fruits and vegetables may be attributed to the limited availability of energy or a low energy production rate. A negative correlation was observed between the senescence extent and ATP content in litchi, longans, apples, pears and sunflower seeds (Jiang et al., 2007; Kibinza, Vinel, Come, Bailly, & Corbineau, 2006; Yi et al., 2010).

Anaerobic treatment can extend the storage life and improve the quality of various harvested fruits such as tomatoes (Fallik, Polevaya, Tuvia-Alkalai, Shalom, & Zuckermann, 2003), kiwifruits (Song et al., 2009), litchi (Liu et al., 2007), loquats (Gao et al., 2009) and bananas (Yi et al., 2006). High oxygen treatment has

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also been shown to be favourable to the maintenance of the postharvest physiology and quality of fruits and vegetables (Kader & Ben-Yehoshua, 2000). Furthermore, both treatments can improve the energy level of harvested litchi fruit (Duan et al., 2004; Liu et al., 2007). However, the molecular mechanisms that are related to the energy level of harvested fruits and vegetables are unclear.

The energy status in fruits is largely associated with the respiration activity and the proteins responsible for the synthesis, dissipation, transport, and regulation of ATP (Geigenberger, Riewe, & Fernie, 2010; Holtzapffel, Finnegan, Millar, Badger, & Day, 2002; Millar, Whelan, Soole, & Day, 2011). As a novel cell death regulator, subunit β (AtpB) of F1Fo-ATP synthase plays a pivotal role in stress response. Upregulation of *AtpB* transcription marks the initiation of the aging process. Respiration rates are reduced, which results in the inhibition of ATP synthesis in the absence of the β subunit (Chivasa, Tome, Hamilton, & Slabas, 2011). Cyanide-resistant respiration and UCP are the two main energy dissipation systems in plant mitochondria and are involved in heat production, reactive oxygen species regulation, stress reaction, energy metabolism and fruit maturity (Smith, Ratcliffe, & Sweetlove, 2004). These systems are closely related to the postharvest physiology of harvested fruits (Fung et al., 2004). Tomato plants with reduced *LeAOX* levels exhibit delayed ripening and respiration (Xu, Yuan, Zhang, Lv, & Lin, 2012). ADP/ATP carrier (AAC) catalyses a counter exchange of cytosolic ADP with matrix ATP, enabling mitochondria to supply the cytosol and, subsequently, other organelles with energy, while sucrose non-fermenting-1-related protein kinase (SnRK) is 'the energy regulator' in the cell (Geigenberger et al., 2010). Transcription levels of proteins synthesising and dissipating ATP have been reported in the senescence progress of several climacteric fruits including bananas, mangoes, papayas, pineapples, apples, strawberries and tomatoes (Borecky et al., 2006; Considine, Daley, & Whelan, 2001; Costa et al., 1999; Tian et al., 2004). However, these studies do not illustrate the physiological function of these proteins, especially in harvested fruits. Very few information is available about the roles of UCP in non-climacteric fruits and the roles of AAC and SnRK in the senescence of fruits and vegetables.

In this study, litchi was exposed to short-term anaerobic and pure oxygen conditions before storage at room temperature. Additionally, litchi fruit was refrigerated and then stored at room temperature. We investigated the effect of these storage conditions on the energy level, the respiratory capacity of the energy dissipation pathway, and the expression levels of the energy-related genes with relation to senescence and the quality deterioration of harvested litchi fruit.

2. Materials and methods

2.1. Fruit material and postharvest treatments

Litchi fruit (*L. chinensis* Sonn.) cv. Huaizhi were harvested at commercial maturation stage from an orchard in Guangzhou city. Fruit were selected for uniformity of shape, colour, and size and for freedom from any blemish or disease. All fruit were dipped in 500 mg/l prochloraz solution (Sportak®, BASF, Ludwigshafen, Germany) for 3 min and air dried before the following two experiments were performed. In the first one, fruit were packaged in low density polyethylene bags (0.015 mm thick; 30 fruit per bag, 27 bags). The bagged fruit were stored for 1, 2 or 3 weeks at 1 °C. After their respective storage time, the fruit were removed from the bags and held at room temperature (25 °C) for up to 48 h, then measurements were taken at shelf time of 0, 24 h and 48 h. In the second one, fruit were divided into three groups for the following treatments. Ninety fruits were put into a 7.2 l glass jar. There were three jars per treatment in a flow-through gas system. Fruit samples were continuously kept in humidified air (control), pure O_2 or N_2 for 2 d, 2 d and 6 h respectively at 25 °C. In our preliminary experiments, O_2 treatment for 2 d or N_2 treatment for 6 h were found effective to postpone browning and senescence of litchi fruit, and therefore were used in the present study. Dry air, pure O_2 and N_2 gases were from the Guangzhou Gas Factory (Guangzhou, China). The treated fruit were packaged in low density polyethylene bags as described above and stored at room temperature (25 °C) for up to 6 d. Additionally, 60 freshly harvested fruits were sampled before treatment and used as 0 day assessments. After 2, 4 and 6 days of storage, three bags of fruits per treatment were used for measurements. Pericarp browning and respiration activities were recorded using fresh fruit. The remaining pericarp tissues were collected, frozen in liquid nitrogen, crushed and stored at −80 °C for ATP determination and RNA extraction. The experiments were conducted three times, and similar results were obtained. The data from one experiment are presented.

2.2. Evaluation of pericarp browning

Pericarp browning was assessed by measuring the extent of the total browned area on each pericarp of 90 fruits on the following scale by an experienced technician: 0 = no browning (excellent quality); 1 = slight browning; 2 = <1/4 browning; 3 = 1/4–1/2 browning; 4 = >1/2 browning (poor quality). The browning index was calculated as: ∑(browning scale × proportion of corresponding fruit within each class).

2.3. Determination of ATP concentration and energy charge

Adenosine triphosphate (ATP), adenosine diphosphate (ADP) and adenosine monophosphate (AMP) were extracted using the method described by Ozogul, Taylor, Quantick, and Ozogul (2000) with a minor modification. The pericarp tissue (2 g) of 30 fruits was ground in liquid nitrogen and homogenised with 5 ml of 0.6 mol/l perchloric acid in an ice bath. The extraction mixture was then centrifuged for 10 min at 10,000 g at 4 °C. The supernatant (3 ml) was adjusted to pH 6.5–6.8 with 1 mol/l potassium hydroxide and was placed in an ice bath for 30 min to precipitate the potassium perchlorate. The solution was diluted to 4 ml and passed through a 0.45 μm filter. Determination of ATP, ADP and AMP were performed by a Waters 2695 HPLC (Waters, Inc., Milford, MA, USA) using a Nucleosil Pinnacle ll-C18 column (4.6 × 250 mm) and a UV detector set at 254 nm. Mobile phase A consisted of 0.06 mol/l dipotassium hydrogen phosphate and 0.04 mol/l potassium dihydrogen phosphate dissolved in deionised water and adjusted to pH 7.0 with 0.1 mol/l potassium hydroxide. Mobile phase B was pure acetonitrile. Elution was carried out by a linear gradient programme with 750–1000 ml/l A solution and 0–250 ml/l B solution for 7 min. The flow rate was 1.2 ml/min. ATP, ADP and AMP concentrations were calculated according to the external standard (Sigma Aldrich, St Louis, MO, USA) program and were expressed as fresh weight (FW). The energy charge (EC) was calculated by $([ATP] + 0.5 \times [ADP])/([ATP] + [ADP] + [AMP])$.

2.4. Analysis of respiration activity

The respiration intensity of intact fruit was measured by infrared gas analysis. Ten fruits were weighed before being sealed in a 4.5 l glass jar with two rubber tubes in the lid. The tubes were connected to a Li-6262 CO_2/H_2O analyser (LI-COR, Inc. Lincoln, NE, USA) to form a flow-through gas system. Increases in the CO_2 concentration in the container were monitored for 5 min by passing

the air stream through the analyser. The respiration rate was expressed as carbon dioxide released per kilogramme per hour of fresh weight (mg CO_2/Kg/h). The analysis was performed in five replicates. The respiration rate was calculated as: $(C2 - C1) \times V \times M \times 1000/1{,}000{,}000 \times V0 \times m \times [(t2 - t1)/60]$, where $C1 = CO_2$ concentration at $t1$, μmol/mol; $C2 = CO_2$ concentration at $t2$, μmol/mol; V = capacity of the airtight container, l; M = Moore quality of CO_2, 44 g/mol; $V0$ = molar volume of CO_2, 22.4 l/mol; M = fruit weight, kg; T = Time interval of record, 5 min.

The respiratory pathway was measured using a method described by Moller, Bérczi, van der Plas, and Lambers (1988) with some modifications. Ten pericarp discs were taken from ten litchi fruits using a puncher with a 10 mm diameter. The pericarp discs were incubated in the cup of an oxygen electrode (Hansatech Instruments Ltd., Kings Lynn, Norfolk, UK) filled with 1 ml of respiratory buffer (buffer I, II, III or IV, respectively as below). The oxygen consumption curve was recorded at 25 °C. The oxygen consumption rate was calculated as nmol of oxygen consumed by 1 cm^2 of pericarp within 1 min. The total respiration activity (Vt) was obtained by applying buffer I (0.065 mol/l potassium phosphate buffer, pH 6.8), the respiratory activity minus cytochromes pathway respiration-remaining and cyanide-resistant respiration ($V_{Res} + V_{AP}$) by buffer II (0.005 mol/l KCN in Buffer I), respiratory activity minus cyanide-resistant respiration ($V_{Res} + V_{CP}$) by buffer III (0.025 mol/l benzohydroxamate (BHAM) in Buffer I) and remaining respiratory activity (V_{Res}) by buffer IV (0.005 mol/l KCN + 0.025 mol/l BHAM in Buffer I), respectively. Respiratory capacity was calculated using the following formula: C = cytochrome pathway capacity (ρV_{CP}) = Vt − ($V_{Res} + V_{AP}$); Alternate pathway capacity (ρV_{AP}) = Vt − ($V_{Res} + V_{CP}$); Contribution of the cytochromes pathway (V_{CP} %) = ρV_{CP}/Vt, contribution of an alternate pathway (ρV_{AP} %) = ρV_{AP}/Vt.

2.5. Determination of gene expression

The transcription levels of the energy related genes were determined by quantitative RT-PCR analysis. Total RNA was extracted from the litchi fruit pericarp using the hot borate method of Wan and Wilkins (1994). The RNA extracts were treated with DNase I (Takara, Dalian, China), and purified according to the manufacturer's instructions. The first-strand cDNA was synthesised from the DNA-free total RNA using Prime-ScriptTM RT-PCR Kit (TaKaRa, Dalian, China), according to the manufacturer's protocol. Quantitative real-time PCR (qPCR) was performed using the LightCycler 480 SYBR Green I Master Mix (Roche Applied Science, Mannheim, Germany) on an ABI 7500 Real-Time PCR system (Applied Biosystems, Carlsbad CA, USA) according to the manufacturer's instructions. The qPCR conditions were 30 s at 95 °C, 40 cycles of 5 s at 95 °C, and 34 s at 58 °C. In this study, the expression of five energy related genes: *LcAtpB* (GenBank ID: JQ349005), *LcAOX1* (GenBank ID: JQ349006), *LcUCP1* (GenBank ID: JQ349009), *LcAAC1* (GenBank ID: JQ349007) and *LcSnRK2* (GenBank ID: JQ349008) were isolated from litchi fruit pericarp and tested. The *LcACTIN* gene was used for quantitative normalisation.

2.6. Statistical analysis

Each experiment was repeated twice. Experiments for respiration intensity were repeated 6 times, while other experiments were repeated 3 times. The results were the mean values ± SD (standard deviation). Significant differences among different treatments and different storage times were determined by a one-way analysis of variance (ANOVA) using SPSS® version 13.0. Differences with p value under 5% were considered significant. The graph was created using SigmaPlot 9.0.

3. Results and discussion

3.1. Browning index

The litchi fruit pericarp browning index increased significantly from the second day of storage at 25 °C (Fig. 1A) and 1 week after cold storage at 1 °C (Fig. 1B). The browning index was decreased under the conditions of pure O_2 and N_2 atmospheres, with a significantly larger decline in the N_2 atmosphere, suggesting a more efficient inhibition of pericarp browning by short-term anaerobic treatment than by pure O_2 exposure. The browning index increased significantly with the extension of storage at room temperature (25 °C) after cold storage. The browning index after 24 h of storage was positively correlated with refrigeration time and was the highest after a storage time of 3 weeks (Fig. 1B). Because browning is the main problem of harvested litchi fruit, the browning index reflects the degree of litchi quality deterioration (Jiang et al., 2006). It has been shown that short-term anaerobic storage can reduce the browning index significantly, which indicates that anaerobic storage has an effective role in the control of browning and senescence in postharvest horticultural crops. For low temperature storage, increased cold storage time resulted in faster browning during room temperature storage.

3.2. ATP and energy charge (EC)

ATP content in litchi fruit pericarp declined significantly during storage at 25 °C, and a less significant decrease in ATP was observed

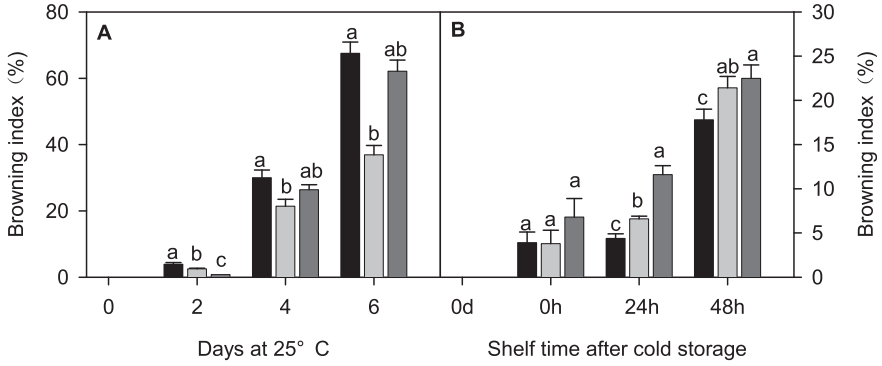

Fig. 1. The browning index of litchi fruit stored at 25 °C (A) for up to 6 days before treatment with air (control; black column), pure N_2 (grey column) and pure O_2 (dark grey column), and at 1 °C (B) for 1 week (black column), 2 weeks (grey column) and 3 weeks (dark grey column) respectively with subsequent 48 h shelf time at 25 °C. Data are the means of measurements ± standard deviation ($n = 3$).

in short-term anaerobic and pure oxygen treated fruit after a storage time of 2 days (Fig. 2A). The energy charge peaked in control and short-term anaerobic treated fruits after a storage time of 2 days. However, the energy charge in pure oxygen treated fruit was moderate and was approximately 0.80 during the entire storage period. Overall, short term anaerobic treatment maintained a higher ATP content and energy charge in litchi fruit pericarp when compared with the control. Pure oxygen treatment improved the ATP content but kept a relatively stable energy charge. Both ATP and EC levels declined under cold storage conditions (Fig. 2C and D). The ATP content increased in the first 24 h after shifting to 25 °C but declined at later time points. The energy charge increased steadily to 0.837 after 48 h at 25 °C in fruit that had been refrigerated for 1 week. In fruits that had been refrigerated for 2 or 3 weeks, the energy charge peaked after 24 h and then declined slightly to 0.821 and 0.842, respectively, after 48 h of shelf life at 25 °C.

Senescence or browning of harvested fruits and vegetables was closely related to the energy status. These results are inconsistent with previous studies that have shown that ATP contents and energy charge was negatively correlated with the aging extent of harvested apples (Saquet, Streif, & Bangerth, 2000), pears (Saquet et al., 2000; Veltman, Lentheric, Van der Plas, & Peppelenbos, 2003) and litchi (Yi et al., 2009). The mechanism could be that ATP was involved in the synthesis of unsaturated fatty acids, which determine the physical and chemical properties of the cell membrane, the integrity of the membrane and the disease resistance of the fruit (Yi et al., 2008). In addition, ATP and the energy charge may induce enzymes that are responsible for eliminating reactive oxygen species (Veltman et al., 2003). The change in the ATP content in this study was consistent with our previous studies, which showed that short-term anaerobic and pure oxygen treatments inhibited pericarp browning and maintained a higher ATP content. However, these results are inconsistent with a previously reported significant decrease in the energy charge from 0.8 to 0.2 (Duan et al., 2004). The energy charge in the current study remained constant at approximately 0.8 without fluctuating more than ±0.1. This result is consistent with results from Meyer and Wagner (1986) that suggested that the aging process was adjusted by the ATP levels and not the energy charge during tobacco leaf senescence. Azad, Ishikawa, Sawa, and Shibata (2008) commented that the ATP level was a key factor in activating apoptosis in tulips petals. This current study also showed a strong negative correlation between the ATP level and the pericarp browning index during the deterioration process of harvested litchi fruit. However, there was no obvious relationship between the energy charge and the browning index. It is quite reasonable that plants no longer require a high energy charge to promote anabolic metabolism when they approach senescence. However, ATP may serve as a signal molecule involved in other physiological manipulations.

3.3. Respiration activities

Respiratory characteristics were significantly different among different atmospheric treatments and cold storage time (Fig. 3). Respiratory intensity of the whole fruit peaked after 2 days of storage at 25 °C, and a similar pattern was observed in the climacteric ripening fruit. The highest CO_2 production was found in pure oxygen treated fruit, followed by short-term N_2 treated fruit and the control fruit, suggesting that pure oxygen and short-term N_2 exposures induce a significant enhancement in respiration (Fig. 3A). The contribution of the cytochrome pathway to the total respiration ($\rho V_{CP}/Vt$) in litchi fruit pericarp increased during the first 4 days and subsequently decreased (Fig. 3B). The contribution of the alternative pathway ($\rho V_{AP}/Vt$) demonstrated a reversed pattern, with an initial decrease followed by an increase (Fig. 3C). Both $\rho V_{CP}/Vt$ and $\rho V_{AP}/Vt$ were higher in pure oxygen treated fruit than in the control fruit, whereas $\rho V_{CP}/Vt$ was significantly lower in short-term N_2 treated fruit than in the control.

The respiration intensity of the whole fruit decreased gradually with the extension of the cold storage time, increased sharply during the first 24 h at room temperature and continued to increase slowly during the next 24 h. An increased refrigeration time resulted in a stronger respiration intensity at room temperature,

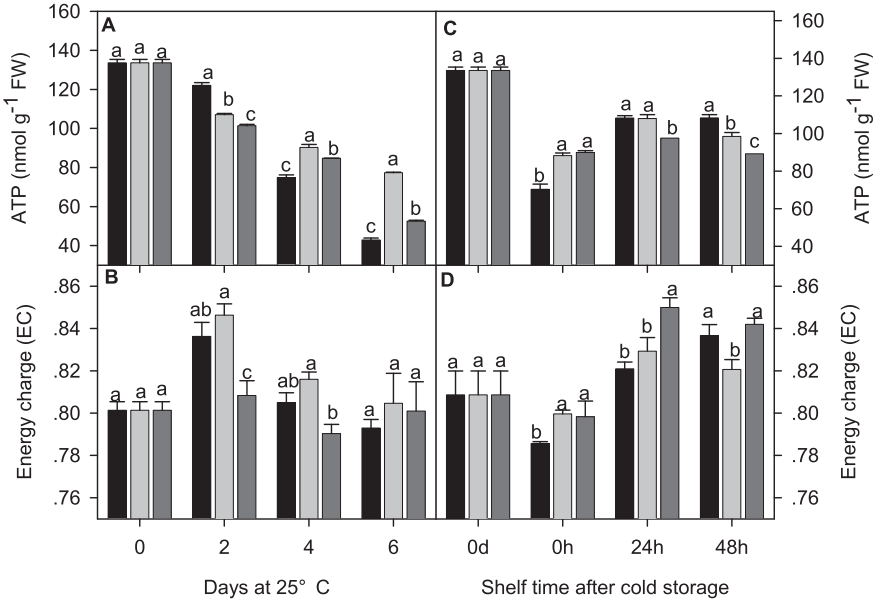

Fig. 2. The energy status of litchi fruit stored at 25 °C (A, B) for up to 6 days before treatment with air (control; black column), pure N_2 (grey column) and pure O_2 (dark grey column), and at 1 °C (C, D) for 1 week (black column), 2 weeks (grey column) and 3 weeks (dark grey column) respectively with subsequent 48 h shelf time at 25 °C. A, C, content of adenosine triphosphate (ATP) expressed as nmol/g FW. B, D, energy charge (EC). FW = fresh weight. Data are the means of measurements ± standard deviation ($n = 3$).

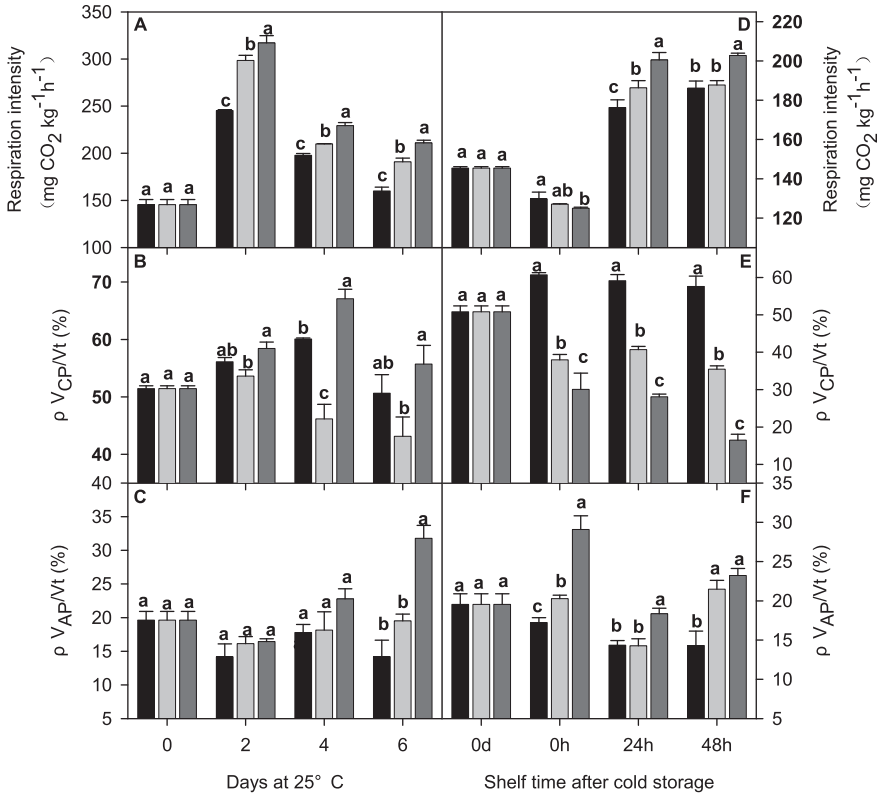

Fig. 3. The respiration of litchi for fruit stored at 25 °C (A, B, C) for up to 6 days before treatment with air (control; black column), pure N_2 (grey column) and pure O_2 (dark grey column), and at 1 °C (D, E, F) for 1 week (black column), 2 weeks (grey column) and 3 weeks (dark grey column) respectively with subsequent 48 h shelf time at 25 °C. A, D, the whole fruit respiration rate; B, E, the contributions of the cytochrome pathway to total respiration ($\rho V_{CP}/Vt$); C, F, the contributions of the alternate oxidative pathway to total respiration ($\rho V_{AP}/Vt$). Data are the means of measurements ± standard deviation ($n \geq 5$).

and the highest respiration intensity was observed in fruit that was stored at the cold temperature for 3 weeks (Fig. 3D). The $\rho V_{CP}/Vt$ value increased after 1 week of cold storage and remained at a high level at 25 °C, while the $\rho V_{AP}/Vt$ value decreased gradually during the entire investigation time (Fig. 3E and F). The $\rho V_{CP}/Vt$ value decreased and then fluctuated slightly in fruit that was stored for 2 or 3 weeks in cold storage, whereas the $\rho V_{AP}/Vt$ value peaked after cold storage but eventually increased. Fruit that was stored for 3 weeks in cold storage showed the lowest $\rho V_{CP}/Vt$ and highest $\rho V_{AP}/Vt$. This result suggests a negative correlation between $\rho V_{CP}/Vt$ and cold duration time and a positive correlation between $\rho V_{AP}/Vt$ and cold duration time.

Measures such as a controlled atmosphere (CA) and low temperature storage for maintaining the freshness of harvested fruits and vegetables are largely based on a mechanism of respiration inhibition. However, pure oxygen treatment has been reported to increase respiration and improve the quality of several harvested fruits (Kader & Ben-Yehoshua, 2000). Meanwhile, short-term anaerobic treatment has been used to delay the decay of post-harvest crops, how short-term anaerobic treatment affects the respiration has not yet been reported. In the present study, both pure oxygen and short-term N_2 treatments enhanced the respiratory intensity of harvested litchi fruit. Cold storage inhibited respiration intensity during refrigeration, but respiration increased rapidly at room temperature after extended cold storage. This intense increase in respiration is unfavourable for the preservation of fruit and vegetables because it consumes more nutrients. However, respiration aids in the transformation of substances. Duan et al. (2004) suggested that pure oxygen treatment increases respiration and then generates more ATP, which is beneficial for maintaining the integrity of the cell membrane and delays browning. A positive correlation between respiration intensity and the ATP content in fruits that were stored at both room temperature and low temperature was observed, which is in agreement with the hypothesis.

If a pair of electrons are oxidised by the cytochrome pathway on the respiratory chain, 2 or 3 molecules of ATP will be produced, but only 1 molecule of ATP is produced by an alternate oxidative pathway. Alternate oxidation can occur before cytochrome oxidation is saturated, which means that the operation of alternate oxidation will reduce the efficiency of energy generation (Millar et al., 2011). In the present study, the respiratory intensity and the $\rho V_{CP}/Vt$ value were higher in fruits treated with pure oxygen; however, the ATP content and energy charge were lower in these fruits than in those subjected to short-term anaerobic conditions. This result is most likely caused by the high contribution of alternate oxidation. We speculate that the alternate oxidation pathway plays a physiological role to balance the generation of reactive oxygen species and energy. We demonstrated in the present study that pure oxygen and anaerobic treatment simultaneously enhanced respiration intensity and increased $\rho V_{AP}/Vt$. If a high ATP level is necessary to suspend the aging process of harvested fruits and vegetables, then the crops must limit the metabolism that consumes ATP to maintain a steady ATP level; otherwise, the plants will require a reasonable respiratory rate to ensure a steady ATP supply. In this case, the corresponding increase of the alternate pathway can adjust the generation of the reactive oxygen species on the busy mitochondria respiratory chain.

3.4. Gene expression

3.4.1. Gene expression in fruit stored at room temperature under short-term anaerobic and pure oxygen conditions

LcAtpB gene expression increased significantly after harvest, and storage under both short-term anaerobic and pure oxygen conditions blocked the expression of *LcAtpB* at room temperature (Fig. 4A). *LcAOX1* expression increased steadily after harvest but increased dramatically in pure oxygen treated fruit and peaked under short-term anaerobic conditions after 2 days of storage. Both short-term anaerobic and pure oxygen treatments promoted transcription of *LcAOX1* (Fig. 4B). There was no significant change in the transcription of *LcUCP1* during storage at 25 °C with no treatment, but both short-term anaerobic and pure oxygen treatments increased transcription of *LcUCP1* (Fig. 4C). *LcAAC1* rose significantly after harvest, was downregulated under short-term

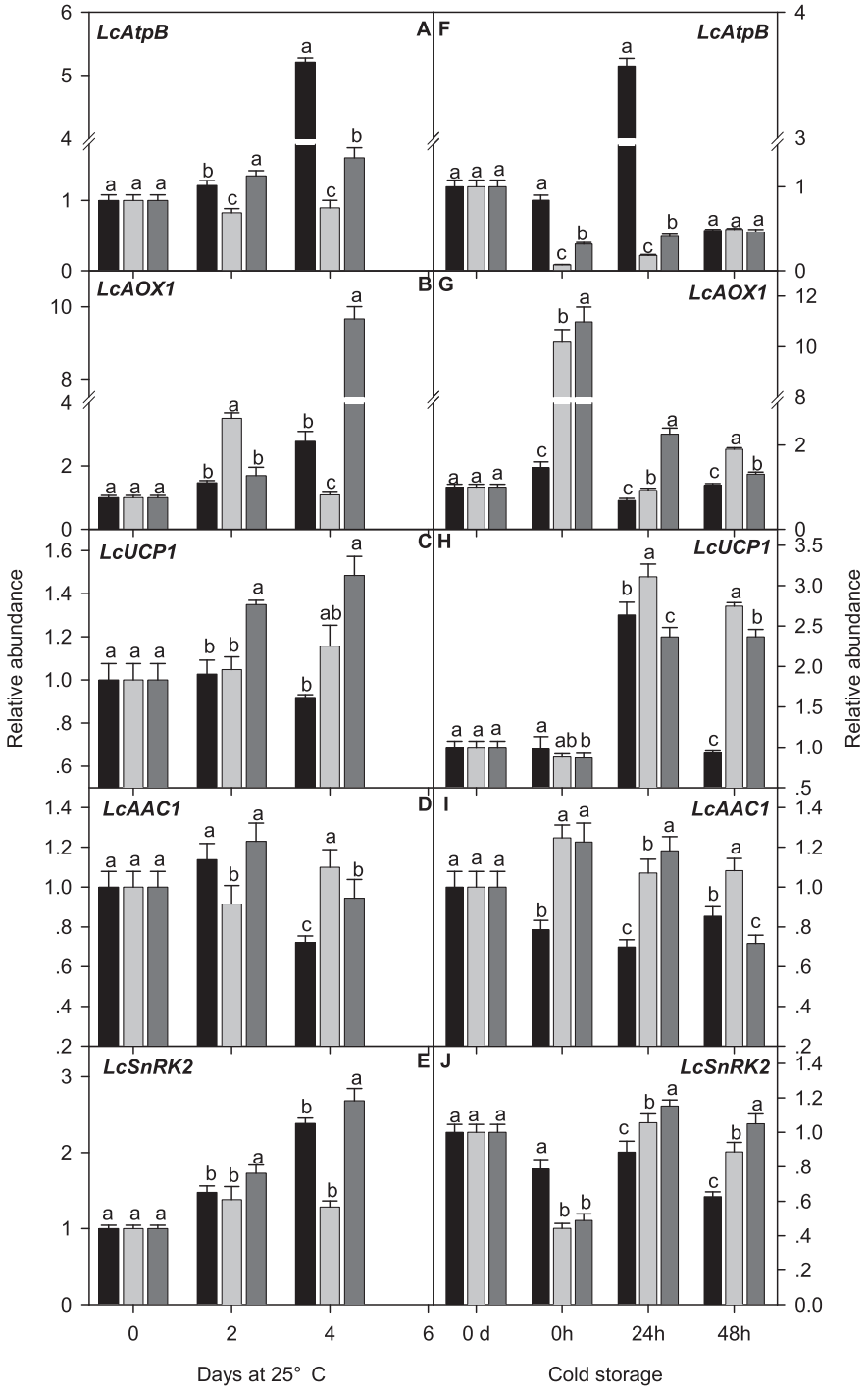

Fig. 4. Relative abundance of *LcAtpB*, *LcAOX1*, *LcUCP1*, *LcAAC1* and *LcSnRK2* in litchi fruit stored at 25 °C (A, B, C, D, E) for up to 6 days before treatment with air (control; black column), pure N_2 (grey column) and pure O_2 (dark grey column), and at 1 °C (F, G, H, I, J) for 1 week (black column), 2 weeks (grey column) and 3 weeks (dark grey column) respectively with subsequent 48 h shelf time at 25 °C. Gene expression was determined using qRT-PCR and was normalised using *LcACTIN*. Data are the means of measurements ± standard deviation ($n = 3$).

anaerobic conditions during the first 3 days of storage but was upregulated during the entire storage time in pure oxygen (Fig. 4D). Transcription of *LcSnRK2* increased significantly after harvest. It was upregulated by pure oxygen but downregulated by short-term anaerobic treatment (Fig. 4E).

3.4.2. Gene expression during cold storage and post cold storage shelf-life under ambient conditions

LcAtpB gene expression decreased after 1 week of cold storage and significantly increased at room temperature, peaking at 24 h after cold storage. *LcAtpB* increased less significantly at room temperature after 2 and 3 weeks of cold storage (Fig. 4F). Transcription of *LcAOX1* significantly increased with the extension of cold storage time (Fig. 4G) especially after 2 weeks cold storage, decreased during the first 24 h at room temperature, and increased in the next 24 h. fruit stored for 3 weeks in cold storage showed a similar gene expression pattern, except the final increase in gene expression was not observed. There was no significant change in *LcUCP1* expression in the fruit during cold storage until the fruit was moved to room temperature. Then, this gene was upregulated significantly after 24 h at room temperature and downregulated during the next 24 h. *LcAAC1* transcription decreased during the first 24 h at room temperature after 1 week of cold storage, while 2 or 3 weeks of cold storage upregulated the transcription significantly during the post-cold storage shelf-life. The transcription of *LcSnRK2* decreased after 1 week of cold storage, slightly increased during the first 24 h at room temperature, and then decreased again. Similar but more robust gene expression patterns were observed in fruit stored for 2 and 3 week in cold storage.

AtpB has been reported to be a cell death regulator and pro-cell death protein (Chivasa et al., 2011). The dramatic increase of *LcAtpB* gene expression after 4 days at room temperature and 24 h of shelf-life under ambient conditions after 1 week of cold storage marked the rapid senescence of litchi fruit, which is consistent with the changes of browning index (Fig. 1A and B). Short-term anaerobic, pure oxygen and cold storage postponed the senescence of litchi fruit, most likely by restraining the transcription of *LcAtpB* (Fig. 4A and F).

Energy dissipation systems play important roles in the development, maturity and aging process of fruits and vegetables. Moreover, it is a challenge to balance the energy and active oxygen metabolism to control the aging and quality deterioration process, which is essential for maintaining cellular homoeostasis. Increased cyanide-resistant respiration during cold storage may reduce the production of hydrogen peroxide and increase the antioxidant enzyme activity (Gualanduzzi et al., 2009). The transcription abundance of *AOX* and *UCP* increased gradually as tomatoes turn red on the mother plants and decreased during later aging phases (Almeida et al., 2002; Navet, Jarmuszkiewicz, Almeida, Sluse-Goffart, & Sluse, 2003). Considine et al. (2001) demonstrated the upregulation of both *AOX* and *UCP* after the climacteric ripening phase in mangoes. While these previous studies focused on expression pattern of *AOX* and *UCP* in climactic fruits, the present investigation attempted to investigate the role of these genes in non-climactic fruits from the aspect of energy metabolism. The above results showed that expression levels of *LcAOX1* were consistent with the contribution of alternate oxidation to the total respiration. The high expression level of this gene may result in a low energy level. The investigation of fruit stored in cold conditions showed that *LcUCP1* transcription started to increase after fruits were moved to room temperature. *LcUCP1* expression increased at room temperature and was upregulated by short-term anaerobic and pure oxygen treatments (Fig. 4C), which may have contributed to a faster decrease in the ATP level during storage (Fig. 2A) and was negatively correlated with the energy level. The higher expression level of *LcUCP1* led to increased operation of the uncoupling pathway to ensure the main use of proton electrochemical potential across the mitochondrial membrane for the synthesis of ATP. The respiration intensity of the cold stored litchi fruits increased sharply with storage time during the post cold storage shelf life at room temperature, which was consistent with the extent of deterioration (Fig. 1B). It was suggested that the higher level of *LcUCP1* transcription may induce the UCP pathway and respiration. However, the increased respiration did not increase the energy level, which could be attributed to the transcription of *LcUCP1* and *LcAOX1*. Excessive activity of these two energy dissipation genes could result in a low energy generation efficiency, which could then accelerate the deterioration of quality.

ADP/ATP carrier (AAC) is the core protein of the mitochondrial adenine nucleotide translocator and the main regulator of mitochondrial ATP concentration in higher organisms. ATP synthesis was inhibited in the 'Arg triplet' AAC signature sequence mutated plants (Klingenberg, 2008). In this study, the transcription of *LcAAC1* peaked after 2 days in fruit stored at room temperature and was lowest during post cold storage shelf-life at room temperature (Fig. 4D and I). Pure oxygen and prolonged cold storage enhanced *LcAAC1* accumulation, which could be attributed to stimulated respiration intensity, as shown in Fig. 3A and D.

SnRK can sense ATP deficiency and trigger downstream transcription of key metabolic enzymes to regulate the energy level in the cell (Baena-Gonzalez, 2010). In this study, the expression level of *LcSnRK2* increased significantly during storage at room temperature and during 24 h post cold storage shelf-life at room temperature (Fig. 4E and J). Furthermore, this gene was upregulated during 4 days of storage in pure oxygen and during room temperature storage after prolonged low temperature storage. This upregulation of *LcSnRK2* was most likely stimulated by decreasing ATP content in the fruit tissue (Fig. 2A and C).

4. Conclusion

The senescence process of the litchi fruit was closely related to the energy status and the expression levels of related genes. Storage under short-term anaerobic and pure oxygen conditions extended the storage life of the litchi fruit, which was attributed to the maintenance of a higher ATP level. Pure oxygen treatment stimulated respiration intensity and upregulated the expression of all five genes except for *LcAtpB*. Refrigerated storage downregulated the expression of all five genes except for *LcAOX1*. *LcAtpB* acted as a marker of senescence. Treatments that downregulated the expression of this gene could postpone the senescence process of litchi fruit. *LcAtpB* might be a good candidate gene for breeding new litchi cultivars with increased ATP supplies and longer storage lives.

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