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Cold storage duration affects litchi fruit quality, membrane permeability, enzyme activities and energy charge during shelf time at ambient temperature

Hai Liu[a,b], Lili Song[b], Yanli You[b], Yuebiao Li[b], Xuewu Duan[b], Yueming Jiang[b,*], Daryl C. Joyce[c], Muhammad Ashraf[d], Wangjin Lu[e]

[a] Modern Biochemistry Center, Department of Experimental Teaching, Guangdong Ocean University, Zhanjiang 524088, PR China
[b] South China Botanical Garden, Chinese Academy of Science, Guangzhou 510650, PR China
[c] School of Land, Crop and Food Sciences, The University of Queensland, Gatton, Qld 4343, Australia
[d] Department of Botany, Faculty of Sciences, University of Agriculture, Faisalabad 38040, Pakistan
[e] Guangdong Key Laboratory for Postharvest Science, College of Horticultural Science, South China Agricultural University, Guangzhou 510642, PR China

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ABSTRACT

Storage and transport of litchi fruit at low temperature is widely practiced commercially. In this study, no pericarp browning was evident at out-turn on litchi fruit stored for 10 and 20 days at cold temperature of 3–5 °C, but fruit stored for 10 days at 3–5 °C gradually browned after 12 h of shelf time at ambient temperature of 25 °C, with the browning index (BI) being elevated to 2.5 at 24 h of shelf life. Furthermore, fruit stored for 30 days began to rot and had a decay incidence of about 30% after 24 h on the shelf. The temperature increment from 3–5 to 25 °C induced marked increases in activities of lipase, phospholipase D (PLD) and lipoxygenase (LOX). Litchi fruit stored for 10 days at 3–5 °C followed by the shelf time at 25 °C had lower activities of lipase, PLD and LOX, and also lower levels of membrane permeability, than did fruit stored for 20 and 30 days. Energy level of the pericarp tissue of cold-stored litchi fruit was similarly dependent on storage time at 3–5 °C plus shelf time at 25 °C. Adenosine triphosphate (ATP) content and adenylate energy charge (AEC) level in pericarp tissues decreased as cold storage progressed. However, ATP and adenosine diphosphate (ADP) contents, and the AEC levels, in cold-stored litchi fruit increased during shelf time at 25 °C, reaching a peak after 6 h, and then decreasing. Fruit stored for 30 days at 3–5 °C had much lower ATP content and AEC level than fruit stored for 10 and 20 days. Increased activities of lipase, PLD and LOX, and energy shortage in cold-stored pericarp tissues during subsequent shelf time at 25 °C suggest that the deterioration of membrane integrity and loss of compartmentation gives rise to accelerated browning and fruit quality deterioration.

1. Introduction

Litchi (*Litchi chinensis* Sonn.) is a subtropical to tropical fruit of strong commercial value on international markets for its bright red skin and sweet, juicy and crisp aril (Holcroft and Mitcham, 1996; Jiang et al., 2006). Production has risen steadily, with increasing exports from both southern and northern hemisphere production centres into North America and Europe (Huang et al., 2005). Most litchi fruit produced in Asia are marketed locally, with some regional exports to other Asian centres, plus lesser exports to Europe and the Arab States (Jiang et al., 2006). Thus, expansion of markets is likely with further development of postharvest handling.

Litchi fruit are highly perishable with a very short postharvest life under ambient conditions. Usually, fruit export can take 1–2 weeks from producer to consumer, which necessitates cold storage. The major quality issues affecting litchi are pericarp browning and fruit decay (Jiang et al., 2006; Sivakumar et al., 2010). Because cold storage is effective, litchi fruit can have a storage life of around 30 days at 3–5 °C (Jiang et al., 2003). Nonetheless, fruit quality can gradually decline during low temperature storage and then can rapidly decline when fruit are subsequently removed to shelf life conditions at ambient temperature (Jiang et al., 2003). Pericarp browning of litchi fruit after harvest is considered due to loss of cellular compartmentalization, where mixing of oxidative enzymes and phenolic substrates yields brown colored products (Zhang et al., 2001; Jiang et al., 2006). Accordingly, membrane integrity plays a pivotal role in pericarp browning of litchi fruit. Changes in membrane fatty acid (FA) synthesis to alter membrane properties may result in cellular de-compartmentalization and ion leakage (Marangoni et al., 1996), whereas energy is essential for maintenance of membrane integrity, and involvement of adenylate nucleotides in lipid metabolism in plants is established (Pradet and Raymond, 1983; Ohlrogge and Browse, 1995). Under anoxia, the depletion of adenosine triphosphate (ATP) leads to reduced

* Corresponding author. Tel.: +86 20 37252525; fax: +86 20 37252831.
E-mail address: ymjiang@scib.ac.cn (Y. Jiang).

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lipid synthesis and diminishes desaturation of acyl chains (Brown and Beevers, 1987; Rawyler et al., 1999). For 'Conference' pear fruit stored under delayed controlled atmospheric conditions, the inhibition of flesh browning has been associated with maintenance of high ATP and adenylate energy charge (AEC) levels (Saquet et al., 2003). Internal browning in pear fruit was suggested by Veltman et al. (2003) and Saquet et al. (2000, 2003) to be the result of limited energy availability and cellular de-compartmentalization. Lower levels of browning and less ion leakage from pure oxygen treated litchi pericarp was correlated with higher ATP and adenosine diphosphate (ADP) levels but was not related to adenosine monophosphate (AMP) content (Duan et al., 2004).

Lipase and phospholipase D (PLD) are important enzymes involved in membrane phospholipid hydrolysis (Paliyath et al., 1987). Burger et al. (1986) reported that lipase and PLD activities increased as cut carnation flowers senesced. Lipoxygenase (LOX) catalyses peroxidation of plasma membrane lipids, increases lipid unsaturation, and thus changes membrane fluidity (Wang, 2001), with direct consequences for membrane integrity and membrane permeability. PLD and LOX are proposed to initiate lipolytic cascades in membrane deterioration during senescence and stress (Paliyath and Thompson, 1987; Paliyath and Droillard, 1992). Increases in membrane associated PLD and LOX have been observed in response to exposure to chilling stress in maize (Pinhero et al., 1998).

With regard to harvested litchi fruit, it is important to discern potential roles of lipid degrading enzymes in association with energy status and quality loss of fruit upon transfer from low temperature storage to ambient temperature shelf life conditions. The specific objective was to understand effects of cold storage periods of 10, 20 and 30 days at 3–5 °C on subsequent shelf life and quality in association with biochemical changes for litchi fruit at 25 °C.

2. Materials and methods

2.1. Plant materials and treatments

Litchi (*Litchi chinensis* Sonn. cv. Huaizhi) fruit at the commercially ripe stage were harvested from a farm in Guangzhou, P. R. China. Blemish and disease free fruit were selected for uniformity of color and shape. A total of 1350 fruit was used in three groups of 450. All fruit were dipped in 0.1% TBZ (thiabendazole, Decco Chemicals, USA) for 3 min and air-dried for 1 h at 25 °C. They were then packed in 0.03 mm thick polyethylene bags (30 fruit per bag) closed with rubber bands. The bagged fruit were stored for 10, 20 and 30 days at 3–5 °C. After their respective storage times, the fruit were removed from the bags and then held for 24 h in a controlled revaluation room at 25 °C and 80–90% RH. Sub-samples were taken for quality evaluation and analyses initially and at 6 h intervals during the shelf time. Pericarp tissues were immediately frozen in liquid nitrogen, crushed and stored at −70 °C until use. Replication with 30 fruit was three-fold ($n = 3$) for each time at 25 °C.

2.2. Quality evaluation

Fruit pulp (aril) quality and other sensory characters were evaluated by 5 trained personnel by the method of Gorny et al. (2002). Specific pulp taste and color scales were conducted by the method of Jiang et al. (2003). For fruit which had been just peeled, the hedonic scale applied for pulp was: 9, excellent (full taste, white and translucent pulp); 7, very good (pleasant mild taste, white and translucent pulp); 5, good (bland taste, white less translucent pulp; lower limit of marketability); 3, fair (faint off odor, white-brown non-translucent pulp, lower limit of acceptability); or 1, poor (distinct off odor, brown non-translucent pulp).

2.3. Pericarp browning

Skin appearance was assessed as the extent of the total browned area on each pericarp for 30 fruit, comprising 10 fruit collected randomly from each of three bags. The browning index (BI) scale was: 1 = no browning (excellent quality); 2 = slight browning; $3 \leq 1/4$ browning; 4 = 1/4–1/2 browning, and, $5 \geq 1/2$ browning (poor quality). BI was calculated as: $\sum$(browning score × proportion (%) of fruit within each class). Fruit with a BI higher than 3.0 were considered unacceptable for marketing (Jiang, 2000).

2.4. Decay

Decayed fruit resulting from natural infection was assessed by observing visible fungal or bacterial growth on the fruit surface. Decay incidence (DI) was scored on a 1–5 scale where 1 = none, 2 = slight (up to 5% surface affected), 3 = moderate (5–20% surface affected), 4 = moderately severe (20–50% surface affected), and, 5 = extreme (>50% surface affected). Decay incidence was calculated as $100 \times \sum$(decay score × fruit within each class)/(total fruit × the highest score).

2.5. Total soluble solids, titratable acidity and ascorbic acid concentrations

Total soluble solids (TSS), titratable acidity (TA) and ascorbic acid concentrations of litchi fruit were analysed during shelf life time at 0, 6, 12, 18, and 24 h. Pulp (20 g) from 15 fruit was homogenised in a grinder and centrifuged at 15,000 × *g* (Beckman J20-2, Palo Alto, USA) for 20 min. The supernatant was used for measuring TSS with a hand refractometer (J1-3A, Guangdong Scientific Instruments, Guangzhou, China) and TA and ascorbic acid by titration with 0.1 M NaOH and 2,6-dichlorophenol indophenol, respectively (Chen et al., 1986).

2.6. Determination of membrane permeability

Membrane permeability was determined on pericarp discs removed with a 5 mm diameter cork borer from the equatorial region of 30 fruit. Fifty discs were rinsed twice in distilled water and then incubated in 30 mL 0.3 M mannitol solution at 25 °C with shaking for 30 min. Electrolyte leakage was measured with a conductivity meter (Model DDS-11A, Shanghai Scientific Instruments, Shanghai, China). Total electrolyte leakage was determined after boiling a duplicate batch of 50 discs for 30 min and then cooling to 25 °C. Relative leakage was expressed as a proportion (%) of total electrolyte leakage.

2.7. Determinations of PLD, LOX and lipase activities

PLD activity was determined by the method of Suttle and Kends (1980). Five grams of fruit pericarp tissues was finely ground in liquid nitrogen and then extracted with 20 mL of 0.1 M sodium acetate buffer (pH 5.6) containing 1% (w/v) polyvinylpyrrolidone. The homogenate was centrifuged at 10,000 × *g* and 4 °C for 20 min and the supernatant used for assaying PLD activity. To prepare the substrate, 40 mg of 1,3-phosphatidyl choline was dissolved in 50 mL of ether and then the mixture dried under a stream of N_2 followed by the addition of 0.1 M sodium acetate buffer (pH 5.6) containing 5 mM DTT and 1 M $CaCl_2$ to obtain 0.4 mg/mL substrate solution. The reaction mixture contained 1 mL of enzyme solution and 3 mL of 0.4 mg/mL prepared substrate. The control contained 1 mL of 0.1 M sodium acetate buffer and 3 mL of 0.4 mg/mL prepared substrate. The enzymatic reaction were performed for 1 h at 28 °C using a shaker and then washed by an addition of petroleum ether for three times. The water phase was collected and followed by

the addition of 3 mL of 1% ammonium reineckete. After centrifugation at 12,000 × *g* for 15 min, the sediment was fully dissolved in propanone and the absorbance of the mixture was measured at 520 nm using a spectrophotometer (UV-2800, Shanghai Spectrotech Instruments Co., Ltd, Shanghai, China). One of unit (U) of PLD activity was defined as a change of 0.001 in absorbance at 520 nm per h. The specific PLD activity was expressed as U/mg protein.

For analysis of LOX activity, fruit pericarp tissues (4.0 g) from eight fruit were ground to a powder in liquid nitrogen and then homogenised with 12 mL of 50 mM potassium phosphate buffer (pH 7.0) containing 1% (w/v) polyvinylpyrrolidone. After centrifugation for 15 min at 10,000 × *g* and 4 °C, the supernatant was collected and then used as the crude enzyme extract. LOX activity was assayed at 25 °C by monitoring the formation of conjugated dienes from linoleic acid at 234 nm by the method of Axelrod et al. (1981). The reaction mixture contained 2.8 mL of 0.05 M sodium phosphate buffer (pH 7.0), 0.1 mL of 0.01 M sodium linoleic acid solution and 0.1 mL enzyme solution. The reaction mixture without enzyme solution was used as a control. The increase in absorbance per minute at 234 nm was recorded. One unit of LOX activity was defined as a change of 0.001 in absorbance per minute at 234 nm. The specific LOX activity was expressed as U/mg protein.

Lipase activity was assayed spectrophotometrically after hydrolysing α-naphthyl acetate according to the method of Aspern (1962). Litchi pericarp tissues (4.0 g) from eight fruit were ground to a fine powder and extracted with 20 mL of 0.2 M phosphate buffer (pH 7.8) containing 0.05 M mercaptoethanol. After centrifugation for 15 min at 10,000 × *g* and 4 °C, the supernatant was collected and then used as the crude enzyme extract. The reaction mixture consisted of 2.3 mL of 0.2 M phosphate buffer (pH 7.8), 0.5 mL of 0.2 mM α-naphthyl acetate solution and 0.2 mL enzyme solution. The control contained 2.5 mL of 0.2 M phosphate buffer (pH 7.8) and 0.5 mL of 0.2 mM α-naphthyl acetate solution. The mixture was incubated for 30 min at 25 °C, followed by the addition of 0.15% Fast Blue B. One unit of lipase activity was defined as the amount that caused a change of 0.001 in absorbance at 520 nm per minute. The specific lipase activity was expressed as U/mg protein.

Protein content was estimated by the method of Bradford (1976), with bovine serum albumin as the standard protein using a calibration curve at 10, 20, 30, 40, 50 and 60 μg/mL against the OD_{595} values.

2.8. ATP, ADP and AMP analyses

ATP, ADP and AMP contents were determined by the method of Liu et al. (2006). Pericarp tissue (3.0 g) from 30 fruit was frozen in liquid nitrogen and then homogenised to a powder. Adenosine phosphates were extracted with 10 mL of 0.6 M perchloric acid for 1 min in an ice bath. The extraction mixture was centrifuged at 4 °C for 10 min at 6000 × *g* (Beckman J20-2 centrifuge, Beckman Instruments Inc., Fullerton, CA, USA). A 6 mL aliquot of the supernatant was quickly neutralised (pH 6.5–6.8) with 1 M KOH. The neutralized supernatant was allowed to stand for 30 min in an ice bath to precipitate most of the potassium perchlorate, which was removed by paper filtration. The solution was filtered again through a 0.45 μm filter and then used for ATP, ADP and AMP measurements. Separation and identification of ATP, ADP and AMP was conducted by high performance liquid chromatography (HPLC) (Gold 125 Solvent System, Beckman Instruments Inc.). The HPLC was equipped with an Ultrasphere ODS EC 250 mm × 4.60 mm column and a Beckman 125 pump system. HPLC separation was achieved using continuous gradient elution by the method of Liu et al. (2006). Peaks were detected at 254 nm using a Gold 168 diode array detector. ATP, ADP and AMP in litchi fruit samples were identified by comparison with retention times of standards. Amounts of ATP, ADP and AMP were determined using the external standard method (Liu et al., 2006). The adenylate energy charge was calculated as: ([ATP] + 0.5 × [ADP]) × 100/([ATP] + [ADP] + [AMP]).

2.9. Data handling

A completely randomised design with three replicates was adopted. Data are presented graphically as means ± standard errors and then analysed by SPSS Version 7.5 (SPSS Inc., Chicago, IL, USA). The Tukey's multiple comparisons were carried out to examine any significant difference between the means at the 5% level.

3. Results and discussion

3.1. Effects of cold storage time on visual acceptance of litchi fruit during shelf time

Acceptance of cold-stored litchi fruit generally declined as storage time at low temperature or shelf time at 25 °C progressed (Table 1). Acceptance of fruit stored for 10 days at 3–5 °C decreased slowly over 24 h of shelf time, being 100% within 12 h. However, for fruit cold-stored for 20 days, a marked drop in acceptance was discerned after 6 h of shelf time, and acceptance was only 41.7% after 12 h at 25 °C. Acceptance of litchi fruit cold stored for 30 days rapidly decreased from 67.4% to 0% over shelf time at 25 °C. After 6 h of shelf time, fruit stored for 20 and 30 days had significantly lower acceptance than litchi fruit for 10 days. Reduced acceptance of cold stored litchi fruit with both increasing storage time at low temperature and shelf life at 25 °C could be due to fruit senescence (Jiang et al., 2006).

3.2. Effects of cold storage time on pericarp browning and decay of litchi fruit during shelf time

Pericarp browning occurred to varying degrees during the 24 h of shelf time after fruit were stored for different durations at low temperature and transferred to 25 °C (Fig. 1A). Litchi fruit cold-stored for 10 days were bright red and showed no browning of the fruit surface within the first 12 h of shelf time. The skin BI gradually increased over the latter stage of shelf time, being 2.4 by 24 h. The BI of fruit cold stored for 20 days gradually increased and reached a higher level than for fruit stored for 10 days, the BI being about 4 by 24 h of shelf time. However, no disease developed on fruit during this period (Fig. 1B). When duration of storage at 3–5 °C was extended to 30 days, some fruit had browned in storage, with a BI of 2.2, but the flesh was still of acceptable eating quality. Upon removal from cold storage, skin BI increased rapidly and seriously to 4.5 by the end of shelf time. Furthermore, a few fruit cold-stored for 30 days showed rots within the first 6 h of shelf time. The DI of these fruit increased after 6 h to be 30% by the end of shelf time (Fig. 1B).

3.3. Effects of cold storage time on eating quality of litchi fruit pulp during shelf time

Eating quality of the pulp of cold stored litchi fruit decreased as storage time at low temperature and shelf time at 25 °C increased (Fig. 1C). There were no changes in eating quality of litchi fruit cold stored for 10 days during subsequent shelf time at 25 °C. However, eating quality of fruit cold stored for 20 days declined slightly during shelf time, and that of fruit stored for 30 days at low temperature decreased rapidly during shelf time, and they were not acceptable after 12 h.

Table 1
Effects of cold storage at 3–5 °C on acceptance (%) of litchi fruit during subsequent shelf time at 25 °C.

Cold storage time (d)	Shelf time (h)				
	0	6	12	18	24
10	100 ± 0.0	100 ± 0.0	100 ± 0.0	60 ± 2.9	44.7 ± 1.5
20	100 ± 0.0	70 ± 4.5	41.7 ± 1.4	25 ± 2.3	15 ± 1.2
30	67.4 ± 5.9	47.5 ± 2.0	15.1 ± 1.4	12.4 ± 0.9	0.0 ± 0.0

Data were expressed as means ± standard errors ($n = 3$).

3.4. Changes in contents of ascorbic acid, TSS and TA in pulp of cold stored litchi fruit during shelf time

Ascorbic acid, TSS and TA are important flavor and nutritional factors in litchi fruit (Jiang et al., 2003). Initial values of ascorbic acid contents in pulp of fruit after storage for 10 and 20 days at 3–5 °C were about 23.7 and 20.7 mg/100 g, respectively. The ascorbic acid content in fruit cold-stored for 30 days was 14.9 mg/100 g, this being just 60% of that in fruit cold-stored for 10 days (Fig. 1D). Thus, the ascorbic acid content decreased with prolonged storage time at low temperature. The fruit stored for 30 days consequently had much lower ascorbic acid content over shelf time at 25 °C than fruit cold stored for 10 or 20 days. TA and TSS of litchi fruit pulp generally decreased during shelf time (Fig. 1E, F). The TA in cold-stored fruit rapidly decreased by 6 h of shelf time and slowly decreased thereafter at 25 °C. Initial TA contents in fruit cold stored for 10, 20 and 30 days at low temperature were 0.296%, 0.278% and 0.259%, respectively, during shelf time. Thus, TA content tended to decline with extended storage duration (Fig. 1F). Fruit cold stored for 30 days had clearly lower TSS and TA contents than those stored for 10 and 20 days (Fig. 1E and F). These effects were consistent with the decline in eating quality (Fig. 1C). Earlier studies also showed that the major problems associated with long-term low temperature storage of litchi fruit include losses of flavor and texture, rapid pericarp browning, and fruit rotting upon transfer to room temperature (Tongdee and Subhadrabandhu, 1992; Jiang et al., 2003).

3.5. Changes in membrane permeability of cold stored litchi fruit pericarp during shelf time

It has been established that pericarp browning of harvested litchi fruit is due to breakdown of cellular compartmentalization as evidenced by high leakage of electrolytes via increased membrane permeability (Jiang et al., 2003, 2006). Relative leakage rates from fruit stored for 10, 20 and 30 days at 3–5 °C were 7.5%, 10.0% and 15.4%, respectively, upon removal from cold storage (Fig. 2A), clearly showing that the membrane permeability of litchi pericarp tissue increased as storage time progressed. However, there were no significant subsequent changes in relative leakage rate from fruit pericarp tissue stored for 10 and 20 days in the first 12 h of shelf time at 25 °C but then there was a slight increase to 24 h of shelf time. In association with rapid pericarp browning and quality loss, relative

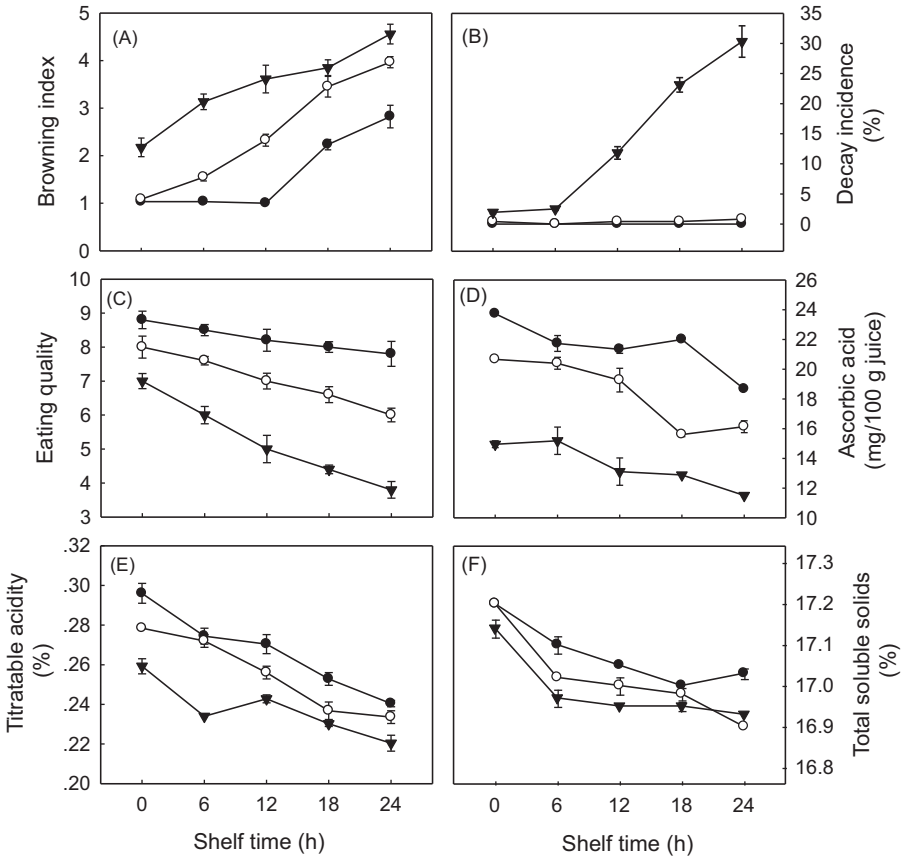

Fig. 1. Changes in pericarp BI (A), DI (B), eating quality (C) and contents of ascorbic acid (D), TA (E) and TSS (F) for cold-stored litchi fruit during subsequent 24 h shelf time at 25 °C. Fruit were stored for 10 (●), 20 (○) and 30 (▼) days at 3–5 °C and then held for 24 h at 25 °C. Each value is the means for three replicates and vertical bars indicate the standard errors where they exceed the symbol size.

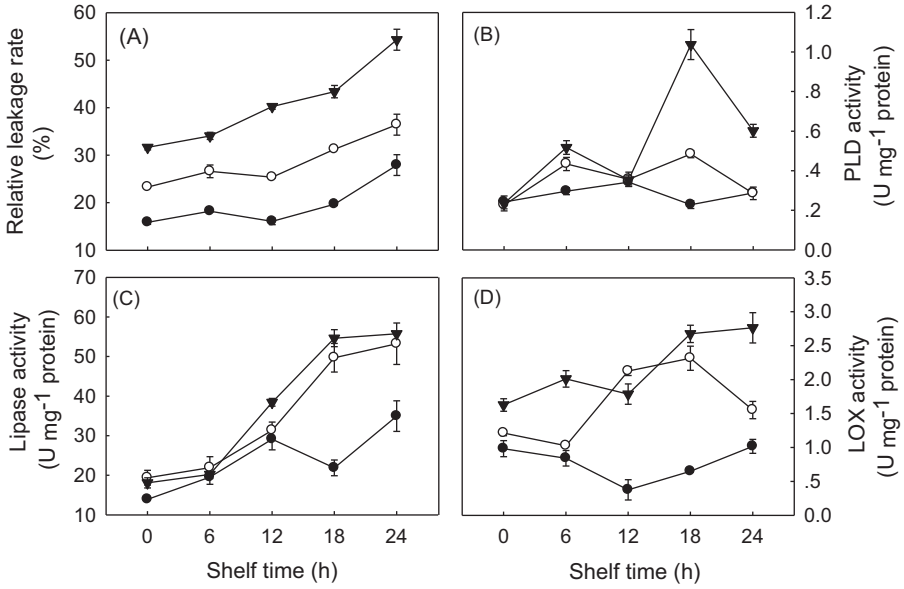

Fig. 2. Changes of relative electrolyte leakage rate (A) and activities of PLD (B), lipase (C), and LOX (D) for cold-stored litchi fruit during subsequent 24 h shelf time at 25 °C. Fruit were stored for 10 (●), 20 (○) and 30 (▼) days at 3–5 °C and then held for 24 h at 25 °C. Each value is the means for three replicates and vertical bars indicate the standard errors where they exceed the symbol size.

leakage from fruit cold stored for 30 days increased significantly ($p \leq 0.05$) during shelf time to reach about 56.7% in the end. Overall, quality deterioration of cold stored litchi fruit was positively correlated with cell membrane damage ($r = 0.9303$; $p \leq 0.05$).

3.6. Changes of PLD, LOX and lipase activities in cold-stored litchi fruit pericarp during shelf time

PLD, LOX and lipase activities are involved in plant senescence and stress through membrane alterations and lipid degradation associated with senescence, injury, drought and chilling (Chapman, 1998; Wang, 2001; Mao et al., 2007). In this study, there was no significant ($p \leq 0.05$) change in PLD activity throughout 24 h of shelf time in litchi fruit cold-stored for 10 days (Fig. 2B), but the enzyme activity in fruit cold stored for 20 and 30 days exhibited increasing trends during shelf time. PLD activity in fruit cold-stored for 30 days increased notably after 12 h of shelf time to a maximum, followed by a decrease and maintenance at a level still significantly higher than for fruit cold-stored for 10 and 20 days (Fig. 2B). This tendency was in general agreement with observed higher levels of relative leakage rate. Similarly, Ruelland et al. (2002) found that PLD activity was rapidly activated and that phosphatidic acid content increased after *Arabidopsis* suspension cells cultivated at low temperature was transferred to ambient temperature.

After cold-stored fruit were transferred from low temperature, lipase activities in fruit stored for 10, 20 and 30 days exhibited similar increasing trends within 12 h of shelf time. However, for fruit cold stored for 10 days, lipase activity stayed relatively constant at a lower level in the latter stage of shelf time than for fruit cold-stored for 20 and 30 days (Fig. 2C).

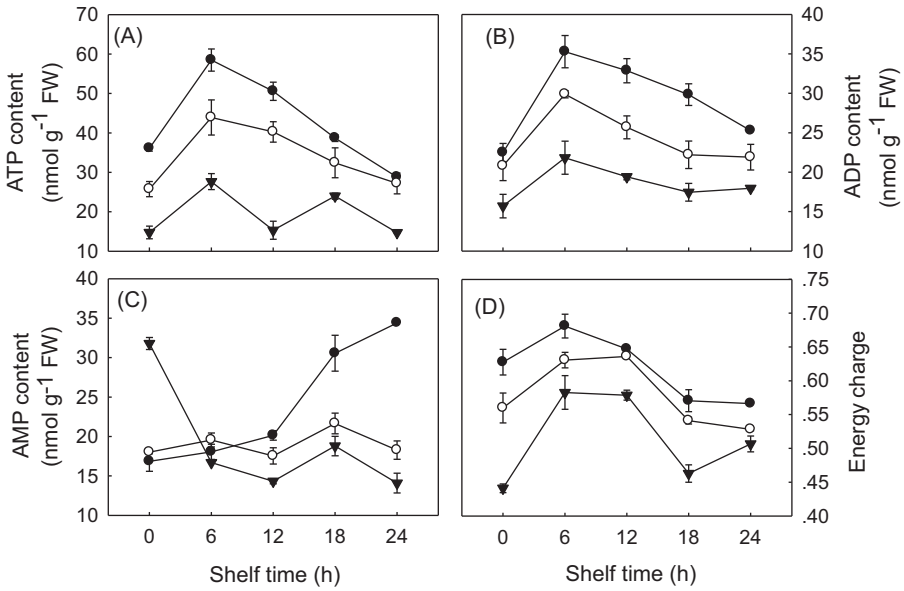

Fig. 3. Changes of ATP (A), ADP (B) and AMP (C) contents and AEC (D) for cold-stored litchi fruit during subsequent 24 h shelf time at 25 °C. Fruit were stored for 10 (●), 20 (○) and 30 (▼) days at 3–5 °C and then held for 24 h at 25 °C. Each value is the means for three replicates and vertical bars indicate the standard errors where they exceed the symbol size.

LOX activity in litchi fruit increased with extension of storage time at low temperature (Fig. 2D). After fruit were transferred to 25 °C, there was no change in LOX activity throughout shelf time in fruit cold-stored for 10 days. However, LOX activity in fruit cold-stored for 20 and 30 days further increased and was maintained significantly ($p \leq 0.05$) higher during shelf life evaluation than in fruit cold-stored for 10 days (Fig. 2D). This difference was consistent with higher relative leakage and deterioration in the former cases.

Thus, extension of storage time at low temperature activated and maintained higher levels of lipase and LOX activities in cold-stored litchi fruit during subsequent shelf time. These increased activities could promote degradation and peroxidation of membrane lipids leading, via increased membrane permeability and deterioration, to rapid pericarp browning. Previous similar studies also reported chilling injury accompanied by increased lipase and LOX activities or increased cell membrane rigidity (Alonso et al., 1997; Mao et al., 2007).

3.7. Changes in ATP, ADP, AMP and AEC in pericarp tissue of cold-stored litchi fruit during shelf time

ATP and ADP contents and AEC in litchi pericarp tissues decreased as storage time progressed (Fig. 3). However, litchi fruit stored for 30 days had a significantly ($p \leq 0.05$) high AMP concentration (Fig. 3C). After fruit were transferred to ambient temperature of 25 °C, ATP and ADP contents (Fig. 3A, B) and AEC (Fig. 3D) increased rapidly to a maximum at 6 h of shelf time, followed by a decline to the end of shelf time. Pericarp tissue of fruit cold stored for 30 days maintained significantly ($p \leq 0.05$) low ATP and ADP contents and AEC throughout shelf time as compared to fruit cold stored for 10 and 20 days. The AMP content in fruit stored for 30 days decreased rapidly during the first 6 h of shelf time and maintained a significantly ($p \leq 0.05$) low level thereafter. However, in pericarp tissue of fruit stored for 10 days, AMP content increased after 12 h of shelf time to significantly ($p \leq 0.05$) higher levels than in fruit cold stored for 20 and 30 days (Fig. 3C). Accordingly, low ATP and ADP contents and low AEC are associated with severe pericarp browning along with high membrane permeability and quality deterioration in litchi fruit cold stored for 30 days. A similar correlation between energy metabolism and incidence of physiological disorders has been established with oat leaves and pear fruit (Trippi et al., 1989; Saquet et al., 2001).

Loss of membrane integrity is generally associated with both peroxidation of membranes and depletion of energy (Trippi et al., 1989). Energy is essential for maintenance of membrane form and function, and, thus, the reduction of ATP levels produced in cells would contribute to a loss of metabolic control and integrity, and incomplete terminal oxidation (Pradet and Raymond, 1983). ATP and total AEC are important in lipid synthesis and de-saturation of acyl chains (Brown and Beevers, 1987; Harwood, 1988). Otherwise, a change in the unsaturation index of fatty acids in membrane lipids adversely affects membrane properties leading to loss of sub-cellular compartmentalization (Marangoni et al., 1996). In the present study, differences in ATP, ADP, AMP and energy charge could be a consequence of the various storage durations (10, 20 or 30 days) of litchi fruit at low temperature. With extending cold storage, reduced ATP and ADP contents and ACE in fruit led to increased membrane permeability. Low ATP content and AEC in fruit cold stored for 30 days was associated with increased membrane permeability (Fig. 2A), which indicates that the loss of energy charge in cells was evidently too pronounced to enable maintenance of membrane integrity and function. That is, there was evidently insufficient ATP to fuel phospholipid synthesis and enable repair of cell membranes damaged by lipid peroxidation, thereby leading to increased membrane permeability and mixing of oxidative enzymes with phenolic substrates manifest as pericarp browning.

In terms of pericarp browning and visual acceptability of litchi fruit, and as a consequence of above-mentioned biochemical processes, the effective shelf life of litchi fruit after 10 and 20 days of storage at 3–5 °C was only about 12 and 6 h, respectively, at 25 °C. However, acceptance of fruit stored for 30 days decreased further to being on 47.5% at 6 h of shelf time, and decay was evident on some fruit. Thus, litchi fruit cannot be cold-stored for 30 days for 6–12 h of shelf time at ambient temperature unless further measures are applied to prevent skin browning and fruit rotting. Overall, loss of ATP and energy depletion appears to limit lipid biosynthesis and accelerate lipid hydrolysis during shelf time at 25 °C leading to rapid pericarp browning and quality loss in the litchi fruit.

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