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ALTERED ENERGY STATUS IN PERICARP BROWNING OF LITCHI FRUIT DURING STORAGE

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Abstract

Browning is a major postharvest problem in litchi fruit which results in reduced shelf life and commercial value. Fruit browning could be related to deficient energy, which could result in a gradual loss of compartmentalization of enzymes and their substrates and then initiates enzyme-catalyzed browning reaction. Experiments were conducted to further examine the energy status in relation to pericarp browning of litchi fruit during storage. Litchi fruit were treated with exogenous adenosine triphosphate (ATP) or water (control) and then stored for up to 5 days at 25°C. Membrane permeability, production of reactive oxygen species, levels of quinine pool and contents of ATP, adenosine diphosphate (ADP) and adenosine monophosphate (AMP) in pericarp tissues of litchi fruit were measured after 0, 1, 3 and 5 days of storage. Treatment with exogenous ATP inhibited significantly pericarp browning index and the accumulation of ROS. Higher ATP level and energy charge (EC) in the ATP-treated fruit than control fruit were observed. Furthermore, the contents of ubiquinol contents and rates of ubiquinone/total ubiquinone in the control fruit maintained a significantly higher level than those in the ATP-treated fruit during storage. It was suggested that reduced energy production could enhance ROS production, damage membrane integrity and then cause loss of compartmentation between enzymes and substrates leading to enzymatic browning reaction in litchi fruit during storage.

Introduction

Litchi (*Litchi chinensis* Sonn.) is a tropical and subtropical fruit with high commercial value in international market for its attractive brightly red skin and sweet, acidic, juicy and soft but crisp pulp (Jiang *et al*., 2006). Litchi fruit is highly perishable and loses rapidly its bright red skin color within 1−2 days at ambient temperature (Huang & Scott, 1985). Rapid postharvest browning of litchi fruit pericarp results from the oxidation of phenolics by polyphenol oxidase (PPO) (Underhill & Critchley, 1995; Jiang & Fu, 1999). Jiang (2000) reported that litchi PPO cannot directly oxidize litchi anthocyanin, but the anthocyanin is degraded rapidly in an anthocyanin-PPO-phenol system. Zhang *et al*., (2005) suggested that enzymatic browning of litchi fruit pericarp caused by peroxidase (POD) may involve the presence of H_2O_2. Vicente *et al*., (2006) reported that reactive oxygen species (ROS) including H_2O_2 accumulates during fruit ripening, which is recognized that its accumulation can accelerate the oxidation of phenolic compounds, resulting in pericarp browning of litchi fruit (Zhang *et al*., 2000).

Energy production in plant tissues depends on two pathways of mitochondrial electron transport branched at the ubiquinone level–the adenosine triphosphate (ATP)

generating largely cytochrome pathway. Electron transfer by the cytochrome pathway couples to the ATP synthesis while the alternative oxidase pathway catalyses the oxidation of reduced ubiquinone without the ATP formation (Vanlerberghe & McIntosh, 1997, McDonald *et al*., 2002). Energy supply is essential for maintenance of membrane integrity and compartmentation between enzymes and substrates of harvested litchi fruit (Jiang *et al*., 2006). Duan *et al*., (2004) found that exposure of litchi fruit to pure O_2 significantly prevented peel browning, maintained high levels of ATP, ADP and energy charge, and reduced the increase in membrane permeability. Furthermore, exogenous supply of ATP improved energy status and inhibited pericarp browning of litchi fruit during storage (Song *et al*., 2006), or increased the resistance of harvested litchi fruit to pathogen invasion (Yi *et al*., 2008; 2009). In addition, some evidences indicated that alternative oxidase activity is controlled by the redox status of the quinine-pool (Hu *et al*., 2006; Millar *et al*., 1998; Szala *et al*., 2003), which affected greatly the ATP production.

In this study, litchi fruit were treated with ATP or water (control) and then stored for up to 5 days at 25°C. Changes in pericarp browning and membrane permeability were measured. Production of ROS, reduced quinine-pool level, contents of ATP, ADP and AMP, and energy charge (EC) level were also determined. The objective of this study was to further investigate the role of the altered energy status in pericarp browning of litchi fruit during storage.

Materials and Methods

Plant materials and treatments: Fruits of litchi (*Litchi chinensis* Sonn.) cv. Huaizhi at 80% maturation stage were obtained from a commercial orchard in Guangzhou, China and transported to the laboratory within 1 h. Fruits were selected for uniformity of shape and color and any blemish or disease fruits were discarded. The fruit were infiltrated for 3 min., under vacuum at 75 Pa using distilled water (control) or 1.0 mM ATP, air-dried for 2 h, packed with 0.03 mm polyethylene bag (15 fruits per bag), and then stored for up to 5 days at 25°C and 95−100% relative humidity. Fruits were sampled and used for the following measurements after 0, 1, 3 and 5 days of storage.

Evaluation of pericarp browning: Pericarp browning was assessed after 0, 1, 3 and 5 days of storage using the following scale (Jiang, 2000): 1, no browning (excellent quality); 2, slight browning; 3, <1/4 browning; 4, 1/4–1/2 browning and 5, >1/2 browning (poor quality). The browning index was calculated as ∑(browning scale × percentage of the corresponding fruit within each class).

Measurement of membrane permeability: Membrane permeability, expressed as relative electrolyte leakage, was determined according to Jiang & Chen (1995). Discs (10 mm in diameter) were removed with a cork borer from the equatorial region of fruit pericarp. Thirty discs (about 2 g) obtained from 30 fruits were rinsed twice in deionized water and incubated for 30 min., in 25 mL of 0.3 M mannitol solution at 25 °C. The initial electrolyte leakage was determined with a conductivity meter (Model DDS-11A, Shanghai Scientific Instruments, Shanghai, China). The discs were then boiled for 15 min., and cooled rapidly to 25°C to determine total electrolytes. The relative leakage was expressed as a percentage of the initial electrolyte to the total electrolytes.

Detection of reactive oxygen species: Production of ROS was measured using the 2',7'-dichlorofluorescein diacetate (HDCF-DA) (Reactive oxygen species Assay Kit, Beyotime, China) (Parsons, 1999). Extraction and determination were conducted by the methods of Wang & Luo (1990) and Li *et al*., (2008) with minor modification. Litchi pericarp tissues (1 g) were ground in liquid nitrogen and the powder was then homogenized with 5 mL of 65 mM phosphate buffer (pH 7.8) and centrifuged at 5000 *g* for 10 min., at 4°C. An aliquot (1 mL) of the collected supernatant was incubated with 10 μM DCFH-DA at 37°C for 30 min. For further processing, the samples were centrifuged for 3 min at 16000 *g* and then the supernatant was diluted 10-fold with deionized water. Fluorescence of the diluted sample was measured immediately with a spectrofluorometer (LS55, PerkinElmer, USA) using an excitation wavelength of 488 nm and an emission wavelength of 525 nm, respectively.

Determinations of ATP, ADP and AMP contents and EC: Extraction and assay of ATP, ADP and AMP contents were conducted by the method of Liu *et al*., (2006). Litchi pericarp tissues (1 g) were ground in liquid nitrogen and the powder was then homogenized with 5 ml of 0.6 M of perchloric acid and centrifuged at 16000 *g* for 10 min., at 4°C. The supernatant (3 mL) was quickly neutralized to pH 6.5–6.8 with 1 M KOH, then diluted to 4 ml and finally passed through a 0.45 μm filter. Measurements of ATP, ADP and AMP contents were performed by HPLC (LC-20AT pump system, Shimadzu, Japan) equipped with a reverse Spherisorb C-18 analytical column (5 μm × 250 mm × 4.6 mm) and an UV detector (SPD-20A detector, Japan) at 254 nm. Mobile phase A consisted of 0.06 M dipotassium hydrogen phosphate and 0.04 M potassium dihydrogen phosphate dissolved in deionized water and adjusted to pH 7.0 with 0.1 M KOH. Mobile phase B was 100% acetonitrile. Elution was conducted using 95% A and 5% B for 15 min., at a flow rate of 1.2 mL/min. Sample aliquots (20 μL) were injected into the HPLC and ATP, ADP and AMP contents were calculated using the external standards and then expressed as on fresh weight (FW) basis. Tissue energy status can be expressed simply by the adenine nucleotide ratio and the energy charge (EC) was then calculated as [ATP + 1/2 ADP] / [ATP + ADP + AMP] (Pradet & Raymond, 1983).

Determinations of ubiquinone (UQ) and ubiquinol (UQr): UQ and UQr levels were assayed by the procedure of Wagner & Wagner (1995) with some modification. Litchi pericarp tissues (1 g) were ground in liquid nitrogen and the powder was then homogenized with 3 mL of 0.2 M $HClO_4$ in 80% methanol and 3 mL of petroleum ether, and then vortexed vigorously for 1 min. The upper petroleum ether phase was removed after the homogenate was centrifuged at 1500 *g* for 2 min. Additional 3 mL of petroleum ether was placed, vortexed and then centrifuged as described above. The upper petroleum ether phase was collected and then combined with the one obtained previously before evaporated to dryness under nitrogen. The dried extract was re-suspended gently in 100 μL of nitrogen-purged methanol before HPLC analysis. The HPLC column was equilibrated with nitrogen-purged ethanol/methanol (9:1, v/v). UQ and UQr contents were detected at 275 nm using nitrogen-purged ethanol/methanol (9:1, v/v) as the mobile phase with a flow rate of 1 mL/min. The amounts of UQ and UQr were calculated based on the peak areas, using external standards (Q_{10} obtained from Sigma Chemical Corpation, USA) ranging from 0 to 800 μM. Ubiquinone was reduced to ubiquinol by the addition of Sodium borohydride and then the amount of total ubiquinone was calculated as the sum of UQ and UQr.

Data handling: Experiments were arranged in a completely randomized design. There were three replicates of each treatment with 30 sample fruit per replicate. All data were expressed as the means and standard errors and then analyzed using SAS 8.1 (SAS Institute Inc., Cary, North Carolina, USA) and the significant differences at the 5% level among the means were determined by the Duncan's multiple range test.

Results and Discussion

Changes in pericarp browning: Pericarp browning of litchi fruit increased markedly with storage time (Fig. 1). A rapid increase in browning index of control fruit was observed during storage. Exogenous ATP treatment inhibited significantly pericarp browning of litchi fruit. A similar result was obtained by Yi *et al*., (2008) who reported that the ATP treatment inhibited disease development and pericarp browning of *P. litchii*-inoculated litchi fruit.

Changes in membrane permeability: A sharp increase in electrolyte leakage occurred in pericarp tissues of litchi fruit after 1 day of storage. The increases in the membrane permeability of control fruit were significantly higher than that of ATP-treated fruit after 3 and 5 days of storage (Fig. 1). Thus, the treatment with ATP reduced the increase in membrane permeability, maintained relatively the membrane integrity and thus, inhibited the enzyme-catalyzed browning reaction (Fig. 1). A similar result was obtained by Duan *et al*., (2004) who used the pure oxygen as a treatment.

Changes in production of ROS: Excessive production of ROS can damage cellular composition such as proteins and lipids and disturb cellular metabolism, leading to the loss of membrane integrity and functionality (Yu, 1994; Xiong *et al.*, 2006; Franck *et al*., 2007; Yang *et al.*, 2008). In this study, generation of ROS in pericarp tissue of litchi fruit increased obviously with storage time (Fig. 2), which was similar to the report of Ruenroengklin *et al*., (2009), who observed that pericarp browning of litchi fruit was associated with the rapid accumulation of ROS. Treatment with exogenous ATP prevented the accumulation of ROS and thus slowed pericarp browning of litchi fruit during storage.

Changes in quinine pool: The alternative oxidase activity is controlled by the redox status of the quinine pool (Wagner *et al*., 2008; Szala *et al*., 2003). The changes in total ubiquinone (UQt), ubiquinone(UQ), reduced ubiquinone (UQr) and UQr/UQt ratio can well indicate the operation of the alternative oxidase activity (McDonald *et al*., 2002; Millenaar & Lambers, 2003). In this study, Fig. 3 shows the changes in levels of UQt, UQ and reduced UQr, and UQr/UQt ratio. Significantly higher levels of UQt and UQr in the non-ATP-treated (control) fruit during storage were observed compared with the ATP-treated fruit. Furthermore, the UQr/UQt ratio in the control fruit exhibited a significantly higher level than the ATP-treated fruit. In addition, the ATP-treated fruit exhibited a significantly lower UQ content than the control fruit after 3 or 5 days of storage. These data show that the ATP-treated fruit had a lower alternative oxidase activity pathway than the control fruit.

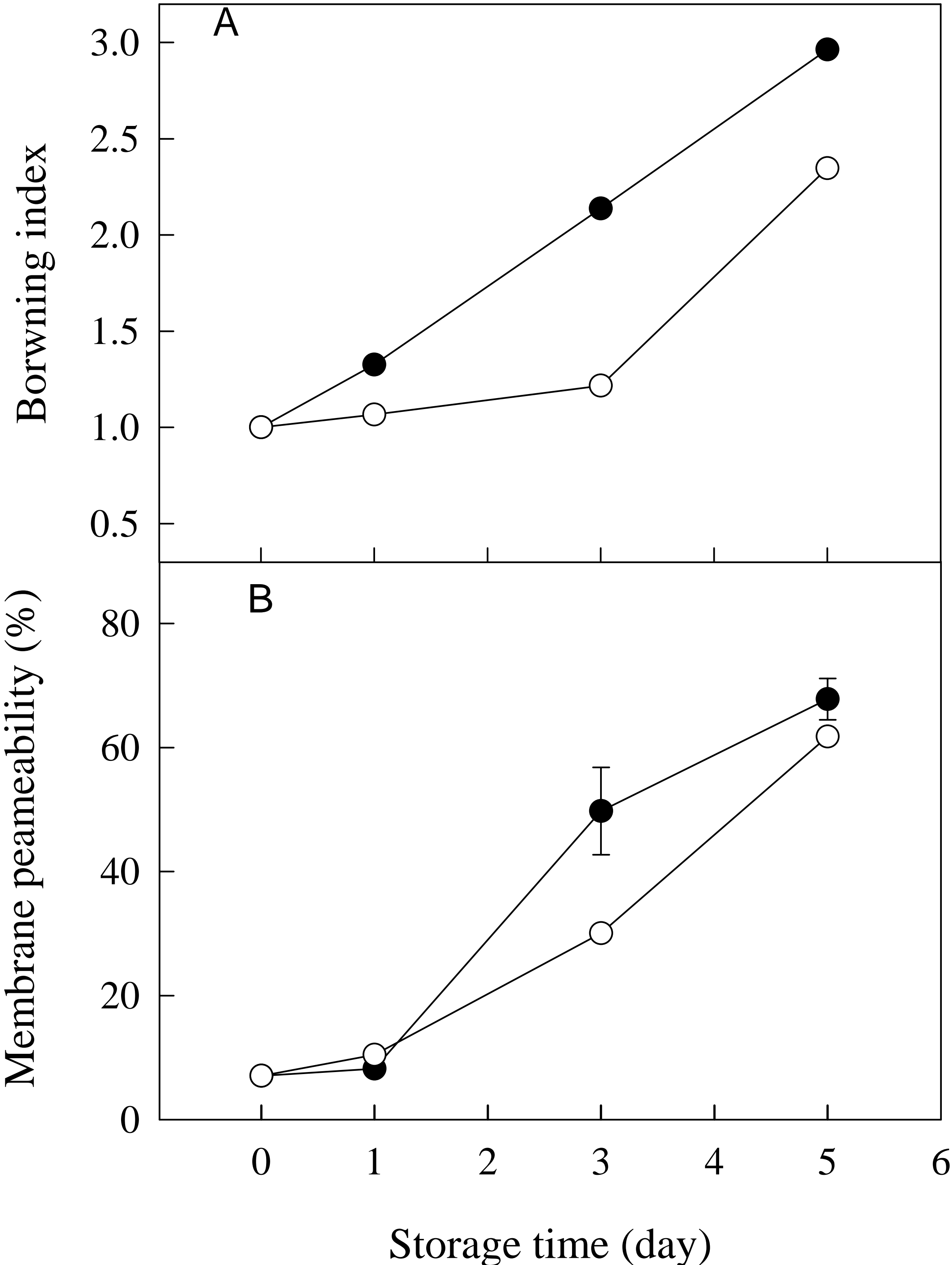

Fig. 1. Effects of exogenous ATP supply on browning index and membrane permeability of harvested litchi fruit.
Each value was the means ± standard error. Vertical bars indicated the standard errors of the means where they exceeded the symbol size. ●, Control and ○, ATP.

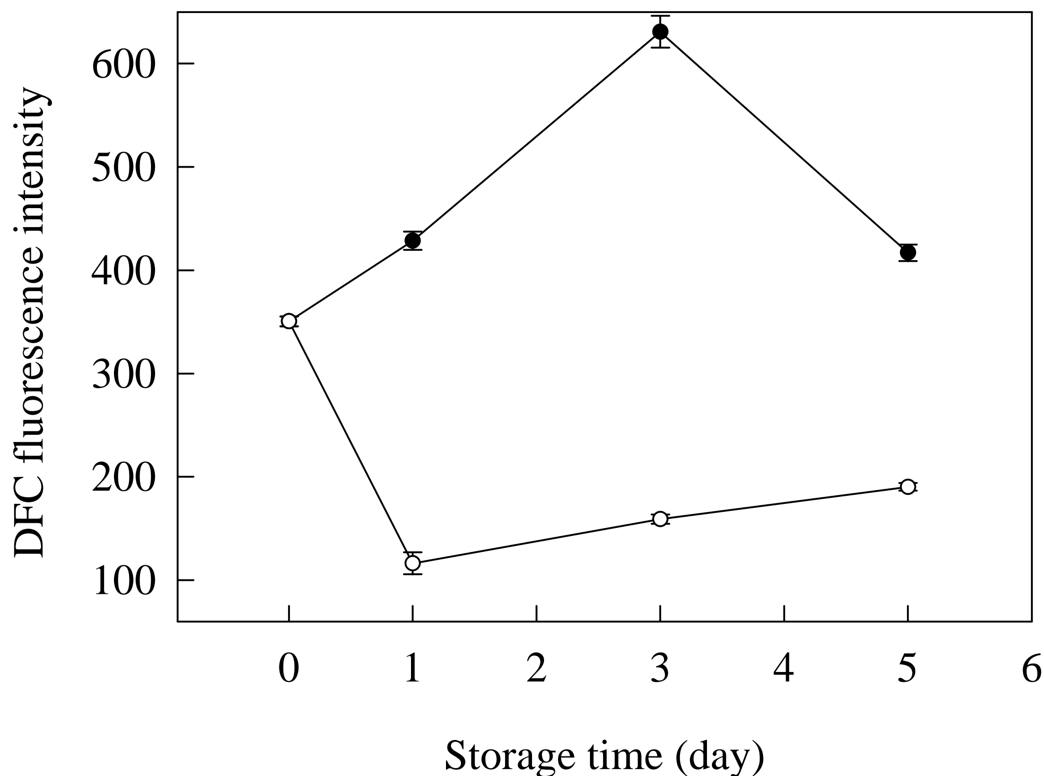

Fig. 2. Effects of exogenous ATP supply on DFC fluorescence of litchi fruit during storage.
Each value was the means ± standard error. Vertical bars indicated the standard errors of the means where they exceeded the symbol size. ●, Control and ○, ATP.

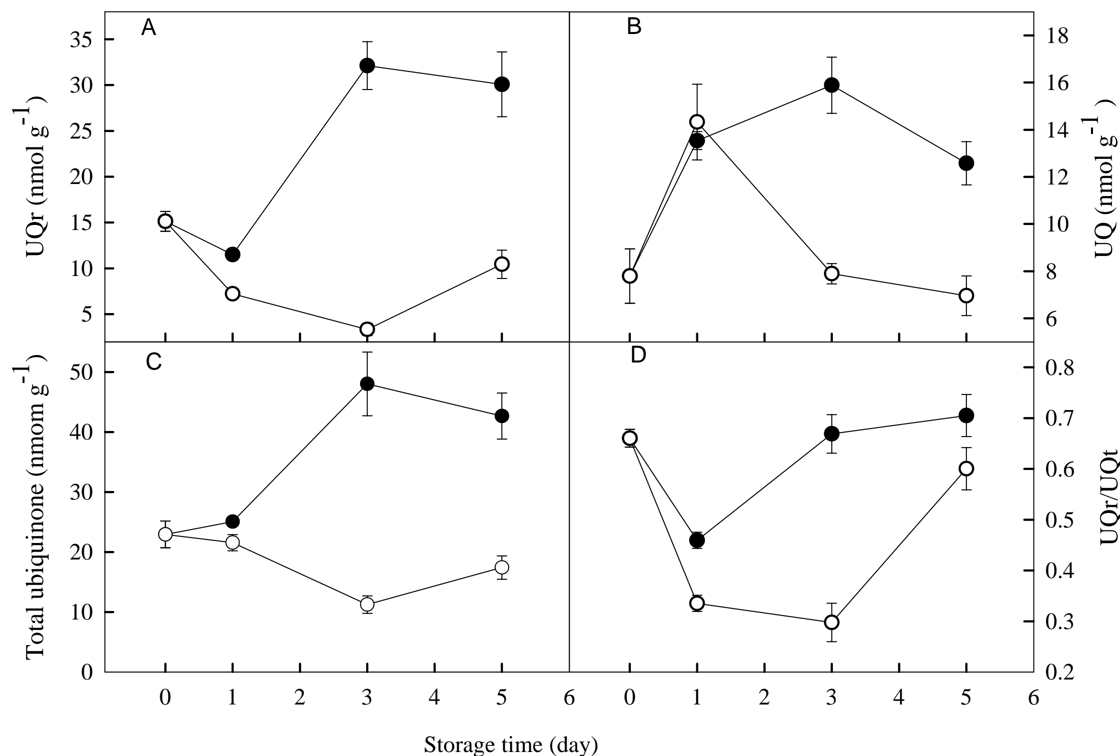

Fig. 3. Effects of exogenous ATP supply on total ubiquinone (UQt) (A), reduced ubiquinone (UQr) (B), ubiquinone (UQ) (C) and UQr/UQt ratio (D) of litchi fruit during storage.
Each value is the means ± standard error. Vertical bars indicated the standard errors of the means where they exceeded the symbol size. ●, Control and ○, ATP.

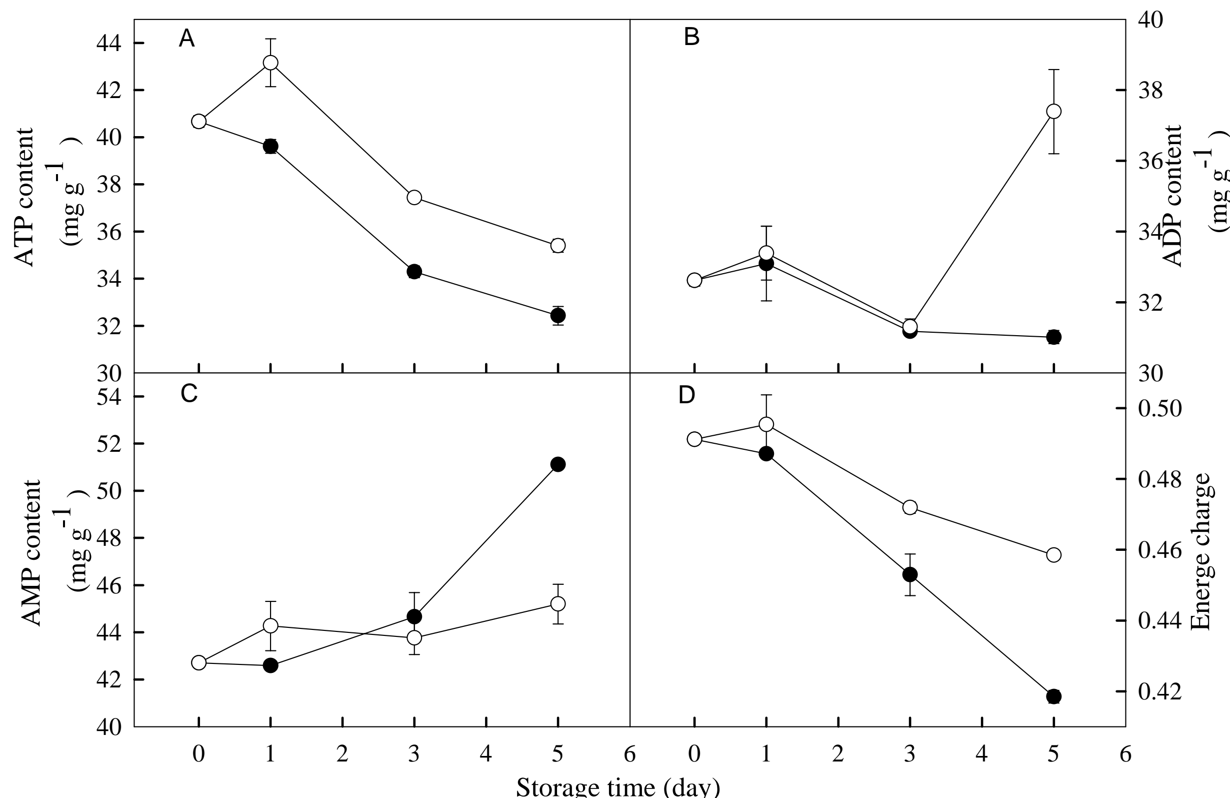

Fig. 4. Effects of exogenous ATP supply on contents of ATP (A), ADP (B) and AMP (C), and energy charge (D) of litchi fruit during storage.
Each value was the means ± standard error. Vertical bars indicated the standard errors of the means where they exceeded the symbol size. ●, Control and ○, ATP.

Changes in contents of ATP, ADP and AMP and EC levels: ATP levels in pericarp tissue of litchi fruit during storage tended to decrease, which was associated with the trend of the energy charge (Fig. 4). The ATP-treated fruit exhibited the significantly higher levels of ATP content and EC as compared with the control fruit. In contrast, the ATP-treated fruit had a significantly lower AMP content than the control fruit by the end of storage. Duan *et al*., (2004), Song *et al*., (2006) and Yi *et al*., (2008) reported that pericarp browning of harvested litchi fruit during storage could be related to deficiency in energy supply (Duan *et al*., 2004; Song *et al*., 2006; Yi *et al*., 2008). This study confirmed further that exogenous ATP supply inhibited effectively the pericarp browning of litchi fruit, which was attributed to the high ATP level and EC.

In conclusion, reduced energy production could enhance ROS production, damage membrane integrity and then cause loss of compartmentation between enzymes and substrates leading to enzyme-catalysed browning reaction in litchi fruit during storage. Exogenous ATP supply reduced ROS production, maintained relatively membrane integrity and, thus, inhibited the pericarp browning of harvested litchi fruit.

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References

Duan, X.W., Y.M. Jiang and X.G. Su. 2004. Role of pure oxygen treatment in browning of litchi fruit after harvest. *Plant Sci*., 167: 665-668

Franck, C., J. Lammertyn, Q.T. Ho, P. Verboven, B. Verlinden and B.M. Nicolai. 2007. Browning disorders in pear fruit. *Postharvest Biol. Technol.*, 43:1-13.

Hu, W.H., K. Shi and X.S. Song. 2006. Different effects of chilling on respiration in leaves and roots of cucumber (*Cucumis sativus*). *Plant Physiol. Biochem*., 44: 837-843.

Huang, P.Y., K.J. Scott. 1985. Control of rotting and browning of litchi fruit after harvest at ambient temperatures in China. *Trop. Agric.*, 62: 2-4.

Jiang, Y.M. 2000. Role of anthocyanins, polyphenol oxidase and phenols in lychee pericarp browning. *J. Sci. Food Agric*., 80: 305–310.

Jiang, Y.M. and F.Chen. 1995. A study on polyamine change and browning of fruit during cold storage of litchi (*Litchi chinensis* Sonn.). *Postharvest Biol. Technol*., 5: 245-250.

Jiang, Y.M. and J.R. Fu. 1999. Postharvest browning of litchi fruit by water loss and its prevention by controlled atmosphere storage at high relative humidity. *LWT- Food Sci. Technol*., 32: 278-283.

Jiang, Y.M., Y. Wang and L.L. Song. 2006. Postharvest characteristics and handling of litchi fruit – an overview. *Australian J. Exp. Agric*., 46: 1541–1556.

Li, Y.C., Y.M. Bao, B. Jiang, Z. Wang, Y.X. Liu, C. Zhang and L.J. An. 2008. Catalpol protects primary cultured astrocytes from *In vitro* ischemia-induced damage. *Int. J. Dev. Neurosci*., 26: 309-317.

Liu, H., Y.M. Jiang, Y. Luo and W.B. Jiang. 2006. A simple and rapid determination of ATP, ADP and AMP concentrations of pericarp tissues of harvested litchi fruit by high performance liquid chromatograph. *Food Technol. Biotechnol*., 44:531-534.

McDonald, A.E., S.M. Sieger and G.C. Vanlerberghe. 2002. Methods and approaches to study plant mitochondrial alternative oxidase. *Physiol. Plant*., 116: 135-143.

Millar, A.H. and K.A. Owen. 1998. Analysis of respiratory chain regulation in roots of soybean seedlings. *Plant. Physiol*., 117: 1083-1093.

Millenaar, F.F. and H. Lambers. 2003. The alternative oxidase: *In vivo* regulation and function. *Plant. Biol*., 2-15.

Parsons, H.L., J.Y.H. Yip and G.C. Vanlerberghe. 1999. Increased respiratory restriction during phosphate-limited growth in transgenic tobacco cells lacking alternative oxidase. *Plant Physiol*., 121: 1309–1320.

Pradet, A. and P. Raymond. 1983. Adenine nucleotide ratios and adenylate energy charge in energy metabolism. *Annu. Rev. Plant. Physiol*., 34:199-224.

Ruenroengklin, N., B. Yang, H.T. Lin, F. Chen and Y.M. Jiang. 2009. Degradation of anthocyanin from litchi fruit pericarp by H_2O_2 and hydroxyl radical. *Food Chem.*, 116: 995-998.

Song, L.L., Y.M. Jiang, H.Y. Gao and C.T. Li. 2006. Effects of adenosine triphosphate on browning and quality of harvested litchi fruit. *American J. Food Technol*., 1: 173-178.

Szala, B., Y. Jolivetb and M.P.H. Sauderb. 2003. Oxygen concentration regulates alternative oxidase expression in barley roots during hypoxia and post-hypoxia. *Physiol. Plant*., 119: 494-502.

Underhill, S.J.R. and C. Critchley. 1994**.** Anthocyanin declorisation and its role in lychee pericarp browning**.** *Australian J. Plant Physiol*., 34:115-122.

Vanlerberghe, G.C. and L. McIntosh. 1997. Alternative oxidase: from gene to function. *Annu. Rev. Plant Physiol. Mol. Biol*., 48: 703-734.

Vicente, A.R., G.A. Martinez, A.R. Chaves and P.M. Civello. 2006. Effect of heat treatment on strawberry fruit damage and oxidative metabolism during storage. *Postharvest Biol. Technol*., 40: 116–122.

Wagner, A.M. and M.J. Wagner. 1995. Measurements of ubiquinone reduction levels in plant cells. *Plant Physiol*., 108: 277-283.

Wagner, A.M., K. Krab and M.J. Wagner. 2008. Regulation of thermogenesis in flowering Araceae: The role of the alternative oxidase. *Biochem. Biophys. Bioenerg*., 1777: 993-1000.

Wang, A. G. and G. H. Luo. 1990. Quantitative relation between the reaction of hydroxylamine and superoxide anion radicals in plants. *Plant Physiol. Commun.*, 6: 55–57.

Xiong, G.M. Xing, C.M. Gong, F.M. Li, S.M. Wang, Z.X. Li and Y.F. Wang. 2006. Dual role of abscisic acid on antioxidative defense in grass pea seedling (*Lathyrus sativus* L.). *Pak. J. Bot*., 38(4): 999-1014.

Yang, S., X. Su, K.N. Prasad, B. Yang, G. Cheng, Y. Chen, E. Yang and Y. Jiang. 2008. Oxidation and peroxidation of postharvest banana fruit during softening. *Pak. J. Bot.*, 40: 2023-2029.

Yi, C., H.X. Qu, Y.M. Jiang, J. Shi and X.W. Duan. 2008. ATP-induced changes in energy status and membrane integrity of harvested Litchi fruit and its relation to pathogen resistance. *J. Phytopathol*., 156: 365-371.

Yi, C., Y.M. Jiang, J. Shi, H.X. Qu, S. Xue, X.W. Duan, J.Y. Shi and N. K. Prasad. 2010. ATP-regulation of antioxidant properties and phenolics in litchi fruit during browning and oathogen infection process. *Food Chem*., 118: 42-47.

Yu, B.P. 1994. Cellular defenses against damage from reactive oxygen species. *Physiol. Rev.*, 74, 139–162.

Zhang, D., P.C. Quantick and J.M. Grigor. 2000. Changes in phenolic compounds in litchi (*Litchi chinensis* Sonn.) fruit during postharvest storage. *Postharvest Biol. Technol.*, 19: 165-172.

Zhang, Z.Q., X.Q. Pang, X.W. Duan, Z.J. Ji and Y.M. Jiang. 2005. Role of peroxidase in anthocyanin degradation in litchi fruit pericarp. *Food Chem.* 90: 47-52.

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