Postharvest Biology and Technology 116 (2016) 26–35

Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

Effects of chlorine dioxide on mitochondrial energy levels and redox status of 'Daw' longan pericarp during storage

Athiwat Chumyam[a], Lalida Shank[b], Jamnong Uthaibutra[a,c], Kobkiat Saengnil[a,c,*]

[a] Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand
[b] Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand
[c] Postharvest Technology Research Institute, Chiang Mai University/Postharvest Technology Innovation Center, Commission on Higher Education, Bangkok 10140, Thailand

ARTICLE INFO

Article history:
Received 31 August 2015
Received in revised form 3 January 2016
Accepted 4 January 2016
Available online xxx

Keywords:
Energy status
Redox status
Respiratory enzymes
Fruit senescence
Dimocarpus longan

ABSTRACT

Mitochondrial redox status and adenosine triphosphate (ATP) production play a central role in responding to physiological and environmental changes and metabolic control. Changes in mitochondrial redox potential are intimately associated with oxidative stress which could lead to plant senescence. This study aimed to demonstrate the involvement of an alteration of the redox status in energy production and fruit senescence of longan (*Dimocarpus longan* Lour. cv. Daw) during storage and to investigate the effects of gaseous chlorine dioxide (ClO_2) both on the redox and the energy status and on delaying senescence. Fresh longan fruit were fumigated with 5, 10 and 25 mg/L ClO_2 for 10 min and then stored in a cardboard box at 25 °C with 82% relative humidity for 7 days. ATP content and energy charge (EC), and activities of respiratory enzymes such as succinate dehydrogenase (SDH) and cytochrome *c* oxidase (CCO) were found to be steadily decreased in the untreated group during storage. These reductions coincided with a decrease in the redox status as determined by pyridine nucleotide (NAD/NADH) and ubiquinone (Q/QH_2) ratios and with fruit senescence including browning and the onset of disease symptoms. However, in the ClO_2 fumigated fruit, ATP content, EC, SDH and CCO activities as well as NAD/NADH ratio were immediately and markedly increased after treatment. The effects can be seen through the fourth day of storage. The Q/QH_2 ratio remained unchanged before rising after Day 4. Higher concentrations of ClO_2 (10 and 25 mg/L) were more effective than the lower one (5 mg/L) in altering the redox balance and increasing energy production. The altered redox state and increased energy by ClO_2 were found to be closely associated with a delay in fruit senescence during storage. These findings support the notion that energy production and redox balance involve directly in longan senescence and demonstrated that ClO_2 could restore ATP and the redox balance, leading to reduction and delay in fruit senescence.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

The oxidation–reduction (redox) status is an important regulator of various metabolic functions of cells. The cellular redox state is made tangible in terms of the redox state or redox status of the individual redox-active molecules in the cell (Potters et al., 2010). Cellular respiration, one of the major redox systems, produces energy needed for various activities of the cell including growth and survival. Electron transport chain (ETC) utilizes the potential energy harvested during the redox reaction steps to produce ATP. ETC enzymes such as reduced nicotinamide adenine dinucleotide (NADH) dehydrogenase, succinate dehydrogenase (SDH), cytochrome *c* oxidase (CCO) and ATP synthase facilitate ATP synthesis from the inner mitochondrial membrane (Soole and Menz, 2013; Schertl and Braun, 2014). Redox status, in respiration, can be envisioned as the ratio of oxidized nicotinamide adenine dinucleotide (NAD) to NADH (NAD/NADH) and ubiquinone (Q) to ubiquinol (QH_2) (Q/QH_2). In a plant cell, both the redox and the energy status as well as respiratory enzyme activities have been shown to be affected by internal and external factors (Yang et al., 2009; Jin et al., 2013, 2014; Ostaszewska et al., 2014).

Considerable evidences suggested that the redox status is a key factor controlling senescence in some horticultural crops (Potters et al., 2010). Wang et al. (2013) reported that alteration in the redox potential and, consequently, the reduction in energy production during various stress conditions leads to senescence of fruits. Stress causes a reduction in the energy status of fruits during

* Corresponding author at: Chiang Mai University, 239 Huaykaew Rd., Chiang Mai, Thailand. Fax: +66 53 892259.
E-mail address: kobkiat_s@hotmail.com (K. Saengnil).

http://dx.doi.org/10.1016/j.postharvbio.2016.01.002
0925-5214/© 2016 Elsevier B.V. All rights reserved.

storage. For example, reduction in ATP content and energy charge (EC) in pear fruit coincided with amplification of flesh browning when the fruit was stored at 0 °C (Saquet et al., 2003). During low temperature storage, it was found that ATP content, EC and SDH as well as CCO activities in pear fruit were significantly decreased while chilling injury was significantly increased (Jin et al., 2013, 2014). Similarly, in other fruits such as mango (Li et al., 2014), longan (Chen et al., 2014), litchi (Yang et al., 2009; Wang et al., 2013) and banana (Wang et al., 2015b), the energy status was found to be greatly reduced in conjunction with an increase in injury or other storage damages.

In addition to the energy status, stress conditions also caused changes in the cellular redox status. For example, an increase in QH_2/total ubiquinone (Qt) ratio of litchi fruit coincided with a decrease in ATP, while browning symptom increased under oxidative stress (Yang et al., 2009). The NAD/NADH ratio of bean seedlings and *Arabidopsis* was found to be significantly decreased in conjunction with a decrease in ATP, while growth was inhibited after sulphur-deficient stress (Juszczuk and Ostaszewska, 2011; Ostaszewska et al., 2014). These evidences suggested that senescence is closely related with the redox status and cellular energy supply. Maintaining a healthy redox status and energy efficiency in the cell could, therefore, delay or inhibit cellular deterioration to a certain extent.

In order to delay or reduce senescence via alteration of energy and redox status, various chemicals and physical methods have been employed. Prior treatment with chemical compounds such as methyl jasmonate (MJ), oxalic acid (OA) (Jin et al., 2013, 2014; Li et al., 2014), nitric oxide (NO) (Wang et al., 2015b) and pure oxygen (Su et al., 2005) had been shown to increase ATP content, EC and some respiratory enzyme activities and lessen purishment during storage of several fruits such as pear, mango, banana and longan. Peach fruit exposed to increased atmospheric pressure exhibited similar outcome during storage (Wang et al., 2015a).

Exogenously added ATP could also tip the cellular energy balance in favor of diminishing and controlling aging and senescence of fruits and vegetables. Yi et al. (2008) reported that application of exogenous ATP on litchi fruit before inoculation with *Peronophythora litchi* and storage at 25 °C for 6 days significantly induced the endogenous ATP level and EC while pericarp browning and disease symptom decreased. Litchi fruit immersed in ATP solution showed significant increase in their endogenous ATP and EC while pericarp browning decreased (Yang et al., 2009). Moreover, ATP-treated litchi fruit was found to have enhanced enzymatic antioxidant and antioxidant capacity while pericarp browning decreased (Yi et al., 2010). Similarly, Yao et al. (2014) reported that application of exogenous ATP on longan fruit before storage at 25 °C for 6 days significantly induced enzymatic antioxidant while pericarp browning decreased.

Chlorine dioxide (ClO_2) is a new and promising chemical found to be able to reduce and delay senescence including browning and disease in fruits during storage. Saengnil et al. (2014) reported that applications of gaseous ClO_2 on longan fruit before storage significantly reduced pericarp browning by reducing the oxidation of phenolic compound, delaying the occurrence of disease and maintaining higher fruit quality. Similarly, Chomkitichai et al. (2014a,b) reported that ClO_2 treatment of longan fruit reduced pericarp browning apparently by enhancing the antioxidant defense system which may involve the alteration of redox status in energy production.

Since overwhelming evidences have shown correlation between cellular energy and redox status with the quality of fruits during storage, it is imperative to explore the possibility that ClO_2 could alter energy producing capacities and cellular redox homeostasis such that senescence is delayed in longan.

2. Materials and methods

2.1. Plant materials

Mature longan fruit were harvested from a commercial orchard in Lamphun province, Thailand and transported to the Postharvest Physiology and Technology Research Laboratory at Chiang Mai University within 2 h. Fruit were individually selected for uniformity in shape, color, size and devoid of defect.

2.2. ATP treatments

Longan fruit were divided into 12 groups of 240 fruits each. A set of 3 groups were submerged in 0 (control), 0.5, 1 and 2 mM ATP solution for 10 min at 25 °C, followed by air drying for 30 min. The submerged fruit in each group were further divided into 8 subgroups of 30 fruits each which was stored in a cardboard box (25 cm (L) × 17 cm (W) × 9 cm (H)). The boxes were stored in a storage room at temperature of 25 ± 1 °C with a relative humidity of 82 ± 5% for 7 days. Every day from the beginning of the experiment, the fruit from each treatment were tested for storage fruit quality including browning index, color of pericarp, disease index and overall quality acceptance.

2.3. ClO_2 treatments

Freshly picked longan fruit were divided into 12 groups of 240 fruits each and exposed to ClO_2 (0, 5, 10 and 25 mg/L) in a fumigation chamber for 10 min. Each concentration was done in triplicate. After fumigation, the chamber was ventilated for 30 min to remove any residual ClO_2 Each group was collected and stored in a cardboard box as described in ATP treatment (2.2). From the beginning until the end of the experiment, samples from each replication of the treatments were taken every day to be quantified for the following parameters; energy status, energy producing enzyme activities, redox status and storage fruit quality including browning index, color of pericarp, disease index and overall quality acceptance.

2.4. Determination of ATP, ADP, AMP contents and energy charge

ATP, ADP and AMP were extracted and evaluated as described by Liu et al. (2006) with some modifications. Longan pericarp (2 g) was sliced and homogenized at 4 °C for 1 min in 10 mL of 0.6 mM perchloric acid. The homogenate was centrifuged at 6000 × g for 10 min at 4 °C and 5 mL of the supernatant was taken and its pH immediately adjusted to 6.5–6.8 with 1 M KOH. The neutralized supernatant was let stand for 30 min in an ice bath before filtration with Whatman® No. 1 filter (Whatman, England) followed by 0.45 μm filter (MS® Nylon Syringe Filter, USA).

ATP, ADP and AMP were separated and measured using an Agilent 1200 high performance liquid chromatography (HPLC) system (Agilent Corporation, USA) using a reserved phase Eclipse XDB-C18 column (5 μm, 4.6 × 150 mm) and a model G1314B diode array detector at 254 nm. Mobile phase A consisted of 50 mM phosphate buffer (pH 7.0). Mobile phase B was pure acetonitrile. HPLC separation was achieved using continuous gradient elution as follows: 0 min 100% A, 0% B; 2 min 95% A, 5% B; 4 min 80% A, 20% B; 5.3 min 75% A, 25% B and 6 min 100% A, 0% B. The flow rate was 1.2 mL/min, while the injection volume was 20 μL. The total retention time was about 5 min and the gradient was run for 6 min to ensure full separation. ATP, ADP and AMP presence in the samples were identified by comparison with retention time of standards, while their concentrations were determined using the external standard method. Energy charge was calculated by [ATP + 0.5ADP]/[ATP + ADP + AMP].

2.5. Energy producing enzyme activities

Crude mitochondria were extracted from longan pericarp by the method of Jin et al. (2013) with the following modifications. Longan pericarp (1 g) was sliced and homogenized at 4 °C for 1 min in 10 mL of 50 mM Tris–HCl buffer (pH 7.5), containing 0.25 M sucrose, 0.3 M mannite, 1 mM EDTA and 0.5 g/L polyvinyl pyrrolidone. The homogenate was then centrifuged at 4000 × *g* for 10 min at 4 °C. The supernatant was collected and centrifuged at 20,000 × *g* for 20 min at at 4 °C. The sediment was washed twice with washing buffer (10 mM Tris–HCl buffer (pH 7.2), containing 0.25 M sucrose, 0.3 M mannite and 1 mM EDTA). The final sediment was dissolved with washing buffer to give the crude mitochondrial extract for the enzyme assays.

The SDH activity was determined by a decrease in absorption at 600 nm, caused by reduction of the artificial electron acceptor dichlorophenolindophenol (DCPIP) (Popov et al., 2010). The reaction was carried out at 30 °C for 5 min in a reaction mixture containing 0.3 mL of crude mitochondrial extract, 3 mL of 0.2 mM potassium phosphate buffer (pH 7.4), 1 mL of 0.2 mM sodium succinate, 0.1 mL of 10 mM sodium azide, 0.1 mL of 10 mM phenazine methosulphate and 0.1 mL of 1 mM DCPIP. One unit of SDH activity was defined as a decrease of 0.01 in absorbance per minute at 600 nm under the assay conditions.

The activity of CCO was assayed according to a modified method of Jin et al. (2013). Crude mitochondrial extract (0.2 mL) was incubated with 0.3 mM cytochrome *c* solution and 20 mM dimethyl phenylene diamine at 37 °C for 5 min. One unit of CCO activity was defined as an increase of 0.01 in absorbance per minute at 510 nm under the assay conditions.

Protein content was determined according to Lowry et al. (1951) using bovine serum albumin as standard.

2.6. Determination of NAD and NADH

NAD and NADH contents were assayed by the procedure of Brugidou et al. (1991) with some modifications. Acid extraction was used for NAD whereas alkaline extraction was used for NADH. Longan pericarp (1 g) was sliced and ground in liquid nitrogen and the powder was then homogenized at 4 °C for 1 min with 10 mL of 0.5 M perchloric acid in 10% methanol (v/v) for acid extraction, or 0.5 M NaOH in 10% methanol (v/v) for basic extraction. The homogenate was then centrifuged at 5000 × *g* for 15 min at 4 °C. The supernatant was adjusted to either pH 5.0 with 6 M KOH for the acid extraction or to pH 8.0 with 1 M HCl for the alkaline extraction. After centrifugation (5000 × *g*) for 10 min at 4 °C, the supernatant was filtered through a 0.45 μm filter (MS® Nylon Syringe Filter, USA). Twenty microliter of the supernatant was used for HPLC (Agilent Corporation, USA) analysis.

Mobile phase A consisted of 0.1 M KH_2PO_4 and 8 mM tetra-*n*-butylammonium hydrogen sulfate (TBAHS), adjusted to pH 6.0 with 0.1 M KOH. Mobile phase B consisted of 0.1 M KH_2PO_4 and 8 mM TBAHS, pH 6.0, containing 20% (v/v) methanol. HPLC separation was achieved using continuous gradient elution as follows; 0 min 100% A, 0% B; 12 min 100% A, 0% B; 16 min 75% A, 25% B; 20 min 50% A, 50% B, 22 min 0% A, 100% B and 30 min 0% A, 100% B. The flow rate was 1.3 mL/min. NAD and NADH contents were detected at 254 nm. NAD and NADH in the samples were identified by comparison with retention time of standards, while the concentrations of NAD and NADH were determined using the external standard method.

2.7. Determinations of Q and QH_2

The concentration of Q and QH_2 were determined using the procedure of Wagner and Wagner (1995) with some modifications. Longan pericarp (1 g) was sliced and ground in liquid nitrogen and the powder was then homogenized at 4 °C for 1 min with 3 mL of 0.2 M perchloric acid in 100% methanol. The mixture was then rapidly transferred to a tube containing 3 mL of petroleum ether, mixed and centrifuged at 1500 × *g* for 2 min. The lower phase was reextracted with 3 mL of petroleum ether. The upper petroleum ether phases from both extractions were combined and evaporated to dryness under a flow of nitrogen. Immediately before use, the extracted Q and QH_2 was resuspended in 100 μL of ethanol and analyzed in a HPLC system (Agilent Corporation, USA). Q and QH_2 were eluted with ethanol:methanol (9:1, v/v) as a mobile phase with a flow rate of 1 mL/min and detected at 275 nm. Respective quantity of Q and QH_2 detected at 275 nm was calculated based on the peak areas comparing to quantified external standards. Ubiquinone was reduced to ubiquinol by the addition of sodium borohydride.

2.8. Browning index

Pericarp browning was estimated using the browning index (BI) as described by Jiang and Li (2001). Browning was estimated visually by measuring the extent of the total brown area on each fruit surface using the following scale: 1 = no browning (excellent quality), 2 = slight browning, 3 = less than 25% browning, 4 = 25–50% browning, and 5 = >50% browning (poor quality). A browning index was calculated using the following formula: $\sum$ (browning scale × percentage of fruit in each class). Fruit having a browning index above 3.0 were considered as unacceptable for visual marketing quality.

2.9. Color measurement

The color of pericarp was measured using a chromameter (Model Miniscan XE plus, Germany) according to the CIELAB scale. The degree of browning was expressed as L^* value which indicates lightness of the pericarp, ranging from black = 0 to white = 100 (Apai, 2010).

2.10. Disease index

Disease index was visually assessed by the lesions or rot on the fruit surface. The severity of fungal development on the surface was given a score from 0 to 4 with 0 = no visual evidence of fungi, 1 = less than 10%, 2 = 10–30%, 3 = 31–70%, and 4 = more than 70% of the surface affected with fungus. A disease index was calculated using the following formula: $\sum$ (disease scale × percentage of fruit in each class) (Thavong et al., 2010).

2.11. Overall quality acceptance

Overall quality acceptance was evaluated by a semi-trained panel of six graduate students from Postharvest Physiology Laboratory at Department of Biology, Chiang Mai University. Overall quality acceptance of fruit was scored based on a modification of the 9-point hedonic scale reported by Wright and Kader (1997) and Apai (2010): 9 = excellent, extremely fresh; 7 = very good, marketable; 5 = good, limit of marketability; 3 = fair, limit of usability; 1 = poor, unusable. Fruit having an overall quality score below 5.0 were considered as unacceptable for marketing quality.

2.12. Statistical analysis

The data collected from each experiment were statistically analyzed by using analysis of variance (ANOVA) and the Duncan's multiple range tests at a significance level of 0.05.

3. Results

3.1. Effects of exogenous ATP on browning, disease and consumer acceptance

Pericarp browning of longan fruit in both control and 0.5 mM ATP treated groups rapidly increased with increasing storage time and became unacceptable on Day 2 with the browning index of more than 3 (Fig. 1A). However, treatment with 1 or 2 mM ATP significantly hindered browning of the pericarp. Browning index slowly increased and reached the unacceptable level on Day 4. Similarly, darkening of pericarp was also significantly delayed under treatment with the higher concentrations of ATP (Fig. 1B).

No disease symptoms were found during the first 4 days of storage in any of the treatments. Subsequently, the symptom was increasingly observed, reaching the maximum level of 2.6 on Day 7. The effects of delaying disease development were more pronounced with prior treatment with 1 and 2 mM ATP (Fig. 1C).

As shown in Fig. 1D, the overall consumer acceptance of the untreated control fruit decreased steadily and the rate of decrease was comparable to the treated ones. However, the score remained higher for 2 days longer in the fruit treated with 1 and 2 mM ATP (Fig. 1D).

3.2. Effects of ClO_2 on energy status

Upon exposure to ClO_2, ATP content was found to be increasing, however, the rate and the extent of which varied with the concentration used in the fumigation. At the lowest concentration (5 mg/L), ATP content was moderately higher than that of the control and started to decrease after 1 day of storage. On the other hand, ATP content of the fruit surged significantly and immediately after fumigation with higher concentrations of ClO_2 (10 and 25 mg/L) and continued to rise until reaching the highest levels on Day 1. It is notable that ATP content in these treatments seemed to decrease more rapidly once the peak was reached (Fig. 2A).

Throughout the storage, ADP content of every group slowly decreased to 71%. However, no significant changes in ADP content were found between the experiment groups (Fig. 2B).

The AMP content increased gradually throughout the storage time, however, the onset of the change depended upon whether the fruit was fumigated. Immediately after ClO_2 treatment a significantly lower level of AMP was found in comparison to that of the control. The content continued to be lower until Day 1 after which it steadily rose until the end of the experiment. No significant difference in the AMP content was found between each concentration (Fig. 2C).

Expectedly, EC changes during the storage followed the same trend as that of ATP content (Fig. 2D). ClO_2 treatment at any

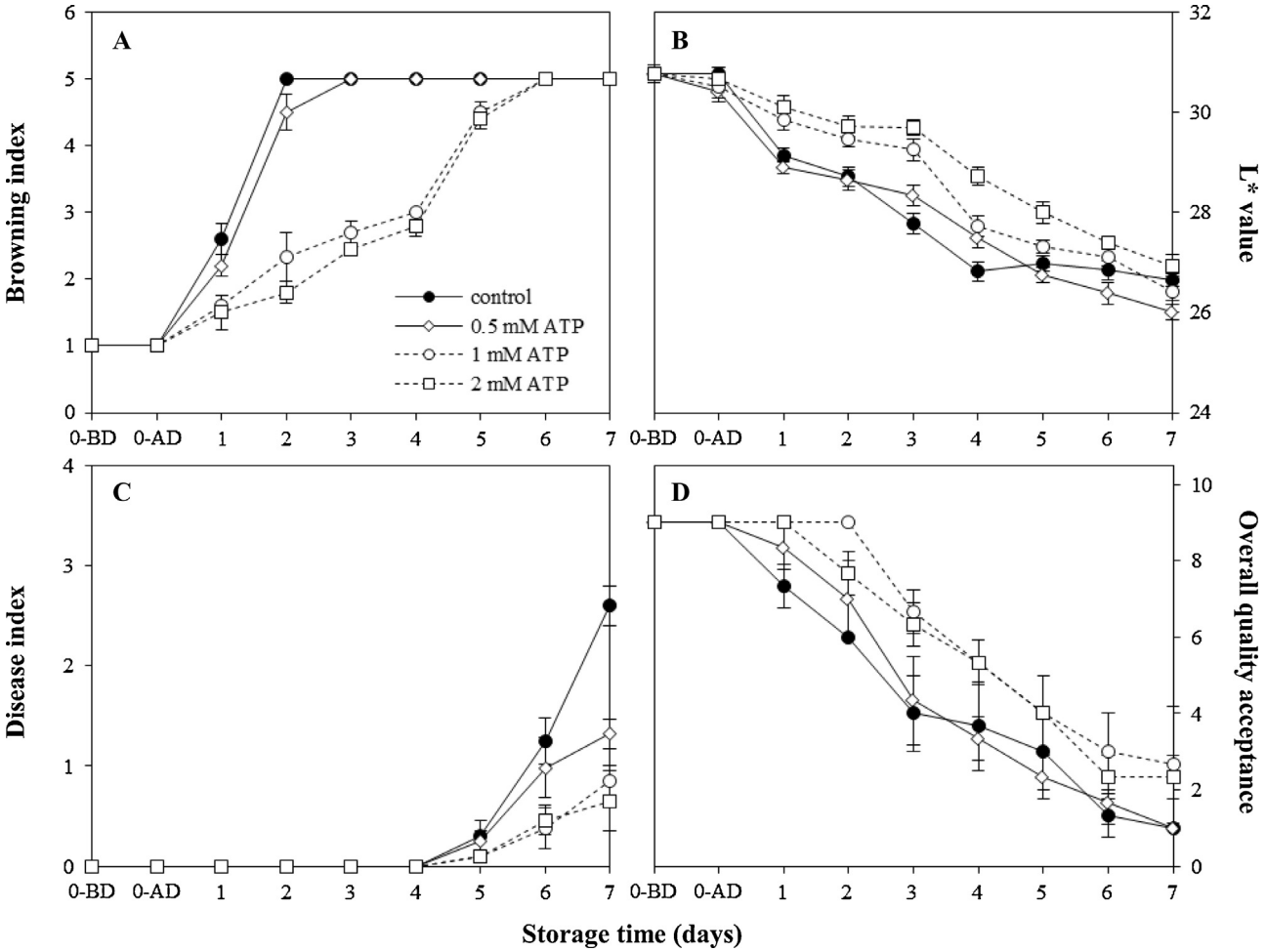

Fig. 1. Effects of exogenous ATP on browning index (A), L^* value (B), disease index (C) and overall quality acceptance (D) of 'Daw' longan pericarp during storage. BD: before dipping. AD: after dipping. Vertical bar indicates standard deviation.

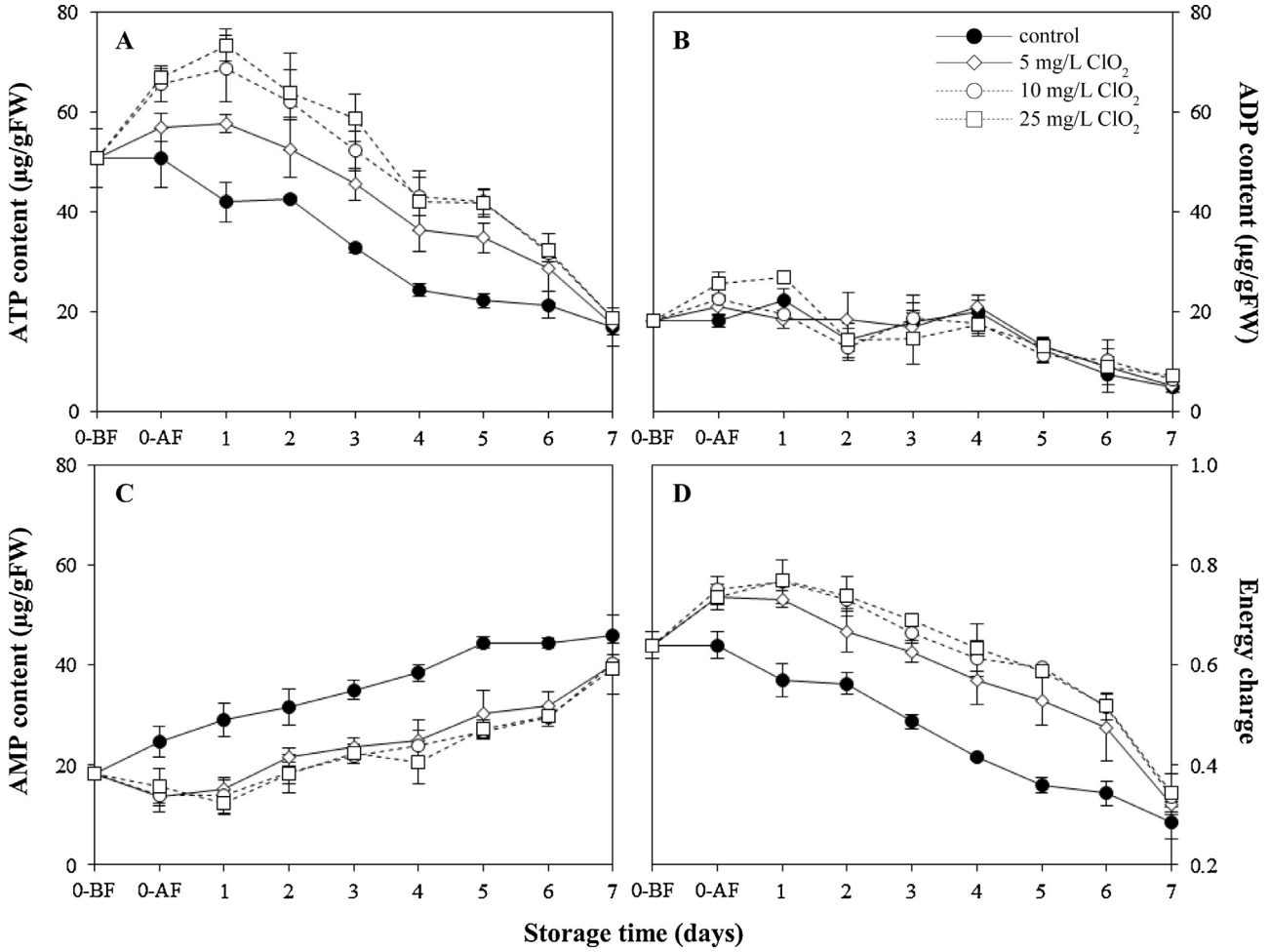

Fig. 2. Effects of chlorine dioxide fumigation on ATP content (A), ADP content (B), AMP content (C) and energy charge (D) of 'Daw' longan pericarp during storage. BF: before fumigation. AF: after fumigation. Vertical bar indicates standard deviation.

concentrations significantly increased EC immediately after fumigation and reached their respected highest EC value on Day 1 before gradually decreased thereafter. No significant difference in EC between the 10 and 25 mg/L treatments.

3.3. Effects of ClO_2 on SDH and CCO activities

As shown in Fig. 3A, SDH activity of the control fruit rapidly declined within the first day of storage and continued gradually

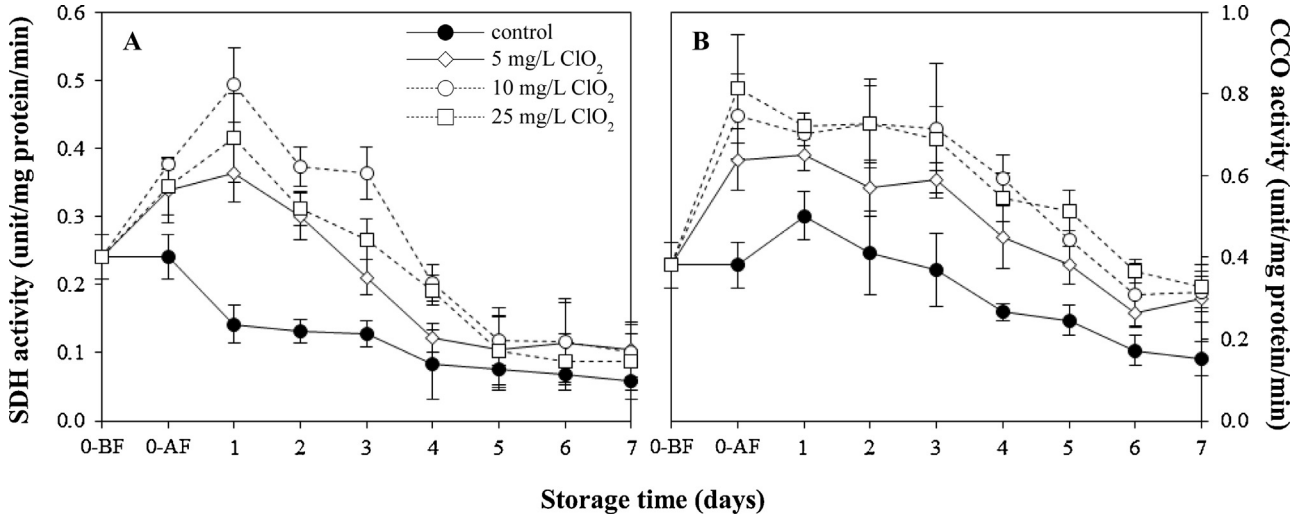

Fig. 3. Effects of chlorine dioxide fumigation on SDH (A) and CCO (B) activities of 'Daw' longan pericarp during storage. BF: before fumigation. AF: after fumigation. Vertical bar indicates standard deviation.

thereafter whereas that of the ClO_2 treated fruit was found to be increasing during the same period. In addition, the activity was significantly higher until Day 3 (5 mg/L) and Day 4 (10 and 25 mg/L). Treatment with 10 mg/L ClO_2 yielded maximum induction of SDH activity approximately 2.5 folds higher than that initially observed.

The changes in the activity of CCO differed from that of SDH. CCO activity of the control fruit increased until reaching the highest value on Day 1, then gradually lessened thereafter (Fig. 3B). In contrast, at all ClO_2 concentrations, CCO activity immediately surged to its respective highest level after fumigation. Treatments with 10 and 25 mg/L ClO_2 resulted in the highest increase in CCO activity (1.9 and 2.1 folds, respectively) during the storage. Interestingly, the rate of reduction in CCO activity was comparable regardless of whether or not the fruit was fumigated.

3.4. Effects of ClO_2 on pyridine nucleotide content and its redox state

In the control, NADH content was found to increase gradually until reaching the highest level on Day 4, after which time the level dropped slightly. The changes were more pronounced in those fumigated with ClO_2. Moreover, higher concentrations of ClO_2 yielded higher NADH contents during the course of storage (Fig. 4A).

As shown in Fig. 4B, the NAD content of control fruit gradually rose and reached the level of 33% higher at the end of storage. However, in all the ClO_2 treatments, NAD content sharply rise until the fourth day, then, fell significantly reaching the level of 66% higher on the last day of the experiment. At its peak, NAD content was 2.5 folds higher when fruit were fumigated with 10 and 25 mg/L ClO_2.

The NAD/NADH ratio of the control only slightly decreased remaining in a more reduced state throughout the time of experiment. In contrast, ClO_2 treatments caused changes in the ratio as the cell was more oxidized. The ratio was highest on Day 4 (10 and 25 mg/L) and Day 5 (5 mg/L) before falling down to about 1.0 at the end (Fig. 4C).

3.5. Effects of ClO_2 on ubiquinone content and its redox state

The QH_2 content of the control fruit increased about 1 fold within 1 day of storage and gradually decreased until Day 3. The level dropped sharply within 1 day before reaching plateau. ClO_2 treatments caused a rapid increase in QH_2 content. The level stayed significantly higher than that of the control during storage. It was interesting to observe that immediately upon reaching the highest respective levels, QH_2 content fell suddenly to the starting level (Fig. 5A).

The Q content of the untreated control showed little fluctuation throughout the experiment. In contrast, ClO_2 treatments resulted in continuous increase in Q content by approximately 3 folds. At 25 mg/L, the final Q concentration was about 3 times the original one (Fig. 5B).

In all experiments, the Q/QH_2 ratio was comparable during the first day. The control fruit exhibited slight decrease before dramatically rose on Day 3 to the level comparable with those treated with 5 mg/L ClO_2 at the end of storage. Treatments with 10 and 25 mg/L ClO_2, significantly higher Q/QH_2 ratios were found especially after the third day of storage (Fig. 5C).

3.6. Effects of ClO_2 on storage fruit quality

Pericarp browning of longan fruit in this experiment showed the same trend as in the ATP treatment experiment. Browning occurred with increasing storage time and became unacceptable on Day 2 of storage in the control group (Fig. 6A). ClO_2 treatments

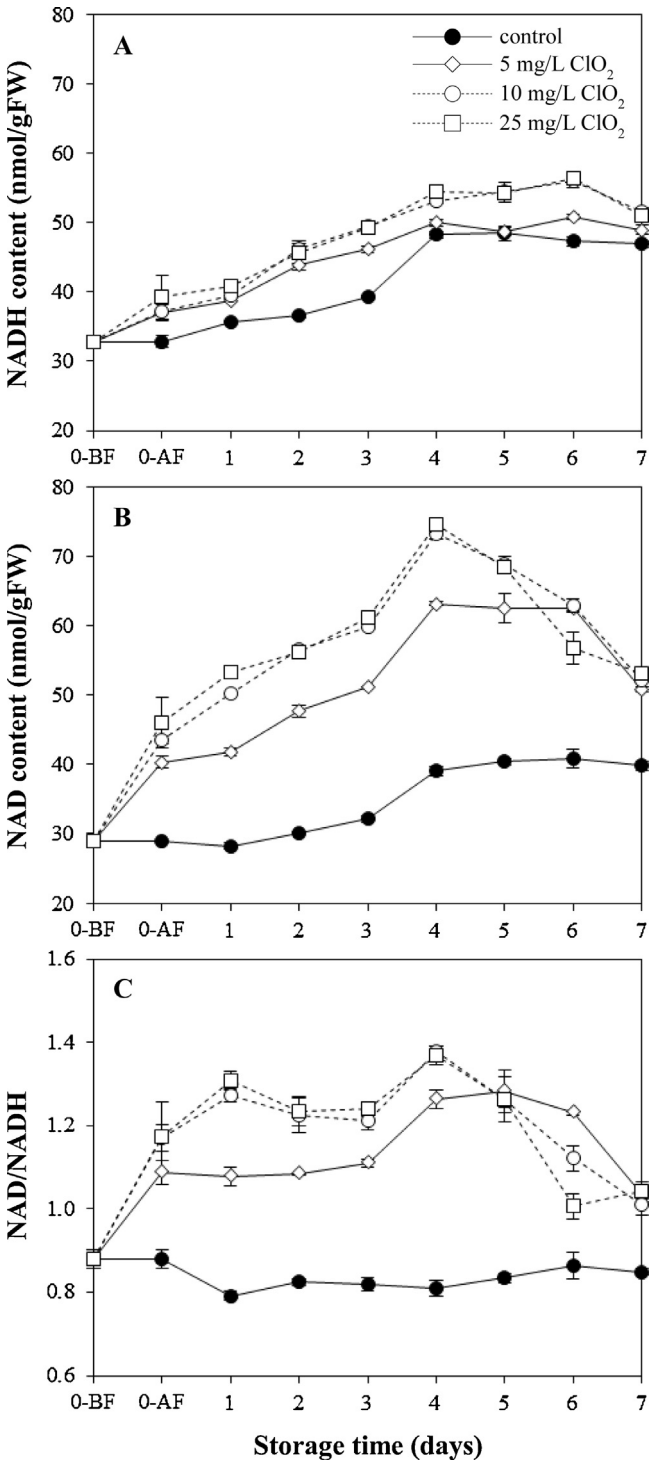

Fig. 4. Effects of chlorine dioxide fumigation on NADH content (A), NAD content (B) and NAD/NADH ratio (C) of 'Daw' longan pericarp during storage. BF: before fumigation. AF: after fumigation. Vertical bar indicates standard deviation.

delayed this further 2 (5 mg/L) or 4 days (10 and 25 mg/L). Changes in the browning index were not dissimilar when higher ClO_2 concentrations were used (Fig. 6A).

The color of pericarp, indicated by L^* value, of the control gradually decreased during storage. ClO_2 fumigation initially and significantly increased L^* value. The effects were prolonged until Day 3. Pericarp color darkened continuously thereafter (Fig. 6B).

As shown in Fig. 6C, no diseases developed during the first 4 days of storage for the control fruit. Subsequently, the disease

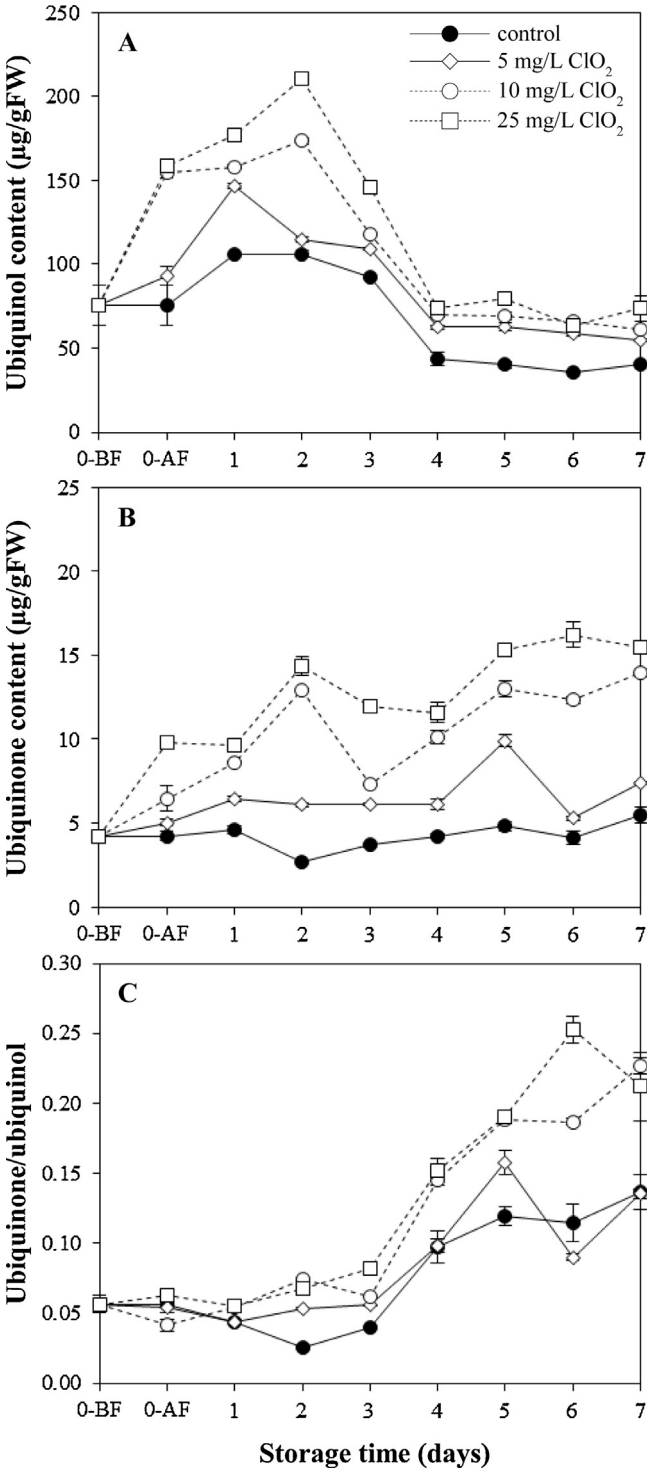

Fig. 5. Effects of chlorine dioxide fumigation on ubiquinol content (A), ubiquinone content (B) and ubiquinone/ubiquinol ratio (C) of 'Daw' longan pericarp during storage. BF: before fumigation. AF: after fumigation. Vertical bar indicates standard deviation.

symptom increased with increasing storage time. ClO_2 fumigation delayed the onset of disease development one day longer and caused the symptom to be less observable (Fig. 6C).

The overall consumer acceptance of untreated control fruit rapidly decreased and became unacceptable on Day 3 of storage with an acceptance score below 5 (Fig. 6D). ClO_2 fumigation at the concentration of 5 and 10 mg/L delayed the decrease in the consumer acceptance score with the latter being the most effective. The acceptance score of fruit fumigated with 25 mg/L ClO_2 was surprisingly lowest reaching the unacceptable level immediately after treatment (Fig. 6D).

4. Discussion

Senescence of longan fruit, characterized by browning of the pericarp, can be seen within 1–2 days after harvest if kept at ambient temperature (Saengnil et al., 2014; Chomkitichai et al., 2014a). Hence, it is important to alter or retard the mechanism of senescence in order to prolong and maintain the market value. ClO_2 was found to be effective in delaying the browning (Saengnil et al., 2014). The mechanism of action, however, has not been thoroughly understood. The regulation of fruit senescence is influenced by various internal factors and environmental stresses (Jiang et al., 2002; Chomkitichai et al., 2014a,b). Since an association between energy metabolism and control of plant senescence has been previously reported in litchi (Yang et al., 2009; Yi et al., 2010) and longan (Yao et al., 2014). It was interesting to see if alteration in cellular energy could be one of the underlying reasons why ClO_2 can delay senescence.

In the present study, prior application of exogenous ATP resulted in maintaining fruit quality and consumer acceptance of 'Daw' longan fruit during storage by lessening and delaying pericarp browning and reducing fruit disease during storage (Fig. 1A–D). The mechanism by which ATP plays a vital role in delaying senescence may comprise of maintaining membrane integrity and activating the accumulation of endogenous antioxidants and natural defense compounds, including phytoalexins and pathogensis-related proteins to survive the stresses. Yi et al. (2008) reported that exogenous ATP maintained membrane integrity by reducing the oxidation and degradation of membrane lipid in litchi fruit, resulting in the delay of senescence and disease. Delaying fruit senescence in litchi and longan by treatment with various compounds has been tied to stabilizing cell membranes (Wu et al., 2011; Wang et al., 2013; Chomkitichai et al., 2014a) and enhancing in natural defense compound biosynthesis (Yi et al., 2008, 2010; Chomkitichai et al., 2014b).

Although, exogenous treatment with ATP has been clearly shown in this work and the others (Yi et al., 2008, 2010; Yang et al., 2009; Yao et al., 2014) to delay fruit senescence during storage, this practice has not been commercially applied. Our previous study demonstrated that ClO_2 fumigation greatly delayed senescence and maintained the quality of longan during storage (Saengnil et al., 2014). In this study, the possibility that ClO_2 treatment alters internal energy and postpone senescence was explored.

It is generally believed that in order to survive either biotic or abiotic stress, plant cells consume generous amount of energy. Parallel decrease in energy status due to various stresses have been reported in peach (Jin et al., 2013, 2014), blueberry (Zhou et al., 2014) and banana (Wang et al., 2015b). ATP was suggested to play an important role in preventing accumulation of reactive oxygen species and maintaining unsaturated fatty acid contents, membrane integrity (Yi et al., 2008; 2010; Yang et al., 2009; Wang et al., 2013) and reduction of senescence or injury in litchi (Yi et al., 2008; Wang et al., 2013), mango (Li et al., 2014) and banana (Wang et al., 2015b). If left unchecked, reactive oxygen species rapidly damage membrane by oxidizing the unsaturated lipids rendering it vulnerable to postharvest pathogens. Moreover, oxidation of phenolic compounds by reactive oxygen species gives rise to browning substance. Both disease development and browning were used as an indicator of fruit senescence in previous reports (Saengnil et al., 2014; Chumyam et al., 2015) as well as in the present study.

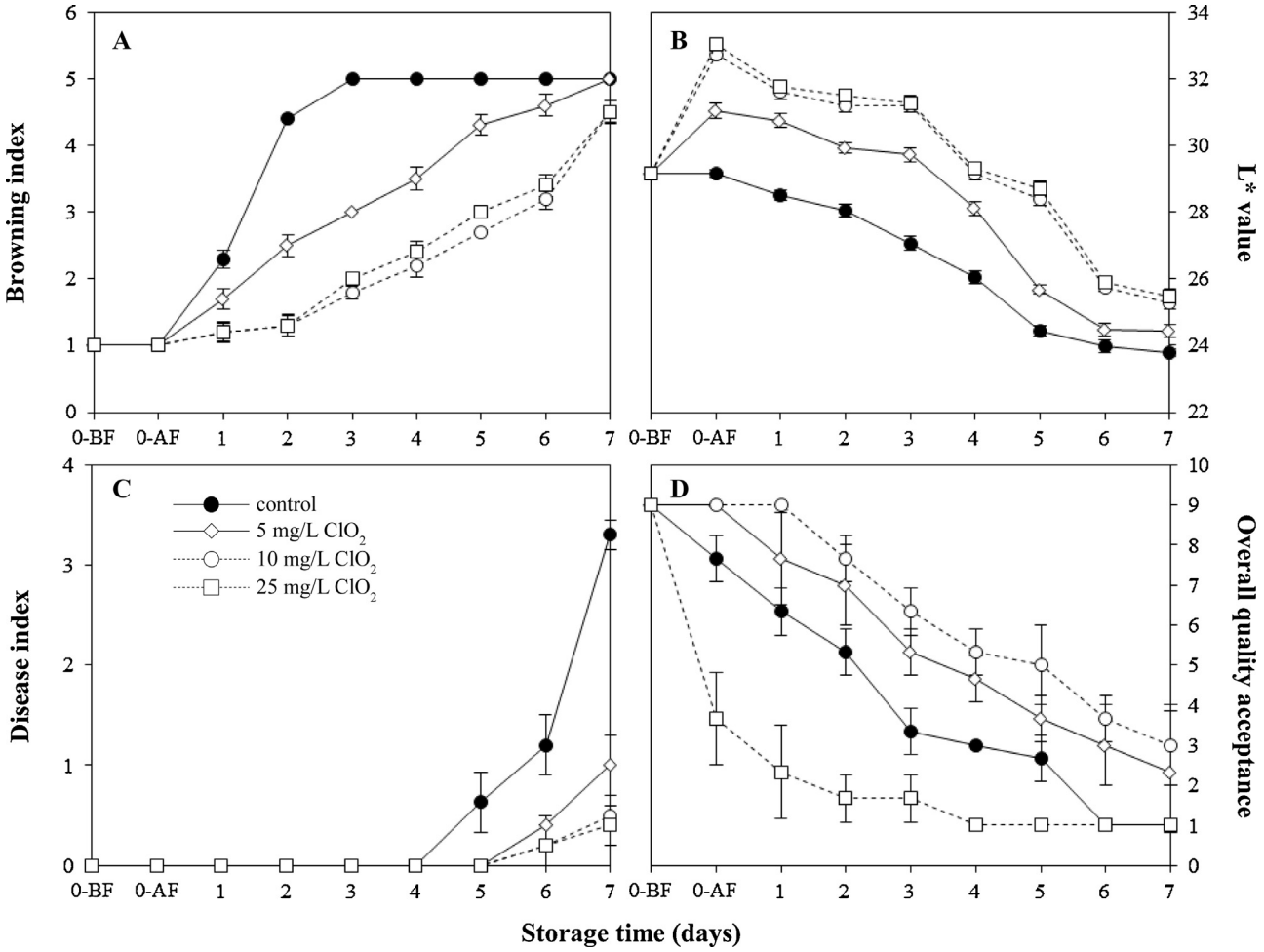

Fig. 6. Effects of chlorine dioxide fumigation on browning index (A), L^* value (B), disease index (C) and overall quality acceptance (D) of 'Daw' longan pericarp during storage. BF: before fumigation. AF: after fumigation. Vertical bar indicates standard deviation.

Our results clearly demonstrated both rapid decrease in ATP and dramatic rise in AMP during the experimental period suggesting elevated energy consumption during the storage. Without ATP or ClO_2 treatment, senescence rapidly ensued (Figs. 1 and 6). ClO_2 treatment immediately increased ATP level and delayed the onset of senescence (Figs. 2 and 6). These findings suggest that ATP plays an important role by which ClO_2 delays senescence of longan during storage. Longan is well-known for its sweetness. Freshly squeezed longan juice has a brix value of as high as 20–25 (20 g/100 g FW) (Yunchalad et al., 2008). Hence, there is ample supply of sugar as fuel for ATP production. From these results, the effect of ClO_2 on the energy state is obvious. It is further investigated by examining the activity of some respiratory enzymes and its outcomes on the redox state.

The possibility that ClO_2 treatments influence the activity of key respiratory enzymes such as SDH and CCO was explored. SDH is the only enzyme that acts on both the tricarboxylic acid (TCA) cycle and the aerobic respiratory chain, simultaneously catalyzing the oxidation of succinate to fumarate and the reduction of Q to QH_2. CCO is the last enzyme in the mitochondrial electron transport chain. The enzyme catalyzes the transfer of electrons to O_2 while creating the proton gradient needed for ATP synthesis (Vedel et al., 1999; van Dongen et al., 2011; Soole and Menz, 2013). It has been shown that chemicals such as MJ, OA and NO promoted SDH and CCO activities which increase the ATP content and EC in peach (Jin et al., 2013, 2014) and banana (Wang et al., 2015b).

Soon after treatment, the SDH activity reached maximum on Day 1 (Fig. 3A) while the CCO activity was found to be significantly higher compared to the untreated group (Fig. 3B). Both stimulations were concentration dependent. Even though the activity of the enzymes increased, it seemed that they responded to ClO_2 differently. The former was solely activated by ClO_2 while the latter responded to both ClO_2 and other cellular stimuli since increasing CCO activity was also found in the control fruit. It is not known if ClO_2 directly acts on the enzymes or cellular receptor is required.

The increase in SDH activity (Fig. 3A) corresponded well with the initial rise in ATP content (Fig. 2A) in all the concentrations used. In addition, CCO activity was also higher at the same time (Fig. 3B). It can be concluded that the surge in ATP content was due to the amplification of these enzymes activities.

The redox state, as indicated by Q/QH_2 ratio, remained steady during the first day regardless of experimental conditions implying that both Q and QH_2 were maintained at approximately the same level (Fig. 5C). The initial increase in QH_2 may not be due to SDH since its activity was declining during that time (Fig. 3A). The effect of ClO_2 on Q content is clearer. It could be concluded that ClO_2 activates Q levels in a concentration-dependent manner.

As one might expect, the greater amount of QH_2 should result in more ATP being produced. However, the present result showed an immediate drop in ATP content in the control, or right after the initial increase in ClO_2 treated fruit (Fig. 2A). Excessive QH_2 changes could tip the redox state of the cell such that the alternative oxidase (AOX) pathway is activated in order to prevent

damage. Similar AOX stimulation by alteration of Q/QH_2 has been shown in bell pepper and cauliflower (Popov et al., 2001) and litchi (Yang et al., 2009).

An increase NAD levels is modulated in response to environmental factors during fruit storage, such as water deficit, nutrition deficit, drought, wound and aging (Ying, 2008; Stein and Imai, 2012). In this study, it was found that NAD in longan pericarp increased during storage regardless of treatments (Fig. 4B), indicating that NAD levels in the tissue was activated. Cells could prepare themselves to cope with the ongoing stress through ATP production, therefore, NAD must be available to be used in glycolysis and TCA cycle. In addition, the extent of NAD levels was dependent on the concentration of ClO_2 being used (Fig. 4B), suggesting that ClO_2 also acts on the mechanism of increase in their levels. The levels ceased on Day 4 in all experimental scenarios indicated that dwindling availability of starting compounds may be the limiting factor.

When comparing between ClO_2 treated and untreated groups, the rate of increment in the NADH content was comparable suggesting that the treatment has no effects on NADH levels. In addition, when NAD levels stopped, the NADH content correspondingly leveled off. It is speculated that ClO_2 treatment does not contribute to any increase in NADH, instead, NAD upregulation of isocitrate dehydrogenase, one of the key enzyme in the TCA cycle, is the main factor in the increase.

It is of interest to note the effects of ClO_2 on the cellular redox status. The NAD/NADH ratio was directly affected by the treatments (Fig. 4C) while the changes in Q/QH_2 ratio seems to arise from both ClO_2 and other unknown cellular component.

ClO_2 has been suggested as a bleaching agent (Tarvo, 2010). Thus, it is possible that the observed delays in senescence may be due to the bleaching effect of ClO_2. L^* value was found to be increased by 13.3, 12.2 and 6.4% immediately after fumigated with 25, 10 and 5 mg/L ClO_2, respectively (Fig. 6B). Thus, the pericarp color became lighter or bleached. However, we do not believe that the reduction in pericarp browning by ClO_2 was only due to bleaching. Studies by Mahovic et al. (2006, 2007),) demonstrated the use of ClO_2 treatment in surface sterilization and prevention of infection on unintact tissue of harvested tomato. The studies also described observable bleaching of the unintact tissue at much higher concentrations (88 and 99 mg/L) than those employed in the present work. In addition, L^* value observed in ClO_2 treatments exhibited similar declining trend as those of ATP treated fruit during the first 3 days after treatment (Figs. 1 B and 6 B). Our results suggested that delaying senescence by ClO_2 may be due to the fact that ClO_2 biochemically increases energy levels and cosmetically lightens the pericarp color.

In conjunction with the previous report by Saengnil et al. (2014), this study confirmed the benefits of ClO_2 during storage by the reduction of browning and disease development and maintaining longan fruit quality (Fig. 6A–C). However, the treated fruit must be subjectively tested before commercial usage is recommended. The overall quality of acceptance clearly showed that, although ClO_2 at 25 mg/L was most effective in maintaining the fruit quality during storage, the treated fruit was unpalatable by the test panel (Fig. 6D). This was due to strong odor in the fumigated fruit. Therefore, comparable effective concentration of 10 mg/L should be employed in the future commercial trials.

In conclusion, this study demonstrated that the availability of ATP directly affected the quality of 'Daw' longan fruit during storage. ClO_2 treatment increased ATP content in the fruit by means of activating the respiratory enzymes, SDH and CCO, as well as altering the cellular redox status by increasing NAD and Q levels.

Acknowledgements

This study was financially supported by a grant from The Science Achievement Scholarship of Thailand (SAST), Office of the Higher Education Commission, Bangkok, Thailand and Faculty of Science and the Graduate School, Chiang Mai University, Chiang Mai, Thailand.

References

Apai, W., 2010. Effects of fruit dipping in hydrochloric acid then rinsing in water on fruit decay and browning of longan fruit. Crop Prot. 29, 1184–1189.
Brugidou, C., Rocher, A., Giraud, E., Lelong, B., Marin, B., Raimbault, M., 1991. A new high performance liquid chromatographic technique for separation and determination of adenylic and nicotinamide nucleotides in *Lactobacillus plantarum*. Biotechnol. Tech. 5, 475–478.
Chen, Y., Lin, H., Jiang, Y., Zhang, S., Lin, Y., Wang, Z., 2014. *Phomopsis longanae* Chi-induced pericarp browning and disease development of harvested longan fruit in association with energy status. Postharvest Biol. Technol. 93, 24–28.
Chomkitichai, W., Chumyam, A., Rachtanapun, P., Uthaibutra, J., Saengnil, K., 2014a. Reduction of reactive oxygen species production and membrane damage during storage of 'Daw' longan fruit by chlorine dioxide. Sci. Hortic. 170, 143–149.
Chomkitichai, W., Faiyue, B., Rachtanapun, P., Uthaibutra, J., Saengnil, K., 2014b. Enhancement of the antioxidant defense system of post-harvested 'Daw' longan fruit by chlorine dioxide fumigation. Sci. Hortic. 178, 138–144.
Chumyam, A., Kunthawun, D., Bussaban, B., Uthaibutra, J., Saengnil, K., 2015. Effects of ClO_2 fumigation on postharvest fungi and disease development of longan fruit. Acta Hortic. 1088, 339–344.
Jiang, Y.M., Li, Y.B., 2001. Effects of chitosan coating on postharvest life and quality of longan fruit. Food Chem. 73, 139–143.
Jiang, Y.M., Zhang, Z., Joyce, D.C., Ketsa, S., 2002. Postharvest biology and handling of longan fruit (*Dimocarpus longan* Lour.). Postharvest Biol. Technol. 26, 241–252.
Jin, P., Zhu, H., Wang, J., Chen, J.J., Wang, X., Zheng, Y.H., 2013. Effect of methyl jasmonate on energy metabolism in peach fruit during chilling stress. J. Sci. Food. Agric. 93, 1827–1832.
Jin, P., Zhu, H., Wang, L., Shan, T., Zheng, Y., 2014. Oxalic acid alleviates chilling injury in peach fruit by regulating energy metabolism and fatty acid contents. Food Chem. 161, 87–93.
Juszczuk, I.M., Ostaszewska, M., 2011. Respiratory activity, energy and redox status in sulphur-deficient bean plants. Environ. Exp. Bot. 74, 245–254.
Li, P., Zheng, X., Liu, Y., Zhu, Y., 2014. Pre-storage application of oxalic acid alleviates chilling injury in mango fruit by modulating proline metabolism and energy status under chilling stress. Food Chem. 142, 72–78.
Liu, H., Jiang, Y.M., Luo, Y., Jiang, W., 2006. A simple and rapid determination of ATP, ADP and AMP concentrations in pericarp tissue of litchi fruit by high performance liquid chromatography. Food Technol. Biotechnol. 44, 531–534.
Lowry, O.H., Rosebrough, N.J., Far, A.L., Randall, R.J., 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193, 265–275.
Mahovic, M.J., Bartz, J.A., Berry, A.D., Sargent, S.A., 2006. Postharvest treatment of tomato fruit with chlorine dioxide gas: dose affects fruit quality. Proc. Fla. State Hort. Soc. 119, 340–342.
Mahovic, M.J., Tenney, J.D., Bartz, J.A., 2007. Applications of chlorine dioxide gas for control of bacterial soft rot in tomatoes. Plant Dis. 91, 1316–1320.
Ostaszewska, M., Juszczuk, I.M., Kołodziejek, I., Rychter, A.M., 2014. Long-term sulphur starvation of *Arabidopsis thaliana* modifies mitochondrial ultrastructure and activity and changes tissue energy and redox status. J. Plant Physiol. 171, 549–558.
Popov, V.N., Eprintsev, A.T., Fedorin, D.N., Igamberdiev, A.U., 2010. Succinate dehydrogenase in *Arabidopsis thaliana* is regulated by light via phytochrome A. FEBS Lett. 584, 199–202.
Popov, V.N., Purvis, A.C., Skulachev, V.P., Wagner, A.M., 2001. Stress-induced changes in ubiquinone concentration and alternative oxidase in plant mitochondria. Biosci. Rep. 21, 369–379.
Potters, G., Horemans, N., Jansen, M.A., 2010. The cellular redox state in plant stress biology—a charging concept. Plant Physiol. Biochem. 48, 292–300.
Saengnil, K., Chumyam, A., Faiyue, B., Uthaibutra, J., 2014. Use of chlorine dioxide fumigation to alleviate enzymatic browning of harvested 'Daw' longan pericarp during storage under ambient conditions. Postharvest Biol. Technol. 91, 49–56.
Saquet, A.A., Streif, J., Banerth, F., 2003. Energy metabolism and membrane lipid alterations in relation to brown heart development in 'Conference' pears during delayed controlled atmosphere storage. Postharvest Biol. Technol. 30, 123–132.
Schertl, P., Braun, H.P., 2014. Respiratory electron transfer pathways in plant mitochondria. Front. Plant Sci. 5, 1–11.
Soole, K.L., Menz, R.I., 2013. ATP in plant mitochondria: substrates, inhibitors, and uncouplers. Encycl. Biol. Chem. 2013, 141–144.
Stein, L.R., Imai, S., 2012. The dynamic regulation of NAD metabolism in mitochondria. Trends Endocrin. Met. 23, 420–428.
Su, X., Jiang, Y., Duan, X., Liu, H., Li, Y., Lin, W., Zheng, Y., 2005. Effects of pure oxygen on the rate of skin browning and energy status in longan fruit. Food Technol. Biotech. 43, 359–365.
Tarvo, V., 2010. Modeling chlorine dioxide bleaching of chemical pulp. School of Science and Technology, Aalto University.

Thavong, P., Archbold, D.D., Pankasemsuk, T., Koslanund, R., 2010. Postharvest use of hexanal vapor and heat treatment on longan fruit decay and consumer acceptance. Thammasat Int. J. Sci. Technol. 15, 54–63.
van Dongen, J.T., Gupta, K.J., Ramírez-Aguilar, S.J., Araújo, W.L., Nunes-Nesi, A., Fernie, A.R., 2011. Regulation of respiration in plants: a role for alternative metabolic pathways. J. Plant Physiol. 168, 1434–1443.
Vedel, F., Lalanne, E., Sabar, M., Chétrit, P., Paepe, R.D., 1999. The mitochondrial respiratory chain and ATP synthase complexes: composition, structure and mutational studies. Plant Physiol. Biochem. 37, 629–643.
Wagner, A.M., Wagner, M.J., 1995. Measurements of ubiquinone reduction levels in plant cells. Plant Physiol. 108, 277–283.
Wang, H., Qian, Z., Ma, S., Zhou, Y., Patrick, J.W., Duan, X., Jiang, Y., Qu, H., 2013. Energy status of ripening and postharvest senescent fruit of litchi (*Litchi chinensis* Sonn.). BMC Plant Biol. 13, 1–16.
Wang, J., You, Y., Chen, W., Xu, Q., Wang, J., Liu, Y., Song, L., Wu, J., 2015a. Optimal hypobaric treatment delays ripening of honey peach fruit via increasing endogenous energy status and enhancing antioxidant defense systems during storage. Postharvest Biol. Technol. 101, 1–9.
Wang, Y., Luo, Z., Khan, Z.U., Mao, L., Ying, T., 2015b. Effect of nitric oxide on energy metabolism in postharvest banana fruit in response to chilling stress. Postharvest Biol. Technol. 108, 21–27.
Wright, K.P., Kader, A.A., 1997. Effect of slicing and controlled-atmosphere storage on the ascorbate content and quality of strawberries and persimmons. Postharvest Biol. Technol. 10, 39–48.
Wu, B., Li, X., Hu, H., Lui, A., Chen, W., 2011. Effect of chlorine dioxide on the control of postharvest diseases and quality of lychee fruit. Afr. J. Biotechnol. 10, 6030–6039.
Yang, E., Lu, W.J., Qu, H.X., Lin, H.D., Wu, E.W., Yang, S.Y., Chen, Y.L., Jiang, Y.M., 2009. Altered energy status in pericarp browning of litchi fruit during storage. Pak. J. Bot. 41, 2271–2279.
Yao, F., Huang, Z., Li, D., Wang, H., Xu, X., Jiang, Y., Qu, H., 2014. Phenolic components, antioxidant enzyme activities and anatomic structure of longan fruit pericarp following treatment with adenylate triphosphate. Sci. Hortic. 180, 6–13.
Yi, C., Jiang, Y., Shi, J., Qu, H., Xue, S., Duan, X., Shi, J., Prasad, N.K., 2010. ATP-regulation of antioxidant properties and phenolics in litchi fruit during browning and pathogen infection process. Food Chem. 118, 42–47.
Yi, C., Qu, H.X., Jiang, Y.M., Shi, J., Duan, X.W., Joyce, D.C., Li, Y.B., 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.
Ying, W., 2008. NAD^+/NADH and $NADP^+$/NADPH in cellular functions and cell death: regulation and biological consequences. Antioxid. Redox Signaling 10, 179–206.
Yunchalad, M., Supasri, R., Boonbamrung, S., Wongkrajank, K., Hiraga, C., Watanasook, A., 2008. Pre-concentration of longan juice extract with microfiltration and reverse osmosis. Asian J. Food Ag-Ind. 1, 17–23.
Zhou, Q., Zhang, C., Cheng, S., Wei, B., Liu, X., Ji, S., 2014. Changes in energy metabolism accompanying pitting in blueberries stored at low temperature. Food Chem. 164, 493–501.