CHAPTER 5

Discussion

Experiment 1 Effects of gaseous ClO_2 fumigation on mitochondrial energy levels and redox status of harvested 'Daw' longan pericarp during storage

Fruit becomes nutrient-starved as soon as it is harvested. It has to turn to a more limited source, its own nutritional components, to maintain basic metabolic activities. This ultimately affects its state of energy production and biosynthesis including antioxidant-related enzymes. This experiment clearly demonstrated that antioxidant capacity of longan pericarp, as determined by ABTS, DPPH and FRAP assays, decreased steadily throughout the storage time (Figures 4.1-4.3).

The results of this present studies are in line with previous studies that both enzymatic and non-enzymatic antioxidant defenses of longan pericarp declined during storage. Chomkitichai *et al*. (2014b) reported that enzymatic antioxidant activity such as APX, SOD and CAT activities, and non-enzymatic antioxidants including total phenolic, ASA, GSH and α-tocopherol contents of longan pericarp decreased during storage. The continuous decrease in ABTS and DPPH antioxidant activities indicated that ROS scavenging capacity in longan pericarp deteriorates with time. The decrease in FRAP also signified the loss of electron donation capacity or reducing power of longan pericarp similar to what found in 'Shixia' (Cheng *et al*., 2009) and 'Daw' longan fruit (Chomkitichai *et al*., 2014b) as well as 'Huaizhi' litchi fruit (Duan *et al*., 2011).

The decrease in antioxidant capacity (Figures 4.1-4.3) coincided with the reduction in overall quality acceptance and increase in pericarp browning and fruit disease (Figures 4.4-4.7). Statistical analyses showed correlation between senescence of harvested fruits during storage and deficiency in energy supply. It was found that antioxidant capacity was significantly and positively correlated with the overall quality

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acceptance (r = 0.916-0.982) as well as negatively correlated with browning index (r = -0.828 - -0.926) during storage. Thus, the reduction in antioxidant capacity is the underlying reason of fruit deterioration. As pericarp appearance is a key factor determining consumer preference, preserving antioxidant capacity is therefore importance to fruit quality during storage.

It is interesting to note that postharvest supplementation of ATP in litchi led to higher intracellular ATP and energy charge levels delaying fruit senescence (Wang *et al*., 2013) and inhibited pericarp browning (Yang *et al*., 2009). Thus, it is possible that ATP supplement may delay senescence through elevated intracellular ATP or cellular energy states. Previous studies indicated that exogenous ATP treatment has a positive impact on preventing microbial growth and delaying senescence in litchi (Yi *et al*., 2008; 2010; Yang *et al*., 2009) and longan fruits (Yao *et al*., 2014; Chen *et al*., 2015). In the present study, exogenous ATP treatment reduced and delayed pericarp browning indicated by lower browning index and higher L* value, reduced fruit disease and maintained higher quality acceptance of 'Daw' longan (Figures 4.4-4.7). Moreover, ATP concentration used was significantly and positively correlated with overall quality acceptance (r = 0.816) as well as negatively correlated with BI (r = -0.634) (Table 4.1), indicating that ATP concentration is important in maintaining fruit quality.

Exposure to ATP rapidly induced antioxidant capacity. The effects were more pronounced with 1 and 2 mM ATP (Figures 4.1-4.3). Statistical analysis of the data showed that the increase was significantly and positively correlated with ATP concentration (r = 0.719-0.823). It is speculated that an ATP receptor such as the plasma membrane-localized P2K1/DORN1 receptor binds to extracellular ATP and triggers Ca^{2+} release from cellular storage (Geigenberger *et al*., 2009; Tanaka *et al*., 2010; 2014). Formation of ROS by membrane-localized NADPH oxidase as well as increase in NO follow (Wu and Wu, 2008; Demidchik *et al*., 2009; Reichler *et al*., 2009; Shang *et al*., 2009; Sun *et al*., 2012). These second messengers cause physiological responses including activation of antioxidant gene expression, including APX, CAT and SOD (Yi *et al*., 2010). This study found the increases in antioxidant capacity in the ATP treated group in a concentration dependent manner (Figures 4.1-4.3). This could be the result of insufficient stimulation of the receptor since the

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minimum concentration at which ATP serves as a signaling molecule is between 1-10 mM (Choi *et al*., 2014).

Statistical analysis showed that treatment with both 1 and 2 mM ATP yield no significant differences in inducing antioxidant capacity and maintaining overall quality acceptance. This is in line with previous studies that the optimum ATP concentration to induce antioxidants or delay senescence in harvested longan (Yao *et al*., 2014; Chen *et al*., 2015) and litchi (Yi *et al*., 2008; 2010; Yang *et al*., 2009; Wang *et al*., 2013) fruit was found to be 1 mM. Though, the optimum concentration varies depending on species, for example, cut carnation flowers require 0.1 mM (Song *et al*., 2008). It is recommended that 1 mM ATP is the optimum concentration for 'Daw' longan fruit. Although, the benefit of exogenous treatment with ATP has been clearly demonstrated both in earlier and this work (Yang *et al*., 2009; Yi *et al*., 2008; 2010; Yao *et al*., 2014), the practice has not seen commercial adoption.

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. 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 pathogenesis-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 lipids in litchi fruit, resulting in the delay of senescence and disease.

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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, to prolong and maintain market value, senescence must be swiftly prevented immediately after picking. Various means have been tried with different levels of success. ClO_2 was found to be effective in delaying browning (Saengnil *et al*., 2014), the mechanism of action of which, however, was not known. 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 is able to delay senescence.

This study clearly demonstrated both rapid decrease in ATP and dramatic rise in AMP during the experimental period (Figures 4.8-4.10) suggesting elevated energy consumption. Without sufficient ATP supply, senescence rapidly ensued (Figures 4.20-4.23). ClO_2 treatment immediately increased cellular ATP level, delaying the onset of senescence (Figures 4.8-4.10 and 4.20-4.23). These findings suggest that ATP plays an important role by which ClO_2 delays senescence. How ClO_2 affects energy state is further investigated.

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. It 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. These increase the ATP content and EC in peach (Jin *et al*., 2013; 2014) and banana (Wang *et al*., 2015b).

It seemed that ClO_2 affects SDH and CCO in a different way. SDH seemed to be solely activated by ClO_2 as its activity only climbed when treated (Figure 4.12). CCO activity, however, was found to be significantly higher compared to that of the control (Figure 4.13). This suggests that there may be additional activators that act on the enzyme in order to increase its activity. The increases in activity of both enzymes ultimately result in more ATP available. In ClO_2 treated fruit, the increase in SDH activity (Figure 4.12) corresponded well with the initial rise in ATP content and EC (Figures 4.8 and 4.11) in all the concentrations used. In addition, CCO activity was also higher at the same time (Figure 4.13). It can be concluded that the surge in ATP content was due to the amplification of these enzymes activities. It should be noted that whether ClO_2 acts directly on the enzymes or any cellular receptor is not known.

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NAD^+ level altered in response to environmental factors during fruit storage, such as water deficit, nutrition deficit, drought, wound and aging (Ying, 2008; Stein and Imai, 2012). The results of this study are consistent with this notion. The level of NAD^+ during the first 4 days in longan pericarp increased during storage regardless of treatments (Figure 4.14). The elevated level may be due to either more NADH was consumed to produce ATP or more NAD^+ was synthesized. It is believed that the latter may be true. Treatment with ClO_2 increased the activity of the key respiratory enzymes, SDH and CCO (Figures 4.12 and 4.13) generating more ATP, thus, requiring more electrons from both NADH and $FADH_2$. However, NADH was produced faster than consumed in both ClO_2 treated and untreated groups (Figure 4.15). TCA cycle is the main NADH producing pathway. One of its main enzymes, isocitrate dehydrogenase was found to be upregulated by higher level of NAD^+. In addition, after Day 4, when NAD^+ had peaked and started to levels off, the level of NADH did so as well. It could be due to the dwindling supply of starting compounds.

The increase in NAD^+ compared to NADH undeniably altered NAD^+/NADH ratio (Figures 4.14-4.16). Alteration of NAD redox level by ClO_2 reached the highest level, 170% higher than the control, on Day 4 (Figure 4.14). The greater increase in NAD redox status lead to an increase in respiratory enzyme activities such as NADH dehydrogenase, cytochrome *c* reductase and CCO, as well as an alternative or nonphosphorylating dehydrogenase such as type II NAD(P)H dehydrogenase (van Dongen *et al*., 2011; Soole and Menz, 2013). The results of this study clearly demonstrated a positive correlation between NAD^+/NADH ratio, CCO activity and ATP production (Figures 4.13, 4.16 and 4.8). The positive relation between NAD^+/NADH ratio and ATP production has also been reported in bean seedlings (Juszczuk and Ostaszewska, 2011) and *Arabidopsis* (Ostaszewska *et al*., 2014).

The redox state, as indicated by Q/QH_2 ratio, remained steady during the first days whether or not ClO_2 was applied. This indicated that both Q and QH_2 were maintained at approximately the same level (Figure 4.19). The initial increase in QH_2 may not be due to SDH since its activity was declining during that time (Figure 4.18). 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.

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As one might expect, the greater amount of QH_2 should result in more ATP being produced. However, the present study found an immediate drop in ATP content in the control, or right after the initial increase in ClO_2 treated fruit (Figure 4.8). 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). 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 while the changes in Q/QH_2 ratio seems to arise from both ClO_2 and other unknown cellular components.

ClO_2 has been used as a bleaching agent (Tarvo, 2010). Fumigation with ClO_2 seemed to lighten the color of pericarp as L* value was found to increase immediately after fumigation (Figures 4.21), hence, the bleached pericarp could be determined as delayed senescence. This is not the case, however. 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 described observable bleaching at much higher concentrations (88 and 99 mg L^{-1}) 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 (Figures 4.8 and 4.21). Thus, delaying senescence by ClO_2 may be due to the fact that ClO_2 biochemically increases energy levels but not cosmetically lightens the pericarp color.

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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 (Figures 4.20-4.23). However, the treated fruit must be subjectively tested for commercial acceptance. The overall quality of acceptance tests clearly showed that, although ClO_2 at 25 mg L^{-1} was most effective in maintaining the fruit quality during storage, the treated fruit was unpalatable (Figure 4.23). This was due to strong odor in the fumigated fruit even though there are not any negative effects on the aril. Therefore, the comparably effective concentration of 10 mg L^{-1} should be employed in the future commercial trials.

From the results of this study, a model by which ClO_2 modulates the redox state of NADH and Q to enhance ATP production was proposed (Figure 5.1).

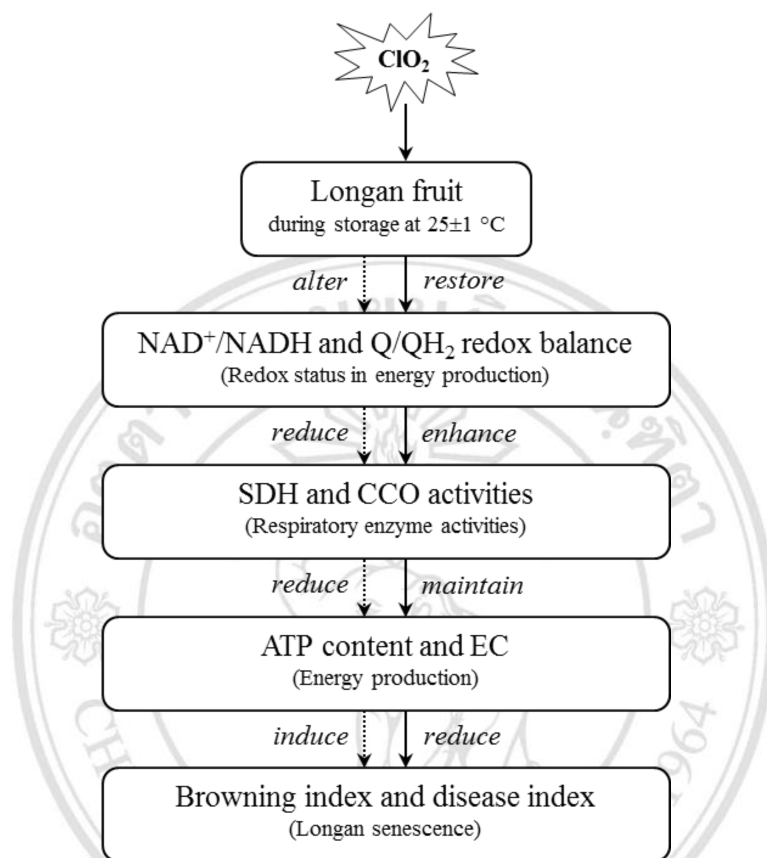

Figure 5.1 Probable mechanism for fruit senescence through energy production (dash arrows) and effects of ClO_2 on fruit senescence alleviation (solid arrows) of 'Daw' longan during storage at 25±1°C

Experiment 2 Effects of gaseous ClO_2 fumigation on redox status in free radical scavenging of harvested 'Daw' longan pericarp during storage

Recent study demonstrated that ClO_2 is able to reduce and delay senescence of longan (Saengnil *et al*., 2014). One of its anti-senescence mechanisms may involve enhancement of antioxidant defense system in order to reduce membrane oxidative damage (Chomkitichai *et al*., 2014a; 2014b). However, the mechanism by which redox regulation of cellular antioxidant defense system of longan maintains fruit quality during storage is not known. An association between redox status and control of plant senescence has been previously reported in many economically important plants such as

broccoli (Mori *et al*., 2009), loquat (Cai *et al*., 2011), tomato (Zhu and Tian, 2012; Manai *et al*., 2014) and plum (Singh and Singh, 2013). It is interesting to see if this alteration in the antioxidant redox balance could be one of the underlying reasons why ClO_2 can delay senescence and maintain the quality of harvested longan.

The redox ratio of ASA/DHA or GSH/GSSG are considered as a marker of cellular redox balance and oxidative stress (Kumari *et al*., 2010). In the present study, the possibility that a disturbance in ASA, GSH and NADPH redox balance may affect the quality of longan during storage was explored. The results clearly demonstrated that ASA/DHA, GSH/GSSG and NADPH/$NADP^+$ ratios markedly decreased during storage (Figures 4.26, 4.29 and 4.32) which coincided with an increase in H_2O_2 and fruit senescence (Figures 4.20-4.23 and 4.39). It is possible that the reduction in these ratios down-regulates reactive oxygen inactivating enzymes. Moreover, browning and disease were significantly and positively correlated with H_2O_2 levels (Appendix: Table 40). As a result, accumulated H_2O_2 accelerates oxidative damage and promotes cellular senescence (Ramakrishna and Rao, 2013; Chomkitichai *et al*., 2014a; 2014b).

The four enzymes (APX, MDHAR, DHAR and GR) in the ASA-GSH pathway work in concert to remove H_2O_2. In non-fumigated fruit, these enzymes behaved differently during the storage. APX activity increased slightly during the first two days whereas MDHAR activity markedly increased in the first day of storage, then, decreased thereafter (Figures 4.33 and 4.35). On the other hand, DHAR and GR activities decreased over time (Figures 4.34 and 4.36). It is possible that water and nutrition stress upon picking longan fruit could trigger production and accumulation of ROS. H_2O_2 produced at the early stage of stress acts as an activation signal for APX to scavenge H_2O_2 and MDHAR to regenerate ASA from MDHA (Gill and Tuteja, 2010; Sharma *et al*., 2012; Chomkitichai *et al*., 2014b). Hence, as the activities of these enzymes generally decreased during the storage of the control fruit (Figures 4.33-4.36), the ability to scavenge H_2O_2 was undeniably diminished resulting in perceivable damage to the fruit. It is well known that MDHAR and DHAR play an important role in maintaining ASA level whereas GR plays important role in maintaining the GSH level for DHAR (Gill and Tuteja, 2010; Foyer and Noctor, 2011; Sharma *et al*., 2012; Pu and Ren, 2014). Although APX and MDHAR activities were stimulated, but the effective

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time was short as well as DHAR and GR activities were relatively low. This caused the reduction of ASA-GSH cycle potential for the detoxification of H_2O_2, resulting in the cellular oxidative damage (Sharma *et al*., 2012; Chomkitichai *et al*., 2014a; 2014b) indicating that APX and MDHAR in longan were mainly important in scavenging H_2O_2, which induced by oxidative stress early in the storage.

Induction of antioxidant defense system by elevated activity of enzymes of the ASA-GSH cycle in association with a well-coordinated cellular redox status of ASA and GSH in reduced form was observed in longan fruit during storage. ASA was oxidized to MDHA and converted to DHA (Figures 3.24 and 3.25). It was found that activities of ASA regenerating enzymes, MDHAR and DHAR, also decreased (Figures 4.34 and 4.35). A marked decline in ASA/DHA ratio and ASA content coincided with a reduction in APX and DHAR activities. When ASA/DHA ratio was lower than 0.25 on Day 3, the APX and DHAR activities sharply dropped and continuously declined during storage time (Figures 4.26, 4.33 and 4.34) indicating that lower ASA/DHA ratio down-regulated the promotion of APX and DHAR activities. The decrease in ASA/DHA ratio associated with activity loss of ASA-DHA pathway enzymes was also reported in broccoli (Mori *et al*., 2009), loquat (Cai *et al*., 2011) and plum (Singh and Singh, 2013).

GSH redox state has an important function in maintaining ASA-GSH cycle capacity (Noctor and Foyer, 1998). In addition, GSH/GSSG ratio and GSH were also found to play an indispensable role in detoxification of ROS (Anjum *et al*., 2012). During storage of non-fumigated longan fruit, GSH/GSSG ratio and GSH level continuously decreased (Figures 4.27 and 4.29). It is possible that GSH was used in ROS detoxification by various antioxidant enzymes such as DHAR, glutathione peroxidase (GPX) and glutathione-S-transferase (GST) (Ramakrishna and Rao, 2013), resulting in a decrease in GSH and increase in GSSG. Moreover, GR activities, the GSH regenerating enzymes, also decreased. This caused a marked decrease in GSH/GSSG ratio. The decrease coincided with a decrease in DHAR activity (Figures 4.29 and 4.34) indicating that imbalance of GSH redox state is a cause of ASA redox state imbalance through the reduction of DHAR and GR activities. The decrease in GSH/GSSG ratio and GSH content associated with activity loss of GSH-GSSG cycle enzymes, leading to

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the alteration of ASA redox state was also reported in broccoli (Mori *et al*., 2009), loquat (Cai *et al*., 2011) and plum (Singh and Singh, 2013).

NADPH is the primary source of reducing equivalents for the ASA-GSH cycle. The NADPH redox state ($NADPH/NADP^+$ ratio) plays a significant role in the regeneration of GSH by GR and the regeneration of ASA by MDHAR (Foyer and Noctor, 2011; Manai *et al*., 2014). In the present study, NADPH redox state declined gradually while the $NADP^+$ pool increased (Figures 4.31-4.32). It is possible that stresses such as water deficit, nutrition deficit, drought, wound or aging elevated $NADP^+$ biosynthesis to provide sufficient reducing power for ROS detoxification (Ying, 2008; Juszczuk and Ostaszewska, 2011; Manai *et al*., 2014). However, NADPH-generating dehydrogenases (G6PDH and 6PGDH) activity was down-regulated during storage time (Figures 4.37-4.38) causing a marked increase in $NADP^+$ level with a decrease in $NADPH/NADP^+$ ratio. Similar decline in NADPH redox balance has been reported in bean seedlings during sulphur-deficient stress (Juszczuk and Ostaszewska, 2011) while the down-regulation of G6PDH, 6PGDH and NADP-isocitrate dehydrogenase has also been reported in rice and tomato under salt stress (Zhang *et al*., 2013; Manai *et al*., 2014) and in lotus under drought stress (Signorelli *et al*., 2013). It was found that a decrease in $NADPH/NADP^+$ ratio closely related to down-regulation of GR and MDHAR activities after Day 3 (Figures 4.33-4.38) indicating that NADPH redox state directly affected GR. The changes in MDHAR may be most broadly regulated by NADPH redox state and other unknown cellular components such as ROS.

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ClO_2 showed a positive effect on both ASA/DHA and GSH/GSSG ratios immediately after fumigation and maintained higher redox level than those of the control (Figures 4.24-4.32) indicating a ClO_2-induced mechanism by which recycling of ASA and GSH is enhanced. The fact that ClO_2 maintained ASA/DHA ratios of longan during storage might be explained by increased activities of DHAR and MDHAR (Figures 4.34-4.35). Moreover, ClO_2 maintained GSH/GSSG ratio might be also explained by increased activities of GR enzymes (Figure 4.36). Higher GSH/GSSG ratio, in turn, up-regulated DHAR activity for maintaining the reduced state of ascorbate as described above. This indicated that enhanced enzyme activity in ASA and GSH metabolism (DHAR, MDHAR and GR) by ClO_2 played an important role in controlling

the redox states of ASA and GSH in longan fruit. Other chemicals such as NO, MJ, H_2S and jasmonic acid have shown similar DHAR, MDHAR and GR activity enhancing ability and their increase in ASA/DHA and GSH/GSSG ratios when applied to chickpea, tomato, maize and wheat (Kumari *et al*., 2010; Zhu and Tian, 2012; Shan *et al*., 2014; Dai *et al*., 2015). Interestingly, heat-shock treatment in mustard also showed similar effects (Hossain *et al*., 2013).

The increase in ASA/DHA and GSH/GSSG redox status by ClO_2 fumigation coincided with lower H_2O_2 content and fruit senescence index (Figures 4.20, 4.22 and 4.39). It is possible that ClO_2-induced alteration of redox status up-regulated APX activity to scavenge H_2O_2. APX stimulation occurred only in the first few days after which its activity declined coinciding with the increase in H_2O_2. These results were in line with the work of Ramakrishna and Rao (2013) that higher GSH redox status enhanced H_2O_2 scavenging capacity through GPX activity. The redox status of ASA and GSH was higher in the stress-tolerant plant than the sensitive one (Zagorchev *et al*., 2013) indicating that high ASA/DHA and GSH/GSSG ratios may play an important role in protection against oxidative stress in longan fruit. Similarly, maintaining higher status of ASA and GSH redox in relation to higher H_2O_2 scavenging capacity was also found in chickpea, tomato, maize, mustard and rice which induced by NO, MJ, heat-shock treatment, H_2S and trehalose, respectively (Kumari *et al*., 2010; Zhu and Tian, 2012; Hossain *et al*., 2013; Shan *et al*., 2014; Mostofa *et al*., 2015). Reduction in ROS such as H_2O_2 through ASA-GSH cycle by ClO_2 treatment could lessen oxidative damage and retard cell senescence, leading to maintained fruit quality. Moreover, ClO_2 could also reduce the production of ROS which cause membrane damage of longan fruit as reported by Chomkitichai *et al*. (2014a).

The effects of ClO_2 on ASA and GSH redox balancing potential of longan was associated with increased NADPH redox state. In the present study, the higher $NADPH/NADP^+$ ratio showed the capacity to up-regulate the ASA and GSH recycling enzyme activity including MDHAR and GR. A higher $NADPH/NADP^+$ ratio in ClO_2 treated fruit coincided with the higher MDHAR and GR activities, resulting in a higher ASA/DHA and GSH/GSSG ratios. Moreover, MDHAR was also activated by thioredoxin through NADPH-dependent thioredoxin reductase (NTR) system which has

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been reported in sweet potato (Huang *et al*., 2008). ClO_2 might induce MDHAR as well as GR through the NTR system for DHA and GSSG reduction. However, the relationship between NADPH redox potential and MDHAR and GR activities in harvested horticultural crops is not clear. The data presented here provide a glimse into this relationship. The effects of ClO_2 on GR activity was greatly induced (4-folds after fumigation) and maintained a higher value than that of the control over storage time (Figure 4.36) indicating that ClO_2 fumigation may enhance the capacity of antioxidative ASA-GSH cycle and the increased capacity of APX, DHAR and MDHAR as well as decrease the level of H_2O_2 via a high GR activity in longan fruit.

ClO_2 fumigation maintained NADPH redox equilibrium of 'Daw' longan as indicated by higher NADPH/$NADP^+$ ratio than that of the control (Figure 4.32). It is possible that ClO_2 modulated NADPH redox potential by the enhancement of NADPH-regenerating enzymes (G6PDH and 6PGDH), resulting in increased thiol and NADPH. G6PDH and 6PGDH has been shown to play a vital role in tolerance to stresses (Hou *et al*., 2007; Liu *et al*., 2013). Enhanced expression of the gene of these enzymes in maintaining NADPH redox homeostasis may be a mechanism of response against oxidative stress (Hou *et al*., 2007; Corpas and Barroso, 2014; Yang *et al*., 2014). The expression of *Zmpdh1* and *Zmpdh2* and increase in its activities has been found to be induced by chemicals such as nitrate in maize (Redinbaugh and Campbell, 1998). Moreover, G6PDH activities are also activated by phosphorylation through Glycogen Synthase Kinase 3 (GSK3) such as ASKα which had been reported in *Arabidopsis* (Santo *et al*., 2012). Thus, ClO_2 might up-regulate G6PDH and 6PGDH activities through gene expression and/or the kinase pathway. Further studies are required to evaluate the effects of ClO_2 on the regulation of NADPH-generating dehydrogenase (G6PDH and 6PGDH) activity of 'Daw' longan fruit.

ClO_2 has been reported in enhancing antioxidant defense system to reduce oxidative stress in 'Daw' longan (Chomkitichai *et al*., 2014a; 2014b). In the present study, the enhancing effects of ClO_2 may be due to increased H_2O_2 scavenging capacity though maintaining redox homeostasis of ASA-GSH. From the results of this study, a model by which ClO_2 modulates the redox state of NADPH and the ASA and GSH redox balance was proposed (Figure 5.2). ClO_2 may govern the redox state of NADPH

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by enhancing G6PDH and 6PGDH activities and may administer the ASA and GSH redox balance by stimulating MDHAR, DHAR and GR. Higher ASA redox ratio promoted APX activity to remove excess H_2O_2 leading to the reduction of oxidative damage and delay senescence of 'Daw' longan fruit during storage.

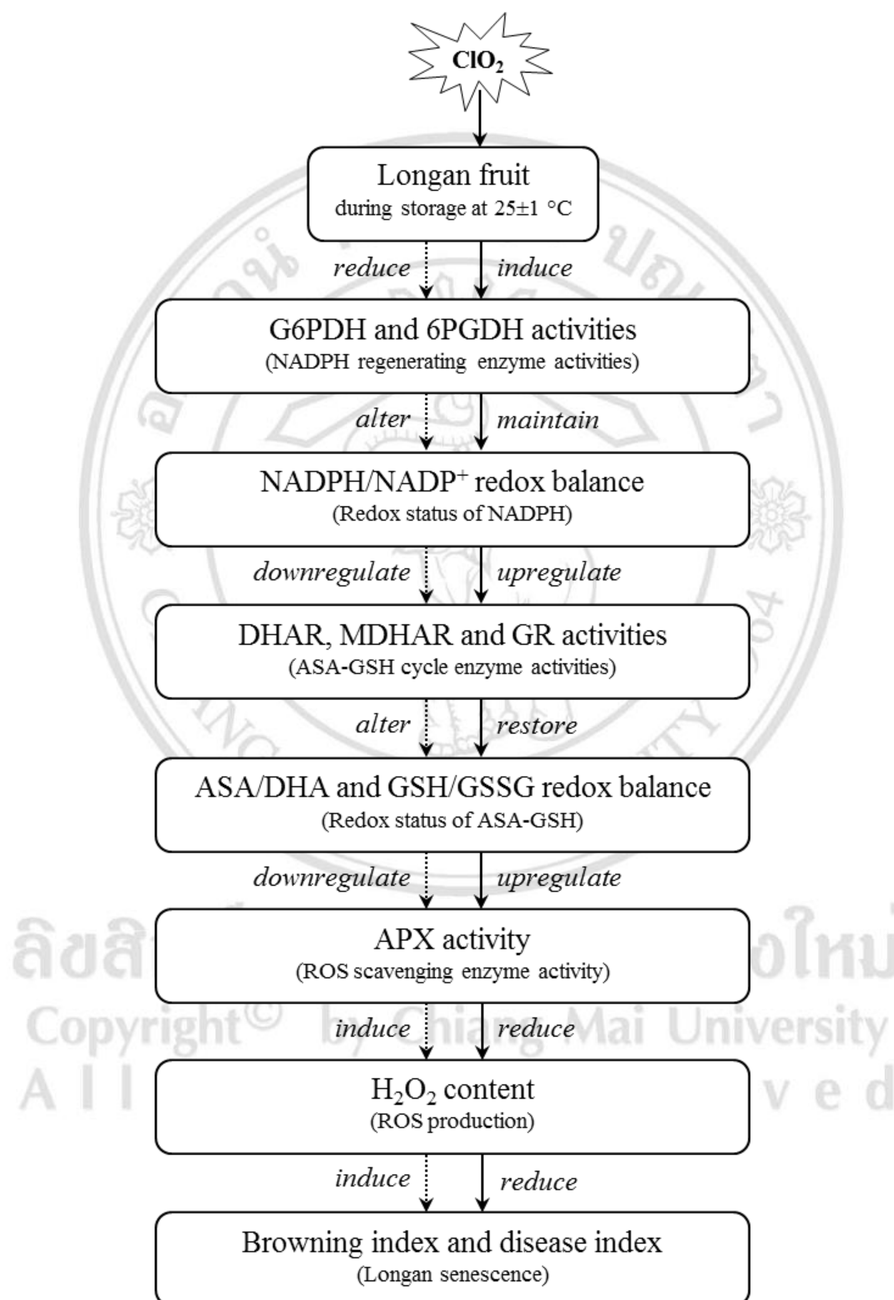

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Figure 5.2 Probable mechanism for fruit senescence through free radical scavenging (dash arrows) and effects of ClO_2 on fruit senescence alleviation (solid arrows) of 'Daw' longan during storage at 25±1°C