# CHAPTER 5

## Discussion

**Effects of SA and MJ on chilling injury and fruit quality of mango fruit cv. Nam Dok Mai No. 4 during low temperature storage.**

Nam Dok Mai No. 4 mango fruits showed CI symptoms under storage at 5 °C. In the cultivar studied, the chilling damage occurred at 4-12 °C (Phakawatmongkol *et al.*, 2004; Chidtragool *et al.*, 2011; Chongchatuporn *et al.*, 2013). Induced CI has been reported with low temperature storage at 5 °C in other mango cultivars such as Kent and Zill (González-Aguilar *et al.*, 2001; Ding *et al.*, 2007). In this study, CI damage appeared in Nam Dok Mai No. 4 mango fruit after 21 days of storage at 5 °C. CI symptoms manifested as exocarp browning occurred on Day 21 and then surface pitting and endocarp browning occurred on Days 35-42. The severity of these symptoms increased rapidly with storage time.

Storage at 5 °C induced exocarp browning during CI of Nam Dok Mai No. 4 mango fruits. This is because low temperature stress stimulates oxidative membrane damage and lead to leakage of browning enzymes that convert leaked phenolic compounds to browning pigment formation. Many studies have shown that low temperature induces phase transition of membrane lipids from liquid-crystalline to solid gel and the change in state would be expected to bring about a contraction that causes cracks or channels, leading to increased membrane permeability, leakage of solutes and loss of compartmentation (Lyons, 1973; Wang, 2010). Low temperature stress also induces ROS initiation and accumulation which causes oxidative membrane damage leading to leakage of PPO from plastids and POD from mitochondrias and cytosols and phenolic compounds from vacuoles (Graham and Paterson, 1982; Martinez and Whitaker, 1995). PPO can promote enzymatic browning by catalysing the oxidation of mono- and di-phenols to *o*-quinones and these quinones polymerise to produce brown pigments (Richard and Gauillard, 1997). POD can oxidise phenols to quinones, then condense tannins to brown polymers in the presence of $H_2O_2$, which may then

contribute to enzymatic browning (Richard and Gauillard, 1997). This result is in agreement with Nguyen *et al.* (2003), van Rooyen and Bower (2003), Tareen *et al.* (2012) and Luo *et al.* (2012) who reported that an increase in browning symptom in banana, avocado, peach and bamboo shoots was associated with an increase in PPO and POD activities and decrease in total phenolic content during low temperature storage at 6, 2, 0 and 1 °C respectively.

The visual symptom frequently occurs in mangoes stored at low temperature is surface pitting. The mango fruits stored at 5 °C also showed this symptom during 35-42 days of storage. The surface pitting which appeared in the exocarp subjected to CI is due to the increased activities of various enzymes involved in fruit softening as well as oxidative membrane damage and collapse of subsurface cells, followed rapidly by invasion of decay organisms (Lyons, 1973). Petracek *et al.* (1995) reported that sporadic or widespread collapse of oil glands near the peel surface caused peel pitting of grapefruit. Such visible pitting is also due to the stimulation of water loss under low temperature stress, reported earlier in some fruits such as grapefruit, cucumber and bell pepper (Alférez and Burns, 2004; Lim *et al.*, 2007).

In this study, endocarp browning of mango fruit also appeared to be adversely affected by chilling temperature (5 °C) but it was found at the end of storage. This symptom may be caused mainly by browning enzymes like an exocarp browning under low temperature storage. CI symptoms were not found in the mesocarp of Nam Dok Mai No. 4 mango fruit. A possible reason might be due to direct contact of exocarp with low temperature. These results are consistent with mango fruit cvs. Nam Dok Mai, Choke Anan, Kaew, Rad, Okrong, Tongdum and Nungklangwun that browning appeared on the exocarp rather than in the mesocarp during low temperature storage at 4, 8 and 12 °C (Phakawatmongkol *et al.*, 2004; Chidtragool *et al.*, 2011).

In this study, Nam Dok Mai No. 4 mango fruits showed CI symptoms as exocarp browning that first appeared on Day 21 with low CI index (0.42) and thereafter rapidly increased. The highest CI index (3.92 and 5.00) appeared in the fruit on Days 35 and 42 with surface pitting and endocarp browning. In Nam Dok Mai Si Thong, exocarp browning and surface pitting occurred after 20 days during storage at 5 °C, while the CI

symptoms appeared after 30 days of storage at 8 °C (Pattanapo *et al.*, 2010). The difference in CI sensitivity between Choke Anan and Nam Dok Mai mango fruits were found during low temperature storage at 4 °C for 30 days. Moreover, Nam Dok Mai mango fruits showed exocarp browning at Day 9 of storage while Choke Anan did not show CI symptoms during all storage time at 4 °C (Chidtragool *et al.*, 2011). Nam Dok Mai cultivar was most sensitive to CI, based on exocarp discoloration, when compared with Kaew, Rad, Okrong, Tongdum and Nungklangwun cultivars during storage at 4, 8 and 12 °C (Phakawatmongkol *et al.*, 2004). These reports indicate that symptoms and sensitivity on CI of mango fruits vary according to the mango cultivar, storage temperature and duration of exposure to low temperature.

Variations of CI sensitivity were also found in other mango cultivars. Tommy Atkins mango fruit showed CI symptom manifested as surface pitting which started to appear after 7 days of exposure to 7 °C and progressed rapidly thereafter (González-Aguilar *et al.*, 2000). Similar CI symptom was also found in Kent cultivar and was greater on fruits stored at 5 °C than on those stored at 10 °C for 14 days (González-Aguilar *et al.*, 2001). Wacheng cultivar showed CI symptoms such as water-soaking pits on the exocarp after 12 days of storage at 2 °C (Zhao *et al.*, 2006). In Tainong cultivar, CI symptoms such as sunken lesions or pitting rapidly appeared after 7 days of storage at 4 °C (Wang *et al.*, 2008).

In this experiment, it was shown that SA and MJ treatments prior to low temperature storage at 5 °C were more effective in reducing CI symptoms of Nam Dok Mai No. 4 mango fruits. They could not delay CI symptoms during low temperature storage. CI symptoms in SA and MJ treated fruits similarly occurred on Day 21 but showed lower injury than the control fruits throughout storage time. SA treatment appeared to be more effective in reducing CI symptoms than MJ treatment in Nam Dok Mai No. 4 mango fruits which reduced CI by 20-80 and 20-40% respectively. The possible mechanism of SA and MJ in reducing CI symptoms of Nam Dok Mai No. 4 mango fruit may be associated with enhancing the antioxidant defense system (discussed in next experiment). SA and MJ treatments induced an increase in antioxidant enzyme activity such as SOD, CAT, APX, POD and GR, resulting in the reduction of CI as reported in peach and loquat fruits (Wang *et al.*, 2006; Cao *et al.*,

2009) and they increased and maintained high contents of ascorbic acid, glutathione, phenolic compounds and anthocyanins as reported in peach, loquat and pomegranate fruits (Wang *et al.*, 2006; Cao *et al.*, 2009; Sayyari *et al.*, 2011a, b). Another possible mechanism of SA and MJ in reducing CI symptoms of Nam Dok Mai No. 4 mango fruit may be associated with enhancing alternative oxidase (AOX) pathways that reduced free radical production resulting in reduction of oxidative stress during low temperature stress as reported in tomato, sweet pepper and cucumber seedlings (Fung *et al.*, 2004, 2006; Lei *et al.*, 2010).

In this study, SA was more effective than MJ in reducing CI in Nam Dok Mai No. 4 mango fruits than MJ. These results are consistent with Siboza and Bertling (2013) who reported that SA reduced CI in lemon better than MJ during storage at -0.5 °C for 35 days. This result suggests that SA is more effective than MJ on enhancing the antioxidant defense system and reducing oxidative stress in mango fruits during low temperature storage (discussed in the next experiments). However, there are many reports that MJ treatment was more effective than SA in reducing CI in tomato and sweet pepper (Ding *et al.*, 2001, 2002; Fung *et al.*, 2004).

SA at the concentrations of 0.1 and 1 mM reduced CI in Nam Dok Mai No. 4 fruits but 1 mM SA was more effective than 0.1 mM SA (Figure 4.2). It might be due to higher SA concentration is more effective than the lower ones in inhibiting enzymatic browning during low temperature storage. SA at higher concentration effectively inhibited PPO activity responsible for reducing browning than the lower ones as reported in peach (Tareen *et al.*, 2012). Induced high PPO activity during stress conditions coincided with membrane damage and enhanced browning and CI symptoms in fruits under this stress (Mayer, 1987). These results agree with Ding *et al.* (2007) that a high concentration of SA at 2 mM was effective in reducing CI in mango fruits cv. Zill during storage at 5 °C for 30 days. Ding *et al.* (2007), Sayyari *et al.* (2009) and Luo *et al.* (2011, 2012) reported that 2, 2, 1.5 and 1 mM were the optimum concentrations of SA for reducing CI in mango cv. Zill, pomegranate, plum and bamboo shoot respectively. It was also reported that the effectiveness of alleviating CI by SA treatment varied with species and tissue type (Cao *et al.*, 2009). SA at the concentration of 1 mM was most effective in reducing CI in Nam Dok Mai No. 4 mango fruits.

Derivatives of SA such as methyl salicylate (MeSA) and acetylsalicylic acid (ASA) also reduced CI in many fruits such as tomato, sweet pepper, pomegranate and loquat fruits (Ding *et al.*, 2001; Fung *et al.*, 2004; Cai *et al.*, 2006; Sayyari *et al.*, 2011a, b). The possible mechanism of ASA and MeSA in reducing CI symptoms may be associated with enhancing the antioxidant defense system both enzymatic and non-enzymatic antioxidants (Ding *et al.*, 2002; Fung *et al.*, 2004; Sayyari *et al.*, 2011a, b). In addition, MeSA also increased expression of AOX genes along with reducing CI symptoms as report in sweet potato during storage at 0 °C for 3 days resulting in CI tolerance in the treated fruits (Fung *et al.*, 2004). This mechanism of chilling tolerance by derivatives of SA is similar to SA in CI reduction of Nam Dok Mai No. 4 mango fruits.

For MJ treatments, 0.1 mM MJ was more effective in reducing CI than 1 mM MJ in Nam Dok Mai No. 4 mango fruits. This result is consistent with mango fruit cv. Tommy Atkins and Kent that 0.1 mM MJ could reduce CI symptoms during storage at 7 and 5 °C respectively (González-Aguilar *et al.*, 2000, 2001). High concentrations above the optimal range was ineffective in reducing CI and increased decay incidence in tomato during storage at 5 °C for 28 days (Ding *et al.*, 2002; Moreira *et al.*, 2009). A higher concentration of MJ at 0.5 mM directly damage the fruit tissue, resulting in more decay and CI in tomato during storage at 5 °C for 4 weeks (Ding *et al.*, 2002). In addition, exogenous application of MJ at high concentration (100 mM) was the cause of local phytotoxicity in plants (Moreira *et al.*, 2009).

Low temperature storage at 5 °C maintained the quality of Nam Dok Mai No. 4 mango fruits as it retards ripening and senescence. The fruit quality of Nam Dok Mai No. 4 fruit slightly changed during storage at 5 °C for 42 days (Figures 4.3-4.8). SA and MJ treated fruits had similar quality as control fruits during storage at low temperature (Figures 4.3-4.8). Mesocarp color, TSS, TA, TSS/TA ratio, firmness, weight loss and disease symptoms of SA and MJ treated fruits were not significantly different during storage at low temperature (Figures 4.4-4.8).

The green color in the exocarp and mesocarp slightly decreased while the yellow color slightly increased at 5 °C (Figures 4.3-4.4). It has been suggested that low temperature storage reduced or inhibited the activity of chlorophyllase, which was

responsible for degreening of the exocarp, resulting in delayed, nonuniform or complete inhibition of yellow color development (Morrelli *et al.*, 2003; Luengwilai and Beckles, 2013). Delay in color damage during ripening as seen in Nam Dok Mai No. 4 mango fruit may be attributed to increased sensitivity of a specific chlorophyllase to low temperature. SA and MJ treatments did not change the exocarp and mesocarp colors during low temperature storage (Figures 4.3-4.4) but maintained high $L^*$ values of the exocarp of mango fruit during storage at low temperature (Figure 4.3a). This indicates that SA and MJ retard the increase in darkness of mango exocarp which is related to browning in CI fruits. SA at 1 mM gave the highest $L^*$ values in the exocarp correlated with the lowest browning of 1 mM SA treated fruits (Figures 4.2-4.3). This result is consistent with Tareen *et al.* (2012) who reported that peach treated with SA had higher $L^*$ values when compared with the control.

TSS slightly increased while TA slightly decreased at 5 °C (Figure 4.5). Low temperature storage reduced or inhibited α-amylase that hydrolysed starch to sucrose, glucose and fructose resulting in retarded increase of TSS (Wang and Wang, 2009; Kami *et al.*, 2011). This result is in agreement with Wu *et al.* (1999), who found that α-amylase activity in sugar apple (*Annona squamosa* L.) stored at 16 °C was lower than that stored at 21 °C and showed lower TSS. Respiration rates can be decreased by reducing fruit storage temperatures (Freitas and Mitcham, 2013). The substrates for cellular respiration including organic acids such as citric, glycolic, malic, tartaric and oxalic acid were maintained at high levels during low temperature storage resulting in delayed decrease of TA. SA and MJ treatments had no effect on TSS, TA and TSS/TA ratio of mango fruits during low temperature storage (Figure 4.5). SA and MJ at 0.1 and 1 mM were not effective in inducing starch hydrolysis or the respiration rate of Nam Dok Mai No. 4 mango fruit resulting in similar TSS, TA and TSS/TA ratio. These results agree with Nilprapruck *et al.* (2008) who reported that TSS, TA, total sugars and reducing sugars of MJ treated pineapple (0.01, 0.1 and 1 mM) were not significantly different from those of the control during low temperature storage.

The firmness of mango fruit slightly decreased when stored at 5 °C (Figure 4.6) indicating that low temperature delayed the decrease of mango firmness due to reduced ethylene production (Villalobos Acuña *et al.*, 2011). This results in reducing the

activities of cell wall degradating enzymes such as polygalactorunase (PG) (Zauberman and Jobin-Decor, 1995; Imsabai *et al.*, 2002), pectinesterase (PE) (Imsabai *et al.*, 2002), pectin methyl esterase (PME) (Zauberman and Jobin-Decor, 1995; Meng *et al.*, 2009), cellulase and β-galactosidase (Zauberman and Jobin-Decor, 1995; Rao *et al.*, 2011). SA and MJ treatments had no effect on the firmness of mango fruits during low temperature storage (Figure 4.6). SA and MJ at 0.1 and 1 mM is not effective in inducing or inhibiting cell wall hydrolytic enzymes of Nam Dok Mai No. 4 mango fruit that resulted in similar firmness as the control fruits. These results are consistent with González-Aguilar *et al.* (2000) who reported that firmness of MJ treated mango fruit cv. Tommy Atkins (0.1 mM) was not significantly different from the control fruit during low temperature storage.

The increase in percentage of weight loss was found in mango fruit during storage at 5 °C but at low percentage (Figure 4.7). It was found that low temperature storage with high humidity reduced water loss from the fruit (Rab *et al.*, 2012). These results are consistent with González-Aguilar *et al.* (2001) who reported that low temperature storage at 5 and 10 °C reduced the percentage of weight loss of Kent mango fruits when compared with those stored at 20 °C. SA and MJ treatments had no effect on the weight loss of mango fruits during low temperature storage (Figure 4.7). SA and MJ at 0.1 and 1 mM was not effective in reducing water lost from Nam Dok Mai No. 4 mango fruit and resulted in a similar percentage of weight loss as the control fruits. These results are consistent with González-Aguilar *et al.* (2000) who reported that weight loss of MJ treated mango fruit cv. Tommy Atkins (0.1 mM) was not significantly different from that of the control fruit during low temperature storage. Tareen *et al.* (2012) reported that SA treatment (0.5, 1, 1.5 and 2 mM) reduced weight loss in peach during low temperature storage. SA and MJ treatments reduced fruit weight loss by closing the stomata which reduced transpiration rate in *Vicia faba*, radish and mandarin fruit (Manthe *et al.*, 1992; Wang, 1998; Zheng and Zhang, 2004) suggesting that the response varies among species.

Disease symptoms were detected in mango after Day 21 of 5 °C storage with low incidence (Figure 4.8). This is because low temperature inhibited growth and development of plant pathogens infecting the fruits (Baiyewu and Amusa, 2005; Rab

*et al.*, 2012). Rab *et al.* (2012) reported that low temperature storage at 10 °C reduced disease incidence and growth of *Penecillium digitatum* and *Penecillium italicum* in sweet orange when compared with those stored at 20 °C. In this study, SA and MJ treatments had no effect on reducing disease symptoms of mango fruits during low temperature storage (Figure 4.8). Thus SA and MJ at 0.1 and 1 mM were not effective in inhibiting growth and development of plant pathogens infecting the mango fruits. It might be due to SA and MJ at these concentrations could not induce the increase in pathogenesis-related (PR) proteins that are reported to control certain fungal disease in mango cv. Matisu (Zeng *et al*., 2006), tomato (Ding *et al*., 2002), cherry (Xu and Tian, 2008) and sweet pepper (Rao *et al*., 2011) fruits.

Mangoes stored at 5 °C ripened when they were transferred to room temperature as indicated by exocarp and mesocarp color, TSS, TA, TSS/TA ratio and firmness. Low temperature at 5 °C reduced metabolism and retarded fruit ripening of Nam Dok Mai No. 4 mango fruits during storage, but warmer temperature (25 °C) could accelerate metabolism and ripening process. These results are consistent with reports in mango cvs. Tommy Atkins, Kent and Zill (González-Aguilar *et al.*, 2000, 2001; Ding *et al.*, 2007).

Mangoes stored at 5 °C require a shorter time for ripening after storage at low temperature for a longer time (Figure 4.9). This is because prolonged low temperature storage accelerates the increases in ethylene production and respiration rate of fruits after transfer to room temperature, resulting in shorter time for ripening (Zaharah and Singh, 2011). It was also shown that ethylene production and respiration rate of mango fruit cv. Kensington Pride stored at 5 °C for 4 weeks rapidly increased and reached their peak at Day 1 after transfer from cold storage to room temperature (21 °C), while ethylene production and respiration rates in 2 weeks cold stored mango fruits gradually increased and reached their peak at Days 3 and 4 respectively. SA and MJ treated fruits could be riped with similar quality as normal control fruits (Figures 4.10-4.14). Mesocarp color, TSS, TA, TSS/TA ratio, firmness and disease symptoms of SA and MJ treated fruits were not significantly different during storage at low temperature (Figures 4.11-4.14).

The quality of ripe mango fruits showed little change throughout storage time (Figures 4.10-4.14). Warmer temperature (25 °C) stimulated fruit ripening by inducing color changes of exocarp and mesocarp. The color change of ripe fruits from green to yellow might be partly explained by the unmasking of existing carotenoids as chlorophylls were degraded. Brightness in the exocarp of ripe fruits significantly decreased after Day 21 of storage. This might be associated with browning that appeared on the exocarp (Figures 4.10a and 4.17). The CI symptoms were also found in ripe fruits on Day 21 and increased with storage time (Figures 4.16-4.17). The lowering of $L^*$ values of exocarp and increasing of browning on the exocarp lead to decreased overall quality acceptance of ripe mango fruits after storage at low temperature for a long time (Figure 4.15). SA and MJ treatments did not change the exocarp and mesocarp color of ripe mango fruit (Figures 4.10-4.11). SA and MJ treatments maintained high $L^*$values in exocarp of ripe mango fruit (Figure 4.10a). This indicates that SA and MJ retarded an increase in darkening of ripe mango exocarp that is related to browning. CI symptoms in ripe fruits were reduced by SA and MJ treatment (Figures 4.16-4.17) which reduced browning in the exocarp of ripe fruits, along with an increase in $L^*$ value in the exocarp (Figure 4.10) and maintained the good appearance and acceptance with high overall quality scores in the treated fruits. So, SA and MJ treatment prolonged storage life of mango that showed higher acceptance scores than the control fruits at the end of storage (Figure 4.15). These results are consistent with González-Aguilar *et al.* (2001) who reported that MJ improved overall quality of ripe mango cv. Kent. In this study, treatment with 1 mM SA was most effective in maintaining fruit quality and prolonging storage life of Nam Dok Mai No. 4 mango fruits associated with lowest CI symptoms.

The increase in TSS of ripe fruits (Figure 4.12a) is associated with starch hydrolysis to sugars by amylase (Wu *et al.*, 1999). The decrease in TA of ripe fruit (Figure 4.12b) is associated with increased respiration rate during fruit ripening (Freitas and Mitcham, 2013). Organic acid such as citric, glycolic, malic, tartaric and oxalic acid were used as substrate for cellular respiration resulting in the decrease in TA. The increase in TSS and decrease in TA resulted in increasing TSS/TA ratio (Figure 4.12c) indicated that ripe fruit was sweeter. SA and MJ treatments did not affect TSS, TA and TSS/TA ratio of ripe mango fruits (Figure 4.12). SA and MJ at 0.1 and 1 mM were not

effective in inducing starch hydrolysis or the respiration rate of ripe mango fruit. These results are consistent with González-Aguilar *et al.* (2000) who reported that MJ treatment (0.1 mM) had no effect on TA of mango cv. Tommy Atkins after transfer to 20 °C for 5 days. On the other hand, TSS of ripe mango cvs. Tommy Atkins and Kent after being removed from cold temperature increased by MJ (0.01 mM) treatments (González-Aguilar *et al.*, 2000, 2001). In addition, SA treatment (2 mM) also increased TSS of ripe mango cv. Zill after removal from cold temperature (Ding *et al.*, 2007).

The decrease in firmness of ripe mango fruit (Figure 4.13) is associated with increased cell wall hydrolytic enzymes such as PG, PE, PME, cellulase and β-galactosidase which are responsible for acceleration of fruit softening in ripe mango (Ketsa *et al.*, 1998; Zaharah and Singh; 2011). SA and MJ treatments had no effect on fruit firmness of ripe mango fruits (Figure 4.13). SA and MJ at 0.1 and 1 mM were not effective in inducing or inhibiting cell wall hydrolytic enzymes of ripe mango fruit. These results are consistent with Jin *et al.* (2009b) who reported that firmness of MJ treated peach (0.001 mM) was not significantly different from that of the control fruit after transferred to 20 °C for 3 days. However, Rao *et al.* (2011) reported that SA treatment (1, 2 and 4 mM) maintained high firmness in sweet pepper at 25 °C by reducing the activity of PG, PME, cellulase and β-galactosidase.

The increase in disease symptom of ripe fruits (Figure 4.14) is correlated with room temperature (25 °C) which accelerated growth and development of plant pathogens that infected the ripe fruits (Baiyewu and Amusa, 2005; Rab *et al.*, 2012). SA and MJ treatments had no effect on reducing disease symptoms of ripe mango fruits (Figure 4.14). SA and MJ at 0.1 and 1 mM are not effective in inhibiting growth and development of plant pathogens in ripe mango fruit that resulted in similar disease index as ripe control fruits. SA or MJ may not affect PR protein induction in Nam Dok Mai No. 4 mangoes stored at 5 °C.

**Effects of SA and MJ on free radical and membrane damage of mango fruit cv. Nam Dok Mai No. 4 during low temperature storage.**

Low temperature stress increased ROS accumulations such as $O_2^{\bullet-}$, $OH^{\bullet}$ and $H_2O_2$ in the exocarp and mesocarp of Nam Dok Mai No. 4 mango fruit during 42 days of storage at 5 °C (Figures 4.18-4.20). The free radical production is caused by plant evolved mechanisms that allow them to adapt and survive periods of low temperature stress. This enhanced ROS production is kept under tight control by a versatile and cooperative antioxidant system. ROS enhancement under the stress functions as an alarm signal that triggers acclamatory/defense responses (Cruz de Carvalho, 2008). In the experiment, mangoes were kept at low temperature and with $O_2$ for a long time, ROS production overwhelmed the scavenging action of the antioxidant system resulting in oxidative damage and CI symptoms (Gill and Tuteja, 2010).

The increase in $O_2^{\bullet-}$, $OH^{\bullet}$ and $H_2O_2$ contents were found in Nam Dok Mai No. 4 mango throughout low temperature storage at 5 °C (Figures 4.18-4.20). These results agree with Cao *et al.* (2009) and Yang *et al.* (2011) who found the increase in $O_2^{\bullet-}$ and $H_2O_2$ contents in loquat during storage at 1 °C for 35 days and in cucumber during storage at 2 °C for 15 days. Jin *et al.* (2009a) also found that $H_2O_2$ content increased in peach during storage at 0 °C for 5 weeks. In seedlings of banana, cucumber and eggplant, the accumulation of $H_2O_2$ contents was also increased during growth under low temperature stress (Kang *et al.*, 2003; Lei *et al.*, 2010; Chen *et al.*, 2011).

The $O_2^{\bullet-}$ content in Nam Dok Mai No. 4 mangoes increased rapidly throughout low temperature storage at 5 °C. Although, $H_2O_2$ showed the highest content but slowly increased when compared with $O_2^{\bullet-}$ and $OH^{\bullet}$. It might be due to relatively stable and long half-life of $H_2O_2$ and it can diffuse at long distance from its site of production whereas, $O_2^{\bullet-}$ and $OH^{\bullet}$ have much shorter half-life and diffuse at shorter distance (Møller *et al.*, 2007; Gill and Tuteja, 2010; Bhattacharjee, 2012). In this study, $H_2O_2$ increased slower than $O_2^{\bullet-}$ and $OH^{\bullet}$ during cold storage which might be due to the presence of many enzymatic antioxidants such as catalase, peroxidase, glutathione peroxidase, guaiacol peroxidase, ascorbate peroxidase and thioredoxin peroxidase and non-enzymatic antioxidants such as ascorbic acid and glutathione for elimination of

$H_2O_2$. $H_2O_2$ can be further converted to $OH^{\bullet}$ in the Fenton reaction that results in slow increase of $H_2O_2$. On the other hand, $O_2^{\bullet-}$ was rapidly increased during cold storage because there are only superoxide dismutase, ascorbic acid and glutathione for scavenging $O_2^{\bullet-}$. While, $OH^{\bullet}$ was rapidly increased due to the absence of any antioxidant enzyme for elimination of $OH^{\bullet}$ but it was only reduced by ascorbic acid, glutathione and proline (Mittler, 2002; Gill and Tuteja, 2010; Sharma *et al.*, 2012).

When compared the content of free radicals between exocarp and mesocarp, it was found that the exocarp showed higher $O_2^{\bullet-}$, $OH^{\bullet}$ and $H_2O_2$ contents than the mesocarp throughout storage period and the increase in $O_2^{\bullet-}$ and $OH^{\bullet}$ contents of the exocarp was higher than the mesocarp (Figures 4.18-4.20). The exocarp might be in direct contact with low temperature. Low temperature stress induced phase transition of lipid bilayer membrane of cell and organelles from liquid-crystalline to solid gel that resulted in changing structural and functional properties of membrane and disrupts normal functioning of membrane bound proteins (Lyons, 1973). Low temperature stress also disturbed electron transport chain by inhibiting cytochrome C oxidase in mitochondrial membrane, leading to leakage of electrons to $O_2$ which contributes to enhancing ROS production (Prasad *et al.*, 1994; Møller, 2001). In addition, the exocarp has many ROS production sources such as chloroplast, mitochondria, endoplasmic reticulum, peroxisome, plasma membrane, cell wall and apoplast. In contrast, the mesocarp cells lack the chloroplast as the main source of ROS production (Gill and Tuteja, 2010; Sharma *et al.*, 2012). Moreover, the exocarp is light exposed part that could stimulate photorespiration in peroxisomes resulting in $H_2O_2$ production (Sharma *et al.*, 2012).

Low temperature stress induced oxidative membrane damage in Nam Dok Mai No. 4 mango fruits during storage at 5 °C. LOX activity, the key enzyme in triggering lipid peroxidation of the plasma membrane and MDA content, one of the final products of peroxidation of unsaturated fatty acids in phospholipids, increased in both exocarp and mesocarp of Nam Dok Mai No. 4 mango fruits throughout storage time (Figures 4.21-4.22). These results were consistent with Ding *et al.* (2007) who found that LOX activity and MDA content increased in mango cv. Zill during storage at 5 °C. Moreover, the increases in LOX activities and MDA contents were also found in many plants such

as lemon (Safizadeh *et al.*, 2007), loquat fruit (Cao *et al.*, 2009), cucumber seedlings (Lei *et al.*, 2010), cucumber fruit (Yang *et al.*, 2011), bamboo shoot (Luo *et al.*, 2012), anthurium flowers (Promyou *et al.*, 2012) and green bell pepper (Wang *et al.*, 2012) during storage at low temperature (1-10 °C).

EL increased in both exocarp and mesocarp of Nam Dok Mai No. 4 mango fruit during storage at low temperature (Figure 4.23). The increase in EL indicates that membrane deterioration occurred in plant tissue during senescence or chilling stress. Biophysical changes in membrane lipids and enzymatic and non-enzymatic lipid peroxidation leads to altered membrane properties and result in ion leakage and cellular decompartmentation were also reported in both senescence and chilling stress tissues (Marangoni *et al*., 1996). In this study, low temperature at 5 °C accelerated membrane deterioration by induced alteration in conformation and structure of membrane and induced ROS resulting in an increase in membrane permeability and EL (Sevillano *et al.*, 2009). These results agreed with González-Aguilar *et al.* (2000) who found that EL increased in mango cv. Tommy Atkins during storage at 7 °C. Moreover, increase in EL was also found in many plants such as guava (González-Aguilar *et al.*, 2004), pineapple (Nilpraprucк *et al.*, 2008), peach (Meng *et al.*, 2009), cucumber seedlings (Lei *et al.*, 2010), bamboo shoot (Luo *et al.*, 2012), anthurium flowers (Promyou *et al.*, 2012) and green bell pepper (Wang *et al.*, 2012) during storage at low temperature stress (1-10 °C). Moreover, low temperature at 5 °C had direct effect on phase transition of lipid bilayer membranes (Lyons, 1973). Low temperatures induced the change in phase transition of lipid bilayers from their normal flexible liquid-crystalline and functional state to a more solid gel-like structure resulting in a decrease in membrane flexibility, so cracking could occur and channels could be formed at the liquid-crystal/gel interface. These cracks and channels lead to membrane leakiness, loss of membrane integrity and loss of solute or ion gradients across the membrane as indicated by increased in EL (Lyons, 1973).

The increase in oxidative membrane damage during storage at 5 °C indicated by LOX activity, MDA content and EL (Figures 4.21-4.23), were associated with the increased ROS levels (Figures 4.18-4.20). High ROS levels damage plants through lipid peroxidation and membrane injury (Cao *et al.*, 2009; Lei *et al.*, 2010; Yang *et al.*, 2011;

Jin *et al.*, 2013). In this study, the increase and overproduction of ROS including $O_2^{\bullet-}$, $OH^{\bullet}$ and $H_2O_2$, might induce membrane lipid peroxidation and membrane destruction in the exocarp and mesocarp of Nam Dok Mai No. 4 mango fruits during low temperature storage. It has been reported that $O_2^{\bullet-}$, $H_2O_2$ and $OH^{\bullet}$ were capable to initiate the lipid peroxidation and $OH^{\bullet}$, which can react with all biological molecules and cause lipid peroxidation and membrane destruction (Møller *et al.*, 2007; Gill and Tuteja, 2010; Bhattacharjee, 2012). In this study, $O_2^{\bullet-}$ and $OH^{\bullet}$ increased in the early storage and continued to increase throughout storage at low temperature (Figures 4.18 and 4.20). The level of $H_2O_2$ increased rapidly after 21 days of storage (Figure 4.19) indicating that the membrane was mostly destroyed by $O_2^{\bullet-}$ and $OH^{\bullet}$ in the early storage and by $H_2O_2$ after 21 days of storage exerting additional damaging effect on the membrane.

LOX activity and MDA level increased with increasing ROS levels in exocarp and mesocarp of Nam Dok Mai No. 4 mango fruits during storage at 5 °C. This indicates that ROS production stimulated membrane lipid peroxidation of mango fruit by enhancing LOX activity, resulting in increased MDA content. These results are consistent with previous studies by Cao *et al.* (2009), Lei *et al.* (2010), Yang *et al.* (2011) and Jin *et al.* (2013) who found that high MDA content and LOX activity were associated with ROS levels in loquat, cucumber and peach fruits and cucumber seedlings at low temperature stress. In this study, LOX activity and MDA contents were positively correlated with ROS levels in the exocarp ($r = 0.903$-$0.972$ and $0.864$-$0.935$ respectively) and the mesocarp ($r = 0.928$-$0.958$ and $0.837$-$0.956$ respectively) (Apendix; Table 23), indicating that the serverity of membrane lipid peroxidation of Nam Dok Mai No. 4 mango depended on the levels of ROS. In this study, EL had a significant positive and high correlation with lipid peroxidation ($r_{exocarp} = 0.853$-$0.872$ and $r_{mesocarp} = 0.912$-$0.975$) and ROS levels ($r_{exocarp} = 0.831$-$0.901$ and $r_{mesocarp} = 0.912$-$0.958$) (Apendix; Table 23). This indicates that membrane lipid peroxidation altered membrane properties and resulted in cell defects such as ion leakage and cellular decompartmentation.

SA and MJ treatments reduced ROS accumulation and membrane damage in Nam Dok Mai No. 4 mango fruits during storage at 5 °C (Figures 4.18-4.23). ROS in SA and MJ treated fruits were lower than those in the control fruits in both exocarp and

mesocarp throughout storage time. These results agreed with Kang *et al.* (2003), Cai *et al.* (2006), Cao *et al.* (2009), Jin *et al.* (2009a), Lei *et al.* (2010) and Chen *et al.* (2011) who reported that treatments with SA and MJ and its derivative reduced ROS accumulation in loquat, peach, cucumber, banana and eggplant seedlings respectively. In this study, the effectiveness in reducing ROS depended on the kind of chemicals and their concentrations. SA treatments were more effective to reduce ROS than MJ treatments (Figures 4.18-4.20). SA at 1 mM was most effective in reducing ROS in both exocarp and mesocarp of Nam Dok Mai No. 4 mango fruits (Figures 4.18-4.20). It has been reported that the efficiency of SA and MJ on reducing ROS production associated with their ability in ROS scavenging (Jin *et al*., 2009a; Siboza and Bertling, 2013). The capacity for ROS scavenging by SA was higher than MJ in SA and MJ treated fruits that is discussed in the next experiment.

There are two possible mechanisms of SA and MJ in reducing ROS during chilling stress. First, they enhance alternative oxidases (AOXs) channel. AOXs, alternative channeling of electrons in the electron transport chains of the chloroplasts and mitochondria, can divert electrons flowing through electron-transport chains and use them to reduce $O_2$ to water (Mittler, 2002). Lei *et al.* (2010) reported that SA treatment enhanced AOX gene expression and their activity that lead to reduce an increase of $H_2O_2$ in cucumber seedlings under low temperature stress at 10 °C. In addition, MeSA and MJ also induced expression of AOX genes in sweet pepper and tomato during low temperature storage at 0 °C which related to increased chilling tolerance (Fung *et al.*, 2004; Fung *et al.*, 2006). However, the effects of SA and MJ on AOX gene expression or activity which relate to reduce ROS production were not examined in this study. Gene expression or activity of AOX might be study in Nam Dok Mai No. 4 mango fruit clearly elucidate SA and MJ mechanism on reducing ROS levels. Another mechanism of SA and MJ for reducing ROS may be associated with enhanced antioxidant defense system to scavenging excess ROS during chilling stress (Mittler, 2002). The scavenging of ROS by function of antioxidant defense system including enzymatic and non-enzymatic antioxidants is discussed in the next experiment.

Reduced oxidative membrane damage during storage at 5 °C of Nam Dok Mai No. 4 mango fruits by SA and MJ treatments was consistent with decreasing ROS levels (Figures 4.18-4.20). This indicates that SA and MJ could maintain membrane integrity of Nam Dok Mai No. 4 mango fruits during low temperature storage by lowering LOX activity, MDA content and EL. These results are consistent with Ding *et al.* (2007), Lei *et al.* (2010), Luo *et al.* (2012) and Promyou *et al.* (2012) who reported that SA treatment reduced membrane damage by lowering LOX activity, MDA content and EL in mango cv. Zill, cucumber seedlings, bamboo shoot and anthurium flowers. Similarly, González-Aguilar *et al.* (2000), González-Aguilar *et al.* (2004), Nilpraprucк *et al.*, (2008), Meng *et al.* (2009) and Cao *et al.* (2009) reported that MJ also reduced LOX activity and EL resulting in maintaining membrane integrity in mango cv. Tommy Atkins, guava, pineapple, peach and loquat fruits during low temperature storage.

In this study, MDA content in Nam Dok Mai No. 4 mango fruit stored at 5 °C was reduced by SA and MJ treatments (Figure 4.22). Reduced free radicals and LOX activity led to reduced lipid peroxidation (Cao *et al.*, 2009; Yang *et al.*, 2011; Jin *et al.*, 2013; Siboza and Bertling, 2013). This indicates that SA and MJ reduced MDA contents by lowering ROS levels and LOX activity, which reduced lipid peroxidation. In addition, the EL in SA and MJ treated fruits was lower than that in the control fruits (Figure 4.23). SA and MJ probably reduced EL of Nam Dok Mai No. 4 mango by reducing membrane lipid peroxidation from free radicals as previously reported in cucumber seedlings and peach (Lei *et al.*, 2010; Jin *et al.*, 2013).

In this study, 1 mM SA was most effective in reducing membrane damage in Nam Dok Mai No. 4 mango fruits during storage at 5 °C (Figures 4.21-4.23). The possible mechanism of SA and MJ treatments in reducing membrane damage or maintaining membrane integrity might be associated with reducing ROS levels and enhancing antioxidant defense system during low temperature storage.

**Effects of SA and MJ on antioxidant defense system of mango fruit cv. Nam Dok Mai No. 4 during low temperature storage.**

Low temperature stress stimulated antioxidant defense system including enzymatic and non-enzymatic antioxidants and their antioxidant capacities in Nam Dok Mai No. 4 mango fruit at the earlier period of storage time and then decreased afterward (Figures 4.24-4.32).

In the part of enzymatic antioxidants, SOD, CAT and APX activities increased in both exocarp and mesocarp of Nam Dok Mai No. 4 mango fruits at initial stage after being exposed to low temperature (Figures 4.24-4.26). Low temperature at 5 °C affected the activities of antioxidant enzymes and expressions of genes related to oxidative stress. The changes in gene expression appeared in the initial stage after exposure to low temperature stress associated with the enzyme activities. The reason for the increase in antioxidant activity might be due to low temperature stress inducing increased expression of antioxidant SOD, CAT and APX genes in the fruit after exposure to low temperature (Figure 4.27). The increase in antioxidant gene expression might be due to cold temperature stress induced signal transduction of stress response genes (Mahajan and Tuteja, 2005; Yadav, 2010; Xiong *et al.*, 2002). Previous studies indicated that cold stress could induce some secondary messenger such as calcium ion ($Ca^{2+}$) that activated some transcription factors by kinases and/or phosphatases reaction. The increase in transcription factors induce expression of various cold-responsive genes which involves the generation of ROS scavenging enzymes and antioxidant compounds (Mahajan and Tuteja, 2005; Yadav, 2010; Xiong *et al.*, 2002). It had been shown that cold stress induced ROS accumulation in the cells (Miura and Tada, 2014). ROS at low or moderate concentration also act as biological signals in plants that mediate systemic activation of gene expression in response to biotic and abiotic stresses (Mittler, 2002; Apel and Hirt, 2004; Yuan and Lin, 2008; Sharma *et al.*, 2012). ROS in higher plants must utilize and/or interfere with other signaling pathways or molecules, forming a signaling network (Foyer and Noctor, 2003; Millar *et al.*, 2003). It was shown that plant stress hormones such as abscisic acid (ABA), ethylene, SA and MJ, were positioned both upstream and downstream of the ROS signal during exposure to biotic and abiotic stresses (Foyer and Noctor, 2003; Apel and Hirt, 2004; Cruz de Carvalho, 2008; Shao

*et al.*, 2008). For example, Zhao *et al.* (2001) reported that ROS production played a role in ABA synthesis in seedling root tips resulting in leaf stomatal closure under drought stress. Moreover, Yuan and Lin (2008) reported that ROS especially $H_2O_2$ directly induced SA during abiotic stress resulting in the induction of abiotic-stress protective genes.

The activities and gene expression of CAT, SOD and APX in Nam Dok Mai No. 4 mango fruits increased and reached their peaks on Days 7, 21 and 28 respectively of 5 °C storage and then decreased afterwards as shown in Figures 4.24-4.27. This could be explained by their triggering subsequent signals like gene expression and the activity changed depending on the intensity of stress stimuli or long period of low temperature stress. This study showed that the effectiveness of free radical scavenging depended on each antioxidant enzyme capacities. CAT had highly effective scavenging of $H_2O_2$ during early period of cold storage while SOD and APX had highly effective scavenging of $O_2^{\bullet -}$ and $H_2O_2$ respectively during the middle period of cold storage. SOD, CAT and APX activities in plant during cold stress storage depended on the degrees of low temperature and plant species. Yang *et al.* (2011) found that SOD, CAT and APX activities in cucumber fruits increased at the first 6 days of cold storage at 2 °C, before decreasing during the latter period of storage. In loquat fruit, CAT and APX activities increased only at the first 7 days of cold storage at 1 °C while SOD activity continuously increased throughout cold storage for 35 days (Cao *et al.*, 2009).

The mesocarp of Nam Dok Mai No. 4 mango fruits showed higher SOD, CAT and APX activities than the exocarp throughout 5 °C storage period and the increase in CAT and APX activities of the mesocarp was higher than those of the exocarp (Figures 4.24-4.26). Thus, the mesocarp showed lower contents of $O_2^{\bullet -}$, $HO^{\bullet}$ and $H_2O_2$ than the exocarp during storage at low temperature (Figures 4.24-4.26) indicating that more potential antioxidants show higher free radical scavenging capabilities.

In the case of non-enzymatic antioxidants, an increase in total phenolic compounds was found in both exocarp and mesocarp of Nam Dok Mai No. 4 mango fruits after exposing to low temperature (Figure 4.28). Chilling temperatures could generally stimulate the biosynthesis of phenolic compounds by enhancing phenylalanine ammonia-lyase (PAL), the first key enzyme, activity. PAL activity had been reported to

be involved in CI development in many plants (Cai *et al.*, 2006; Cao *et al.*, 2010; Luo *et al.*, 2012). Luo *et al.* (2012) reported that an increase in PAL activity associated with an increase in total phenolic content in bamboo shoot during low temperature storage at 1 °C. In loquat fruit, PAL activity increased after exposing to low temperature concomitant with an increase in lignin content during low temperature storage (Cai *et al.*, 2006; Cao *et al.*, 2010). These results are similar to the report by Dokhanieh *et al.* (2013) that the PAL activity increased in cornelian cherry fruits stored at 4 °C concomitant with an increase in total flavonoids.

Similarly, in this study total phenolic content increased during the first 21 days of cold storage and decreased afterwards (Figure 4.28). These results indicate that total phenolics had high potential in free radical reduction during early period of cold storage at 5 °C, before decreasing during the remaining time as a result of imbalance in the antioxidant defense system. However, phenolic compound might not be involved in chilling injury in some plants as reported by Jin *et al.* (2009a) who found that total phenolic content decreased during storage time at 0 °C for 5 weeks. Moreover, during low temperature stress at 4 °C cornelian cherry fruit had stable total phenolic content throughout chilling temperature storage time for 3 weeks (Dokhanieh *et al.*, 2013).

The exocarp of Nam Dok Mai No. 4 mango fruits showed higher total phenolic content than the mesocarp throughout storage period (Figure 4.28). This is because mango exocarp is a rich source of PAL and phenolic compounds (Ketsa and Chidtragool, 2005). These results are consistent with Ribeiro *et al.* (2008) and Zulkifli *et al.* (2012) who reported that the exocarp of mango fruit cvs. Chakonan and Ubá contain a high content of phenolic compounds such as flavonols, xanthone-C-glycoside, gallic acid and chlorogenic acid. Chidtragool *et al.* (2011) also reported higher phenolic content in the exocarp of Nam Dok Mai and Choke Anan mango fruits, along with higher PAL activity in the exocarp than that in the mesocarp. A high activity of PAL correlated with high synthesis of phenolic compounds in the exocarp than the mesocarp.

This study also showed that the ascorbic acid (AsA) content in both exocarp and mesocarp of Nam Dok Mai No. 4 mango fruits decreased throughout storage time at 5 °C (Figure 4.29). This might be due to AsA reduced excess ROS that accumulated during low temperature storage (Gill and Tuteja, 2010). Previous studies showed that

AsA was utilized by APX to reduce $H_2O_2$ to water with concomitant generation of MDHA and DHA (Gill and Tuteja, 2010). These studies indicated that the activity of APX rapidly increased at the first 28 days of storage and associated with rapid decrease in AsA. Moreover, AsA can directly react with $O_2^{\bullet -}$ and $H_2O_2$ and regenerate α-tocopherol from tocopheroxyl radical (Sharma *et al.*, 2012). MDHA and DHA can be regenerated to AsA by the enzymes MDHAR, DHAR and use reducing equivalents from GSH in AsA-GSH cycle (Gill and Tuteja, 2010; Sharma *et al.*, 2012).

The exocarp of mango is a rich source of bioactive compounds including AsA exhibited ROS elimination (Ajila *et al.*, 2007). It has higher AsA content than the mesocarp (Figure 4.29) since it is a main source for AsA synthesis. Smirnoff (2000) also reported that AsA mostly remained available in reduced form in leaves and chloroplasts under normal physiological conditions and higher AsA content was found in the mango exocarp than in the mesocarp. However, the exocarp of mango showed higher decrease in AsA content throughout storage time than the mesocarp. It might have higher ROS than the mesocarp that lead to the use of higher AsA for ROS scavenging than the mesocarp. The response of AsA after exposure to low temperature also varied differently depending on plant species. This study showed that low temperature stress at 5 °C induced the decrease in AsA content of Nam Dok Mai No. 4 mango fruits throughout storage time at 5 °C (Figure 4.29). This result agrees with Cai *et al.* (2011) who reported that AsA content in loquat fruit decreased after exposed to low temperature at 1 °C for 35 days. In contrast, Chen *et al.* (2011) found that low temperature stress at 4 °C enhanced the AsA content in eggplant seedlings throughout storage time.

For the last non-enzymatic antioxidant, the glutathione (GSH) content in the exocarp and mesocarp of Nam Dok Mai No. 4 mango fruits also rapidly decreased throughout storage time at low temperature like AsA (Figure 4.30). GSH reduced $O_2^{\bullet -}$, $HO^{\bullet}$ and $H_2O_2$ and regenerated AsA from its oxidized to reduced form by enzyme DHAR resulting in a decrease of GSH during storage at 5 °C. This result agrees with Cai *et al.* (2011) who reported that GSH content in loquat fruit decreased after exposing to low temperature at 1 °C for 35 days.

Apart from the activities of enzymatic antioxidants and contents of non-enzymatic antioxidants, the function of antioxidant defense system was measured by using total antioxidant capacity (TAC) during low temperature stress. In this study, the methods of DPPH and ABTS radical scavenging activities were used to evaluate TAC of Nam Dok Mai No. 4 mango fruit. It was found that both DPPH and ABTS methods gave similar results (Figures 4.31-4.32). The TAC in both DPPH and ABTS methods in the exocarp and mesocarp increased during exposure to low temperature stress for 21 days and decreased later (Figures 4.31-4.32). The increase in TAC associated with the increase in SOD, CAT and APX activities or with an increase in total phenolic content during expose to low temperature (Figures 4.24-4.26, 4.28, 4.31-4.32). The increase in SOD, CAT and APX activities and total phenolic content, led to an increase in the capacity for scavenging ROS and protected the cells from oxidative damage. These results agree with Sayyari *et al.* (2011a, b) who reported that low temperature stress induced an increase in antioxidant content such as ascorbic acid, flavonoids and phenolic compounds such as anthocyanins resulted in increasing TAC in pomegranate fruits. However, TAC in the exocarp and mesocarp by both methods decreased after 21 days of cold storage, opposite to that in the earlier period (Figures 4.31-4.32). Thus, TAC indicates a balance between the production of ROS and antioxidant defense system. Activities of SOD, CAT and APX and levels of phenolic compounds, AsA and GSH declined as storage time continued. Thus, the decline in the ability of these compounds in the protective antioxidant system during the prolonged stress of low temperature storage might be one of the major causes of mango CI, a decrease in antioxidant defense system coupled with the decrease in TAC.

When compared TAC of both methods between exocarp and mesocarp, the exocarp showed higher TAC than the mesocarp throughout storage period (Figures 4.31-4.32). The changes of TAC in the exocarp and mesocarp of mango were similar to changes in SOD activity or total phenolic content during low temperature storage (Figures 4.24, 4.28, 4.31-4.32). The exocarp showed higher TAC similar to higher total phenolic content than the mesocarp (Figures 4.28, 4.31-4.32). These results indicate that phenolic compounds are the main antioxidants which have important role to scavenge ROS in the exocarp and mesocarp of Nam Dok Mai No. 4 mango fruit during storage at 5 °C.

In this study, it was shown that SA and MJ treatments enhanced antioxidant enzyme activities in the exocarp and mesocarp of Nam Dok Mai No. 4 mango fruit during low temperature storage (Figures 4.24-4.26). These results are consistent with Tareen *et al.* (2012), Promyou *et al.* (2012) and Chen *et al.* (2011) who found that SA induced some antioxidant enzymes such as SOD, CAT, APX and MDHAR during low temperature storage at 0-4 °C. MJ also enhanced SOD, CAT and APX activities during low temperature storage at 0-1 °C (Cao *et al.*, 2009; Jin *et al.*, 2009b).

SA and MJ enhanced and maintained antioxidant enzyme activities of Nam Dok Mai No. 4 mango during storage at low temperature. These might be because SA and MJ increased their antioxidant enzyme gene expression. Treatment with 1 mM SA enhanced expression of SOD, CAT and APX genes in the exocarp of Nam Dok Mai No. 4 mango and expression of those genes are higher than the control fruits during all storage time at low temperature (Figure 4.27). The increase in the expression of antioxidant enzyme genes in SA treated fruits led to the increase in antioxidant enzymes activities. The expression of SOD, CAT and APX genes were highly significant ($P = 0.01$) and positively correlated with SOD, CAT and APX activities ($r = 0.879$, 0.678 and 0.886 respectively). These results are consistent with the work of Fung *et al.* (2004) who reported that higher SOD, CAT and APX genes expression in sweet pepper during storage at 0 °C were activated by MeSA treatment. The results are also compatible with Ding *et al.* (2002) who found that CAT gene expression of MeSA treated tomato was higher than the control fruit after 3 days of storage at 5 °C. SA treatment also enhanced the induced expression of stress-responsive genes under chilling stress such as GST1, GST2, GPX1, GPX2, GSH, MDHAR, GR and DHAR encoding to ascorbate-glutathione cycle enzymes in eggplant seedlings (Chen *et al.*, 2011). In this experiment, expression of SOD, CAT and APX genes were not studied in MJ-treated fruits. MJ can enhance SOD, CAT and APX genes expression in many fruits (Ding *et al.*, 2002; Fung *et al.*, 2004).

The possible mechanism of SA and MJ capable of inducing antioxidant gene expression is that they act as signaling molecules which had ability to activate some antioxidant genes expression that response to chilling stress. SA and MJ induced abiotic stress protective genes by activating mitogen-activated protein kinases (MAPKs) (Yuan

and Lin, 2008; Hu *et al.*, 2009). MAPK cascades had been proposed as major pathways for signal transduction into intracellular response to activated transcription factors then induced gene expression that response to stress (Yuan and Lin, 2008; Miura and Tada, 2014). Moreover, SA induced an increase in cytosolic $Ca^{2+}$ concentrations (Wang and Li, 2006). $Ca^{2+}$ acts as a secondary messenger that transfers extracellular signals (Mahajan and Tuteja, 2005; Yadav, 2010; Xiong *et al.*, 2002). In addition, pretreatment with SA at low concentration (0.1-0.5 mM) caused low levels of ROS accumulation (Harfouche *et al.*, 2008). SA increased ROS accumulation by inducing SHAM-sensitive guaiacol peroxidases in guard cells (Mori *et al.*, 2001; Khokon *et al.*, 2011). SA also inhibited CAT, APX and carbonic anhydrase which act as ROS scavenger (Chen *et al.*, 1993; Conrath *et al.*, 1995; Durner and Klessig, 1995; Slaymaker *et al.*, 2002). The inhibition of these enzymes by SA induced an increase in the ROS levels. Low ROS levels act as secondary signal molecules to enhance the activities of cellular protective enzymes, including APX, CAT, SOD, GPX and GR (Janda *et al.*, 1999; Kang and Saltveit, 2002; Taşgín *et al.*, 2003; He *et al.*, 2005; Shi *et al.*, 2006). The increase in signal transduction molecules by SA or MJ treatment might lead to activate some antioxidant genes.

In addition, to enhance antioxidant enzyme activities, SA and MJ treatments also increased or maintained non-enzymatic antioxidant contents. SA and MJ enhanced total phenolic content in exocarp and mesocarp of Nam Dok Mai No. 4 mango fruit during storage at low temperature (Figure 4.28). These results are consistent with Luo *et al.* (2012) who found that total phenolic content in bamboo shoots treated with SA. ASA and MeSA, a derivative of SA, also enhanced total phenolic content in pomegranate during postharvest storage (Sayyari *et al.*, 2011a, b). MJ treatment also enhanced total phenolic content in peach and pomegranate during cold storage (Jin *et al.*, 2009b; Sayyari *et al.*, 2011a).

The possible mechanism of SA and MJ to increase total phenolic content in Nam Dok Mai No. 4 mango might be associated with SA and MJ enhanced phenolic compound biosynthesis. Wen *et al.* (2005) and Dokhanieh *et al.* (2013) reported that SA and MJ acted as signal molecule to induce gene expression or activities of PAL enzyme. Consequently, the increase in PAL activities led to activated phenolic compounds

biosynthesis via phenylpropanoid and flavonoid pathways (Ali *et al.*, 2007; Dokhanieh *et al.*, 2013). SA and MJ also induced biosynthesis of phenolic compounds which have antioxidant properties due to the stimulation of PAL activity. The phenolic compounds include chlorogenic acid, caffeic acid, quercetin, ferulic acid, gallic acid, protocatechuic acid, myricetin, quercetin-3-*O*-rutinoside and cyanidin-3-glucoside, showing a high antioxidant capacity (Ali *et al.*, 2007; Wang *et al*., 2009; Dokhanieh *et al.*, 2013). Thus SA and MJ might positively affect phenolic biosynthesis resulting in enhanced antioxidant capacity and reduced CI in Nam Dok Mai No. 4 mango fruit during storage.

SA and MJ treatment maintained the content of ascorbic acid in exocarp and mesocarp of Nam Dok Mai No. 4 mango during storage at low temperature (Figure 4.29).These results are consistent with Sayyari *et al.* (2009, 2011b) who reported that treatment with SA and ASA maintained higher ascorbic acid content in pomegranate during storage at low temperature. MJ also maintained high levels of ascorbic acid in peach and loquat fruits during storage at low temperature (Jin *et al.*, 2009b; Cai *et al.*, 2011).

The possible mechanism of SA and MJ to keep higher ascorbic acid content in Nam Dok Mai No. 4 mango might be caused by maintaining the high reduced form of ascorbic acid (AsA). SA and MJ enhanced activities of MDHAR and DHAR which catalyzed the regeneration of AsA from MDHA and DHA respectively (Wang and Li, 2006; Cai *et al.*, 2011). The increase in MDHAR and DHAR activities were found in grape plant treated with SA (Wang and Li, 2006). The activities of MDHAR and DHAR also increased in loquat fruit treated with MJ (Cai *et al.*, 2011).

Finally, SA and MJ treatments also helped keeping higher content of total glutathione in exocarp and mesocarp of Nam Dok Mai No. 4 mango during storage at low temperature (Figure 4.30). These results are consistent with Chen *et al.* (2011) who reported that treatment with SA increased glutathione content in eggplant seedlings after exposed to chilling stress. MJ also maintained higher glutathione content in loquat fruit during storage at low temperature (Cai *et al.*, 2011).

The possible mechanism of SA and MJ in keeping higher glutathione content in Nam Dok Mai No. 4 mango might be associated with maintaining the higher reduced

form of glutathione (GSH) by SA and MJ. SA and MJ enhanced activities of GR which catalyzed the regeneration of GSH from GSSG (Wang and Li, 2006; Cai *et al.*, 2011). The increase in GR activities were found in grape treated with SA (Wang and Li, 2006) and in loquat fruit treated with MJ (Cai *et al.*, 2011). The increase in GR activity leads to more content of reduced glutathione that has an ability to scavenge ROS.

An increase in TAC by SA and MJ treatments during the first 21 days and delayed the decrease of TAC during 21-42 days of storage of Nam Dok Mai No. 4 mango related to the changes in the enzymatic antioxidants (Figures 4.31-4.32). These results are consistent with Sayyari *et al.* (2011a, b) who reported that treatment with ASA, MeSA and MJ increased TAC in pomegranate during chilling stress. SA and MJ treatments also increased TAC in lemon during storage at low temperature (Siboza and Bertling, 2013). The increase in TAC by SA and MJ treatments associated with an increase in total phenolic content by SA and MJ in Nam Dok Mai No. 4 mango stored at 5 °C. SA and MJ treatments increased the content of many phytochemical compounds with antioxidant activity, including ascorbic acid, flavonoids, and phenolic compounds such as anthocyanins that are the main compounds contributing to the antioxidant capacity of the pomegranate arils during storage at low temperature (Sayyari *et al.*, 2011a, b).

The results from experiments 1, 2 and 3 indicated that the CI development and some fruit quality changes in Nam Dok Mai No. 4 mango fruits were induced by low temperature storage. Low temperature stress induced ROS accumulation, antioxidant defense system was activated to scavenge ROS. Unless, storage at low temperature stress was prolonged over a certain extent (Day 21), ROS production overwhelmed the scavenging action of the antioxidant defense system resulting in oxidative membrane damage. These increases in damage of cell and organelle membrane led to CI appearance in the fruits during storage at 5 °C for 21 days (Figure 5.1). SA and MJ treatment reduced CI symptoms in Nam Dok Mai No. 4 mango during storage at 5 °C by enhancing antioxidant defense system including both enzymatic and non-enzymatic antioxidants. The higher antioxidant defense system of SA and MJ treated fruits led to more effective action in scavenging excess ROS resulting in lowering ROS content, oxidative membrane damage and CI development during storage at 5 °C (Figure 5.2).

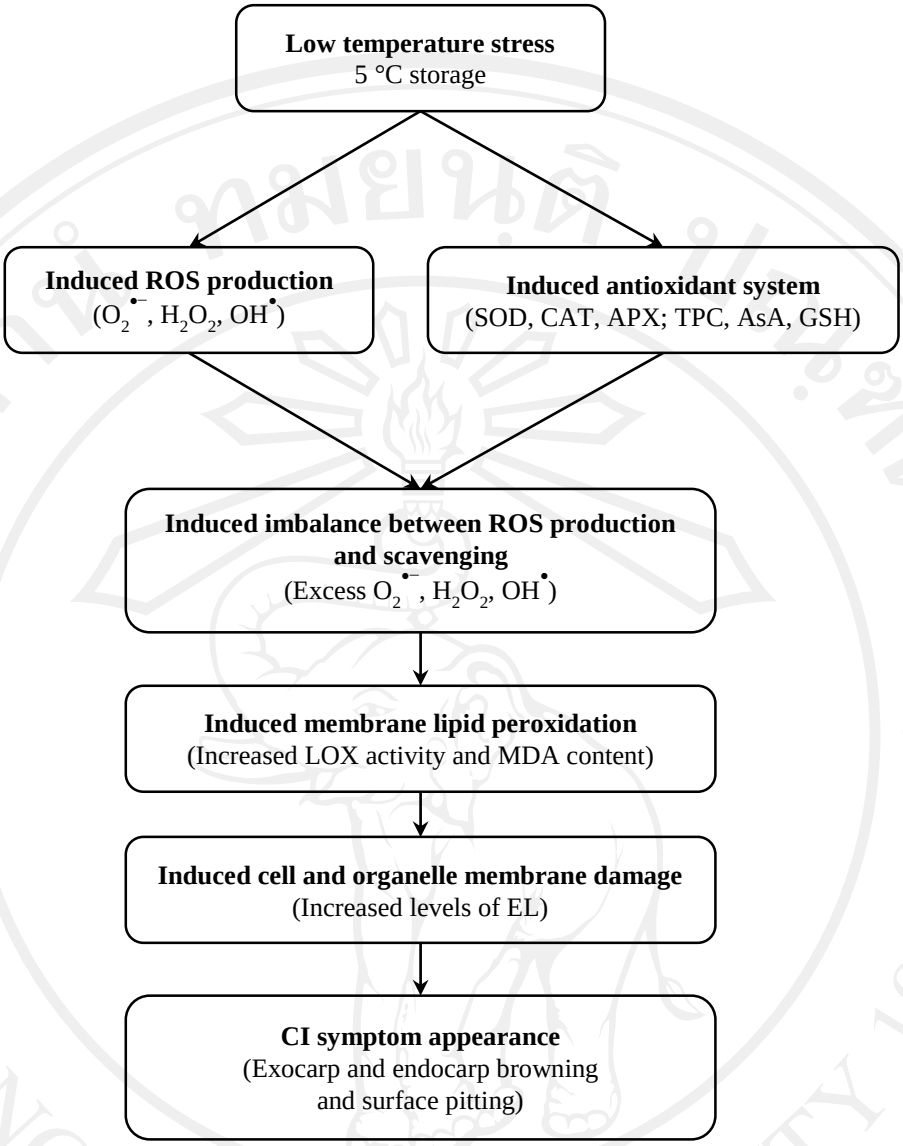


Figure 5.1 Schematic model for initiation and development of chilling injury in Nam Dok Mai No. 4 mango fruit during storage at 5 °C.

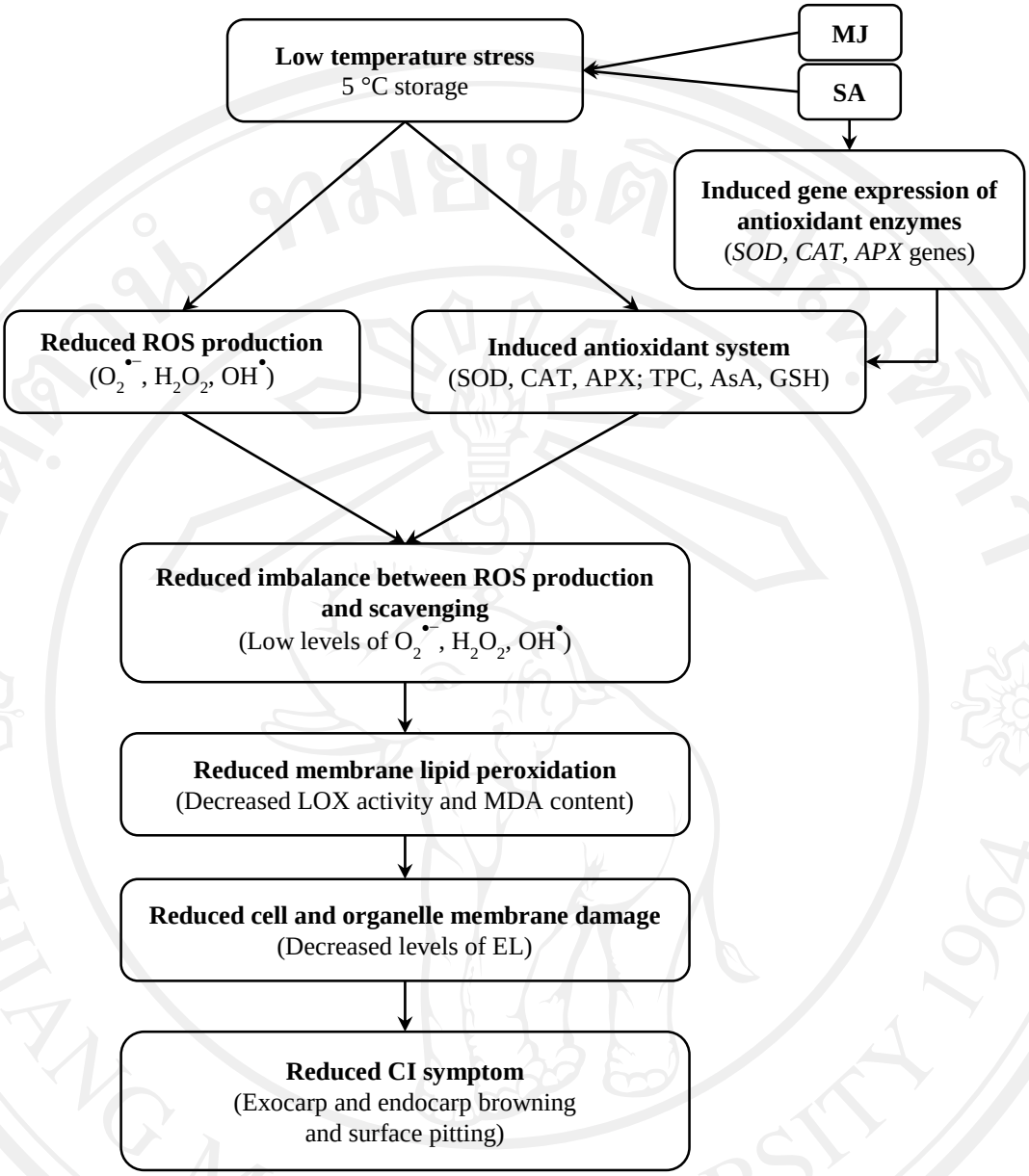


Figure 5.2 Schematic model for reduction of chilling injury by SA and MJ in Nam Dok Mai No. 4 mango fruit during storage at 5 °C.