CHAPTER 5

DISCUSSION

5.1 Experiment I Effect of UV-B irradiation on chlorophyll-degrading enzyme activities and postharvest quality in stored lime (*Citrus latifolia* Tan.) fruit

The yellowing of leaves, florets and fruit pericarp is an important factor indicative of quality deterioration in stored horticultural products. Obviously, in lime, the most visible deterioration is the loss of peel greenness that usually occurs with Chl degradation (Win et al. 2006; Srilaong et al., 2011). The maintenance of green color in the peel of lime during storage is required if fruit are to add value prices (Pranmornkith et al., 2005). Many studies have shown the deleterious effect of UV irradiation on fruits and vegetables to maintain the postharvest quality. Specifically, UV-C irradiation can reduce decay in grapefruit (D'hallewin et al., 2000) or maintain the postharvest quality of strawberry (Erkan et al., 2008) and in suppressing Chl degradation in stored broccoli florets (Costa et al., 2006a). Also, UV-B is an alternative UV range that may maintain postharvest quality of fresh produce. Aimala-or et al. (2010) found that UV-B treatment effectively delayed Chl degradation of broccoli during storage. Previously, Srilaong et al. (2011) reported that UV-B treatment effectively suppressed Chl degradation in mature green lime during storage. In the present study, lime fruit were irradiated with UV-B at 19.0 kJm^{-2} and the results were compared with those of untreated fruit. Base on the results of hue angle and Chl *a* and Chl *b* contents, we suggested that UV-B treatment effectively delayed the chlorophyll breakdown in the lime peel. The delay of Chl

degradation with UV-B treatment may have the same effect in broccoli florets as 1-MCP induced reduction in Chl-degrading peroxidase and Chlase activities (Gong and Mattheis, 2003). Heat treatment reduce the Chl degradation through the suppression of activities of Chl-degrading enzymes, including Chlase, MD, Chl-degrading peroxidase and Chl oxidase (Costa et al., 2006a; Funamoto et al., 2002; Kaewsuksaeng et al., 2007). UV-C (Costa et al., 2006b) and UV-B irradiation (Aimala-or et al., 2010) which also suppressed Chl-degrading enzyme activities as Chlase, MD, MDS and Chl-degrading peroxidase. In this study, a UV-B treatment at 19.0 kJm^{-2} effectively suppressed the activities of Chl-degrading enzymes such as Chlase, Chl-POX, MDS and PPH in lime fruits. During storage, Chlase activity gradually increased in lime fruit with or without UV-B treatment. In contrast, it was previously reported that Chlase activity decreased with degreening of *Citrus nagato-yuzukichi* (Yamauchi et al., 2003), *Citrus aurantifolia* Swingle cv. Paan (Win et al., 2006) and broccoli florets (Aimala-or et al., 2010). In the study, we found that Chlase activity was tentatively suppressed in stored lime fruit throughout storage by UV-B treatment. The mature green lime was shown to have a high level of Chlide *a* which suggests that the high level of Chlide *a* is caused by Chlase action in the flavedo. This enzyme is involved in the first step of the chlorophyll catabolic pathway, which catalyzes the conversion of Chl *a* to Chlide *a* and phytol (Harpaz-Saad et al., 2007). This result agrees with a report on lime fruits that the level of Chlide *a* was retained at high levels in limes with UV-B treatment (Srilaong et al., 2011). This outcome could be attributed to the UV-B treatment, which effectively inhibits the activity of Chlase, resulting in the retention of the Chlide *a* levels. Our results showed that Chl-POX activity was markedly increased in lime fruit during

storage, but its activity was clearly suppressed throughout the storage life of lime treated with UV-B. In lime fruits, Chl *a* can be degraded by Chl-POX (Win et al., 2006) with 13^2-hydroxychlorophyll *a* is formed as an intermediate and does not accumulate by UV-B treatment (Aimala-or et al., 2010). MDS activity was also determined by using Chlide *a* as native substrate in lime fruits. MDS, which is small molecule and heat stable substance, was required to remove the magnesium atom from Chlide *a* (Suzuki et al., 2005; Kaewsuksaeng et al., 2010). We found that MDS activity was a little increased in control stored lime fruits and UV-B treatment also effectively suppressed MDS activity. These findings were similar to the finding by Aimala-or et al. (2010). Our result also correlates with Srilaong et al., (2011) found that the levels of Pheide *a* were lower in UV-B-treated limes than in the control fruit. This indicates that the degradation of Chlide *a* to Pheide *a* may be suppressed by UV-B treatment. Accordingly, we suggest that MDS could be involved in Mg-dechelation from Chlide *a* in lime fruits. In addition, a new enzyme in relation Chl degradation as PPH activity was determined in lime fruit. PPH specially dephytilates Phein *a* to produce Pheide *a* (Schelbert et al., 2009). Our result showed the PPH activity gradually increased during storage of lime fruits. While, the UV-B treatment effectively suppressed PPH activity even though activation after treatment. Sequential degradation of Chl by PPH activity implies formation of Pheide *a* in lime fruit is possibly included. Several studies have detected Phein *a* accumulation during storage of broccoli florets (Costa et al., 2006a; Kaewsuksaeng et al., 2006; Aiamla-or et al., 2010) and lime fruit (Srilaong et al., 2011), which suggest the presence of enzymatic that release Mg^{2+} from Chl *a* as Phein *a* and thus provides substrates for PPH. However, Phein *a* was accumulated at a higher rate in the UV-B-treated limes

than in the control fruit (Srilaong et al., 2011). Suggesting that the high accumulation of Phein *a* in UV-B treatment initiate also non-enzymatic formation to produce substrate for PPH. Further study needs to clarify the role of PPH in Chl degradation of lime fruit.

Besides Chl degradation, the composition changes in relation to the internal quality occur during storage of lime fruits. The citric acid and malic acid contents in lime fruit almost no changed during storage. However, theirs were higher in UV-B treatment than in the control. UV-B treatment may suppress the respiration rate, which are necessary to maintain organic acids in lime fruit. As a consequence, the level of fructose, glucose and sucrose contents showed a decrease during storage, but the decrement was higher in UV-B treatment of lime fruit. In this case, UV-B may have inhibited enzymes that inter-convert different carbohydrates such as invertase or enzymes that are normolly enhance during postharvest senescence. The results agreed with effect of heat treatment on reducing sugar (Lemoine et al., 2008). Thus, UV-B treatment seems to be a useful treatment for maintenance of internal quality in lime fruit.

The UV-B treatment that also affects on stomatal closure of epidermal peels of lime fruit during storage. We agreed with Dai et al. (1995) reported UV-B irradiation reduced the opening of stomata on the surface of rice leaves. In addition, Nogués et al. (1999) also found that UV-B irradiation can cause stomatal closure in plants. Our result supporting with Srilaong et al. (2011) showed UV-B treatment was markedly reduced weight loss and shriveling in the lime fruit.

5.2 Experiment II Hot water treatment delays chlorophyll degradation and postharvest quality in lime (*Citrus aurantifolia* Swingle cv. Paan) fruit

For postharvest horticultural crops such as leafy vegetables, broccoli florets, and limes, one of the main factors related to quality deterioration is the loss of green color with Chl degradation (Win et al. 2006a; Srilaong et al., 2011; Kaewsuksaeng et al., 2011). The maintenance of green color in the peel of Thai limes during storage and shelf life are required for fruit to maintain their value prices (Pranmornkith et al., 2005). Due to consumers' interest in decreasing the postharvest use of chemicals, heat treatments such as hot water, hot air, and vapor heat treatment have gained interest for the control the quality. Heat treatment has also been demonstrated to show physiological effects on the control of ripening and senescence and the tolerance to chilling injury in postharvest fruits and vegetables (Fallik, 2004; Lurie and Mitcham, 2007).

The effects of postharvest stress treatments such as heat and UV treatments on yellowing or degreening were determined in stored horticultural produce (Yamauchi, 2013). Broccoli floret yellowing was effectively retarded during storage treated with hot air at 50 °C for 2 h (Funamoto et al., 2002). Additionally, heat treatment at 45 °C for 2.5 and 3 h had an inhibitory effect on Chl degradation, but the effect was less than that of 50 °C for 2 h (Funamoto et al., 2003). Yamauchi et al. (2003) reported that the heat treatment at 50 °C for 3 min with the solution of 2% sucrose laurate ester delayed the degreening in Nagato-yuzukichi (*Citrus nagato-yuzukichi* hort. Ex Y. Tanaka). Green yuzu (*Citrus junos* Siebold ex Tanaka) and Nagato-yuzukichi hot water treatments at 40 and 45 °C efficiently suppressed the decline of hue angle values during storage at 25 °C. Yuzu fruit treated with hot water at 40 °C for 5 and 10

min and Nagato-yuzukichi fruit treated at 45 °C for 5 min kept their green peel color (Ogo et al., 2011). In the present study, Thai lime fruits were treated with hot water at 50 °C for 3 and 5 min, and the results were compared with those of untreated fruit. We found that hot water treatment at 50 °C for 5 min retarded the Chl breakdown with delayed decline of hue angle value and Chl *a* and *b* contents in the lime fruit peel.

Heat treatment has been known to reduce the Chl degradation through the suppression of activities of Chl-degrading enzymes, including Chlase, Mg-dechelation activity, Chl-degrading peroxidase, and Chl oxidase (Costa et al., 2006; Funamoto et al., 2002; Kaewsuksaeng et al., 2007). UV-B irradiation in Tahitian lime (*Citrus latifolia* Tan.) (Kaewsuksaeng et al., 2011) also suppressed the activities of Chl-degrading enzymes such as Chlase, Mg-dechelation, Chl-degrading peroxidase and PPH. In this study, hot water treatment at 50 °C for 3 and 5 min also effectively suppressed the enhancement of Chlase and Chl-POX activity during storage in lime fruit, especially the latter. Mg-dechelation and PPH activities in hot water-treated lime fruit at 50 °C for 5 min reduced after a temporary increase during storage. Chlase activity in the control gradually increased with senescence during storage in Thai lime (*Citrus aurantifolia* Swingle cv. Paan), the same as in Tahitain lime fruits (Kaewsuksaeng et al., 2011). In contrast, it was previously reported that Chlase activity decreased with degreening of *Citrus nagato-yuzukichi* (Yamauchi et al., 2003) and broccoli florets (Aiamla-or et al., 2010). We also found that Chlase activity was tentatively suppressed in lime fruit throughout storage by hot water treatment at 50 °C for 5 min. The mature green lime was shown to have a high accumulation level of Chlide *a* during storage (Srilaong et al., 2011), which suggests

that the high level of Chlide *a* is due to the increased Chlase action in the flavedo. This enzyme is involved in the first step of the chlorophyll catabolic pathway, which catalyzes the conversion of Chl *a* to Chlide *a* and phytol (Harpaz-Saad et al., 2007).

Our results indicated that Chl-POX activity markedly increased in lime fruit during storage, but its activity was clearly suppressed throughout the storage period with hot water treatment at 50 °C for 5 min. In lime fruit, Chl *a* can be degraded by Chl-POX (Win et al., 2006a) to form the oxidized Chl *a*, 13^2-hydroxychlorophyll *a*, which did not accumulate during storage by UV-B treatment (Aimala-or et al., 2010). Mg-dechelation activity by MDS was also determined by using Chlide *a* as native substrate in lime fruit. MDS, which is a small molecule and heat-stable substance, was required to remove the magnesium atom from Chlide *a* (Suzuki et al., 2005; Kaewsuksaeng et al., 2010). We found that Mg-dechelation activity increased sharply in day 5 after hot water treatment due to the stress condition from high temperature and after that hot water treatment at 50 °C for 5 min also effectively inhibited Mg-dechelation. These results were similar to those by Kaewsuksaeng et al. (2011) that Mg-dechelation activity in Tahitian lime increased slightly in the control and that UV-B treatment also effectively suppressed the enhancement of Mg-dechelation. Srilaong et al. (2011) demonstrated that the Pheide *a* level declined in UV-B-treated fruit, especially during the development of yellowing. This indicates that the degradation of Chlide *a* to Pheide *a* might be suppressed by UV-B. Further study needs to clarify the Chl derivatives and Chi-degrading enzymes in heat-treated lime fruit. These findings show that Mg-dechelation activity is significantly involved in the Chl-degrading process in lime fruit peel.

Recently reported as a new Chl-degrading enzyme, PPH dephytylates Phein *a* to form Pheide *a*. It is inferred that PPH activity measured in this study might include some activity of chlorophyllase, since it can also dephytylate Phein *a* along with Chl *a* (McFeeters et al., 1971; Mínguez-Mosquera et al., 1994). Kaewsuksaeng et al. (2011) showed that PPH activity gradually increased during storage in Tahitian lime fruit, while the UV-B treatment effectively suppressed PPH activity. For broccoli florets, it was found that PPH activity with or without UV-B treatment also increased during storage at 15 °C, but UV-B did not significantly affect PPH activity (Aiamla-or et al., 2012). In contrast, our results found a temporary increase in PPH activity in Thai lime after heat treatment, after that a decline during storage of lime fruits. However, hot water treatment at 50 °C for 5 min could greatly inhibit PPH activity in lime fruit. These findings seems to be due to the suppression of Chl-degrading enzymes such as Chlase, Mg-dechelation, Chl-degrading peroxidase and PPH activities by stress treatment such as hot water in Thai lime fruit. Further study needs to be carried out to clarify the characterization of Chl-degrading enzymes in relation to Chl degradation of lime fruit.

Besides Chl degradation, the physiological effects were determined in ethylene production and respiration rate. The results showed that the endogenous ethylene production slightly increased from mature green to full yellow in control of lime fruit, while the hot water-treated fruit at 50 °C for 5 min did not change during storage. Application of heat treatment such as hot water at 50 °C for 5 min significantly reduced ethylene production in lime fruit. This might be because of the suppression of ethylene production by hot water treatment due to the decrease of ACC (1-aminocylocpropane-1-carboxylic acid) oxidase action in the ethylene

pathway in lime fruit (Win et al., 2006b). The composition changes in relation to quality occur during the storage of lime fruit. The citric acid content, determined to be titratable acidity in lime fruit, showed a slight change during storage. This content was higher in hot water- treated fruit at 50 °C for 5 min than any other during storage, suggesting that this acid might be maintained due to the suppression of respiration rate by hot water treatment. Hot water treatment was similar to that UV-B treatment in maintaining the acid content in lime fruit (Kaewsuksaeng et al., 2011). Hot water treatment at 50 °C for 5 min also suppressed the changes in total soluble solid during storage. On the other hand, total soluble solids in the control increased with the advance of senescence. The results agreed with the effect of heat treatment on reducing sugar level changes (Lemoine et al., 2008). Thus, hot water treatment seems to be a useful treatment for the maintenance of postharvest internal quality in lime fruit.