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Histological and Biochemical Traits of Chilling-injured Pulp Tissues as Affected by Cold Storage of Mango Fruit

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***Abstract*. Green mango (*Mangifera indica* L.) 'Nam Doc Mai See Thong' fruit were dipped in 2-chloroethylphosphonic acid solution (50 ppm) for 5 minutes, kept at 25 °C for 3 days, cold stored at 5 °C for 35 days and then transferred to 25 °C for 7 days. The skin color of the cold-stored fruit partly changed to dark-brown with surface depression. In addition, desiccated white-corky pulp tissues developed mainly along to the dark-brownish skin. Histological and biochemical analyses revealed that the formation of white-corky pulp tissues was correlated with starch accumulation in the hypodermal cells. Cell wall polymers of the white-corky pulp tissues were characterized by both a lower amount of solubilized pectins and higher amount of hemicelluloses than those of normally ripened (NR) tissues. The highest fatty acid unsaturation was observed in the NR pulps under chilling conditions followed by the white-corky pulp tissues under chilling conditions and the NR tissues without chilling. These results suggested that the disordered membrane caused by chilling inhibited the subsequent cascade of secondary reactions, such as the cell wall degradation. The skin damage derived from chilling injury (CI) is a direct factor inducing abnormal desiccation in the adjacent pulp, resulting in the formation of white-corky pulp tissues.**

Thailand produces more than 3 million t/year of mangoes and exports the fresh fruit to Japan, Malaysia, Indonesia, Singapore, and Hong Kong (Chomchalow and Songkhla, 2008). Harvested fruit is commonly refrigerated to delay ripening because the storage life is limited to 2–3 weeks in air at 10 to 15 °C (Sivakumar et al., 2011). However, CI often occurs, especially when green mangoes are stored at low temperatures (Singh et al., 2013). The CI of postharvest mango fruit is characterized by high starch (Chhatpar et al., 1971) and ascorbic acid (Thomas and Joshi, 1988) contents, low sugar (Mohammed and Brecht, 2002) and carotenoid (Thomas and Oke, 1983) contents, skin pitting or browning (Chaplin et al., 1991; Thomas and Joshi, 1988; Thomas and Oke, 1983), and the inhibition of fruit softening (Chaplin et al., 1991; Medlicott et al., 1990). However, desiccated white-corky pulp tissues, which are similar to those of spongy disorder (SD), were also recently identified along with the damaged skin tissues.

SD in mango fruit is known as a ripening disorder which usually occurs in the fruit-ripening stage (Katrodia, 1988; Sivakumar et al., 2011). SD is formed on trees (Katrodia, 1988; Lima et al., 2001; Raja, 2009), especially in fruit with a large size and high gravity (Krishnamurthy, 1980). In a fruit, SD is more frequently formed near the seed rather than near the skin (Burondkar et al., 2009). As for the environmental conditions, SD develops frequently under high-temperature conditions (Raja, 2009).

SD in mango fruit is also characterized by low soluble sugar and high starch accumulations (Chhatpar et al., 1971; Katrodia, 1988; Lima et al., 2001; Vasanthaiah et al., 2006) because of the disturbance of gluconeogenesis (Yashoda et al., 2006).

Some researchers have suggested that the damage of cell membranes derived from lipid peroxidation leads to the formation of SD in mangoes (Janave, 2009; Janave and Sharma, 2008; Shivashankar et al., 2007). Cell membranes in the pulp tissues at the fruit-ripening stage are oxidized by some factors such as high temperatures (Vasanthaiah et al., 2006), desiccation due to water movement from the pulp to the seed (Ravindra and Shivashankar, 2004, 2006; Shivashankar et al., 2007), and infection by *Staphylococcus xylosus* (Janave and Sharma, 2008; Machhindra and Sharma, 2008).

Therefore, if the damage of cell membranes is the key factor leading to SD, it may also be caused under low-temperature storage conditions because the antioxidant capacities of mango fruit also decrease during cold storage (Ding et al., 2007; Miguel et al., 2011; Shivashankara et al., 2004), which may result in the disturbance of physiological functions in the cell membranes (Kane et al., 1978). Chilling sensitivity to the cell membranes in tropical fruit such as mangoes is often shown by the unsaturation of fatty acids (Kane et al., 1978).

When mango fruit are stored at lower than 10 °C, malfunction or disruption of the membrane function takes place and results in CI (Singh et al., 2013; Sivakumar et al., 2011). Typical physiological changes in chilling-injured pulp tissues are similar to those of SD which are formed on trees at the fruit-ripening stage, i.e., high water loss, high starch accumulation, and a low soluble sugar content (Chaplin et al., 1991; Chhatpar et al., 1971; Gupta and Jain, 2014; Medlicott et al., 1990; Mohammed and Brecht, 2002).

Histological observation revealed that starch granules in the pulp cells rapidly changed their shape from round at the green mature stage to irregular as the fruit matures and eventually almost disappears at the ripening stage (Simão et al., 2008). Such a decomposition process of starch granules in mango fruit is also closely associated with rapid fruit softening caused by cell wall depolymerization (Yashoda et al., 2006).

Immature to mature green mangoes are more sensitive to CI compared with ripe fruit and fail to ripen adequately (Sivakumar et al., 2011). In addition, the CI symptom primarily appears on the skin as grayscales and pitting, and then eventually appears in the pulp (Lizada, 1991), indicating that pulp tissues are more tolerant to CI than the skin.

The preapplication of exogenous ethylene has been used to avoid the ripening inhibition, especially when green mangoes are stored at a low temperature (Montalvo et al., 2007). The preapplication of 2-chloroethylphosphonic acid (ethephon) solution has been commercially

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used as an alternative method to ethylene treatment because it is decomposed to ethylene in the absorbed plant tissues (Singh and Janes, 2001).

In our study, green mangoes were stored under low-temperature conditions after being treated with 0.05% ethephon (50 ppm 2-chloroethylphosphonic acid) to determine if chilling disorder also developed in the pulp tissues due to postharvest cold storage.

In addition, soluble sugars, starch, cell wall polysaccharides, and fatty acid unsaturation were compared with those of NR tissues.

Materials and Methods

Plant materials and storage conditions. One-hundred mango (*Mangifera indica* L. cv. Nam Doc Mai See Thong) fruit were harvested at 120 d after fruit set from an orchard of Chiang Mai, Thailand in June 2011. The fruit were dipped in tap water and floated fruit (64 of 100 fruit), which were in hard-green stage (Saranwong et al., 2004), were used for the experiment. Half (32) of the fruit were then dipped in 0.05% ethephon which contains 50-ppm 2-chloroethylphosphonic acid for 5 min, kept at 25 °C for 3 d, chilled at 5 °C for 35 d and then replaced again at 25 °C for 7 d to complete fruit ripening. The other half of the fruit were immediately kept at 25 °C for 8 d without ethephon treatment to promote normal ripening.

Preparation of pulp tissues for histological and chemical analyses. Each fruit was cut into three pieces longitudinally along the seed for measuring the incidence of spongy tissues, bilateral sections were trisected transversely, and the pulp tissues in the proximal sections were used for histological and chemical analyses. Each tissue sample was taken as follows:

1. Disordered pulp tissue in the chilled fruit (chilled-D).
2. NR tissue adjacent to the chilled-D tissue in the chilled fruit (chilled-NR).
3. NR tissue in the non-chilled fruit (non-chilled).

The chilled-NR tissue was obtained from the inner portion adjacent to the chilled-D tissue (about 5-mm thickness), and the non-chilled tissue was obtained from the portion equivalent to the chilled-NR tissue.

Microscopic observation. About 5-mm cubic samples were taken from the chilled-D and chilled-NR tissues, fixed in 37% formaldehyde:pure acetic acid:pure ethanol: distilled water = 5:5:45:45 (v/v) and then post-fixed in 1% osmium tetroxide, The tissues were dehydrated using a mixed-solution series of *t*-butanol, ethanol, and water, dried at 5 °C using a lyophilizer (JFD-310, JEOL, Tokyo, Japan), and coated with a 10-nm Pt layer by a fine coater (JFC-1200; JEOL, Tokyo, Japan). Surface structures and starch granules of the tissues were examined using a scanning electron microscope (SEM) (TM3000; Hitachi, Tokyo, Japan).

Soluble sugars. Pulp tissues taken from the chilled-D, chilled-NR, and non-chilled fruit were lyophilized at –50 °C and powdered in a mill. Then, 150 mg of the powdered sample was extracted by 10 mL of 80% methanol (MeOH) at 80 °C for 10 min to inactivate enzymes. The homogenate was centrifuged at 5000 *g* for 10 min at 5 °C, and the supernatant was decanted. The MeOH-insoluble residue was added to 8 mL of 80% MeOH, stirred at 6 rpm for 30 min, and then centrifuged at 5000 *g* for 10 min at 5 °C. This procedure was repeated until soluble sugars were not detected using High-performance liquid chromatography (HPLC) (Shimazu 18-A, Kyoto, Japan) equipped with a Cosmosil sugar-D packed column (Nacalai Tesque, Kyoto, Japan), as described previously (Nishizawa et al., 2000).

All of the supernatants were combined and brought to a final volume of 100 mL by adding 80% MeOH, dried by N_2 gas, resuspended in distilled water and then filtered through a 0.2-µm glass filter. Then, 10 µL of the filtrates was injected into the abovementioned HPLC for determining glucose, fructose, and sucrose contents. The MeOH-insoluble residue (AIS) was washed with 3 mL of pure acetone, dried at 60 °C using N_2 gas, and used for the analysis of cell wall polysaccharides.

Cell walls. A total of 0.2 g of the powdered sample was used to obtain AIS, as described above. The AIS samples were sequentially extracted with 8 mL of H_2O (water-soluble fraction), 50-mM *trans*-1.2-cyclohexanediamine-*N,N,N′,N′*-tetraacetic acid monohydrate (CDTA) containing 50 mM sodium acetate (pH 4.5) (CDTA-soluble fraction), 25-mM $NaBH_4$ containing 50-mM Na_2CO_3 (Na_2CO_3-soluble fraction), and 4M KOH (KOH-soluble fraction) by stirring the test tubes at 6 rpm for 6 h. Each homogenate was centrifuged at 5000 *g* for 10 min at 5 °C after each extraction. The supernatant was then neutralized, dialyzed against distilled water at 20 °C for 48 h using 6000–8000 MW cut-off dialysis membrane (Spectrum Laboratories, Rancho Dominguez, CA), and brought to 25-mL with distilled water (Nishizawa and Ito, 2007). The content of uronic acids (UA) and neutral sugars (NS) in each fraction was determined by the *m*-hydroxydiphenyl (Blumenkrantz and Asboe-Hansen, 1973) and phenol-sulfuric acid (Dubois et al., 1956) methods, respectively.

A 5 mL of each fraction was then boiled at 100 °C for 1 h, cooled in iced water, added to 6 mL of amyloglucosidase (EC 3.2.1.3. 8 unit/mL: Wako Pure Chemical, Osaka, Japan) in 1-M acetic acid buffer (pH 4.5), and incubated at 55 °C for 2 h. The homogenate was then centrifuged at 5000 *g* for 10 min at 5 °C, and the glucose content in the supernatant was measured by the glucose oxidase method for evaluating starch content in each fraction (Nishizawa et al., 2002). The starch content subtracted from AIS was regarded as the crude cell wall materials (CWM), and cell wall content in each fraction was shown as an amount per CWM.

Fatty acids. Fatty acids were analyzed using the method of Prates et al., (2011) with some modifications. A 20-mg powdered sample was taken from the three abovementioned pulp tissues and methylated using a methylation kit of fatty acids (Nacalai Tesque, Kyoto, Japan). A total of 1 µl of the methylated fatty acids was analyzed by a gas chromatograph (GC-18A; Shimadzu, Kyoto, Japan) equipped with a flame ionization detector and a 0.53-mm × 1-µm × 30-m (external diameter × internal diameter × length) capillary column (Fused silica mega-bore Column DB-225; Agilent Technologies J&W, CA). The temperature of both the injector and detector was kept at 220 °C. The oven was programmed as follows: 150 °C for 3 min followed by 7.5 °C·min to 190 °C, which was then maintained. The total analytical time was about 90 min. Fatty acids were identified by comparison with the retention time of authentic standards (F.A.M.E. Mix C8-C24, Supelco, PA).

Membrane lipid unsaturation is often shown as the double bound index (DBI), which is calculated as follows (Zhang and Tian, 2009):

$$\mathrm{DBI} = [3(\%18:3) + 2(\%18:2)] / [(\%16:0) + (\%18:0) + (\%18:1)]$$

Statistical analysis. Data were subjected to an analysis of variance and the multi-comparison of means was assessed by Tukey's Honestly Significant Difference Test at $P < 0.05$ using SAS for windows (version 6.12; SAS system, Cary, NC).

Results and Discussion

Symptom of CI. Non-chilled fruit ripened normally with yellow pulp and skin (Fig. 1A and B), whereas chilled fruit partially showed a dark-brownish skin color and surface depression, which were reported as typical symptoms of CI previously (Fig. 1C and D) (González-Aguilar et al., 2000; Han et al., 2006; Kane et al., 1982; Pesis et al., 2000; Singh et al., 2013). In addition, white-corky pulps that were similar symptom to SD also developed along with the injured skin (Fig. 1C and D). Such a desiccated white-corky pulp tissue was not found at the end of cold storage (data not shown) indicating that the CI rapidly developed after transferring the fruit from chilling to 25 °C.

SD on trees is characterized by desiccated white-corky tissues (Katrodia, 1988), and such desiccation of pulp tissues at the fruit-ripening stage mainly occurs around the seed (Burondkar et al., 2009), probably because of water movement from the pulp to the seed (Ravindra and Shivashankar, 2004, 2006; Shivashankar et al., 2007).

In the chilled fruit, however, white-corky tissue developed near the fruit surface along the dark-brownish skin, which was also associated with surface depression (Fig. 1C and D). SEM showed that the injury was

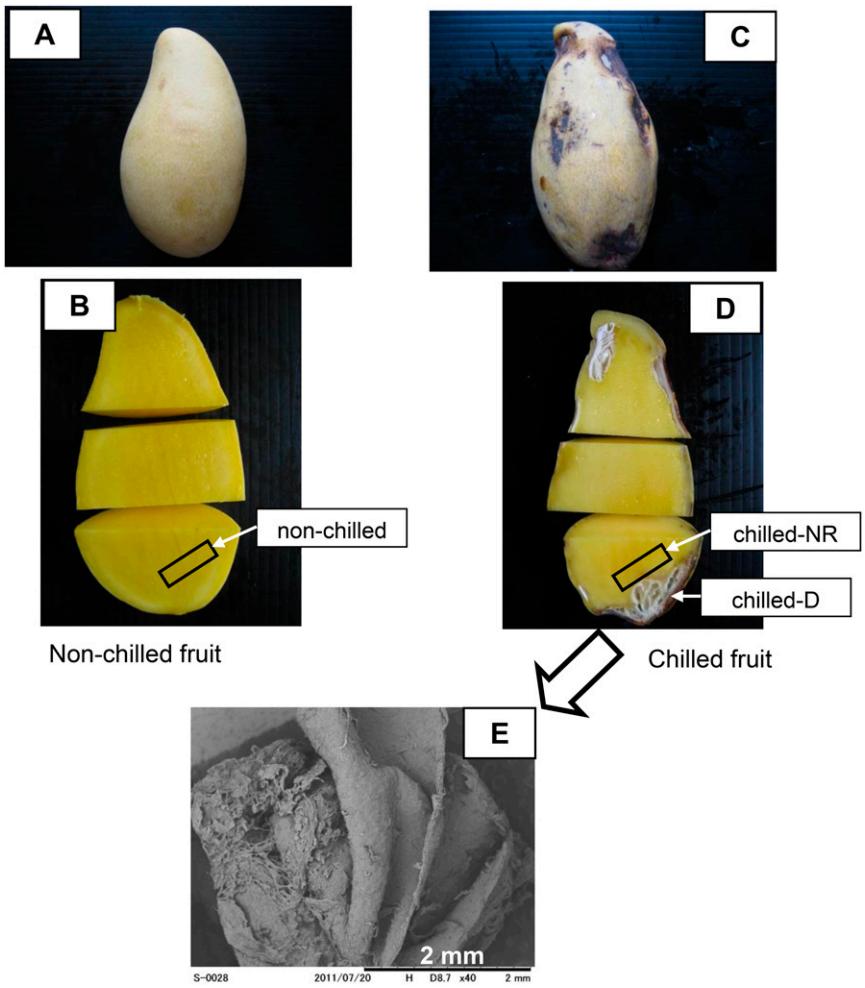

Fig. 1. Comparison of the appearance of the skin and cut surface in the non-chilled and chilled 'Nam Doc Mai See Thong' mango fruits. The non-chilled fruit were kept at 25 °C for 8 d directly after harvest to induce normal ripening (NR), while the chilled fruit were treated with 2-chloroethylphosphonic acid solution (50 ppm), kept at 25 °C for 3 d, stored at 5 °C for 35 d, and then transferred to 25 °C for 7 d to observe chilling injury. (**A** and **B**) Non-chilled fruit, (**C** and **D**) chilled fruit, and (**E**) SEM image of chilling-injured pulp tissue. The rectangles show the portions in which the non-chilled and chilled-NR tissues were taken.

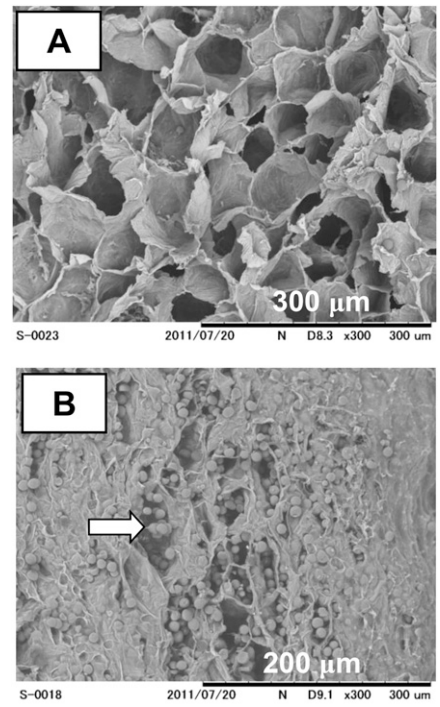

Fig. 2. Comparison of SEM images between normally ripened and chilling-injured 'Nam Doc Mai See Thong' mango pulps. Arrow: starch granules, (**A**) normally ripened pulp at 25 °C, and (**B**) chilling-injured pulp with starch granules.

Table 1. Soluble sugar contents of chilling-injured 'Nam Doc Mai See Thong' mango pulps.

	Soluble sugars (mg·g^{-1} DW)^{z}			
Tissue	Glucose	Fructose	Sucrose	Total
Chilled-D	38.8	110.6 b	235.2	384.6 b
Chilled-NR	49.5	197.0 a	398.1	644.6 a
Non-chilled	50.8	166.4 ab	547.3	764.5 a
Significance	NS	*	NS	*

Data are presented as means (n = 5). Values followed by the same letter do not differ significantly (P < 0.05) among tissues according to Tukey's test.

^{z}The measurements were taken after 35 d at 5 °C plus 7 d at 25 °C in the chilled fruit, and 8 d at 25 °C in the non-chilled fruit.

$^{NS, *}$Nonsignificant or significant at P < 0.05, respectively.

characterized by a wavy form involving cavities (Fig. 1E).

Young mango skin is more sensitive to CI (Singh et al., 2013; Sivakumar et al., 2011), and ion leakage from the pulp increases as the period of cold storage lengthens (González-Aguilar et al., 2000) because of membrane disintegration through lipid peroxidation (Sivakumar et al., 2011; Wang et al., 2008), which will result in water loss from the fruit surface. Therefore, water in the pulp adjacent to the skin would be lost through the damaged skin because of the chilling temperature, resulting in the formation of an irregular pulp structure (Fig. 1E) and surface depression (Fig. 1C).

Changes in soluble sugars and starch. High starch and low soluble sugar accumulations have been reported in both spongy disordered (Katrodia, 1988; Lima et al., 2001) and chilling-injured (Chhatpar et al., 1971; Singh et al., 2013) mango fruits because of inhibition of the solubilization of starch granules into glucose (Simão et al., 2008). In our experiment, SEM showed that all of the non-chilled fruit contained almost no starch granules (Fig. 2A). In the chilled-D tissues, however, most of the chilling-injured tissues contained many starch granules (Fig. 2B). This was also true for the soluble sugar content; the chilled-D tissue showed the lowest content among the three different tissues although both glucose and sucrose contents were nonsignificant (Table 1). These results indicate that the accumulation of soluble sugars was inhibited due to the reduced starch breakdown under chilling conditions (Rahman et al., 2011; Thinh et al., 2013).

Changes in the content of cell wall polysaccharides. Fruit ripening of mangoes after harvest is highly correlated with pectin-degrading enzymes such as *exo-* and *endo-*polygalacturonase, pectin methylesterase, and *endo*-1,4-β-D-glucanase, which are also often activated by ethylene, resulting in fruit softening (Zaharah and Singh, 2011). Therefore, if desiccated white-corky pulp tissues are formed by the inhibition of normal ripening through the inhibition of ethylene action and/or biosynthesis (Nagamani et al., 2010), it will also involve compositional changes in the primary cell walls (Chourasia et al., 2006; Han et al., 2006).

In our experiment, a significant difference in the content of cell wall polysaccharides was observed only between the chilled-D and other two tissues (Table 2). Among each fraction, both UA and total cell wall (sum of UA and NS) contents of the chilled-D in the KOH-soluble fractions were significantly higher than those in the other two tissues whereas those in the water-soluble fractions were vice versa. The patterns of noncellulosic polysaccharide contents in the Na_2CO_3-soluble fractions were similar to those of the KOH-soluble fractions, but no significant difference was observed among the three different pulp tissues. Cell wall polymers in the water-, 50 mM CDTA-, and 50 mM Na_2CO_3-soluble fractions are considered as soluble, ionically-bound, and covalently bound pectins, respectively, whereas those in the KOH-soluble fraction are considered as hemicelluloses (Rose et al.,

1998). In normally ripening mango fruit, an apparent decrease in the cell wall polymers is often observed in the ionically and covalently bound pectin fractions rather than hemicelluloses (Chourasia et al., 2006; Mitcham and McDonald, 1992). In our experiment, however, the difference among the three different pulp tissues was more apparent in the hemicelluloses rather than the pectins, suggesting that the inhibition of cell wall degradation might be more pronounced in hemicelluloses when the white-corky pulp tissues are formed by CI.

Total amounts of UA and NS did not differ significantly among the three different pulp tissues (Table 2) as reported in ripening mango fruit (Mitcham and McDonald, 1992) indicating that the formation of white-corky pulp tissues was not associated with the total amount of noncellulosic cell wall polysaccharides.

Changes in fatty acid unsaturation. Damage of the cell membrane caused by oxidative stress such as active oxygen has been recognized as a factor inducing CI (Ding et al., 2007; Wang et al., 2008). Chilling in mangoes affects the formation of reactive oxygen species (ROS), such as the superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) (Wang et al., 2008). The ROS scavenging system incorporates both nonenzymatic and enzymatic mechanisms (Ding et al., 2007). Nonenzymatic compounds include ascorbate, glutathione, tocopherol, flavonoids, alkaloids, and carotenoids. The enzymatic scavenging mechanism includes superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (POD), and ascorbate peroxidase (APX). SOD dismutates O_2^- to H_2O_2, then CAT, POD, and APX detoxify H_2O_2. These results suggest that fatty acid compositions might be changed between white-corky pulp and NR pulp tissues because of oxidative stress, which is caused by CI (Kane et al., 1982).

In some horticultural crops, both fatty acid unsaturation and membrane permeability increase at lower temperatures as a response to chilling stress to prevent CI (Antunes and Sfakiotakis, 2008; Forney, 1990; Shewfelt and del Rosario, 2000). In our experiment, eight fatty acids were found by GC analysis (Table 3), as reported previously in ripening mangoes (Bandyopadhyay and Gholap, 1973). Among them, three fatty acids, palmitic acid (C16:0), oleic acid (C18:1), and linolenic acid (C18:3), were the main components (20.7% to 34.0%), followed by linoleic acid (18:2) and stealic acid (18:0) (2.5% to 17.4%). The other minor fatty acids were palmitoleic acid (16:1), lauric acid (C12:0), and myristic acid (C14:0), which were 7.0% to 13.5% in total.

The disordered membrane functions that were derived from CI would result in the disturbance of the cascades of secondary reactions, such as sugar metabolism and cell wall depolymerization (Zhang and Tian, 2009).

Membrane lipid unsaturation is often shown as the DBI (Zhang and Tian, 2009). In our experiment, a significant difference was observed in DBI among the three different pulp tissues, and the chilled-NR tissue showed the highest value (2.05) followed by the chilled-D (1.56) and non-chilled tissues (1.07), respectively (Table 3). The chilled-NR tissue was taken from the inner portion adjacent to the chilled-D tissue. Therefore, the inner pulps might respond well to chilling temperatures and increased DBI, whereas the pulp near the skin did not increase the fatty acid unsaturation probably because of marked desiccation through rapidly damaged skin, resulting in a lower DBI.

Table 2. Uronic acid and noncellulosic neutral sugar contents among cell wall polymer fractions of chilling-injured 'Nam Doc Mai See Thong' mango pulps.

Tissue	Cell wall polysaccharides (mg·g^{-1} CWM)^z		
	Uronic acids	Neutral sugars	Total
		Water-soluble fraction	
Chilled-D	109.8 b	65.7	175.5 b
Chilled-NR	193.5 a	95.7	289.2 a
Non-chilled	243.2 a	105.1	348.3 a
Significance	*	NS	*
		CDTA-soluble fraction	
Chilled-D	51.3	22.4	73.7
Chilled-NR	58.3	18.2	76.5
Non-chilled	53.3	22.3	75.6
Significance	NS	NS	NS
		Na_2CO_3-soluble fraction	
Chilled-D	55.8	27.6	83.4
Chilled-NR	52.9	26.4	79.3
Non-chilled	34.2	16.0	50.2
Significance	NS	NS	NS
		KOH-soluble fraction	
Chilled-D	85.7 a	81.1	166.8 a
Chilled-NR	41.2 b	79.5	120.7 b
Non-chilled	44.8 b	73.2	118.0 b
Significance	*	NS	*
		Total	
Chilled-D	302.6	196.8	499.4
Chilled-NR	345.9	219.8	565.7
Non-chilled	375.5	216.6	592.1
Significance	NS	NS	NS

Data are presented as means (n = 5). Values followed by the same letter do not differ significantly (P < 0.05) among tissues according to Tukey's test.

[z]The measurements were taken after 35 d at 5 °C plus 7 d at 25 °C in the chilled fruit, and 8 d at 25 °C in the non-chilled fruit.

[NS, *]Nonsignificant or significant at P < 0.05, respectively.

Table 3. Fatty acid compositions of chilling-injured 'Nam Doc Mai See Thong' mango pulps.

Tissues	Fatty acid composition (%)^z						Double bound index
	16:0	18:0	18:1	18:2	18:3	Others	
Chilled-D	32.8	3.9	20.7 b	17.4	18.2	7.0	1.56 b
Chilled-NR	30.9	2.8	18.8 b	10.9	28.4	8.1	2.05 a
Non-chilled	26.8	2.5	34.0 a	3.3	20.1	13.5	1.07 c
Significance	NS	NS	**	NS	NS	NS	**

Data are presented as means (n = 5). Values followed by the same letter do not differ significantly (P < 0.05) among tissues according to Tukey's test. The measurements were taken after 35 d at 5 °C plus 7 d at 25 °C in the chilled fruit, and 8 d at 25 °C in the non-chilled fruit.

[z]The measurements were taken after 35 d at 5 °C plus 7 d at 25 °C in the chilled fruit, and 8 d at 25 °C in the non-chilled fruit.

[NS, **]Nonsignificant or significant at P < 0.01, respectively.

Conclusions

Desiccated white-corky pulp tissues developed as the result of CI when ethephon-treated 'Nam Doc Mai See Thong' green mangoes were chilled at 5 °C for 35 d followed by incubation at 25 °C for 7 d. The chilling-injured pulp tissues were characterized by the insolubilization of cell walls. The skin damage derived from CI will be a direct factor inducing abnormal desiccation in the adjacent pulp, resulting in the formation of white-corky pulp tissues along the skin. Plant extracts (Gupta and Jain, 2014), 2–4-dichlorophenoxy acetic acid (Wang et al., 2008), methyl jasmonate (González-Aguilar et al., 2000), polyamines (Nair and Singh, 2004), salicylic acid, and oxalic acid (Ding et al., 2007), hot water and heat treatments (Jacobi and Wong, 1992; McCollum et al., 1993; Nair et al., 2001), and CA storage (Pesis et al., 2000) have been reported to reduce CI in mango fruit. Therefore, these techniques should be adopted in future studies to confirm whether they can also prevent the formation of white-corky pulp tissues under chilling storage conditions.

Literature Cited

Antunes, M.D.C. and E.M. Sfakiotakis. 2008. Changes in fatty acid composition and electrolyte leakage of 'Hayward' kiwifruit during storage at different temperatures. Food Chem. 110:891–896.

Bandyopadhyay, C. and A.S. Gholap. 1973. Changes in fatty acids in ripening mango pulp (variety Alphonso). J. Agr. Food Chem. 21:496–497.

Blumenkrantz, N. and G. Asboe-Hansen. 1973. New method for quantitative determination of microgram quantities of uronic acids. Anal. Biochem. 54:484–489.

Burondkar, M.M., B.B. Jadhav, and M.B. Chetti. 2009. Effect of plant growth regulators, polyamine and nutrients on fruit quality and occurrence of spongy tissue in Alphonso mango. Acta Hort. 820:689–696.

Chaplin, G.R., S.P. Cole, M. Landrigan, P.A. Nuevo, P.F. Lam, and D. Graham. 1991. Chilling injury and storage of mango (*Mangifera indica* L.) fruit held under low temperature. Acta Hort. 291:461–471.

Chhatpar, H.S., A.K. Mattoo, and V.V. Modi. 1971. Biochemical studies on chilling injury in mangoes. Phytochemistry 10:1007–1009.

Chomchalow, N. and P.N. Songkhla. 2008. Thai mango export: A slow-but-sustainable development. AU J.T. 12:1–8.

Chourasia, A., V.A. Sane, and P. Nath. 2006. Differential expression of pectate lyase during ethylene-induced postharvest softening of mango (*Mangifera indica* var. Dashehari). Physiol. Plant. 128:546–555.

Ding, Z.-S., S.-P. Tian, X.-L. Zheng, Z.-W. Zhou, and Y. Xu. 2007. Responses of reactive oxygen metabolism and quality in mango fruit to exogenous oxalic acid or salicylic acid under chilling temperature stress. Physiol. Plant. 130:112–127.

Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers, and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28:350–356.

Forney, C.F. 1990. Ripening and solar exposure alter polar lipid fatty acid compostition of 'Honey Dew' muskmelons. HortScience 25:1262–1264.

González-Aguilar, G.A., J. Fortiz, R. Cruz, and C.Y. Wang. 2000. Methyl jasmonate reduces chilling injury and maintains postharvest quality of mango fruit. J. Agr. Food Chem. 48:515–519.

Gupta, N. and S.K. Jain. 2014. Storage behaviour of mango as affected by post harvest application of plant extracts and storage conditions. J. Food Sci. Technol. 51:2499–2507.

Han, J., S.-P. Tian, X.-H. Meng, and Z.-S. Ding. 2006. Response of physiologic metabolism and cell wall structures in mango fruit to exogenous methyl salicylate under low-temperature stress. Physiol. Plant. 128:125–133.

Jacobi, K.K. and L.S. Wong. 1992. Quality of 'Kensington' mango (*Mangifera indica* Linn.) following hot water and vapour-heat treatments. Postharvest Biol. Technol. 4:349–359.

Janave, M.T. 2009. Profile of peroxidase isoforms influenced by spongy tissue disorder in Alphonso mango (*Mangifera indica* L.) fruits. Acta Physiol. Plant. 31:1176–1184.

Janave, M.T. and A. Sharma. 2008. Spongy tissue development in Alphonso mango: Association with *Staphylococcus xylosus*. Eur. J. Plant Pathol. 122:335–348.

Kane, O., M. Boulet, and F. Castaigne. 1982. Effect of chilling-injury on texture and fungal rot of mangoes (*Mangifera indica* L.). J. Food Sci. 47:992–995.

Kane, O., P. Marcellin, and P. Mazliak. 1978. Incidence of ripening and chilling injury on the oxidative activities and fatty acid compositions of the mitochondria from mango fruits. Plant Physiol. 61:634–638.

Katrodia, J.S. 1988. Spongy tissue in mango—Causes and control measures. Acta Hort. 231:814–826.

Krishnamurthy, S. 1980. Internal breakdown during ripening of Alphonso mango (*Mangifera indica* Linn.) in relation to specific gravity of the fruit. J. Food Sci. Technol. 17:198–199.

Lima, L.C.O., A.B. Chitarra, and M.I.F. Chitarra. 2001. Changes in amylase activity starch and sugars contents in mango fruits pulp cv. Tommy Atkins with spongy tissue. Brazil. Arch. Biol. Technol. 44:59–62.

Lizada, M.C. 1991. Postharvest physiology of the mango - A review. Acta Hort. 291:437–453.

Machhindra, T.J. and A. Sharma. 2008. Spongy tissue development in Alphonso mango: Association with *Staphylococcus xylosus*. Eur. J. Plant Pathol. 122:335–348.

McCollum, T.G., S. D'Aquino, and R.E. McDonald. 1993. Heat treatment inhibits mango chilling injury. HortScience 28:197–198.

Medlicott, A.P., J.M.M. Sigrist, and O. Sy. 1990. Ripening of mangoes following low-temperature storage. J. Amer. Soc. Hort. Sci. 115:430–434.

Miguel, A.C.A., J.F. Durigan, and A.S. Ferraudo. 2011. Multivariate analysis that can be used to evaluate the chilling injury in mangoes cv. Tommy Atkins (in Portoguese). Rev. Bras. Fruticu. 33:371–376.

Mitcham, E.J. and R.E. McDonald. 1992. Cell wall modification during ripening of 'Keitt' and 'Tommy Atkins' mango fruit. J. Amer. Soc. Hort. Sci. 117:919–924.

Mohammed, M.A. and J.K. Brecht. 2002. Reduction of chilling injury in 'Tommy Atkins' mangoes during ripening. Sci. Hort. 95:297–308.

Montalvo, E., H.S. García, B. Tovar, and M. Mata. 2007. Application of exogenous ethylene on postharvest ripening of refrigerated 'Ataulfo' mangoes. LWT 40:1466–1472.

Nagamani, J.E., K.S. Shivashankara, and T.K. Roy. 2010. Role of oxidative stress and the activity of ethylene biosynthetic enzymes on the formation of spongy tissue in 'Alphonso' mango. J. Food Sci. Technol. 47:295–299.

Nair, S. and Z. Singh. 2004. Chilling injury in mango fruit in relation to biosynthesis of free polyamines. J. Hortic. Sci. Biotechnol. 79:515–522.

Nair, S., Z. Singh, and S.C. Tan. 2001. Heat treatments affect development chilling injury, respiration, ethylene production and fruit quality of mango. Acta Hort. 553:549–550.

Nishizawa, T., A. Ito, Y. Motomura, M. Ito, and M. Togashi. 2000. Changes in fruit quality as influenced by shading of netted melon plants (*Cucumis melo* L. 'Andesu' and 'Luster'). J. Jpn. Soc. Hort. Sci. 69:563–569.

Nishizawa, T. and A. Ito. 2007. Analysis of cell wall polysaccharides during storage of a local melon accession 'Wasada-uri' compared to the melon cultivar 'Prince'. J. Hort. Sci. Biotechnol. 82:227–234.

Nishizawa, T., S. Nagasawa, J.B. Retamales, A. Lavín, and Y. Motomura. 2002. Comparison of cell wall components between *Fragaria* X *ananassa* and *Fragaria chiloensis* grown in Chile. J. Hort. Sci. Biotechnol. 77:404–410.

Pesis, E., D. Aharoni, Z. Aharon, R. Ben-Arie, N. Aharoni, and Y. Fuchs. 2000. Modified atmosphere and modified humidity packaging alleviates chilling injury symptoms in mango fruit. Postharvest Biol. Technol. 19:93–101.

Prates, J.A.M., C.M. Alfaia, S.P. Alves, and R.J.B. Bessa. 2011. Fatty acids, p. 137–161. In: L.M.L. Nollet and F. Toldra (eds.). Handbook of analysis of edible animal by-products. CRC Press, New York.

Rahman, M.M., M.M. Rahman, N. Absar, and M.A. Ahsan. 2011. Correlation of carbohydrate content with the changes in amylase, invertase and β-galactosidase activity of ripe mango pulp during storage under different temperatures. Bangladesh J. Sci. Ind. Res. 46:443–446.

Raja, M.E. 2009. Investigation on causes and correction of spongy tissue in Alphonso mango (*Mangifera indica* L.). Acta Hort. 820:697–706.

Ravindra, V. and S. Shivashankar. 2004. Spongy tissue in Alphonso mango – significance of in situ seed germination events. Curr. Sci. India 87:1045–1049.

Ravindra, V. and S. Shivashankar. 2006. Spongy tissue in Alphonso mango. II. A key evidence for the causative role of seed. Curr. Sci. India 91:1712–1714.

Rose, J.K.C., K.A. Hadfield, J.M. Labavitch, and A.B. Bennett. 1998. Temporal sequence of cell wall disassembly in rapidly ripening melon fruit. Plant Physiol. 117:345–361.

Saranwong, S., J. Sornsrivichai, and S. Kawano. 2004. Prediction of ripe-stage eating quality of mango fruit from its harvest quality measured nondestructively by near infrared spectroscopy. Postharvest Biol. Technol. 31:137–145.

Shewfelt, R.L. and B.A. del Rosario. 2000. The role of lipid peroxidation in storage disorders of fresh fruits and vegetables. HortScience 21:951–956.

Shivashankara, K.S., S. Isobe, M.I. Al-Haq, M. Takenaka, and T. Shiina. 2004. Fruit antioxidant activity, ascorbic acid, total phenol, quercetin, and carotene of Irwin mango fruits stored at low temperature after high electric field pretreatment. J. Agr. Food Chem. 52:1281–1286.

Shivashankar, S., V. Ravindra, and L. Louis. 2007. Biochemical changes in seed and mesocarp of mango (*Mangifera indica* L.) cv. 'Alphonso' and their significance during the development of spongy tissue. J. Hort. Sci. Biotechnol. 82:35–40.

Simão, R.A., A.P.F.B. Silva, F.H.G. Peroni, J.R.O. Nascimento, R.P. Louro, F.M. Lajolo, and B.R. Cordenunsi. 2008. Mango starch degradation. I. a microscopic view of the granule during ripening. J. Agr. Food Chem. 56:7410–7415.

Singh, Z. and J. Janes. 2001. Effects of postharvest application of ethephon on fruit ripening, quality and shelf life of mango under modified atmosphere packaging. Acta Hort. 553:599–602.

Singh, Z., R.K. Singh, V.A. Sane, and P. Nath. 2013. Mango - postharvest biology and biotechnology. Crit. Rev. Plant Sci. 32:217–236.

Sivakumar, D., Y. Jiang, and E.M. Yahia. 2011. Maintaining mango (*Mangifera indica* L.) fruit quality during the export chain. Food Res. Intl. 44:1254–1263.

Thinh, D.-C., J. Uthaibutra, and A. Joomwong. 2013. Effect of storage temperatures on ripening behavior and quality change of Vietnamese mango cv. Cat Hoa Loc. IJBTR 3:19–30.

Thomas, P. and M.R. Joshi. 1988. Reduction of chilling injury in ripe Alphonso mango fruit in cold storage by temperature conditioning. Intl. J. Food Sci. Technol. 23:447–455.

Thomas, P. and M.S. Oke. 1983. Improvement in quality and storage of 'Alphonso' mangoes by cold adaptation. Sci. Hort. 19:257–262.

Vasanthaiah, H.K.N., K.V. Ravishankar, K.S. Shivashankara, L. Anand, P. Narayanaswamy, G. Mukunda, and T.G. Prasad. 2006. Cloning and characterization of differentially expressed genes of internal breakdown in mango fruit (*Mangifera indica*). J. Plant Physiol. 163:671–679.

Wang, B., J. Wang, H. Liang, J. Yi, J. Zhang, L. Lin, Y. Wu, X. Feng, J. Cao, and W. Jiang. 2008. Reduced chilling injury in mango fruit by 2,4-dichlorophenoxyacetic acid and the antioxidant response. Postharvest Biol. Technol. 48:172–181.

Yashoda, H.M., T.N. Prabha, and R.N. Tharanathan. 2006. Mango ripening: Changes in cell wall constituents in relation to textural softening. J. Sci. Food Agr. 86:713–721.

Zaharah, S.S. and Z. Singh. 2011. Mode of action of nitric oxide in inhibiting ethylene biosynthesis and fruit softening during ripening and cool storage of 'Kensington Pride' mango. Postharvest Biol. Technol. 62:258–266.

Zhang, C. and S. Tian. 2009. Crucial contribution of membrane lipids' unsaturation to acquisition of chilling-tolerance in peach fruit stored at 0°C. Food Chem. 115:405–411.