

## Enhanced chilling tolerance in heat-treated mangosteen

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### Abstract

Mangosteen is sensitive to low temperature and develops chilling injury (CI) when stored at temperature below 13°C. Postharvest heat treatment has been applied to alleviate CI in various fruits. In this research, induction of CI resistance in mangosteen affected by heat treatment was investigated. Mangosteen was heated with hot air at 35°C for 3, 6, 12 and 24 hours, prior to storage at 7°C. Thereafter, fruit was held for 3 days at 25°C and determined for weight loss, heat injury and CI symptoms. All heat treatments resulted in retarding abnormal ripening. Fruit heated for 3 and 6 hours exhibited smaller extents of browning and shrinkage of calyx and stem after the long-term cold storage, whereas heat treatment for 12 and 24 hours imparted undesirable appearance of calyx and stem, in addition to increased weight loss. The highest extent of pericarp hardening was detected in non-heated and 3-hour-heated fruit after 9-15 days of cold storage. During the cold storage, the lowest electrolyte leakage was found in 6-hour-heated fruit. Heat treatment at 35°C for 6 hours effectively delayed CI occurrence, in terms of abnormal ripening, browning and shrinkage of calyx and stem, hardening of pericarp and electrolyte leakage, with smallest extents of weight loss and heat injury.

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### Introduction

Mangosteen (*Garcinia mangostana* L.) is one of the important economic fruit crop in Thailand, the world's leading producer and major exporter of mangosteen. With an increasing demand of mangosteen fruit being exported abroad, its storage life is an important concern. Mangosteen is a climacteric fruit and usually harvested at different ripeness stages. The maturity stage of fruit can be categorized by the pericarp color into six stages, from light greenish yellow with few scattered pink spots (stage 1) to dark purple (stage 6) (Palapol *et al.*, 2009). After harvest, the purple color continues to develop very quickly. Therefore, the maturity stage of mangosteen is important when harvesting for commercial scale. The ripeness stage 1 is generally recognized as the proper fruit maturity for export.

To maintain the quality of harvested horticultural crops including mangosteen fruit, temperature is one of the most predominant factors. Physiological disorder resulting from exposure to low temperatures above freezing is termed chilling injury (CI) (Purvis, 2004). This phenomenon limits storage life and leads to significant degradation of produce quality. Mangosteen fruit is sensitive to low temperature and develops CI when stored at temperature below 13°C. Unacceptable CI symptoms were found in 5 days at 3 and 6°C (Choehom *et al.*, 2003). The most

common symptoms of CI in mangosteen fruit are browning and hardening of pericarp, shrinkage and browning of stem and calyx, brown discoloration and off-flavor of edible aril and higher susceptibility to decay (Uthairatanakij and Ketsa, 1996). Most of the time these symptoms reach the aril, resulting in a reduction in both internal and external fruit qualities and subsequent consumer acceptance. For this reason, storage of mangosteen at low temperatures limits safe transporting, shipping and marketing of the fruit, resulting in a decrease in sales volume of fruit producer. However, use of storage temperature above 13°C is not a solution as it permits commercially mature mangosteen to ripen during storage.

In recent years, there has been an increasing interest in the use of postharvest heat treatments (hot water dips, forced hot air and vapor) to delay ripening (Mitchell, 1986) and to induce CI resistance (Barkai-Golan and Phillips, 1991; Lurie, 1998) in many fruits. For instance, heat treatments were used to prevent CI in sweet persimmon (Woolf *et al.*, 1997) tomatoes (McDonald *et al.*, 1999; Henríquez *et al.*, 2005; Saltveit, 2005) pomegranate (Artés *et al.*, 2000; Mirdehghan *et al.*, 2007) and avocado (Florissen *et al.*, 1996; Woolf *et al.*, 2004). One proposed mechanism for induced tolerance is that of induction and action of heat shock proteins (HSP). Heat-treatments induce HSP, which protects fresh produce from both heat injury and CI. HSP

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also called stress proteins, are a group of proteins that present in all cells in all life forms. These HSP could act as chaperons to protect chilling sensitive components of the cell, e.g. enzymes and membranes (Vierling, 1991). However, many fruits are sensitive to high temperature. Heat-treatment also develops external or internal heat injuries in many cultivars. Damage can be detected in both external and internal parts of fruit. External heat damage is generally peel browning and pitting. Internal heat damage symptoms include flesh darkening, abnormal softening and poor color development (Lurie, 1998). There are several effective combinations of temperature and duration of heat treatments, which range from 34 to 48°C and from a few minutes up to many hours, depending on a number of factors including species, stage of fruit maturity, fruit size and type of application (Jacobi *et al.*, 2001).

In spite of the increasing importance of this technology as a way to alleviate CI and control postharvest diseases, no information is available on the use of heat treatments for mangosteen, neither on the fruit quality nor their influence on CI symptoms. Thus, the purpose of this present work was to investigate the effect of heat treatment on physiological changes and CI occurrence of mangosteen during storage at low temperature.

## Materials and Methods

### Fruit

Mangosteen from an orchard in Nakhon Si Thammarat province, Thailand, was used in this experiment. Fruit at ripeness stage 1 (light greenish yellow pericarp with few pink spots) was selected by uniformity of shape, color and size.

### Hot air heat treatment

Mangosteen (1,050 fruit) was randomly divided into replicate of 10 fruit for each treatment. The fruit was enclosed in a perforated linear low density polyethylene bag (30 cm x 40 cm, 6 holes of 1.3 cm diameter per bag), to prevent water loss during the heat treatment (Florissen *et al.*, 1996), before heat treatment. The heat treatments were conducted using hot air oven at 35°C for various durations including 0, 3, 6, 12 and 24 hours. Thereafter, the fruit was transferred from the bag and stored at 7 ± 2°C and 80-90% relative humidity. All treatments were replicated three times. At 3-day intervals during 15 days of the cold storage, the fruit was removed to ripen at 25°C for 3 days, prior to quality assessment.

## Quality measurement

### Pericarp color

Development of pericarp color was judged by the following ripeness stage, according to Papapol *et al.* (2009): 1 = light greenish yellow with 5 - 50% scattered pink spots, 2 = light greenish yellow with 51-100% scattered pink spots, 3 = spots not as distinct as in stage 2 or reddish pink, 4 = red to reddish purple, 5 = dark purple, 6 = purple black. Ripeness stage index for each treatment was calculated using the following formula (Ratanachinakorn, 2003):

$$\frac{(\text{Ripeness stage}) \times (\text{Number of fruit at each stage})}{\text{Total number of fruit in the treatment}}$$

### Appearance of calyx and stem

The stem and the calyx freshness of each fruit were assessed on a scale of 1 - 5 according to the appearance of stem and calyx: 5 = green color, 4 = degreening, 3 = slight browning, 2 = moderate browning and shrinkage, 1 = severe browning and shrinkage. Calyx or stem freshness index for each treatment was calculated using the following formula (Ratanachinakorn, 2003):

$$\frac{(\text{Freshness score}) \times (\text{Number of fruit at each freshness score})}{\text{Total number of fruit in the treatment}}$$

### Weight loss

Weight loss was evaluated by weighing fruit before and after the storage. Weight loss (%) was calculated using the following formula (Zhou *et al.*, 2002):

$$\frac{\text{Weight after the storage} \times 100}{\text{Weight before the storage}}$$

### Heat injury symptoms

Fruit was examined and the heat injury symptoms were assessed on a scale of 0 - 3 according to the amount of browning on the fruit surface (external damage), or the surface of equatorial cut fruit (internal damage): 0 = no damage, 1 = slight browning (< 25% of the surface area), 2 = moderate browning (about 50% of the surface area), 3 = severe browning (> 75% of the surface area) (Woolf *et al.*, 1997). The heat damage index for each treatment was calculated using the following formula (modified from Porat *et al.*, 2000):

$$\frac{(\text{Injury score}) \times (\text{Number of fruit at each injury score})}{\text{Total number of fruit in the treatment}}$$

### Pericarp firmness

Pericarp firmness of a whole fruit was determined with a Texture analyzer (Model LR 5K, Lloyd Instrument, Hampshire, UK) equipped with a ball probe (0.5 cm diameter head) through the peel in two locations around the equator of each fruit (Manurakchinakorn *et al.*, 2008).

### Electrolyte leakage

To determine the rate of electrolyte leakage, the method described by McCollum and McDonald (1991) was used. For each treatment, five discs of pericarp tissue were taken with a cork borer (15 mm diameter). The disks were washed twice with deionized water to eliminate the electrolyte at the cut surface. The five samples were then placed in a flask containing 30 ml of 0.4 M mannitol. After incubating at 25°C for 3 hours, the electrical conductivity of the solution was measured with a conductivity meter (Model inoLab multi IDS, WTW, Germany) as an initial reading. After readings were taken, the vials were autoclaved at 121°C for 30 min, held overnight and conductivity was measured again for total electrolytes. Electrolyte leakage (%) was calculated using the following formula (Mirdehghan *et al.*, 2007):

$$\frac{\text{Initial conductivity} \times 100}{\text{Total conductivity}}$$

## Results and Discussion

### Development of pericarp color

Heat treatment at 35°C for 6, 12 and 24 hours resulted in the development of pericarp color of some mangosteen fruit to ripeness stage 2. On the other hand, the pericarp color of all fruit heat-treated at 35°C for 3 hours remained at ripeness stage 1 (data not shown).

After heat treatment, the fruit was stored at 7°C for various durations and then transferred to room temperature for 3 days, prior to evaluation of pericarp color. Figure 1 showed that the pericarp color of non-heated fruit, without cold storage, progressively developed to ripeness stage 5. However, the non-heated fruit exhibited abnormal ripening after 6 days of the storage at 7°C. The abnormal ripening of non-heated mangosteen might be attributed to the decrease of ethylene production caused by CI. The reduction of ethylene concentration was detected in chilling-injured peach (Valero *et al.*, 1997) and mango (Mohammed and Brecht, 2002). The decrease of ethylene production could be related with the accumulation of polyamines during the cold storage

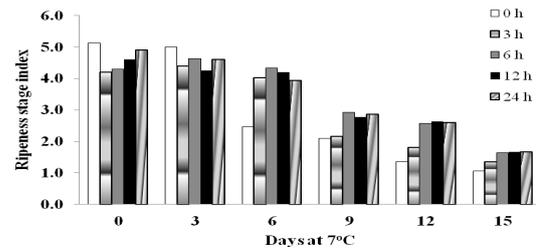


Figure 1. Ripeness stage index of mangosteen heat-treated at 35°C for various durations and stored for up to 15 days at 7°C, prior to storage at 25°C for 3 days (score: 1 = stage 1, 2 = stage 2, 3 = stage 3, 4 = stage 4, 5 = stage 5 and 6 = stage 6) (n = 30)

(Artés *et al.*, 1995). In plant tissues affected by stress condition caused by CI, an increase in polyamines concentration has been reported (Serrano *et al.*, 1996; Martínez-Romero *et al.*, 2003).

Heat treatment of mangosteen at 35°C for 3, 6, 12 and 24 hours, without cold storage, resulted in retarding the fruit ripening during storage at 25°C for 3 days, compared with the non-heated fruit. Disruption of ethylene synthesis may mediate heat-induced ripening inhibition (Lurie, 1998). Heat treatment might inactivate ethylene forming enzymes such as 1-aminocyclopropane-1-carboxylic acid (ACC) synthase and ACC oxidase, and destroy ethylene receptors (Metzidakis and Sfakiotakis, 1993). However, mangosteen exposed to long periods of heat treatment (12 and 24 hours) tended to recover its ability for ethylene synthesis, compared with the shorter durations. Long term of heat exposure has been revealed to stimulate the respiration and ethylene synthesis of kiwifruit (Antunes and Sfakiotakis, 2000).

After the cold storage of heat-treated mangosteen for 6 days, all heat-treated fruit progressively developed to ripeness stage index at about 4, whereas the ripeness stage index of the non-heated fruit was 2.5. These results indicated that protection against chilling injury arose from the heat treatment of mangosteen. Beneficial effects of heat treatment in reduction of chilling injury have also been reported in pomegranate (Mirdehghan *et al.*, 2007), avocado (Woolf *et al.*, 2004) and tomato (Henríquez *et al.*, 2005). The chilling tolerance is thought to work through the induced synthesis and accumulation of specific heat-shock proteins (Lafuente *et al.*, 1991; Sabehat *et al.*, 1996). These heat-shock proteins could act as chaperons to protect chilling sensitive components of the cell, e.g. enzymes and membranes (Vierling, 1991). Alternatively, heat shock-induced changes in protein synthesis may protect against symptoms arising from chilling-induced alterations in protein synthesis since heat shock-induced protein

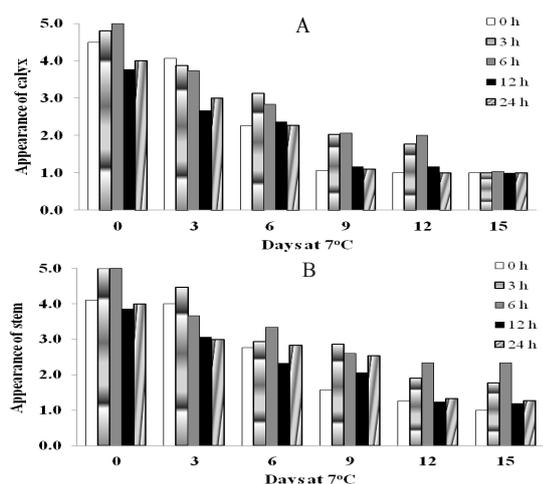


Figure 2. Appearance of calyx (A) and stem (B) of mangosteen heat-treated at 35°C for various durations and stored for up to 15 days at 7°C, prior to storage at 25°C for 3 days (score: 1 = severe browning and shrinkage, 2 = moderate browning and shrinkage, 3 = slight browning, 4 = degreening, 5 = green color) (n = 30)

synthesis appears to subordinate other stress-induced protein synthesis (Campos-Vargas *et al.*, 2004). After storage under low temperature for 9 days, the mangosteen heat-treated for 3 hours exhibited abnormal ripening similarly to the non-heated fruit.

#### Appearance of calyx and stem

One of the CI symptoms in mangosteen is browning and shrinkage of calyx and stem (Choehom *et al.*, 2003). As presented in Figure 2, the extent of browning and shrinkage of the calyx and stem of mangosteen increased with the increase of storage duration at 7°C. Color changes of calyx and stem could be related to the degradation of chlorophyll by chlorophyllase during the storage (Azuma *et al.*, 1999). In addition, CI of fruit may result in membrane alterations of plant tissue and stimulate the activity of chlorophyllase. It was found that long exposure (12 and 24 hours) of mangosteen to heat treatment at 35°C induced degreening of calyx and stem after storage at 25°C for 3 days, compared with other treatments. The extent of symptom of these treatments increased with the increase of storage duration at 7°C. Browning and shrinkage of calyx (score = 4.07) and stem (score = 4.00) in non-heated fruit apparently appeared after 3 days of the cold storage. On the other hand, 3- and 6-hour-heated mangosteen markedly exhibited less extent of symptom of browning and shrinkage of calyx and stem when stored under low temperature. Furthermore, heat treatment of mangosteen for 6 hours tended to be the most effective condition for suppressing the browning and shrinkage of calyx and stem of the mangosteen fruit. Heat during hot air treatment of mangosteen might inactivate chlorophyllase. However, long period of heat

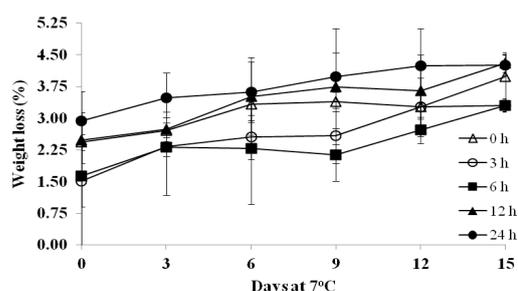


Figure 3. Weight loss of mangosteen heat-treated at 35°C for various durations and stored for up to 15 days at 7°C, prior to storage at 25°C for 3 days. The vertical bar represents the standard deviation of the relevant mean (n = 3)

treatment might adversely affect the appearance of calyx and stem by stimulating the degradation of chlorophyll. This response has been found in apple (Klein and Lurie, 1990) and tomato (Lurie and Klein, 1991). Besides CI, the shrinkage of calyx and stem may be linked with water loss of calyx and stem during heat treatment and cold storage. Bower and Cutting (1988) reported that water loss has been associated with CI on the skin of avocado.

#### Weight loss

As shown in Figure 3, heat treatment of mangosteen at 35°C for different durations resulted in various extents of weight loss, however, there was no significant difference among treatments ( $p > 0.05$ ). During storage at 7°C for 15 days, weight loss of mangosteen of all treatments tended to increase with the increasing storage time. The weight loss of mangosteen during storage could be attributed to water loss through transpiration (Ben-Yehoshua, 1987). After the cold storage for 15 days, the lowest extent of weight loss ( $p \leq 0.05$ ) was detected in 3- and 6-hour heated fruit (3.30%), compared with other treatments. Higher extent of weight loss in non-heated (3.97%), and 12- and 24-hour heated fruit (4.29 and 4.25%, respectively) could be attributed to higher extent of CI and heat injury, respectively. Severe CI in non-heated mangosteen could adversely affect the membrane integrity, resulting in the leakage of ions and weight loss (Gómez-Galindo *et al.*, 2004). On the other hand, long durations of heat treatment could damage the plant tissue. Cellular membranes have been stressed with respect to heat injury (Bernstam, 1978), and the role of membranes in cell death induced by heat has been reviewed (Blum, 1987). Heating fruit also changes the cuticle structure resulting in enhanced water loss in cactus pear fruit (Dimitris *et al.*, 2005) and grapefruit (Porat *et al.*, 2000). In contrast, heat treatment of mangosteen at 35°C for 3 and 6 hours might induce heat shock protein synthesis, resulting in strengthening and maintaining membrane integrity

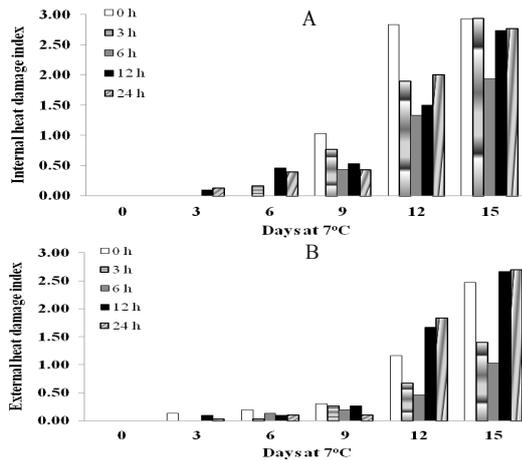


Figure 4. Internal heat damage index (A) and external heat damage index (B) of mangosteen heat-treated at 35°C for various durations and stored for up to 15 days at 7°C, prior to storage at 25°C for 3 days (0 = no damage, 1 = slight browning (< 25% of surface area), 2 = moderate browning (about 50% of surface area) and 3 = severe browning (> 75% of surface area)) (n = 30)

and subsequent reduced weight loss.

#### Heat injury

After heat treatment of mangosteen at 35°C for various durations, no internal and external heat damage was noticed in all treatments as shown in Figure 4. However, after the cold storage at 7°C for 3 days, mangosteen heat-treated for 12 and 24 hours exhibited small extent of internal heat damage index (0.10 and 0.13, respectively). Most concepts for explanation of heat injury involve protein denaturation, disruption of protein synthesis and loss of membrane integrity (Paull and Chen, 2000). Similar response of heat injury has been reported in persimmon (Woolf *et al.*, 1997). On the other hand, non-heated mangosteen also showed browning on the fruit surface and the surface of equatorial cut fruit, indicating that the browning symptoms could be attributed to the CI, in addition to the heat injury as evident by the increased extent of browning of both internal and external part of the fruit pericarp with the increased time during the cold storage. Choehom *et al.* (2003) reported that symptoms of chilling-injured mangosteen included browning of the pericarp. Mangosteen heat-treated at 35°C for 6 hours exhibited the lowest extent of internal and external pericarp browning (ranged from 0.43 to 1.93 and 0.13 to 1.03, respectively) throughout the cold storage. The results in this study indicate that 6 hours of heat treatment seems to be an optimal duration for mangosteen which minimize both CI and heat injury (internal and external browning of the pericarp). Heat treatment could affect the capacity of biological systems to synthesize proteins, resulting in accumulation of HSP that might induce thermo-

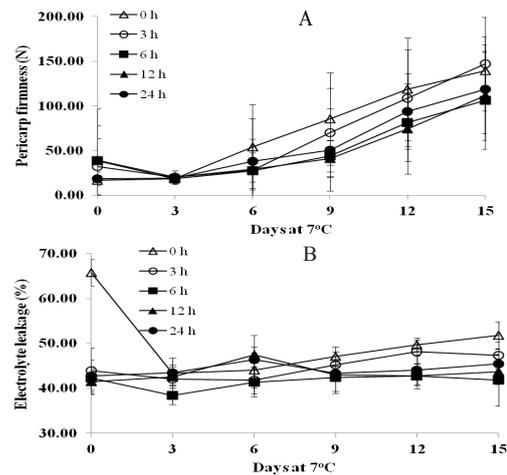


Figure 5. Pericarp firmness (A) and electrolyte leakage (B) of mangosteen heat-treated at 35°C for various durations and stored for up to 15 days at 7°C, prior to storage at 25°C for 3 days. The vertical bar represents the standard deviation of the relevant mean (n = 30 and 6, respectively)

tolerance and resistance to CI (Vierling, 1991; Lurie, 1998).

#### Pericarp hardening

An increase in pericarp firmness of mangosteen indicates the hardening of pericarp. Without cold storage at 7°C, the lowest pericarp firmness ( $p \leq 0.05$ ) was detected in non-heated (17.13 N) and 24 hour-heated fruit (18.87 N) (Figure 5A). Heat treatment of mangosteen for 3, 6 and 12 hours might inhibit ethylene synthesis of mangosteen fruit, resulting in the decreased extent of ripening and subsequent pericarp softening, whereas heat treatment for 24 hours might stimulate ethylene production. Disruption of ethylene synthesis may mediate heat-induced ripening inhibition (Lurie, 1998). The conversion of 1-aminocyclopropane-1-carboxylic acid to ethylene is apparently highly susceptible to heat damage above 30°C (Yu *et al.*, 1980). However, fruit exposed to long periods at high temperature quickly recover their ability for ethylene synthesis (Dunlap *et al.*, 1990).

After the cold storage of heat-treated mangosteen for 6 days, pericarp firmness of all heat-treated fruit tended to be comparable, whereas the increased pericarp firmness of non-heated fruit ( $p \leq 0.05$ ) was detected. During 9 - 15 days of storage at 7°C, the highest extent of pericarp hardening ( $p \leq 0.05$ ) was found in non-heated (ranged from 85.71 to 139.74 N) and 3-hour-heated mangosteen (ranged from 70.38 to 147.01). Pericarp hardening (loss of flexibility of pericarp tissue) was reported to be correlated with occurrence of CI in mangosteen (Uthairatanakij and Ketsa, 1996). Furthermore, Choehom and Ketsa

(2003) found that pericarp hardening caused by CI in mangosteen correlated with increased lignin synthesis. Similarly, the increased lignin synthesis was detected in pericarp hardening of loquat fruit during storage in low temperature (Zheng *et al.*, 2000). The increased activity of phenylalanine ammonia lyase was correlated with the lignin accumulation in pericarp of loquat fruit (Cai *et al.*, 2006).

#### *Electrolyte leakage*

Without the cold storage at 7°C, electrolyte leakages of all heat-treated mangosteen (ranged from 41.50 to 43.88%) were lower than the non-heated fruit (65.77%) ( $p \leq 0.05$ ) (Figure 5B). Electrolyte leakage demonstrates tissue damage, a possible loss of membrane integrity that is very common during plant senescence and fruit ripening (Ferguson, 1984). However, the drastic decrease of electrolyte leakage of the non-heated mangosteen after 3 days of cold storage might be attributed to the decreased development of ripening caused by CI. On the other hand, it has been reported that low temperatures induce changes in cell membrane lipids from a liquid-crystalline to a solid-gel state, which lead to an increase in membrane permeability and leakage of ions (Gómez-Galindo *et al.*, 2004). CI symptoms include increased membrane permeability and a resultant increase in leakage of cellular constituents (Murata, 1990; Sharom *et al.*, 1994). The rate of ion leakage from excised tissue into an isotonic aqueous solution is a useful measure of the severity of chilling-induced increase in membrane permeability (Saltveit, 2002).

During the cold storage, the lowest electrolyte leakage ( $p \leq 0.05$ ) was noticed in 6-hour-heat-treated mangosteen, suggesting the beneficial effect of proper heat treatment on maintaining membrane integrity. Heat treatment is thought to exert their protective effects through the induction, synthesis and accumulation of heat shock proteins (Lafuente *et al.*, 1991; Sabehat *et al.*, 1996). The heat-shock treatment protected membrane components involved in ion movement across the membrane from chilling-induced damage, such that ion leakage only increased a fraction of that in non-heat-shocked tissue (Saltveit, 2005). Mirdehghan *et al.* (2007) found that heat treatment could induce acclimation of pomegranate to low temperature, and in turn reduce CI, possibly by a mechanism involving an increase in both polyamine levels and the unsaturated/saturated fatty acid ratio. The results in this study indicated that heat treatment at 35°C for 6 hours effectively minimize the CI symptoms, whereas 3 hours of the heat treatment seemed to be inadequate to induce CI tolerance of

mangosteen fruit.

#### **Conclusion**

Heating of mangosteen fruit at ripeness stage 1 at 35°C for 6 hours provided an effective protection from CI incidence such as abnormal ripening, browning and shrinkage of calyx and stem, pericarp hardening and electrolyte leakage, during storage at 7°C. Longer exposure of mangosteen to the heat treatment resulted in the heat damage including weight loss, pericarp browning, and shrinkage of calyx and stem. However, shorter period of the heat treatment had only a marginal effect on inhibition of CI. The results revealed that postharvest heat treatment at 35°C for 6 hours had great potential to enhance chilling resistance in mangosteen and prolong storage life during the low temperature storage.

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