

Alleviation of Chilling Injury in Jujube Fruit (*Ziziphus jujuba* Mill) by Dipping in 35 °C Water

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ABSTRACT

The purpose of this study was to investigate the effect of mild hot water immersion on the chilling injury and certain physicochemical changes in jujube fruit (*Ziziphus jujuba* Mill) during refrigerated storage. The fruit were immersed in hot water at 35 °C for 0, 10, 20 and 30 min and then stored at 4 ± 1 °C for 20 d. The chilling injury score and electrolyte leakage of hot-water-treated jujube fruit were lower than those of the untreated fruit. The weight loss of all treatments increased throughout storage and immersion in hot water reduced the fresh weight loss and improved the texture. The lightness (L^* value) of the hot-water-treated fruit was higher and the greenness (a^* value) of the hot-water-treated fruit was slightly lower than that of the untreated fruit over the storage period. Hot water immersion for 10 min inhibited the increase in activity of polyphenol oxidase (PPO) and lipoxygenase (LOX) and inhibited the increase in the malondialdehyde (MDA) content in the peel of the jujube fruit during storage when compared to the untreated fruit. The total phenolic content and the total antioxidant capacity decreased with storage time. The total phenolic content and the antioxidant capacity of the jujube fruit immersed in the hot water for 10 min were higher than those of other treatments. In conclusion, water immersion at 35 °C for 10 min effectively maintained the quality and reduced the chilling injury of jujube fruit during refrigerated storage.

Keywords: Jujube fruit (*Ziziphus jujuba* Mill), hot water immersion, chilling injury

INTRODUCTION

Jujube fruit (*Ziziphus jujuba* Mill) is a subtropical fruit tree native to the northern hemisphere (Al-Niami *et al.*, 1992). Recently, jujube has been grown commercially in many countries such as China (Lin *et al.*, 2004), Turkey (Kamiloğlu *et al.*, 2009) and Taiwan (Ma *et al.*, 2009). The demand for jujube fruit in the market has increased substantially as the fruit is a rich source of ascorbic acid, carotenoids, total phenolic

compounds, antioxidant capacity, and minerals, especially potassium and iron (Abbas *et al.*, 1988; Al-Niami *et al.*, 1992; Li *et al.*, 2007; Xue *et al.*, 2009). Jujube fruit is classified as a climacteric fruit (Abbas and Fandi, 2002) and is very perishable being susceptible to skin browning, decay and water loss (Lin *et al.*, 2004; Qiuping and Wenshui, 2007). In Thailand, skin browning and decay due to chilling stress during refrigeration are the main problems associated with jujube fruit in the market and these symptoms normally appear

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on the fruit within 10 d.

It is widely accepted that tropical fruit and vegetables are very susceptible to chilling injury when exposed to low temperatures above freezing point, causing a loss commercial quality (Promyou *et al.*, 2008). The chilling injury symptoms manifest as skin browning, failure to ripen, off-flavor, surface pitting and higher susceptibility to decay (Mirdehghan *et al.*, 2007; Yang *et al.*, 2009). The appearance of these symptoms is thought to be associated with the transition of polar lipid membranes which is the primary physical event related to the loss of membrane permeability and membrane-bound enzyme activity. Changes in the membrane integrity and fluidity caused by chilling stress are also associated with membrane peroxidation, a reduction in the unsaturated/saturated fatty acids ratio and an increase in saturated fatty acids of membrane components (Mirdehghan *et al.*, 2007; Promyou *et al.*, 2008). These are concomitant with an increase in the lipoxygenase (LOX) activity, leading to an increase in the malondialdehyde (MDA) content under chilling stress (Promyou *et al.*, 2008). Mild hot water immersion is one of the most promising methods for the postharvest control of decay and insect infestation (Vigneault, 2007). Heat treatments, hot air, vapor heat and hot water treatments are also used to improve postharvest quality, delay ripening, induce resistance to chilling injury and extent the shelf life of many commodities (Lurie, 1998; Paull and Chen, 2000; Fallik, 2004; Lu *et al.*, 2010). Paull and Chen (2000) reported that heat treatment can inhibit external skin damage to fruit during storage at chilling temperature and in marketing. The induction of chilling tolerance by heat treatments has been reported in cucumber (McCollum *et al.*, 1995), persimmon (Lay-Yee *et al.*, 1997), avocado (Woolf, 1997), pepper (Fallik *et al.*, 1999; González-Aguilar *et al.*, 2000), plum (Abu-Kpawoh *et al.*, 2002), pomegranate (Mirdehghan and Rahemi, 2005; Mirdehghan *et al.*, 2007), banana (Chen *et al.*, 2008; Promyou *et al.*, 2008), cherry tomato (Yang *et al.*, 2009) and orange (Bassal and El-Hamahmy, 2011). Lin and Shiesh (2010) suggested that an optimal storage temperature for Indian jujube fruit was 5 °C. Preliminary work to the current study identified chilling injury symptoms (skin browning) in jujube fruit (a local variety called cv. Nomsoad) within 1 wk when it was stored at 4 ± 1 °C. Thus, the purposes of this study were to investigate the effect on jujube fruit of immersion in mild-to-hot water before refrigerated storage and the subsequent chilling-induced physicochemical changes.

MATERIAL AND METHODS

Plant materials

Jujube fruit (*Ziziphus jujuba* Mill, locally called cv. Nomsoad) samples were obtained from a commercial orchard, Phanna Nikhom district, Sakon Nakhon province, Thailand. The fruit were harvested at the commercially mature stage and delivered to the laboratory within 2 hr. The fruit were selected for uniformity, (free from physical damage and disease). The fruit were then sanitized in a 200 µL.L⁻¹ sodium hypochlorite solution for 5 min, rinsed with tap water and air dried at ambient temperature.

Treatment

The jujube fruit were dipped in hot water at 35 °C for either 10, 20 or 30 min and the untreated fruit were used as the control treatment. After hot water dipping, the fruit were air-dried at room temperature. Seven fruit were placed in a polypropylene (PP) bag. The bag was folded to prevent the loss of moisture. Twelve bags per treatment were stored at 4 ± 1 °C for 20 d.

Chilling injury score measurement

Chilling injury of the fruit during storage was estimated using the score of chilling injury symptoms on the peel of the fruit. The chilling

injury severity was rated on a relative scale of 0 to 4 where: 0 = no occurrence, 1 = trace, 2 = slight, 3 = moderate and 4 = severe.

Weight loss and firmness measurement

The fruit were weighed on the initial day after packing and then during storage every 5 d. The results were expressed as the percentage of the loss of fresh weight relative to the initial weight.

The firmness of the fruit without peeling was determined using a TA.XT Plus Texture Analyzer (Stable Micro System Ltd., UK). A 2 mm cylindrical probe was used to measure the fruit firmness at a rate of 1 mm.s^{-1} . Each fruit was punctured three times in the middle part of the fruit.

Electrolyte leakage determination

Electrolyte leakage from the jujube fruit peel (exocarp) disks was monitored by removing tissue plugs with a 15 mm diameter cork borer and excising the exocarp 1 mm above the exocarp/mesocarp interface. A sample of 5 g of the exocarp was rinsed with deionized water and incubated in 30 mL of 0.4 M mannitol at room temperature ($28 \pm 2^\circ \text{C}$) and shaken for 1 hr. The conductivity of the solution (final conductivity) was immediately measured using a conductivity meter (sensION5, Hach Company, USA). Then, the sample was frozen for 24 hr and then thawed. After thawing, the sample was boiled for 10 min. The conductivity was again measured after the temperature of the solution reached room temperature (total conductivity). The percentage conductivity was calculated as (final conductivity divided by the total conductivity) $\times 100$.

Surface color measurement

The color of the jujube fruit peel (exocarp) was measured using a HunterLab MiniScan XE Plus (Hunter Associates Laboratory Inc., USA). The peel color was recorded using the L^* value (lightness) and a^* value (greenness) of

the middle part of the fruit. Three points on each fruit were measured.

Total phenolic content and antioxidant capacity analysis

A sample of 5 g of jujube fruit peel was homogenized with 50 mL cold distilled water and then filtered using Whatman filter paper No. 42. The supernatant was collected and kept in an ice bath.

The total phenolic content was assayed using the method described by Slinkard and Singleton (1977). The reaction began when 1 mL of the supernatant was added into a solution of 1 mL 50% (v/v) Folin-Ciocalteu reagent solution and 2 mL saturated Na_2CO_3 solution. The mixture was left at room temperature for 30 min. The absorbance at 750 nm was recorded. The total phenolic content was expressed in term of micrograms of gallic acid per gram of fresh weight (μg gallic acid/g FW).

The antioxidant capacity of the sample was determined using a ferric reducing antioxidant potential (FRAP) assay according to the method described by Benzie and Strain (1996). The FRAP reagent was a mixture of 25 mL acetate buffer pH 3, 2.5 mL 10 mM 2,4,6-trioryridyl-1,3,5-triazine (TPTZ) and 2.5 mL 20 mM ferric chloride hexahydrate. The reaction started when 0.3 mL of the supernatant was added into 3 mL of FRAP solution. The mixture was incubated at room temperature for 30 min and then the absorbance was measured at 630 nm. The antioxidant capacity was expressed as micro moles of Trolox equivalents per gram fresh weight (μmole Trolox equivalent/g FW).

Polyphenol oxidase (PPO) activity assay

A sample of 3 g of jujube fruit peel was homogenized in 10 mL of 0.1 M sodium phosphate buffer pH 7.0 containing 0.1 g of polyvinylpyrrolidone (PVPP) and then filtered through cheesecloth. The extract was centrifuged

at 10,000×g for 10 min. The supernatant was collected. The PPO activity was performed using the method described by Galeazzi *et al.* (1981) with slight modification. The reaction began when 1 mL of aliquot of the supernatant was mixed into 3 mL of 0.1 M sodium phosphate buffer pH 7.0 and then 1 mL of 0.6 M 4-methylcatechol was added. Changes in absorbance at 420 nm were measured using a UV-visible spectrophotometer (Thermo Scientific, France). One unit of PPO activity was defined as a change of 0.001 in absorbance per minute. The data were expressed as units per gram fresh weight (units/g FW).

Lipoxygenase (LOX) activity assay

A sample of 3 g of jujube fruit peel was homogenized in 10 mL of Tris-HCl buffer pH 7.0 containing 0.1 g of polyvinylpyrrolidone (PVPP) and then filtered through cheesecloth. The extract was centrifuged at 10,000×g for 10 min. The supernatant was collected. The LOX activity was determined using linoleic acid sodium salt as a substrate, according to the method described by Pérez *et al.* (1999) with slight modification. A 3 mL aliquot of the supernatant was mixed with 2 mL of 0.1 M sodium phosphate buffer pH 6.0 and the reaction began when 20 µL of 0.01 M linoleic acid sodium salt was added. The increase in absorbance at 234 nm was recorded using the UV-visible spectrophotometer. The LOX activity was expressed as units per gram fresh weight (units/g FW) where one unit was present as 1 µmol hydroperoxide formed per minute at room temperature (29 ± 1 °C).

Malondialdehyde (MDA) content assay

A sample of 3 g of jujube fruit peel was homogenized in 10 mL of 5 % trichloroacetic acid (TCA) and then filtered through cheesecloth. The extract was centrifuged at 10,000×g for 10 min. The supernatant was collected. The MDA content assay was slightly modified from the method described by Heath and Packer (1968), with a 1 mL aliquot of supernatant being mixed with 2

mL of 15 % TCA containing 0.5 % thiobarbituric acid. The mixture was heated at 60 °C for 30 min and immediately cooled using an ice bath. The absorbance of the mixture was then measured at 532 and 600 nm, respectively. The MDA content was calculated using an extinction coefficient of 1.55 mM.cm⁻¹ as follows: MDA content = [(OD₅₃₂ - OD₆₀₀) × 2 mL × (total volume of extract (mL) × 1 mL)] / (1.55 × 10⁻¹ × 3 g). The data were expressed as nanomoles of MDA per gram fresh weight (nmol/g FW).

Statistical analysis

All experiments were performed according to a completely randomized design and replicated three times. Data were analyzed using analysis of variance which was performed with the SPSS software. Significant differences between means of data were compared using the least significant difference (LSD) at the 5% level.

RESULTS

Chilling injury score and electrolyte leakage

Chilling injury in the jujube fruit was observed as susceptibility to skin pitting and browning. As shown in Figure 1A, chilling injury of the jujube fruit in all treatments occurred at day 10 and continuously increased throughout storage. The chilling injury score of the control fruit increased sharply during storage and was higher than that of other treatments. The lowest chilling injury score throughout storage was present in the fruit immersed in hot water for 10 min. The increase in electrolyte leakage of jujube fruit is shown in Figure 1B. The electrolyte leakage of jujube fruit was delayed during storage by using hot water immersion when compared to the control sample. Jujube fruit immersed in the mild hot water for 10 min showed a significantly ($P \leq 0.05$) lower electrolyte leakage at days 10 and 15 when compared to the other treatments. The highest electrolyte leakage during storage was detected

in the control fruit.

Weight loss and texture

The loss of fresh weight and the change in texture of the jujube fruit immersed in hot water during refrigerated storage ($4 \pm 1^\circ\text{C}$) are shown in Figure 2. The weight loss of the fruit increased throughout storage. The lowest weight loss throughout storage was found in the fruit immersed in hot water for 10 min (Figure 2A). The weight loss of the control fruit increased rapidly and was

higher than other hot water treatments at day 15 and 20, respectively. As shown in Figure 2B, the firmness of the jujube fruit immersed in hot water for all durations remained constant throughout storage whilst the firmness of the control fruit decreased continuously. At the end of storage, the lowest weight loss and the highest firmness were present in the fruit immersed in hot water for 10 min.

Peel color

The lightness (L^* value) and greenness (a^* value) of the jujube fruit peel are shown in

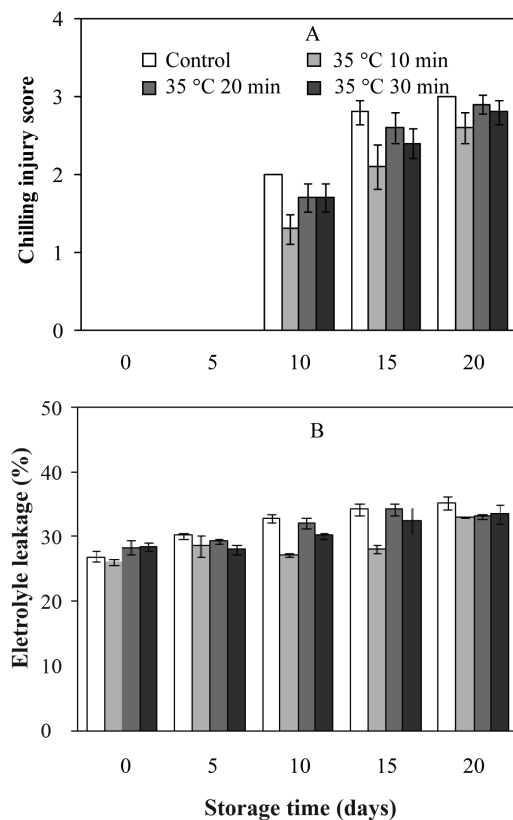


Figure 1 Chilling injury score (A) and electrolyte leakage (B) of the peel of jujube fruit immersed in hot water at 35°C for 10, 20 and 30 min compared to untreated fruit (Control) during storage at $4 \pm 1^\circ\text{C}$ for 20 d. Vertical bars indicate the standard error of the means ($n = 7$) and least significant differences at $P \leq 0.05$.

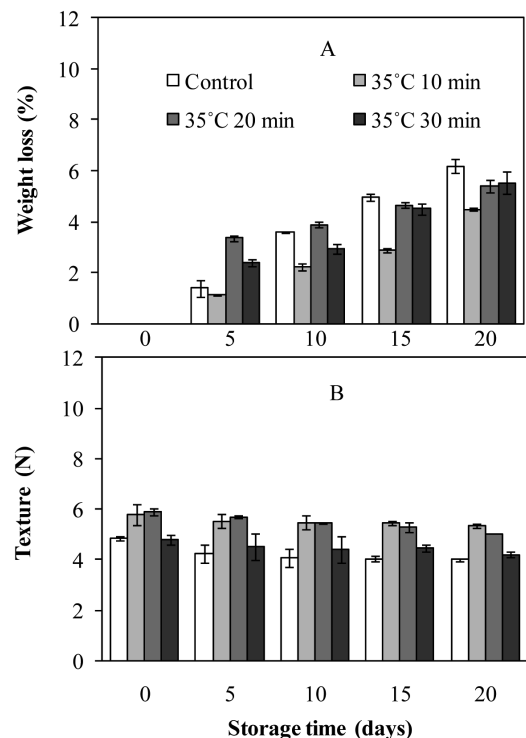


Figure 2 Weight loss (A) and texture (B) of jujube fruit immersed in hot water at 35°C for 10, 20 and 30 min compared to untreated fruit (Control) during storage at $4 \pm 1^\circ\text{C}$ for 20 d. Vertical bars indicate the standard error of the means ($n = 7$) and least significant differences at $P \leq 0.05$.

Figure 3. A decrease in lightness was found in both hot-water-treated fruit and the untreated fruit over the storage period (Figure 3A). The L^* value of the hot-water-treated fruit was higher than that of the control fruit. A significant ($P \leq 0.05$) difference in lightness between the hot-water-treated fruit and the control fruit was detected at the end of storage (day 20); however, no significant difference in hot-water-treated fruit was found over the storage period. The $-a^*$ value of peel from both hot-water-treated and untreated fruit indicated a green color. The a^* value of all treatments slightly increased throughout storage, resulting in a reduction of

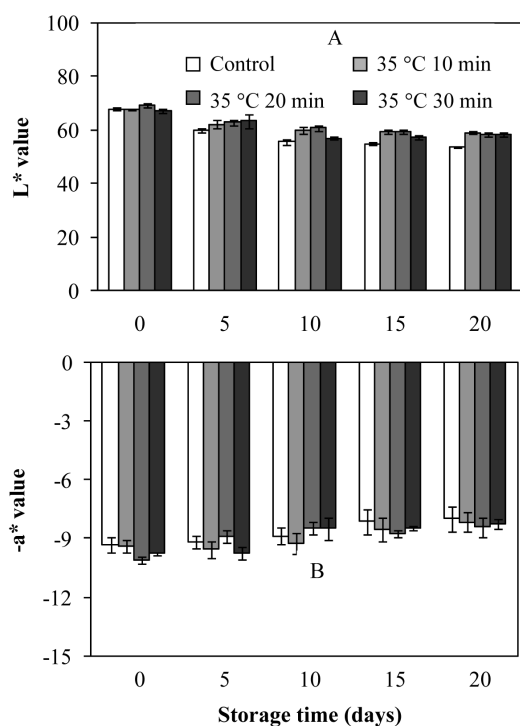


Figure 3 Lightness (L^* value) (A) and greenness (a^* value) (B) of the peel of jujube fruit immersed in hot water at 35 °C for 10, 20 and 30 min compared to untreated fruit (Control) during storage at 4 ± 1 °C for 20 d. Vertical bars indicate the standard deviation of the means ($n = 7$) and least significant differences at $P \leq 0.05$.

the greenness (Figure 3B). The a^* value of the hot-water-treated fruit was lower than that of the control fruit; however, no significant differences were found throughout the storage period. These findings indicated that the hot water treatment could maintain the lightness of jujube fruit during chilling storage.

Total phenolics content and antioxidant capacity

The reduction of both the antioxidant capacity and the total phenolics content during storage are presented in Figure 4. A significant

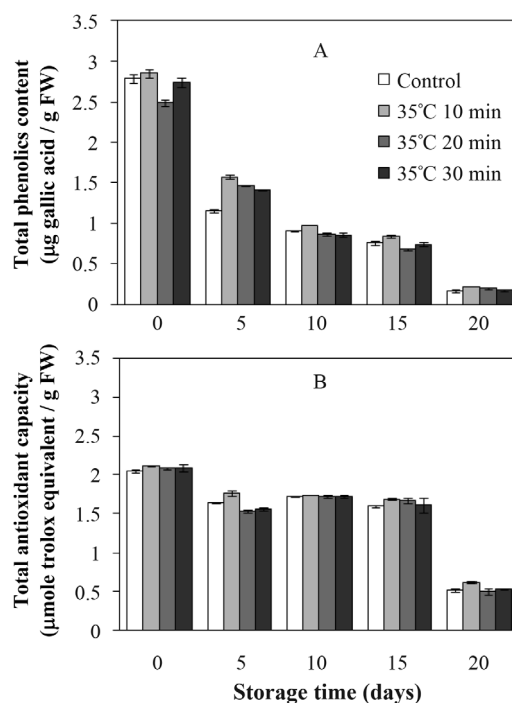


Figure 4 Total phenolics content (A) and total antioxidant capacity (B) on a fresh weight (FW) basis in the peel of jujube fruit immersed in hot water at 35 °C for 10, 20 and 30 min compared to the untreated fruit (Control) during storage at 4 ± 1 °C for 20 d. Vertical bars indicate the standard error of the means ($n = 3$) and least significant differences at $P \leq 0.05$.

($P \leq 0.05$) decrease in the total phenolics content was detected in all treatments throughout storage (Figure 4 A). The lowest total phenolics content was observed in the control fruit throughout storage. All hot water treatments retarded the reduction in the total phenolics content. The total phenolics content of the jujube fruit treated with hot water for 10 min during storage for 15 d was significantly ($P \leq 0.05$) higher than that of other treatments and at the end of storage, no difference in the total phenolics content was found among the hot water treatments. As shown in Figure 4B, the antioxidant capacity of all treatments decreased throughout storage. At the end of storage, the antioxidant capacity of the fruit immersed in hot water for 10 min was significantly ($P \leq 0.05$) higher than that of other treatments and no significance differences between the control fruit and the fruit immersed in hot water for 20 and 30 min were detected.

PPO and LOX activity and malonaldehyde content

Figure 5 shows the changes in the activities of PPO and LOX in the peel of the jujube fruit immersed in hot water for 10 min compared with the untreated fruit. The activities of PPO and LOX of both treatments increased over storage. Both activities (PPO and LOX) in the fruit peel immersed in hot water were significantly ($P \leq 0.05$) lower than those of the untreated fruit throughout storage. As shown in Figure 6, the MDA content in the peel of both treated and untreated fruit increased continuously throughout storage. A rapid increase in the MDA content in both treatments was detected at day 10 of storage. It was clear that the malonaldehyde content in the peel of the fruit immersed in hot water at 35 °C for 10 min was significantly ($P \leq 0.05$) lower than that of the untreated fruit over the period of refrigerated storage.

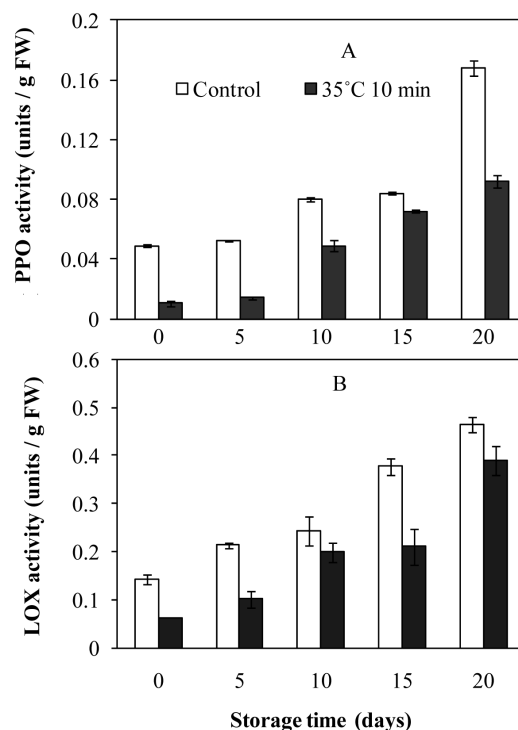


Figure 5 Polyphenoloxidase (PPO) activity (A) and lipoxygenase (LOX) activity (B) on a fresh weight (FW) basis in the peel of jujube fruit immersed in hot water at 35 °C for 10 min compared to the untreated fruit (Control) during storage at 4 ± 1 °C for 20 d. Vertical bars indicate the standard error of the means ($n = 3$) and least significant differences at $P \leq 0.05$.

DISCUSSION

Pre storage heat treatments (both hot air and hot water treatments) are widely accepted as effective in the control of decay and insect activity in fresh commodities (Lurie, 1998). In addition, heat treatments have been reported as a potential method to reduce chilling injury in cucumber (McCollum *et al.*, 1995), avocado (Woolf, 1997), persimmon (Lay-Yee *et al.*, 1997), pepper (Fallik *et al.*, 1999; González-Aguilar *et al.*, 2000),

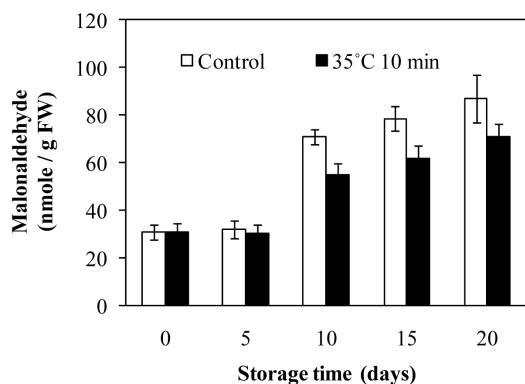


Figure 6 Malonaldehyde (MDA) content on a fresh weight (FW) basis in the peel of jujube fruit immersed in hot water at 35 °C for 10 min compared to the untreated fruit (Control) during storage at 4 ± 1 °C for 20 d. Vertical bars indicate the standard error of the means ($n = 3$) and least significant differences at $P \leq 0.05$.

pomegranate (Mirdehghan and Rahemi, 2005) and tomato (Lu *et al.*, 2010; Bassal and El-Hamahmy, 2011). This work showed that the optimal water temperature and time of exposure to alleviate chilling injury in jujube fruit was 35 °C for 10 min. As shown in Fig 1, the chilling injury symptoms in the jujube fruit appeared in the form of pitting and skin browning. The hot water treatment at 35 °C alleviated chilling injury and reduced the increase in ion leakage in the jujube fruit during refrigerated storage. It is generally accepted that chilling injury is associated with an increase in cell membrane permeability (Gómez-Galindo *et al.*, 2004) and a decrease in the unsaturated/saturated fatty acid ratio of the cell membrane (Mirdehghan *et al.*, 2007). The alleviation of chilling injury and a reduction of any ion leakage increase caused by the heat treatment could be related to a mechanism involving increases in the membrane integrity, the ratio of unsaturated/saturated fatty acid in the cell membrane (Mirdehghan *et al.*, 2007; Promyou *et al.*, 2008) and polyamine levels (Abu-Kpawoh *et*

al., 2002; Mirdehghan *et al.*, 2007). González-Aguilar *et al.* (2000) reported that the reduction in chilling injury of hot-water-treated pepper fruit was clearly related to the high levels of polyamines. A similar result was also reported in zucchini squash (Wang, 1994), lemon (Valero *et al.*, 1998), plum (Serrano *et al.*, 2004) and peach (Xu *et al.*, 2005). Moreover, the application of heat treatments to reduce chilling injury in fruit has been reported for ‘Hass’ avocado (Woolf, 1997), ‘Valencia’ oranges (Erkan *et al.*, 2005; Bassal and El-Hamahmy, 2011), cherry tomato (Yang *et al.*, 2009), and pomegranate (Mirdehghan and Rahemi, 2005; Mirdehghan *et al.*, 2007). However, the high chilling injury score which results in skin browning and electrolyte leakage of the jujube fruit immersed in hot water for 20 and 30 min might have been due to the excessive heat.

As shown by the results in Figure 2, the loss of firmness was associated with an increase in weight loss of the jujube fruit during storage and the low weight loss in the hot-water-treated fruit was also concomitant with the low severity of chilling injury. The results revealed that hot water treatment alleviated chilling injury, reduced the loss of fresh weight and maintained the firmness of the jujube fruit, with the best result being shown with the jujube fruit immersed in hot water for 10 min. The mechanism of injury could be described as due to a reduction in the cell membrane integrity and properties that result in a decrease in the turgor pressure of tissues (Brummell, 2006) which leads to a loss of firmness and an increase in the weight loss. Heat treatment could alleviate the damage to the cell ultrastructure under chilling stress, with membrane disassembly and disorganized cell wall middle lamellae (Zhang *et al.*, 2005). In a similar vein, Rodov *et al.* (1995) and Erkan *et al.* (2005) suggested that hot water dipping reduced the loss of fresh weight in citrus fruit which might be associated with an improvement in the membrane function or in the cuticular properties at the fruit surface. Schirra *et al.* (1995),

Mirdehghan and Rahemi (2005) and Bassal and El-Hamahmy (2011), also reported that hot water dipping reduced the weight loss of kumquat, pomegranate and Valencia orange fruit during storage. Conway *et al.* (1994) and Vincente *et al.* (2006) suggested that heat treatment would delay the action of certain cell wall hydrolases and activate endogenous Ca to form calcium-pectate. Mirdehghan *et al.* (2007) considered that heat treatment could induce polyamine levels which might be related to maintaining the firmness of fruit. Serrano *et al.* (2003) reported that exogenous polyamine treatment reduced the softening of plum fruit through a decrease in cell wall hydrolases and the maintenance of cell wall structure by cross-linking to pectin. Moreover, a reduction of the softening process by mild heat treatments has been reported in 'Fuyu' persimmon (Lay-Yee *et al.*, 1997), pomegranate (Mirdehghan *et al.*, 2007) and peach (Budde *et al.*, 2006).

The reduction in the L^* value and the increase in the a^* value of the jujube fruit skin (Figure 3) were related to the severity of chilling injury and weight loss as a result, as is shown in Figures 1 and 2. There was a loss of skin brightness and greenness in the jujube fruit stored at a chilling temperature that was concomitant with the severity of the chilling injury, while the hot water treatments prevented the reduction in lightness and greenness of the fruit skin. Similarly, Chen *et al.* (2008) reported that heat treatment delayed the onset of chilling injury and decreased the lightness and chroma of banana. Moreover, previous studies have suggested that hot water immersion is a potential alternative method to reduce the loss of green color in vegetables by maintaining the chlorophyll content (Wang, 2000; Dong *et al.*, 2004; Koukounaras *et al.*, 2009).

The increase in chilling injury during storage was also related to the reduction in the total phenolics content and the antioxidant capacity and the increase in PPO activity, as described by Galli *et al.* (2009) and Jaitrong *et al.* (2006), where the

loss of antioxidant protection including a reduction in the total phenolics content in fruit during cold storage may be the cause of chilling injury. As shown in Figure 4, the levels of the total phenolics content and total antioxidant capacity were related to the chilling injury of the jujube fruit. Interestingly, the higher total phenolics content and total antioxidant capacity of the hot-water-treated jujube fruit for 10 min were concomitant with the lower chilling injury score and electrolyte leakage compared to the untreated sample, even though both suffered from a decrease over the storage period. In general, the development of chilling injury symptoms is associated with the peroxidation of the membrane components (Berger *et al.*, 2001; Mirdehghan *et al.*, 2007; Promyou *et al.*, 2008) which produces and scavenges reactive oxygen species (Toivonen, 2004). Under this stress, fruit normally triggers antioxidant protection systems which included phenolics and antioxidant enzymes to ameliorate the reactive oxygen species (Mittler, 2002; Tsantili *et al.*, 2010). The current work showed that hot water treatment retarded the reduction of antioxidant capacity in the fruit compared to the untreated fruit and this might be related to the lower chilling injury score, electrolyte leakage, LOX activity and MDA content of hot-water-treated fruit. Chen *et al.* (2008) also reported that heat pretreatment at 38 °C induced the accumulation of total phenolics in banana fruit and alleviated the damage caused by subsequent chilling injury.

It is widely accepted that under chilling stress, the damage and modification of the membrane are key indicators (Mirdehghan *et al.*, 2007; Promyou *et al.*, 2008). The formation of a gel phase, a decrease in the unsaturated/saturated fatty acid ratio and an increase in the saturated fatty acids of the membrane during chilling stress have been investigated by Mirdehghan *et al.* (2007), Promyou *et al.* (2008) and Yang *et al.* (2009). The results of the increase in membrane permeability and dysfunction in membrane

properties are related to the increase in electrolyte leakage and the MDA content and are due to visual chilling injury symptoms, such as skin pitting and browning. The study showed that the increase in electrolyte leakage (Figure 1B) and the decrease in lightness (Figure 3A) were obviously related to the severity of chilling injury in jujube fruit (Figure 1A). It was found that these were associated with the increases in the PPO and LOX activity (Figure 5) and the MDA content (Figure 6). In a similar vein, Berger *et al.* (2001) reported that the processes of cell membrane fatty acid degradation and peroxidation are attributed to LOX activity, resulting in an increase in the MDA content under chilling stress. Hot water treatment for 10 min at 35 °C markedly lowered both the PPO and LOX activity and the MDA content of jujube fruit when compared with those of the untreated fruit. These findings revealed that the heat treatment effectively reduced the membrane damage and skin darkening of jujube fruit under chilling storage. Promyou *et al.* (2008) reported that the application of a hot water treatment reduced the activity of PPO and LOX in 'Gros Michel' and 'Namwa' banana varieties and a reduction in gene encoding of PPO was detected in the hot-water-treated fruit. Similar results have been reported for 'Brazil' banana (Chen *et al.*, 2008) and grape berries (Zhang *et al.*, 2005).

CONCLUSION

Hot water immersion for 10 min at 35 °C was an effective treatment to alleviate chilling injury in jujube fruit during refrigerated storage. The hot water treatment reduced the electrolyte leakage, LOX activity and MDA content, which are the key indicators used to estimate chilling stress, and provided a higher total phenolics content and antioxidant capacity compared to the untreated fruit. The hot water treatment maintained the lightness (L^* value) and greenness (a^* value) of the fruit skin. A reduction of lightness in untreated

fruit was due to the skin darkening. The increase in the PPO activity was delayed by the hot water treatment. Moreover, the hot water treatment also reduced the loss of fresh weight and maintained the firmness of jujube fruit during refrigerated storage.

ACKNOWLEDGEMENT

The authors thank the Research and Development Institute of Kasetsart University (KURDI) for financial support and the Faculty of Natural Resources and Agro-Industry for the use of their facilities.

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