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Wu, Chun-Ta

Department of Horticulture, National Taiwan University

Roan, Su-Feng

Department of Horticulture and Biotechnology, Chinese Culture University

Hsiung, Tung-Chuan

Department of Horticulture and Biotechnology, Chinese Culture University

Chen, Iou-Zen

Department of Horticulture, National Taiwan University

他

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Effect of Harvest Maturity and Heat Pretreatment on the Quality of Low Temperature Storage Avocados in Taiwan

Chun-Ta WU¹, Su-Feng ROAN², Tung-Chuan HSIUNG², Iou-Zen CHEN¹,
Jeng-Jung SHYR^{3*} and Akira WAKANA

Laboratory of Horticultural Science, Division of Agronomy and Environmental Science,
Department of Agro-environmental Sciences, Faculty of Agriculture,
Kyushu University, Fukuoka 812-8581

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The present study aimed to find out the effects of different fruit maturities and heat pretreatments before storage on the quality of climacteric avocado fruit during low temperature storage. Two local cultivars, 'Chanan' and 'Ching-Jin 2', were chosen to analyze the relationship between the degree of fruit maturity at harvest and the quality of the fruits after low temperature (1 °C) storage. Quality changes were observed in terms of color of skin, color of flesh, fruit hardness, chilling injury and rottenness. Fruits of high maturity at harvest showed better storage quality in the two cultivars. Under the storage temperature of 1 °C, fruit can be stored for 21 days for 'Chanan' and for 14 days for 'Ching-Jin 2', and retained fully ripening quality after three days of ripening treatment at 21 °C. Pretreatments of fruits were conducted by immersion of them in 38 °C water for 5, 15, 30 and 60 minutes to decrease the low temperature injury on avocado during 1 °C storage. The pretreatment for 30 minutes resulted in the optimum quality of 'Ching-Jin 2' fruit after four-week-storage at 1 °C. Post-treatment of fruit for six hours under 38 °C air is recommended to reduce chilling injury in 'Chin-Jin 2' avocado before the 1 °C storage. Heat shock protein HSP 70 showed highly negative correlation with the chilling injury of fruit stored at 1 °C.

Keywords: avocado, maturity, heat pretreatment, low temperature, storage quality

INTRODUCTION

Avocado, a climacteric type of fruit, can not be preserved for long time after harvest at room temperature, because it autonomously ripens 5–10 days after harvest, depending on the cultivars, under 25 °C temperature condition. A significant delay in fruit ripening from the time of harvest is necessary for consumption of ripened fruit; without this delay fruit may be over-ripened when shipped to an overseas market. Fruit may be stored during the time of overproduction in a domestic market. Generally, the storage period of fresh fruit products can be extended by low temperature, which reduces the rate of respiration of the fruit. However, chilling injury is often induced during low temperature storage of avocados, and resulted in a tremendous quality loss. The typical symptoms of chilling injury in avocado fruit include mesocarp discoloration, hardening of vascular strands and off flavors (Woolf, 1997).

Fruit maturity at harvest is a very important factor that determines storage life and fruit quality. Immature fruit is more likely to shrivel, and has inferior quality when ripens. Overripe fruit is likely to become soft and mealy soon after harvest. Fruit harvested too early or too late in the season is more likely to have a shorter storage life than those harvested at the proper season.

Fruit maturity has been broadly studied in relation to the low temperature tolerance in horticultural crops. 'Hass' avocado fruit with high maturity, or after ripening fruit, showed better storage result under low temperature conditions (Hofman *et al.*, 2002). Reduction of chilling injury can permit extended storage period. Better maturity is desirable for climacteric kiwifruit destined for long-term storage at 0 °C (Gordon *et al.*, 1992). However, it is not always true for all situations. For example, climacteric 'Jonathan' apple at the most advanced stage in maturity at harvest has the highest incidence of low-temperature breakdown and the lowest incidence of storage scald. Apples at the least advanced stage of fruit maturity at harvest have the lowest incidence of low-temperature breakdown and the highest incidence of scald (Blanpied and Little, 1991).

Heat treatment has been shown to be generally effective as a postharvest treatment on reducing chilling injury in tomato fruit (McDonald *et al.*, 1999), maintaining quality of strawberry (Vicente *et al.*, 2002), zucchini squash (Wang, 1994), grapefruit (Porat *et al.*, 2000), avocado (Woolf *et al.*, 1995), pear (Abreu *et al.*, 2003) and peach (Zhou *et al.*, 2002) during cold storage. Tolerance to low temperature in 'Hass' avocado can be increased by pretreatment with high temperature such as 38 °C hot air (Florissen *et al.*, 1996; Woolf *et al.*, 1995) and hot water treatments (Woolf, 1997). Heat treatment could protect the ultra-structure of the pericarp cells in the heat-pretreated grape berries under chilling stress (Zhang *et al.*, 2005). Heat pretreatment was also effective for decrease in the fruit decay rate during low temperature storage. Sabehat *et al.* (1996) showed that a two-day-pretreatment with hot tempera-

¹ Department of Horticulture, National Taiwan University, Taiwan

² Department of Horticulture and Biotechnology, Chinese Culture University, Taipei, Taiwan

³ Department of Horticulture, National Ilan University, Sheng-Loung Rd., I-Lan, Taiwan

* Corresponding author (E-mail: jjshyr@niu.edu.tw)

ture of 38 °C to tomato fruit could reduce its chilling injury and rots during the low temperature storage at 2 °C. The same pretreatment was also proven to be effective for controlling microbial levels or decay in citrus low temperature storage (Porat *et al.*, 2000). The analysis of heat shock protein (HSP) and postharvest responses showed that HSP transcripts remain elevated for considerable long periods at low temperature subsequent to the heat treatment: Sabehat *et al.* (1996) found the persistence of heat-induced HSP in tomato fruit stored for 21 days at 2 °C after hot temperature treatment. Both HSP 70 and HSP 17 mRNAs and new heat-induced proteins were found in apple fruit under direct sunlight. In in-vitro-cultured apple cells, HSP 70 and low molecular weight HSP transcripts were maintained at elevated levels for 4 days at 1 °C after 1h pretreatment at 38 °C, whereas the levels returned to those in control cells within 24 h when the heat-treated cells were maintained at 25 °C (Wang *et al.*, 2001).

These results provided useful references to this study for prolonging the low temperature storage limits of the local variety of avocados in Taiwan. However, most of previous works focused on 'Hass' avocado, whose results may not be applicable to other cultivars. Therefore, this study focused on the effects of fruit maturity and heat pretreatment on the quality of fruit stored at low temperature in other avocado cultivars. Fruits of two important local avocado cultivars at three different maturity stages were investigated to increase their quality during low temperature storage and extend their low temperature storage life.

MATERIALS AND METHODS

Materials

Two avocado cultivars, 'Chanan' and 'Ching-Jin 2', were selected for this experiment. Fruits were harvested by hand in the orchard managed according to the standard procedure by local farmers in Cha-I County, Taiwan. The harvested fruits were packed in carton boxes maintained at 4–7 °C and transferred to laboratory within 12 h. Fruits were graded for size by weight. The graded fruits without damage and sunburn symptoms were randomly chosen for further analysis.

Low temperature storage of fruits with different maturity

Fruit was harvested 10 days intervals over a 30-day period from the end of July to the end of August (the best season for harvest) in 2005 and 2006. The harvested fruits were packed in carton boxes, cooled immediately and air-stored at 1 °C without ethylene, and monitored at 0, 7, 14, 21 and 28 days of cold storage. After the treatment had finished, the fruits were allowed to ripen at 21 °C for three days.

Hot water pretreatment

The fruits were immersed in a water bath tank adjusted at 38 °C for the following periods: 0, 5, 15, 30 and 60 min. Thermometer was placed into the tank and

was used to monitor temperature change during the heat treatment. Following the heat pretreatment, fruits were allowed to recover at 25 °C for 2 h. Before low temperature storage at 1 °C, all fruits were individually wiped by clean tissues to dry their surface.

Hot air pretreatment

The fruits were loaded in an air convection oven adjusted at 38 °C for heat pretreatment by air for the following periods: 0, 6, 12, 24 and 48 h. To prevent water loss during the heat treatment, the fruits were individually sealed in perforated polyethylene bags. Following heat pretreatment, the fruits were allowed to recover at 25 °C for 2 h. Polyethylene bags were removed before stored at 1 °C.

Quality analysis

In all experiments, quality analyses were based on the three replicates of five fruits. Quality parameters, including color of skin, color of flesh, and hardness of flesh, symptoms of chilling injury and decay of the fruit, were assessed objectively.

Measurement of color

Skin color of the fruit was measured using a color differential meter (ZE-2000, Nippon Denshoku, Japan) to determined Hunter Lab's L* value (lightness or brightness), a* value (redness or greenness), and b* value (yellowness or blueness) by averaging four measurements of the fruit equator. The color of flesh was determined on the surface of flesh (0.5 cm beneath the skin). Measurements were taken for three samples and the average of L*, a*, and b* values were obtained. The colorimeter was warmed up for 30 min and calibrated with a white standard tile: L*=95.87, a*=-0.86 and b*=2.47.

Measurement of hardness

Texture measurement was made by using a texture analyzer (TA-XT2 Texture analyzer, Stable Micro Systems (SMS), England). Samples were subjected to a puncture test at a constant speed of 2 mm/sec, using a 5 mm diameter round tipped puncture probe or plunger. Four measurements were taken on each fruit at different locations of 0.5 cm beneath the skin around the fruit equator. Measurements were taken for three samples and the average values were obtained.

Assessment of chilling injury

The degree of ripening of each fruit was determined using a subjective assessment of softness determined by hand touch (Florissen *et al.*, 1996). Once ripe, the fruit appearance was checked first, and then the fruit was longitudinally cut in half and examined for symptoms of chilling injury. The degree of chilling injury observed on the fruit surface was revealed as the relative score of 0–3 as described by Woolf and Lay-Yee (1977), i.e., score 0 is the degree of surface injury for 0% (no surface injury), 0.5 for <10%, 1.0 for 10–19%, 1.5 for 20–49%, 2.0 for 50–74%, 2.5 for 75–89%, 3.0 for ≥90% of the fruit surface injury.

SDS-PAGE and Western blot analysis

The total protein of avocado samples was extracted

with Tris-HCl buffer (60 mM; pH 8.5; containing 2% SDS, 2.5% glycerol, 0.13 mM EDTA and 1% protease inhibitor cocktail). The protein amount was measured by using DC Protein Assay reagents (Bio-Rad) with bovine serum albumin as the standard. For immunoblot analysis, protein was separated by SDS-PAGE with a precast minigel assembly (NuPAGE 4%–12% BisTris gel 1 MOPS SDS running buffer; Invitrogen) and transferred onto a nitrocellulose membrane for antibody probing. The amount of antigen was detected by using the Super Signal West Dura Extended Duration Substrate system (Pierce). The polyclonal antibodies against HSP70 and sHSP were kindly provided by Dr. Yee-yung Charng (Academia Sinica, Taipei, Taiwan). Following the chemiluminescence detection, the membrane was stained with 0.1% (w/v) Amido Black to ensure equal loading of protein. The intensity of HSP was quantified by scanning, and analyzed the immune signals with the Image J 1.37V software.

Statistical analysis

One-way analysis of variance (ANOVA) was used to detect significant differences among avocado samples with different treatments. The significant level used was $p \leq 0.05$. Duncan's multiple range test was conducted to compare the mean values of different storage days. Statistical analysis was carried out using SAS software (SAS Institute, Cary, NC, USA).

RESULTS AND DISCUSSION

Effect of harvest maturity on the quality of low temperature storage fruit

Low temperature storage of 'Chanan' avocado fruit

Avocado fruits with different maturities were stored at 1 °C for 7–28 days and transferred from 1 °C cold room to 21 °C warm room for three days to enhance their ripening, and then observation was carried out about the symptoms of chilling injury, and the quality of ripened fruit was analyzed. Figures 1 and 2 show the color changes of the flesh of 'Chanan' exposed at 21 °C for three days after 1 °C cold storage. When fruits of 'Chanan' cultivars with low and mid-range maturity at harvest were preserved for seven days and 14 days under the cold temperature, no serious chilling injury was visible (Table 1). The fruits were able to maintain good quality both on appearance and in flesh during ripening. Normal ripening was found on the fruits.

In the treatment of 21 days of low temperature storage, the appearance of fruit was still good; however, serious chilling symptoms including browning and inability of normal softening were found in the internal flesh of the fruit (Table 2). The brown spots were also found in the peel. The chilling symptoms were more serious in the fruits cold-treated for 28 days (Tables 1 and 2).

In the low temperature storage of fruit with high

Table 1. Changes for fruit skin chilling injury of 'Chanan' and 'Ching-Jin 2' avocado fruits harvested in three different degree of maturity of low, mid-range and high and stored at 1 °C for 7 to 28 days followed by three days exposure at 21 °C. The degree of chilling injury of the fruit skin was divided into seven groups, revealed as the relative score of 0–3 (Woolf and Lay-Yee, 1977) and averaged in each treatment. Here, score 0 is for 0% (no chilling injury), 0.5 for <10%, 1.0 for 10–19%, 1.5 for 20–49%, 2.0 for 50–74%, 2.5 for 75–89%, and 3.0 for $\geq 90\%$ of the fruit surface.

Storage period (day)	Score of chilling injury in 'Chanan' avocado fruit with indicated maturity at harvest			Score of chilling injury in 'Ching-Jin 2' avocado fruit with indicated maturity at harvest		
	Low	Mid-range	High	Low	Mid-range	High
7	0.67 ^{ay}	0.67 ^{ay}	0.25 ^{ax}	0.58 ^{az}	0.25 ^{ay}	0.08 ^{ax}
14	1.83 ^{by}	0.58 ^{ax}	0.33 ^{ax}	1.33 ^{by}	0.41 ^{ax}	0.42 ^{bx}
21	1.83 ^{by}	1.33 ^{by}	0.33 ^{ax}	1.25 ^{bx}	0.25 ^{ax}	0.58 ^{by}
28	3.00 ^{cz}	1.83 ^{by}	1.08 ^{bx}	1.67 ^{cz}	1.08 ^{by}	1.83 ^{bx}

^{a, b, c} Different superscripts within the same column indicate significant different at 5% level.

^{x, y, z} Different superscripts within the same row indicate significant different at 5% level.

Table 2. Changes for flesh hardness of 'Chanan' and 'Ching-Jin 2' avocado fruits harvested with low, mid-range and high maturity, stored for 7 to 28 days at 1 °C, and exposed for 3 days at 21 °C. Values are expressed as average \pm standard deviation (n=5).

Storage period (day)	Flesh hardness of 'Chanan' fruit with indicated maturity at harvest (g/cm ²)			Flesh hardness of 'Ching-Jin 2' fruit with indicated maturity at harvest (g/cm ²)		
	Low	Mid-range	High	Low	Mid-range	High
7	108.8 \pm 28.4	82.5 \pm 6.8	74.6 \pm 3.8	106.4 \pm 11.6	79.0 \pm 11.4	74.4 \pm 121.9
14	172.5 \pm 24.3	99.7 \pm 9.0	302.3 \pm 42.9	142.6 \pm 58.9	62.0 \pm 15.0	72.0 \pm 15.9
21	131.1 \pm 30.0	178.1 \pm 53.3	187.8 \pm 53.7	591.0 \pm 522.5	95.1 \pm 3.0	81.2 \pm 210.9
28	660.6 \pm 403.5	867.4 \pm 770.5	163.3 \pm 76.8	658.4 \pm 356.4	336.3 \pm 397.8	740.6 \pm 584.0

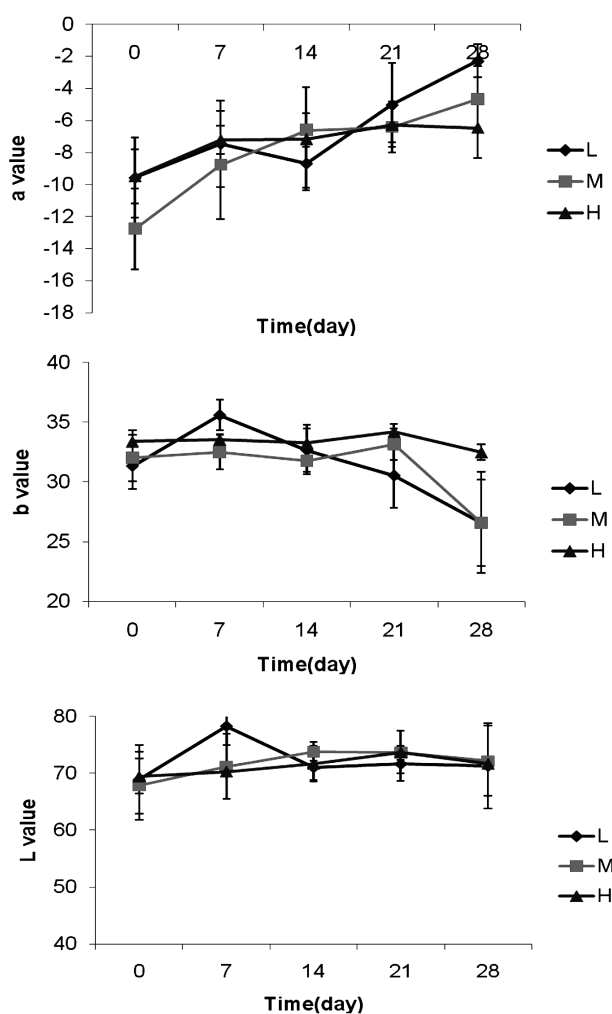


Fig. 1. Changes of L^* , a^* and b^* color values in the flesh of low-temperature-stored 'Chanan' avocado fruit with low (L), medium (M), or high (H) maturity at harvest. The fruits were stored at 1 °C, collected seven days intervals and exposed at 21 °C for three days in each collection, and then their flesh color measurements were carried out.

maturity at harvest, no serious chilling symptoms were found in the fruits stored for 7, 14 and 21 days. After the low-temperature-stored fruits were exposed at 21 °C for three days, their overall quality was also good: the peel and flesh showed desirable appearance, suggesting the ripening process of fruit was completed. At the day 28 of storage at 1 °C, the fruit harvested with high maturity showed good appearance; however, serious chilling symptoms appeared in the flesh after the three days ripening treatment at 21 °C. The major symptoms of the chilling injury were internal browning and inability of flesh softening (Table 2), followed by small dark brown spots found on the skin. Because of the difficulty to control full softening, the hardness of flesh varied among fruit individuals with high standard deviations (Table 2). *Low temperature storage of 'Ching-Jin 2' avocado fruit*

Change of flesh color in 'Ching-Jin 2' fruits, which had low maturity at harvest, stored for 7 days at 1 °C and exposed for three days at 21 °C, was not obvious in any internal parts (Fig. 2), and softening of the fruits

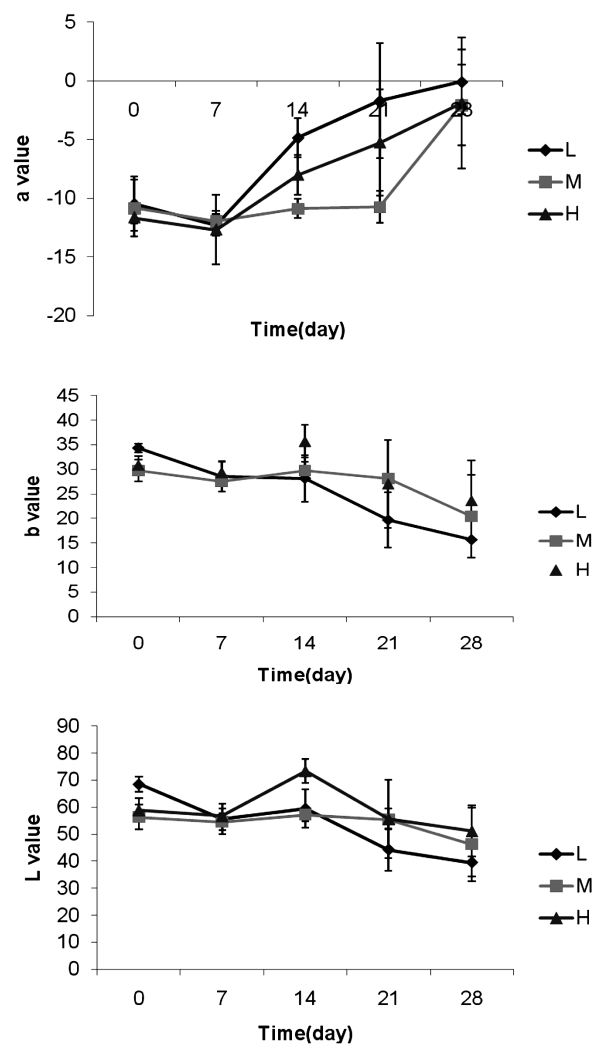


Fig. 2. Changes of L^* , a^* and b^* color values in the flesh of low-temperature-stored 'Ching-Jin 2' avocado fruits with low (L), medium (M), or high (H) maturity at harvest. The fruits were stored at 1 °C, collected seven days intervals and exposed at 21 °C for three days in each collection, and then their flesh color measurements were carried out.

was normal (Table 1), suggesting chilling injury did not occur in the seven days storage as has been observed in 'Chanan'. Low temperature storage for 14 days and subsequent exposure at 21 °C for three days in 'Ching-Jin 2' fruits resulted in serious chilling injury in the flesh, although their skin appearance was normal when they were taken out from the cold storage room. The longer the low temperature storage, the worse the chilling symptoms were. They were internal browning and incomplete softening that occurred during ripening at 21 °C. Brown spots were also found on the skin. High standard deviation of the hardness of flesh was also found for 'Ching-Jin 2' fruit with serious chilling injury (Table 1). Twenty-one days storage for mid-mature 'Ching-Jin 2' and 14 days for high-mature 'Ching-Jin 2' at 1 °C showed good ripening quality. Chilling injury became obvious if the storage period reached 28 days in 'Ching-Jin 2' avocado fruit of mid maturity at harvest, which showed the highest low temperature storage ability in this experiments.

Cutting *et al.* (1988) pointed out that the potential

and incidence of physiological disorders like vascular browning and mesocarp discoloration increased with increase in maturity of 'Fuerte' avocado. Cutting and Wolstenholme (1992) reported that the browning disorder was very serious problem in fruit harvested early, but decreased thereafter; however, the incidence of disorder rose rapidly as the fruit reached high maturity. There is a relation between the extent of maturity at harvest and the chilling injury due to cold storage in 'Ching-Jin 2' avocado fruit. In 'Chanan' avocado, however, the symptoms for the chilling injury were reduced with the decrease of fruit maturity. These results in the both cultivars are not perfectly consistent with the report by Cutting and Wolstenholme (1992).

Natural pretreatment of fruit with high temperature before harvest

In New Zealand, Woolf *et al.* (1999) studied the difference of flesh temperatures of fruit individuals setting on the various parts of a tree, which was growing under the direct sun light and high temperature conditions frequently exceeding 35 °C. The fruit exposed to direct sun light in the tree and stored at 0 °C showed lower chilling injury than that harvested from a shaded part of the tree and stored at 0 °C. They suggested that the high temperature, particularly, at the time of harvest season might induce fruit with low temperature tolerance during postharvest storage. The field temperature in central Taiwan area is always over 35 °C in summer, in which the best time of harvest season is from the end of July to the end of August. This high temperature conditions in harvest season in Taiwan is considered to be one of the reasons why high-matured fruit could store for relatively long time at 1 °C in this study. Bramlage and Weis (1977) have reported the similar result in apple that reduction in chilling injury (scald) susceptibility in 'Delicious' was related to high temperature in late harvest season.

An integrating high temperature more than 35 °C during the harvest season with long time sunlight exposure with diurnal fluctuation affects on the internal quality, such as sugar, oil and mineral contents and tissue firmness. It has been also known that with increase of avocado fruit maturity oil content increases and water content decreases (Pearson, 1975). Polyphenol oxidase activity (Kahn, 1975) and the concentration of abscisic acid (Cutting *et al.*, 1988) increase with the increase in maturity of avocado fruits. To sufficiently understand the correlation between fruit maturity and low temperature storage tolerance in avocado fruit, these constituents for fruit quality and maturity may be very helpful markers for finding the optimum maturity for low temperature storage of avocado.

Effect of hot water pretreatment on the quality of fruit stored at 1 °C

'Ching-Jin 2' fruit was selected for pretreatment with hot water to prolong the term of low temperature storage, since it showed lower patience for low temperature storage at 1 °C. The fruits immersed in hot water for 5 min appeared to be good quality after 7, 14 and 21

days of storage at 1 °C, and ripened normally during three days of ripening treatment at 21 °C. No chilling injury was seen in the skin and flesh of the fruits. The appearance of fruit stored for 28 days at 1 °C was normal; however, serious chilling injury with some symptoms mentioned above appeared after three days of ripening treatment at 21 °C.

The fruits pretreated in hot water of 38 °C for 15 min showed bad storage behavior except for those stored for two weeks at 1 °C. Some of the fruits stored for 21 day at 1 °C showed chilling injury symptoms during ripening at 21 °C. All fruits stored for 28 days at 1 °C showed serious chilling injury symptoms after the three days of ripening at 21 °C.

The fruits pretreated in hot water of 38 °C for 30 min showed good appearance and internal qualities after 28 days of storage at 1 °C. The fruits were normally ripe and showed a similar texture and flesh color to the control samples (Fig. 3).

The fruits pretreated in hot water of 38 °C for 60 min extended storage life at 1 °C up to 21 days without serious chilling injury after fully ripe. Storage of the fruits for 28 days showed internal browning and incomplete ripening during three days storage at 21 °C. In summary, pretreatment with hot water at 38 °C for 30 minutes is the optimum condition for 'Ching-Jin 2' avocado fruit to prolong the storage period to 28 days.

Hot water treatment significantly reduced skin damage of 'Hass' avocado due to disinfestation; the highest effect was with treatment of 40 °C for 30 min, 41 °C for 20–30 minutes and 42 °C for 25–30 min. Hot water treatment also reduced body rots in ripe avocado fruit: the most common and effective method was with treatment fruit at 40 and 41 °C for 30 minutes (Hofman, 2003). Woolf (1997) reported that 'Hass' avocado fruit

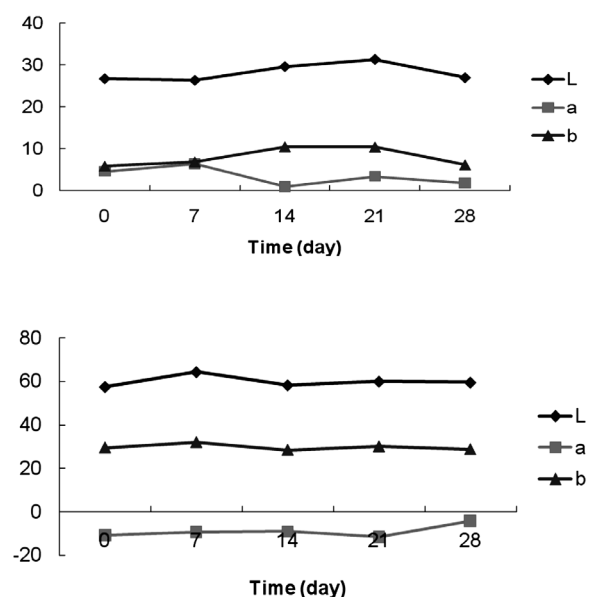


Fig. 3. Changes for L*, a* and b* color values of the skin (upper) and flesh (lower) of 'Ching-Jin 2' avocado fruit pretreated with 38 °C water for 30 minutes, stored for 7, 14, 21 and 28 days at 1 °C, and exposed for 3 days at 21 °C.

heated with hot water at 38 °C for 60 minutes generated the optimal result of elimination of external chilling injury, and was able to store for 28 days at 0.5 °C. The optimal heating conditions to reduce chilling injury in ‘Hass’ avocado fruit were longer time or higher temperature than those of ‘Ching–Jin 2’. The average weight of ‘Hass’ is about 250 g, while that of ‘Ching–Jin 2’ is about 650 g. These results suggest that the heat sensitivity of ‘Ching–Jin 2’ to increase low temperature tolerance should be higher than that of ‘Hass’ avocado.

Effect of hot air pretreatment on the quality of fruit stored at 1 °C

The fruits were placed in an air convection oven at 38 °C for 6, 12, 24 and 48h. Most pretreated fruits were unable to undergo normal ripening after three weeks of storage at 1 °C (Table 3). The shorter the time of pretreatment, the longer the time of storage was. Fruit pretreated with hot air for 6h maintained ripening ability after 21 days of storage at 1 °C. For 12 h–pretreated fruit, internal tissue disruption was seen on the 7 days of storage and serious internal browning was observed after 14 days of storage at 1 °C. Fruits pretreated with hot air for 24 and 48 h showed the serious browning of skin (Fig. 4) and the tough texture of flesh. Thus, these fruits were not stored at 1 °C and their data lacked in Table 3.

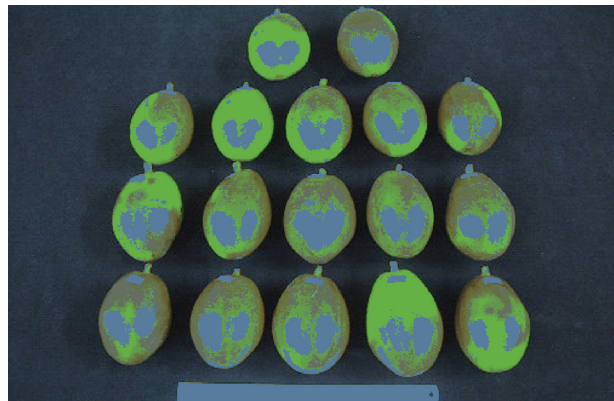


Fig. 4. Heat damage in ‘Ching–Jin 2’ avocado fruit pretreated by 38 °C air for 48 h.

Florissen *et al.* (1996) demonstrated that short time (6–12 h) of heat treatment applied to pre-climacteric ‘Hass’ avocado fruit provided partial protection from chilling injury. In ‘Sharwil’ avocado, heat pretreatment time of 8 to 12 h was effective in reducing chilling injury symptoms if the flesh temperature was kept ≤ 2.2 °C during 16 days of storage (Nishijima *et al.*, 1995). Chilling injury symptoms were also reduced when ‘Sharwil’ avocados were pretreated by 37–38 °C for 17–18 h, and then air-cooled at 20 °C for 4 h before storing at 1.1 °C for 14 days or more (Sanxter *et al.*, 1994). In the present study, however, the protection effect of 38 °C air pretreatment from chilling injury was not observed in the treated fruits, except for those treated for 6 h (a very short time). It seems that the chilling injury protection effect was lost if fruits were heated in excess of limit of hot air pretreatment. Woolf *et al.* (1995) reported a similar result.

For ‘Ching–Jin 2’ avocado, pretreatment with hot air at 38 °C for 6 h is the optimal duration, which is much shorter than that of ‘Hass’ or ‘Shirwil’ avocado. No matter which we use (hot water or hot air treatment), ‘Ching–Jin 2’ avocado shows shorter preheating time or lower preheating temperature than other cultivars of avocado, for the quality maintenance at low temperature.

Relationship between HSP and chilling injury

A close correlation between HSP production and protection effect from chilling injury during cold storage of fruit was found in grape berry (Zhang *et al.*, 2005) and avocado (Florissen *et al.*, 1996; Woolf *et al.*, 1995). Florissen *et al.* (1996) used short heat treatment to improve chilling injury, and investigated the heat shock proteins of avocado fruit synthesized at 38 °C for 6, 12, 24, 36, or 48 h prior to transferring them to 0 °C. Results showed that heating for 6–12 h provided a significant degree of protection from chilling injury. The minimal conditions needed to induce maximal production of HSPs in samples of mesocarp tissue were to expose to 38 °C for 4 h, and the most intense band had molecular weight of 74 kDa. Based on this report, two heat shock proteins in the mesocarp tissue of ‘Ching–Jin 2’ avocado fruits were analyzed.

Table 3. The effect of pretreatment of ‘Ching–Jin 2’ avocado fruit with hot water or hot air on hardness (g/cm²) of the flesh. Fruit was preheated with 38 °C water or air for 5 minutes to 12 hours before storage, stored for 7, 14, 21 and 28 days at 1 °C and exposed for 3 days at 21 °C. Each value of flesh hardness is the mean of five replicates

Storage period (day)	Flesh hardness (g/cm ²) of fruit preheated with 38 °C water for indicated time (minute)				Flesh hardness (g/cm ²) of fruit preheated with 38 °C air for indicated time (hour)	
	5	15	30	60	6	12
7	109.97 ^b	121.41 ^b	197.70 ^b	105.24 ^b	120.99 ^b	200.42 ^a
14	70.40 ^a	73.93 ^a	80.88 ^a	71.74 ^a	69.13 ^a	1263.10 ^b
21	80.40 ^a	202.60 ^c	81.69 ^a	74.08 ^a	81.63 ^a	1248.06 ^b
28	163.50 ^c	293.30 ^c	74.97 ^a	298.13 ^c	294.30 ^c	1257.78 ^b

^{a, b, c} Different superscripts within same column indicate significant different at 5% level.

Production of HSP70 in fruit treated with hot water (38 °C) increased with the increase in time of the heat treatment up to 30 min and slightly decreased in fruit treated for 60 min. Those treated by hot air (38 °C) for 6 to 24 h showed relatively high content of HSP70 but no significant difference in HSP70 contents were detected among them ($p>0.05$). The fruits treated with hot air for 48 h resulted in a tremendous decrease of HSP70 content compared to control samples. One of the possible reasons for this is that long time heat treatment would cause serious denaturation of HSP70.

In comparison to control, heat treatment caused significant increases of sHSP in the treated fruits irrespective of water or air treatments ($p<0.05$). The intensity of bands in hot water-treated fruits was almost same among 5 to 30 min treatments and then increased in 60 min treatment. The intensity of bands in hot air-treated fruits showed different trend, i.e., the longer the heat treatment, the lower the sHSP content was. The optimum treatment for increasing protection is considered to be 30 min immersion in 38 °C water, since longer duration would deteriorate the level of chilling injury. The serious browning on the skin and tough texture of flesh observed in fruits just after hot treatment at 38 °C air for 24 or 48 h have not correlation to chilling injury. It could be ascribed as heat injury. Florissen *et al.* (1996), suggested that there is a 'crossover' point after which the protection from chilling injury is lost, and that the heat treatment has a detrimental effect on fruit cells. In their trial, the 'crossover' point was between 12

and 24 h at 38 °C; this coincided with the point of samples treated by 38 °C air in this experiment. For fruits treated by hot water, the 'crossover' point is considered to be between 30 and 60 min.

Sabehat *et al.* (1996) found that HSP70 antibody reacted more strongly with proteins from heated and chilled tomato fruit than with those from chilled tomato. HSP18.1 antibody reacted strongly with proteins from heated fruit but not with those from unheated fruit. The increased intensities of HSP18.1 were greater than those of HSP70. They suggested that the increase of low molecular HSP is responsible for the good low temperature storage ability. In 'Ching-Jin 2' avocado fruit, the increasing rates in the intensity of sHSP by heat treatment was larger than that of HSP70 (Fig. 5). As compared with sHSP, however, HSP70 showed relative high intensity and good correlation with storage time ($r=0.90$ for HSP70 and $r=0.63$ for sHSP). It means that the intensity of HSP70 reflects the low temperature tolerance of 'Ching-Jin 2' avocado fruit.

Based on our results, it is proposed that the protection effect of heat treatment on chilling injury may be caused by the production of heat shock proteins in tissues. The protection effect improves as the intensity of heat shock proteins increases, but the effect is lost under long time heating even when the HSP intensity increased. This study demonstrates that the increase of HSP70 is responsible for the good low temperature storage tolerance.

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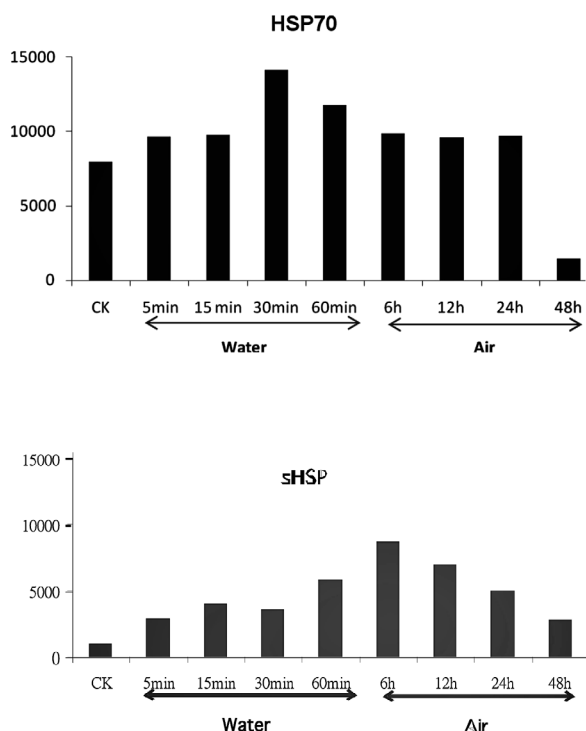


Fig. 5. Relative intensity of heat shock proteins HSP70 (upper) and sHSP (lower) of 'Ching-Jin 2' avocado fruits pre-heated with 38 °C water for 5, 15, 30 and 60 minutes and those pretreated with 38 °C air for 6, 12, 24 and 48 h. CK: control, non-pretreated.

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