Reduction of Chilling Injury in Stored 'Hass' Avocado Fruit by 38 °C Water Treatments

Allan B. Woolf

The Horticulture & Food Research Institute of New Zealand, Mt Albert Research Centre, Private Bag 92169, Auckland, New Zealand

Abstract: 'Hass' avocado (*Persea americana* Mill.) fruit were heat treated in water at 38 °C for 0 to 120 minutes, and stored at 0.5 °C for up to 28 days. After storage, fruit were ripened at 20 °C and their quality evaluated. External chilling injury (CI) developed during storage in nonheated fruit. Skin (exocarp) sectioning showed that browning developed from the base of the exocarp, and with longer storage, this browning moved outwards toward the epidermis. Longer durations of hot water treatment (HWT) progressively reduced CI; 60 minutes was the optimal duration that eliminated external CI, while best maintaining fruit quality. Concomitantly, electrolyte leakage of heated skin tissue increased ≈70% during storage, whereas electrolyte leakage of nonheated skin tissue increased ≈480% over the same period. Thus, significant protection was conferred by HWTs against low temperature damage to avocados and these effects are reflected in the morphology and physiology of the skin tissue.

Low-temperature storage of avocado would be desirable to increase storage/shelf life and possibly as a disinfestation technique (Sanxter et al., 1994). However, low temperature storage (<6 °C) results in chilling injury (CI) of many avocado cultivars. CI of avocado often results in browning/blackening of the skin, and internally, flesh browning or graying (Vakis, 1982). Changes to avocado skin (or peel) tissue at chilling temperatures (2 °C for 21 d) have been examined in the cultivar 'Fuerte' (Pesis et al., 1994). However, these changes do not appear to have been studied in the skin of 'Hass', nor in response to heat treatments (HTs) that reduce low temperature damage.

Hot air HTs reduce CI of 'Sharwil' avocado (Sanxter et al., 1994) and Woolf et al. (1995) demonstrated that the most effective treatments for 'Hass' are 40 °C for 0.5 h, or 38 °C for 3 to 10 h. However, these treatments are relatively long, particularly when time to heat fruit to the target temperature is included (>2 h). Thus, shorter duration treatments, that are more easily achieved using hot water treatments (HWTs), may be commercially feasible.

The experiments presented here involve defining an optimum 38 °C HWT duration for reducing external CI of 'Hass' avocado during storage at 0.5 °C. The development of CI in the skin during storage was then examined in relation to this optimum treatment.

Materials and Methods

Fruit handling and hot water treatment. Export grade avocado fruit ('Hass') were obtained from a commercial orchard in Whangarei, New Zealand, where they were sorted by mass (190 to 220 g), then packed and transported to the laboratory. Fruit were further graded for freedom from blemishes, and held in a 20 °C room overnight, prior to HWT (carried out 2 d after harvest). Hot water treatments were carried out at 38 ± 0.2 °C in large water baths as described by Woolf and Lay-Yee (1997).

Duration of HWT and CI. (Expt. 1). Nonheated fruit, and fruit immersed in 38 °C water for 2.5, 5, 15, 30, 60, or 120 min were stored at 0.5 °C for 28 d. A set of control fruit was held in air at 20 °C during treatment. External blackening was rated weekly on the same fruit during, and upon removal from storage. Fruit were then ripened at 20 °C and assessed as described below. Each treatment consisted of seven fruit, and the experiment was carried out twice, using fruit from the same harvest.

External CI development. (Expt.2). The optimum treatment duration for reducing CI determined above (60 min) was applied to half of each fruit by laying fruit sideways on plastic mesh so that one longitudinal half of each fruit was submerged in the same 38 °C waterbaths described above, while the other half was exposed to rapidly circulated air at 20 °C (Woolf and Laing, 1996). Three replications

of seven half-treated fruit were then either ripened at 20 °C without storage, or stored up to 21 d at 0.5 °C, then ripened at 20 °C. Fruit not stored were sampled to determine electrolyte leakage on day 3, 5, and 7, and were ripe by day 7. Similarly, the group of half-treated fruit stored at 0.5 °C were sampled in storage at 4, 7, 11, 14, 17, and 21 d, and 3, 5, and 7 d after removal to 20 °C. For each treatment, four additional fruit were included for skin sectioning (see below).

In addition, to verify the whole-fruit quality results of Expt. 1, four replications of eight fruit were either completely immersed in 38 °C water, or held at 20 °C in air (relative humidity 65% ± 10%), then either ripened without storage, or after 21 d storage at 0.5 °C.

Fruit assessment. After treatment, fruit were dried with cotton towels, placed in open commercial cardboard trays in storage at 0.5 ± 0.5 °C, within 15 min of treatment. The following day, tray lids were closed, and fruit stored for up to 28 d, depending on the experiment. Upon removal from storage, external damage (browning/blackening) was rated on a relative scale of 0 to 3 (0 = no occurrence, 0.5 = <10%, 1.0 = ≥10% ≤20%, 1.5 = ≥20% ≤50%, 2 = ≥50% ≤75%, ≥2.5 = 75% ≤90%, 3.0 = >90% of the fruit surface). Fruit were then held in a 20 ± 2 °C controlled environment room (relative humidity 55% to 75%) and allowed to ripen. Fruit ripeness was determined by firmness, as assessed daily by gentle hand-squeezing by one trained assessor. When each fruit became "ripe to eat" (equivalent to a "firmometer" (Swarts, 1981) reading of ≈99), the number of days to become ripe was recorded (shelf life). The fruit was cut longitudinally into quarters, and the following factors evaluated: hard skin (hardness or brittleness of the skin determined when cutting and peeling back the skin), tissue breakdown (breakdown of emerald green flesh adjacent to the skin such that tissue adhered to the skin when peeled), body rots (rots entering through the skin), stem-end rots (rots entering only through the fruit peduncle), uneven ripening (uneven flesh softening such that flesh tissue adhered to the seed when fruit was cut in half), vascular browning (browning of the vascular strands running longitudinally through the fruit tissue), and flesh browning (browning of the fruit tissue not due to disease or vascular browning). Each factor was rated on a scale of 0 to 3 where 0 = no occurrence; 1 = slight; 2 = a level at which the consumer would notice, and possibly reject the fruit; 3 = severe.

Electrolyte leakage. Electrolyte leakage from avocado skin (exocarp) disks was determined by removing tissue plugs with a 12 mm diameter cork borer, and excising the exocarp 0.5 mm above the exocarp/mesocarp interface. Ten disks per treatment were rinsed and incubated in 10 mL of 0.4 M mannitol at 30 °C in a shaking waterbath, and conductivity measured (model CG875; Schott-Gerate Conductivity Meter, Hofheim, West Germany) after 3 h. Data presented are the average of three replications. Percent conductivity was calculated as final conductivity divided by the total

conductivity (measured after freezing, thawing, and boiling the disks for 15 min).

Skin microscopy. Representative fruit were selected for microscopy immediately after treatment, and at weekly intervals during storage. Cross-sections of the exocarp were made by hand. Photographs of representative sections were taken less than 15 min after treatment or removal from storage.

Statistics. Each response was analyzed using ordinal logistic regression (McCullagh and Nelder). Different location parameters were calculated for each of the two treatments, and compared using a *t* test. Any differences detected between treatment distributions were for $P \le 0.05$.

Results

Duration of HWT and CI (Expt. 1)

External damage. Nonheated fruit had developed external damage (blackened regions) after 14 d of storage at 0.5 °C and it increased in size and intensity to an average rating of 1.8 during 28 d of storage (Fig. 1). Some pitting of the epidermis developed around the lenticels. After removal from storage, the blackened areas tended to turn brown or "bronzed," and did not markedly increase in size. Although short durations of 38 °C HWT (2.5 and 5 min) had little effect on external damage, a duration of 15 min and longer reduced damage to <1 ("slight") by 28 d (Fig. 1). A HWT of 120 min was the most effective treatment, but both 60 and 120 min maintained damage levels below 0.5.

Fruit quality. After 28 d at 0.5 °C and during subsequent ripening at 20 °C, a range of disorders developed. Nonheated fruit exhibited severe (≥2) tissue breakdown, body and stem end rots, moderate (1 to 2) vascular browning, and slight (≤1) uneven ripening, flesh browning, and hard skin (Table 1). Shorter duration 38 °C HWTs had little effect on fruit quality, while longer durations reduced the severity of many disorders. A HWT of 60 min most effectively maintained fruit quality following storage, and resulted in lower damage levels than either 30- or 120-min treatments. The 60 min HWT significantly reduced tissue breakdown, vascular browning and uneven ripening compared to control fruit (Table 1).

Time to ripen at 20 °C (shelf life) was slightly delayed by HWT. Nonheated fruit took 10.0 ± 0.3 d to ripen, 60 min hot water treated fruit 10.9 ± 0.6 d, and 120 min treated fruit 11.2 ± 0.9 d.

External CI development (Expt. 2)

Skin morphology. Low-power microscopy of skin (exocarp) sections of nonheated fruit revealed that as storage time increased, slight browning of the base of the exocarp (at the exocarp–mesocarp interface), became apparent after 7 d (Fig. 2E) and increased by 14 d (see arrow of Fig. 2G). Browning advanced toward the exocarp surface and first reached the epidermis at the lenticels (see arrow of Fig. 2I). Immediately after treatment, the exocarp
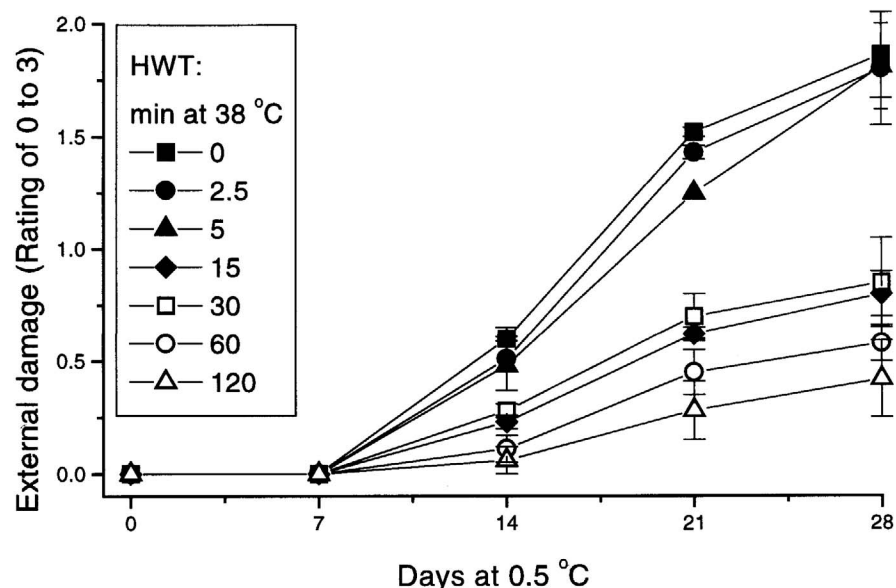

Fig. 1. Effect of hot water treatment (HWT; 38 °C for 0 to 120 min) on external damage (rating scale; 0 = no occurrence, 3 = damage of >90% of the surface) of 'Hass' fruit. Fruit were stored for 28 d at 0.5 °C following HWT (Expt. 1). Vertical bars represent SEM values.

Table 1. Quality of 'Hass' avocado fruit following hot water treatment (HWT; 38 °C for 0 to 120 min) and subsequent storage at 0.5 °C for 28 d, and finally ripening at 20 °C (Expt. 1).

HWT duration (min at 38 °C)	Fruit quality factor[z]						
	Tissue breakdown	Body rots	Stem end rots	Vascular browning	Uneven ripening	Flesh browning	Hard skin
0	2.4[y]	2.3	1.9	1.7	1.0	1.0	0.8
2.5	2.6	2.9	1.7	1.3	1.1	1.4	1.1
5	2.5	2.5	2.0	1.0	0.9	0.6	1.0
15	2.7	2.8	2.4	1.6	1.6	1.0	0.9
30	2.2	2.3	1.8	1.5	0.7	1.3	1.1
60	1.1	1.5	1.4	0.7	0.2	0.8	0.3
120	1.3	2.1	1.9	1.2	0.8	1.1	0.8
Test of significance[y]	*	NS	NS	*	*	NS	NS

[z]Fruit quality factors were rated on a scale of 0 (no occurrence) to 3 (severe) and data are presented as average severity.
[y]Tests of significance for pair-wise comparison of control (0 min), and 60 min HWT at 38 °C are presented.
[NS, *]Nonsignificant or significant at $P \le 0.05$, respectively

colors of control (Fig. 2A) and hot water-treated fruit (Fig. 2B) were similar, but with increased storage, browning remained absent in heated exocarp (Figs. 2 F, H, and J). Exocarp of fruit ripened without storage developed a purple pigmentation, with no apparent difference between nonheated (Fig. 2C) and heated fruit (Fig. 2D). However, following storage, the entire exocarp of nonheated fruit became "corky" (Fig. 2K). Exocarp of stored, heated fruit (Fig. 2L) ripened in the same manner as nonheated fruit without storage (Fig. 2C).

External damage. The fruit half treated at 38 °C for 60 min was undamaged after 21 d at 0.5 °C (Fig. 2M, right half), whereas the left half of the fruit (nonheated) exhibited severe external damage. External damage was not obvious after 7 d of storage, but became evident after 14 d and increased in intensity up to 21 d.

Fruit quality. When control fruit were ripened without storage the main disorders were tissue breakdown and body and stem end rots, while other disorders were slight (Table 2). Hot water treatment of 38 °C for 60 min significantly reduced the severity of tissue

breakdown, while all other disorders were slight (<1), thus indicating acceptable fruit quality. However, following ripening after 21 d at 0.5 °C, external damage, tissue breakdown, hard skin and body and stem-end rots were severe in control fruit (Table 2). Hot water-treated fruit had significantly lower levels of most disorders, although stem-end rots were not significantly reduced and body rots remained at moderate levels. All other disorders were slight.

Days to ripen of nonstored fruit were similar for control and hot water treatments, while after storage, HWT slightly delayed ripening (6.8 ± 0.2 d vs. 8.3 ± 0.2 d).

Electrolyte leakage. As fruit ripened at 20 °C without cold storage, electrolyte leakage of nonheated exocarp tissue (expressed as conductance), increased slightly, from 7% to 10% over the first 3 d, after which leakage increased markedly to 30% when fruit were ripe at 7 d (Fig. 3). Electrolyte leakage of nonheated skin tissue measured during storage increased with time in cold store from 7% at time zero, to 34% after 21 d (Fig. 3). In contrast, leakage of the skin of hot water-treated fruit increased only slightly (7% at time zero, to 12% after 21 d).
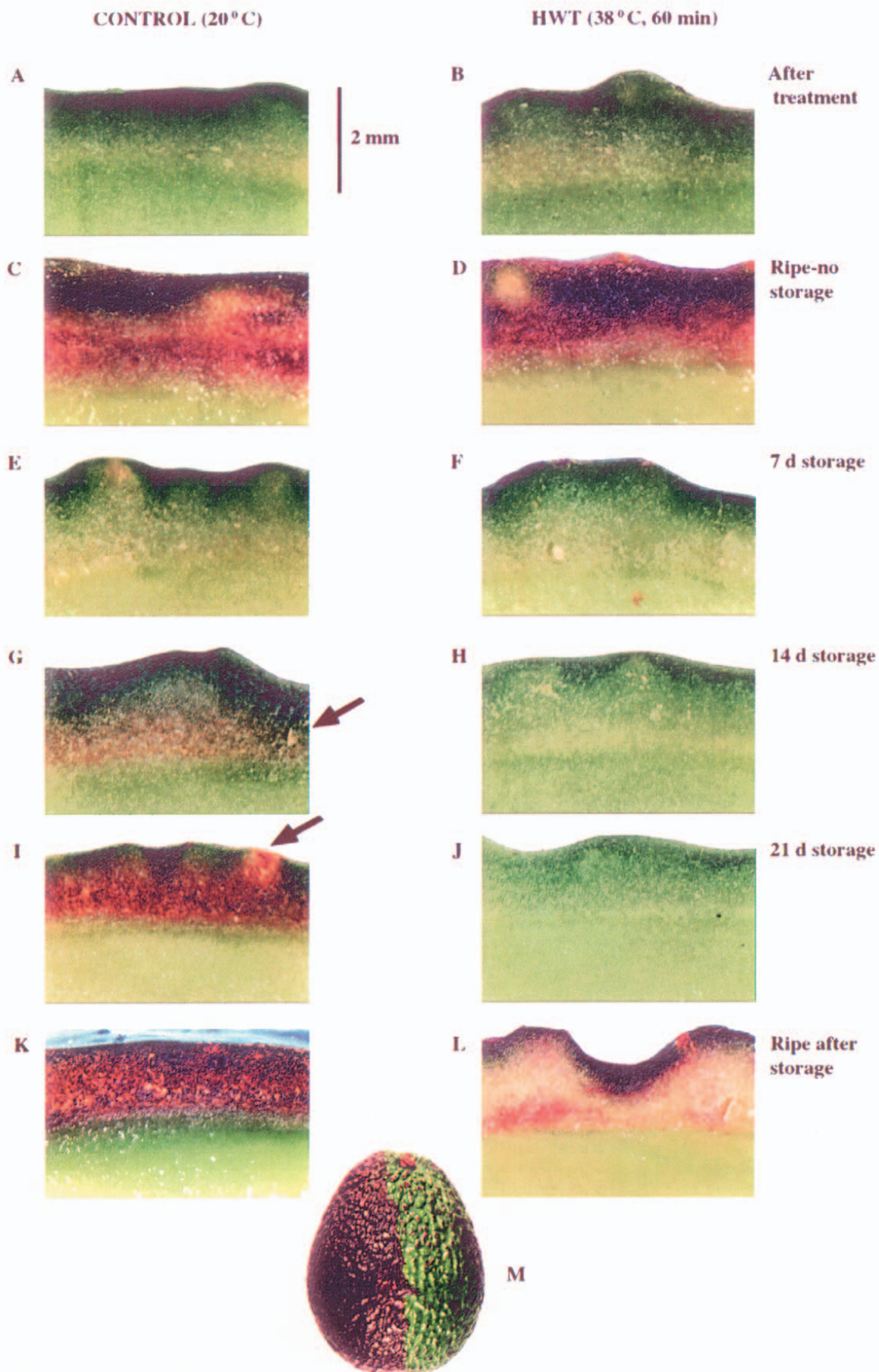
Fig. 2. Cross section of skin of nonheated fruit (**left column**), and hot-water treated fruit (**right column**). Photographs were taken immediately following treatment (**A** and **B**), after ripening without storage (**C** and **D**), after storage at 0.5 °C for up to 21 d (**E** to **J**), or ripened after storage (**K** and **L**). Whole fruit (**M**): Effect of hot water treatment (38 °C for 60 min) on half of an avocado fruit, and subsequently storing the fruit for 21 d at 0.5 °C. The left, nonheated half exhibits severe chilling injury, while the right half (heated), is green and continued to ripen to a natural purple/black color (Expt. 2).

Table 2. Quality of 'Hass' avocado fruit following either control (20 °C) or hot water treatment (HWT; 38 °C for 60 min). Fruit were either ripened at 20 °C without storage, or ripened following storage at 0.5 °C for 21 d (Expt. 2).

	Severity rating[z] (means and significance[y])					
	Duration of storage (days)					
	0			21		
	Heat treatment					
Fruit quality factor	–	+		–	+	
External damage	0.0	0.0	NS	2.3	0.3	*
Tissue breakdown	1.4	0.8	*	2.7	0.9	*
Body rots	1.2	0.8	NS	2.4	1.7	*
Stem end rots	0.6	0.3	NS	2.4	2.4	NS
Vascular browning	0.1	0.0	NS	0.7	0.8	NS
Uneven ripening	0.0	0.0	NS	1.3	0.1	*
Flesh browning	0.0	0.0	NS	0.7	0.4	NS
Hard skin	0.0	0.0	NS	2.2	0.6	*

[z]Fruit quality factors were rated on a scale of 0 (no occurrence) to 3 (severe) and data are presented as average severity.

[y]Tests of significance for pair-wise comparison of control (20 °C), and 60 min HWT at 38 °C are presented. [NS,] [*]Nonsignificant or significant at $P \leq 0.05$, respectively
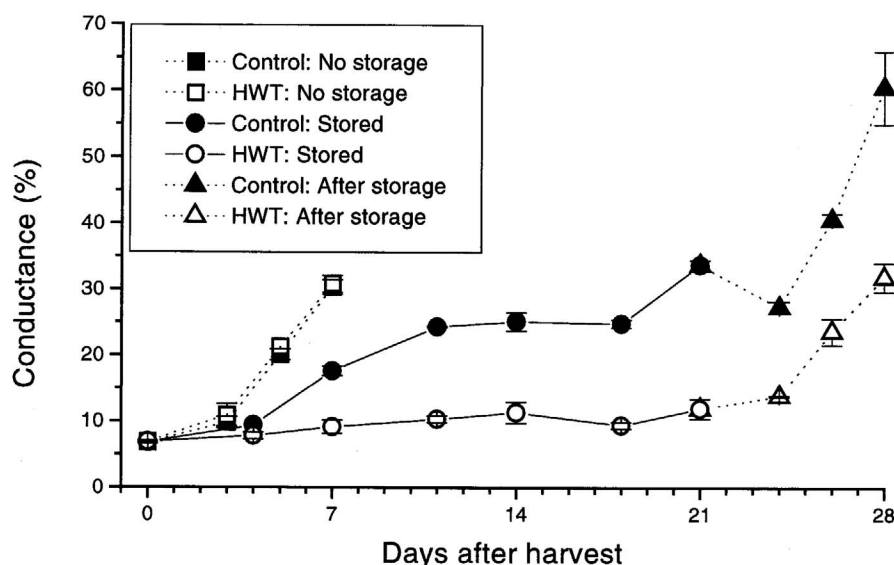

Fig. 3. Electrolyte leakage of skin disks for hot water treated (38 °C for 60 min) or control (20 °C) halves of fruit. Fruit were either ripened at 20 °C without storage, sampled during storage at 0.5 °C, or ripened at 20 °C after 21 d storage at 0.5 °C (Expt. 2). Vertical bars represent SEM values.

After removal from 21 d storage, electrolyte leakage of control fruit skin first showed a slight decrease, then increased to 61% when ripe, 7 d later (Fig. 3). However, for skin of stored hot water-treated fruit, leakage increased in a pattern nearly identical to that of fruit not stored, with a conductance at 32% when ripe.

Discussion

CI development. The increased CI of avocado with longer storage time at 0.5 °C is consistent with observations of Fuchs et al. (1989) and Vakis (1982) who examined 'Hass' stored at 0 °C and 2.2 °C, respectively. In our work, external CI of intact fruit did not become visible until more than 7 d at 0.5 °C. However, internal browning commenced before this time at the base of the exocarp, and moved upwards to the epidermis. Browning development moves most rapidly to the lenticels (present at the most raised portions of the exocarp); thus, lenticel damage is one of the earlier externally visible symptoms of CI.

At removal from 28 d of storage, the fruit appear black/green because the epidermis is mostly undamaged, while the exocarp below the epidermis is brown. However, during ripening at ambient temperatures, the entire exocarp (including the epidermis) dies and the fruit therefore, takes on a brown or "bronzed" appearance, rather than the purple/black of normal ripening. This process may explain the observations of Vakis (1982), who found that skin discoloration increased following removal of fruit from storage. Such skin damage appears "corky" in nature in ripe fruit, and results in the increased hardening of the skin, as noted when fruit are cut (hard skin).

Electrolyte leakage increased in nonheated skin tissue exposed to chilling temperatures. The increased browning in skin tissue was correlated with the increase in electrolyte leakage of nonheated fruit ($R = 0.81$). This change is similar to that found in skin tissue of 'Fuerte' under a similar storage regime (Pesis et al., 1994). Thus, although Fuchs et al. (1989) observed a poor correlation between electrolyte leakage and CI of the mesocarp of 'Hass' avocados, leakage of exocarp tissue appears to

reflect the level of external CI symptoms (blackening or browning) of the fruit. Increased leakage may result from breakdown in cellular compartmentation, thus mixing substrate and browning enzymes such as polyphenoloxidase or peroxidase (Vamos-Vigyazo, 1981) with browning as a consequence.

Hot water treatments. A hot water treatment of 60 min at 38 °C was the most effective treatment for reducing the levels of external and internal damage factors following storage at 0.5 °C (the dramatic effect of this treatment on skin browning is demonstrated in Fig. 2M). The efficacy of HWTs observed here is supported by results in citrus (*Citrus sinensis*, L.; Wild and Hood, 1989), cucumber (*Cucumis sativus* L.; McCollum and McDonald, 1993), and persimmon (*Diospyros kaki* L.; Lay-Yee et al., 1997), and parallels that for 'Hass' avocados in response to 38 °C hot air treatments (Woolf et al., 1995). The decrease in external damage visible after storage was supported by reduced browning of skin tissue observed under low-power microscopy, and a concomitant reduction of leakage levels during storage. The low severity of hard skin and pattern of electrolyte leakage after storage for 21 d at 0.5 °C suggest that the skin of hot water-treated fruit remained healthy and ripened normally. The amelioration of CI symptoms and electrolyte leakage in 'Hass' skin tissue by HWTs I observed is similar to the effect of anaerobic shock treatments on 'Fuerte' skin tissue (Pesis et al., 1994).

As found with hot air HTs (Woolf et al., 1995), HWTs also reduced internal damage associated with cold storage at chilling temperatures. Many disorders were reduced to low levels, such as vascular and flesh browning, and tissue breakdown. However, body and stem-end rot severities remained unacceptably high, suggesting that use of a fungicide may be beneficial if HWTs are used commercially. Similarly, uneven ripening was reduced to only moderate levels in Expt. 2, suggesting that slightly higher storage temperatures may be more favorable. The slight extension of shelf life by the 60 min HWT ($\approx$1 d), is similar to that found in response to hot air HTs (Woolf et al., 1995).

In conclusion, a HWT of 38 °C for 60 min confers significant tolerance to chilling at 0.5 °C in 'Hass' avocados. However, further research to examine the optimal storage temperature, and possible postharvest treatments to reduce rots is required.

Literature Cited

Fuchs, Y., G. Zauberman, I. Rot, and A. Weksler. 1989. Chilling injury and electrolyte leakage in cold stored mango and avocado fruits. Acta Hort. 258:303–308.

Lay-Yee, M., S. Ball, S.K. Forbes, and A.B. Woolf. 1997. Hot water treatment for insect disinfestation and reduction of chilling sensitivity of 'Fuyu' persimmon. Postharvest Biol. Technol. 10:81–87.

McCollum, T.G. and R.E. McDonald. 1993. Tolerance of cucumber fruit to immersion in heated water and subsequent effects on chilling tolerance. Acta Hort. 343:233–237.

McCullagh, P. and J.A. Nelder. 1989. Generalized linear models. 2nd ed. Chapman and Hill, London.

Pesis, E., R. Marinansky, G. Zauberman, and Y. Fuchs. 1994. Prestorage low-oxygen atmosphere treatment reduces chilling injury symptoms in 'Fuerte' avocado fruit. HortScience 29:1042–1046.

Sanxter, S.S., K.A. Nishijima, and H.T. Chan, Jr. 1994. Heat-treating 'Sharwil' avocado for cold tolerance in quarantine cold treatments. HortScience 29:1166–1168.

Swarts, D.H. 1981. The Firmometer—For monitoring ripening of avocados (and other fruit). S. African Avocado Growers Assn. 4:42–46.

Vakis, N.J. 1982. Storage behaviour of Ettinger, Fuerte and Hass avocados grown on Mexican rootstock in Cyprus. J. Hort. Sci. 57:221–226.

Vamos-Vigyazo, L. 1981. Polyphenol oxidase and peroxidase in fruits and vegetables. CRC Crit. Rev. Food Sci. Nutr. 6:49–127.

Wild, B.L. and C.W. Hood. 1989. Hot dip treatments reduce chilling injury in long-term storage of 'Valencia' oranges. HortScience 24:109–110.

Woolf, A.B. and A.L. Laing. 1996. Avocado fruit skin fluorescence following hot water treatments and pretreatments. J. Amer. Soc. Hort. Sci. 121:147–151.

Woolf, A.B., C.B. Watkins, J.H. Bowen, M. Lay-Yee, J.H. Maindonald, and I.B. Ferguson. 1995. Reduction of external chilling injury in stored 'Hass' avocado fruit by dry heat treatments. J. Amer. Soc. Hort. Sci. 120:1050–1056.

Woolf, A.B. and M. Lay-Yee. 1997. Pretreatments at 38 °C of 'Hass' Avocado Confer Thermotolerance to 50 °C Hot Water Treatments. HortScience 32:705–708.