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ABSTRACT
Heat treatment shows promise for maintaining avocado fruit quality during storage, and as a non-chemical disinfection technique. We have found that a range of both hot-air and hot-water heat treatments can reduce chilling injury of avocados stored at low temperatures. We have examined the response of Hass avocado fruit (Persea americana Mill.) to a range of hot-air treatments (25 °C to 46 °C for 0.5 to 24 h and stored at 0, 2 or 6 °C), and to hot-water treatments (38 °C for 5 to 120 mins and stored at 0 °C). Hot-air heat treatments of 38 °C for 3, 6, or 10 h and 40 °C for 0.5 h were the most effective treatments for reducing external chilling injury induced by storage at 2 °C. Following a heat treatment of 38 °C for 6 h, a delayed time into storage after heat treatment of 2 days resulted in some loss of effectiveness of the heat treatment to reduce chilling injury. Longer durations of hot-water treatment (HWT) progressively reduced chilling injury with 60 min being the optimal duration which almost eliminated chilling injury, and maintained fruit quality at the highest level. Analysis of the expression of heat shock proteins (hsp's) and their genes suggests that reduction of chilling injury may be related to induction of these proteins by heat treatments.

INTRODUCTION
Storage of avocados at low temperatures (0-2 °C) could provide a number of commercial benefits such as reduced ripening rate (increased shelf life), and as a disinfection technique for certain insects (Sanxter et al., 1994). However, storage temperatures lower than 4-6 °C tend to result in chilling injury of many avocado cultivars.

Heat treatment of fruit, typically at 34-50 °C, has been shown to reduce chilling injury of a range of fruits including mango (McCollum et al., 1993), tomato (Lurie & Klein, 1991) and orange (Wild & Hood, 1989). In addition, heat treatments (both hot air and hot water) are employed commercially in a number of countries as a disinfection technique (e.g. papaya in Hawaii and the Cook Islands, and mango in Australia and South Africa).

Compared with the amount of research carried out into the general fruit response to heat treatments (e.g. overall acceptability, browning etc.), relatively little work has been
carried out into the physiological and biochemical nature of fruit response to heat treatments. The mechanism(s) by which chilling injury is reduced by heat treatments is not known, although a possible role for heat shock proteins has been proposed (Lurie & Klein, 1991; Collins et al., 1995). Heat shock proteins (hsp) are proteins whose synthesis is characteristically increased by heat treatments, while the synthesis of the majority of other proteins is markedly reduced by such a treatment. The expression of hsp genes (as measured by RNA levels), has been shown to increase following heat treatments. A greater understanding of these mechanisms is important as it can assist us with modification of the heat treatment process and prediction of fruit responses to such changes.

At the Mt. Albert Research Centre of Horticultural Research, we have been examining both the practical implications of hot-air and hot-water heat treatments, and possible mechanisms which may be involved in these responses.

HOT-AIR HEAT TREATMENTS

Methods

Heat treatment systems. Fruit temperature was measured by inserting a thermistor temperature probe (CM-UU-V5-1; Grant Inc, Cambridge, UK) into the stem-end of the fruit to the seed surface. Heat treatment duration was defined as the time at which the internal fruit temperature reached the target temperature. For simultaneous heat treatments at a range of temperatures, six small identical chambers were employed as described by Woolf et al., (1995). Larger-scale treatments at one temperature were carried out in the High Air Flow Controlled Atmosphere and Temperature (HAFCAT) facility as described by Dentener et al., (1996).

Experiments

A range of experiments have been carried out. The first three (to define an optimum heat treatment) involved heat treatments at 25, 34, 38 or 42 °C for 6 or 24 h, prior to stored at either 0 or 6 °C for 3 weeks. Two further experiments were carried out at 34, 36, 38, 40 or 42 °C for durations of 3, 6, 10 or 24 h, and at 40, 42, 44 or 46 °C for durations of 0, 1, 3 or 6 h, followed by storage at 2 °C for 4, 5 weeks (Woolf et al., 1995). A larger-scale heat treatment of 38 °C for 6 h was carried out and fruit stored for up to 5 weeks at 0 °C (Ball et al., in preparation). In this experiment, fruit were also placed into storage at 3 weeks at 0 °C immediately after heat treatment or with delays after heat treatment of up to 4 days, during which time fruit were held at 20 °C.

Fruit quality measurements. Fruit quality was assessed as described by Woolf et al., (1995) where external injury (blackening of the skin surface) was rated on a relative scale of 0 to 3 (0 = no damage, 3 = blackening of > 90 % of the fruit surface) immediately after removal from storage. A range of other quality factors were evaluated after ripening at 20 °C and rated on a scale of 0 to 3 (0 = no occurrence; 1 = slight; 2 = a level to which the consumer would notice and possibly reject the fruit; 3 = extreme).
Results and discussion

Lower temperature heat treatments (34-36 °C) resulted in some reduction of chilling injury, but heat treatments of 38 °C for 3, 6 or 10 h, and 40 °C for 0.5 h were the most effective treatments which minimized external damage, increased shelf life compared to non-heated stored controls, and resulted in levels of sound fruit equivalent to those of fruit stored under standard industry conditions (6 °C; Woolf et al., 1995). Heat treatments at higher temperatures and longer durations resulted in heat damage to the fruit surface (browning similar to that observed with chilling injury).

One of these heat treatments (38 °C for 6 h) was used and fruit subsequently stored at 0.5 °C for up to 5 weeks (Ball et al., in preparation). External damage (chilling injury) was maintained at low levels (< 1) while control fruit exhibited high levels of damage (average severity rating of 2.9). However, this treatment could not maintain adequate fruit quality after 5 weeks storage at 0.5 °C. Sanxter et al., (1994) have also reported beneficial effects of air heat treatments on quality of Sharwil avocados stored at below 6 °C. Also, as found in this work, heat treatment did not completely alleviate the detrimental effects of chilling injury on fruit quality.

On a commercial basis, a shorter duration heat treatment would be advantageous in maximizing the volume of fruit treated. Although a wide range of heat treatment temperatures and durations have been examined in this work, it is likely that a heat treatment shorter than 38 °C for 6 h could be used (e.g. 38 °C for 3 h, or possibly, 39 °C for some duration shorter than 3 h). In addition to the heat treatment conditions, the subsequent storage temperature is clearly an important variable. Here we have examined 0.5 °C and 2 °C and observed large differences in fruit response. It is likely that a storage temperature of 1 to 1.5 °C would result in significant improvements in the quality of heat treated-fruit relative to that of 0.5 °C. However, this should be balanced against the temperatures and times required for low temperature disinfestation of key insect pests.

Delaying the timing of placing fruit into storage after heat treatment influenced the levels of chilling injury observed after 3 weeks storage at 0.5 °C. Heat-treated fruit placed directly into cool storage for 3 weeks exhibited low levels of damage (0.2), but after a delay of 1 day, damage increased 500%. However, even after 4 days delay, the level of external injury found on heat treated fruit was not as great as that of non-heated fruit placed directly into storage (1.5 cf 1.8, respectively). This loss of benefit of heat treatment on chilling injury has important implications in commercial terms, suggesting that fruit should be placed into storage as soon as is practicable following heat treatment, although the effect of delay durations of less than 24 h are yet to be examined.

HOT-WATER HEAT TREATMENTS

Methods

Water baths. Hot-water treatments (HWTs) were carried out in large experimental water baths (volume 82 ℓ) as described by Woolf & Lay-Yee (1996). Fruit were hot-water-treated at 38 °C for up to 120 min in plastic netting baskets which were weighted to
submerge fruit during treatment. Fruit were then dried and immediately placed in storage for up to 4 weeks at 0 to 0.5 °C.

In a second experiment, the optimum treatment duration for reducing chilling injury (60 min) was then applied to half of each fruit by laying fruit sideways on plastic mesh so that one longitudinal half of each fruit was submerged in 38 °C water, while the other half was exposed to highly circulated air at 20 °C as described by Woolf & Laing (1995).

Results and discussion

As the duration of time in storage increased, the level of external chilling injury in non-heated fruit increased. No damage was evident following 1 week in storage, but by 4 weeks there was an average severity of 1.8.

Skin sections examined under a low-powered microscope showed slight browning of the skin tissue evident at the skin/flesh interface after 7 days, and as time in storage increased, the level of browning intensified and moved up to the skin surface. This pattern of development explains why chilling injury is not evident from the outside of the fruit after short storage periods, but becomes evident with longer storage durations.

Progressively longer HWTs reduced external damage evident after 28 days storage at 0 °C. Durations less than 15 min decreased external damage slightly, while HWTs of 15 min or longer all reduced damage to less than 1 (‘slight’ on a scale of 0 to 3). Hot-water treatments of 60 and 120 min were the most effective treatments, maintaining damage levels below 0.5 (Woolf et al., 1996).

Hot-water treatments also reduced the levels of uneven ripening and vascular and flesh browning to low levels (< 1). Although the levels of body and stem end rots were reduced, these remained at moderate levels (~1.5), suggesting that some further means of disease control may be required if adequate quality is to be maintained after 4 weeks storage.

The HWT which was most effective as a means of reducing external damage and maintained the highest fruit quality was the 60 min treatment. This HWT also increased shelf life by about 1 day.

The effectiveness of a 38 °C HWT of 60 min as a means of reducing chilling injury is clearly demonstrated in figure 1 where the right half of the fruit is undamaged after 21 days at 0 °C, whereas the left half of the fruit exhibits extreme external damage. External damage was not obvious after 1 week in storage, but became evident after 2 weeks and increased in intensity up to 3 weeks.

In the examination of the effects of HWT, we selected a treatment temperature of 38 °C on the basis of fruit response to hot-air treatments. However, to minimize the treatment time, a slightly higher temperature (e.g. 39 or 40 °C) and/or, a shorter duration (30 to 60 mins) may be as, or possibly even more effective. Similarly, as noted previously, a slightly higher storage temperature (1 to 1.5 °C) may result in improvements to fruit quality of hot-water-treated fruit.
HEAT SHOCK PROTEINS

Methods
We examined RNA levels immediately after treatment and following storage in response to both hot-air and hot-water heat treatments. In addition, protein synthesis was studied following heat treatment and storage for fruit treated in air at 38 °C for 6 h. RNA analysis

Skin and flesh tissue were sampled and stored at -80 °C. RNA extraction and northern analysis was carried out for flesh tissue as described by Woolf et al., (1995), and skin tissue, Woolf & LayYee (1996). Northern blots were hybridized with 32P-labelled inserts from pFS1968 (soybean hsp 17 cDNA; Schoffl et al., 1984) and pMON9575 (petunia hsp70 cDNA; Winter et al., 1988).

Protein analysis
Skin disks were removed from the fruit, washed and labelled with 35S-methionine.
Protein was extracted, precipitated, equal protein cpm loaded on 13 % SDS-PAGE gels, and the resulting gel autoradiographed as described by Ball et al., (in preparation).

Results and discussion

In Hass avocado, hot-air heat treatments which are the most effective for reducing chilling injury are 38 °C for 3 to 10 h, and 40 °C for 0.5 h. In flesh tissue sampled directly after selected heat treatments, the levels of hsp17 and hsp70 mRNA increased to a maximum at 40 °C, and declined at higher temperatures. These increases in gene expression coincided with the extent to which heat treatments prevented chilling injury (Woolf et al., 1995).

For hot-water treatments, even short durations (5 min at 38 °C) increased the levels of hsp7 mRNA observed immediately after HWT, and levels further increased with longer durations to a maximum at 120 min (Woolf & Lay-Yee, 1995). This pattern of mRNA levels present immediately after HWTs also parallels the effectiveness of these treatments in reducing chilling injury (Woolf et al., 1996).

In addition to being elevated immediately after HWT, increased hsp RNA levels are maintained in fruit stored at low temperatures following heat treatments with both hot-air followed by storage at 2 °C (Woolf et al., 1995), and hot-water and storage at 0 °C (Woolf et al., 1996). The fact that hsp RNA levels are maintained in avocado tissue during storage suggests that hsp gene products (i.e. proteins) could play a role in chilling tolerance of heat treated fruit during storage.

Immediately after a hot-air heat treatment, synthesis of many proteins was elevated, and at ambient temperatures, synthesis of some of these bands decreased between 1 and 3 days after heat treatment (Woolf et al., 1996). This is typical of the response observed in other plant tissues (DeRocher et al., 1991). The decrease in protein synthesis paralleled the loss of some heat treatment-induced chilling injury protection. The continued presence of hsps (although hsp synthesis was declining) may explain why beneficial effects of heat treatment were not completely lost over the 4 days after heat treatment prior to being placed in storage (Ball et al., in preparation).

Although these results lend support to a role for hsps in chilling injury reduction, it should be noted that the mechanism(s) of chilling injury remain unknown. Thus, considering the wide range of physiological and biochemical effects of heat on plant tissues, it would be overly simplistic to suggest that hsps alone are the mechanism of chilling injury reduction by heat treatments.

CONCLUSION

These results clearly demonstrate that both hot-air and hot-water heat treatments have potential as a means of reducing chilling injury in avocado fruit during storage at low temperatures (0 to 2 °C). Storage at these temperatures also maintained shelf life (compared with fruit stored at 6 °C) and may be effective as a means of low temperature disinfestation.
REFERENCES


