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HOT AIR AND HOT WATER TREATMENTS REDUCE CHILLING INJURY OF AVOCADOS DURING STORAGE

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Abstract

Heat treatment is a promising technology for postharvest disinfestation and maintaining fruit quality during storage. We have found that a range of heat treatments (HTs) confer tolerance to low temperatures (chilling tolerance) of 'Hass' avocados.

The influence of a range of 38°C hot water treatments (5 to 120 mins) on chilling injury and heat shock protein (hsp) gene expression were examined after subsequent 4 weeks storage at 0°C. The hot water treatment (HWT) most effective for reducing chilling injury without adversely affecting fruit quality was 60 min at 38°C. Northern analysis of RNA extracted from avocado skin after the range of HWTs and storage, indicated that steady state levels of hsp17 RNA increased progressively with longer HWT durations. The reduction of chilling injury was inversely related to the increased levels of hsp17 RNA.

On the basis of our previous work, a hot air HT of 38°C for 6 h was employed to examine the effect of delaying storage after treatment (up to 4 days) on chilling injury and hsp synthesis. A delay of 2 days before coolstorage after HT resulted in some loss of effectiveness of the HT to reduce chilling injury. The synthesis of some hsps decreased from 1 day after HT.

The similarity in the reduction of chilling injury and the patterns of gene expression and protein synthesis suggest that hsps may play a role in reducing the symptoms of chilling injury in 'Hass' avocados.

1. Introduction

Chilling injury is a limitation to the long distance export of New Zealand avocados. Heat treatment is a promising method of control. In 'Hass' avocado, hot air HTs which are the most effective for reducing chilling injury are 38°C for 3 to 10 h, and 40°C for 0.5 h (Woolf et. al., 1995). Whilst there are no reports of the response of avocados to HWTs, they have proved effective in reducing chilling injury in citrus (Wild and Hood, 1989).

The mechanism(s) by which chilling injury is reduced by HTs is not known, although a possible role for hsps (proteins whose synthesis is characteristically induced by heat shock), has been proposed (Lurie and Klein, 1991; Collins et al., 1995).

Temperatures (38 and 40°C) and durations (3 to 10 h) of hot air HTs wherein hsp gene expression is highest, coincides with HTs which most effectively reduce chilling injury (Woolf et. al., 1995). Transfer of plant tissue to ambient temperatures after a HT results in the levels of RNA encoding hsps, and the levels of the hsps themselves, decreasing over time (DeRocher et al., 1991). Thus, another means of examining the possible role of hsps in chilling injury

reduction is to delay the time into coolstore and examine the levels of hsp synthesis and resulting chilling injury.

We have examined the effect of 38°C hot water treatments on chilling injury and hsp gene expression, and determined the effect of delaying time'into storage after hot air treatments on chilling injury and hsp protein synthesis.

2. Materials and methods

2.1. Hot water heat treatments

2.1.1. Heat treatment

'Hass' avocados *(Persea americana* Mill.) were hot water treated by immersing fruit in water at 38°C for a range of durations (5 to 120 mins) using water baths as described in Woolf and Lay-Yee (1995). After treatment, fruit were placed immediately (<10 min) into coolstore for 4 weeks at 0°C.

2.1.2. RNA extraction and northern analysis

When fruit were removed from storage, skin tissue was sampled and stored at -80°C. RNA extraction and northern analysis was carried out as described by Woolf and Lay-Yee (1995). Northern blots were hybridised with ^{32}P-labelled inserts from pFS1968 (soybean hspl7 cDNA; Schoffl et al., 1984).

2.2. Delayed time to coolstore after hot air heat treatments

2.2.1. Heat treatment

Hot air HT was carried out in a computer-controlled, semi-commercial unit. Internal temperature was increased over 2 h to 38°C and held for 6 h (relative humidity = 85 ± 5%). After treatment, heated and nonheated (control) fruit were placed in storage (0°C for 3 weeks) either immediately (a delay of 0 days), or after a delay of 1, 2, 3 or 4 days at 20°C.

2.2.2. Protein labelling

At the time fruit were placed into storage (0 to 4 days after HT), flesh disks were cut from immediately below the skin and 5 µl (50µCi) ^{35}S-methionine was spotted onto the disk surface. After 2 h incubation the disk was ground in liquid N_2 with 20 mg PVPP. After solubilizing with 0.5 ml l00mM Hepes pH 8 (1mM PMSF + 1mM DTT), protein was precipitated with cold acetone and resolubilized in 100 µl SDS sample buffer and boiled for 5 min. Equal amounts of labelled protein were loaded onto 10% gels for SDS-PAGE analysis and visualized by exposure of dried gels to Kodak XAR film at -80°C.

2.3. Fruit assessment

In both experiments, external chilling injury was rated directly after fruit were removed from coolstore. Fruit were then ripened at 20°C until they reached eating ripeness, as determined by finger pressure. A range of fruit quality factors were examined including body rots (rots invading through the skin) and tissue breakdown (presence of green fruit tissue adhering to the skin when peeled away from the flesh). Each factor was rated on a scale of 0 = none, 1 = slight, 2 = moderate, and 3 = severe, (Woolf et. al., 1995).

3. Results

3.1. Hot water heat treatments

On the basis of work carried out with hot air HTs where 38°C appeared to be the most effective treatment, we examined fruit response to a range of 38°C HWTs. Increasing duration at 38°C progressively reduced external chilling injury of 'Hass' avocados following storage at 0°C for 4 weeks (Table 1). Following storage, assessment of fruit when ripened at 20°C revealed that the HWT duration which minimized chilling injury and resulted in the best fruit quality was 60 min.

Previous work indicated that the levels of RNA homologous to pFS1968 (hspl7) observed immediately after HWT increase after only 5 min at 38°C, and further increase with longer durations to a maximum at 120 min (Woolf and Lay-Yee, 1995). In the present study, hspl7 homologous RNA was found to be at elevated levels in fruit hot water treated for 15 min or longer after storage for as long as 4 weeks at 0°C (Fig. 1).

3.2. Delayed time to coolstore after hot air heat treatments

The levels of chilling injury observed after 3 weeks storage, in fruit placed immediately into coolstore after HT, was low (0. 15). However, if fruit were placed into coolstore 1 and 2 days after HT, chilling injury ratings increased to 0.9 and 1.1, respectively. These levels were, however, still lower than nonheated control fruit which exhibited levels of 1.8, 2 and 2.1 when placed into coolstore 0, 1 and 2 days, respectively, after the time of HT.

Immediately after HT, synthesis of many proteins was elevated (Fig. 2A). However, at ambient temperatures, synthesis of some of these bands decreased between 1 and 3 days after HT (Fig. 2B).

4. Discussion

These results clearly demonstrate that both hot air and hot water HTs have potential as a means of reducing chilling injury in avocado fruit during storage at 0°C. This is consistent with results found in response to hot air HTs of 'Hass' stored at 2°C (Woolf et. al., 1995). As found with the response of many fruit crops to HTs, the range of effective treatments is small with lower temperatures and/or shorter durations failing to induce the beneficial effect (reduced chilling injury in this case), while higher temperatures and/or longer durations induce damage and reduce fruit quality.

Woolf et. al. (1995) demonstrated a correlation between increased hsp expression and reduction in susceptibility to chilling injury. Similarly, in response, HWTs at 38°C, the progressive reduction of susceptibility to chilling injury increased HWT duration parallels the increase in levels of RNA homologous to hsp present immediately after HWT (Woolf and Lay-Yee, 1995) and at the end of the storage period (Table 1 versus Fig. 1).

Increased hsp RNA levels are maintained if fruit is stored at low temperatures following HTs with both hot air (Woolf et. al., 1995), and hot water (Fig. 1). At ambient temperatures, hsp RNA levels decrease rapidly after HT of pea tissue (DeRocher et al., 1991). The fact that hsp homologous RNA levels are maintained in avocado tissue during storage invites the speculation that hsp gene products may play a role in protecting tissue from chilling injury damage during storage.

When heat treated fruit are not placed immediately into storage, synthesis of specific hsps which are high immediately following HT (Fig. 2A), decreases over time (Fig. 2B). This is paralleled by a loss of some HT-induced chilling injury protection. The continued presence of hsps (even with declining hsp synthesis) may explain why the beneficial effects of HT were not completely lost over the 4 days after HT prior to being placed in storage. For example in pea, hsp protein levels were still detectable 5 days after HT (DeRocher et al., 1991).

In summary, it is clear that both hot air and hot water heat treatments reduce chilling injury in 'Hass' avocados, and concomitantly increase hsp RNA levels, and hsp synthesis. It is worth investigating whether the hsps themselves may play a role in this response.

References

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Table 1- Quality factors of 'Hass' avocados after 4 weeks storage at 0°C with a range of durations of 38°C water treatments. Values are the mean levels of chilling injury (external browning evident immediately after storage) and body rots and tissue breakdown (examined when fruit were ripe). SE values are in subscript.

	Duration of HWT (mins at 38°C)					
Quality factor	0	5	15	30	60	120
Chilling injury	$2.1_{0.2}$	$2.0_{0.1}$	$0.9_{0.2}$	$0.7_{0.2}$	$0.5_{0.1}$	$0.3_{0.1}$
Body rots	$3.0_{0.0}$	$3.0_{0.0}$	$2.8_{0.2}$	$2.8_{0.2}$	$1.8_{0.4}$	$1.8_{0.3}$
Tissue breakdown	$2.4_{0.3}$	$2.9_{0.1}$	$2.8_{0.2}$	$2.4_{0.4}$	$1.2_{0.4}$	$2.1_{0.3}$

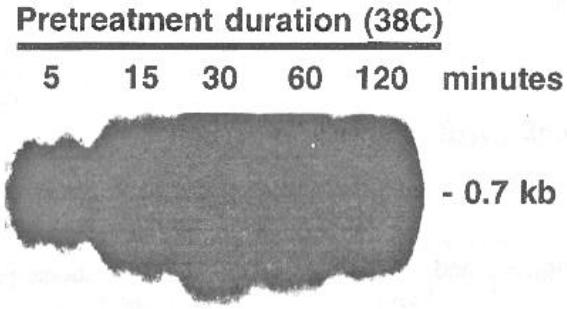

Fig. 1. Northern analysis of RNA extracted from 'Hass' avocado skin after a range of durations of 38°C water treatment and storage at 0°C for 4 weeks. Northern blots were probed with the ^{32}P-labelled insert of pFS1968 (soybean hsp17 cDNA).

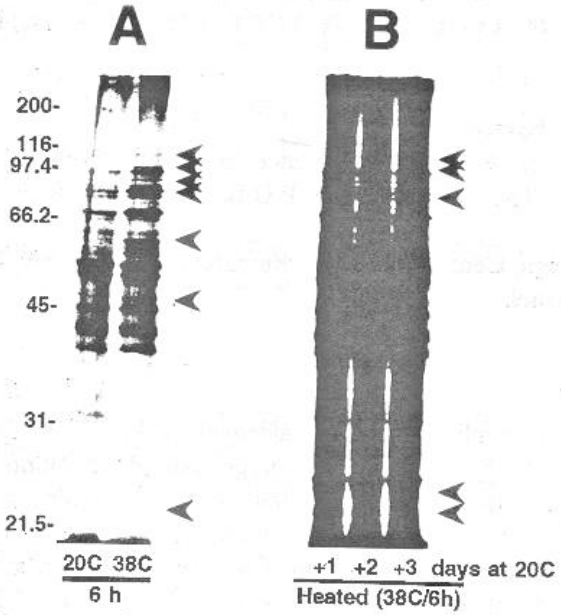

Fig. 2. SDS-PAGE gel of ^{35}S-methionine labelled protein extracted from avocado flesh disks. Labelling was carried out immediately after the HT period (**A**), or, for heated tissue; 1, 2 and 3 days after HT (**B**). Arrows indicate bands of interest.