Enhanced cold-tolerance in pineapple (*Ananas comosus* 'Mauritius') by combined cold- and heat-shock treatments or intermittent warming

By H. L. D. WEERAHEWA* and N. K. B. ADIKARAM¹

Department of Botany, The Open University of Sri Lanka, Nawala, Nugegoda, Sri Lanka ¹Department of Botany, University of Peradeniya, Peradeniya, Sri Lanka (e-mail: weerahewa@gmail.com) (Accepted 8 October 2010)

SUMMARY

Internal browning (IB) is a physiological disorder that develops in harvested pineapple (*Ananas comosus*) 'Mauritius' fruit during prolonged periods of cold storage. A brief exposure of fruit to low temperature (i.e., 4°C for 60 min) prior to cold storage at 10°C reduced the incidence of IB by 40% in both core and flesh tissues. When subjected to 4°C for 60 min before or after a heat-shock (38°C for 60 min), pineapple fruit developed 88% and 40% less IB in the flesh and core areas, respectively, than in control fruit which were not subjected to 4°C for 60 min. Intermittent warming (IW) involving the exposure of pineapple fruit to 28° – 30°C for 8 h every 6 d during 21 d of storage at 10°C and 85% RH, reduced the incidence of IB by \geq 80% in the core, and by \geq 50% in flesh tissue. Fruit subjected to IW showed only isolated areas of IB in their flesh tissue, and this was found in only 40% of the total fruit reated. Heat-shock (38°C for 60 min) before or after a low temperature (i.e., 4°C for 60 min) treatment, slowed fruit ripening slightly, but this effect was not observed in fruit subjected to IW. Cell damage was less in fruit tissues showing no symptoms of IB when fruit were given a heat-shock treatment, before or after a low temperature treatment.

Internal browning (IB) is a physiological disorder of pineapple, also known as "black heart" or "endogenous brown spot". Pineapple is a chillingsensitive fruit. IB develops when harvested fruit are exposed to low temperatures (8° – 15°C) during storage or transport, or when developing fruit are exposed to cool Winter periods in the field (Wills *et al.*, 1985). Characteristic symptoms of IB are the formation of translucent, water-soaked spots at the base of the fruitlets, which later turn brown. In severe cases, these brown areas turn black and spread to neighbouring tissue (Wills *et al.*, 1985). Internal browning is a major problem for the export of fresh fruit from Sri Lanka *via* sea freight.

Various attempts have been made to control IB; however, complete control has not been possible. Methods have included the waxing of fruit, which restricts the availability of oxygen (Rohrbach and Paull, 1982), post-harvest heat-shock treatment (Weerahewa and Adikaram, 2005), modified atmosphere storage (Abdullah *et al.*, 1985), treatment with 1-methyl cyclopropene (1-MCP; Selvarajah *et al.*, 2001), or pre-harvest application of calcium (Hewajulige *et al.*, 2003).

Low temperature (LT)-conditioning (i.e., exposing a commodity to temperatures slightly above the critical chilling range) can increase their tolerance to later chilling (Wang, 1994). A combination of hot water and LT-conditioning treatments synergistically reduced the development of chilling injury (CI) in grapefruit (*Citrus paradisi* 'Star Ruby'; Sapitnitskaya *et al.*, 2006) and plum

fruit (Sun *et al.*, 2010) during later cold storage at 2°C. Pre-conditioning of fruit prior to heat treatment was examined as a means to reduce CI in avocado (Woolf *et al.*, 2003; Sanxter *et al.*, 1994), loquat (Cai *et al.*, 2006), and mango (Joyce and Shorter, 1994; Zhao *et al.*, 2006) fruit.

Intermittent warming (IW), the interruption of low temperature storage with one or more periods of higher temperature, has also been examined as a means to reduce CI in many fruit (Wang, 1994). Intermittent warming has been used successfully in commercial lemon fruit production in Israel. Warming lemons to 13°C for 7 d every 21 d during cold storage at 2°C reduced the incidence of CI (Cohen, 1988). When apples were subjected to IW at 20°C for 24 h every 1, 2, or 4 weeks during 0.5°C cold storage for 16 weeks, the development of scald was reduced; however, the magnitude of the effect varied among cultivars (Alwan and Watkins, 1999). Subjecting tomato fruit to four cycles of IW for 6 d at 9°C and 1 d at 20°C prevented CI and decay, enhanced the surface colour, but increased the loss of firmness, delayed shrivelling, and resulted in the lowest losses of marketable fruit at the end of storage and at the post-storage ripening stage (Artes and Escriche, 1994). Intermittent warming delayed the onset of CI by approx. 10 weeks, and greatly enhanced resistance to the development of CI in orange (Schirra and Cohen, 1999).

This paper reports on the ability of LT-conditioning (i.e., a cold-shock treatment) alone, or in combination with a heat-shock treatment or periodic IW during cold storage to reduce the development of IB in 'Mauritius' pineapple during cold storage for 3 weeks.

^{*}Author for correspondence.

MATERIALS AND METHODS *Fruit*

Mature, fully-green fruit of the pineapple (*Ananas comosus*) cultivar 'Mauritius', harvested 3 months after anthesis in the Gampaha District (Western Province, Sri Lanka), were used in all the experiments described in this paper. Fruit devoid of any mechanical damage or disease symptoms were selected, packed in cardboard boxes, and transported to the Department of Botany, University of Peradeniya. Fruit were cleaned with a soft brush and the stalks were trimmed to approx. 6 cm in length before being used for the experiments.

Development and assessment of internal browning (IB)

Five samples of pineapple, each containing four replicate fruit, were placed in a cold room at 10°C and 85% RH. Another sample was kept at room temperature $(28^{\circ} - 30^{\circ}C)$ for 48 h as a control. One sample was withdrawn from cold storage at 7, 10, 14, 18, and 21 d and allowed to stand for 48 h at room temperature (28° – 30°C). Individual fruit were then cut longitudinally into two halves, and the intensity of IB symptoms was assessed using the seven-point scale developed by Teisson et al. (1979) after slight modification: 0, good flesh/core with no sign of browning; 1, brown spots near the stalk-end of the flesh/core; 2, brown spots coalesced, but covering $\leq 10\%$ of the flesh/core; 3, 25% of the flesh/core turned brown; 4, 50% of the flesh/core turned brown; 5, 75% of the flesh/core turned brown; and 6, complete browning of the flesh/core.

Effect on internal browning (IB) of brief chilling prior to cold storage

Four samples of pineapple, each containing four fruit, were obtained. Three samples were pre-cooled in a cold room at 2°C, and one sample (i.e., four fruit) was withdrawn after 30, 60, and 75 min. The fourth sample, without exposure to low temperature, served as a control. All treated and control fruit were subsequently stored for 3 weeks in a cold room at 10°C and 85% RH. The fruit were then transferred to room temperature $(28^{\circ} - 30^{\circ}C)$ and allowed to stand for 48 h. The extent of IB was assayed and scored as above. The experiment was repeated at pre-cooling temperatures of 4°C or 6°C using two fresh batches of fruit, each containing four samples of pineapple with four fruit per sample. All experiments were repeated three times.

Heat-shock treatment before or after a brief cold treatment

Five samples of pineapple, each containing four fruit, were obtained. One sample of fruit was immersed in a 48 l water bath at 38°C for 60 min (heat-shock). A second sample was exposed to 4°C for 60 min (cold-shock). A third sample of pineapple fruit was initially treated at 38°C for 60 min, left at room temperature $(28^{\circ} - 30^{\circ} \text{ C})$ for 15 min, then treated at 4°C for 60 min in a cold room. The fourth sample of pineapple fruit was initially treated at 4°C for 60 min, allowed to stand for 15 min at 28° – 30°C, then immersed in a water bath at 38°C for 60 min. The fifth sample of fruit, with no cold or heat treatments, was maintained as a control. All treated and control fruit were then stored in a cold room at 10°C and 85% RH. Fruit were withdrawn after 21 d of cold storage and

allowed to stand for 48 h at room temperature $(28^{\circ} - 30^{\circ}C)$. Fruit were cut longitudinally into two halves and the intensity of IB was assessed and scored as described above. The experiments were repeated three times.

Intermittent warming (IW) of 'Mauritius' pineapple

Two samples of fruit, each containing ten pineapples, were stored in a cold room at 10°C and 85% RH for 21 d. The low storage temperature was interrupted for one sample by removing all ten fruit from cold storage every 6 d and exposing them to room temperature $(28^{\circ} - 30^{\circ}C)$ for 8 h. The second sample was kept in cold storage continuously for 21 d. After 21 d, both samples of fruit were removed from the cold store, exposed to room temperature $(28^{\circ} - 30^{\circ}C)$ for 48 h, and the intensity of IB was assessed and scored as described previously.

Determination of the physicochemical parameters of fruit subjected to heat-shock treatment before or after a cold-shock treatment or intermittent warming

Four samples, each containing four pineapple fruit, were obtained and treated separately as follows: (i) precooled in a cold room at 4°C for 60 min; (ii) treated at 38°C for 60 min (heat-shock); (iii) pre-cooled at 4°C for 60 min and allowed to stand for 15 min at $28^{\circ} - 30^{\circ}$ C, then treated at 38°C for 60 min; or (iv) given no treatment (control). All treated and control samples were then stored at 10°C and 85% RH for 21 d. Another sample, containing ten pineapple fruit, was subjected to IW during 21 d of storage at 10°C and 85% RH, as described above. All fruit were removed after 21 d storage and hand-peeled. Flesh tissue (100 g) was cut separately from the region surrounding the central core in each fruit, weighed, and stored in sealed polythene bags at -20°C. Each tissue sample was then cut into smaller square pieces $(3 \text{ cm} \times 3 \text{ cm})$ and homogenised, separately, for 3 min in a blender without adding water. The resulting slurry was squeezed through a muslin cloth to obtain a clear extract.

Total soluble solid (TSS) contents were measured using a hand-held refractometer (Model 10430; Leica, Solms, Germany). Titratable acidity (TA; as a %) was determined according to Askar and Trepow (1993). pH was measured using a pH meter (TOA Electronics Ltd, Tokyo, Japan). Firmness (in N) was measured using a hand-held penetrometer (Forestry Suppliers Inc., Jackson, MS, USA). Skin colour development was assessed using a six-point scale where: 0 = green; 1 = 10%yellow; 2 = 25% yellow; 3 = 50% yellow; 4 = 75% yellow; and 5 = 100% yellow.

Electrolyte leakage

Electrolyte leakage in tissue from the core/flesh interface was used as a measure of the extent of cellular damage in pineapple fruit subjected to the various treatments. Fruit subjected to the different treatments (i.e., cold- or heat-shock) were removed from cold storage (10°C and 85% RH) and exposed to room temperature at 28° – 30°C for 48 h. Cylindrical pieces (5 cm \times 1 cm; 2 g) were cut separately from the core-flesh interface region of each replicate fruit. Four tissue pieces were taken from each fruit. Two pieces of tissue were dipped separately in 25 ml deionised water and left for 3 h. The conductivity of the solution (EC₁)was measured

		TABI	le I			
Development	of internal	browning in	ı 'Mauritius'	pineapple	fruit	during
	cold.	storage (10°	C and 85% I	$\bar{R}H)$		-

Duration of	Severity of interna	al browning (IB)‡
storage (d)	Flesh tissue	Core tissue
0	0 b	0 b
7	0 b	$1.3 \pm 0.0 \text{ b}$
10	0 b	$2.7 \pm 0.1 \text{ a}$
14	$3.3 \pm 0.1 \ a^{\dagger}$	$3.3 \pm 0.1 \text{ a}$
18	$4.3 \pm 0.1 a$	$4.0 \pm 0.1 \text{ a}$
21	5.0 ± 0.1 a	5.0 ± 0.1 a

[†]Mean values (n = 12) ±SE followed by the same lower-case letter in each column did not differ significantly at $P \le 0.05$ by the Kruskal-Wallis test.

[‡]Severity of IB scores were based on the extent and intensity of the brown colour in flesh and core tissues using a seven-point scale (see text).

using a conductivity meter (WPA CM 3; Linton, Cambridge, UK). The remaining tissue was frozen for 24 h at -20° C, then allowed to stand at room temperature to thaw for 3 h. The tissue was then rinsed in H₂O for 3 h, as above, and the conductivity of the solution (EC₂) was measured. The relative leakage of electrolytes from tissues (expressed as a %) was determined by taking the ratio of the two EC measurements before and after freezing the sample.

Statistical analysis

The non-parametric data (i.e., score values) were subjected to analysis using the Kruskal-Wallis test. Parametric values were subjected to ANOVA using Tukey's Studentised Range (HSD) test at $P \le 0.05$.

RESULTS

Internal browning in 'Mauritius' pineapple

Symptoms of IB appeared first at the periphery of the core of each fruit as small, light-brown, translucent, diffused areas, within 7 d of cold storage (Table I). These areas gradually spread along the core into the flesh, affecting approx. 75% of the flesh and core after 21 d in cold storage.

Effect on internal browning (IB) of a cold-shock treatment prior to cold storage

Samples of fruit were subjected, separately, to cold

 TABLE III

 Development of internal browning symptoms in 'Mauritius' pineapple

 fruit subjected to intermittent warming treatment during a 21 d period of

 cold storage (10°C and 85% RH)

		Severity of IB [‡]		
Experiment	Sample	Flesh	Core	
1	Treated	$2.0 \pm 0.3 a^{\dagger}$ $4.0 \pm 0.5 b$	$0.5 \pm 0.1 \text{ b}$ $4.2 \pm 0.5 \text{ a}$	
2	Treated	$2.0 \pm 0.3 \text{ a}$ 4.0 ± 0.5	$0.5 \pm 0.1 \text{ b}$ $4.0 \pm 0.5 \text{ a}$	
3	Treated Control	4.0 ± 0.5 2.0 ± 0.3 a 4.0 ± 0.5 b	$4.0 \pm 0.5 a$ $0.6 \pm 0.1 b$ $4.0 \pm 0.5 a$	

†Mean values (n = 4) ±SE of the severity of internal browning (assessed separately for core and flesh tissues) followed by the same lower-case letter in each row did not differ significantly at $P \le 0.05$ by the Mann-Whitney test.

^{*}Internal browning (IB) severity scores are based on the extent and intensity of the brown colour in flesh and core tissues using a sevenpoint scale (see text).

treatments at 2°C, 4°C, or 6°C for different times (30, 60, or 75 min) prior to cold storage at 10°C. Fruit that were treated at 2°C for 30, 60, or 75 min showed increased symptoms over 10% of their core tissue, compared to the untreated controls. A 10% increase in IB symptoms in core tissue was also observed in fruit treated at 6°C for 30, 60, or 75 min. However, fruit first subjected to a cold shock treatment at 4°C for 60 min, developed 40% less IB in both their flesh and core tissues, compared to the untreated controls (Table II).

Heat-shock treatment before or after a cold treatment

Fruit subjected to a cold treatment at 4°C for 60 min before a heat-shock treatment developed 87% less IB in the flesh and 40% less in the core, compared to the controls. Fruit treated at 4°C for 60 min after a heatshock treatment showed similar IB symptoms, namely 88% less in the flesh and 37% less in the core, compared to the controls. These combined treatments were slightly more effective at reducing IB than heat-shock treatment (38°C for 60 min) alone (Table II).

Development of internal browning (IB) in pineapple fruit subjected to intermittent warming (IW) during cold storage

Control fruit that were stored at 10° C continuously for 21 d developed IB symptoms in 50% of their flesh and core tissues (Table III). Fruit that had been exposed to

TABLE II

Effect of a cold-shock treatment alone, or in combination with a heat-shock treatment, on the incidence of internal browning (IB) in 'Mauritius' pineapple fruit during 21 d cold storage (10°C and 85% RH)

	Severity	Increase/Reduction (%) in IB [§]			
Treatment	Flesh	Core	Flesh	Core	
2°C for 30 min	$5.0 \pm 0.1 \ a^{\dagger}$	6.0 ± 0.1 a	0	-10	
2°C for 60 min	5.0 ± 0.1 a	6.0 ± 0.1 a	0	-10	
2°C for 75 min	5.0 ± 0.1 a	6.0 ± 0.1 a	0	-10	
4°C for 30 min	4.0 ± 0.1 a	5.0 ± 0.1 a	20	0	
4°C for 60 min	3.0 ± 0.1 a	3.0 ± 0.1 a	40	40	
4°C for 75 min	5.0 ± 0.1 a	5.0 ± 0.1 a	0	0	
6°C for 30 min	5.0 ± 0.1 a	6.0 ± 0.1 a	0	-10	
6°C for 60 min	5.0 ± 0.1 a	6.0 ± 0.1 a	0	-10	
6°C for 75 min	4.0 ± 0.1 a	6.0 ± 0.1 a	20	-10	
Heat-shock treatment (38°C, 60 min)	$0.7 \pm 0.0 \text{ b}$	$3.2 \pm 0.1 \text{ b}$	83	34	
Cold-shock treatment (4°C, 60 min)	2.6 ± 0.1 a	4.1 ± 0.1 a	40	15	
Heat-shock treatment (38°C, 60 min) before a cold-shock treatment (4°C, 60 min)	$0.6 \pm 0.0 \text{ a}$	2.8 ± 0.1 b	87	40	
Heat-shock treatment (38°C, 60 min) after a cold-shock treatment (4°C, 60 min)	$0.5 \pm 0.0 \text{ b}$	3.2 ± 0.1 b	88	34	
Control	$4.3 \pm 0.1 \text{ a}$	$4.7 \pm 0.1 \text{ a}$	-	-	

[†]Mean values (n = 12) ±SE followed by the same lower-case letter within each column did not differ significantly at $P \le 0.05$ by the Kruskal-Wallis test. [‡]Internal browning (IB) scores are based on the extent and intensity of the brown colour in flesh and core tissues using a seven-point scale (see text).

[§]Values (%) are relative to untreated control fruit.

	TABLE	IV	
	 	. 7	

Effect of a heat-shock treatment before or after a cold-shock treatment on the physicochemical parameters and electrolyte leakage in 'Mauritius' pineapple fruit tissue

						Electrolyt	e leakage (%) [¶]
Treatment	TSS (°Brix)	TA (%)	pН	Firmness (N)	Skin colour#	Affected	Healthy tissue
Heat-shock treatment (38°C for 60 min)	$10.2\pm0.2~a^{\dagger}$	$0.6\ \pm 0.0\ b$	3.6 ± 0.1 a	4.2 ± 0.1 a	2.4 ± 0.2 b	79.7 ± 1.9 a	42.3 ± 1.0 b
Cold-shock treatment (4°C for 60 min)	11.6 ± 0.3 a	$0.6 \pm 0.0 \text{ ab}$	3.7 ± 0.1 a	$4.2 \pm 0.1 \text{ a}$	4.0 ± 0.0 a	82.5 ± 1.9 a	64.3 ± 1.5 b
Heat-shock treatment (38°C for 60 min) before							
a cold shock treatment (4°C for 60 min)	10.5 ± 0.2 a	$0.7 \pm 0.0 \text{ ab}$	$3.8 \pm 0.1 \text{ a}$	$4.4 \pm 0.1 \text{ a}$	$2.0 \pm 0.0 \text{ b}$	77.6 ± 1.8 a	44.7 ± 1.0 b
Heat-shock treatment (38°C for 60 min) after a cold shock treatment (4°C for 60 min)	11.2 ± 0.3 a	0.8 ± 0.0 a	3.5 ± 0.1 a	4.33 ± 0.1 a	2.0 ± 0.0 b	77.3 ± 1.8 a	37.4 ± 0.9 b
Control	$11.5\pm0.3~\mathrm{a}$	0.8 ± 0.0 ab	$3.6\pm0.1~a$	$3.8\pm0.1~b$	$4.5\pm0.1~\mathrm{a}$	$84.0\pm2.0~\mathrm{a}$	_ [§]

[†]Mean values (n =12) ±SE followed by same lower-case letter within each column did not differ significantly at $P \le 0.05$ by Tukey's Studentised Range (HSD) test.

[#]Skin colour data were analysed using the Kruskal-Wallis test.

[¶]Mean electrolyte leakage values. Four replicate fruit were used for each experiment and the experiment was repeated three times.

[§]No healthy tissue was found in control fruit.

 $28^{\circ} - 30^{\circ}$ C for 8 h every 6 d during 21 d of cold storage at 10° C showed $\ge 80\%$ less IB in their core and $\ge 50\%$ less IB in their flesh tissue, compared to the controls (Table III). Approx. 60% of treated fruit had IB symptoms present as small, isolated patches in the flesh tissue. The areas of IB in the remaining 40% of fruit were much larger (Table III).

Physicochemical parameters in fruit subjected to different treatments

Fruit subjected to heat-shock treatment alone, or in combination with a cold-shock treatment, showed slightly lower TSS contents than untreated control fruit after 21 d of cold storage (Table IV). However, the differences were not significant. Fruit subjected to heatshock treatment alone had the lowest TA, but again, this was not significantly different from fruit that were subjected to a heat-shock treatment before or after a cold-shock treatment. pH values were lowest in fruit subjected to a heat-shock treatment after a cold-shock treatment, and highest in fruit that had been subjected to a heat-shock treatment before a cold-shock treatment (Table IV).

Fruit subjected to a heat-shock treatment alone, or in combination with a cold shock (4°C for 60 min) had significantly higher firmness values than the controls at the end of 21 d of cold storage (Table IV). After 21 d of cold storage, the skin colour of these fruit was 25% yellow, compared to 75% yellow in the untreated controls. Fruit subjected to a heat-shock treatment alone developed slightly more yellowish coloured skin. Cold treatment of fruit at 4°C for 60 min did not affect skin colour development, which remained similar to that of the untreated controls (Table IV). There were no

TABLE V			
Effect of intermittent warming (IW) during 21 d of cold s	storage	on	the
physicochemical parameters of 'Mauritius' pineappl	le fruit		

	· · ·	<u>^</u>
Parameter	Fruit subjected to IW	Control fruit
TSS content (°Brix)	11.7 ± 0.2 a	$12.7 \pm 0.3 \text{ a}$
Titratable acidity (%)	$0.5 \pm 0.0 \text{ a}$	0.6 ± 0.01 a
pH	4.0 ± 0.1 a	$3.9 \pm 0.1 a$
Skin colour**	$4.5 \pm 0.1 a^{\dagger}$	4.5 ± 0.1 a
Firmness (N)	$4.2 \pm 0.1 \text{ a}$	$4.2\pm0.1~\mathrm{a}$

[†]Mean values of skin colour (\pm SE) followed by same lower-case letter in a row did not differ significantly at $P \le 0.05$ by the Mann-Whitney test. (n = 10).

Other mean values followed by the same lower-case letter within a row did not differ significantly at $P \le 0.05$ by the Student's t-test.

**Assessed using a five-point scale (see text).

significant differences in TSS content, TA, pH, skin colour, or firmness between fruit subjected to IW and control fruit after 21 d cold storage (Table V).

Electrolyte leakage in tissues of fruit subjected to the different treatments

Relative electrolyte leakage (%) was used as a measure of cellular membrane damage in this study. Although heat-shock treatment alone, or in combination with a cold-shock treatment, reduced the extent of IB symptoms, the relative level of electrolytic leakage was similar in IB-affected tissues of both treated and control fruit. Flesh tissue adjacent to the IB-affected areas, that had no symptoms of IB, showed less electrolyte leakage than tissues showing IB symptoms (Table IV).

DISCUSSION

The work reported in this paper was carried out to investigate the effects of different low temperature and heat pre-treatments on the development of IB in 'Mauritius' pineapple during prolonged cold storage. In order to optimise the treatments, pineapple fruit were first subjected to several temperature-time treatment combinations: namely 2°C, 4°C, or 6°C, for 30, 45, or 60 min, prior to storage at 10°C for 21 d. Pre-treatment of fruit at 4°C for 60 min resulted in a slight reduction in the incidence of IB; however, fruit treated at 2°C or 6°C for different periods showed an increased intensity of IB. Weerahewa and Adikaram (2005) previously reported a significant reduction in IB when 'Mauritius' fruit were exposed to a pre-storage heat-shock treatment at 38°C for 60 min.

When fruit were treated at 4°C for 60 min, then exposed to a moderately higher temperature (10°C) for 60 min, a further reduction in IB symptoms occurred, particularly in flesh tissue. However, this treatment did not eliminate the symptoms of IB completely. Chilling injury (CI) was reduced in zucchini squash when the fruit were "conditioned" at 15°C for 2 d after a hot water treatment at 42°C for 30 min, prior to storage at 5°C (Wang, 1994). Heat-shock treatment at 55°C for 2 min and cold acclimatisation by "conditioning" the fruit at 8°C for 5 d prior to storage at 2°C effectively alleviated CI in plum fruit (Sun *et al.*, 2010).

'Kensington Pride' mangoes that were conditioned by heating for 7 h to a core temperature of 37°C, maintained for 12 h, showed less pulp injury on ripening following a hot water treatment (Joyce and Shorter, 1994). Heatshock treatment, in combination with air-cooling, had beneficial effects on CI in avocado (Sanxter *et al.*, 1994). The symptoms of CI were reduced when 'Sharwil' avocados were held at $37^{\circ} - 39^{\circ}$ C for 17 - 18 h compared to those that were air-cooled at 20° C for 4 h before storage at 1.1°C for 14 d. In contrast, non-heated avocado fruit developed severe discolouration and pitting (Sanxter *et al.*, 1994). A combination of a hot water rinse at 62°C for 20 s and "conditioning" treatment (i.e., pre-storage at 16°C for 7 d) synergistically reduced the incidence of CI in grapefruit (*Citrus paradisi* 'Star Ruby') during subsequent cold storage at 2°C (Sapitnitskaya *et al.*, 2006).

In the present work, a heat-shock treatment at 38°C for 60 min alone, or in combination with a low temperature (4°C for 10 min) treatment, resulted in a significant reduction in IB symptoms, irrespective of whether the low temperature treament was applied before or after the heat-shock. The fact that combining a low temperature treatment with the heat-shock did not result in any additional reduction in IB indicates that the low temperature treatment, applied in either way, provides no extra benefit. None of these treatments resulted in any apparent cellular damage, as the tissues in IB-affected areas in treated or control fruit had higher but similar levels of electrolyte leakage. However, the extent of cellular damage in healthy areas of fruit was less in fruit that had been given a heat-shock treatment before or after a cold-shock treatment, compared to fruit that were only given a low temperature treatment. This may be due to cellular repair mechanisms in the heat-treated fruit, or to a "conditioning" effect on fruit by the low temperature treatment applied before or after the heat-shock. Low temperature (LT) "conditioning" has been reported to be associated with maintaining high levels of phospholipids in membranes, increased levels of unsaturation in membrane fatty acids, suppression of the increase in the sterol:phospholipid ratio, increased concentrations of polyamines, squalene, and long-chain aldehydes (Wang, 1994), and the expression of membrane lipid modification enzymes (Sapitnitskaya *et al.*, 2006). All these factors may contribute to a reduced incidence of CI (Wang, 1994).

Heat treatment, before or after a low temperature treatment and prior to cold storage resulted in a delayed softening of pineapple flesh, altered development of flesh colour, and lower TSS contents, indicating that the treatments had a negative effect on fruit ripening. Similar findings were reported when pineapple fruit were subjected to only a heat-shock treatment at 38°C for 60 min (Weerahewa and Adikaram, 2005).

Intermittent warming (IW; the interruption of low temperature storage by one or more periods of higher temperature) has been used extensively as a means to reduce physiological disorders in many fruits, including apple, tomato, lemon, zucchini, mango, pomegranate, and peach. The mechanism underlying IW is that it interrupts the processes of CI by warming the fruit intermittently during cold storage. Chilling injury is a result of two successive events. Those primary events taking place in the cells are reversible, but the secondary events are irreversible (Wills et al., 2007). Therefore, periodic interruptions of cold storage by exposing fruit to a higher temperature are thought to help avoid the secondary events and thus inhibit the process of CI. In the present study, pineapple fruit were warmed periodically by exposing them to room temperature every 6 d during 21 d of storage at 10°C and 85% RH. In most IW-treated fruit, only patches of IB were observed. On average, only 40% of all fruit showed isolated brown areas in their flesh. The IW treatment did not have an adverse effect on fruit ripening. IW-treated fruit developed their skin colour and flesh firmness in a manner similar to control fruit. IW treatment is therefore not beneficial in terms of extending the shelf-life of fruit. However, IW-treated fruit did not have significantly lower TSS contents than control fruit. Titratable acidity was lower in IW-treated fruit due to a higher flesh pH. Chilling injury in peach was reduced by IW, and the mechanism underlying this was related to reduced ethylene emissions from fruit during the CI latency period (Fernández-Trujillo and Artes, 1998). A similar mechanism may be involved in the reduced incidence of IB observed in 'Mauritius' pineapple subjected to IW during cold storage.

REFERENCES

- ABDULLAH, H., ROHAYA, M. A. and ZAIPUN, M. Z. (1985). Effect of modified atmosphere on black heart development and ascorbic acid contents in 'Mauritius' pineapple (*Ananas comosus* 'Mauritius') during storage at low temperature. *Asian Food Journal*, 1, 15–18.
- ALWAN, T. F. and WATKINS, C. B. (1999). Intermittent warming effects on superficial scald development of 'Cortland', 'Delicious', and 'Low Rome' apple fruit. *Postharvest Biology* and Technology, 16, 203–212.
- ARTES, F. and ESCRICHE, A. J. (1994). Intermittent warming reduces chilling injury and the decay of tomato fruit. *Journal of Food Science*, **59**, 1053–1056.
- ASKAR, A. A. and TREPOW, H. (1993). In: *Quality Assurance in Tropical Fruit Processing*. Springer-Verlag, New York, USA. 9–25.
- CAI, C., XU, C., SHAN, L., LI, X., ZHOU, C., ZHANG, W., FERGUSON, I. and CHEN, K. (2006). Low temperature conditioning reduces postharvest chilling injury in loquat fruit. *Postharvest Biology* and *Technology*, **41**, 252–259.
- COHEN, E. (1988). Commercial use of long-term storage of lemon with intermittent warming. *HortScience*, **23**, 400.
- FERNÁNDEZ-TRUJILLO, J. P. and ARTÉS, F. (1998). Intermittent warming during cold storage of peaches packed in perforated polypropylene. *Lebensmittel-Wissenschaft und Technologie*, **31**, 38–43.
- HEWAJULIGE, I. G. N., WIJERATNAM, R. S. W., WIJESUNDARA, R. L. C. and ABEYSEKARE, M. (2003). Fruit calcium concentration and chilling injury during low temperature storage of pineapple. *Journal of the Science of Food and Agriculture*, 83, 1451–1454.

- JOYCE, D. C. and SHORTER, A. J. (1994). High temperature conditioning reduces hot water treatment injury of 'Kensington Pride' mango fruit. *HortScience*, 29, 1047–1051.
- ROHRBACH, K. G. and PAULL, R. E. (1982). Incidence and severity of chilling-induced internal browning of waxed smooth cayenne pineapple. *Journal of the American Society for Horticultural Science*, **107**, 453–457.
- SANXTER, S. S., NISHIGMA, K. A. and CHAN, H. T., JR. (1994). Heat treating 'Sharwil' avocado for cold tolerance in quarantine cold treatments. *HortScience*, 29, 1166–1168.
- SAPITNITSKAYA, M., MAUL, P., MCCOLLUM, G. T., GUY, C L., WEISS, B., SAMACH, A. and PORAT, R. (2006). Postharvest heat and conditioning treatments activate different molecular responses and reduce chilling injuries in grapefruit. *Journal of Experimental Botany*, **57**, 2943–2953.
- SCHIRRA, M. and COHEN, E. (1999). Long-term storage of 'Olinda' oranges under chilling and intermittent warming temperatures. *Postharvest Biology and Technology*, **16**, 63–69.
- SELVARAJAH, S., BAUCHOT, A. D. and JOHN, P. (2001). Internal browning in cold-stored pineapples is suppressed by a postharvest application of 1-methylcyclopropene. *Postharvest Biology and Technology*, 23, 167–170.
- SUN, J. H., CHEN, J-Y., KUANG, J-F., CHEN, W-X. and LU, W-J. (2010). Expression of sHSP genes as affected by heat-shock and cold acclimation in relation to chilling tolerance in plum fruit. Postharvest Biology and Technology, 55, 91–96.

- TEISSON, C., COMBRES, J. C., MARTIN-PREVEL, P. and MARCHAL, J. (1979). Internal browning of pineapples: II – Symptoms; IV – Biochemical approach. *Fruits*, 34, 315–338.
- WANG, C. Y. (1994). Combined treatment of heat-shock and low temperature conditioning reduces chilling injury in zucchini squash. *Postharvest Biology and Technology*, **4**, 65–73.
- WEERAHEWA, D. and ADIKARAM, N. K. B. (2005). Heat-induced tolerance to internal browning of pineapple (*Ananas comosus* 'Mauritius') under cold storage. *Journal of Horticultural Science* & *Biotechnology*, **80**, 503–509.
- WILLS, R. B. H., HASSAN, A. and SCOTT, K. J. (1985). Effect of storage time at low temperature on the development of black heart in pineapple. *Tropical Agriculture*, **62**, 199–200.
- WILLS, R. B. H., MCGLASSON, W. B., GRAHAM, D. and JOYCE, D. C. (2007). Postharvest: An Introduction to the Physiology and Handling of Fruit, Vegetables and Ornamentals. 5th Edition. CAB International, Wallingford, Oxon, UK. 121–125.
- WOOLF, A. B., COX, K. A., WHITE, A. and FERGUSON, I. B. (2003). Low temperature conditioning treatments reduce external chilling injury of 'Hass' avocados. *Postharvest Biology and Technology*, 28, 113–122.
- ZHAO, Z., JIANG, W., CAO, J., ZHAO, Y. and GU, Y. (2006). Effect of cold-shock treatment on chilling injury in mango (*Mangifera indica* L. 'Wacheng') fruit. *Journal of the Science of Food and Agriculture*, 86, 2458–2462.