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Heat-induced tolerance to internal browning of pineapple (*Ananas comosus* cv. 'Mauritius') under cold storage

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SUMMARY

Pineapple (cv. 'Mauritius') fruit, under low temperature (10°C) and 85% RH storage, develops internal browning symptoms. Fruit acidity, polyphenol oxidase (PPO) and peroxidase (POD) enzyme activities increased sharply, and phenylalanine ammonia-lyase (PAL) activity increased slightly during low temperature storage, concurrent with the development of internal browning. Harvesting fruit early reduced both the incidence and severity of internal browning. Post-harvest heat-treatment, in the form of a hot water dip, induced pineapple fruit tolerance to cold injury and, in turn, reduced internal browning during prolonged low temperature storage. Several temperature-time combinations were effective, but the best was 38°C for 60 min. Pineapple fruits treated at 38°C for 60 min developed 70% and 45% less browning than untreated controls in the flesh and core regions, respectively. The results also indicate that an internal tissue temperature of 37°C is a prerequisite for the induction of cold tolerance. Heat treatment, however, slowed down fruit ripening and increased water loss. Wrapping heat-treated fruits in polythene, exposing only the crown, prior to cold-storage reduced internal browning by a further 10% and water loss by 8.5%, giving fruit a better appearance.

Low temperature storage, although the most effective method to maintain the quality of fruit, can be detrimental to cold-sensitive tropical commodities due to chilling injury. Fruit tolerance to chilling injury can be induced or enhanced by heat treatment prior to storage (Klein and Lurie, 1992), by temperature conditioning (Porat *et al.*, 2000), intermittent warming (Cohen *et al.*, 1983), controlled atmosphere storage (Paull and Rohrbach, 1982) and/or by the application of growth regulators (Kawada *et al.*, 1979). Reduced chilling injury occurs either through increasing the tolerance of the commodity to cold, or by retarding the development of the symptoms of chilling injury (Wang, 1994). Moderate levels of heat treatment allow fruit to tolerate both high and low temperatures (Lurie, 1998). Protection against chilling injury conferred by moderate heat-shock has been achieved in several cold-sensitive fruits such as avocado (*Persea americana*), tomato (*Lycopersicon esculentum*), papaya (*Carica papaya*), mango (*Mangifera indica*), oranges (*Citrus sinensis*), lemon (*Citrus limon*), lime (*Citrus aurantifolia*), grapefruit (*Citrus paradisi*) and persimmon (*Diospyros kakai*) (Lurie, 1998; Woolf, 1997). Schirra *et al.* (1997) reported that hot water treatment at 53°C for 3 min, prior to cold storage at 3°C for 10 weeks, reduced chilling injury in oranges. Salveit (1991) found that exposure to warm air at 38°–42°C for several hours affected the chilling sensitivity of tomato disks. When warm air (38°C) treatment was applied for 2–3 d, the sensitivity of tomato fruit to low temperatures was reduced, enabling storage for up to 1 month at 2°C without chilling injury (Lurie and Klein, 1991; Sabehat *et al.*, 1995; Lurie and Sabehat, 1997).

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Internal browning, a physiological disorder that develops when pineapples are exposed to low temperatures during storage or in the field, has been reported from many countries including Australia (Smith, 1983), Taiwan (Sun, 1988), Hawaii, Ivory Coast (Teisson, 1979), South Africa (Van Lelyveld and De Bruyn, 1976; 1977) Japan (Mizuno *et al.*, 1982), India and Malaysia (Abdullah and Rohaya, 1983; Abdullah *et al.*, 1986). The characteristic symptoms are initial formation of translucent, water-soaked spots at the base of the fruitlets which turn brown at later stages. In severe cases, these areas turn black and spread to neighbouring tissues. The severity of the disorder is clearly influenced by chilling temperature, the duration of storage and the duration of exposure to higher temperature (Wills *et al.*, 1985). Symptoms are most apparent in fruits stored first at temperatures between 10°–12°C, then exposed to higher temperatures, 18°–30°C (Paull and Rohrbach, 1985). Internal browning limits prolonged storage of fruit following harvest and long-distance transport. The severity of internal browning depends on certain factors such as harvest maturity, size and weight of fruit (Botrel and De Carvalho, 1993), external appearance of the crown and certain fruits metabolites (Teisson *et al.*, 1979). Although, the extent of internal browning can be reduced by manipulation of these factors, no complete control of this disorder has been possible. This paper reports the ability of post-harvest hot water treatment to induce cold tolerance in pineapple fruit to reduce the development of internal browning during prolonged cold storage.

MATERIALS AND METHODS

Fruits

Fruits of pineapple (*Ananas comosus*) cv 'Mauritius' harvested from the Gampaha district (Western Province

of Sri Lanka) were used in all the experiments reported in this paper. Mature (3 months after anthesis), fully green pineapples, devoid of any mechanical damage or disease symptoms, were selected, packed in cardboard boxes and transported to the Department of Botany, University of Peradeniya. Fruits were cleaned and stalks were trimmed to about 6 cm in length before being used for the experiments.

Development of internal browning and associated changes in acidity, phenylalanine ammonia-lyase (PAL), peroxidase (POD) and polyphenol oxidase (PPO) activities during cold storage

Five samples of pineapples, 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°–30°C) for 48 h as a control. One sample was withdrawn from cold storage after 7, 10, 14, 18 and 21 d and allowed to stand for 48 h at room temperature (28°–30°C). Individual fruit were cut longitudinally into two halves and the intensity of internal browning was assessed visually using a seven-point scale developed by Teisson (1979) with slight modification: 0 = good flesh/core with no sign of browning; 1 = brown spots near the stalk end of the flesh/core; 2 = 10% of the flesh/core turned brown; 3 = 25% of flesh/core turned brown; 4 = 50% of flesh/core turned brown; 5 = 75% of flesh/core turned brown; 6 = complete browning of flesh/core.

Frozen samples (100 g) of flesh were withdrawn from fruit after different periods of cold storage, cut into small pieces and homogenised separately for 3 min in a blender. The resulting slurry was squeezed through a muslin cloth to obtain the juice.

To determine the percentage titratable acidity (TA), 10 ml aliquots of extracts were diluted five-fold with distilled water. Aliquots (10 ml) were then titrated against 0.1 M NaOH, in triplicate, in the presence of phenolphthalein as an indicator. Titratable acidity (%) for each sample was calculated using the equation (Askar and Trepow, 1993):

$$\frac{\text{Volume of NaOH} \times \text{Molarity} \times 100}{\text{Tissue weight equivalent (g)}}$$

°Brix values were obtained using a hand-held refractometer (Leica Model 10430; Solms, Germany) within the range of 0°–30°Brix. For PAL, PPO and POD enzyme assays, tissue samples (5 g) collected from the area immediately outside the core of each replicate fruit, were pooled and frozen. Twenty g of frozen tissue were ground into a fine powder in liquid N_2. The powder was extracted with 150 ml cold (–20°C) acetone for 1 min with constant stirring. The extract was filtered through Whatman No 2 paper under vacuum and the residual powder was washed twice with 100 ml portions of fresh cold acetone. Extracts were further clarified and PAL, PPO and POD activities were assayed as described by Riov *et al.* (1968) and Dubery and Schabort (1986). Each experiment was repeated three times. Non-parametric data were subjected to the Kruscal-Wallis test and parametric data were analysed using a SAS statistical package.

Effect of harvest maturity on the development of internal browning

Samples of pineapple, each containing four fruit, were harvested at five different stages of maturity: Maturity stage 1 (immature fruit with green shell, 1 month after anthesis); Maturity stage 2 (immature fruit with fully green shell, 1.5 months after anthesis); Maturity stage 3 (immature fruit with fully green shell, 2 months after anthesis); Maturity stage 4 (mature fruit with fully green shell, 3 months after anthesis); and Maturity stage 5 (ripening fruit, shell with 25% of yellow eyes, 3.5 months after anthesis).

Fruit were stored at 10 C and 85% RH for 21 d and, after exposure to ambient temperature (28 C–30°C) for 48 h, the intensity of internal browning was assessed as described previously. °Brix values and titratable acidity (%) of fruit at the end of cold storage were determined.

Effect of heat treatment on the development of internal browning during cold storage

A 48 l plastic container with 36 l hot water was used to heat-treat fruit. The water temperature was maintained by heating every 15 min with an electric immersion heater. The water was stirred continuously to maintain a uniform temperature in the bath. To determine the best temperature and duration for heat treatment, thirteen samples of pineapple, each containing four fruit, were used. Ten samples were immersed separately in hot water maintained at different temperatures for different times: 38°C for 60 min; 40 C for 60 min; 42 C for 60 min; 44°C for 60 min; 46°C for 30 min; 48 C for 30 min; 50 C for 10 min; 52°C for 10 min; 55 C for 10 min; or 60 C for 10 min. The remaining three samples of fruit were immersed in water at room temperature (28°–30 C) for 10, 30 or 60 min and maintained as controls. After treatment, each sample of fruit was allowed to stand for 15 min at room temperature, until the water had dripped from the surface, then transferred to a cold room maintained at 10°C and 85% RH.

To determine the optimum exposure time, heat-shock treatment at 38°C was applied for different time periods. Six samples each containing four pineapples were used. Four samples were immersed separately in water at 38°C for 30, 45, 60 or 90 min. Another sample was first immersed in hot water at 38°C for 60 min, then removed and immersed immediately in hot water at 50°C for 10 min. This treatment was described as "double hot water" treatment. The sixth pineapple sample was immersed in water at room temperature (28°–30°C) for 60 min and maintained as a control. After treatment, all fruit were first stored at low temperature (10°C) for 3 weeks. Fruit were then withdrawn and exposed to room temperature (28°–30°C) for 48 h and evaluated for internal browning as described previously. The experiment was repeated twice.

Determination of internal tissue temperature of heat-treated fruit

The internal tissue temperature of heat-treated fruit was measured using a hand-held thermometer (RKC, Japan) attached to a thermocouple (Crom-Alum Thermocouple, Omega Engines, USA). One sample of four fruit was immersed in a water bath at 38°C for

TABLE I
Development of internal browning in pineapple during cold storage and some associated changes in enzyme activities and titratable acidity

Period of cold storage (d)	Internal Browning[1]		PPO activity (units min^{-1} g^{-1} FW)	POD activity (units min^{-1} g^{-1} FW)	PAL activity (μmoles cinnamic acid h^{-1} g^{-1} FW)	Titratable acidity (%)
	Flesh	Core				
0	*0b	0b	1.8a	1.8b	9.0a	0.7c
7	0b	1.3b	2.0b	1.8b	9.1a	0.8bc
10	0b	2.7a	11.1ba	3.7b	9.2a	0.8bc
14	3.3a	3.3a	13.3ba	3.7b	6.6b	0.9ba
18	4.3a	4.0a	19.8ba	4.4b	8.7a	0.9a
21	5.0a	5.0a	22.2a	9.4a	10.5c	1.0a

Mean values (n = 4) followed by the same letter within each column do not differ significantly at $P \leq 0.05$ (Duncan's Multiple Range Test; *Kruscal-Wallis test for internal browning data).
[1]Internal browning scores based on extent and intensity of colour.

60 min. Fine holes (0.50 mm) were made in the middle portion of each fruit using a needle, and the thermocouple was inserted. Internal temperatures were measured in the shell (1 cm from the surface), in flesh tissue (2 cm from the surface) and in the core tissue (3 – 3.5 cm from the surface) of each replicate fruit, separately. The procedure was repeated for fruit treated at 42°C for 60 min, 55°C for 10 min or 60°C for 10 min. The average temperature for shell, flesh and core for each temperature-time combination was determined. The experiment was repeated twice.

Effect of heat treatment on fruit ripening

Fruit treated at different temperature-time combinations, 38°C for 30, 45, 60 or 90 min, 38°C for 60 min then 50°C for 10 min ("double hot water dip"), and their respective controls, were first stored for 21 d at 10°C, then exposed to room temperature (28°–30°C) for 48 h as described previously. Shell colour was recorded using a 6-point scale where: 0 = green shell; 1 = 10% yellow; 2 = 25% yellow; 3 = 50% yellow; 4 = 75% yellow; and 5 = 100% yellow. Fruit firmness was measured in N using a penetrometer (Forestry Supplies Inc., Jackson, MS, USA) with a 6.4 mm probe and a 6.4 mm loading depth. Individual fruit were hand-peeled and 100 g of tissue was cut from the region surrounding the central core and stored in sealed polythene bags at –20°C. °Brix values and titratable acidity (%) were determined. The pH of each extract was measured for replicate fruit using a pH meter (HM 205; TOA Electronics Ltd, Japan) and the average pH value was determined. The experiment was repeated twice.

Effect of polythene wrapping of heat-treated fruit on the development of internal browning

Three pineapple samples, each containing four fruit, were treated at 38°C for 60 min as described previously. Individual fruit in one sample were wrapped in polythene covers (350 μm thick × 30 cm × 40 cm) leaving only the leafy-crown exposed. In another sample, the entire fruit was wrapped with polythene (350 μm × 30 cm × 50 cm). The third sample of fruit was not wrapped with polythene. Three further, non-heat-treated samples of pineapple, each containing four fruit, were wrapped as described above and maintained as controls. All heat-treated and control fruit were stored at 10°C and 85% RH for 21 d. The polythene covers were then removed and the fruits were allowed to stand for 48 h at room temperature (28°–30°C). The intensity of internal browning was assessed in individual fruit as described previously, and average values were calculated. TSS content, TA (%), shell colour score, firmness (N) and percentage weight loss of treated and control fruits were also determined.

RESULTS

Development of internal browning of pineapple fruit during cold storage

Symptoms of internal browning first appeared at the periphery of the fruit core as small, light brown, translucent and diffused areas within 7 d of cold storage (Table I). These areas gradually spread along the core and into the flesh, affecting about 75% of the flesh and core 21 d after cold storage. Titratable acidity increased progressively by about 43% of its original level during the 3 week storage period. PPO activity was low during the first week and increased five-fold from day-7 to day-10 of cold storage, reaching its highest level 21 d after storage (Table I). Peroxidase activity, increased gradually during the first 2 weeks, then rose sharply in the third week. PAL activity was steady during the first 10 d and, following a brief decline, increased reaching its highest level after 21 d (Table I).

Effect of harvest maturity on the development of internal browning

There was an inverse relationship between fruit maturity at harvest and internal browning development during cold storage (Table II). Fruit harvested 1–1.5 months after anthesis developed the least internal browning among all maturity stages examined, and the symptoms were seen only in the core. Internal browning was more intense in fruit harvested 3 months after anthesis compared to fruit harvested 1, 1.5 or 2 months after anthesis. The highest browning intensity was observed in fruit harvested 3.5 months after anthesis, when the fruit were 25% yellow (Table II). TSS contents were higher in fruit harvested at more mature stages.

TABLE II
Intensity of internal browning and TSS content in pineapple fruit, harvested at different stage of maturity, after 21 d storage at 10°C and 85% RH

Harvest maturity (months after anthesis)	Intensity of Internal Browning[1]		TSS (°Brix)
	Flesh	Core	
1	0c	1.3b	7.0b
1.5	0c	2.0b	7.2b
2	2.0b	2.7b	8.3b
3	4.7a	4.3a	13.7a
3.5	5.0a	5.0a	15.7a

Mean values (n = 4) followed by the same letter within each column do not differ significantly at $P \leq 0.05$ (Duncan Multiple Range Test; *Kruscal-Wallis test for internal browning data).
[1]Internal browning scores based on the extent and intensity of colour.

TABLE III
Effect of post-harvest heat treatment on development of internal browning in pineapple during cold storage

Treatment (temp. time)	Intensity of Internal Browning*	
	Flesh	Core
28°C, 10 min	4.7ab	5.1 a
28°C, 30 min	5.1 a	5.3 a
28°C, 60 min	5.0 a	5.3 a
38°C, 60 min.	1.6 e (68)†	2.9 d (46)
40°C, 60 min.	3.6 bcd (28)	4.4 b (17)
42°C, 60 min.	3.2 cd (36)	3.6 c (33)
44°C, 30 min.	4.3 ab (14)	5.3 a (0)
46°C, 30 min.	4.5 ab (10)	5.0a (6)
48°C, 30 min.	4.2 abc (16)	4.4 b (17)
50°C, 10 min.	4.9 a (–5)	5.4 a (–6)
52°C, 10 min.	4.6 ab (3)	5.4 a (–6)
55°C, 10 min.	2.6 dc (45)	3.9 bc (24)
60°C, 10 min.	2.9 d (39)	4.3 b (16)

Mean values (n = 4) followed by the same letter within each column do not differ significantly at $P \leq 0.05$ (Duncan's Multiple Range Test).
†Values in parentheses represent the % reduction in internal browning compared to their respective controls.
*Internal browning scores based on the extent and intensity of colour.

Effect of heat treatment on the development of internal browning during cold storage

Prolonged treatment of pineapples for 60 min in water at 38°C, 40°C or 42°C, or treatment in moderately hot water at 55°C or 60°C for 10 min, significantly reduced the development of internal browning in both the flesh and the core during 21 d of cold storage compared to the respective control fruits (Table III). Treatment at 38°C for 60 min was most effective, reducing internal browning in the flesh by 68% and in the core by 57%, compared to controls (Table III). In fruit given this treatment, the brown areas were mostly confined to the core-flesh interface whereas, in control fruit, browning was seen in both the core and the flesh. While treatment at moderate heat (55°C or 60°C) for 10 min significantly reduced internal browning, severe crown damage also occurred in these fruits.

To optimise treatment at 38°C, different exposure times were tested. The results showed that exposure for 30, 45 or 60 min reduced internal browning (Table IV),

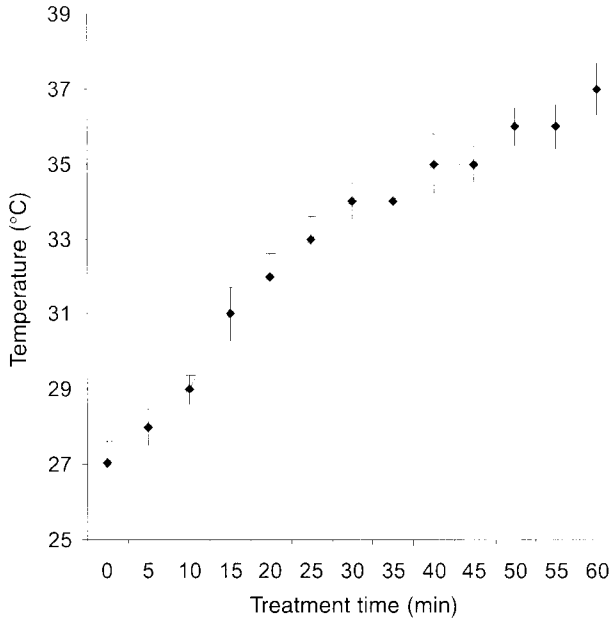

FIG. 1.
Average change in internal flesh tissue temperature (± SE error bars) during 60 min exposure of pineapple to 38°C in a water bath.

TABLE IV
Effect of treatment at 38°C for different time intervals on internal browning during cold storage of pineapple

Treatment time (min)	Intensity of Internal Browning*	
	Flesh	Core
30	3.7a (14)	4.7a (67)
45	3.3a (23)	4.3a (14)
60	1.7b (60)	3.0b (40)
60 (plus 50°C, 10 min)	2.7a (37)	4.0a (20)
90	5.3a (–19)	5.3a (–07)
Control (60 min; 28°–30C°)	4.3a	5.0a

Mean values followed by a same letter within each column do not differ significantly at $P \leq 0.05$ (Kruscal-Wallis Test).
†Values in parentheses represent the % reduction of internal browning compared to controls.
*Internal browning scores based on the extent and intensity of colour.

but that a significant reduction was observed only in fruit treated for 60 min (Table IV).

Internal temperature of heat-treated fruit

The internal flesh and core temperatures in the middle portion of fruit increased progressively with time when fruit were treated at 38°C (Figure 1) reaching 37°C and 34.8°C, respectively after 60 min (Table V). The average internal temperatures of fruit treated at 55°C for 10 min or at 60°C for 10 min were 37.4°C and 38.7°C, respectively.

Effect of heat treatment on fruit ripening

Fruit heat-treated at 38°C for 60 min had a significantly ($P = 0.0027$) lower TSS content after 21 d of cold storage, compared to untreated control fruit or fruit treated at 38°C for 30, 45, 60 or 90 min. TSS values of fruit treated at 38°C for 30, 45 or 90 min were not, however, significantly different from control fruit (Table VI). The TSS value of fruit subjected to 38°C for 60 min then 50°C for 10 min ("double hot water dip") was 13.3°, while the °Brix value was similar to the control fruit.

Heat-treatment at 38°C for 30, 45 or 60 min resulted in slightly, but significantly ($P = 0.0001$) less acidity development during ripening compared to control fruit (Table VI). The pH values of tissue extracts taken from fruit heat-treated at 38°C for 30, 45 or 60 min were significantly ($P = 0.0001$) higher than in control fruit (Table VI). But treatment at 38°C for 90 min, or fruit subjected to 38°C for 60 min followed by 50°C for 10 min ("double hot water dip") had no significant effect on the pH values. "Double hot water dip-treated" fruit had a significantly lower pH compared to fruit treated at 38°C for 30 or 45 min (Table VI).

Influence of polythene wrapping of fruits treated at 38°C for 60 min, on internal browning during cold storage

Wrapping in polythene prior to cold storage slightly enhanced the effectiveness of heat treatment, further reducing the development of internal browning

TABLE V
Mean internal temperatures in the shell, flesh and core of pineapples (n = 4) after fruit were treated for different time and temperature combinations

Treatment (temp. time)	Tissue		
	Shell	Flesh	Core
38°C, 60 min.	38.0 ± 0.35†	37.0 ± 0.32	34.8 ± 0.27
55°C, 10 min.	41.7 ± 0.21	37.4 ± 0.25	28.9 ± 0.36
60°C, 10 min.	41.6 ± 0.30	38.7 ± 0.34	28.3 ± 0.31

†Standard error in mean temperatures.

TABLE VI
Selected physico-chemical parameters of fruit treated at 38°C for different times and assayed after 21 d cold storage

Treatment	TSS (°Brix)	pH	Titratable acidity (%TA)	Shell colour[a]	Firmness
38°C, 30 min	13.0a[*]	3.8a	0.8b	n.d.	n.d.
38°C, 45 min	13.5a	3.9a	0.8b	n.d.	n.d.
38°C, 60 min	11.0b	3.7ab	0.8b	0.00a	4.2a
38°C, 60 min plus 50°C, 10 min	13.3a	3.5bc	1.0a	n.d.	n.d.
38°C, 90 min	13.5a	3.4c	0.9a	n.d.	n.d.
Control (28°–30°C; 60 min)	13.3a	3.3c	0.9a	3.0b	3.5a

[*]Values followed in same letter within each column do not differ significantly at $P \leq 0.05$ (Duncan Multiple Range Test).
[a]Shell colour scores based on percentage yellow eyes.
n.d., not determined.

(Table VII). Covering only the fruit in polythene, leaving the crown exposed, had a greater effect than covering the whole fruit. Polythene wrapping, while significantly reducing water loss in heat-treated fruits, slightly improved shell colour development. However, this treatment had no effect on other physicochemical parameters (Table VII).

DISCUSSION

It has been shown that heat-shock (Saltveit, 1991) or prolonged heat-treatment (Sabehat *et al.*, 1995) confers resistance to chilling injury in a number of plants. The work reported in this paper was carried out to investigate the effectiveness of heat as a post-harvest treatment to reduce internal browning in pineapples during cold storage.

Green, freshly harvested pineapples, when treated for different temperature-time combinations and stored at low temperature, showed varied responses. Certain temperature-time combinations increased the severity of internal browning during cold storage, while others significantly reduced internal browning. Several moderate temperature and exposure time combinations proved to be beneficial. The most effective combination was 38°C for 60 min, which reduced internal browning significantly in fruit, particularly in the flesh.

The results of our study indicate that selection of the correct treatment temperature and exposure time for heat treatment are crucial. What appears to be most critical is the precise temperature of the internal tissue where browning actually takes place, and tolerance would be induced following heat-treatment. The observation that pre-treatment at 38°C for 60 min induced greater cold-tolerance in flesh than in core tissue may indicate that the optimum internal tissue temperature for induction of cold tolerance is 37°C. The internal temperature of the core region was 2°C lower than the flesh as treatment created a temperature gradient across the fruit. The reason for the reduced effectiveness of heat-treatment in core tissue could therefore be that the core did not reach the critical temperature. Treatment at 55°C for 10 min, or 60°C for 10 min which elevated the internal flesh temperature to 37°C also reduced internal browning development, but these treatments caused severe crown damage. A similar reduction of internal browning was recorded in pineapple cv. 'Kew' following heat-shock treatment at 38°C for 60 min (Weerahewa, 2002). Exposure of pineapple cv. 'Smooth Cayenne' to warm-air at 37.2°C for 24 h, prior to cold storage at 7°C for 6 d, significantly reduced internal browning without any significant weight loss, crown browning, senescence, decay or pulp translucence (Akamine, 1976). Substantial evidence is available on the beneficial effect of heat treatment in reducing chilling injury in apples, avocado, mango, citrus and tomato. Heat-treatment at 38°C for 4 h provided protection against chilling injury in mango (McCollum *et al.*, 1993) and in citrus (Rodov *et al.*, 1996). Chilling injury, and external browning of avocado, was reduced by heat-treatment at 38°C for 120 min prior to storage at 0.5°C for up to 28 d (Woolf, 1997). Chilling injury has also been reduced by treatment at higher temperatures such as 53°C for 3 min in oranges prior to cold storage at 3°C for 10 weeks (Wild, 1993).

Pineapples treated at 38°C for 60 min exhibited delayed softening of flesh, altered shell colour development, and lower TSS contents during cold storage, indicating that heat-treatment has a negative effect on fruit ripening. Reduction of TSS content, lowering the sweetness, is a disadvantage of heat-treatment. Heat-treatment has also been reported to slow softening of strawberry (Civello *et al.*, 1997), 'Tommy Atkins' mango (Mitcham and McDonald, 1993), pears (Maxie *et al.*, 1974), avocado (Earks, 1978),

TABLE VII
Effect of heat treatment followed by polythene wrapping on internal browning during cold storage

Treatment	Intensity of Internal Browning[a]		TSS (°Brix)	% TA	Firmness (N)	Weight loss (%)	Shell colour[b]
	Flesh	Core					
Polythene wrap (crown exposed)	3.6a (17%)[**]	4.1a (15%)	11.9a	0.8a	4.2a	2.9d	4.2a
Polythene wrap (entire fruit)	3.6a (18%)	4.2a (15%)	10.4b	0.7ab	4.2a	0.4e	4.0a
Heat treatment + polythene wrap (crown exposed)	0.7b (85%)	3.0b (40%)	10.3b	0.8 a	4.3a	4.2c	3.1b
Heat treatment + polythene wrap (entire fruit)	1.0b (76%)	3.2b (37%)	10.3b	0.7ab	4.3a	2.5d	3.3b
Heat treatment only	1.1b (75%)	3.5b (30%)	10.2b	0.6bc	4.4a	12.7a	2.4b
Control	4.2a	5.0a	12.9a	0.8a	3.7b	11.3b	4.5a

[*]Mean values followed by the same letters within each column do not differ significantly at $P \leq 0.05$ (Kruscal-Wallis test).
[**]Values given in parentheses represent the % reduction in internal browning compared to controls.
[a]Internal browning scores based on the extent and intensity of colour.
[b]Shell colour scores based on percentage yellow eyes.

tomatoes (Biggs *et al.*, 1988), and apple cvs. 'Golden Delicious' and 'Granny Smith' (Ben-Shalom *et al.*, 1993; Klein and Lurie, 1990; Lurie and Klein, 1990).

A gradual buildup of acidity in the flesh was observed in pineapple fruit during cold storage; however, fruit treated at 38°C for 60 min had lower levels of acids after 3 weeks cold storage. Acid levels in cv. 'Kew', a pineapple cultivar more resistant to internal browning, remained lower compared to cv. 'Mauritius', and did not increase significantly during cold-storage (Weerahewa, 2002). Increased acid levels in cv. 'Mauritius' might be an important factor in the development of increased internal browning. Among other changes were PPO and POD enzyme activities, which increased steadily during 3 weeks of cold storage, the initial rise in which coincided with the onset of internal browning symptoms. Heat-treatment did not appear to affect the levels of these enzymes.

Fruit wrapped in polythene packing after heat-treatment showed reduced internal browning by a further 10%. Browning of fruit is due to the formation of polyphenolic compounds and this reaction required the presence of oxygen. Another advantage of polythene packing is reduced water loss following heat-treatment, giving a better appearance to the fruit. Heat-treatment perturbs surface wax, accelerating desiccation while, on the other hand, polythene packaging maintains humidity and protects the fruit. Polythene wrapping of fruit also slightly delayed ripening of heat-treated fruits. However, the small increase in pulp TSS content caused by this treatment could not be explained.

In conclusion, harvesting pineapple fruit at a mature, but 100% green stage and a dip treatment in water at 38°C for 60 min immediately after harvest, followed by polythene wrapping, reduced internal browning during cold-storage. Large-scale commercial trials would be needed prior to recommending these as widespread control measures.

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