



Inhibition of chilling injury and quality changes in pineapple fruit with prestorage heat treatment

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Abstract

Chilling injury limits refrigeration of tropical fruits. The development of injury symptoms during and after slight to extreme chilling treatment and the effects of 24-hour exposure to 38°C prior to chilling were determined in this study. Mature-green pineapple (*Ananas comosus*) fruits cv. Smooth Cayenne with crowns intact were chilled at 0, 5 or 10°C for 15 days and transferred to 25°C for 6 days. Relative humidity during heat treatment and storage was maintained at 85%. During the chilling period, shell discoloration was minimal. After transfer to 25°C, it increased dramatically and was more severe at lower chilling temperatures. Flesh browning similarly developed after transfer to 25°C only in fruits chilled at 0-5°C, affecting more than 75% of the core and fruitlets. Fruit cracks were unexpectedly observed at 0-5°C and did not increase during subsequent storage at 25°C. Heat treatment remarkably reduced shell and flesh discoloration and totally prevented fruit cracking. However, it did not affect chill damage in the crown. Colorimetric attributes L* (lightness), b* and C* (chroma) provided good quantitative measures of shell color while only L* was consistent with flesh color. Respiration rate and weight loss increased after heat treatment but during chilling, these decreased to levels lower than that of unheated fruits. Shell yellowing was inhibited at 0-10°C and later induced at 25°C. Fruits continuously held at 25°C turned full yellow after 9 days. Sugar content, juiciness and juice pH were not markedly affected by the treatments while fruit firmness, titratable acidity and ascorbic acid content decreased as a consequence of chilling. Heat treatment improved shell color quality and maintained firmer texture of fruits chilled at 5°C and higher ascorbic acid content of fruits chilled at 10°C.

Key words: Pineapple, *Ananas comosus*, cold storage, chilling injury, fruit quality.

Introduction

Chilling injury is the primary factor limiting storage and transport of fresh tropical fruits at refrigerated temperatures. Pineapple (*Ananas comosus*), for example, can be stored only at 8-13°C since lower temperatures (i.e. above freezing point of fruit tissues) would induce the disorder usually manifested as dull coloration or browning of shell, wilting, drying and discoloration of the crown and breakdown and browning of internal tissues¹⁻⁶. Increasing chilling tolerance enables the use of much lower temperatures for more effective control of fruit quality and shelf-life and for greater flexibility in marketing and utilization. Recently, heat treatment is receiving increased attention as a non-chemical and non-polluting method to improve the keeping quality of fresh fruits, including chilling tolerance induction. In fruits like mango, avocado, tomato, apple, peach, nectarine and oranges, chilling injury was reduced by pre-storage exposure to 36-46°C air for 8 hours to 4 days or dipping in 45-60°C water for 3-60 minutes⁷⁻¹⁴. The same effect was produced by heat treatment involving warming of fruits for 1-3 days at 10-20°C after 4 days to 2 weeks of cold storage¹⁵⁻¹⁷. Aside from its inhibitory effect on chilling injury, heat treatment with cold storage was found to slow down fruit ripening, decrease disease infection and improve cold tolerance in quarantine cold treatment for fruit fly disinfestation^{11-14,18}. In pineapple, exposure to 32-37°C for 24 hours minimized the internal brown spotting of fruits stored at 7°C, with the post-storage heat treatment being more effective than the prestorage treatment¹⁹. However, in a

later study using 12-hour exposure to 45°C prior to chilling storage, development of injury symptoms was promoted³. The present study re-examined the effects of prestorage heat treatment using constant temperature exposure on injury development and quality changes in the pineapple fruit during and after slight to extreme chilling storage.

Materials and Methods

Fruit sampling: Freshly harvested 'Smooth Cayenne' pineapple fruits at the mature-green stage and with crowns intact were obtained from a commercial orchard. The fruits measured 15.7±1.0 cm in length and 12.2±0.5 cm in mid-portion diameter and weighed 1.5±0.2 kg. For each treatment per replicate, the fruit samples were divided into two groups, one group with four fruits for non-invasive sampling and the other group for internal fruit temperature and physicochemical measurements requiring destructive sampling. All treatments were replicated four times.

Heat treatment: The fruits were exposed to 38°C at 85% relative humidity (RH) for 24 hours in a constant temperature-humidity chamber (Tabai PSL-2E). The treatment was adapted from previous works^{10,19}. Timing of heat treatment was started when the fruit temperature reached the desired level. Temperature was monitored using a T-type thermocouple wire inserted into the mid-portion of

the fruit and recorded with a data logger (Chino Digital Recorder DR-015). After heat treatment the fruits were examined for heat injury.

Chilling and storage conditions: The heated fruits together with the unheated ones were chilled for 15 days at 0°C (extreme chilling), 5°C (moderate chilling) or 10°C (slight chilling) using cold chambers (Hitachi CR-14C) and then transferred to 25°C chamber (Tabai PR-3G) for 6 days to allow ripening and simulate retail marketing. RH during and after chilling was maintained at 85%. Air temperature and humidity inside the chambers were measured with a hygrometer (ESPEC Thermo Recorder RS-10). Internal fruit temperature was also monitored as described above. Unheated fruits continuously held at 25°C and 85% RH were included for comparison.

Chilling injury determination: Fruit surface or shell discoloration was rated using an index of 0-3, with 0 none, 1 slight <10%, 2 moderate 10-25% and 3 severe >25% surface area discolored. Flesh discoloration was measured after slicing the fruit into half along its core, using an index of 0-5 with 0 none, 1 trace, 2 slight up to 25%, 3 moderate up to 50%, 4 severe up to 75% and 5 very severe more than 75% of flesh (core and fruitlets) discolored. Quantitative color readings were obtained using Minolta CR-200b Chromameter, taking the lightness (L^*) value and the a^* and b^* color space coordinates which were used to calculate the chroma (C^*) and hue angle (h°)^{20,21}. Values presented for shell color are means of readings taken from the upper half (towards the crown) and lower half of each fruit while those for flesh color are means of readings taken from the top (near the crown), middle and basal part of the fruit cut into half. The presence of other chilling injury symptoms was noted and rated using a scale of 0 (none) to 3 (severe symptom).

Respiration and weight loss measurement: The KOH absorption method⁵ of measuring CO₂ production was followed with slight modification. Each fruit was placed inside a fixed-volume respiration container together with 25 ml 2.0 N potassium hydroxide (KOH) contained in a 40 ml beaker. A respiration container with the KOH solution only was included as control. The containers were sealed, wrapped with black polyethylene sheet to simulate dark condition and placed inside the respective storage or treatment chamber. After 2 hours, the KOH solution was dispensed into a 250 ml volumetric flask containing 10 ml 2.0 N barium chloride (BaCl₂), mixed thoroughly, and the volume was made to 250 ml with distilled water. After 24 hours, 5 ml supernatant was titrated with 0.2 N hydrochloric acid using 1% phenolphthalein as indicator and an automatic titration system (Metrohm Multi-Dosimat Model E645) until the solution became colorless. Respiration rate in CO₂ mg kg⁻¹ h⁻¹ was calculated accordingly.

Weight loss after 1 day corresponding to the end of the heat treatment period was calculated as percentage of the initial weight while that at the end of the 15-day chilling and 6-day post-chilling at 25°C as percentage of the weight at the start of chilling and post-chilling period, respectively. The sum of the weight loss at these 3 periods was taken as the total weight loss.

Fruit quality parameters: Shell yellowing was rated using a scale of 1-6, with 1 green, 2 breaker, trace of yellow, 3 more green than yellow, 4 more yellow than green, 5 yellow with trace of green and 6 full yellow, in addition to colorimetric readings described

earlier. Fruit firmness was measured using an universal testing machine (Toyo-Boldwin Tensilon UTM-4-100) fitted with 8 mm-diameter plunger. The maximum force required to compress the shell at a constant speed of 10 mm/min. was recorded. Values presented are means of readings from the top, middle and base of each fruit. Quality attributes of the flesh were determined. Juiciness was approximated based on the moisture content determined by oven-drying at 110°C for 24 hours. Soluble solids content was taken as a rough measure of the sugar content using a digital refractometer (Atago PR-1). Titratable acidity as percent citric acid was measured by the titrimetric method using 1.0 N sodium hydroxide and 1% phenolphthalein as indicator while pH of a sample juice was taken using an electronic pH meter (Horiba F-11). Vitamin C content was analyzed using a Boehringer-Mannheim F-kit and expressed as mg/l L-ascorbic acid.

Statistical analysis: Variance analysis for completely randomized design experiments and mean comparison by the Duncan's multiple range test (DMRT) were performed using MSTAT program.

Results and Discussion

Chilling injury development: Injury symptoms during the 15-day chilling period were minimal and visible as dull coloration and darkening of the green shell (Table 1). After transfer to 25°C, symptom development dramatically increased. Brown patches appeared and some portions of the darkened green shell failed to turn yellow. The symptoms were more severe at lower chilling temperatures. It was rated highest at 0°C, with the maximum score of 3.0 (severe or more than 25% of fruit surface affected), moderate to severe (score of 2.3) at 5°C, and slight to moderate (score of 1.6) at 10°C. Heat treatment remarkably decreased symptom severity to slight to moderate (score of 1.2-1.7) at 0-5°C and very slight (score of <1.0) at 10°C. No heat injury was noted, except for the drying of some bracts and small brown spotting in fruits held at 0°C. The latter symptom was apparently not chill-induced since it developed during the first 3 days of chilling and did not increase thereafter even after transfer to 25°C. Fruits stored continuously at 25°C did not show any off-color of the shell which turned full yellow (yellowing index of 6.0) after 9 days. Shell yellowing was inhibited during chilling at 0-10°C and developed only after transfer to 25°C (Table 1). In fruits chilled at 0°C, heated fruits showed more yellow coloration (4.8) because of less tissue browning than that of unheated fruits (4.0). In fruits chilled at 5°C, heat treatment retarded shell yellowing, indicated as greener shell (4.2) than that of unheated fruits (5.2). Heat treatment did not significantly affect yellowing of fruits chilled at 10°C. Heated and unheated fruits had a yellowing index of 5.7-6.0 comparable to that of unchilled fruits held at 25°C.

L^* , b^* and C^* quantitatively measured shell color (Table 1). The heated fruits, which had reduced shell discoloration, had higher L^* values, indicating lighter color and less color darkening than the unheated fruits. Differences in L^* values among treatments were very pronounced after 6 days holding at 25°C when the shell turned yellow and L^* readings increased. The magnitude of increase in L^* was greater in heated fruits than in unheated ones. Similarly, the heated fruits were more chromatic (higher C^*) than the unheated fruits. High L^* and C^* values indicate that the fruits developed a bright yellow shell. Furthermore, it can be seen from Table 1 that chromaticity of unheated fruits chilled at 0-5°C did

Table 1. Shell color attributes of pineapple fruit at the end of 15-day chilling at 0-10°C and 6-day post-chilling at 25°C.

Treatment	Discoloration index ¹	L*	b*	C*	h°	Yellowing index ¹
1. After 15d chilling						
Heated fruits						
0°C	0.7ef	28.3ef	15.8d	16.0d	97.5ab	1.0d
5°C	0.5f	28.0ef	12.5def	13.3de	104.2a	1.2d
10°C	0.1g	32.8d	20.1c	21.2c	99.7ab	1.6d
Unheated fruits						
0°C	1.0de	26.2f	10.3efg	11.0ef	106.3a	1.0d
5°C	0.7ef	27.0f	13.2def	13.4de	105.2a	1.0d
10°C	0.4f	28.0f	14.0de	14.9d	106.0a	1.0d
2. After 6d post-chilling						
Heated fruits						
0°C	1.7c	38.1bc	22.5c	23.0c	75.5cd	4.8bc
5°C	1.2cd	37.0bc	22.2c	21.8c	84.3bc	4.2c
10°C	0.7fe	46.4a	35.0a	36.0a	71.0cd	6.0a
Unheated fruits						
0°C	3.0a	27.0f	7.9g	8.1f	67.0d	4.0c
5°C	2.3b	35.3cd	14.0de	15.0d	67.2d	5.2ab
10°C	1.6c	41.5b	29.0b	30.3b	71.5cd	5.7a
Continuous 25°C*	0.0g	48.5a	37.8a	39.8a	74.0cd	6.0a
Before storage (initial)	0.0g	31.5de	10.3efg	10.3f	99.0ab	1.0d

¹Discoloration index: 0 (none) to 3 (severe, >25% surface discolored); Yellowing index: 1 (green) to 6 (full yellow)

*Taken after 9d storage when fruits turned full yellow. Mean separation within columns by DMRT, 5%.

Table 2. Flesh color attributes of pineapple fruit at the end of 15-day chilling at 0-10°C and 6-day post-chilling at 25°C.

Treatment	Discoloration index ¹	L*	b*	C*	h°	Fruit cracking ¹
1. After 15d chilling						
Heated fruits						
0°C	0.0c	68.5ab	20.5ab	20.5abcd	94.0efg	0c
5°C	0.0c	73.4ab	18.7abc	18.7bcd	94.7def	0c
10°C	0.0c	75.5a	19.4abc	19.6abcd	98.2abc	0c
Unheated fruits						
0°C	0.0c	68.9ab	15.6c	15.7d	96.5cde	2a
5°C	0.0c	75.6a	18.7abcd	18.8abcd	96.9bcd	1b
10°C	0.0c	75.6a	16.3bc	16.5cd	100.4a	0c
2. After 6d post-chilling at 25°C						
Heated fruits						
0°C	3.0b	63.6b	21.3a	21.3abc	92.8fg	0c
5°C	2.8b	65.1ab	19.2abc	19.2abcd	93.4fg	0c
10°C	0.0c	76.6a	20.1abc	20.3abcd	96.5cde	0c
Unheated fruits						
0°C	5.0a	53.4c	19.8abc	19.8abcd	93.1fg	2a
5°C	5.0a	53.3c	19.4abc	19.4abcd	91.9g	1b
10°C	0.0c	72.7ab	22.8a	22.9ab	95.2def	0c
Continuous 25°C*	0.0c	73.3ab	23.4a	23.7a	98.2abc	0c
Before storage (initial)	0.0c	76.5a	20.6ab	20.9abc	99.1ab	0c

¹Discoloration index: 0 (none) to 5 (>75% surface discolored); Fruit cracking index: 0 (none) to 3 (severe)

*Taken after 9d storage when fruits turned full yellow. Mean separation within columns by DMRT, 5%.

not increase even if some shell parts turned yellow. This implies that shell color was not only brown but also dull yellow, both of which are low in chroma. The b* values showed very similar trend as C*. The hue angle (h°) did not compare well with shell color changes (Table 1) similar to that of a* values (results not shown).

Flesh color appeared normal after 15 days chilling at 0-10°C (Table 2). After 6 days at 25°C, the fruits chilled at 0-5°C showed massive flesh browning, with more than 75% of the core and fruitlets turning brown (score of 5). With heat treatment, flesh browning significantly decreased to 2.8-3.0 (25-50% browning). Fruits chilled at 10°C with or without heat treatment had no flesh

browning similar to fruits continuously held at 25°C. Among the colorimetric parameters only L* showed variations consistent with qualitative indexing (Table 2). L* was distinctly lowest in unheated fruits chilled at 0-5°C after 6 days at 25°C due to the massive browning while heated fruits had higher L* values due to reduced browning. Fruits from the other treatments where flesh browning was absent had comparably high L* values, but C*, b* and h° had no clear relationship with flesh color.

Fruit cracking, which seemed to separate the fruitlets from each other, was noted during chilling at 0-5°C, affecting only the unheated fruits with more severe symptom at 0°C than at 5°C

(Table 2). It developed at the later part of the chilling period and did not increase during subsequent storage at 25°C. In the crown, injury symptom likewise developed only at 0-5°C as water-soaked areas that later turned brown. Heat treatment had no marked influence on crown injury development (results not shown).

The inhibitory effect of prestorage heat treatment on the development of chilling injury symptoms in the fruit shell and flesh seems to concur with previous findings in other fruits^{10, 13, 14} and in pineapple exposed to non-constant high temperature treatment¹⁹. Colorimetric measurements enabled quantification of symptom severity. For shell color, L*, b* and C* could be good objective measures. In a previous study, L* and C* were considered as reliable quantitative measures of chilling damage on green and ripe peel²². In the present study, using b* and C* seems to be redundant since both have the same trend. Perhaps, b* is already sufficient. Computed from the a* and b* values, the C* values will not differ much from the b* values if the a* would only be 50% less than the b* values, as observed in this study. For flesh color, only L* provided a very good quantitative measure of chilling damage while the other colorimetric parameters did not give variations consistent with the qualitative indexing. These results partly agree with earlier findings that L* and b* coordinates could provide good objective measures of flesh color²³. Furthermore, the development of fruit cracks as a response to chilling at 0-5°C was not anticipated. There is no available report describing the occurrence of this phenomenon. The presence of fruit cracks was observed to promote mold growth.

Respiration rate and weight loss: Initial respiration rates were lowest at 0°C and highest at 38°C, the latter being exhibited by fruits exposed to the heat treatment prior to chilling (Fig. 1A). The same respiratory trend was obtained during the chilling period, that is, fruits held at higher temperatures had also higher rates of respiration (Fig. 1B). However, heated fruits showed much reduced rates of about 50% lower than that of unheated fruits. After transfer to 25°C, respiration rates increased and did not differ much with treatment, except for fruits chilled at 0°C in which the rates were considerably higher in unheated fruits than in heated ones (Fig. 1C).

Weight loss increased also after the 24-hour heat treatment to about 3-4% from less than 1% at 0-10°C (Fig. 2A). During the

15-day chilling period and similar to the trend in respiration, the heated fruits incurred lower weight loss than the unheated fruits (Fig. 2B). Weight loss of fruits chilled at 0°C was the highest among treatments. The same trend was repeated during the 6-day post-chilling period at 25°C (Fig. 2C). In terms of total weight loss, it was highest in fruits chilled at 0°C (18.4%) and was reduced by heat treatment (15.9%) (Table 3). Fruits chilled at 5-10°C had comparable weight loss regardless of heat treatment (10.8-13.2%). A major portion (25-30%) of the total weight loss of heated fruits was due to the weight loss after the 24-hour heat treatment. Unchilled fruits held at 25°C had the lowest weight loss among treatments (5.9%) as a natural consequence of shortened storage period of 9 days. In contrast, the chilled fruits had a total holding period of 21 days, with the 6-day post-chilling being necessary to allow sufficient shell yellowing to occur.

The rate of respiration or carbon dioxide production serves as a physiological measure of enzyme-mediated cellular processes while weight loss physically reflects the intensity of metabolic activities in fruit tissues including non-enzymatic processes (e.g. transpiration rate). Respiration and weight loss would normally increase with increasing temperature, as also observed in the present study. When faced with stress, such as chilling, and to cope with it, respiration and energy generation must continue to support vital life processes in cells and maintain metabolic balance. If tissues possess a stress-coping mechanism, which seemed to be induced by prestorage heat treatment as implied in the results, the needed metabolic and respiratory reaction to counter the stress is lowered. This may explain the reduced respiration rates and weight loss of heated fruits during the chilling period. Similar reduction in respiration rate and weight loss in response to heat treatment was obtained in previous studies^{9, 10, 14}. When tissues could not cope with the stress, metabolic aberrations and tissue breakdown set in. In this study, these were manifested as abnormally high levels of respiration rate and weight loss of fruits chilled at 0°C, with the heat treatment exerting a modulating effect (Figs 1B, 2B). In extreme condition, total loss of tissue integrity occurs. In unheated fruits kept at 0°C, this resulted to cell death, which later turned into brown patches and cracking. This fruit condition favored secondary causes of deterioration, particularly mold invasion which grew and proliferated during subsequent

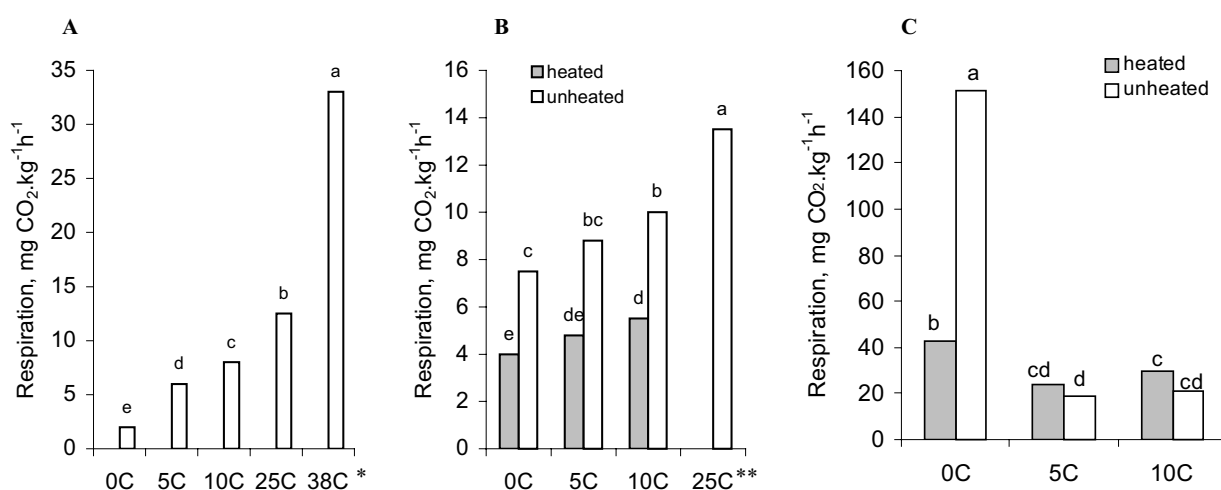


Figure 1. Respiration rate of pineapple fruit after 1 day (A) and at the end of 15-day chilling at 0-10°C (B) and 6-day post-chilling at 25°C (C). (*Taken after heat treatment and before chilling. **Taken after 9d storage when fruits turned full yellow. Mean separation by DMRT, 5%).

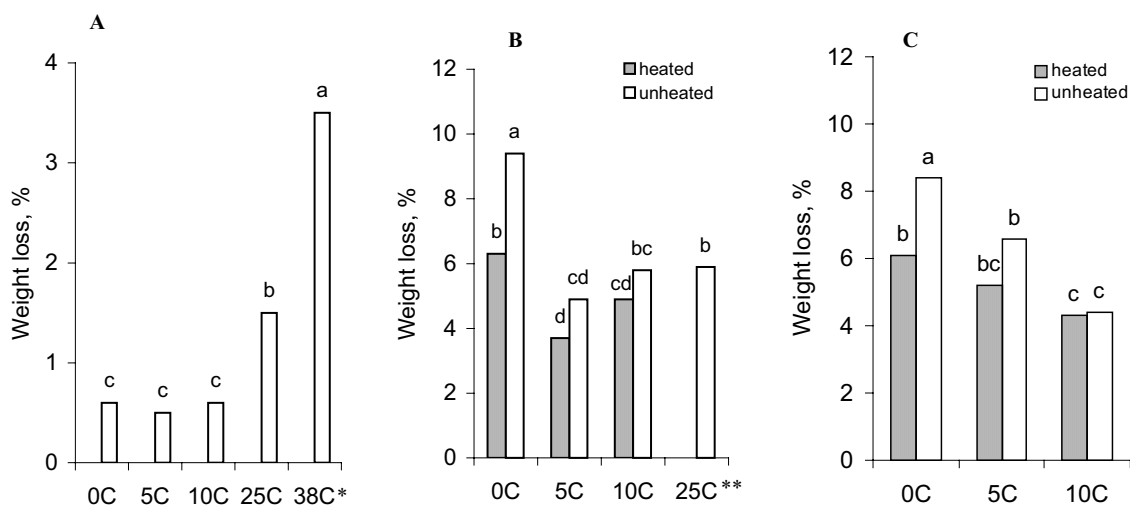


Figure 2. Weight loss of pineapple fruit after 1 day (A) and during the 15-day chilling at 0-10°C (B) and 6-day post-chilling at 25°C (C). (*Taken after heat treatment and before chilling. **Taken after 9d storage when fruits turned full yellow. Mean separation by DMRT, 5%.)

holding at 25°C. As a consequence, respiration rate and weight loss sharply increased (Figs 1C, 2C).

Physicochemical quality: Firmness of chilled fruits was generally lower than that of fruits held continuously at 25°C (Table 3). Firmness loss increased with decreasing chilling temperatures. Heat treatment improved firmness retention only in fruits chilled at 5°C. Quality attributes of the flesh, particularly moisture content as a measure of juiciness, soluble solids content as a measure of sugar content and sweetness and juice pH did not greatly vary with treatment (Table 3). Moisture content ranged from 85.5-87.6%, soluble solids content from 12.8-14.8°B and pH from 3.4-3.6. However, titratable acidity and ascorbic acid levels significantly decreased in response to chilling at 0-5°C which was not altered by heat treatment (Table 3). With 10°C chilling, titratable acidity was not adversely affected while ascorbic acid content significantly decreased in unheated fruits. The heated fruits had ascorbic acid levels comparable to that of fruits continuously held at 25°C.

The results demonstrate that the prestorage heat treatment had no deleterious effects on quality. Instead, it was the chilling exposure that caused the losses in some quality attributes, in addition to its destructive effect on shell and flesh color. The

heat treatment, which minimized fruit discoloration, had an added benefit particularly during slight chilling (i.e. 10°C). It maintained ascorbic acid levels similar to that of unchilled fruits. This effect has important nutritional quality implication. The application of heat treatment prior to 10°C storage would be more advantageous if weight loss during the 24-hour treatment at 38°C is reduced. Weight loss reduction increases economic gains in marketing situations where the fruits are traded by weight basis. On the other hand, heat treatment prior to moderate (i.e. 5°C) or extreme (i.e. 0°C) chilling storage is promising as it markedly decreased injury symptoms. However, as a single postharvest intervention, heat treatment could not possibly secure the successful storage at 0-5°C since it did not reverse the detrimental effects of chilling on some quality attributes (e.g. ascorbic acid loss), aside from the fact that it did not completely inhibit chilling injury. It would probably be more feasible to combine heat treatment with other safe techniques (e.g. modified atmosphere systems). This integrated approach could produce additive or synergistic effects on increasing chilling tolerance and enhancing quality and shelf-life of the fruit.

Table 3. Physicochemical attributes of pineapple fruit after 6-day post-chilling at 25°C.

Treatment	Total weight loss, (%)	Firmness (kg)	Moisture content (%)	Soluble solids (°B)	Titratable acidity (% citrate)	pH	Ascorbic acid (mg/l)
Heated fruits							
0°C	15.9b	7.8cd	86.2	13.6	0.46b	3.5	77.4cd
5°C	12.0c	10.3ab	86.5	13.0	0.50b	3.6	91.5bc
10°C	13.2bc	8.4bc	87.6	12.8	0.61a	3.5	146.3a
Unheated fruits							
0°C	18.4a	6.2d	87.3	13.0	0.52b	3.4	57.7d
5°C	12.0c	7.9cd	85.5	14.8	0.50b	3.5	104.1b
10°C	10.8c	8.4bc	86.9	14.4	0.63a	3.4	112.6b
Continuous 25°C*	5.9d	10.9a	87.1	13.1	0.66a	3.4	166.0a

*Taken after 9d storage when fruits turned full yellow. Mean separation within columns by DMRT, 5%.

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