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Original Article

Delayed softening of "Hom Thong" banana fruits during postharvest storage following hot water treatment

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Abstract

This study investigated the effects of hot water treatment on the texture of banana fruits. Fruits were dipped in hot water at 50 ºC and 55 ºC for 10 min before being stored at 25 ºC. The firmness of fruits treated at 50 ºC was higher than that of untreated fruits, and physiological changes including fresh weight loss, ethylene production, total soluble solid content, and peel color were also prevented. Treatment at 55 ºC slowed softening, but induced peel damage and failure to develop a normal color. Investigation of the cell wall composition and structure following treatment at 50 ºC showed a delay in the increase of water-soluble pectin, correlated with fruit firmness. A higher level of HCl-soluble pectin was detected in treated fruits, indicating that cell wall polymer solubilization had been reduced. Structural alteration to the peel was also slower than in untreated fruits. The study clarified the effect of hot water treatment on alteration of the cell wall structure and composition of banana fruit during postharvest storage.

Keywords: banana, hot water treatment, fruit softening, cell wall composition, cell wall structure

1. Introduction

Banana fruit is one of the most economically important and nutritious fruits. Several changes occur during banana fruit ripening: an increase in ethylene production, respiration, chlorophyll breakdown, starch degradation, and sugar biosynthesis (Amnuaysin, Jones, & Seraypheap, 2012). A significant additional important change is a softening in the texture of the fruit (Lurie, 1998). Excessive softening shortens storage life, encourages physical damage during handling, and increases vulnerability to postharvest pests and diseases (Brummell & Harpster, 2001).

Fruit softening is correlated with modifications to the polysaccharide components of the middle lamella and primary cell wall during ripening, leading to the alteration of the cell wall structure (Brummell, 2006). Changes to the cell wall of several fruits during ripening have been observed. Harker, Redgwell, Hallett, Murray, and Carter (1997) reported that these were related to structural changes in the walls of the parenchyma cells. Biochemical studies of cell wall changes during fruit ripening indicate that modifications of pectin, hemicellulose, and cellulose are the main factors. These are three groups of cell wall polysaccharide. Pectin is the main component of the middle lamella and primary cell wall, and plays a role in the adhesion between cells. Ilker and Szczesniak (1990) reported that alterations in pectin composition are involved in the reduction of cell adhesion, resulting in textural changes. Softening of ripening fruit is associated with pectin solubilization in the cell wall (Brummell & Labavitch, 1997). As fruits ripen, there is an increase in water-soluble pectin and a decrease in insoluble pectin (Rosli, Civello, & Martinaz, 2004). Solubilization and depolymerisation of pectin have been reported in tomatoes (Brummell & Labavitch, 1997). Depolymerization of pectin

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polysaccharides is responsible for softening of banana fruit (Duan *et al.*, 2008). Though attention has been focused on pectin modification as the main factor in fruit softening, hemicellulose and cellulose are also thought to play important roles in the process. Hemicelluloses bind to cellulose microfibrils via hydrogen bonds and crosslinking between cellulose microfibrils, and the resulting network provides strength to the cellular wall (Cheng *et al.*, 2009). Degradation of hemicellulose polysaccharides is therefore believed to be a factor in fruit softening. The reduction of hemicellulose has been observed in fruits including papayas (Manrique & Lajolo, 2004), strawberries (Lara, Garcia, & Vendrell, 2006), and bananas (Cheng *et al.*, 2009). Loss of hemicellulose and cellulose has also been reported in strawberries, and of cellulose in grapes (Deng, Wu, & Li, 2005). The modification of cell wall polysaccharides results from the combined action of multiple enzymes, including pectin methylesterase (PME), polygalacturonase (PG), and pectate lyase (PL). These alter the cell wall properties.

Postharvest heat treatment has been used to maintain fruit quality during storage, and has been shown to be effective in delaying softening of many fruits (Lurie, 1998). The firmness of cherries has been preserved by exposure to a temperature of 70 ^{o}C before freezing (Alonso, Canet, & Rodriguez, 1993). The use of hot water treatment to reduce spoilage and microbial development also delayed softening of banana fruits (Hassan, 2000). A study of the apple cell wall demonstrated that treatment with 38 ^{o}C air for four days reduced the levels of soluble pectin and increased those of insoluble pectin, compared with untreated fruit, indicating the inhibition of uronic acid degradation (Ben-Shalom, Hanzon, Pinto, & Lurie. 1996). The dissolution of the middle lamella and destruction of the papaya cell wall were inhibited by treatment with water at 49 ^{o}C for 120 min (Bacay-Roldan & Serrano, 2005). In the study of strawberries by Vicente, Costa, Martinaz, Chaves, and Civello (2005), heat treatment delayed hemicellulose degradation and affected pectin solubilization. Heat treatment modifies cell wall polysaccharides, affecting enzyme degradation (Lurie, 1998). Temperatures up to 45 ^{o}C disrupt these enzymes and slow softening (Paull & Chen, 2000).

The role of hot water treatment (HWT) in enhancing the postharvest shelf life of bananas has been elucidated by a number of groups. However, its role in the modification of cell wall composition and structure of Thai 'Hom Thong' banana has not been examined. To clarify the physiological and biochemical bases of cell wall stability enhancement by postharvest HWT, we investigated the association between banana fruit softening, cell wall composition, and structural change in 'Hom Thong'. We provide evidence that, in addition to modifying ethylene production, the HWT prolongs shelf life by regulating processes including fruit softening, delaying solubilization of cell wall polymers, in particular pectin, and slowing structural changes in cell shape and size, peel thickness, and formation of intercellular spaces.

2. Materials and Methods

2.1 Plant material and hot water treatment

Hands of banana fruits (*Musa acuminata*, AAA group, Gros Michel subgroup, cultivar 'Hom Thong') were harvested at the mature green stage from a local farm and transferred to the laboratory within two hours. The fruits were sorted to ensure uniformity of size and color and lack of noticeable defects. The fruits were cut into fingers and classified into three groups. A control group was dipped in water at 25 ^{o}C for 10 min. The two test groups were dipped in hot water at 50 and 55 ^{o}C, again for 10 min. After treatment, the fruits were air-dried and stored in corrugated cardboard boxes at 25±1 ^{o}C until ripening. Measurements were performed every two days.

2.2 Physiological measurements

2.2.1 Fresh weight loss

Loss of fresh weight was measured by weighing individual fruits before and after treatment and was expressed as percentage loss.

2.2.2 Firmness

The firmness of banana peel was determined using a penetrometer hardness tester with 12 mm cylindrical probe (FHM-1, Takemura, Japan). Firmness readings were made at three points: the blossom end, middle, and stem end. Data were recorded as kilogram-force and converted to Newton (N).

2.2.3 Total soluble solids (TSS)

Pulp tissue was extracted and the TSS was analyzed using a hand refractometer (model N-1E; Atago Co, Ltd., Japan). The fruit pulp was homogenized in distilled water (1:2 ratio) and the mixture was centrifuged at 8,000 x g for 5 min. A drop of the clear supernatant was then placed on the prism glass of the refractometer to obtain the %TSS value. This was multiplied by the dilution factor to recover the original %TSS of the pulp.

2.2.4 Peel color

The color was determined from the lightness (L value) and hue angle (h°), using a Color Konica Minolta CR-10 (Konica Minolta Sensing, Inc., Japan). Measurements were taken at the same three points used in the firmness measurements, and a mean value was calculated for each individual fruit. The peel color was expressed as L value and hue angle.

2.2.5 Ethylene production rate

Banana fruits were placed in an airtight glass jar (2.4 liters volume) equipped with a rubber stopper, and air samples were taken by syringe after 1 h of incubation. Ethylene was determined using a Shimadzu G-14A gas chromatograph (Shimadzu, Japan) equipped with an activated Poropak 80/100 column and a flame ionization detector. Ethylene levels were expressed in microliters per kilogram per hour.

2.3 Cell wall analysis

2.3.1 Isolation of cell wall polysaccharides

Cell wall polysaccharides were isolated using a method adapted from Rosli, Civello, and Martinez (2004). Approximately 10 g of frozen peel was homogenized with 40 mL of absolute ethanol, and then boiled for 30 min while shaking to inactivate cell wall degrading enzymes. The homogenate was filtered and the residue was washed three times with 15 mL of absolute ethanol. The residue was incubated overnight at 37 °C to evaporate the solvent then weighed. Cell wall fractions were extracted from the dried residue or alcohol insoluble residue (AIR).

2.3.2 Extraction and quantification of cell wall fractions

AIR extraction was done by a sequential chemical method that allowed separation into cell wall fractions, following Rosli, Civello, and Martinez (2004) and Figueroa *et al.* (2010) with slight modification. A 100 mg aliquot of AIR was suspended in 100 ml of water and shaken overnight at 20 °C. The homogenate was filtered and washed three times with 10 mL of water. The filtrates were pooled and collected as water-soluble pectin fraction (WSP). The water-insoluble residue was shaken in 100 mL of 0.05 M sodium acetate (pH 4.5) containing 0.04 M EDTA for 4 hrs at 20 °C. The homogenate was filtered and washed three times with 10 mL of the same buffer. The filtrates were pooled and collected as EDTA-soluble pectin fraction (ESP). The residue was again suspended in 100 ml of 0.05M HCl at 100 °C with stirring for 1 hr. The homogenate was cooled to room temperature, filtered, and then washed three times with 7 mL of 0.05M HCl. The filtrates were pooled and collected as HCl-soluble pectin fraction (HSP). The residue remaining from pectin extraction was extracted in 100 mL of 4M NaOH and shaken for 8 hrs at 20 °C. The homogenate was filtered and washed three times with 5 mL of 4M NaOH. The filtrates were pooled and collected as hemicellulose. The final residue was hydrolyzed in 12 mL of 72% (w/w) H_2SO_4, shaken for 1 h at 30 °C, then diluted to 4% (w/w) H_2SO_4 (d' Amour, Gosselin, Arul, Castaigne, & Willemot, 1993). The uronic acid concentrations in WSP, ESP, and HSP were quantified using the m-hydroxydiphenyl method (Blumenkrantz & Asboe-Hansen, 1973) with galacturonic acid as the standard. Hemicellulose and cellulose concentrations were quantified using the anthrone method (Nigam & Ayyagari, 2007) with glucose as the standard.

2.4 Microscopy observation

Small pieces (5x5 mm) of peel were cut from the mid zone of test and control group fruits and fixed in formalin-aceto-alcohol (FAA). The fixed tissue was dehydrated in ethanol and embedded in paraffin wax, following the method of Ruzin (1999). The paraffin-embedded tissues were sectioned to a thickness of approximately 10 µm using microtome and attached to microscope slides. The sections were stained with safranin and fast green, and examined for structural change under a light microscope. The tissue sections were chosen randomly. Epidermal cells, parenchyma cells, fiber, and vascular tissues were classified as either collapsed or normal. A collapsed cell was identified by the presence of a collapsed cell wall and large intercellular space. Tissue sections of 2x2 mm were evaluated for peel thickness, number of layers, and percentage air space.

2.5 Statistical analysis

A completely randomized design (CRD) with four replications was used. Analysis of variance (ANOVA) was applied to identify significant treatment effects, with an alpha of 0.05. Means were compared using an LSD test and independent sample t-tests. Statistics were run on SPSS software.

3. Results and Discussion

3.1 Physiological changes

Postharvest HWT at 50 and 55 °C delayed ripening of the 'Hom Thong' banana fruits. The storage life of treated fruits was longer by four days than that of controls (data not shown). HWT slightly increased fresh weight loss of banana fruits during storage at 25 °C (Table 1) which has also been reported for tropical fruits such as papayas and mangoes. The increase was greater in fruits that were heat treated before storage, but there were no significant differences between control and treated fruits.

In the course of ripening the firmness of the fruits declined slowly, resulting in softening. Firmness decreased gradually in the first stage, then rapidly. Fruits treated with hot water before storage remained firmer than the control fruits in a temperature-dependent manner (Table 1). Heat treatment has been reported to delay softening of bananas (Hassan, Shipton, Coventry, & Gardiner, 2004), strawberries (Vicente, Costa, Martinaz, Chaves, & Civello, 2005) and mei fruit (Luo, 2006). This effect may be due to changes in the activity of cell wall-degrading enzymes such as PG and PME altering the cell wall components (Lurie, 1998).

TSS reflects the concentration of sugar, which is a major component of juice. The TSS content increased rapidly in the first four days of storage of both control and treated fruits, then became constant (Table 1). HWT had no effect on the TSS content of the pulp tissue. Similar results have been reported for tomatoes treated with air at 38-48 °C for 1 hr to 3 days (Paull & Chen, 2000).

The peel color changed from green to yellow during fruit development. The color was expressed as both L value and hue angle; the L value increased over the ripening period whereas the hue angle decreased. The L value increased slowly in the first stage and then became constant (Table 1). No significant difference in L values was found between control and fruits treated at 50 °C, but the L value of fruits treated at 55 °C was significantly lower than that of control.

Fruits treated with hot water at 55 °C showed the highest hue angle, followed by fruits treated at 50 °C, then the untreated fruits (Table 1). A previous study reported that higher temperatures delayed chlorophyll breakdown in banana peel by inhibiting Mg-dechelatase activity (Yang, 2009). Nevertheless, fruits heated at 55 °C for 10 min had darker skin (lower L value and higher hue angle). This darkening occurs

Table 1. Physiological change to bananas water treated at 50 ºC and 55 ºC and untreated fruits during storage at 25 ºC.

Parameter	Storage time (days)					
	0	2	4	6	8	10
Fresh weight loss (%)						
Control	0 ± 0.00	2.43 ± 0.22^{ns}	4.94 ± 0.51^{ns}	7.79 ± 0.79^{ns}	10.82 ± 0.99^{ns}	14.00 ± 1.27^{ns}
HWT 50ºC	0 ± 0.00	2.74 ± 0.18^{ns}	5.54 ± 0.31^{ns}	8.34 ± 0.49^{ns}	11.43 ± 0.64^{ns}	14.89 ± 0.81^{ns}
HWT 55ºC	0 ± 0.00	2.61 ± 0.04^{ns}	5.50 ± 0.05^{ns}	8.99 ± 0.18^{ns}	12.46 ± 0.36^{ns}	15.97± 0.53^{ns}
Firmness (N)						
Control	7.22 ± 0.25^{ns}	6.80 ± 0.18^{ns}	5.60 ± 0.24^{b}	5.46 ± 0.68^{ab}	3.72 ± 0.20^{b}	2.77 ± 0.19^{b}
HWT 50ºC	7.33 ± 0.21^{ns}	6.35 ± 0.17^{ns}	6.05 ± 0.07^{b}	5.25 ± 0.18^{b}	5.17 ± 0.98^{ab}	3.56 ± 0.42^{b}
HWT 55ºC	7.15 ± 0.16^{ns}	6.74 ± 0.17^{ns}	6.98 ± 0.07^{a}	6.89 ± 0.43^{a}	6.84 ± 0.26^{a}	6.74 ± 0.44^{a}
TSS (ºBrix)						
Control	7.13 ± 2.24^{ns}	11.85 ± 4.11^{ns}	29.81 ± 0.39^{a}	20.96 ± 5.55^{ns}	26.70 ± 1.14^{ns}	23.70 ± 0.32^{ns}
HWT 50ºC	6.86 ± 1.84^{ns}	21.75 ± 5.07^{ns}	29.25 ± 0.78^{a}	27.60 ± 1.16^{ns}	22.50 ± 3.48^{ns}	24.08 ± 0.65^{ns}
HWT 55ºC	6.11 ± 1.35^{ns}	13.80± 2.47^{ns}	24.98 ± 0.63^{b}	21.38± 4.02^{ns}	22.65± 4.46^{ns}	25.39± 1.01^{ns}
L value						
Control	54.13 ± 1.40^{ns}	60.20 ± 2.49^{a}	68.97 ± 4.15^{a}	68.11 ± 3.22^{a}	69.26 ± 1.19^{a}	65.82 ± 3.78^{a}
HWT 50ºC	53.83 ±1.36^{ns}	55.38 ± 1.23^{ab}	64.28 ± 3.37^{a}	70.20 ± 0.55^{a}	68.78 ± 0.43^{a}	64.11 ± 2.30^{a}
HWT 55ºC	52.78± 1.07^{ns}	52.90 ± 1.01^{b}	48.49 ± 1.74^{b}	48.33 ± 1.03^{b}	40.18 ± 3.70^{b}	38.04 ± 2.72^{b}
hue angle						
Control	118.50±0.67^{ns}	114.32 ±2.66^{ns}	98.21 ±6.41^{ns}	96.13 ± 5.80^{ab}	89.03 ± 1.22^{b}	86.73 ± 1.42^{ns}
HWT 50ºC	119.68±0.64^{ns}	117.38± 0.51^{ns}	103.10±4.94^{ns}	90.41 ± 1.08^{b}	88.43 ± 0.74^{b}	85.18 ± 0.98^{ns}
HWT 55ºC	119.59±0.45^{ns}	117.76± 0.39^{ns}	113.56±0.96^{ns}	106.06 ±1.11^{a}	96.96 ± 1.20^{a}	90.53± 2.26^{ns}
C_2H_4 production rate (nl/kg.h)						
Control	2.04 ± 0.51^{ns}	3.07 ± 1.40^{ns}	3.82 ± 0.71^{a}	10.36 ± 3.50^{a}	9.41 ± 1.41^{ab}	2.49 ± 0.41^{ab}
HWT 50ºC	1.05 ± 0.20^{ns}	2.88 ± 0.89^{ns}	2.76 ± 0.28^{a}	4.64 ± 1.26^{ab}	10.39 ± 4.24^{a}	3.11 ± 0.34^{a}
HWT 55ºC	1.00 ± 0.18^{ns}	1.50 ± 0.58^{ns}	0.90 ± 0.31^{b}	2.55 ± 0.66^{b}	1.56 ± 0.34^{b}	1.60 ± 0.30^{b}

Different letters within column for each parameter in same day indicate significant difference ($P<0.05$). ^{ns} not significantly different.

at temperatures above 45 ºC (Paull & Chen, 2000). Surface browning has been observed in apples exposed to HWT at 65 ºC or higher for 2 min (Zuo, Lee, & Lee, 2004).

The ethylene production rate was low at the onset of ripening, then increased sharply. This is the defining characteristic of climacteric fruits. Ethylene production peaked on Day 6 for the control group, Day 8 for the 50 ºC treatment group, and Day 12 for the 55 ºC group (Table 1). All treated fruits had lower ethylene production than controls. Fruits treated at 55 ºC maintained low ethylene production throughout storage at 25 ºC. HWT decreased the rate of ethylene production and delayed the appearance of the ethylene climacteric peak in 'Hom Thong' banana, slightly retarding ripening. Disruption of ethylene production may mediate heat-induced inhibition of ripening. Previous study has shown that heat treatment can inhibit ethylene production in banana (Chopsri, Sekozawa, & Sugaya, 2018). Inhibition of ethylene production by HWT in mango has been attributed to inhibition of ACC synthase and ACC oxidase activities (Ketsa, Chidtragool, Klein, & Lurie, 1999).

3.2 Cell wall composition

From the results reported above, HWT at 50 and 55 ºC for 10 min may delay softening and maintain the physio-chemical quality of 'Hom Thong' bananas. However, treatment at 55 ºC produced negative effects including increased weight loss, peel damage, and failure to develop a normal color.

It is assumed that changes in fruit texture arise from modification of the cell wall components, producing disassembly of the middle lamella and primary cell wall structure. The effect of 50 ºC HWT on the cell wall components was therefore investigated further.

Cell wall materials were sequentially extracted using water, EDTA, and HCl. This yielded both loosely ionically- and covalently-bounded pectin fractions. NaOH and H_2SO_4 were used to solubilize hemicellulose and cellulose fractions, respectively. The WSP content increased throughout the ripening period. The water-soluble fraction in the control did not differ from that in treated fruits, but the latter showed a significantly lower WSP level (Figure 1A), indicating that HWT led to a decrease in pectin solubilization. This supported the findings of Woolf, MacRae, Spooner, and Redgwell (1997) for persimmons, that heat treatment delayed pectin solubilization. A similar decrease in soluble pectin and increase in insoluble pectin has been reported for hot water treated strawberries (Lara, Garcia, & Vendrell, 2006).

ESP attaches to the wall by ionic calcium bonds, and is thought to be partially homogalacturonan from the middle lamella (Brummell, Cin, Crisosto, & Labavitch, 2004). Moderate changes in ESP levels were observed during fruit development (Figure 1B), but remained at a low level. HWT had no effect on ESP content in 'Hom Thong' banana, in contrast with a study of strawberry fruit, which found higher levels of chelating soluble fraction after heat treatment (Lara, Garcia, & Vendrell, 2006).

HCl removed covalently bound pectin rich in branched rhamnogalacturonan I. HSP gradually increased, reaching a maximum on Day 6 in control fruits and on Day 8 in treated fruits. The fraction then decreased (Figure 1C). The HSP content of hot water treated fruits was higher than that of control fruits. HWT preserved a high level of HSP in 'Hom Thong' banana, as previously reported for strawberries

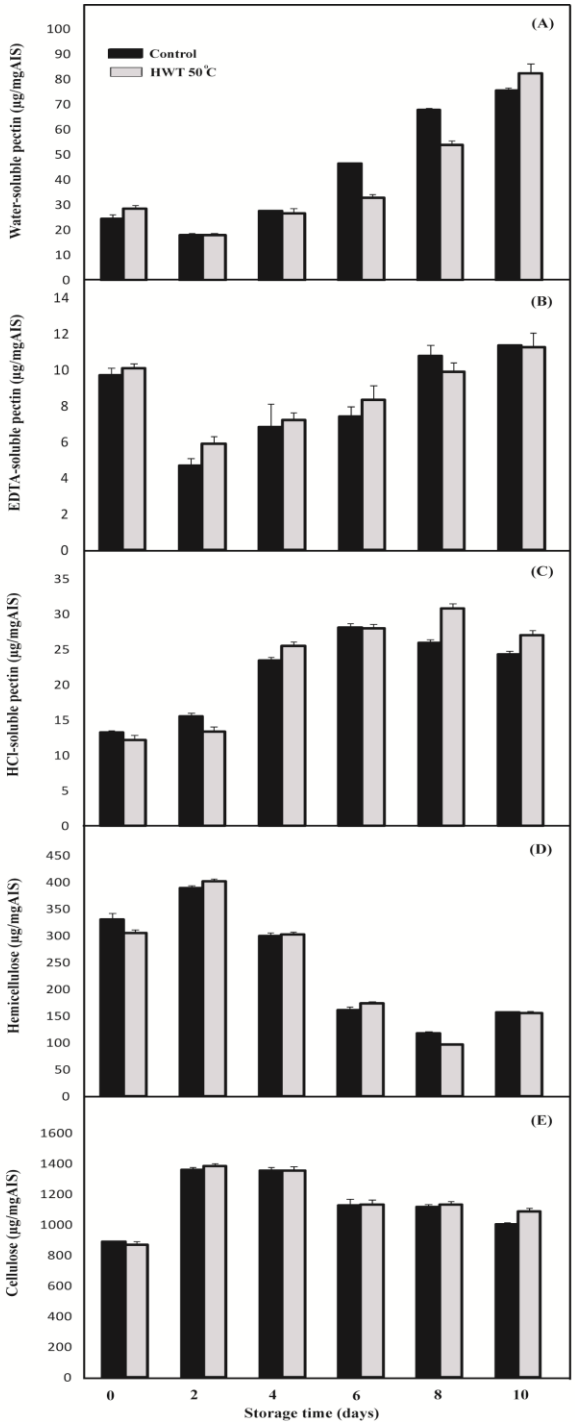

Figure 1. Changes in content of water-soluble pectin (A), EDTA-soluble pectin (B), HCl-soluble pectin (C), hemicelluloses (D), and cellulose (E) of banana following HWT at 50 ºC and of untreated fruits during storage at 25 ºC

(Vicente, Costa, Martinaz, Chaves, & Civello, 2005). The decline in the covalently bound pectin level was accompanied by a rapid increase in WSP. This suggests that the increase in WSP content during ripening is due to the release of covalently bound pectin, which becomes soluble (Figueroa *et al.*, 2010).

The hemicellulose level increased initially then decreased as ripening progressed in both control and treated fruits (Figure 1D). This is consistent with a study of 'Brazil' bananas, which reported a decreasing hemicellulose yield during ripening (Cheng *et al.*, 2009). However, the loss of hemicellulose in hot water treated bananas appeared slower than that of unheated fruit, though the only significant difference was found on Day 8. Hot air treatment at 45 ºC for 3 hrs has been reported to delay hemicellulose solubilization in strawberries, with treated fruits also maintaining a higher level of hemicellulose (Vicente, Costa, Martinaz, Chaves, & Civello, 2005).

Cellulose is a major component of the primary and secondary cell walls of plants. In this study, the cellulose level declined gradually throughout storage at 25 ºC, beginning on Day 2 (Figure 1E). The decrease may be due to an increase in cellulase activity. Prabha and Bhagyalashmi (1998) reported a decrease in cellulose content during ripening of banana fruit, accompanied by an increase in cellulase activity. However, the change in cellulose was slight, as its crystalline structure makes it robust against degradation by enzymes or chemicals. No significant difference in cellulose level was found between control and treated fruits, though the level was higher in treated fruit.

These results suggest that 50 ºC exposure delayed solubilization of some polysaccharide components, perhaps due to disruption of the activity of enzymes associated with cell wall degradation. Vicente, Costa, Martinaz, Chaves, and Civello (2005) found that polygalacturonase, β-galactosidase, β-xylosidase, and glucanase activity were reduced in strawberries following heat treatment, with consequent changes to the solubilization of pectin and hemicelluloses.

3.3 Anatomical alteration

Any structural changes in the peel of 'Hom Thong' fruits following 50 ºC HWT were investigated using light microscopy. As shown in Figure 2A, the peel comprised an epidermis, ground tissue, and a vascular bundle. The peel thickness and number of cell layers continuously decreased over the course of ripening. The peel thickness was approximately 3,000-3,500 µm. The peel thickness of 50 ºC treated fruits did not initially differ from that of the control fruits, but a significant difference was observed after storage (Table 2). Treated fruits also showed a significantly higher number of cell layers than control fruits from Day 4 of incubation (Table 2), corresponding to the retardation of firmness loss observed following HWT.

The epidermis remained at a single layer throughout development, in line with the findings of Santhakumari and Krishnamurthy (1991) for 'Poovan' banana. The cuticle exhibited cracks and scaling as ripening proceeded in both treated and untreated fruits. Changes in the ground tissue cell became more apparent on Day 4 of storage, as separation of the parenchyma cell led to an increase in intercellular space. The size and number of these spaces increased continuously as the fruit ripened, confirming previous studies of several fruits, including bananas (Prabha & Bhagyalashmi, 1998). The formation of intercellular spaces was more frequent in the inner ground tissue, and especially in the interior region. The arrangement and shape of cells started to change, and cell damage was also observed in this region. The cells in the outer

Table 2. Peel thickness, number of cell layers, and intercellular space area of bananas treated at 50 ºC and untreated fruits during storage at 25 ºC.

Parameter	Storage time (days)					
	0	2	4	6	8	10
Peel thickness (mm)						
Control	3.421±0.122	3.347±0.034	2.716±0.060	2.651±0.017	2.529±0.094	2.030±0.056
HWT 50ºC	3.264±0.038	3.218±0.077	2.976±0.058*	2.910±0.063*	2.657±0.037	2.426±0.045*
Number of cell layer						
Control	64.56±2.48	64.36±0.42	58.75±0.82	54.72±0.94	50.31±1.18	49.31±0.76
HWT 50ºC	65.58±0.74	64.83±1.22	62.78±1.05*	60.06±0.87*	54.61±1.07*	52.33±0.98*
Intercellular space area (m^2)						
Control	0.317±0.014	0.344±0.008	0.505±0.023	0.579±0.043	0.811±0.039	0.923±0.070
HWT 50ºC	0.305±0.022	0.384±0.020	0.428±0.035	0.462±0.034*	0.709±0.005	0.776±0.041

Asterisks within a column indicate a significant difference ($P<0.05$).

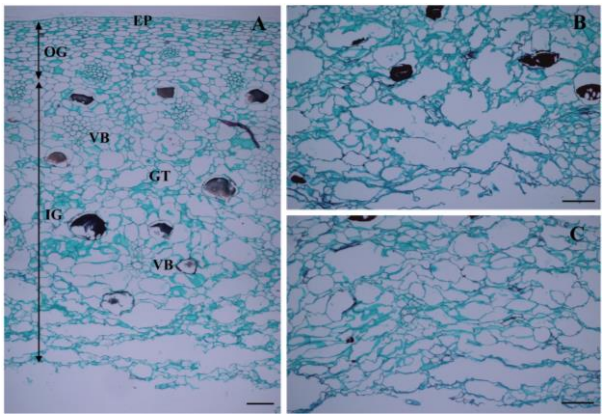

Figure 2. Structure of 'Hom Thong' banana peel at the mature green stage (A) and changes in inner ground tissue of untreated fruits (B) and fruits treated at 50 ºC (C) after storage at 25 ºC for 10 days. EP, epidermis; GT, ground tissue; VB, vascular bundle; OG, outer ground tissue; IG, inner ground tissue. Scale bars: A, B, C = 200 µm

zone exhibited no change in arrangement or shape in the early stage of ripening, but changes began to be observed in the later stage. These included the formation of intercellular spaces, a process that involves polysaccharide modification in the middle lamella and cell wall degradation by enzymes. The walls of some cells collapsed, while other cells clumped together, forming larger spaces. Measurements demonstrated that intercellular space formation in the peel of treated fruits was lower than that in control fruits, though this was only significant on Day 6 (Table 2). This may be due to the inhibition of cell wall degrading enzymes by heat treatment, as reported for strawberries (Vicente, Costa, Martinaz, Chaves, & Civello, 2005) and mei fruit (Luo, 2006).

Cells in the ground tissue were damaged and collapsed after prolonged ripening. At the end of the experiment, serious damage was found to cells in the innermost region, close to the banana pulp. Damage and changes in the arrangement of cells in the ground tissue were delayed by HWT. Cell collapse was more severe in control fruits than in treated fruits after incubation at 25 ºC for 10 days (Figure 2B and 2C). This may be explained by the inhibition of polysaccharide component changes following treatment, and especially of the pectin fraction. This is one of the main components of the middle lamella and primary cell wall, and acts as an important adhesive between cells. Ratule, Osman, Saari, and Ahmad (2007) reported that bananas ripened at 37 ºC retained the integrity of the peel cell wall, and especially of the middle lamella, better than those ripened at 25 ºC.

4. Conclusions

Application of 10 min postharvest hot water treatment at 50 and 55 ºC retarded the softening of 'Hom Thong' banana fruit and delayed physiological changes. However, peel discoloration observed at the higher temperature means that it is not recommended. Treatment at 50 ºC affected compositional changes by delaying the solubilization of cell wall polymer, especially pectin, and slowed structural changes to cell shape and size, peel thickness, and the formation of intercellular spaces. Our results suggest that hot water treatment can prevent cell wall degradation of 'Hom Thong' bananas, extending maintenance of firmness.

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