

Enzymatic Activity and Microstructural Changes of Hot Water Treated Banana during Ripening

A. Mirshekari^{1*}, Ph. Ding², and H. M. Ghazali³

ABSTRACT

Berangan banana were treated with hot water at 50 °C for 0 (control), 10, and 20 min and then kept at room temperature (25±2 °C), 75-80% RH to observe the ripening progress. Firmness and activity of cell wall degradation enzymes such as polygalacturonase (PG), pectin methylesterase (PME), and pectate lyase (PL) were determined for treated and untreated banana. The microstructure of banana peel cell wall was observed by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Treated fruit was firmer than the control fruit during ripening process. PG, PME, and PL activities were reduced in the treated bananas when compared with the control treatment. Hot water treatment disturbed cell wall degrading enzymes activity and delayed the disassembling of pectin fraction of Berangan banana. The micrographs of SEM and TEM exhibited lower changes in cell compartment and less loosening of cell wall fibril fractions in treated fruits. The electrolyte leakage in Berangan banana decreased with the increase of exposure time. The results of this study indicated that hot water treatment at 50 °C slowed down the ripening process and extended shelf life of banana.

Keywords: Berangan, Cell wall; Fruit firmness; Heat treatment, Shelf life.

INTRODUCTION

Banana is globally important as a food crop and considered as one of the main fruit with high consumer demand in the world. In Malaysia, banana is the second extensively-cultivated fruit crop, and Berangan is one of the most popular cultivars (Chai *et al.*, 2004). Banana is characterized by rapid softening once ripening is initiated, which reflect a typical climacteric fruit (Duan *et al.*, 2007). Fruit softening is a main determinant of ripening in banana and the rate of softening is high during the later stages of ripening. It causes major postharvest deterioration and economical losses in banana industry. Significant softening of the banana fruit is the result of

extensive cell wall degradation (Asif and Nath, 2005) associated with the disassembly of primary cell wall and middle lamella and changes in the pectin fraction (Seymour and Gross, 1996) that take place in the cell wall during ripening. Also, a large increase in pectin solubilization has been correlated with softening (Brummell and Harpster, 2001).

Cell wall degeneration occurs due to the action of hydrolases, including pectolytic enzymes such as polygalacturonase (PG), pectin methylesterase (PME) and pectate lyase (PL) (Brummell and Harpster, 2001). PG, an important hydrolytic enzyme, is the primary enzyme playing a significant role in pectin dissolution *in vivo* (Brownleader *et al.*, 1999) which would result in textural

¹ Department of Agronomy and Plant Breeding, Faculty of Agriculture, Yasouj University, Islamic Republic of Iran.

* Corresponding author; e-mail: a_mirshekari@yu.ac.ir

² Department of Crop Science, Universiti Putra Malaysia, Malaysia.

³ Department of Food Science, Universiti Putra Malaysia, Malaysia.



softening and loosening of cell structure. PG acts on pectic acid (polygalacturonic acid) and hydrolyses α -1,4-linked D-galacturonic acid, following de-esterification of pectin by PME (Cheng *et al.*, 2011). On the other hand, PME catalyses the hydrolysis of pectin methylester groups resulting in de-esterification (Ren and Kermode, 2000). PG and PME cooperatively regulate the breakdown of pectin (Duan *et al.*, 2008). The combined effect of PG and PME in fruit softening has been extensively reported (Nikolic and Mojovic, 2007; Prasanna *et al.*, 2007; Verlent *et al.*, 2005). PL catalyses the cleavage of (1 \rightarrow 4) galacturonan linkages of pectate by β -elimination reaction (Payasi *et al.*, 2006).

Heat treatments were originally used for insect disinfestation and disease control. Also, heat treatment has been shown to be effective in delaying softening in many fruits (Benitez *et al.*, 2006; Lurie, 1998; Paull and Chen, 2000; Varit and Songsin, 2011). A relatively short treatment of hot water at about 50 °C prolongs the shelf life of several fruit crops such as banana, mango and papaya (Lurie, 1998). Heat treatment also causes changes in fruit ripening, such as inhibition of ethylene synthesis and reduction of cell wall degrading enzymes activity, due to changes in gene expression and protein synthesis (Paull and Chen, 2000). Thus, the possible mechanism by which heat treatment delays softening in Berangan banana during ripening is the reduction of cell wall degrading enzymes activity.

Paull and Chen (2000) postulated that the application of heat treatments at non-lethal conditions causes a moderate stress in fruits, resulting in a momentary stop of the regular metabolism that is recovered once the fruit is returned to normal temperature. Application of hot water after harvest reduced the activities of cell wall degrading enzymes of fruits. Also, the ultrastructural and biochemical changes are correlated with the synthesis of the cell wall hydrolases enzymes (Fischer and Bennett, 1991). Apparently, hot water treatment has a

potential effect in reducing PG, PME, and PL activity in the fruits including banana. The physiological reactions of cultivars of fruit species to heat treatments can be different by season and growing location due to variation in climate, soil type, season, production practices, and fruit maturity at harvest (Schirra *et al.*, 1997; Jacobi *et al.*, 2001). Therefore, in this work, the effect of hot water treatments on firmness, activity of cell wall degrading enzymes (PG, PME and PL) and cell wall ultrastructure of Berangan banana during ripening process was elucidated.

MATERIALS AND METHODS

Plant Material and Heat Treatment

Mature green (first stage of ripening) banana (*Musa* AAA cv. Berangan) used in this study were purchased from a wholesale market. Fruits with uniform size and color and lacking noticeable defects were selected. An electric water bath (Stuart, SBS40, OSA, UK) with digital temperature regulator profile was used for treatment. The fruits were dipped in hot water at 50 °C for 10 and 20 min, respectively, and the untreated fruits were used as the control. Treated fruits were cooled in distilled water for 30 min, and then left for air-drying. All fruits were placed in an air-tight container and treated with 100 μ L/L ethylene for 24 h at 25 \pm 2 °C to initiate uniform ripening. The ethylene treated fruits were kept in the plastic basket at laboratory of 25 \pm 2 °C and 75-80% RH for ripening to take place. Fruits were randomly sampled on day 0, 1, 3, 5 and 7, and analyzed for firmness, enzymes activity, and electrolyte leakage. The peel specimens were prepared on days 1 and 5 for evaluation of electron microscopy.

Determination of Firmness

The fruit pulp firmness was measured by an Instron Universal Testing Machine (5540,

USA) by using a probe diameter of 5 mm, with speed of 50 mm/min, and a load range from 0 to 100 N load cells. The compression force was measured at the maximum peak of the recorded force on the chart and expressed in Newton.

Determination of Electrolyte Leakage

Electrolyte leakage (EL) was determined as membrane permeability according to the method of Gonzalez-Aguilar *et al.* (2004). The initial conductivity was determined using a conductivity meter (AB-300, Fisher Scientific Instruments, Singapore). The peel discs were autoclaved at 121 °C for 30 min and then cooled to room temperature (25±2 °C) prior to assessing the total conductivity. The EL was expressed as a percentage of total conductivity.

Enzyme Extraction

The procedure of enzyme extraction was adopted from Ali *et al.* (2004) and Lazan *et al.* (1989) with some modifications. All steps were done at 4 °C. Ten grams of fresh pulp tissues were homogenized in a blender (MX-799S, Panasonic, Malaysia) for 2 min with 20 mL cold 0.1 mol/L sodium citrate, pH 4.6, buffer containing 1 mol/L NaCl, 13 mmol/L EDTA, 10 mmol/L β-mercaptoethanol, and 1% (w/v) polyvinylpyrrolidone (PVP). Then, the mixture was incubated at 4 °C for 60 min with occasional stirring. The supernatant was subsequently recovered by centrifugation at 15,000 x g for 30 min at 4 °C in a refrigerated centrifuge (Avanti J-25, Beckman Coulter, USA). The clear supernatant (crude enzyme extract) was used to determine the enzyme activity.

Polygalacturonase Assay

Polygalacturonase activity was assayed based on the method described by Lohani *et*

al. (2004) and Pathak and Sanwal (1998) with slight modifications. The reaction mixture contained 0.4 mL sodium acetate (200 mmol/L, pH 4.5) buffer, 0.1 mL NaCl (200 mmol/L), 0.4 ml polygalacturonic acid (PGA, 1% aqueous solution adjusted to pH 4.5) and 0.1 mL of enzyme extract in a total volume of 1.0 mL. The control comprised the same components but with the enzyme extract boiled for 5 min. The reaction was initiated by the addition of the PGA substrate. The mixture was incubated at 37 °C for 1 h and followed by addition of 0.1 mL of 3,5-dinitrosalicylate (DNS) reagent. The reaction mixture was then heated in boiling water bath for 5 min. When the mixture reached room temperature (25±2 °C), the absorbance was measured at 540 nm using a spectrophotometer (WPA, Biochrom, Cambridge, England). The formation of reducing groups was estimated against D-galacturonic acid as a standard. One unit of enzyme activity is defined as the amount of enzymes required to liberate 1 nmol of galacturonic acid per min per g of original fresh weight of fruit pulp (Miller, 1959).

Pectin Methylsterase Assay

Pectin methylsterase activity was measured according to the method described by Lohani *et al.* (2004) and Hangermann and Austin (1986). The reaction mixture was prepared in a 3 mL glass cuvette and was composed of 1 mL pectin solution (0.01% aqueous solution adjusted to pH 7.5 using 0.1 mol/L NaOH), 0.2 mL NaCl (0.15 mol/L), 0.1 mL bromothymol blue solution (0.01%), 0.2 mL sterilized water and 0.1 mL crude enzyme extract. After adding the enzyme preparation, the cuvette was shaken gently. The absorbance of the reaction mixture was measured immediately at 620 nm using a spectrophotometer (WPA, Biochrom, Cambridge, England). The absorbance was again measured after 3 min. The difference in absorbance between 0 and 3 min was the measure of PME activity.



Calculation of the activity was carried out against a standard curve of galacturonic acid constructed as described by Hangermann and Austin (1986). One unit is defined as the amount of the enzyme required for liberating 1 μmol of methyl ester per min per g of the original fresh weight of fruit pulp.

Pectate Lyase Assay

Pectate lyase activity was measured according to the methods by Lohani *et al.* (2004) and Moran *et al.* (1968). The assay mixture comprised 0.6 mL of 4 mmol/L sodium acetate buffer (pH 4.5), 0.3 mL polygalacturonic acid solution (PGA, 1% aqueous solution adjusted to pH 4.5) and 0.1 mL crude enzyme preparation in 1 mL total reaction volume. The control assay contained the same components but with the enzyme extract was first boiled for 2 min. The reaction mixture was incubated at 37 °C for 30 min followed by heating in boiling water bath for 2 min to stop the reaction and then allowing the mixture to reach room temperature (25 \pm 2 °C). The absorbance of the reaction mixture was measured at 235 nm using a spectrophotometer (WPA, Biochrom, Cambridge, England). The increase in the absorbance against the control was taken as a measure of the PL activity. All calculations were made according to Moran *et al.* (1968) and one unit of PL activity was expressed as the amount of enzyme required to liberate 1 nmol of aldehyde groups from PGA per min per g of original fresh weight of fruit pulp.

Preparation of Specimen for Scanning Electron Microscopy

Samples (1 cm²) were incised from the mid-region of each banana fruit peel, and then fixed in 4% glutaraldehyde fixative for 24 h at 4 °C (Karnovsky, 1965). The samples were washed in three changes of 0.1 mol/L sodium cacodylate buffer (pH=7.6) for 30 min and post-fixed in 1% (w/v) osmium

tetraoxide for 2 h. The samples were then washed again and dehydrated in graded series of alcohol at 30%, 50%, 70%, 80%, 90%, and 95% for 30 min each, and two changes in absolute alcohol for 30 min each. Drying was completed in a critical point dryer (Samdri-780-A, Tousimic corp. Rockville, Japan). The samples were coated with Au/Pd using Hummer V sputter coater (Technic, Alexandria, USA), and viewed and photograph under scanning electron microscope (SEM) (JEOL, JSM- 5610LV, Tokyo, Japan).

Preparation of Specimen for Transmission Electron Microscopy

Samples (1.5 mm³) were fixed, washed and dehydrated as described before for SEM. The dehydrated samples were infiltrated gradually with acetone: resin mixture at graded series of 1:1 and 1:3 for overnight and two changes in 100% resin for 12 h and then finally polymerized at 60 °C for 2 days. Ultra-thin sections (60-90 nm) of the tissue were cut using a diamond knife (Diatome 45°, Switzerland) on a Reichert Jung Ultracut-S ultramicrotome (Austria) and mounted on 200 mesh copper grids. Sections were examined under transmission electron microscope (TEM) (Hitachi, H-7100, Japan).

Experimental Design and Statistical Analysis

The experiment was conducted using a completely randomized design with a factorial arrangement. Data were analyzed using ANOVA (SAS version 9.2). Mean comparisons were made using Duncan's multiple range tests when the F values of ANOVA showed significance at $p \leq 0.05$. Correlation analysis by means of Pearson's correlation matrix was performed to establish the associations between enzymes activity and firmness.

RESULTS AND DISCUSSION

Fruit Firmness

Hot water treatment at 50 °C for 10 and 20 min had a positive effect on maintenance of fruit firmness in comparison to the control during ripening process as shown by significant higher values than the control (Table 1). In the control, the fruit firmness decreased as ripening progressed. However, as dipping time increased, the pulp firmness increased indicating delay in fruit softening. The softening was greatly reduced with 20 min dipping time. Varit and Songsin (2011) reported that hot water dip treatment at 50 °C for 10 min was able to maintain the firmness of Kluai Khai banana. Firmness is a main feature that dictates the postharvest life and quality of fruits. Lara *et al.* (2006) mentioned that heat treatment retarded solubilization of cell wall polymers fractions by inhibiting some related enzymes activity. It is suggested that heat treatment disrupt cell wall breakdown and lead to delay or poor softening (Rose *et al.*, 1998). Thus, the results of this study demonstrated that hot

water temperature at 50 °C for 10 and 20 min dipping time could retard softening progress and delayed ripening process of Berangan banana.

Electrolyte Leakage

Electrolyte leakage (EL) of the hot water treated banana peel tissues decreased as dipping time increased (Table 1) and there were no significant differences between 10 min with the control and the 20 min treatments. Dipping fruit in hot water for 20 min significantly decreased EL as compared to the control. In contrast, EL increased as ripening progressed. There were no interaction effects between dipping time and ripening day on EL of Berangan banana. EL is often used as an indicator of membrane plasma rupture, as ions leak out from the cells (Kamdee *et al.*, 2009). Therefore, increase leakage may result from breakdown in cellular compartment. EL in husks of pomegranate was significantly higher in the control than fruits treated in hot water of 45 °C for 4 min (Mirdehghan *et al.*, 2007). The mechanism of heat tolerance in response to

Table 1. Main and interaction effects of 50 °C hot water treatment on fruit firmness, electrolyte leakage, polygalacturonase (PG), pectin methylesterase (PME) and pectate lyase (PL) activity of Berangan banana fruit during ripening.

Factors	Firmness (N)	Electrolyte leakage (%)	PG activity (U/g f w)	PME activity (U/g f w)	PL activity (U/g f w)
Dipping time (D), min					
0 (control)	8.28 c ^z	28.89 a	1.62 a	0.48 a	1.88 a
10	10.15 b	26.73 ab	1.36 b	0.45 b	1.60 b
20	12.20 a	25.35 b	1.02 c	0.41 c	1.44 c
Ripening day (R)					
0	26.76 a	9.14 e	0.06 e	0.06 e	0.42 e
1	17.90 b	15.94 d	0.36 d	0.22 d	1.35 d
3	5.39 c	27.08 c	1.09 c	0.70 b	2.11 b
5	3.44 cd	34.21 b	2.42 b	0.74 a	2.36 a
7	2.01 d	48.25 a	2.75 a	0.52 c	1.97 c
Interaction					
DxR	ns	ns	**	*	**

f w = fresh weight.

^zMean followed by the same letter in the same column within factors are not significantly different by Duncan's multiple range tests.

ns, *, ** = Non significant, significant at $p \leq 0.05$ or highly significant at $p \leq 0.01$, respectively.



heat stress in fruits include synthesis of heat shock and other proteins, effect on wall degrading enzymes, EL, ethylene synthesis, etc. (Lurie, 1998). The results of this study suggested that hot water dipping of Berangan banana at 50 °C for 20 min alleviated cell membrane permeability as compared to the control treatment.

Polygalacturonase Activity

PG activity and dipping time were significantly inversely related (Table 1). This indicated that hot water temperature at 50 °C for 10 and 20 min could slow down cell wall degradation by retarding PG activity. In contrast, as ripening progressed, the PG activity increased significantly. There was a significant interaction effect between dipping time and ripening day (Table 1). In the control, PG activity of Berangan banana increased gradually and achieved maximum activity on day 5 and decreased thereafter (Figure 1). On the other hand, on day 5, hot water treatment for 10 and 20 min dipping time significantly reduced PG activity by about 30 and 40%, respectively, as compared to the control.

It was observed that heat treatment

inhibited cell wall degradation enzymes activity and delayed the softening of Berangan banana. The application of hot water dip treatment at 50 °C for 20 min dipping time suppressed PG activity immediately after treatment (Figure 1). Obviously, by dipping fruit in 50 °C for 20 min, the activity of PG were delayed for 1 day and, thus, slowed down the cell wall degrading process in Berangan banana fruit.

PG is an enzyme responsible for pectin breakdown in fruits, which catalyzes the hydrolytic cleavage of galacturonide linkages, and removes single galacturonic acid units from the polygalacturonic acid (Ali *et al.*, 2004; Brummell and Harpster, 2001). The degradation of cell walls leads to fruit softening. PG activity has been reported to increase in banana fruit during ripening (Pathak *et al.*, 2000). Hot water dip treatment at 50 °C for 10 min reduced PG activity in Hom Thong banana (Amnuaysin *et al.*, 2012). High temperatures inhibit the activity of PG and slow down fruit softening (Ketsa *et al.*, 1998). In this study, hot water dip treatment at 50 °C for 10 and 20 min could delay Berangan banana softening as a result of disrupting the PG activity in fruit.

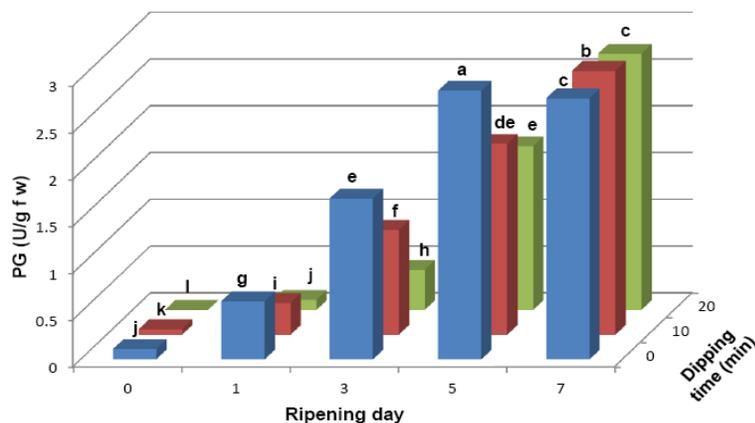


Figure 1. Interaction effects of 50 °C hot water dipping time and ripening day on polygalacturonase (PG) activity of Berangan banana during ripening. f w = fresh weight. Different letters within columns denote significant differences by Duncan's multiple range tests at $p \leq 0.05$.

Pectin Methylesterase Activity

The PME activity of Berangan banana decreased significantly as dipping time of hot water increased (Table 1). The activity of the enzyme increased significantly from day 0 until 5 as banana fruit ripened, then followed a decreasing trend as fruit ripened to day 7. There were significant interaction effects between dipping time and ripening day on PME activity of Berangan banana (Table 1). In the control and the fruits dipped in hot water for 10 min, maximum PME activity was recorded on day 3 during the ripening process (Figure 2). However, PME activity significantly decreased by about 45% on day 3 in fruits treated with 50 °C hot water for 20 min as compared to 0 min dipping time. This indicates that PME activity was suppressed by hot water treatment.

Also, Ali *et al.* (2004) reported that PME activity of Mas banana fruits increased during ripening. The present results are in accordance with those reported by Amnuaysin *et al.* (2012) where hot water at

50 °C and dipping time of 10 min reduced PME activity in Hom Thong banana during ripening. Hot water dip at 50 or 55 °C for 5 min reduced Nam Dok Mai mango PME activity during storage at 25 °C (Benitez *et al.*, 2006). Demethylesterification of cell wall as catalyzed by PME is a prerequisite for hydrolysis by PG (Micheli, 2001). The activity of these cell wall degradation enzymes contribute to fruit softening (Bennett, 2002; Carrillo-Lopez *et al.*, 2002). It has been proposed that heat treatment interrupt cell wall hydrolytic enzymes activity as the reason of delay or poor softening in fruit (Paull and Chen, 2000; Rose *et al.*, 1998). Inhibition of PME activity by hot water treatment at 50 °C for 20 min reduced PME activity during ripening, thereby delaying softening of Berangan banana fruit.

Pectate Lyase Activity

The different treatments showed significant differences in PL activity of Berangan banana as affected by hot water

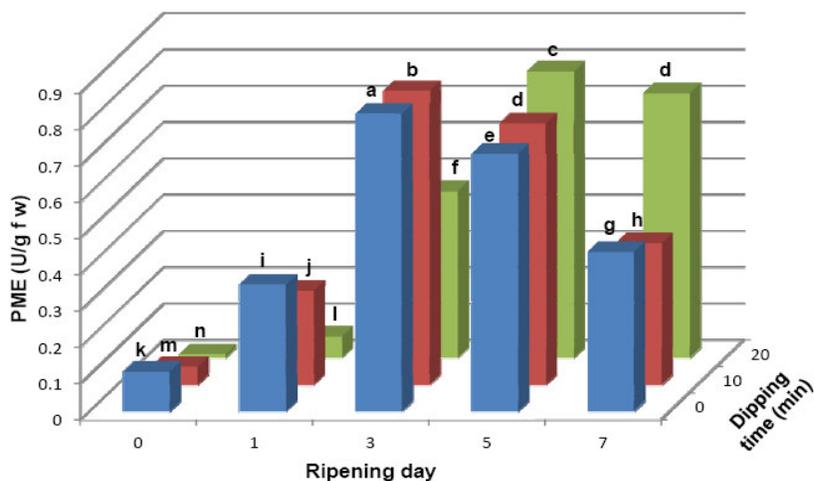


Figure 2. Interaction effects of 50 °C hot water dipping time and ripening day on pectin methylesterase (PME) activity of Berangan banana during ripening. f w = fresh weight. Different letters within columns denote significant differences by Duncan's multiple range tests at $p \leq 0.05$.



dipping time and ripening day (Table 1). The result showed that the activity of PL decreased as dipping time increased. Dipping time of 20 min could reduce PL activity of Berangan banana more than 10 min. PL activity increased significantly as ripening day progressed and achieved maximum by day 5, then decreased by day 7.

There were significant interaction effects between dipping time and ripening day (Table 1). The activity of banana PL in 0 min dipping time increased to maximum by day 3 over the ripening period (Figure 3). After day 3, the PL activity declined slowly. A similar trend with a delay in PL activity was observed in banana dipped for 10 min in hot water. At day 3, PL activity significantly decreased by about 40% in fruits dipped for 20 min as compared to 0 min. There was no significant difference in PL activity of Berangan banana between day 3 of 0 min and day 5 of 20 min hot water dipping (Figure 3). The result showed that PL activity of Berangan banana dipped in 50 °C hot water for 20 min was suppressed at least for 2 days as compared to the control.

It appears that in addition to PG and PME, PL is likely to be involved in

softening associated with ripening in banana fruit (Pathak *et al.*, 2000). Payasi and Sanwal (2003) mentioned that PL activity in banana fruit appeared at early climacteric stage and increased steadily, with the maximum activity coinciding with the climacteric peak and decreasing throughout the post-climacteric stage. This finding was consistent with previous studies in Hom Thong banana by Amnuaysin *et al.* (2012). The reduction in PL activity in the present study could be due to inhibition of pectin degrading enzyme activity as affected by hot water treatment. This study indicated that hot water at 50 °C for 20 min could retard PL activity and delay cell wall degradation of Berangan banana.

Correlation between Firmness and Enzyme Activity

In order to assess the relationship between the firmness and various cell wall degrading enzymes (PG, PME and PL) activity, a correlation matrix of all the variables for Berangan banana was obtained as shown in Table 2. From the analysis of data, there was a significant ($p \leq 0.01$) negative correlation

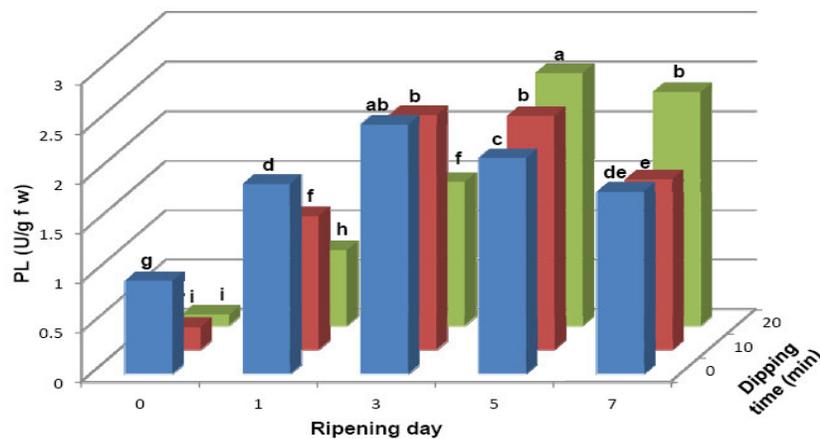


Figure 3. Interaction effects of 50 °C hot water dipping time and ripening day on pectate lyase (PL) activity of Berangan banana during ripening. f w = fresh weight. Different letters within columns denote significant differences by Duncan's multiple range tests at $p \leq 0.05$.

Table 2. Correlation coefficients between firmness and cell wall degrading enzymes (PG, PME and PL) of Berangan banana during ripening.

	PG	PME	PL	Firmness
PG	--			
PME	0.76**	--		
PL	0.73**	0.64**	--	
Firmness	-0.88**	-0.85**	-0.79**	--

** = significant at $p \leq 0.01$

between firmness and activity of PG ($r = -0.88^{**}$), PME ($r = -0.85^{**}$) and PL ($r = -0.79^{**}$). Negative values of correlation coefficient (r) indicate that when enzyme activity increased, firmness decreased. These results support the correlation between cell wall degrading enzymes activity and softening in Berangan banana fruit. It is suggested that fruit softening property, indicated by the decrease in firmness, could be reliably predicted from enzyme (PG, PME and PL) activities.

Most of the studies in fruit ripening have concluded that the level of cell wall degrading enzymes activity has negative correlation with fruit firmness (Fischer and Bennet 1991). Although all enzyme (PG, PME and PL) activities could be used to predict fruit softening, in the present study, the highest correlation coefficient ($r = -0.88^{**}$) was observed between firmness and PG activity. This indicates that PG activity is the best indicator of the occurrences of fruit softening in Berangan banana during ripening. There were also significant correlations between PG and PME activity ($r = 0.76^{**}$) and between PG and PL activity ($r = 0.73^{**}$). This indicates that the increase in PG activity of Berangan banana during ripening was also followed by a significant increase in PME and PL activity. However, the correlation coefficient between PG and PME activity ($r = 0.76^{**}$) was higher as compared to the correlation coefficient between PG and PL activity ($r = 0.73^{**}$). This suggests that, besides PG activity, PME activity could also be considered as an

enzyme activity related with fruit softening in Berangan banana.

Evaluation of Scanning Electron Microscopy Micrographs

The effects of hot water dip treatment at 50 °C for 0 (control), 10, and 20 min dipping time on fruit peel cell wall structure was observed using a SEM. As shown in Figure 4a, there was no obvious change in the cell packing, and cells had an angular polyhedral shape with only limited intercellular space visible in the treated and control fruits by day 1 of ripening process as shown by micrograph of the control fruits. By day 5, the cellular profile became rounded and intercellular spaces increased as tissue softened in the control fruit (Figure 4b). This was due to intensive loss of cell structure, which induced loss of cell compartment. Fruits treated for 10 min showed less spherical shaped cells (Figure 4c), while banana dipped for 20 min showed partially polyhedral shape cells by day 5 of ripening (Figure 4d).

Harker *et al.* (1997) reported that in most fruits, the softening process during ripening is mainly related with microstructural modification in the cell wall of the parenchyma cells. Reduction in cell wall rigidity was mainly due to dissolution and depolymerization of pectin, hemicellulose and cellulose (Brummell and Harpster, 2001; Lohani *et al.*, 2004). It is often considered as a loss of membrane integrity resulting in tissue injury (Fan and Sokorai,

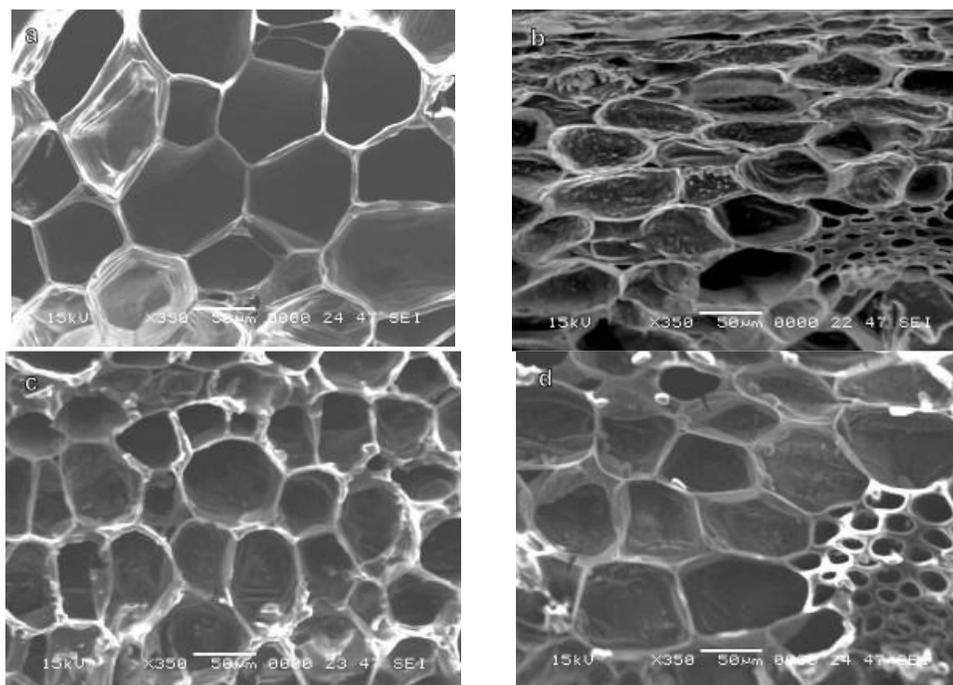


Figure 4. SEM micrographs (Bar= 50 µm, x350) of Berangan banana peel tissue structure as affected by hot water at 50 °C during ripening process. (a) Tissue of fruits dipped for 0 min (control) on day 1. Cells have an angular polyhedral interlocking profile with small intercellular spaces. (b) Tissue of fruit dipped for 0 min (control) on day 5. Cells have a rounded profile with prominent intercellular spaces. (c) Tissue of fruits dipped for 10 min on day 5. Cells became rounded and intercellular spaces are not visible. (d) Tissue of fruits dipped for 20 min on day 5. Cells retain with an angular polyhedral profile.

2005). The loosening of the cell wall structure was observed by day 5 in the control Berangan banana fruit, while fruit dipped in 50 °C water for 20 min did not show sign of cell structural loosening yet. The combination of 50 °C and 20 min dipping time slowed down fruit tissue softening by retaining cell packing. These observations supported that hot water treatment of banana at 50 °C for 10 or 20 min would retard cell wall degradation enzymes activity. Hence, the treated fruits remain firmer than the control fruit.

Evaluation of Transmission Electron Microscopy Micrographs

The effects of hot water dip treatment at 50 °C on cell wall structure of Berangan

banana peel was examined using TEM at day 1 and 5, and the micrograph is shown in Figure 5. The cell wall displayed intensive longitudinal fibers on day 1 for both the control and treated fruits as shown by micrograph of the control fruit (Figure 5a). The cell wall contains tightly packed fibrillar material on day 1. On day 5, the middle lamella and primary cell wall of the control fruits dissolved by showing losses on the fibril lines (Figure 5b). The cell wall showed a loosening of the fibrils and electron dense in the middle lamella. However, cell wall loosening was also observed in the fruit tissue subjected to hot water dipping for 10 min (Figure 5c) but the looseness of cell wall fibril lamination was slightly less than that of the cell wall of the control fruits. Fruits dipped for 20 min showed the least loosening in cell wall as

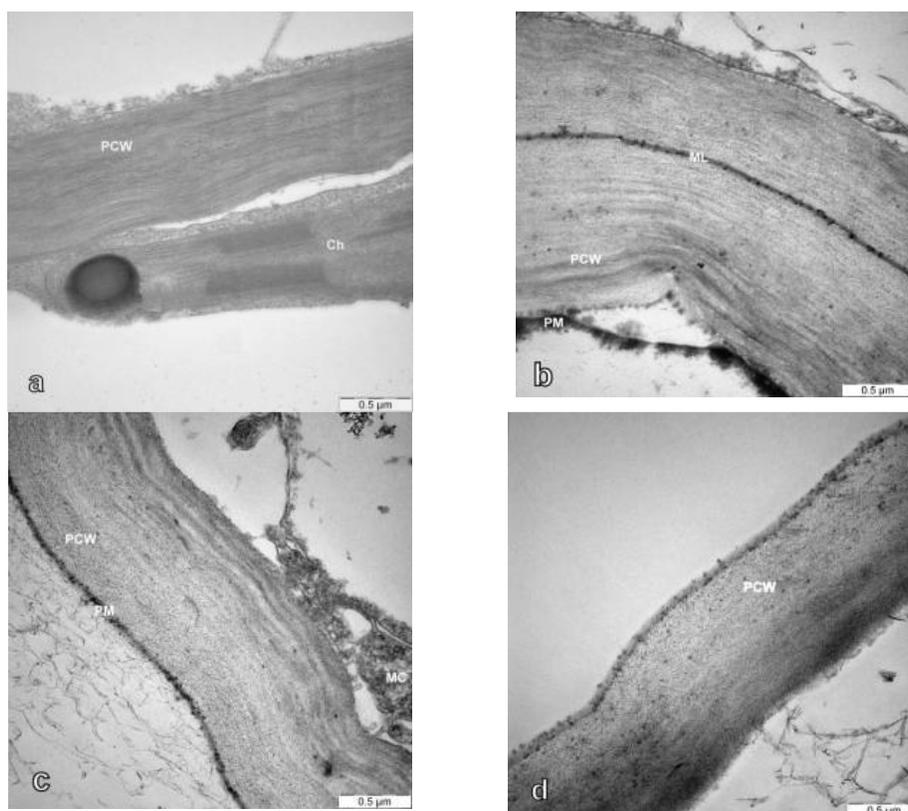


Figure 5. TEM micrographs (Bar = 0.5 μm , $\times 40000$) of cell wall of Berangan banana peel as affected by hot water at 50 $^{\circ}\text{C}$ during ripening. (a) Cell wall of fruits dipped for 0 min (control) on day 1. Cell wall shows a compact array of fibrils. (b) Cell wall of fruits dipped for 0 min on day 5. Cell wall has loose, its network of fibers and electron dense in middle lamella. (c) Cell wall of fruit dipped for 10 min on day 5. Cell wall shows less loose fibril. (d) Cell wall of fruit dipped for 20 min on day 5. Cell wall has dissolution of fibers fractions. (PCW = primary cell wall, ML = middle lamella, PM = plasma membrane, MC = mitochondria, Ch = chloroplast).

compared to the other treatments of the fruit on day 5 (Figure 5d).

The stability of tissue decreased due to losing of fibril fractions in cell wall and middle lamella. Miller and Fry (2001) reported that firmness in fruit decreased as activity of hydrolases cell wall increased. The losing of fibril fraction in Berangan banana cell wall was in parallel with the increase of hydrolases cell wall enzyme as reported earlier. The results indicated that hot water at 50 $^{\circ}\text{C}$ for 10 and 20 min delayed fibril loosening in cell wall tissues of Berangan banana. This might be caused by lower activity of the cell wall degrading

enzymes due to suppression of activity by hot water treatment. The TEM micrographs supported that hot water treatment using 50 $^{\circ}\text{C}$ for 10 and 20 min would retain cell wall rigidity and delay softening of Berangan banana fruit.

CONCLUSION

Hot water dip treatment at 50 $^{\circ}\text{C}$ for 10 and 20 min retarded softening of Berangan banana fruit as compared to the control. Hot water treatment at 50 $^{\circ}\text{C}$ for 20 min was more effective than 10 min. Concomitant



with softening, the treated fruits exhibited reduced cell wall degrading enzymes activity such as PG, PME and PL. Hot water treatment at 50 °C for 20 min generally resulted in lower PME and PG activity than that for 10 min. The results from SEM and TEM showed that hot water treatment also retarded cell loosening. Hot water treatment at 50 °C for 20 min caused less dissolution of pectin fractions as compared to the control treatment. Hence, these combinations of the aforementioned temperature and dipping time could slow down ripening of Berangan banana.

REFERENCES

1. Ali, Z. M., Chin, L. H. and Lazan, H. 2004. A Comparative Study on Wall Degrading Enzymes, Pectin Modifications and Softening during Ripening of Selected Tropical Fruits. *Plant Sci.*, **167**: 317-327.
2. Amnuaysin, N., Jonesc, M. L. and Seraypheap, K. 2012. Changes in Activities and Gene Expression of Enzymes Associated with Cell Wall Modification in Peels of Hot Water Treated Bananas. *Sci. Hortic.*, **142**: 98-104.
3. Asif, M. H. and Nath, P. 2005. Expression of Multiple Forms of Polygalacturonase Gene during Ripening in Banana Fruit. *Plant Physiol. Biochem.*, **43**: 177-184.
4. Benitez, M. M., Acedo Jr., A. L., Jitareerat, P. and Kanlavanarat, S. 2006. Induction of Disease Resistance in Mango Fruit by Postharvest Heat. *Acta Hortic.*, **712**: 785-792.
5. Bennett, A. B. 2002. Biochemical and Genetic Determinants of Cell Wall Disassembly in Ripening Fruit: A General Model. *HortSci.*, **37**: 447-450.
6. Brownleader, M. D., Jackson, P., Mobasheri, A., Pantelides, A. T., Sumar, S. and Trevan, M. 1999. Molecular Aspects of Cell Wall Modifications during Fruit Ripening. *Crit. Rev. Food Sci. Nutr.*, **39**: 149-164.
7. Brummell, D. A. and Harpster, M. H. 2001. Cell Wall Metabolism in Fruit Softening and Quality and Its Manipulation in Transgenic Plants. *Plant Mol. Biol.*, **47**: 311-340.
8. Carrillo-Lopez, A., Cruz-Hernandez, A., Carabez-Trejo, A., Guevara-Lara, F. and Paredes-Lopez, O. 2002. Hydrolytic Activity and Ultrastructural Changes in Fruit Skins from Two Prickly Pear (*Opuntia* sp.) Varieties during Storage. *J. Agric. Food Chem.*, **50**: 1681-1685.
9. Chai, M., Ho, Y. W., Liew, K. W. and Asif, J. M. 2004. *Biotechnology and In vitro Mutagenesis for Banana Improvement*. Accessed on 20 May 2011, <http://www.fao.org/docrep/007/ae216e/ae216e08.htm>.
10. Cheng, G., Jiang, Y., Chen, Y., Yang, S., He, S., Liang, H. and Duan, X. 2011. Combined Effects of Pectic Enzymes on the Degradation of Pectin Polysaccharides of Banana Fruit. *Pak. J. Bot.*, **43**: 1501-1505.
11. Duan, X., Cheng, G., Yang, E., Yi, C., Ruenroengklin, N., Lu, W., Luo, Y. and Jiang, Y. 2008. Modification of Pectin Polysaccharides during Ripening of Postharvest Banana Fruit. *Food Chem.*, **111**: 144-149.
12. Duan, X. W., Joyce, D. C. and Jiang, Y. M. 2007. Postharvest Biology and Handling of Banana Fruit: Review. *Fresh Produce*, **1**: 140-152.
13. Fan, X. and Sokorai, K. J. B. 2005. Assessment of Radiation Sensivity of Fresh-cut Vegetable Using Electrolyte Leakage Measurement. *Postharvest Biol. Technol.*, **36**: 191-197.
14. Fischer, R. L. and Bennett, A. B. 1991. Role of Cell Wall Hydrolases in Fruit Ripening. *Ann. Rev. Plant Physiol. Plant Mol. Biol.*, **42**: 675-703.
15. Gonzalez-Aguilar, G. A., Tiznado-Hernandez, M. E., Zavaleta-Gatica, R. and Martinez-Teliez, M. A. 2004. Methyl Jasmonate Treatments Reduce Chilling Injury and Activate the Defense Response of Guava Fruits. *Biochem. Biophys. Res. Commun.* **313**: 694-701.
16. Hangermann, A. E. and Austin, P. J. 1986. Continuous Spectrophotometric Assay for Plant Pectin Methyl-esterase. *J. Agric. Food Chem.*, **34**: 440-444.
17. Harker, F. R., Redgwell, R. J., Hallett, I. C., Murray, S. H. and Carter, G. 1997. Texture of Fresh Fruit. *Hortic. Rev.*, **20**: 121-224.
18. Jacobi, K. K., MacRae, E. A. and Hetherington, S. E. 2001. Effect of Fruit Maturity on the Response of 'Kensington' Mango Fruit to Heat Treatment. *Aust. J. Exp. Agric.*, **41**: 793-803.

19. Kamdee, C., Ketsa, S. and Doorn, W. G. V. 2009. Effect of Heat Treatment on Ripening and Early Peel Spotting in cv. Sucrier Banana. *Postharvest Biol. Technol.*, **52**: 288-293.
20. Karnovsky, M. J. 1965. A Formaldehyde-glutaraldehyde Fixative of High Osmolarity for Use in Electron Microscopy. *J. Cell Biol.*, **27**: 137-138.
21. Ketsa, S., Chidtragool, S., Klein, J. D. and Lurie, S. 1998. Effect of Heat Treatment on Changes in Softening, Pectic Substances and Activities of Polygalacturonase, Pectinesterase and β -galactosidase of Ripening Mango. *J. Plant Physiol.*, **153**: 457-461.
22. Lara, I., Garcia, P. and Vendrell, M. 2006. Post-harvest Heat Treatments Modify Cell Wall Composition of Strawberry (*Fragaria x ananassa* Duch.) Fruit. *Sci. Hortic.*, **109**: 48-53.
23. Lazan, H., Ali, Z. M., Liang, K. S. and Yee, K. L. 1989. Polygalacturonase Activity and Variation in Ripening of Papaya Fruit with Tissue Depth and Heat Treatment. *Physiol. Plant.*, **77**: 93-98.
24. Lohani, S., Trivedi, P. K. and Nath, P. 2004. Changes in Activities of Cell Wall Hydrolases during Ethylene-induced Ripening in Banana: Effect of 1-MCP, ABA and IAA. *Postharvest Biol. Technol.*, **31**: 119-126.
25. Lurie, S. 1998. Postharvest Heat Treatments of Horticultural Crops. *Hortic. Rev.*, **22**: 91-121.
26. Micheli, F. 2001. Pectin Methylsterases: Cell Wall Enzymes with Important Roles in Plant Physiology. *Trends Plant Sci.*, **6**: 414-419.
27. Miller, G. L. 1959. Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. *Ann. Chem.*, **31**: 426-428.
28. Miller, J. G. and Fry, S. C. 2001. Characteristics of Xyloglucan after Attack by Hydroxyl Radicals. *Carbohydr. Res.*, **332**: 389-403.
29. Mirdehghan, S. H., Rahemi, M., Martinez-Romero, D., Guillen, F., Valverde, J. M., Zapata, P. J., Serrano, M. and Valero, D. 2007. Reduction of Pomegranate Chilling Injury during Storage after Heat Treatment: Role of Polyamines. *Postharvest Biol. Technol.*, **44**: 19-25.
30. Moran, F., Nasuno, S. and Starr, M. P. 1968. Extracellular and Intracellular Polygalacturonic Acid Trans-eliminase of *Erwinia carotovora*. *Arch. Biochem. Biophys.*, **123**: 298-306.
31. Nikolic, M. V. and Mojovic, L. 2007. Hydrolysis of Apple Pectin by the Coordinated Activity of Pectic Enzymes. *Food Chem.*, **101**: 1-9.
32. Pathak, N., Mishra, S. and Sanwal, G. G. 2000. Purification and Characterization of Polygalacturonase from Banana Fruit. *Phytochem.*, **54**: 147-152.
33. Pathak, N. and Sanwal, G. G. 1998. Multiple Forms of Polygalacturonase from Banana Fruits. *Phytochem.*, **48**: 249-255.
34. Paull, R. E. and Chen, N. J. 2000. Heat Treatment and Fruit Ripening. *Postharvest Biol. Technol.*, **21**: 21-37.
35. Payasi, A., Misra, P. C. and Sanwal, G. G. 2006. Purification and Characterization of Pectate Lyase from Banana (*Musa acuminata*) Fruits. *Phytochem.*, **67**: 861-869.
36. Payasi, A. and Sanwal, G. G. 2003. Pectate Lyase Activity during Ripening of Banana Fruit. *Phytochem.*, **63**: 243-248.
37. Prasanna, V., Prabha, T. N. and Tharanathan, R. N. 2007. Fruit Ripening Phenomena: An Overview. *Critical Rev. Food Sci. Nutr.*, **47**: 1-19.
38. Ren, C. and Kermode, A. R. 2000. An Increase in Pectin Methylsterase Activity Accompanies Dormancy Breakage and Germination of Yellow Cedar Seeds. *Plant Physiol.*, **124**: 231-242.
39. Rose, J. K. C., Hadfield, K. A., Labavitch, J. M. and Bennett, A. B. 1998. Temporal Sequence of Cell Wall Disassembly in Rapidly Ripening Melon Fruit. *Plant Physiol.*, **117**: 345-361.
40. Schirra, M., Agabbio, M., D'Hallewin, G., Pala, M. and Ruggiu, R. 1997. Response of Tarocco Oranges to Picking Date, Postharvest Hot Water Dips, and Chilling Storage Temperature. *J. Agric. Food Chem.*, **45**: 3216-3220.
41. Seymour, G. B. and Gross, K. C. 1996. Cell Wall Disassembly and Fruit Softening. *Postharvest News Information*, **7**: 45-52.
42. Varit, S. and Songsin, P. 2011. Effects of Hot Water Treatments on the Physiology and Quality of 'Kluai Khai' Banana. *Int. Food Res. J.*, **18**: 971-974.
43. Verlent, I., Smout, C., Duvetter, T., Hendrickx, M. E. and Loey, A. V. 2005. Effect of Temperature and Pressure on the Activity of Purified Tomato



فعالیت آنزیمی و تغییرات ریزساختاری درمیوه موز تیمار شده با آب گرم در طول دوره رسیدن

۱. میرشکاری، ف. دینگ، وح. م. غزالی

چکیده

میوه های موز رقم برانگان با آب گرم در دمای ۵۰ درجه سلسیوس به مدت صفر (شاهد)، ۱۰ و ۲۰ دقیقه تیمار گردید و در دمای اتاق (۲۵±۲ درجه سلسیوس) و رطوبت نسبی ۷۰ تا ۸۰ درصد جهت بررسی فرایند رسیدن نگهداری شدند. سفتی میوه، فعالیت آنزیم های تخریب کننده دیواره سلولی مثل پلی گالاکتروناز، پکتین متیل استراز و پکتین لیاز در میوه های تیمار شده و شاهد اندازه گیری شد. ریزساختار دیواره سلولی پوست موز بوسیله میکروسکپ های الکترونی نگاره و گذاره مشاهده گردید. در طی فرایند رسیدن میوه های تیمار شده سفت تر از میوه های تیمار نشده بودند. در مقایسه با شاهد فعالیت هر سه آنزیم دیواره سلولی در دو گروه میوه های موز تیمار شده کاهش یافت. تیمار آب گرم فعالیت آنزیم های تخریب کننده دیواره سلولی میوه موز برانگان را مختل نموده و انحطاط رشته های پکتین را به تاخیر انداخت. در میوه های تیمار شده ریزنگاره های میکروسکپ الکترونی تغییرات کمتری در از هم گسیختگی و شل شدن رشته های دیواره سلولی را نمایان نمود. با افزایش مدت زمان غوطه ور شدن در آب گرم نشت الکترولیتی در میوه موز کاهش یافت. نتایج این مطالعه بیانگر این است که تیمار آب گرم ۵۰ درجه سلسیوس فرایندهای رسیدن را کند کرده و می تواند عمر قفسه ای را در موز برانگان افزایش دهد.