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Microstructure of peel cell wall and selected physico-chemical characteristics of 'Berangan' banana (*Musa* cv. Berangan [AAA]) ripened at high temperature

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Abstract. Peel of mature-green 'Berangan' banana (*Musa* cv. Berangan AAA) was investigated by scanning and energy filtering transmission electron microscopy. Fruits were ripened at 25ÚC and 37 °C, and micrographs of the fruits were compared at three different stages of ripening namely green, turning yellow and full yellow. Increased of loss of cell wall integrity was exhibited by the fruit ripened at 25 °C as compared to that ripened at 37 °C, especially at turning yellow and full yellow stages because of pectin solubilization. This is supported by a greater percentage decrease on firmness in banana exposed to 25 °C. Transmission micrographs showed empty regions of the middle lamella at 25 °C at turning yellow and full yellow stages. However, a higher linear increase in soluble solids concentration at 25 °C enhanced the turgor loss in the peel. This could have contributed to the loss of firmness at 25 °C that was indicated by the higher linear increase of pulp to peel ratio. Results of this study indicated that 37 °C retains the integrity of cell wall, especially of the middle lamella as compared to 25 °C.

Keywords. 'Berangan' banana, peel cell wall, electron microscopy, temperature, physico-chemical properties.

INTRODUCTION

In Malaysia, banana is the second largest cultivated fruit crop after durian. This tropical fruit is grown in all states and available throughout the year. The popular cultivars are 'Mas' (*AA*), 'Embun' (*AAA*), 'Rastali' (*AAB*), and 'Berangan' (*AAA*) (Abdullah *et al*., 1990). Berangan, a major cultivated banana for local consumption, occupies 39 % of total banana cultivated area of 31,917.7 ha (Ministry of Agriculture, 2004). The fruit is commonly harvested at the mature-green stage and ripened off the tree to produce a yellow peel colour.

In retailer, banana was usually ripened at room temperature (27 ± 2 °C) induced by using calcium carbide (CaC_2). Smith et al. (1986) reported that the application of exogenous ethylene stimulators such as CaC_2 accelerates ripening in bananas. The banana was ripened in high densities with good air circulation. However, this ripening method results in a poor peel colour of ripening fruit. Therefore, the alternative ripening method was needed to obtain extremely high quality banana with proper colour development. It is known that high temperature could also initiate ripening of banana. Lewis (1976) found that 2-days exposure to 35 °C could initiate ripening of bananas. Seymour *et al*., (1987) also reported that bananas have been shown to be more sensitive to ethylene at 35 °C than at 20 °C.

Generally, a characteristic of the ripening process common to most fruits is a decrease in fruit firmness. In banana, the relative firmness of the fruits is greatly determined by physical and chemical attributes both peel and the pulp. Loss in firmness is shown to be associated with the activity of cell-wall degrading enzymes (Pilnik and Voragen, 1970; Ali *et al*., 2004) and it is reasonable to hypothesis that this activity could lead to structural alterations in cell walls. Harker *et al*. (1997) reported that many fruit soften during ripening, a process primarily associated with microstructural changes in the cell walls of the parenchyma cells. The parenchyma cells have non-lignified walls and are separated from the neighbouring cells by a morphologically distinct region known as the middle lamella which is rich in pectins.

Loss of turgor also affects firmness of fruits. According to Harker *et al*., (1997), the excess internal pressure of cells provides the hydrostatic component of cell and tissues

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strength and also influences the brittleness of the cell wall. Clearly, cell turgor has a major function in determining tissue strength, and changes in turgor are an integral part of fruit softening. Increasing the sugar content in the pulp could allow the water to move from peel to the pulp. This is indicated by the increase in the pulp to peel ratio of fruit. The consequence of this event would cause loss of turgor in the peel as indicated by accelerated softening process.

This paper reports a study on the changes in microstructure of peel cell wall and selected physico-chemical characteristic of Berangan banana **(***Musa* cv. Berangan AAA**)** ripened for 24 hours at 25 and 37 ^{o}C followed by further ripening at 25 ^{o}C.

RESULTS AND DISCUSSION

The obvious change in the cell packing related to the change of the cell wall was observed using SEM study. At mature green stage, there were no obvious cell ruptures indicated with cells having an angular polyhedral shape at both temperatures (Figure 1a and 1b). However for banana ripened at 25^{o}C, there were thickening of some cell walls within the tissue (Figure 1a). Cell rupture started to appear at turning yellow stage that was shown by peel of bananas ripened at 25^{o}C. Meanwhile some cell thickening was observed in cell walls of bananas ripened at 37^{o}C (Figure 1c and 1d). At 25^{o}C, the interfaces between cells were indistinct. This is due to the extensive loss of cell integrity which induces loss of cell compartment as compared to bananas exposed to 37^{o}C. Even when cells became rounded in banana ripened at 37^{o}C, large areas of the cell walls remained in close apposition. The cell ruptures at turning yellow stage ripened at 25^{o}C were more severe as compared to previous stages (Figure1c) meanwhile cell wall of peel from bananas ripened at 37^{o}C exhibited swelling cell wall followed by a spot rupture of the cells. At full yellow stage, peel cells collapsed but visible intact of the wall were still apparent in bananas ripened at 37^{o}C as compared to those bananas ripened at 25^{o}C. The disappearance of cell packing at these stages (Figure 1e and 1f) could be caused by senescence. It is often thought to involve a loss of membrane integrity resulting in tissue injury (Fan and Sokorai, 2005).

Swelling of cell wall were observed much earlier in bananas ripened at 25^{o}C as compared to that ripened at 37^{o}C. These could be due to inhibition of pectin degradation at 37^{o}C. Klein *et al.* (1990) reported that heated apple fruit contained less soluble pectin and more insoluble pectin as compared to non-heated fruit. The lower pectin concentration found at 37^{o}C gives an indication of inhibition of polyuronide degradation, especially at turning yellow stage, which could decrease fruit softening by maintaining the cell packing. As shown at Figure 2a, there was a significant linear decrease in peel firmness of banana ripened at both

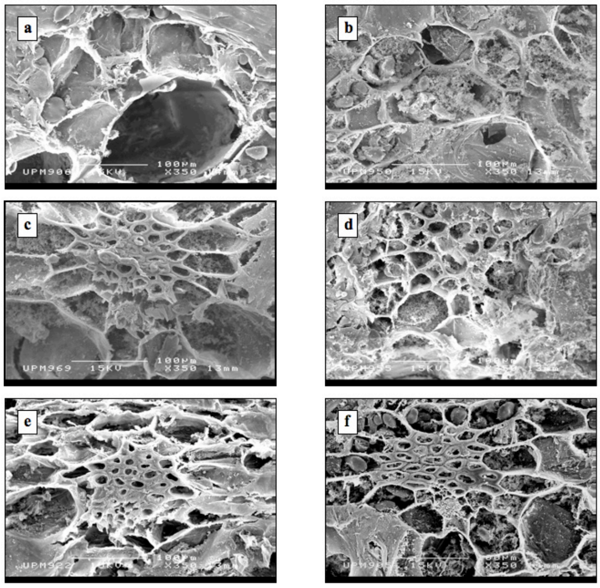

Figure 1. SEM micrographs of cell wall changes of fresh banana sample (Bar =100 μm, x350): (a) cell wall of mature green fruit ripened at 25°C. Cells have an angular polyhedral interlocking profile, (b) cell wall of mature green fruit ripened at 37 oC. Cells have an angular polyhedral interlocking profile with appearance of lipids bodies in the cells, (c) cell wall at turning yellow fruit ripened at 25°C. Cells begin to show signs of cell rupture, (d) peel cell wall of turning yellow fruit ripened at 37°C. Cells retain rounded profile; walls are ruptured slightly and showing cell wall thickening, (e) cell wall of full yellow fruit ripened at 25°C. The cells showed more ruptured cells, (f) cell wall of full yellow fruit ripened at 37°C. Irregular cell wall packing with slight rupturing of the cells. Cells retain rounded profile.

25 and 37^{o}C. The significant linear decrease indicated that peel firmness decreased with increasing ripening time. The peel firmness decreased for 42.64 % and 40.89 % at 25 and 37^{o}C, respectively during further ripening for 144 hours. This indicated that the rate of softening of banana ripened at 37^{o}C was slower as compared to softening of banana ripened to 25^{o}C. The decrease in the rate of softening could be due to the inhibition of the synthesis of cell wall hydrolytic enzymes such as polygalacturonase. In tomato, mRNA for polygalacturonase was absent in fruit during heat treatment and only appeared after it was removed from the heat treatment (Lurie *et al.*, 1996).

However, the softening of banana could not be solely affected by dissolution of cell wall pectin. The texture of fruits and vegetables is also affected by biochemical constituents and water content or turgor (Sams, 1999). Fruit softening could rise from loss of turgor, degradation of starch, or breakdown of the fruit cell walls. The excessive loss of water content was mainly caused by releasing water vapour into the surrounding atmosphere by transpiration. Excessive

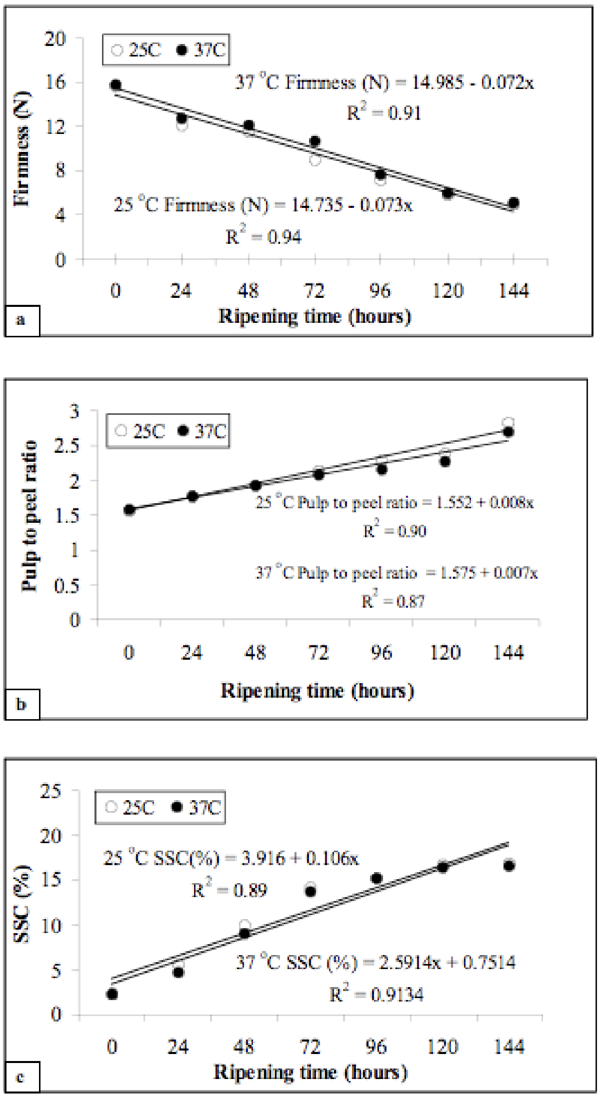

Figure 2. (a) Firmness, (b) Pulp to peel ratio and (c) SSC of 'Berangan' banana ripened at 25 and 37°C.
———— , Significant linear response

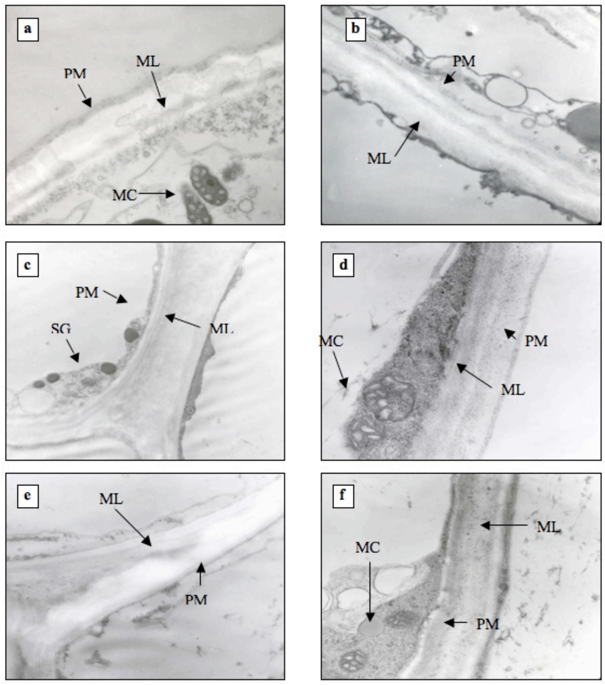

Figure 3. EFTEM micrographs of cell wall changes of fresh banana sample: (a) cell wall of mature green fruit ripened at 25°C; x 20.000. Middle lamella appears to undergo initial breakdown and are thicker, (b) cell wall of mature green fruit ripened at 37°C; x20.000. Middle lamella is tightly packed, (c) cell wall of turning yellow fruit ripened at 25°C; x8.000. Middle lamella is lightly stained and beginning to disintegrate, (d) peel cell wall of turning yellow fruit ripened at 37°C; x31.500. Middle lamella showing darker stained, fibrils in state of dispersal, and formation empty region, (e) cell wall of full yellow fruit ripened at 25°C; x12.500. Cell separation due to loss of the middle lamella is evident, (f) cell wall of full yellow fruit ripened at 37°C; x20.000. Middle lamella retained darker stained but showed more empty region both at the lamella and membrane plasma. (PM, Plasma Membrane; ML, Middle lamella; MC, Mitochondria; SG, Starch Granule).

water loss from fruits can result in a shriveled appearance (Hagenmaier and Baker, 1993). Even though, there was no shriveling in Berangan banana observed, however, the loss of water could contribute to the textural change. Ben Yehoshua (1987) reported that even in the absence of visible wilting or shriveling, water loss cause undesirable textural changes. It was expected that the loss of water content in the peel is due to the withdrawing of water from the peel to the pulp. Lizada *et al.* (1990) reported that water is withdrawn from the peel to the pulp, consequently, water content decreases in the peel but not in the pulp during ripening. As shown at Fig. 2b, there was a significant increase in pulp to peel ratio of the banana ripened at 25ºC and 37ºC. However, banana ripened at 25ºC showed more rapid increase in pulp to peel ratio as compared to that at 37ºC for as high as 42.60% and 39.02 %, respectively. This indicated that more water was withdrawn to the pulp at 25ºC than 37ºC. This phenomenon could be explained by the more rapid increase of the SSC found in banana ripened at 25ºC as compared to 37ºC (Figure 2c). The SSC increased for 82.31 % and 79.58 % at 25 and 37ºC, respectively. In this study, the increase of pulp to peel ratio coincided with the decrease of SSC indicating the increase of pulp to peel ratio promoted the increase of SSC. The SSC in fruit indicate the degree of sweetness (Sapii, 1991). The SSC of ripe Berangan banana tends to follow the same trend as the sugar content (Abdullah *et al.,* 1987).

In breadfruit, the rise of sugars at ambient temperature is consistent with reported increases in soluble solids during breadfruit ripening (Maharaj and Sankat, 1990). As reported by Wrolstad and Shallenberger (1981), sugars constitute about 80-90% of soluble solids in ripe strawberry. Therefore, the increase of SSC is generally manifested as increased of sugar content and then influenced the increase in pulp to peel ratio. Charles and Tung (1973) reported that the rise in pulp to peel ratio is related to the sugar concentration in the two tissues. There was rapid increase in sugars in the pulp during ripening as compared to that in the peel which contributes to a differential change in the osmotic pressure (Salunkhe and Desai, 1984).

The dissolution of pectin in the middle lamella could be examined obviously by using EFTEM. At the mature green stage, cell walls from banana ripened at 25°C and 37°C were rather similar in structure and both displayed a conspicuous darked stained middle lamella (Figure 3a and 3b). However, the middle lamella of ripening bananas began to dissolve at 25°C by showing incompactness of the middle lamella line. Some fibrillar materials were seen to be more tightly packed to the primary wall of banana ripened at high temperature, even though, it showed little empty regions at these sites. The disappearance of tightly packed middle lamella was seen both at turning yellow and full yellow stages. At turning yellow stage, the middle lamella had undergone dissolution and showed a flaky structure especially for ripened bananas (Figure 3c and 3d) at 25°C. Even though, some electron densed regions could still be seen at the cell wall, it mainly showed an empty region at the region previously occupied by the middle lamella. At full yellow stage, a more advanced dissolution of the middle lamella dissolution was exhibited at both ripening temperatures (Figure 3e and 3f).

In conclusion, banana fruit ripened at 25°C showed more loss in integrity of the peel cell wall as compared to those ripened at 37°C as examined using SEM and EFTEM. Pectin solubilization in the middle lamella was probably the causal factor for cell wall disintegration both at turning yellow and full yellow stages. The sequential effect of this phenomenon was a more advanced softening at 25°C as compared to 37°C. The advanced softening process at 25°C was also caused by higher loss of turgor in the peel due to the movement of more water to the pulp that was contributed by the higher SSC at 25°C as compared to 37°C.

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