

Cellular Structure and Related Physico-Chemical Changes During Ripening of *Musa* AAA 'Berangan'

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ABSTRACT

Ripening is the result of complex changes and it turns an unpalatable fruit to be palatable. The distinct changes in eating quality of ripening fruits are determined by changes in their cellular structure and composition. This work was carried out to relate structural changes of *Musa* AAA 'Berangan' with related physico-chemical quality characteristics during ripening. The cell structure of Berangan banana was examined using scanning electron microscopy while pulp firmness, soluble solids concentration and starch pattern were determined as fruit ripened from mature green stage (ripening stage 1) until full yellow (ripening stage 6) at 27°C. As ripening progressed, the cellular structure of Berangan banana showed dramatic changes and coincided with changes in the physico-chemical characteristics. The epicuticular wax and stomatal complex of peel degenerated while cell wall integrity was lost as fruit ripened. The starch began to degrade from the central core outward and concomitant with a decrease in starch granules sizes and increase of soluble solids concentration as ripening took place. The loss of cell wall integrity and conversion of starch into sugars as ripening progressed contributed to fruit softening. It is concluded that as ripening progressed, the increase in soluble solids concentration and decrease in firmness of Berangan banana fruit was closely related to the changes in the cellular structure.

Keywords: Epicuticular wax, firmness, soluble solids concentration, starch granules, starch distribution pattern

INTRODUCTION

In Malaysia, bananas are the second largest cultivated fruit crops, after durian (*Durio zibethinus*). The major commercial bananas cultivated by smallholders are 'Berangan', 'Mas' and 'Rastali', while the larger plantations grow Cavendish. Cavendish banana is the most commercially important banana in global trade; however, it does not degreen naturally under tropical temperatures such as in Malaysia (Ding *et al.*, 2007). To degreen Cavendish banana under Malaysian conditions, cool ripening rooms have to be built to provide temperatures of 18-20°C. This has resulted in increased production costs for smallholders who are the major banana producers in Malaysia. In light of these difficulties, smallholders prefer to plant traditional cultivars of bananas which can degreen naturally under tropical conditions

although Cavendish banana is a major international trading banana.

Ripening is the result of complex changes. During ripening, green peel of banana will turn to yellow with the breakdown of chromoplast grana-thylakoid membranes (Ding *et al.*, 2007). The texture of fruit softens progressively. The relative firmness of the fruit is greatly determined by physical and chemical attributes such as peel thickness and starch content (Lizada *et al.*, 1990). The starch in the pulp of banana will be hydrolysed into sugar (Marriot *et al.*, 1981) and organic acids, which give desirable sugar-to-acid balance, will change. Malic acid will be the main acid during ripening, followed by oxalic and citric acids (Marriott, 1980).

Among local cultivars, Berangan banana is of highest popularity. The market potential of Berangan banana increased by 5% per annum

to 39,970 metric tonnes in 2006 (Anon, 2006). Malaysia exports Berangan banana mainly to Singapore, Brunei, Germany and UAE. Unlike Cavendish banana whose genome make up is also AAA, the peel of the Berangan banana naturally changes from green to golden yellow as ripening progresses under tropical conditions. Information on the changes in structural aspects during ripening has importance in relation to fruit quality, storage and commercial value. There is very little information on the cellular structure of this Berangan banana cultivar during ripening. This study attempts to relate structural changes in the peel and pulp of Berangan banana using scanning electron microscopy with selected physico-chemical quality characteristics during ripening.

MATERIALS AND METHODS

Plant Materials

Mature green *Musa* AAA 'Berangan banana' bunch were obtained from a fruit distributor and transported to the laboratory. Hands of second and third from the proximal end of the peduncle of a bunch were used in the experiment. A total of 4 bunches of banana were used and each bunch served as a replicate. Each hand containing 16-18 fingers, with each finger weighing 80-90 g, were placed in a box and gassed with acetylene from CaC_2 (with an equivalent of 10 g CaC_2 kg^{-1} fruit) at room temperature of $27 \pm 2^\circ\text{C}$ with relative humidity of 85% for 24 h. The fruits were allowed to ripen in the same environment after 24 h of ripening initiation. Fruit ripening was visually assessed based on the following ripening stages (RS) as established by the Federal Agricultural Marketing Authority: 1 = mature green; 2 = tinge of yellow; 3 = more green than yellow; 4 = more yellow than green; 5 = yellow with green tips and 6 = full yellow. Two fingers from each RS of fruit were analysed with each fruit taken from the upper and lower row of a hand.

Scanning Electron Microscopy (SEM) Studies

Samples blocks measuring 0.5 cm x 0.5 cm x 1 cm were taken from the mid region of fruit and fixed in Bouin's fixative for 4 h under vacuum. The samples were washed in 1% cacodylate buffer, post-fixed in 1% osmium tetroxide in 0.1 mol L^{-1} cacodylate buffer for 2 h, washed in 0.1 mol L^{-1} cacodylate buffer before dehydration

through graded series of ethanol to absolute ethanol, and critical-point-dried using a Balzer CPD 030 (Balzer Union, Furstentum, Liechtenstein). The fruit tissues were mounted on stubs, sputter coated in gold and viewed in a JEOL 6400 SEM (JEOL Ltd., Tokyo, Japan) at an acceleration voltage of 15 kV and at working distance of 15 mm. Dimension of cells at the five upper layers of peel and starch granules in pulp were measured using Semafore (digital slow scan image recording system) release 3.04. The length was the longest distance between two ends and the width was the distance transversely across at the middle of a cell and starch granule. Fifty cells at the uppermost layer of peel and 50 starch granules in pulp per replicate were determined from each RS and replicated twice.

Physico-chemical Characteristics

(i) Determination of flesh firmness

Flesh firmness was evaluated using the Bishop Penetrometer FT 327 (Alfonsine, Italy). The force required for an 11-mm probe to penetrate to the depth of 5 mm of the pulp was recorded. The penetration force was expressed in kg cm^{-2} . Two fingers of each RS were analyzed at each time and replicated four times.

(ii) Determination of soluble solids concentration (SSC)

One centimeter thick of banana pulp was cut transversely from mid region of a banana finger, and a total of 2 fingers for each RS were macerated with a chopping knife. Ten grams of the macerated tissue was homogenised with 40 mL of distilled water by using a kitchen blender. The mixture was filtered with cotton wool. A drop of the filtrate was then placed on the prism glass of a refractometer (Atago Co, Ltd., Model N1, Tokyo, Japan) to obtain the %SSC. The readings were corrected to a standard temperature of 20°C by adding 0.28% to obtain %SSC at 27°C . The determination was repeated four times.

(iii) Determination of starch distribution pattern

A 2-cm-thick cross-sectioned of pulp cut from the mid-region of a fruit was stained with 1% of KI/I_2 for 1 min. The intensity and area of stain was photographed with a camera (Canon EOS 888, Japan) and total of four replications were carried out.

Statistical Analysis

The experiments were conducted using a randomized complete block design with each RS replicated four times using 2 fingers at a time except for SEM studies which were repeated twice. Data were analyzed using analysis of variance (SAS Institute, Cary, NC) and means were separated with Duncan's multiple range test (DMRT) at $P \leq 0.05$.

RESULTS

The peel of Berangan banana fruit was bounded by epidermis. The epidermal cells were hexagonal in shape and covered by lamellae-like strands of wax that fused together into a compact mound forming papillae topography (Fig. 1A). Elliptical-shaped stomata were distributed randomly among epidermal cells and raised slightly above the surface. The stomata density and stomatal aperture have been reported by Ding *et al.* (2006). At RS 1, the lamellae-like strands of wax were distinct in appearance (Fig. 1B). When green fruit began to turn yellow, the wax started to degenerate and became completely degenerated in full yellow ripe fruit, RS 6 (Fig. 2A). The guard cells, subsidiary cell and extended wings of stomata were also deformed at RS 6 (Fig. 2B). The stomata were no longer found to be raised in later stage of ripening, especially at RS 6. This could be due to the loss of guard cells turgidity and lead to the sunken appearance of the stomatal complex as senescence started to take place at the end of the ripening process.

The integrity of cell walls was intact in mature green RS 1 cells (Fig. 3A). As ripening progressed, the breakdown of cell wall was clearly noticed in the micrograph of the peel at RS 6 (Fig. 3B). The characteristic of the pulp region of Berangan

banana was the starch granules containing cells. The granules were irregular in shape with spheroid and oval elongated form being predominant (Fig. 4A). In green bananas, the surface of the starch granules was smooth and the cell wall and membrane were well organized. As ripening advanced, the cell wall and membrane broke down. At RS 6, parallel striation appeared on the previously smooth surface of starch granules (Fig. 4B). The length and width of starch granules decreased significantly ($P \leq 0.05$) as ripening progressed (Table 1).

The firmness of Berangan banana flesh decreased significantly ($P \leq 0.05$) by 83% as fruits ripened from RS 1 to 3 (Table 2). Thereafter, there was no significant decrease in flesh firmness until RS 6. The SSC of fruits was closely associated with ripeness stage where it increased significantly ($P \leq 0.05$) as ripening progressed (Table 2). RS 1 had the lowest while RS 6 had the highest SSC value. The SSC values increased by 648% as fruits ripened from stage 1 to 6. Hydrolysis of starch into sugar increases the SSC value. The increase in SSC with hydrolysis of starch into sugar could be further explained by the starch iodine pattern (Fig. 5).

Before fruit ripening initiation (RS 1), the tissue was not well stained with iodine solution and showed lightly stained blue-black colour. Probably the exuded latex in green mature fruits prevented the starch from being stained by iodine solution. However, some areas of peel tissues were stained with iodine solution. Once the ripening had been initiated (RS 2), the iodine solution began to stain well with the banana pulp starch and some area of peel tissues. At this RS, the whole transversal surface was stained with blue-black colour. At RS 3, the blue-black

TABLE 1
Effects of ripening stage on pulp starch granules length and width of Berangan banana fruit

Ripening stage	Starch granules length ^z (µm)	Starch granules width ^y (µm)
1	29.26 a ^x	15.12 a
2	27.78 a	13.73 b
3	26.21 bc	12.76 bc
4	24.66 cd	12.17 c
5	23.61 de	12.00 c
6	22.79 e	11.50 c

^{z,y} Mean of 100 observations

^x Mean separation within column by DMRT at $P \leq 0.05$.

TABLE 2
Effects of ripening stage on soluble solids concentration and firmness of
Berangan banana fruit pulp

Ripening stage	Soluble solids concentration (%SSC)	Firmness (kg cm ⁻²)
1	2.63 e ^z	10.60 a
2	10.25 d	2.97 b
3	14.00 c	1.78 c
4	16.61 b	1.44 c
5	17.53 b	1.27 c
6	19.67 a	1.16 c

^z Mean separation within column by DMRT at $P \leq 0.05$.

stain at the central core of the fruits started to clear while the peel tissue was no longer stained by iodine solution. The stain clearing of the pulp central core became obvious as ripening advanced. At the end of evaluation (RS 6), the ovarian cavity of Berangan banana was not stained with iodine solution and only the peripheral of the transverse section was stained in blue-black colour. The pattern indicated that the starch degradation started at the central core of the fruits and advanced towards the outer borders as ripening progressed.

DISCUSSION

The surface morphology of Berangan banana was papillae with a well-organized pattern. Burdon *et al.* (1993) found that the surface sculpture of banana fruits differ among fruit genome. To the best of the author's knowledge, there is little literature reported in changes of epicuticular waxes as ripening progressed. Bally (1999) observed changes in epicuticular surface structure during fruit development of 'Kensington Pride' mango. However, there was no study on the changes of epicuticular surface as ripening progressed. The epicuticular wax with lamellae-like strands of Berangan banana degenerated as ripening progressed (*Fig. 2B*). Epicuticular waxes play an important role in regulating water loss, uptake or release of chemicals (Riederer and Schreiber, 1995) and act as barriers to insects and pathogens (Juniper and Cox, 1973). In cranberry fruit, the thicker wax accumulation at calyx end was related to microorganism entry retardation into the fruit during wet harvest (Ozgen *et al.*, 2002). The degenerated epicuticular waxes at RS 6 (*Fig. 2A*)

of Berangan banana indicated the physical barrier had weakened and the fruits are prone to microorganism infection as compared to RS 1.

The loss of cell wall integrity and disappearance of starch granules as observed under SEM in Berangan banana was similar to the findings of Prabha and Bhagyalakshimi (1998) for 'Robusta' banana. Microscopic examination of jackfruit (*Artocarpus heterophyllus L.*) showed loss of cell wall integrity and starch granules as ripening progressed (Rahman *et al.*, 1995). A similar observation was also reported in ripening of 'Harumanis' mango where the cells lose their integrity and starch granules were degraded as ripening progressed (Muhammad and Ding, 2007). This indicated cellular structures of fruits undergo degradation as ripening progressed and this shortens the postharvest life of fruits if poor postharvest management is being practised.

As ripening progressed, the starch granules hydrolysed and sugar accumulated as reflected by high %SSC and fading of starch-iodine staining pattern. Hydrolysis of starch granules could be noticed with striation appearing on its surface as observed under SEM (*Fig. 4B*). This striated structure is due to the action of amylase during ripening which hydrolysed polysaccharides of starch granules into monosaccharides (Fuwa *et al.*, 1979). As a consequence of this action, the length and width of starch granules decreased significantly ($P \leq 0.05$) as ripening progressed from stage 1 to 6 (Table 2), indicating starch granules turned from large to small size. In addition, the density of starch granules in pulp cells decreased as ripening progressed from green to full yellow stage. Starch constituted 85-

95% of the dry matter in green banana pulp, which is reduced to 5-15% and consequently sugar content increased to 70-80% as fruits ripened (Hubbard *et al.*, 1990). The enzymatic transformation of starch granules to soluble sugars (e.g. sucrose, fructose and glucose) appeared as a reduction in granular size in ripe banana as evidenced in Table 1. Prabha and Bhagyalakshmi (1998) observed that more than 80% of the radio-activity of [¹⁴C] starch was incorporated into soluble sugars in Robusta banana, indicating active sugar interconversions. The transformation of starch is a complex process involving several enzymes and more than one pathway as proposed by Beatriz and Lajolo (1995).

The loss of starch from the central core of banana has also been reported by Blankenship *et al.* (1993) and Garcia and Lajolo (1988) in Cavendish banana. This indicated that Berangan banana had the similar starch-iodine staining pattern as Cavendish banana. The starch degradation advanced towards the outer border of the peel as ripening progressed until the starch was completely degraded at RS 6. The blue-black stain was due to the presence of amylose. Garcia and Lajolo (1988) noted that banana starch consists of 16% amylose which is the unbranched chain polysaccharide component of starch and has a strong affinity for iodine to produce a deep blue-black complex.

As ripening progressed, fruit firmness of Berangan banana decreased markedly (Table 3). During ripening, pectins and hemicelluloses of cell wall undergo solubilization and depolymerization thus leading to softening (Redgwell *et al.*, 1992). The insoluble pectins of banana decreased from 0.5 to 0.2% fresh weight with a corresponding rise in soluble pectins (Marriot *et al.*, 1981). The interconversion of pectic substances is presumed to lead to cell wall breakdown and results in loss of turgidity and firmness of banana fruits during ripening. It is also reported that the softening of banana fruits during ripening is a result from the concerted action of at least four polygalacturonase genes (Asif and Nath, 2005). The decrease of fruit firmness is also related to starch hydrolysis. Starch could contribute to the structural properties of fruits (John and Marchal, 1995) and create resistance to the penetrating force. Seymour (1993) stated that the loss of starch likely plays a major role in textural changes in ripening

bananas. Recently, Sane *et al.* (2007) found out that expression of multiple expansin genes in Dwarf Cavendish banana might be required for softening of monocot fruits too.

In conclusion, once ripening is initiated, Berangan banana showed an irreversible structural and physico-chemical change. The cellular structure changes observed under SEM were closely associated with physico-chemical changes. As ripening took place, fruits soften due to cell wall breakdown and conversion of starch into sugar. As a result, fruits become palatable with soft and sweet pulp. Senescence happening at the end of ripening will degenerate the structure of fruit cells and cause the fruit to lose quality.

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