Tropical Natural History 12(2): 127-136, October 2012
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Anatomical Changes in Peel Structure of 'Hom Thong' Banana during Fruit Development and Ripening

NUANKAMOL AMNUAYSIN[1,2], KANOGWAN SERAYPHEAP[2] AND MANIT KIDYOO[2*]

[1]*Biological Science Program, Faculty of Science, Chulalongkorn University, Bangkok 10330, THAILAND*
[2]*Department of Botany, Faculty of Science, Chulalongkorn University, Bangkok 10330, THAILAND*
* Corresponding author. E-mail: kidyoo_m@yahoo.co.th
Received: 18 February 2012; Accepted: 6 June 2012

ABSTRACT.– **The cellular structure of fruit peel is one of the important factors determining fruit texture, a primary component of fruit quality. In this study, the anatomical alteration in peel of 'Hom Thong' banana (*Musa acuminata*, AAA group, Gros Michel subgroup) during fruit development and ripening was investigated. The result showed that 'Hom Thong' banana peel is composed of epidermis, ground tissue, and vascular bundle. The structure of peel changed with regard to number and size of cells during fruit maturation leading to an increase of peel thickness and number of cell layers. Cells in the epidermis, ground tissue, and vascular tissue increased in size with fruit development. In contrast, the reduction of peel thickness, cell layer number, and cell size was observed when ripening initiated. Furthermore, formation of air space increased during ripening. Cell damage and rupture were also found following fruit ripening. Therefore, the alteration in internal structure of peel was associated with changes in external symptoms especially the loss of peel firmness which lead to fruit softening during banana ripening.**

KEY WORDS: **banana, structural alteration, fruit development, ripening**

INTRODUCTION

Banana (*Musa* L.) which belongs to the family Musaceae is cultivated for different purposes, such as a vegetable, fruit, medicine, and for cosmetics. Banana is cultivated in more than 100 countries throughout tropical and subtropical areas. The annual world production of banana is around 98 million tonnes and a third is produced in African, Latin American, Asia-Pacific, and the Caribbean regions (Frison and Sharrock, 1999). Most edible bananas originated from two species, *M. acuminata* Colla and *M. balbisiana* Colla. The cultivars are either hybrids among subspecies of *M. acuminata* or between *M. acuminata* and *M. balbisiana*. Edible cultivars are mainly triploids of the acuminata genome (AAA) with varying degrees of parthenocarpy and sterility (Cheesman, 1947; Simmonds, 1962).

In Thailand, at least 110 *Musa* species and cultivars have been found including 'Hom Thong' banana (Wongniam et al., 2010). 'Hom Thong' (*Musa acuminata*) is an edible triploid cultivar. It belongs to the AAA genomic group and Gros Michel subgroup. Homogenomic AAA triploid is derived from hybridization within *Musa acuminata* (AA), a wild diploid species. The AAA genomic group is the main cultivar of international commerce (Fortescue and Turner, 2005).

Banana fruit, called a finger, is characterized as a berry and developed from an inferior ovary of female flowers. The outer protective layer of the banana fruit is known as the peel which is derived from the ovary wall. The triploid sweet banana cultivars include 'Hom Thong' banana which is a parthenocarpic fruit developed without fertilization. In principal, growth of the ovary in parthenocarpic fruit occurs in two ways; the first is the inward growth of the pericarp tissue, and the second is the expansion of the central floral axis, placenta, and septa. Finally, the ovarian cavity is obliterated completely by filling up with a soft, fleshy tissue, but there is no development of ovules into seeds (Mohan Ram et al., 1962; Fortescue and Turner, 2005).

According to the Office of Agricultural Economic (2011), 'Hom Thong' banana is an important export cultivar from Thailand. Softening is a major aspect of the ripening process which affects shelf life, pathogen infection, and physical injury during postharvest handling and storage (Goulao and Oliveira, 2008). This event relates to change in fruit texture, an important quality feature for consumer acceptance. Textural quality is of commercial importance in fleshy fruits including banana. Changes in texture and firmness of banana peel have a direct impact on consumer preference. However, cellular structure of banana peel, the factor determining the fruit texture, has been described infrequently. Therefore, the objective of this study was to examine changes in cellular structure of 'Hom Thong' banana peel during fruit development and ripening. This descriptive account provides basic information that could be applied in the development of post-harvest treatment to improve texture and shelf life by combining physiological studies and conventional breeding to develop new cultivars. Anatomical characteristics also provide supporting data for the classification of cultivars.

MATERIALS AND METHODS

Plant Material.– *Musa acuminata*, AAA group, Gros Michel subgroup, cultivar 'Hom Thong' was used in this experiment. The study was divided into two periods, fruit development and ripening period. Various stages of development of flower and fruit of banana, with weeks numbered from the day the flower cluster's bracts were shed, were obtained from a local farm in Chachoengsao province. The fruits without noticeable defects were sorted for each developmental stage. For the ripening period, banana fruits at the mature green stage were stored at room temperature in laboratory until ripening.

Determination of Anatomical Structures.– Small pieces of peel were cut from the mid zone of banana fruits of each developmental stage and sectioned by Automatic plant microtome MT-3 at 70 μm thickness without embedding. The sections were stained with safranin O. In addition, banana peel at various ripening stages was fixed in formalin-aceto-alcohol (FAA). The fixed tissue was dehydrated in ethanol dehydration series and embedded in paraffin wax using the method of Ruzin (1999). The tissues embedded in paraffin were sectioned at 10 μm on a rotary microtome and attached to slides. The sections were then stained with safranin O and fast green. All stained sections were observed under a light microscope.

Images of banana peel were captured in high resolution by Multipurpose Microscope (Olympus BX-51). Peel thickness, number of cell layer, and area of air space in transverse section were measured. Measurements of epidermal cell, parenchyma cell, vessel, and fiber widths were made to record changes in size.

Thickness of fiber wall was also measured. Peel surface imprints were made using clear nail polish to study stomata shape and size, and density of stoma were calculated.

Determination of Peel Firmness.– To determine peel firmness of banana fruit, a penetrometer (Hardness tester FHM-1, Takemura, Japan) firmness tester (0.5-cm tip) was used. Readings were taken on three regions of peel and were recorded in Newtons. The mean of the three readings was taken as a measure of firmness for each fruit.

Determination of Peel Color.– Change of banana peel color was measured in three regions of each fruit using the Color Konica Minolta CR-10 (Konica Minolta Sensing, Inc., Japan), and the average of three values was calculated for individual fruit. The peel color was expressed as L and hue values. L value represented lightness which range from 0 = black to 100 = white. Hue value was calculated to interpret the color changes, the angle of tangent -1b*/a* which a* showed red (+) or green (-) and b* showed yellow (+) or blue (-). A low hue value meant yellow coloration and high value indicated green coloration.

RESULTS AND DISCUSSION

Anatomical Peel Structure of 'Hom Thong' Banana at the Mature Green Stage.– Anatomical analysis revealed that the peel is composed of three main parts; epidermis, ground tissues, and vascular bundle (Fig. 1A). The epidermis consists of one layer of small rectangular and square cells without intercellular space (Fig. 1B). The epidermal cells had a thin cell wall and were covered by a thin layer of cuticle on the outer surface. The scattered stomata were observed in the epidermis and the stomatal shape was elliptical with kidney-shaped guard cells surrounded by 4 subsidiary cells (Fig. 1C). The structure of the subsidiary cells was similar to the surrounding epidermal cells. An elliptical shaped stomata is called an anomocytic type, the same type that was found in the stem and leaf of banana (Fahn, 1990; Sumardi and Wulandari, 2010).

Ground tissue was the main part of banana peel that could be divided into two regions, outer and inner ground tissues (Fig. 1A). The outer ground tissue consisted of seven to twelve layers of pentagonal to polyhedral parenchyma cell. The arrangement of cells was orderly with small triangular intercellular spaces. Most of the cells in this zone were found to contain chloroplasts providing green coloration to banana peel.

In addition, we found that the inner ground tissue of the peel contained loose parenchyma consisting of isodiametric cells which were less orderly compared to the outer zone. The formation of air spaces was also observed in the inner ground tissue (Fig. 1A). Furthermore, starch-containing parenchyma cells were found in this region, and the starch granules were mainly rod shaped. There was evidence that the accumulation of starch granules increased in the maturation period, and starch granules started to diminished when the fruit turned yellow (data not shown). The reduction of starch was due to the conversion of starch to sugar during fruit ripening, which gives the ripe fruit a sweet taste (Goulao and Oliveira, 2008).

The other component distributed throughout ground tissue was the vascular bundles (Fig. 1A). They comprised of phloem which were arranged adjacent to the xylem inside (Fig. 1D). Phloem was made

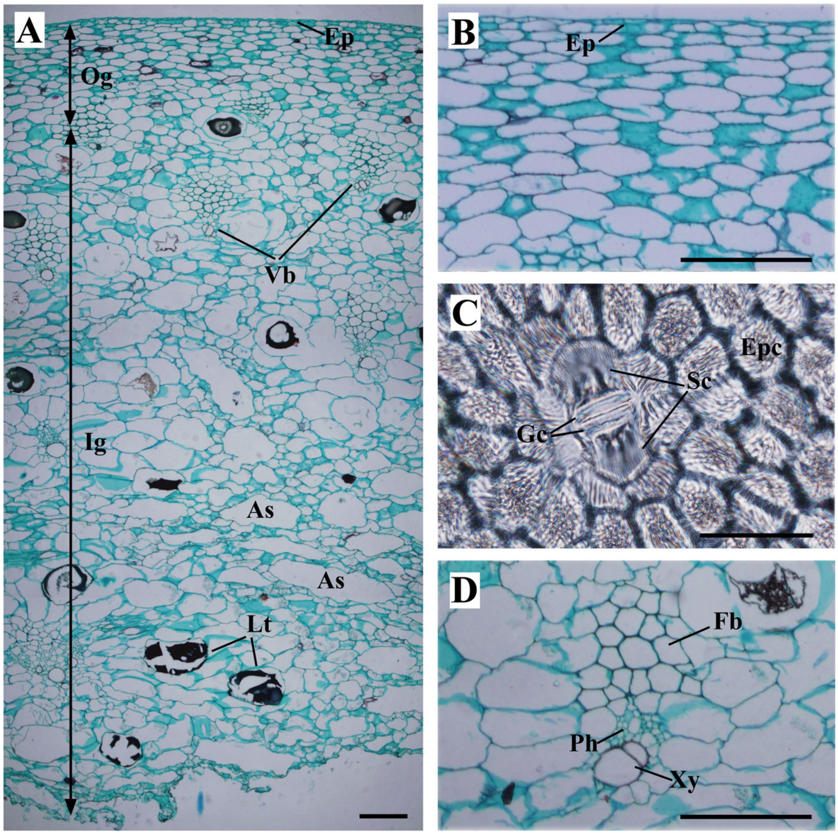

FIGURE 1. Peel structure of 'Hom Thong' banana at the mature green stage (A); Epidermis (B), Stoma (C); Vascular bundle (D). Abbreviations: Ep, Epidemis; Og, Outer ground tissue; Ig, Inner ground tissue; Vb, Vascular bundle; As, Air space; Lt, Laticiferous; Gc, Guard cell; Sc, Subsidiary cell; Epc, Epidermal cell; Xy, Xylem; Ph, Phloem; Fb, Fiber. Scale bars: A, B, C and D = 0.2 mm.

up of small rectangular to pentagonal cells with a thin cell wall, whereas, xylem contained a vessel that had large circular cells with a thick secondary cell wall. Fiber was located above the phloem and had a polyhedral shape in transverse section (Fig. 1D). The outer vascular bundles seemed to be more fibrous than the inward vascular bundles, but vascular elements of inward bundles were more prominent.

The vascular bundles observed in the mature green stage of banana were surrounded by laticiferous or latex vessels that formed rings and were separated by thin cell wall parenchyma (Fig. 1A). The latex of banana clearly demonstrated stained tannin (Lt; Fig. 1A). However, Kallarackal et al. (1986) reported that in addition to tannin banana latex had three kinds of colloidal suspension including lipid globules, lutoid, and cytoplasmic fragments. It was known that tannin, which was defined as polyphenolic compounds, caused astringency in fruit (Goldstein and Swain, 1963). Tannin could be converted into insoluble compounds leading to the reduction of astringent taste in banana fruit during ripening (Lizada et al., 1990)

Anatomical Peel Structure and Firmness of 'Hom Thong' Banana During Maturation and Ripening.– Microscopic examination of the transverse section of 'Hom Thong' banana at various stages during fruit development from ovary demonstrated an alteration in cellular structure of banana peel both in qualitative and quantitative attributes during the maturation and ripening period. The thickness of the peel ranged from 1.1 to 3.8 mm, and it increased considerably from the flower stage to one week after the opening of the flower clusters and increased slightly thereafter (Figs. 2 and 3A). The number of cell layers tended to follow the same trend as peel thickness (Fig. 3B). This might be due to the increase in cell number by cell division in the early stage of fruit development and growth by cell

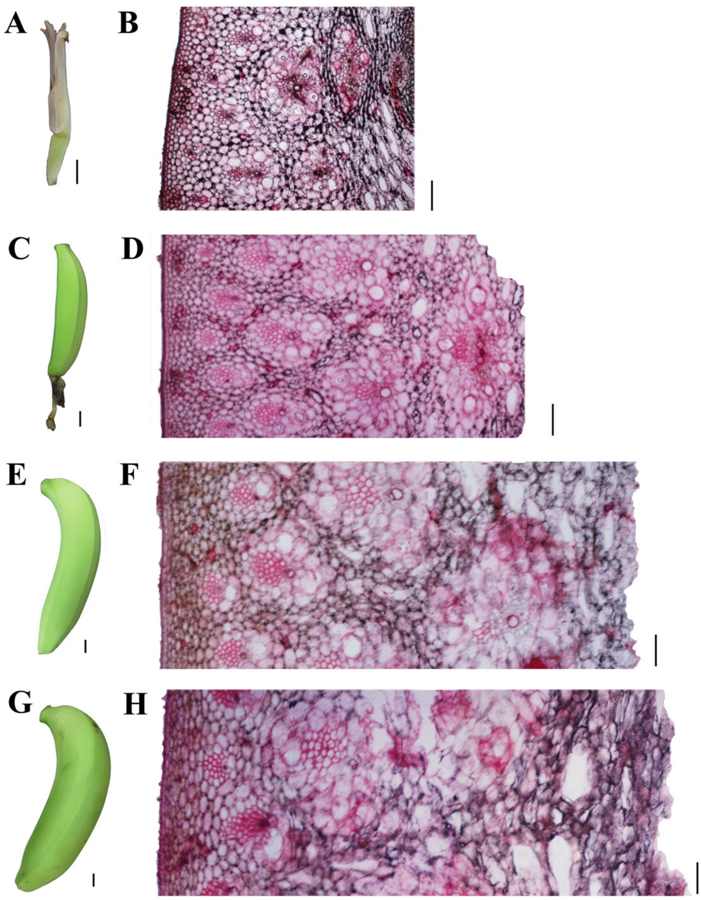

FIGURE 2. The development of 'Hom Thong' banana fruit from flower and peel structure in transverse section. Flower and its transverse section (A and B); Banana fruit at 1 week and its transverse section (C and D); Banana fruit at 4 week and its transverse section (E and F); Banana fruit at 8 week and its transverse section (G and H). Scale bars: A, C, E and G = 1.0 cm; B, D, F and H = 0.2 mm.

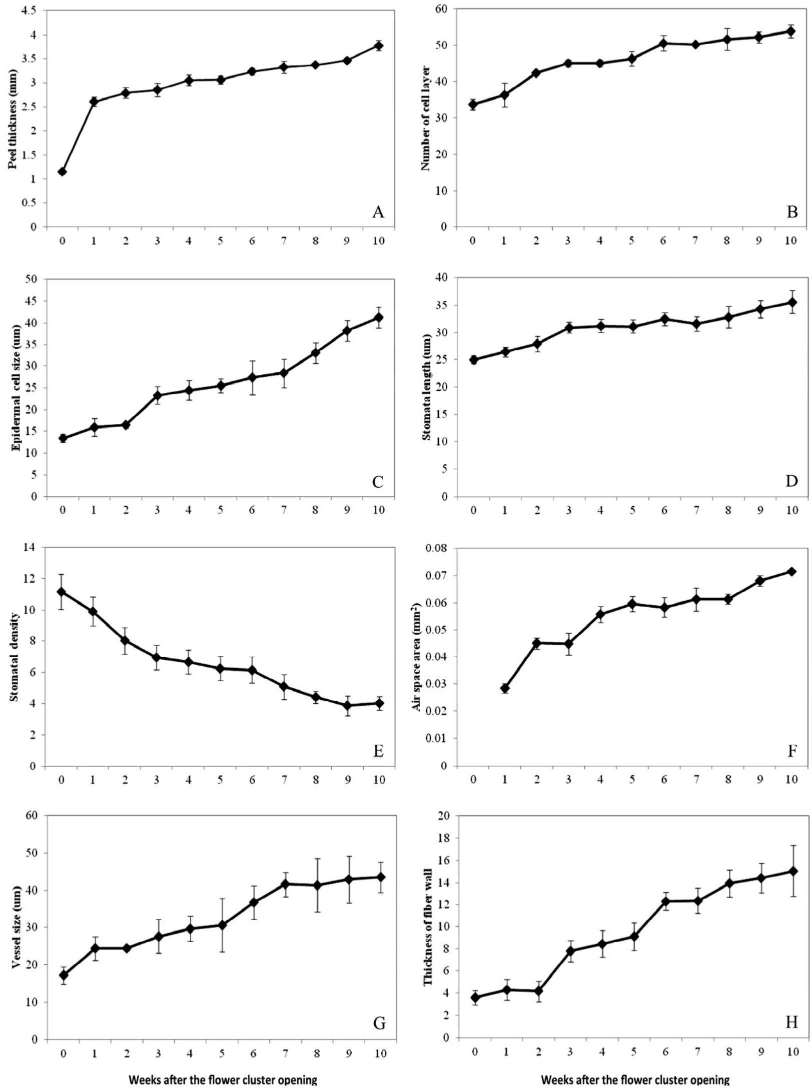

FIGURE 3. Changes in peel thickness (A), cell layer number (B), epidermal cell size (C), stomata length (D), stomatal density (E), air space area (F), vessel size (G) and fiber wall thickness (H) during maturation of 'Hom Thong' banana peel. Data represents the mean ± standard deviation.

enlargement in later stages. However, peel thickness and cell layer number decreased continuously when ripening was initiated (Figs. 4, 5A and 5B). This reduction could be explained by modifications occurring in the cell wall and middle lamella during ripening leading to change in cell wall structure; the walls of some cells collapsed while some cells had joined with other cells. Loss of cell wall integrity was observed in banana peel during ripening, and the formation of an empty region in the middle lamella was also found (Ratule et al., 2007). Moreover, the decrease in peel thickness and number of cell layers was associated with peel firmness that declined with further ripening (Fig. 5C).

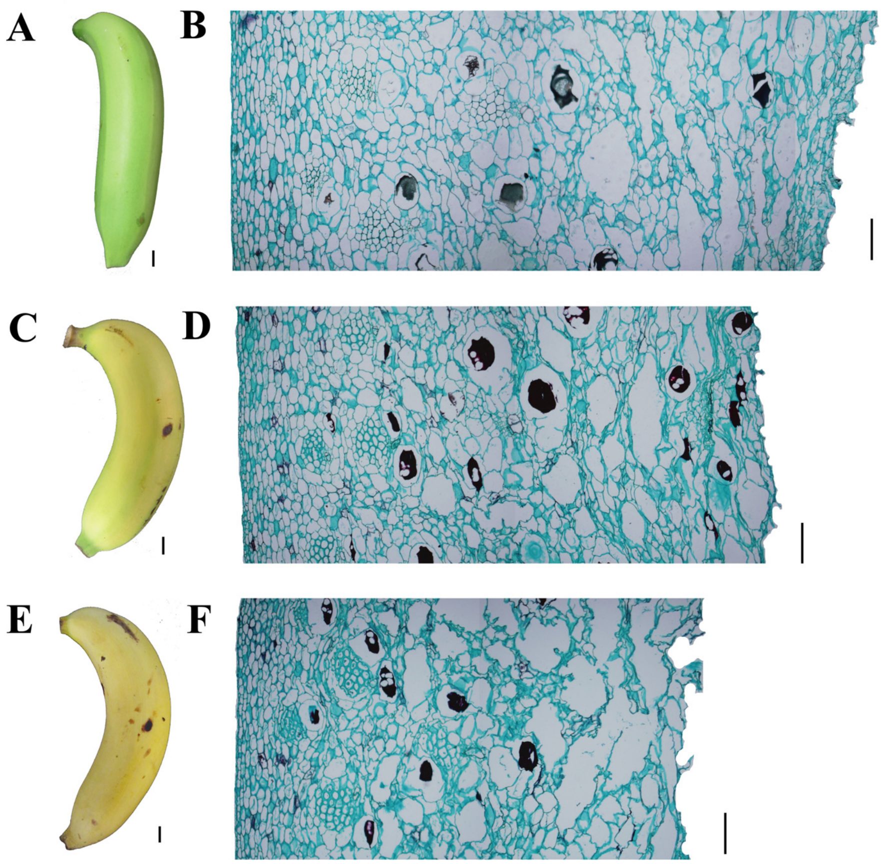

FIGURE 4. The ripening of 'Hom Thong' banana fruit and peel structure in transverse section. Banana fruit at mature green stage and its transverse section (A and B); Banana fruit at yellow more than green stage and its transverse section (C and D); Banana fruit at full yellow stage and its transverse section (E and F). Scale bars: A, C and E = 1.0 cm; B, D and F = 0.2 mm.

The epidermis remained as one layer throughout fruit development and epidermal cells increased in size with fruit maturation (Figs. 2 and 3C). Santhakumari and Krishnamurthy (1991) reported that in 'Poovan' banana expansion of epidermal cells mainly ocurred in a tangential direction. Size of guard cells increased in a longitudinal direction with fruit enlargement (Fig. 3D). The calculation of stomatal number per mm^2 showed that stomatal density declined as bananas age and ripen (Figs. 3E and 5D). This reduction is due to the increase of epidermal and stomatal cell size. A similar result was reported in 'Poovan' banana (Santhakumari and Krishnamurthy, 1991).

The size of the cell in ground tissue was found to increase as fruit development, but cell size reduced after initiation of ripening (Figs. 2 and 4). Cell rupture and collapse were detected when bananas ripened, which could be a cause of decreased cell size. The measurement of air space area in peel

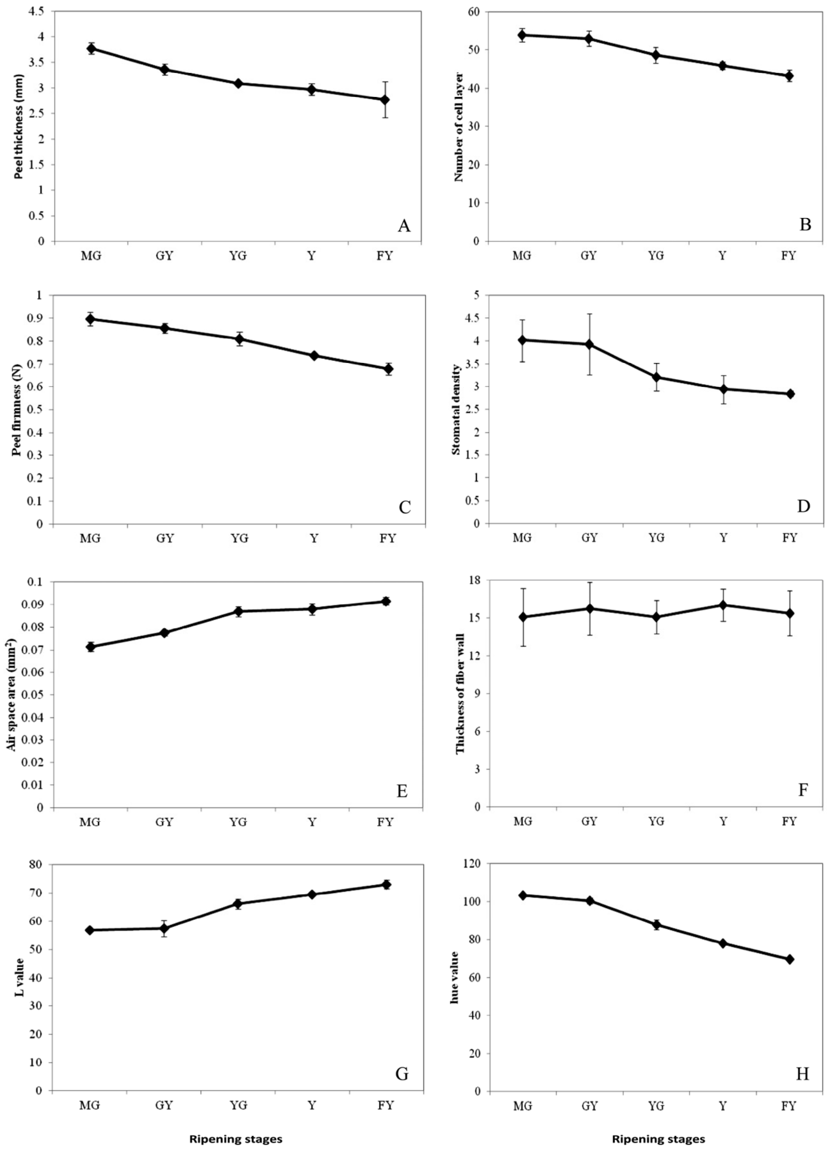

FIGURE 5. Changes in peel thickness (A), cell layer number (B), peel firmness (C), stomatal density (D), air space area (E), fiber wall thickness (F), L value (G) and hue value (H) during ripening of 'Hom Thong' banana peel. Data represents the mean ± standard deviation. Abbreviations: MG, Mature green; GY, Green more than yellow; YG, Yellow more than green; Y, Yellow; FY, Full yellow.

indicated that the formation of air space increased throughout fruit development (Fig. 2 and 4). Air space formation was higher in the ripening period (Fig. 5E) than in the maturation period (Fig. 3F). The formation of air space suggested that polysaccharide modification in the middle lamella and the cell wall was induced by increased activity of cell wall degrading enzymes, which led to damaged cell walls and cell separation, thus forming larger spaces. Several reports noted the increase of cell wall degrading enzyme activities during banana ripening such as polygalacturonase, pectate lyase, and β-galactosidase (Payasi and Sanwal, 2003; Lohani et al., 2004; Zhuang et al., 2006).

Furthermore, vessels tended to increase in diameter during fruit maturation (Fig. 3G). An increase in cell size of fiber was observed in the early stage of development, cell enlargement rate then reduced gradually and stopped when fruit reached maturity. Increased thickness of the fiber wall, caused by deposition of a secondary cell wall, helped to provide strength to the peel (Fig. 3H). Fiber wall was also found to slightly increase in size during ripening (Fig. 5F). However, thickening of the wall of the fiber cell that occurred in ripening did not seem to involve mechanical support. It might be related to the alteration of pectin which could be a component in the fiber cell wall (Esau, 1978). When fruit ripens, insoluble pectin is converted into a soluble form resulting in cell wall swelling (Rosli et al., 2004).

Peel Color of 'Hom Thong' Banana during Maturation and Ripening.– A change in peel color of banana from green to yellow occurred during ripening (Fig. 4). This was a result of chlorophyll breakdown unmasking carotenoids in the plastids (Yang et al., 2009). The measurement of banana peel color revealed that L value and hue value hardly changed during fruit development but L value increased during ripening (Fig. 5G) whereas hue value decreased (Fig. 5H). Alteration in pigment synthesis and accumulation is one of are physiological and biochemical modifications that take place during fruit ripening (Goulao and Oliveira, 2008).

CONCLUSIONS

Peel structure of 'Hom Thong' banana changed during fruit development in both qualitative and quantitative attributes. Cell proliferation was observed in early stages of development followed by differentiation and enlargement of cells in later stages. The alteration in cell shape and arrangement proceeded throughout fruit maturation and continued in the ripening period. However, the changes during ripening did not relate to fruit growth and development but were the result of modifications in cell wall and the middle lamella. Cell damage and collapse and the formation of air space and cell separation in peel structure increased proportionally with ripening. The internal changes that occur in banana peel affect visual appearance and firmness resulting in the unique character of ripe banana.

ACKNOWLEDGEMENTS

This research was supported financially by the Institute for the Promotion of Teaching Science and Technology (IPST) through the Development and Promotion of Science and Technology Talents Project (DPST).

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