รายงานวิจัยฉบับสมบูรณ์

โครงการการศึกษาบทบาทของ cell wall hydrolases องค์ประกอบของผนังเซลล์ และการแสดงออกของยีน ที่เกี่ยวข้องกับเอนไซม์ในการหลุดร่วงของผลกล้วยระหว่างการสุก

A Study on the Role of Cell Wall Hydrolases, Cell Wall Components and Gene Expression of Enzyme(s) Involved in Finger Drop of Ripening Bananas

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การศึกษาเกี่ยวกับการหลุดร่วงของผลกล้วยระหว่างการสุก ประกอบด้วย 5 การทดลองย่อยคือ 1) การเปลี่ยนแปลงเอนไซม์ cell wall hydrolases และองค์ประกอบของผนังเซลล์ในเปลือกและเนื้อของผลกล้วยไข่ระหว่างอ่อนตัว 2) บทบาทของเอนไซม์ที่ทำให้เกิดการอ่อนตัวของเนื้อเยื่อต่อการหลุดร่วงของผลกล้วยระหว่างการสุกภายใต้ความชื้นสัมพัทธ์ต่ำและสูง 3) การศึกษาเปรียบเทียบการหลุดร่วงของกล้วยหอมและกล้วยน้ำหว้าระหว่างการสุก 4) การศึกษาชั้นบริเวณการหลุดร่วงของผลกล้วยระหว่างการสุก และ 5) การแสดงออกของยีนที่ควบคุมเอนไซม์เกี่ยวข้องกับการหลุดร่วงของผลกล้วยระหว่างการสุก ในการศึกษาครั้งนี้พบว่า การอ่อนตัวของเปลือกและเนื้อของผลกล้วยไข่ระหว่างการสุก เกิดขึ้นในลักษณะที่คล้ายกัน เพคตินที่ละลายน้ำได้มีการเพิ่มขึ้นในเนื้อแต่ไม่ได้เพิ่มในเปลือก กิจกรรม pectin methylesterase ลดลงในเปลือกแต่เพิ่มในเนื้อ ขณะที่กิจกรรม polygalacturonase ลดลงในเปลือกแต่เพิ่มในเนื้อ กิจกรรม β-galactosidase มีการเพิ่มในเปลือกมากกว่าในเนื้อ ส่วนกิจกรรม cellulase ทั้งในเปลือกและเนื้อไม่มีการเปลี่ยนแปลงระหว่างการสุก

การศึกษาการหลุดร่วงของผลกล้วยไข่และกล้วยหอมทองระหว่างการสุกพบว่า ทั้งผลกล้วยไข่และกล้วยหอมทองที่บ่มให้สุกภายใต้ความชื้นสัมพัทธ์สูง (90%) มีการหลุดร่วงของผลมากกว่าและเร็วกว่าผลกล้วยที่บ่มให้สุกภายใต้ความชื้นสัมพัทธ์ต่ำ (60%) กิจกรรม polygalacturonase และ pectin methylesterase ในเปลือกตรงกลางผล และตรงขั้วผลบริเวณที่เกิดการหลุดร่วง ของผล

กล้วยไข่ที่บ่มภายใต้ความชื้นสัมพัทธ์สูง มีมากกว่าผลกล้วยที่บ่มให้สุกภายใต้ความชื้นสัมพัทธ์ต่ำ และเนื้อเยื่อบริเวณตรงขั้วผลมีกิจกรรม polygalacturonase และ pectin methylesterase มากกว่าในเนื้อเยื่อเปลือกบริเวณตรงกลางผล ขณะที่การเปลี่ยนแปลงกิจกรรม β-galactosidase ในเนื้อเยื่อบริเวณกลางผลและขั้วผลของผลกล้วยไข่ที่บ่มภายใต้ความชื้นสัมพัทธ์ต่ำและสูง ไม่สัมพันธ์กับการหลุดร่วงของผล กิจกรรม polygalacturonase ในเปลือกผลตรงกลางและตรงขั้วผลของผลกล้วยหอมทองบ่มให้สุกภายใต้ความชื้นสัมพัทธ์สูงมีมากกว่าในผลกล้วยหอมทองที่บ่มให้สุกภายใต้ความชื้นสัมพัทธ์ต่ำ ขณะที่กิจกรรม pectin methylesterase ในเนื้อเยื่อบริเวณดังกล่าวของผลกล้วยไข่หอมทองที่บ่มให้สุกภายใต้ความชื้นสัมพัทธ์ต่ำและสูงไม่มีความแตกต่างกัน กิจกรรมทั้ง polygalacturonase และ pectin methylesterase ในบริเวณขั้วผลมีมากกว่าในเนื้อเยื่อของเปลือกบริเวณกลางผลของผลกล้วยที่บ่มให้สุกภายใต้ความชื้นสัมพัทธ์ต่ำและสูง ขณะที่การเปลี่ยนแปลงกิจกรรม β-galactosidase ในเนื้อเยื่อบริเวณทั้งสองส่วน ไม่สัมพันธ์กับการหลุดร่วงผลกล้วยหอมทองที่บ่มให้สุกภายใต้ความชื้นสัมพัทธ์ต่ำและสูง

การศึกษาเปรียบเทียบการหลุดร่วงผลกล้วยหอมทองและกล้วยน้ำว้าระหว่างการบ่มให้สุกภายใต้ความชื้นสัมพัทธ์ 85% พบว่าผลกล้วยหอมทองมีการหลุดร่วง 100% ขณะที่ผลกล้วยน้ำว้าไม่มีการหลุดร่วงของผล ภายในเวลา 5 วันระหว่างการบ่มให้สุก ผลกล้วยน้ำว้ามีความแน่นเนื้อเปลือกและแรงต้านทานการเกิดหลุดร่วงของผลมากกว่าผลกล้วยหอมทอง กิจกรรม polygalacturonase ในเนื้อเยื่อขั้วผลบริเวณการหลุดร่วงของผลกล้วยหอมทอง มีการเพิ่มขึ้นอย่างรวดเร็วและมีมากกว่าในบริเวณเปลือกตรงกลางผลของผลกล้วยน้ำว้าและหอมทอง และกิจกรรม polygalacturonase ในบริเวณขั้วผลของผลกล้วยหอมทอง มีมากกว่าของผลกล้วยน้ำว้า กิจกรรม pectin methylesterase ในเปลือกผลกล้วยหอมทองเพิ่มขึ้นเล็กน้อยขณะที่ในผลกล้วยน้ำว้ากลับลดลงในระหว่างการสุก ขณะที่กิจกรรม pectin methylesterase ในขั้วผลบริเวณหลุดร่วงของผลกล้วยน้ำว้ามีมากกว่าในผลกล้วยหอมทอง

การศึกษาชั้นของเนื้อเยื่อบริเวณที่เกิดการหลุดร่วงของผลกล้วยหอมทองพบว่า ไม่มีการสร้างชั้นของบริเวณการร่วง (abscission zone) เกิดขึ้นในบริเวณเนื้อเยื่อที่จะเกิดการหลุดร่วงของผล การศึกษาการแสดงออกของยีนที่ควบคุมเอนไซม์ polygalacturonase พบว่า การแสดงออกของยีนในบริเวณขั้วผลที่เกิดรอยหลุดร่วงในผลกล้วยหอมทองมีมากกว่าในผลกล้วยน้ำว้าและการแสดงออกของยีนในบริเวณขั้วผลมีมากกว่าในบริเวณเปลือกผล

คำสำคัญ : การหลุดร่วงของผล, การสุก, กล้วยไข่, กล้วยหอมทอง, กล้วยน้ำว้า, บริเวณการร่วง, การอ่อนตัว, polygalacturonase, pectin methylesterase, β-galactosidase

Abstract

Project Code : BGJ / 11 / 2543

Project Title : A Study on the Role of Cell Wall Hydrolases, Cell Wall Components and Gene Expression of Enzyme(s) Involved in Finger Drop of Ripening Bananas

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The study of finger drop in ripening bananas comprised of 5 experiments as following : 1) changes in cell wall hydrolases and cell wall components of the peel and pulp of banana during softening, 2) the role of cell wall hydrolases in finger drop of banana ripened under low and high relative humidities, 3) comparative study of finger drop in 'Hom Thong' and 'Namwa' bananas during ripening, 4) anatomical study of abscission zone in rupture area of finger drop and 5) gene expression of enzymes involved in finger drop of banana.

Changes of pectin fractions (water-soluble, alkali-soluble, and ammonium oxalate soluble) and activities of polygalacturonase (PG), pectin methylesterase (PME), β-galactosidase (GAL) and cellulase in the peel and pulp were studied during ripening of 'Khai' (*Musa* AA Group) banana. Soluble pectin increased in the pulp, not in the peel. PME activity decreased in the peel and increased in the pulp, whereas PG activity increased in both parts. GAL activity increased much more in the peel than in the pulp. Cellulase activity in both peel and pulp did not change. The results indicate that cell wall degradation in the peel and pulp is quite different.

'Khai' (*Musa* AA Group) banana ripened at 25°C (90% RH) for 24 h had low finger drop, while those kept continuously at 25°C (90%RH) until they were fully ripe

had high finger drop. The degree of finger drop increased as bananas advanced ripening. Peel at the pedicel of ripened bananas contained higher activities of PME and PG and water-soluble pectin than the peel at the middle fruit of ripened bananas. Bananas with high finger drop had greater activities of PME and PG than those with low finger drop. Change of GAL activity apparently had no relationship with finger drop in ‘Khai’ banana.

‘Khai’ (*Musa* AA Group) and ‘Hom Thong’ (*Musa* AAA Group) bananas were ripened under low and high relative humidities. Finger drop of ‘Khai’ and ‘Hom Thong’ bananas occurred more rapidly under high relative humidity (RH) than low RH condition. Pedicel rupture force of ripened bananas was the same under low and high RH. PG activity in the peel at the middle of the fruit and in the pedicel adjacent to the rupture areas of bananas ripened under high RH was higher than that of banana ripened under low RH. Change in PME activity of ‘Khai’ and ‘Hom Thong’ bananas ripened under low and high RH was similar. PG and PME activities in the peel at the pedicel adjacent to the rupture area under both low and high RH were higher than in the middle of the fruit. In contrast, GAL activity in the middle of the fruit ripened under both low and high RH was higher than in the pedicel adjacent to the rupture area.

‘Hom Thong’ and ‘Namwa’ bananas were ripened at 25°C (~85% RH). Finger drop of ‘Hom Thong’ bananas rapidly reached 100%, whereas ‘Namwa’ banana did not show finger drop 5 days after peel colour change to yellow. The rupture force and fruit firmness of both banana cultivars decreased while ripening advanced. ‘Namwa’ bananas had greater rupture force, peel firmness and resistance to finger drop than ‘Hom Thong’ bananas. PG activity in the pedicel adjacent to the rupture area rapidly increased and was higher than in the peel of both ‘Hom Thong’ and ‘Namwa’ bananas. PG activity in the pedicel adjacent to the rupture area of ‘Hom Thong’ bananas was higher than that of ‘Namwa’ bananas. PME activity in ‘Hom Thong’ bananas slightly increased while PME activity in ‘Namwa’ bananas gradually decreased during ripening. In contrast, PME activity in the pedicel adjacent to the rupture area of ‘Namwa’ bananas was much higher than that of ‘Hom Thong’ bananas.

Abscission zone was not formed before and during finger drop in the rupture area. In northern blotting analysis found that *MAPG* transcript in the pedicel adjacent to the rupture area in ‘Namwa’ banana was lower than that in ‘Hom Thong’ banana. The abundance of *MAPG* transcript in the pedicel adjacent to the rupture area was higher

than that in the middle of the fruit in both bananas. It is suggested that *MAPG* transcript is related to PG activity and it may be involved in finger drop of ripening banana.

Keywords : Hom Thong, Namwa, Khai, finger drop, polygalacturonase, pectin methylesterase, β-galactosidase, abscission zone, ripening

A STUDY ON THE ROLE OF CELL WALL HYDROLASES, CELL WALL COMPONENTS AND GENE EXPRESSION OF ENZYME (S) INVOLVED IN FINGER DROP OF RIPENING BANANAS

Introduction

'Hom Thong' (*Musa* AAA Group), 'Khai' (*Musa* AA Group) and 'Namwa' (*Musa* ABB Group) bananas are widely grown in Thailand. Only 'Hom Thong' and 'Khai' bananas are exported. In the past, 'Hom Thong' banana was the main export variety but the problems such as its thin peel (which causes the fruit easily bruising), and fruit (finger) dropping resulted in a decline in its overall export potential (Silayoi, 1991). From 1994 there has been a recovery of the export market for 'Hom Thong' bananas, with Japan as the main destination (Department of Promotion Agricultural data, 1998).

Senescent bananas develop spotting in the peel and the fingers are often drop during the later phase of ripening process. Finger drop is a physiological disorder of cooking banana, plantain and banana. It was defined by Baldry *et al.* (1981) as a 'physiological softening and weakening of the pedicel which causes the individual fruit in a hand to very easily separate from the crown'. This condition limits the marketability of many banana varieties such as 'Dwarf Brazilian' (Paull, 1996) and 'Hom Thong' (Silayoi, 1991). Finger drop has also been reported in the triploid Cavendish AAA Group (Semple and Thompsom, 1988) and tetraploids as well (Marriott, 1980). Prayurawong (1999) reported that finger drop also occurred in 'Khai' banana, which is diploid. Susceptibility varies widely among cultivars. For example, Valery is considerably more prone to finger drop than Gros Michel (New and Marriott, 1983).

Finger drop is affected by relative humidity (RH), ethylene, and ripening temperature. Low RH during the end of the ripening process reduced finger drop (Semple and Thompson, 1988). Ethylene treatment for one day at 25°C resulted in less finger drop than control fruit (Paull, 1996). In addition, more mature hands were more susceptible to finger drop than less mature ones (Paull, 1996). Cultivars with a long preclimacteric period and low ripening might have less finger drop (Marriott, 1980; Semple and Thompson, 1988).

Pedicel rupture force is related to finger drop (New and Marriott, 1983; Semple and Thompson, 1988), while peel puncture force at the full ripe stage is not a good indicator of finger drop (Paull, 1996). This suggests a localized weakening of peel at the pedicel. Softening has been described as a change of the pectic substance in primary cell wall and middle lamella (Seymour, 1993). The changes of cell wall composition involve the action of cell wall hydrolases such as polygalacturonase (PG), pectin methylesterase (PME), β-galactosidase (GAL) and cellulase (Huber, 1983).

At present, there is no information about the role of cell wall hydrolases and the changes of cell wall components involved in finger drop in 'Hom Thong' banana.

This study was set to determine the role of cell wall hydrolases in association with finger drop. The changes of cell wall components and expression of cell wall hydrolases genes involved in finger drop were determined.

As banana fruit ripens, the peel and pulp soften and the peel color changes from green to yellow (Marriott, 1980). Late in the ripening process, the senescent peel develops spotting and finger drop may occur, depending on the cultivar (Semple and Thompson, 1988). Finger drop is a physiological disorder that occurs as a result of the softening and weakening of the pedicel. The individual fruit of a hand separates very easily from the crown during ripening (Baldry et al., 1981; New and Marriott, 1983; Semple and Thompson, 1988). Finger drop is believed to be associated with rapid ripening at a too high temperature in the ripening room (New and Marriott, 1983). Finger drop increases with ripening. Some cultivars, particularly triploid (Semple and Thompson, 1988) and tetraploid ones, are rather susceptible (Marriott, 1980). Finger drop is not associated with phytopathology of the fruit pedicel because the levels of fungi isolated from fruit pedicel with and without finger drop are not different (Coursey et al., 1974).

Paull (1996) showed that total weight loss is not related to finger drop. However, bananas ripened at 40°C showed a decrease in finger drop. This was associated with a high weight loss, which resulted in a thin dry leathery skin (Semple and Thompson, 1988). High relative humidity (RH) during the later phase of banana ripening was found to promote finger drop (Semple and Thompson, 1988). Banana fruits treated with ethylene (100 $\mu l.l^{-1}$) for one day had reduced finger drop (Semple and Thompson, 1988; Paull, 1996), and prolonged exposure (two days or more) to ethylene increased finger drop (Semple and Thompson, 1988). Finger drop was reported to be relate to a second peak of ethylene production in 'Dwarf Brazilian' banana (Paull, 1996). More mature fruits are more susceptible to finger drop than less mature ones (Paull, 1996). Cultivars with long preclimacteric periods and slow ripening seem to have less finger drop (Marriott, 1980; Semple and Thompson, 1988). Ripening temperature affected the development of finger drop (Thompson, 1974). Paull (1996) found that finger drop of 'Dwarf Brazillian' banana was reduced from 100% to less than 10% when banana was ripened at 17.5°C or one day at 25°C after that transferring at 17.5°C.

The peel puncture force at the fully ripe stage is not a good indicator of finger drop (Paull, 1996), but pedicel rupture force is closely correlated. The development of finger drop occurs during peel and pulp softening (Semple and Thompson, 1988). This suggests a localized weakening of the peel at the pedicel. Banana peel softening during ripening has been described as a change in the cell wall pectic components (Seymour, 1993). Changes in the pectic components resulted from the action of cell wall hydrolases, i.e. polygalacturonase (PG), pectin methylesterase (PME), β-galactosidase (GAL), cellulase and pectate lyase (PEL or PL) (Huber, 1983; Fisher and Bennett, 1991).

PG degrades polyuronides, together with PME (Figure 1). PG is more active in degrading demethylated pectin than methylated pectin. PME catalyzes demethylation of the C-6 carboxyl group of galacturonic acid residues, it may play an important role

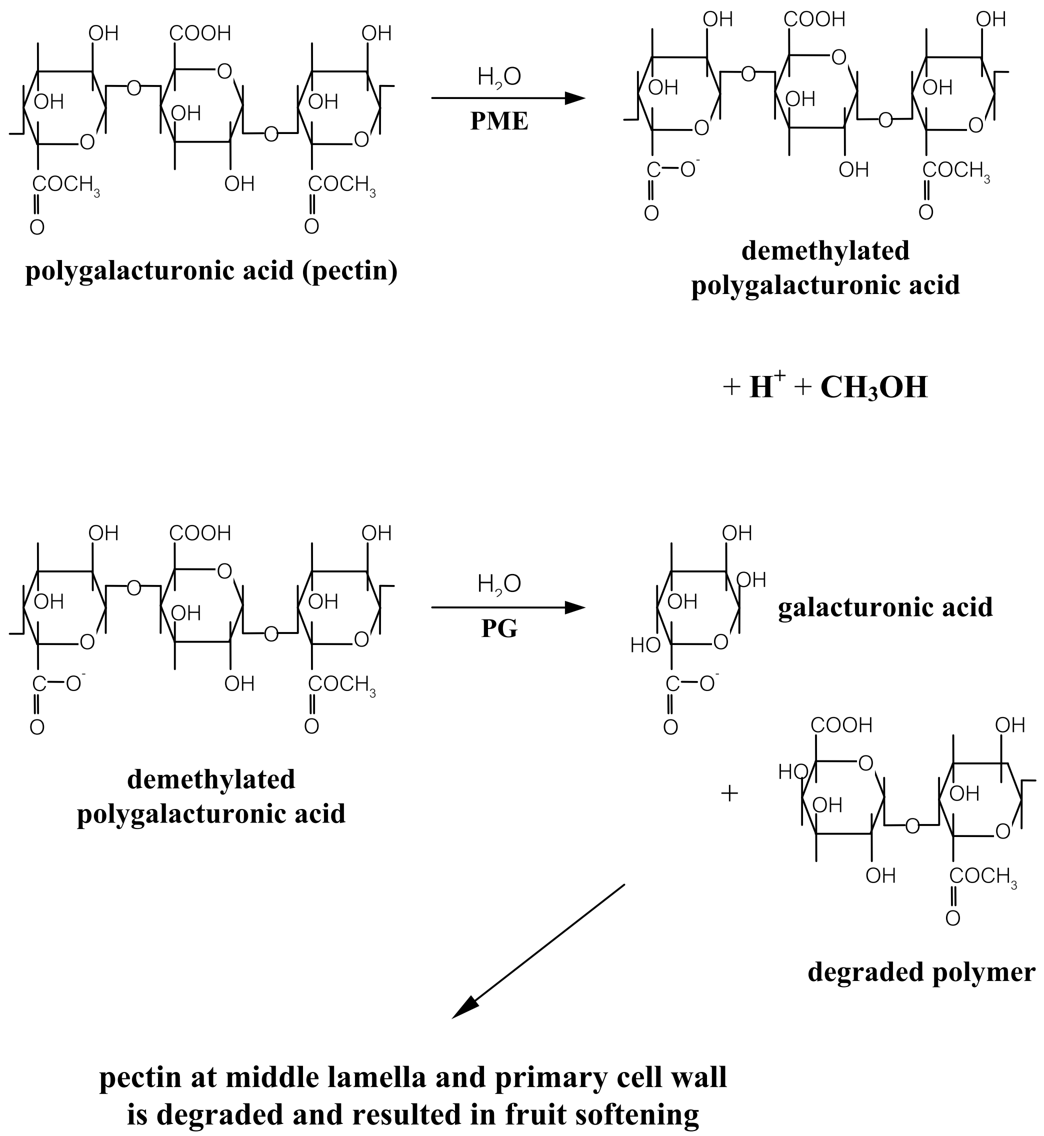

Figure 1 Fruit softening involves PG and PME activity (Source : Modified from Tucker, 1993; Fischer and Bennett, 1991).

in determining the extent to which pectin is accessible to degradation by PG (Fischer and Bennett, 1991).

Pectate is the de-esterified (demethylated) product of pectin and is the major component maintaining the structural integrity of the cell wall in higher plants (Carpita and Gibeaut, 1993). It can be depolymerized by PG and PL. PL catalyses the cleavage of α-1-4-galacturonan linkages by a β-elimination reaction resulting in tissue maceration (Fisher and Bennett, 1991).

In banana, ripening was associated with an increase in polyuronide extractability. During ripening, the degree of depolymerisation of polyuronides remained unchanged. Both endo-PG (EC 3.2.1.15) and exo-PG (EC 3.2.1.67) were reported to be present in bananas (Wade et al., 1992). The increase in low molecular size uronic acid in ripening banana 'Williams' fruit was consistent with hydrolysis of cell wall polyuronides by exo-PG (Wade and Satyan, 1993). Hultin and Levine (1965) reported that PE or PME activity strongly increased in the pulp of banana fruit during ripening. However, in the of presence of polyvinylpyrolidone (PVP), which bound phenolic compounds, PME (EC 3.1.1.11) activity was constant throughout the ripening period (Wade et al., 1992). PL activity in banana pulp showed a substantial increase during the ripening. The enchanced levels of PL activity corresponded with an increase in soluble polyuronides (Marin-Rodrigez et al., 2003).

There are several other enzymes that may involved in softening during fruit ripening, such as GAL and cellulase. GAL has been implicated in the removal of galactoses in side chains of pectin. An increase in GAL activity during fruit ripening was reported in the tomato cultivars 'Roma' and 'Rutgers' (Carrington and Presey, 1996), mango (Ali et al., 1995) and papaya (Paull and Chen, 1973). Cellulase is an enzymes characterized by its ability to degrade carboxymethylcellulose (C_x-cellulose) (Fischer and Bennett, 1991). Changes in cellulose during tomato softening have been associated with changes in C_x-cellulase activity (Pharr and Dickinson, 1973). Cellulase activity increased during softening, both in avocado and papaya (Paull and Chen, 1983; O' Donoghue et al., 1994).

Prayurawong (1999) reported an increase in PG, PME and GAL activities in the peel of 'Khai' banana during ripening and prior to finger drop. The peel of the pedicel had higher activities of PG and PME and water-soluble pectin (WSP) than the peel in the middle of ripening bananas. Bananas with most finger drop had highest PG and PME activity. GAL activity was not related with finger drop in 'Khai' bananas.

Tomato fruit softening are accompanied by an increase in *PG* mRNA abundance, immunologically detectable protein, and PG activity (Grierson and Tucker, 1983). The increase in PG activity is related to an increase in cell wall pectin solubilisation and a decrease in polyuronide (Huber, 1983; Smith et al., 1990). In the mesocarp of ripening fruit, cellulase activity was correlated with the accumulation of cellulase mRNA (transcripts) and *de novo* synthesis of the cellulase protein (Christoffersen et al., 1984; Tucker and Laties, 1984). Cell wall degradation during ripening in this fruit was apparently linked to the expression of genes encoding enzymes degradation (cell wall hydrolases), in this case cellulase.

Finger drop may result from the formation of an abscission zone at the fruit pedicel. Changes in cell wall during fruit abscission leading to separation of cells involve (1) hydrolysis of the middle lamella; (2) dissolution of the lamella plus breakdown of all or part of the cellulosic cell wall; and (3) mechanical breakage of non-living elements (Baird and Webster, 1976). The process of cell separation in fruit abscission has been primarily focused in both histochemical and enzymatic changes in the layers. Anatomical observation in cantaloupe suggested that chemical change in the walls of abscission zone cells might accompany with the structural changes (Webster, 1975).

The objectives of this study were:

1. To determine the activities of cell wall hydrolases in the peel and pulp during softening of ripening bananas.
2. To determine the activities of cell wall hydrolases during finger drop in ripening bananas.
3. To identify the formation of the abscission zone involved in finger drop of ripening bananas.
4. To comparison of gene(s) expression of the cell wall hydrolases involved in the development of finger drop in bananas during ripening.

Materials and Methods

Experiment I: Changes of cell wall hydrolases and pectin substances in the peel and pulp of 'Khai' banana during ripening

Fruit material

Bananas 'Khai' (*Musa* AA Group) used in this study were purchased from a banana plantation in Petchaburi Province in western Thailand. Bananas were harvested at 80 percent maturity. Bunches were placed in corrugated cardboard boxes and transported by truck to the laboratory within 2 h of harvest. Bunches were selected for uniformity of size and cleaned in water containing 5 % $MgSO_4$ to remove latex from the cut surface. They were then dipped in 500 ppm thiabendazole for 2-3 min to control fruit rot and were let to dry at ambient conditions. In the laboratory, individual hands of banana were placed in plastic baskets and held at ambient conditions (28.9^o C and 72% RH). For analysis, peel and pulp of 15 bananas were separated, pooled and frozen at –80^{o}C until use.

Peel colour, and firmness solude solids content

Every other day, 15 bananas were randomly sampled for determination of peel color, firmness and soluble solids content. Peel colour was determined using a colour meter (Dr. Lang Tricolor LFM 3) to record 'b' value (Hunter scale). Banana firmness with and without peel was determined with an Effegi firmness tester using a spherical plunger 1.1 cm in diameter. The plunger was inserted to a depth of 5 mm and the necessary force was recorded in newtons (N). Soluble solids content of the pulp was measured using an Atago hand refractometer.

Pectic substances and cell wall hydrolases

Pectin fractions were separated by successive extractions with distilled water, ammonium oxalate and sodium hydroxide using the procedure described by Robertson (1979).

Extract and assay of pectinesterase, polygalacturonase, β-galactosidase and cellulase were described in Hagerman and Austin (1986), Yoshida et al. (1984) Ross et al. (1993) and Abeles and Takeda (1990), respectively. Protein content was determined according to Bradford (1976). The analysis was repeated 5 times for each treatment.

Experiment II: Development of finger drop in 'Khai' and 'Hom Thong' bananas ripening at low and high relative humidities

Plant material

Bananas 'Khai' (*Musa* AA Group) and 'Hom Thong' (*Musa* AAA Group) used in this study were purchased from a banana plantation in Petchaburi Province in western Thailand. Banana bunches were harvested at 80% materity in the morning based on their shape development, sorted for uniformity of fimger size and color, and returned to the laboratory within 2 h after harvest. The bunches were dehanded and immediately cleaned in water containing 5% $MgSO_4$ to remove latex from the cut surface. Bananas were then treated with 500 ppm benomyl and allowed to air dry before dipping in 500 ppm ethephon for 1 mon and allowed to air dry. Bananas 'Khai' were divided into two groups. One group was held at 25° (90% RH) for 24 h and transferred to ambient conditions (29°C, 65%RH) and other group was held 25°C (90%RH) until they were fully ripe. While banana 'Hom Thong' were held continuously at low RH (65%RH) and high RH (90%RH) until they were fully ripe. Peel tissue at the middle fruit and at the pedicel of bananas was pooled and frozen at -80°C until use.

The following parameters were recorded:

Finger drop

The method was modified from Semple and Thompson (1988). A hand of banana was picked up to 15 cm above the table for 10 second and the number of dislodged fingers was recorded, and expressed as a percentage of total number of fingers.

Firmness of the peel

Firmness of the peel at the middle of fruit was measured by Effigi. The probe (0.5 cm) was penetrated 5 mm into the fruit. The results were expressed in newtons (N).

Pedicel rupture force

Pedicel rupture force was measure by pushing down the pedicel until it separated from the fruit. It was expressed in newtons.

Resistance to finger drop

The method to measure resistance to finger drop was modified from Cerqueira et al. (2000). Banana fruit was inserted in a hole and held by a big clip which connected with a spring weight. As the pedicel of banana was pulled, the piston of the spring weight and a marker move together. The marker on the spring weight stopped when the pedicel broke. The force at the moment of rupture is indicated on the marker. Resistance to finger drop was expressed in kg.

Water content

Peel at the middle of banana fruit was collected and oven-dried at 60°C for at least 5 days and it was weighed until the weight did not change. The percentage of water content was calculated as following:

$$\text{Water content (\%)} = \frac{(\text{Fresh weight} - \text{Dry weight})}{\text{Fresh weight}} \times 100$$

Polygalacturonase (PG) activity

Enzyme extraction modified from Yosida et al. (1984). The peel at the middle of the fruit and at the pedicel adjacent to the rupture area (10 g) was homogenized for 2 min in 20 ml of chilled distilled water in a homogenizer. The homogenate was centrifuged at 15,000 rpm for 20 min at 4°C and the supernatant was discarded. The pellet was resuspended in 10 ml of 0.2 M Tris-HCl buffer (pH 9.0) containing 5% NaCl and added 2% (w/w) polyvinylpolypyrolidone (PVPP) then stirred for 2 hr at 4° C. The mixture was centrifuged at 15,000 rpm for 30 min at 4°C, and the supernatant was collected. Ammonium sulfate (0.561 g/ ml) was added to the supernatant to give 80% saturation. Protein was collected by centrifugation (18,000 rpm, 30 min at 4°C) then dissolved in a small amount of cool water and dialysed against 20mM sodium acetate buffer (pH 6.0) at least 14 h. The dialysate was centrifuged 15,000 rpm for 20 min at 4°C. The supernatant was used for enzyme assay and protein content.

PG activity was assayed by measuring the formation of reducing groups in a reaction mixture at 37°C, which initially contained 0.25 ml of 0.5% polygalacturonic acid, 0.25 ml of 0.4 M NaCl, 0.25 ml of sodium acetate buffer (pH 4.5) and 0.25 ml of the enzyme solution. The reaction was terminated by the addition of 0.5 ml of 30% K_2CO_3 solution containing 5% $Na_2S_2O_4$. A solution (0.5 ml) of 0.3% 3,6-dinitrophthalic acid monopyridiniumsalt was added to the reaction and the whole boiled for 10 min. After it cooled, 8 ml distilled water was added and the absorbance at 450 nm was measured. One unit of enzyme activity was defined as the amount of enzyme that liberated 1 ηmol of galacturonic acid per min per mg protein under the above conditions.

Pectin methylesterase (PME) activity

Enzyme extraction modified from Miller et al. (1987). Banana tissue at the middle of the fruit and at the pedicel adjacent to the rupture area (2 g) was homogenized for 2 min in 20 ml of 1 M NaCl in a homogenizer. 2% (w/w) of PVPP was added to the homogenate then stirred for 2 h at 4°C. The mixture was filtrated through two layers of cheesecloth and then centrifuged at 15,000 rpm for 20 min at 4° C. The supernatant was used for enzyme assay and protein content. The supernatant was adjusted with NaOH to pH 7.5 before enzyme assay. The supernatant was collected for protein assay also.

PME was assayed spectrophotometrically according to Hagerman and Austin (1986). Two milliliters of 0.5% (w/v) pectin (pH 7.5), 0.2 ml of 0.01% bromthymol blue (in 0.003 M, pH 7.5, potassium phosphate buffer) and 0.7 ml of distilled water (pH 7.5) were incubated at 25°C. The reaction was started by adding 100 ml of enzyme solution, and the rate of decreased in absorbance 260 nm (ΔA_{260}/ min) was recorded after 1 min using distilled water as the blank.

β-Galactosidase (GAL) activity

Enzyme extraction used the method of Ross et al. (1993). Banana tissue at the middle of the fruit and at the pedicel adjacent to the rupture area (10 g) was mixed with 2% (w/v) PVPP and homogenized for 2 min in 20 ml of 25 mM sodiun acetate buffer containing 10 mM sodium tetrathionate (NaTT) in a homogenizer. The homogenate was centrifuged at 15,000 rpm for 20 min at 4°C and the supernatant was discarded. The pellet was washed twice with 5 mM NaTT. The pellet was resuspended in 6 ml of 25 mM sodium acetate, 100 mM NaCl, 2 mM NaTT, pH 4.5. This mixture was stirred for 1 h at 4°C and centrifuged at 15,000 rpm for 20 min at 4° C. The supernatant was collected for enzyme assay and protein content.

Samples to be assayed for enzyme activity were incubate in test tube for 60 min at 30°C, as a part of a 2-ml mixture also containing 25 mM sodium acetate (pH 4.0), 0.3% β-mercaptoethanol, 2 mM *p*-nitrophenyl-β-D-galactopyranoside and 0.8 ml of enzyme solution. Incubations were terminated by addition of 2 ml of 200 mM Na_2CO_3 and absorbance (410 nm) measured. Change in absorbance was related to η mol *p*-nitrophenol released per min using a standard curve constructed with know amount of *p*-nitrophenol.

Protein content

Protein content was detected for calculating specific enzyme activity using the method described by Bradford (1976). 4 ml of Coomassie blue were added to 1 ml of enzyme solution. The samples were measured at 595 nm and protein concentration determined for each sample with a bovine serum albumin standard curve.

Experiment III: Cell wall hydrolase activities in relation to finger drop in 'Hom Thong' and 'Namwa' bananas

The experiment had the same designate the same as Experiment II. 'Hom Thong' (greater finger drop) and 'Namwa' (no finger drop) were used. The parameters recorded were the same as in Experiment II.

Experiment IV: Histochemical study of the formation of an abscission zone in relation to finger drop of 'Hom Thong' banana

The pedicel adjacent to the rupture area of 'Hom Thong' bananas on day 4 (0% finger drop) and day 6 (100% finger drop) after peel colour change were fixed in formalin acetic acid (FAA) at least 12 h. The fixed samples were washed by 50% ethanol for three times. Then the samples were replaced with tertiary butyl alcohol (TBA) series at 50, 70, 85 and 100% for 12 h per each step. After that the samples were transferred into 1:1 (v/v) of TBA mixed with paraffin oil and hard paraffin following with three times in pure hard paraffin for 12 h per each step before embedding. Longitudinal 10 μm sections were cut by rotary microtome. Before staining the tissues, paraffin was removed by dripping in xylene for 1 hr. To observe the abscission zone, the tissues were stained with safranin-O and fast green, sections were mounted in, cover with the cover slip and observed under a light microscope. Specific staining of pectic substances (Johansen, 1940), placed sectin in 0.05% ruthenium red (fresh preparation) for 10-30 min, mounted in water and observed under a light microscope.

Experiment V: Gene expression of cell wall hydrolase(s) involved in finger drop in 'Hom Thong' and 'Namwa' bananas

The study comprises of 2 treatments similar to Experiment III. Gene expression of PG was investigated.

Total RNA extraction

The protocol was modified from Chang et al. (1993) to make it suitable for extracting RNA from banana peel samples. Approximately 3 g of tissue was ground in liquid nitrogen. Ground tissue was added to 15 ml of extraction buffer containing 2% hexadecyltrimethylammonium bromide (CTAB), 2 M NaCl, 100 mM Tris-HCl (pH 8.2), 25 mM EDTA, 2% β-mercaptoethanol, previously warmed to 65°C. The mixture was incubated at 65°C for 10 min after that it was shaken vigorously for 10 min by vortexing. Then it was centrifuged at 4,500 rpm for 30 min (4°C). The supernatant was colleted and extracted twice with chloroform:isoamyl alcohol (24:1, v/v). RNA was precipitated with 2 M lithium chloride. After centrifugation, the RNA pellet was dissolved in DEPC-water. It was extracted once using chloroform:isoamyl alcohol. The aqueous phase was collected and added two volumes of ethanol was added, then allowed to precipitated at -20°C for 4 h. The mixture was centrifuged in a microcentrifuge at 12,000 rpm at 4°C. Discarded ethanol and vacuumed dry for 10 min and resuspended the RNA pellet in cool DEPC-water.

RT-PCR

The first strand cDNA was synthesized from 1 μg of total RNA using Superscript III H^- Reverse Transcriptase kit (Invitrogen) and used as a template to amplify the targeted genes by PCR. cDNA encoding for PG was amplified by PCR with AmpliTaq Gold (Roche). Specific PG primers were designed from accession number AF311882, using the Primer3 program for designing primer. The sequence of the upstream (forward) primer was 5' AAG ACA TGG CAG GGT GGT AG 3' and that of the downstream (reverse) primer was 5' GGG GTG CAT TCC ATG TGT A 3'. The conditions for PCR amplification were 45 cycles of 94°C for 30 seconds, 56°C for 30 seconds and then 72°C for 30 seconds.

The amplified cDNA fragments of approximately 545 bp were cloned in pGEM-T Easy Vectors (Promega) and the sequences were determined using a DNA sequencer. The sequence was identified and the corresponding clone termed *MAPG*.

RNA blotting and hybridisation

Ten-microgram samples of total RNA isolated from banana skins were separated by electrophoresis on 1% agarose gels containing 0.5X TBE and transferred to nylon membranes (Amersham Biosciences). Membranes were prehybridized at 65° C for 1 hr in Church Buffer (0.5 M sodium phosphate buffer, pH 7.2 and 5% SDS) and the hybridization was performed overnight in the same buffer containing the *MAPG* ^{32}P-labelled probe at 65°C. Probes were prepared with a Rediprime II kit, Random Primer Labelling System (Amersham Biosciences) according to the manufacturer's instructions. Following hybridization, membranes were washed with 2X SSC containing 0.1% SDS for 15 min at room temperature, 2X SSC containing 0.1% SDS for 15 min at 50°C and 0.2X SSC containing 0.1% SDS for 15 min at 50°C. The hybridized ^{32}P-labelled probe was detected using phosphorimager (Kodak).

Statistical Design

Experimental data are means and least significant different (LSD). Variance analysis using *t*-test and *F*-test was performed to determine differences between means of the treatments, at $P < 0.05$.

Results

Experiment I: Changes of cell wall hydrolases and pectin substances in the peel and pulp of 'Khai' banana during ripening

Peel colour and soluble solids content

The 'b' value of peel colour of banana remained unchanged for the first 4 days and then increased rapidly to a maximum on day 6. It decreased thereafter. Soluble solid content of the pulp of unripe banana was low. It increased slightly from day 0 to day 4, then increased sharply over the ripening period (Figure 2A).

Firmness

Changes in firmness of banana with and without peel were similar. Little change occurred for the first 4 day, then firmness decreased rapidly from day 4 to day 6 and became stable thereafter (Figure 2B).

Pectic substances

Water-soluble pectin content of the peel remained unchanged from day 0 to day 4, then increased to a maximum of day 6 and decreased thereafter (Figure 3A). Water-soluble pectin content of the pulp increased steadily from day 0 to day 6, then increased rapidly to a maximum at day 8 and decreased thereafter (Figure 3B). The peel ammonium oxalate-soluble pectin content remained unchanged from day 0 to day 8 and incraseed slightly thereafter (Figure 2A), while ammonium oxalate-soluble pectin content of the pulp increased steadily throughout the ripening period (Figure 3B). Alkali-soluble pectin content of the peel increased slightly from day 0 to day 2, then decreased steadily thereafter (Figure 3A), while alkali-soluble pectin content of the pulp increased progressively to a maximum at day 8 and decreased thereafter (Figure 3B).

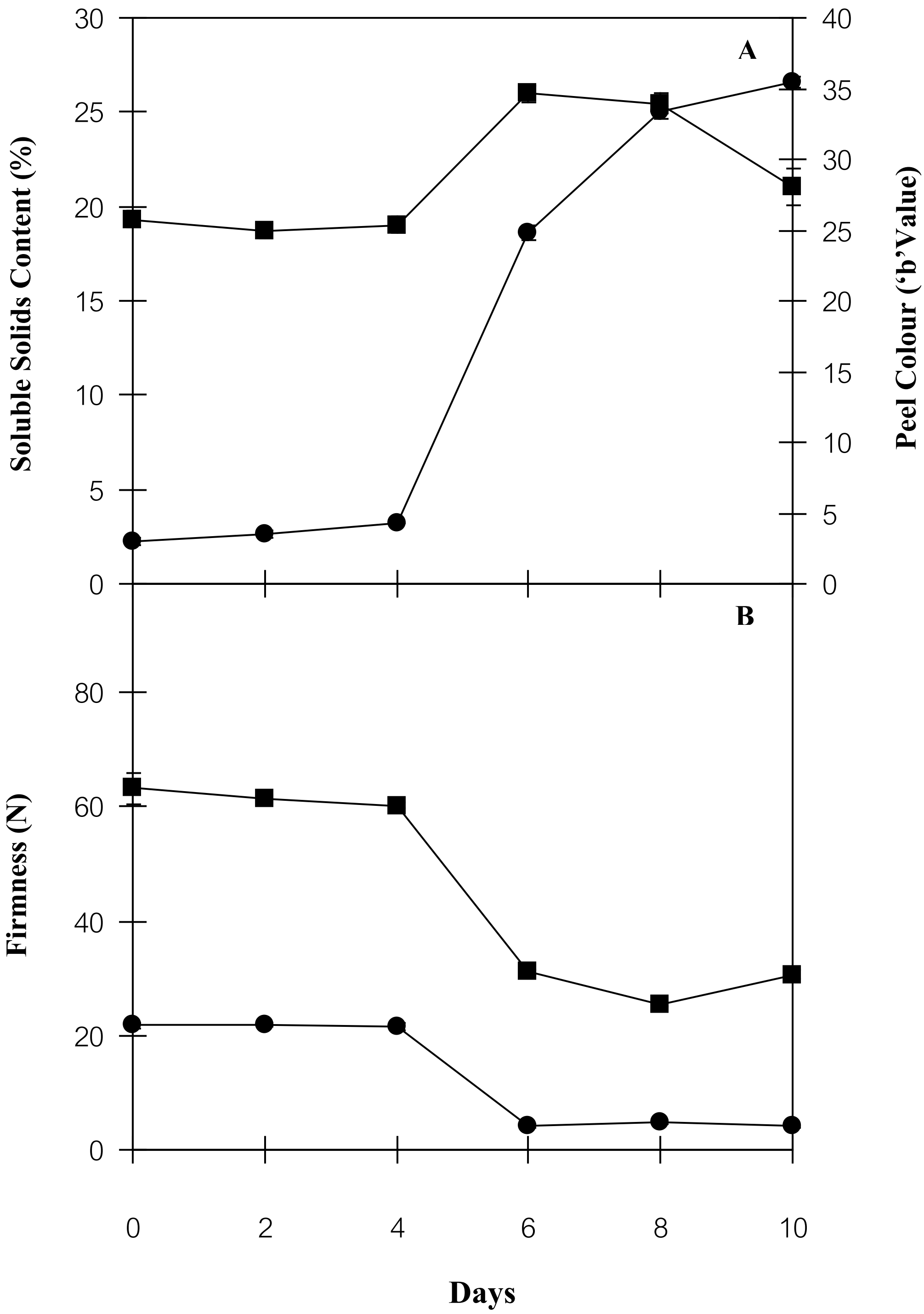

Figure 2 Changes in (A) soluble solids content (●) in the pulp, peel colour (■) and (B) firmness of ‘Khai’ bananas with (■) and without (●) the peel during ripening. Bars indicate standard errors of means.

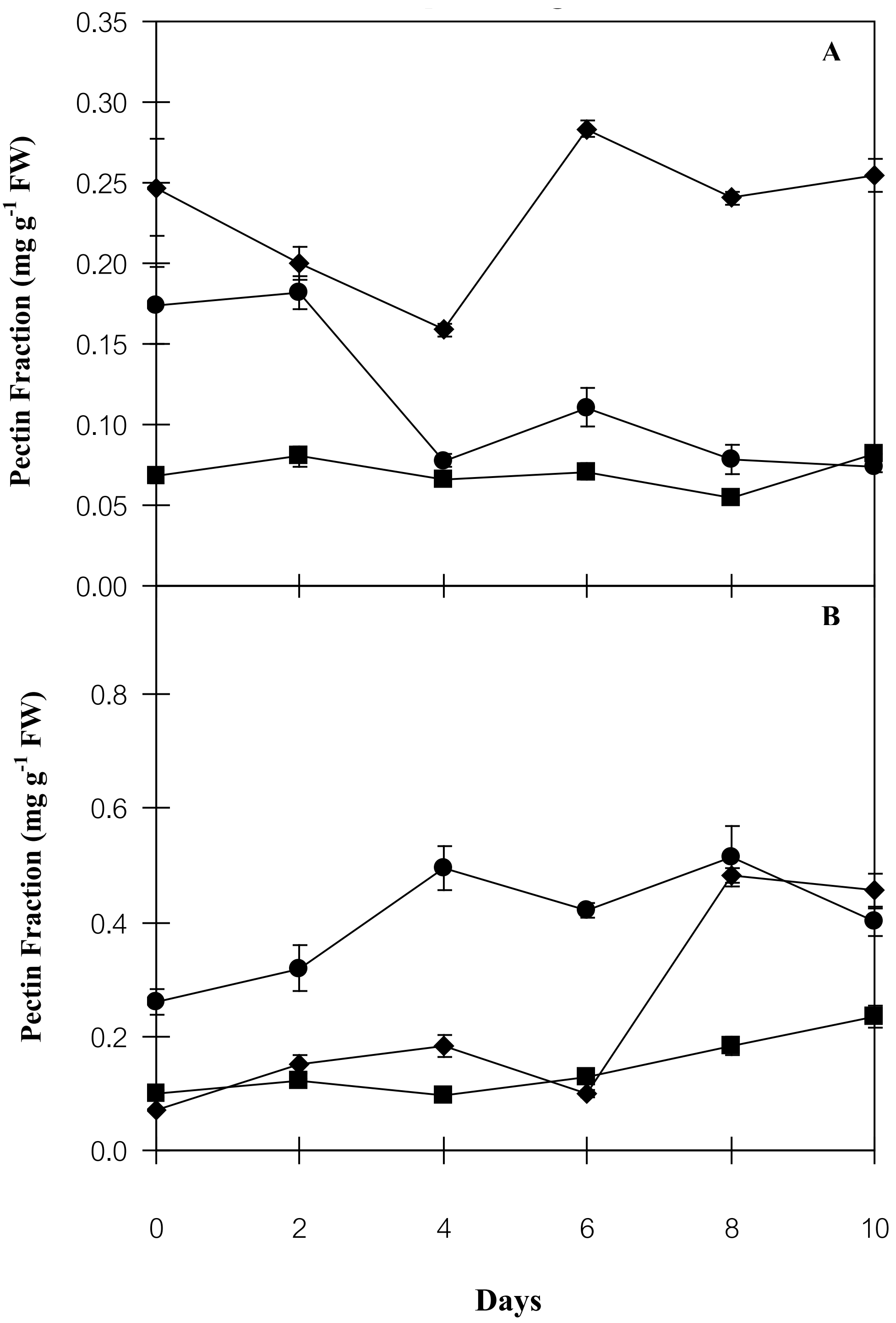

Figure 3 Changes in water-soluble pectin (◆) ammonium oxalate – soluble pectin (●) and alkali-soluble pectin (■) in the peel (A) and pulp (B) of ' Khai' bananas during ripening. Bars indicate standard errors of means.

Cell wall hydrolase activity

Pectinesterase activity of the peel of unripe banana, was higher (day 0) and decreased rapidly from day 0 to day 2, and slowly decreased thereafter. Pectinesterase activity of the pulp of unripe banana was low and increased steadily to a maximum on day 4, then decreased slowly (Figure 4A). Polygalacturonase activity of the peel of unripe banana increased to a maximum on day 4, then decreased. Polygalacturonase activity of the pulp of unripe banana was lower and increased progressively to a maximum on day 8, and decreased rapidly thereafter (Figure 4B).

β-Galactosidase activities of the peel and pulp of unripe banana were low, but the activity in the peel was higher than those in the pulp. β-Galactosidase activity of the peel increased to a maximum on day 8 and then rapidly decreased. β-Galactosidase activity in the pulp increased slightly to a maximum on day 4 and then also decreased (Figure 5A). Cellulase activity of the peel of unripe banana decreased rapidly from day 0 to day 2, then decreased more slowly, while Cellulase activity of the pulp of unripe banana remained unchanged (Figure 5B).

Experiment II: Development of finger drop in 'Khai' and 'Hom Thong' bananas ripening at low and high relative humidities

Bananas 'Khai' ripened at 25°C/90% RH for 24 h showed less finger drop than those ripened at 25°C/90% RH for 72 h. Bananas ripened at 25°C/90% RH for 24 h developed finger drop later than those ripened at 25°C / 90%RH (Figure 6).

Pectin methylesterase activities in peel at the middle fruit and peel at the pedicel of bananas increased to a maximum at day 6 and decreased thereafter and pectin methylesterase activities at a maximum of peel at the middle fruit was greater than that of peel at the pedicel. Pectin methylesterase activities in peel at the middle fruit and at the pedicel of bananas ripened at 25°C/90%RH for 72 h were greater than those ripened at 25°C/90%RH for 24 h (Figure 7).

Similary polygalacturonase activities in peel at the middle fruit and at pedicel of bananas increased to a maximum at day 6 and decreased thereafter and polygalacturonase activities in peel at the pedicel were greater than in peel at the middle fruit. polygalacturonase activities in peel at the middle fruit of bananas ripened at 25°C/90% RH for 24 and 72 h were not significatnly different while polygalacturonase activities at maximum in peel at the pedicel of bananas ripened at 25°C/90% RH for 24 h (Figure 8).

β-Galactosidase activities in peel at the middle fruit and at the pedicel of bananas increased throughout the study period. But β-galactosidase activities in peel at the middle fruit and at the pedicel of bananas ripening at 25°C /90%RH for 24 h increased more rapidly at days 5 and 6, respectively, than those ripened at 25°C /90%RH for 72 h (Figure 9).

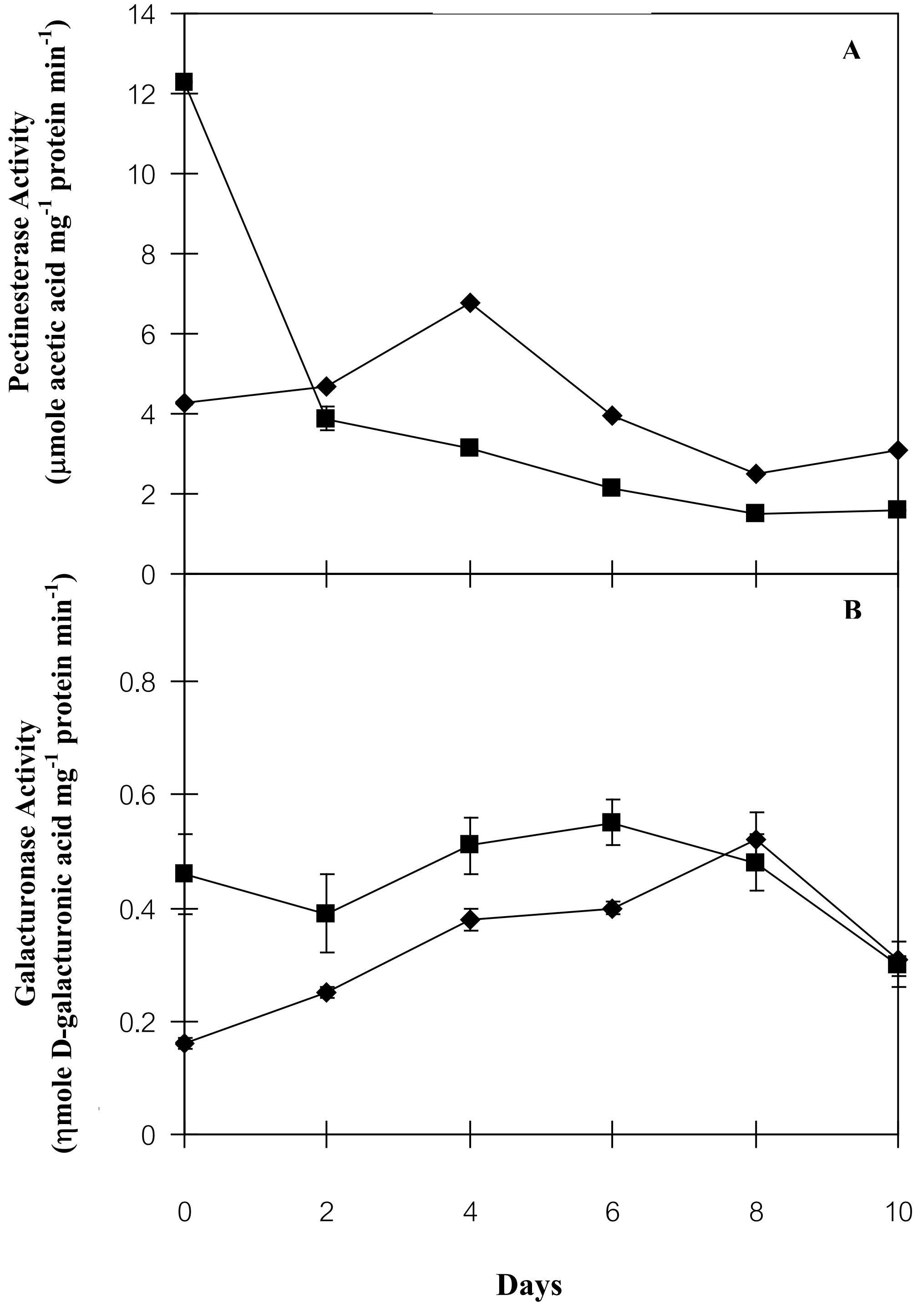

Figure 4 Changes in pectinesterase (A) and polgalacturonase (B) activities in the peel (■) and pulp (◆) of 'Khai' bananas during ripening. Bars indicate standard errors of means.

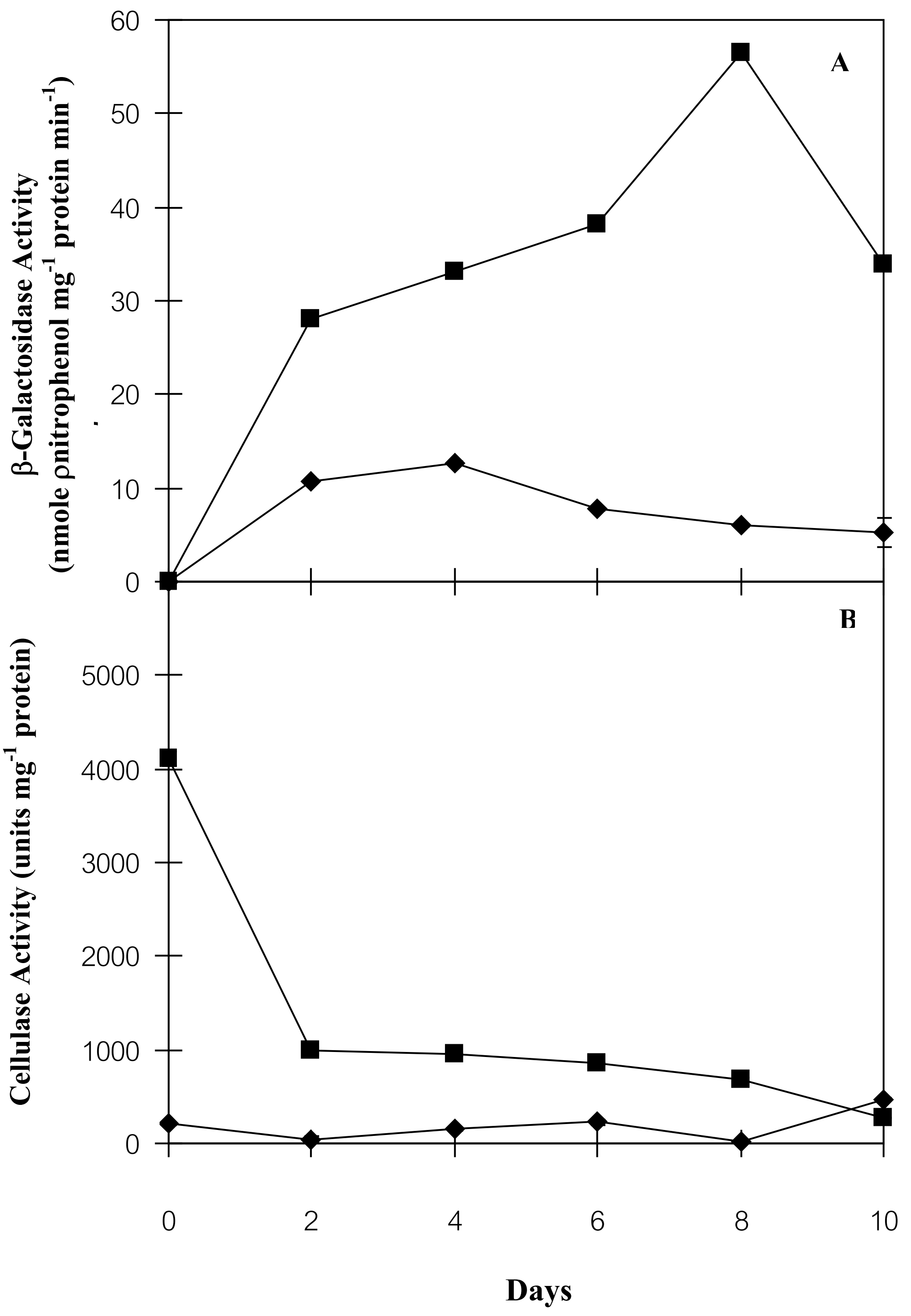

Figure 5 Changes in β-galactosidase (A) and cellulase (B) activities in the peel (■) and pulp (◆) of 'Khai' bananas during ripening. Bars indicate standard errors of means.

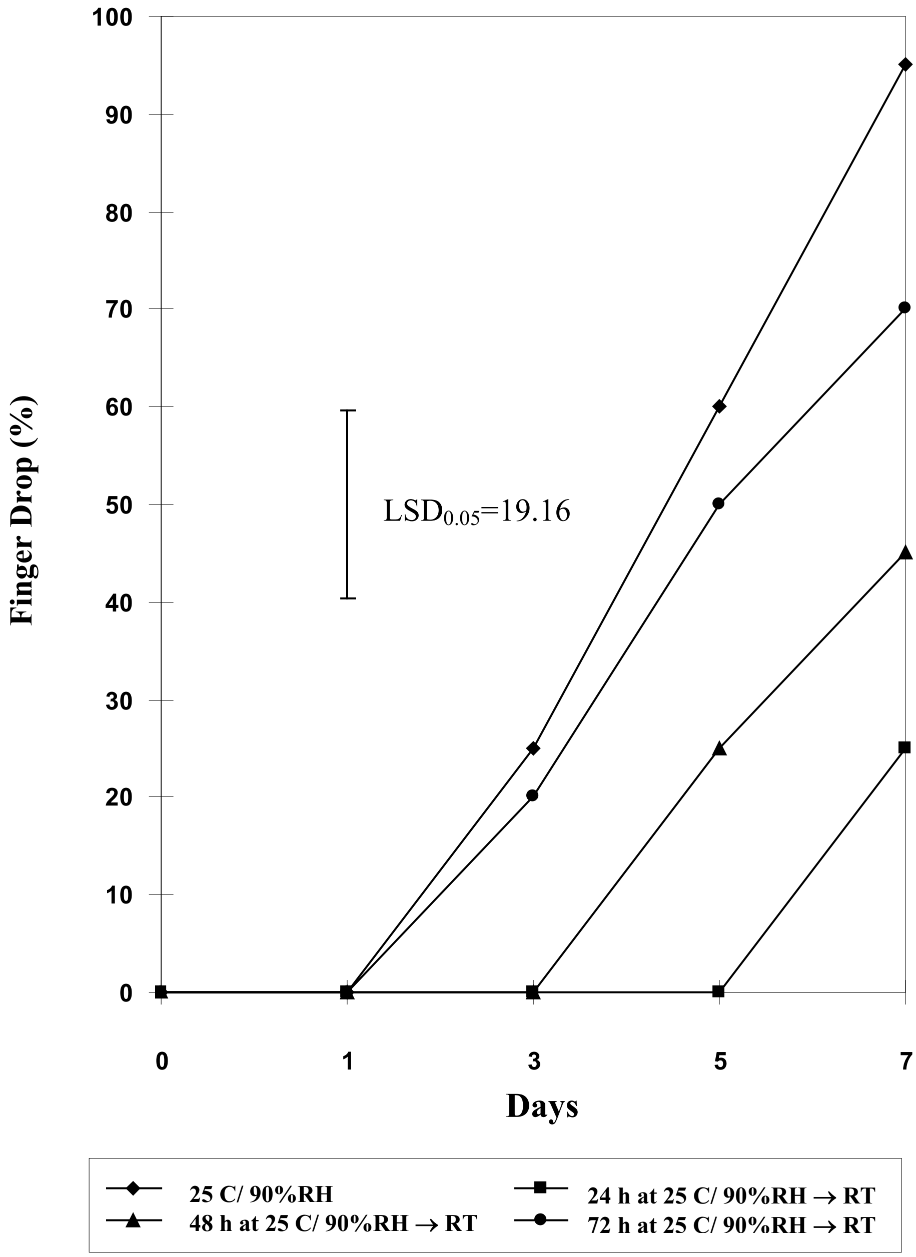

Figure 6 Finger drop of 'Khai' bananas ripened at 25 °C (90%RH) for 24, 48 or 72 h and transferred to room temperature (RT).

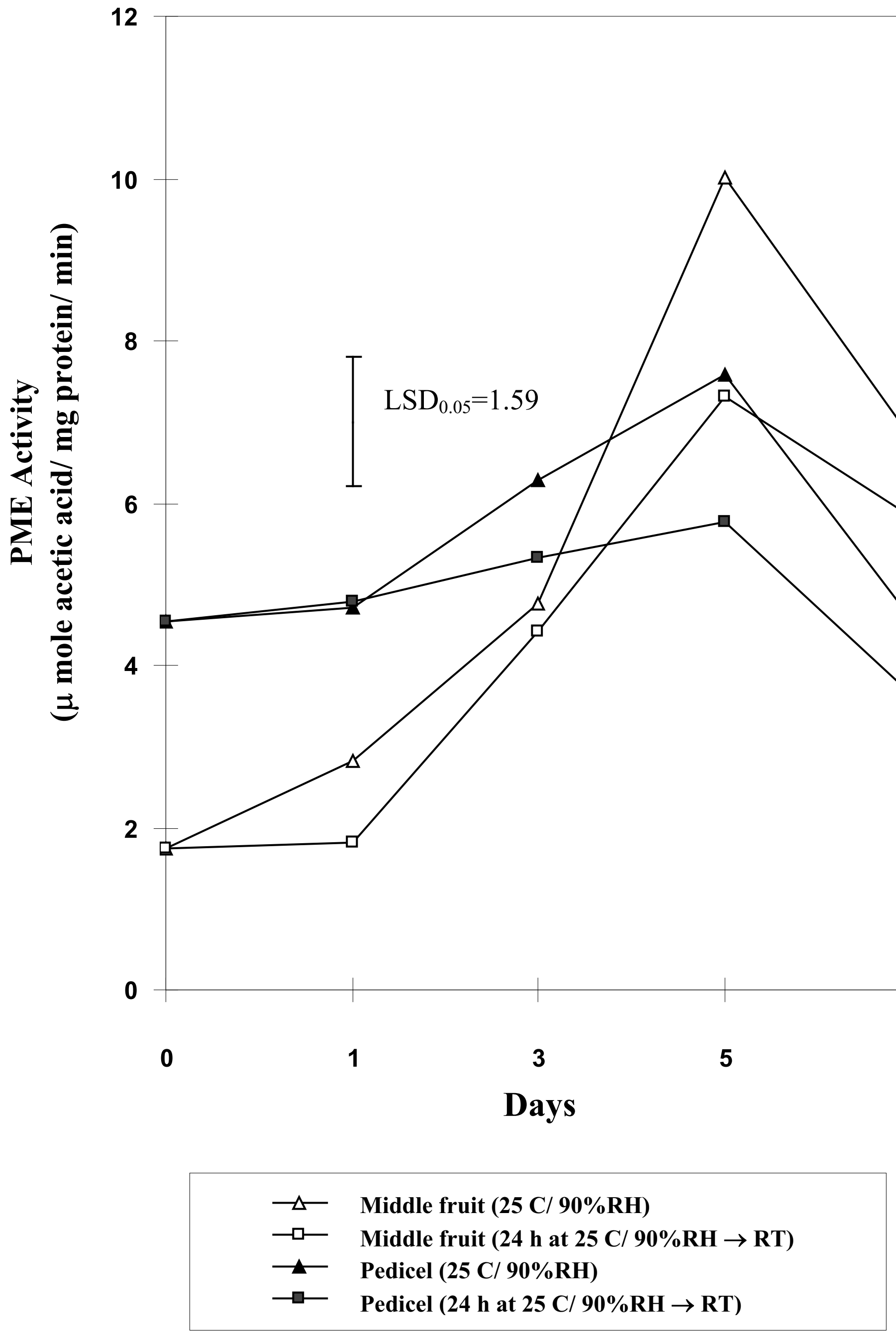

Figure 7 Changes in activity of pectinemethylesterase in peel of 'Khai' bananas at the middle fruit (△, □) and at the pedicel (▲, ■).

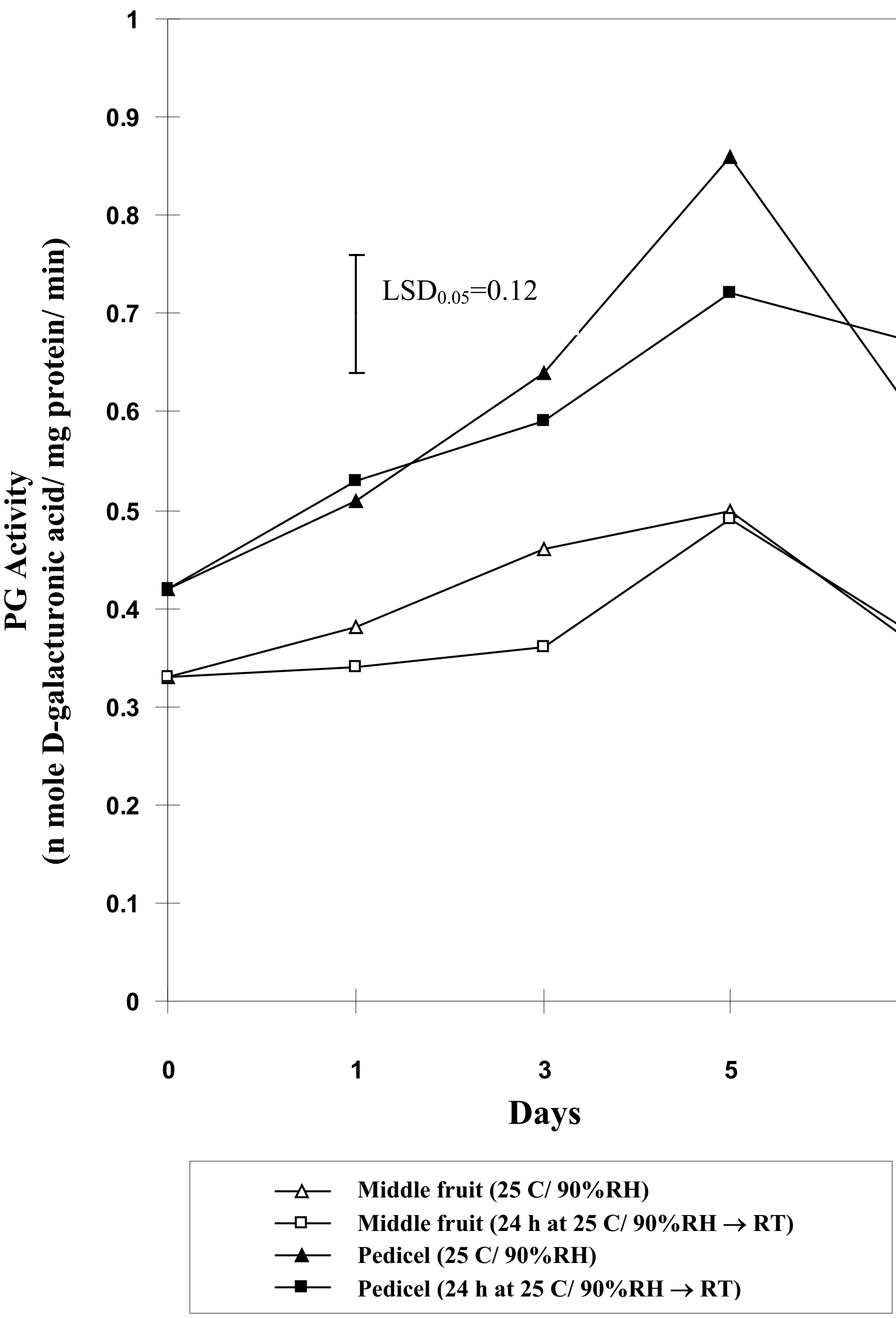

Figure 8 Changes in activity of polygalacturonase in peel of 'Khai' bananas at the middle fruit (△ ,□) and at the pedicel (▲ ,■).

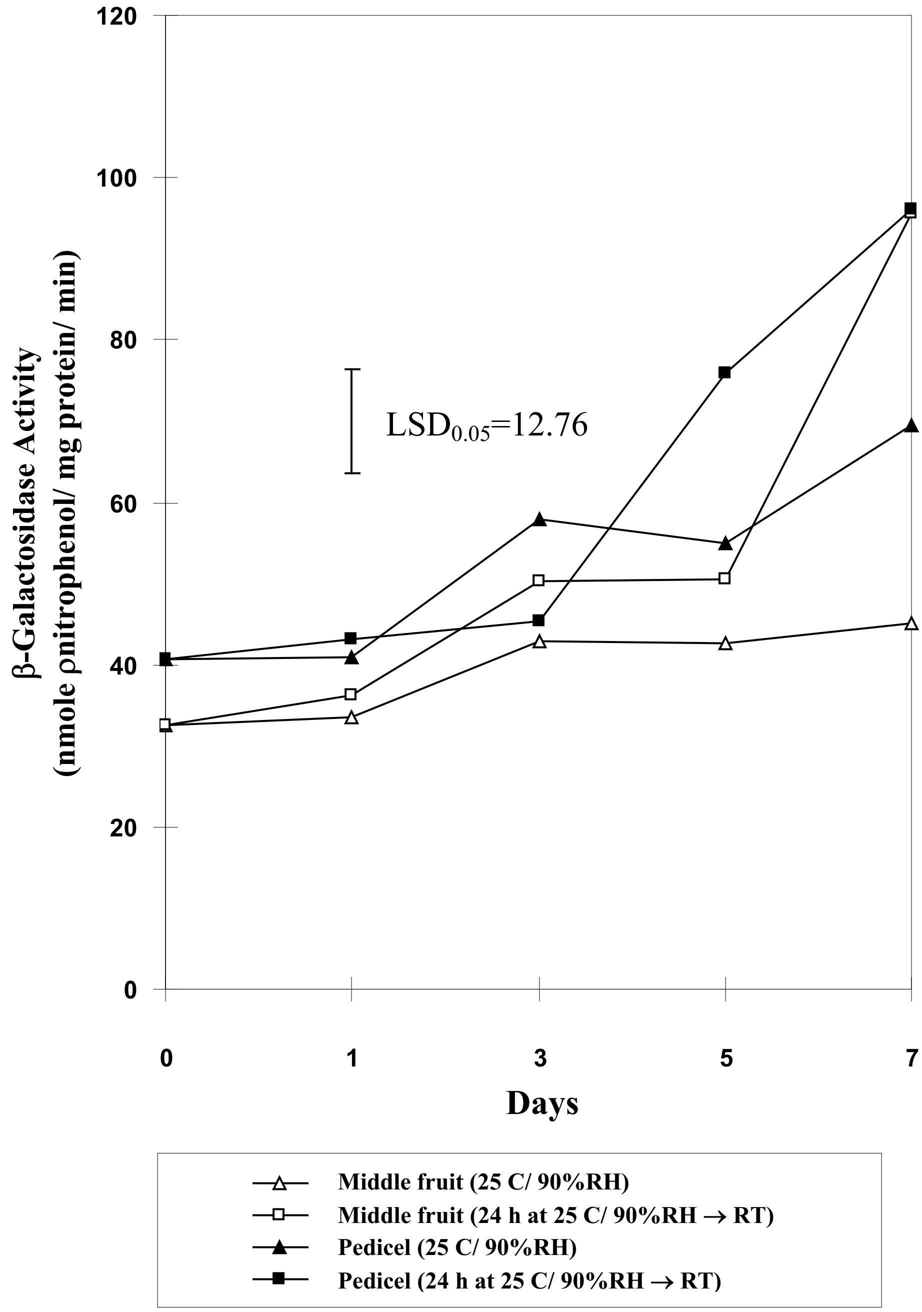

Figure 9 Changes in activity of β–galactosidase in peel of 'Khai' bananas at the middle fruit (△ ,□) and at the pedicel (▲ ,■).

Water-soluble pectin in peel at the pedicel increased to a maximum at day 5 and decreased sharply thereafter, while water-soluble pectin in peel at the middle fruit slightly increased throughout the study period. Water-soluble pectin in peel at the middle fruit and at the pedicel of banana ripened at 25°C /90%RH for 24 and 72 h was not significatly different (Figure 10).

Finger drop

In 'Hom Thong' bananas which ripened under high relative humidity (RH) finger drop was 100% already on day 3 (Figure 11). 'Hom Thong' bananas ripening under low RH had 0% finger drop until day 3, but after that finger drop increased remarkably and reached 100% on day 5.

Firmness of the peel

Firmness of bananas ripened under low and high RH decreased rapidly to 5 newtons on day 4. At the first day, the firmness with peel of bananas ripened under low RH was slightly higher than bananas ripened under high RH (Figure 12). Later on firmness of bananas ripened under both low and high RHs was not significantly different.

Pedicel rupture force

The pattern of pedicel rupture force of banana ripened under low and high RH was similar to that of peel firmness (Figure 13).

Water content

Water content of the peel in the middle of the fruit was the same at both RH levels. Water content was around 88% at the first day. Later on, water content slightly decreased (Figure 14).

polygalacturonase activity

Polygalacturonase activity in the peel from the middle of fruits ripened under low and high RH, at both RHs was lower than at the pedicel adjacent to the rupture area. Polygalacturonase activity in the peel in the middle of the fruits slightly increased during ripening while polygalacturonase activity in the peel at the pedicel adjacent to the rupture area markedly increased on day 4 (Figure 15). Polygalacturonase activity in the peel of both areas of bananas ripened under high RH was higher than that of bananas ripened under low RH.

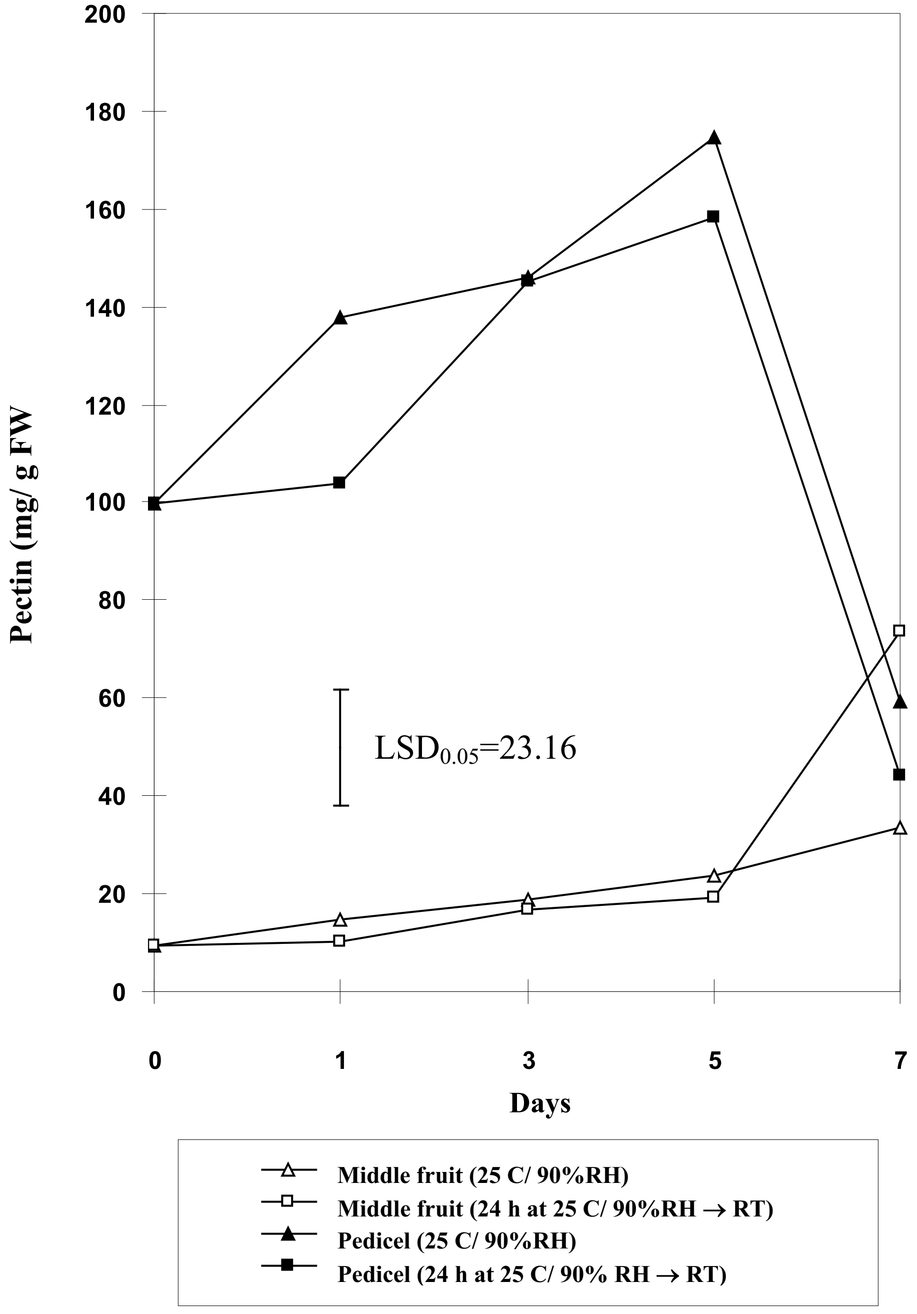

Figure 10 Changes water-soluble pectin in peel of 'Khai' bananas at the middle fruit (▲,■) and at the pedicel (△,□).

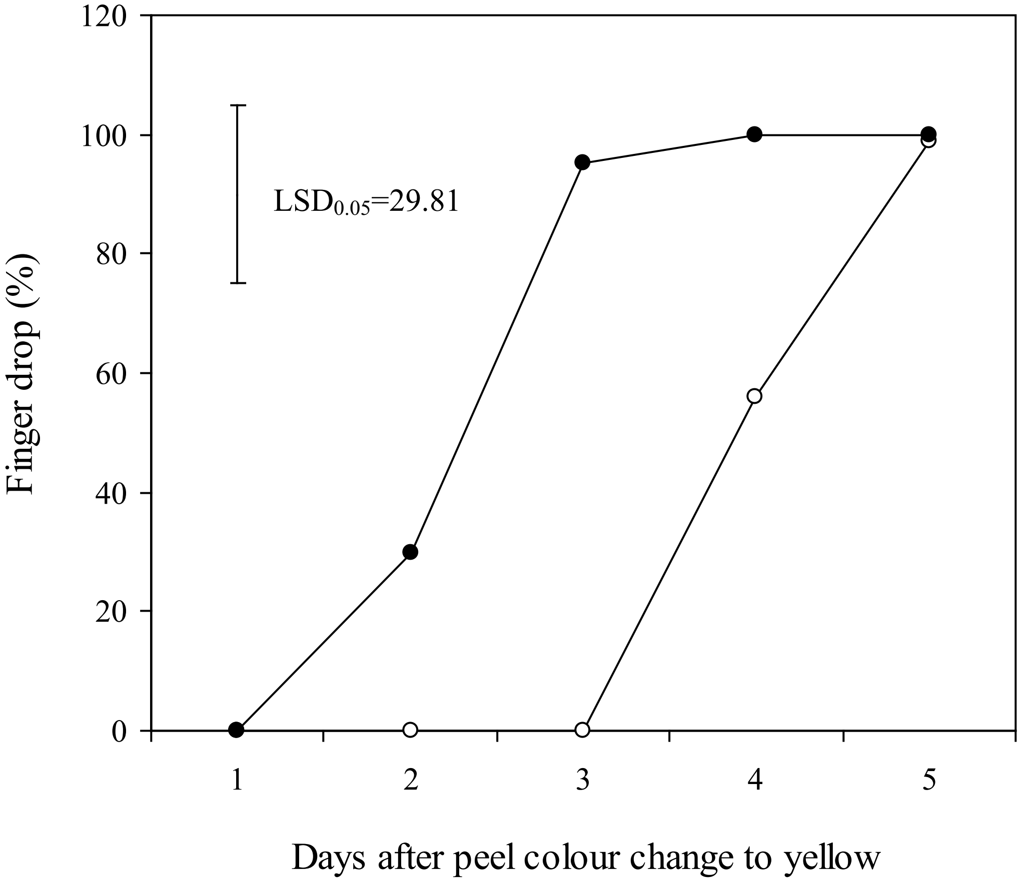

Figure 11 Finger drop of 'Hom Thong' bananas ripened under low (○) and high (●) RH.

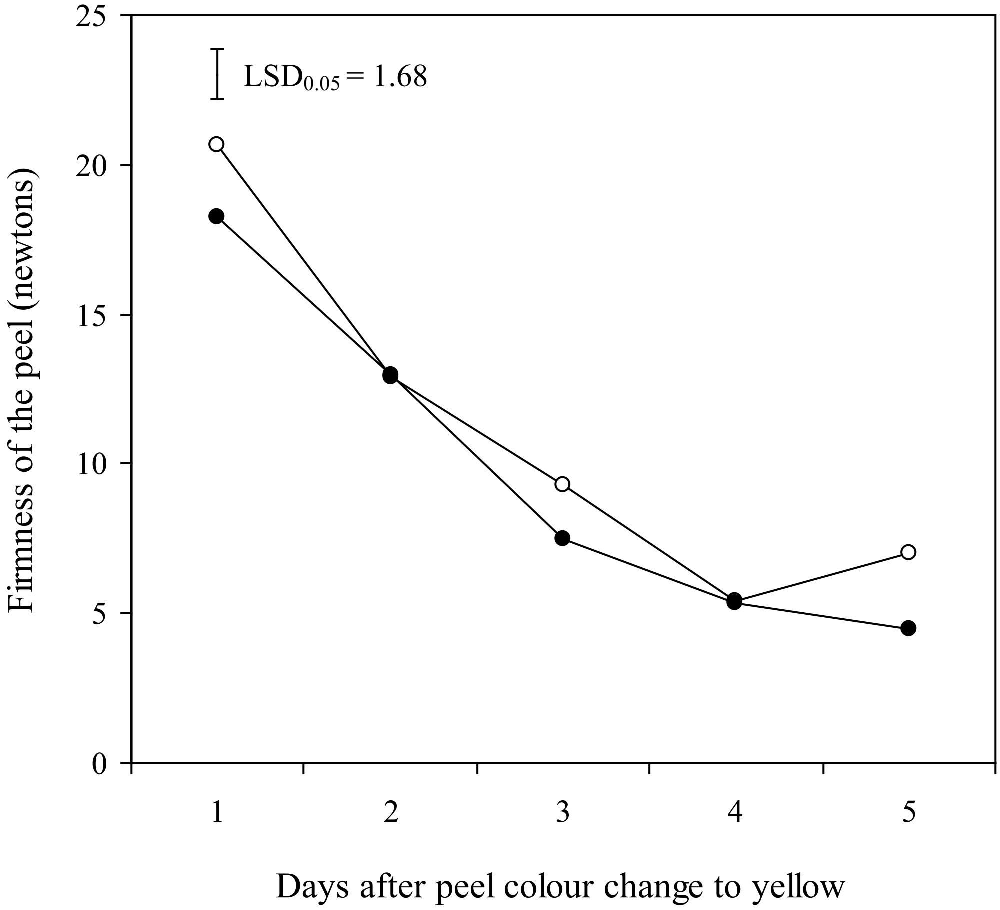

Figure 12 Firmness of the peel of 'Hom Thong' bananas ripened under low (○) and high (●) RH.

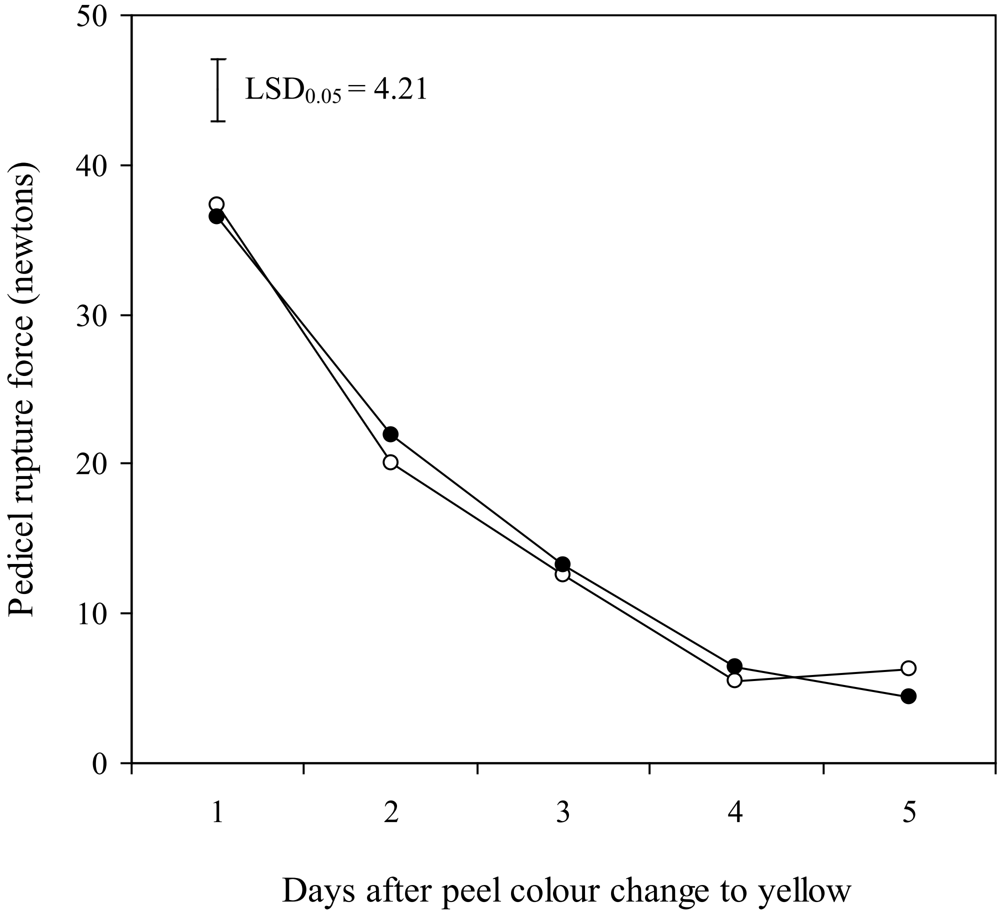

Figure 13 Pedicel rupture force of 'Hom Thong' bananas ripened under low (○) and high (●) RH.

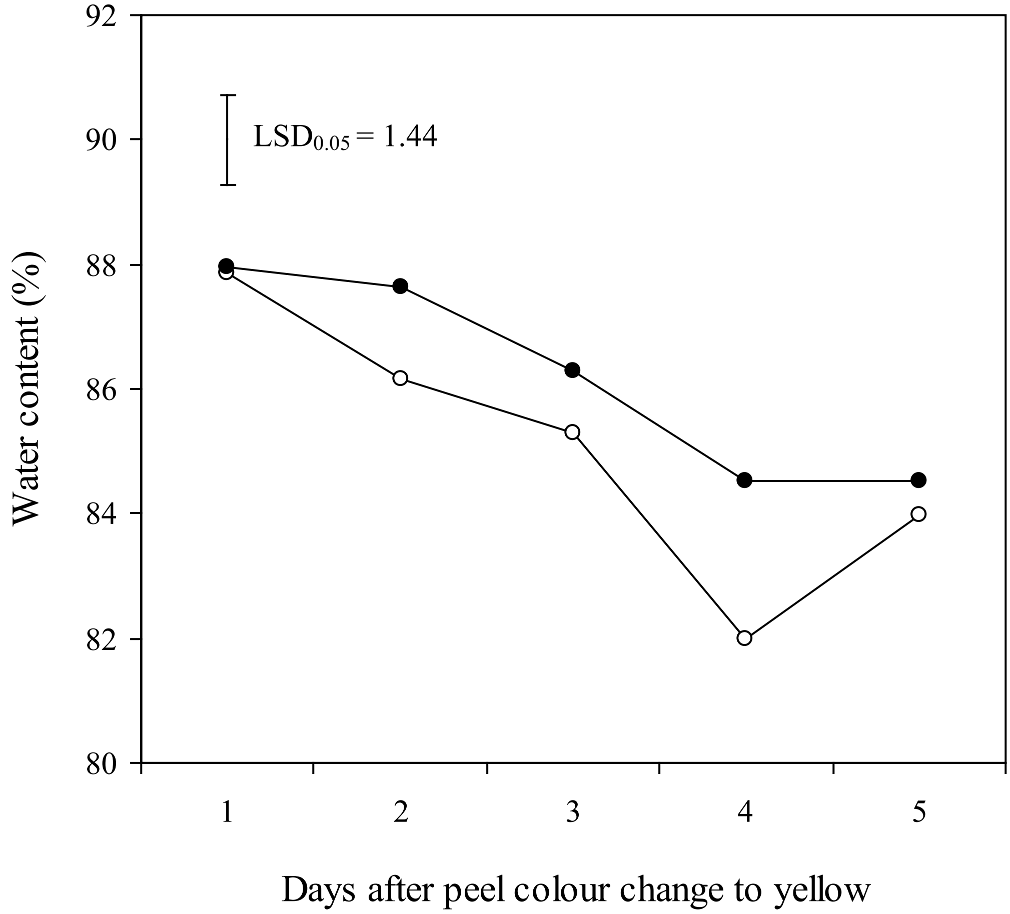

Figure 14 Water content in peel at the middle of the fruit of 'Hom Thong' bananas ripened under low (○) and high (●) RH.

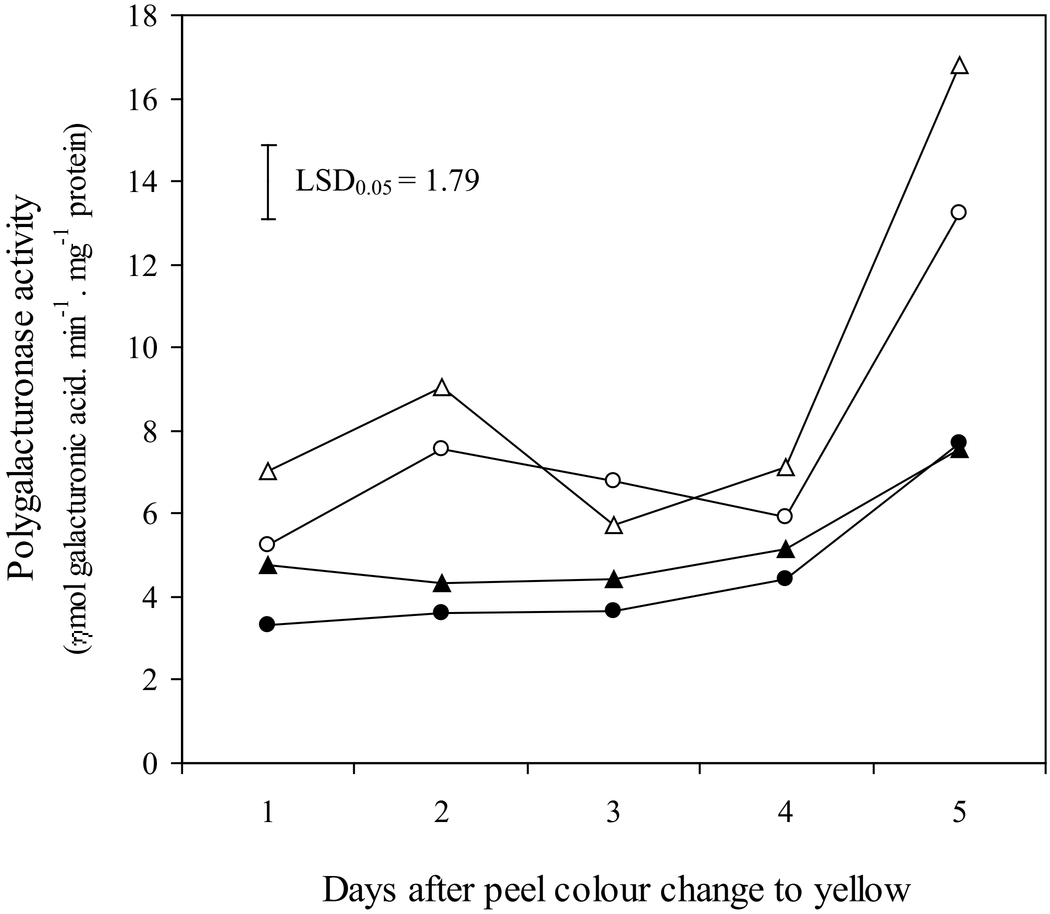

Figure 15 PG activity in the peel at the middle (●,▲) and in the pedicel (○,△) of 'Hom Thong' bananas ripened under low (○ ,●) and high (△,▲) RH.

Pectin methylesterase activity

Pectin methylesterase activity in the peel at the middle of the fruit and at the pedicel adjacent to the rupture area in bananas ripening at high RH increased to a maximum on day 3 and then decreased. Pectin methylesterase activity in peel of both areas showed a little change in bananas fruit ripened at low RH (Figure 16). Pectin methylesterase activity in the peel from the pedicel adjacent to the rupture area was higher than in the peel from the middle of the fruit of bananas ripened under both low and high RH. However, pectin methylesterase activity in the peel of both areas was not significantly different. The activity in the peel at both areas of bananas ripened under low RH increased to a maximum on day 2 and 4, then decreased thereafter. Pectin methylesterase activity in the peel of both areas was higher at high RH than at low RH, but they were not significantly different.

β-Galactosidase activity

β-Galactosidase activities in the peel at the middle of the fruit and at the pedicel adjacent to rupture area of banana ripened under low and high RH were slight different. β-Galactosidase activities were high on day 1 and decrease to a minimum on day 2. After that β-Galactosidase activities increased markedly to a maximum on day 3 and decreased rapidly thereafter (Figure 17).

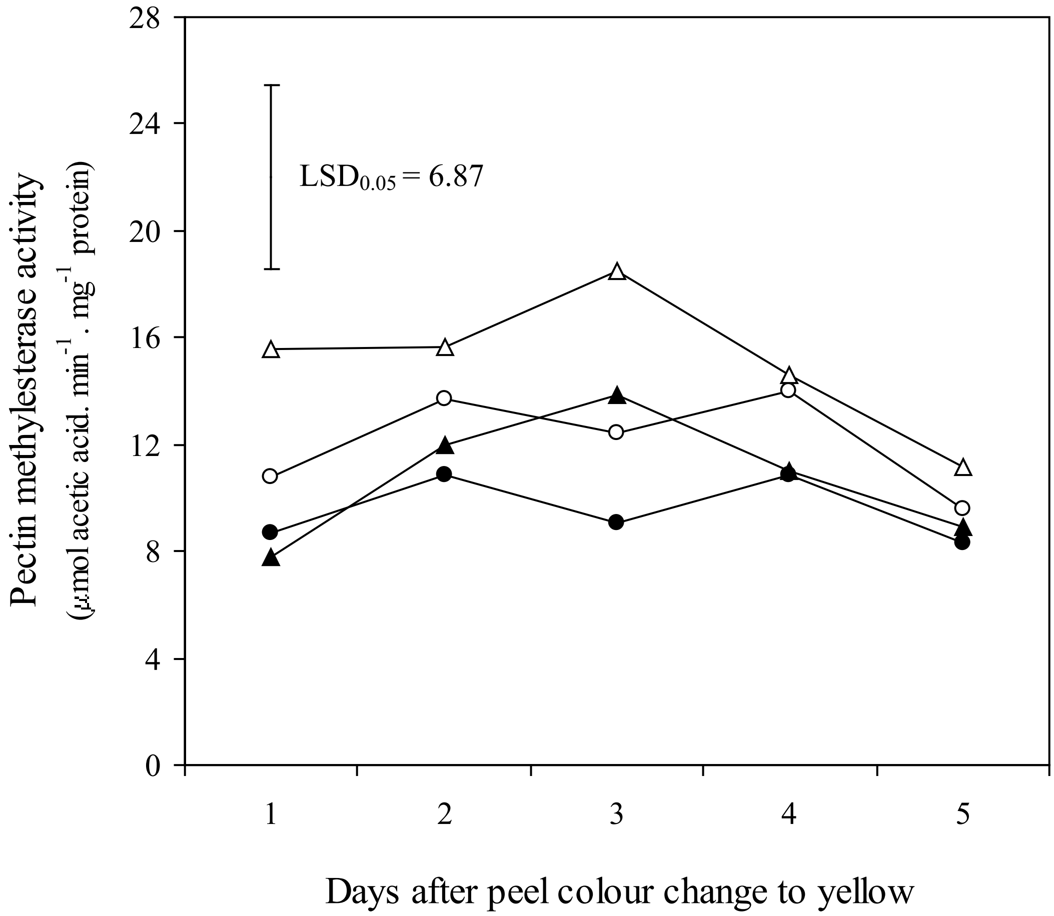

Figure 16 PME activity in the peel at the middle (●,▲) and in the pedicel (○,△) of 'Hom Thong' bananas ripened under low (○,●) and high (△,▲) RH.

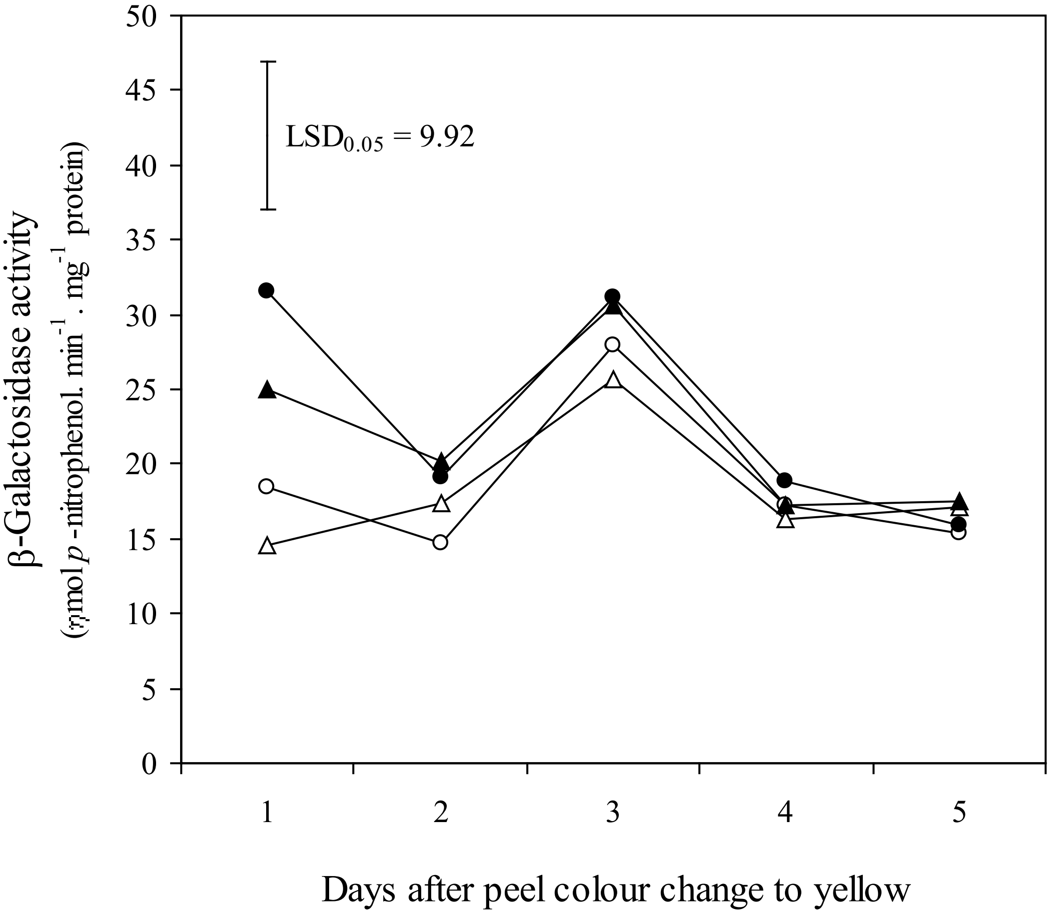

Figure 17 GAL activity in peel at the middle (●, ▲) and at the pedicel (○, △) of 'Hom Thong' bananas ripened under low (○, ●) and high (△, ▲) RH.

Experiment III: Cell wall hydrolase activities in relation to finger drop in 'Hom Thong' and 'Namwa' bananas

Finger drop of 'Hom Thong' bananas started on day 2 and increased rapidly to 100% at the end of experiment while 'Namwa' bananas did not show finger drop during ripening. Finger drop of 'Hom Thong' bananas rapidly increased and reached 100% at day 4 (Figure 18).

Firmness of the peel in both 'Namwa' and 'Hom Thong' bananas decreased rapidly during ripening. At the early stage of ripening (day 1-2), 'Hom Thong' bananas were firmer than 'Namwa' bananas. The peel firmness of 'Hom Thong' bananas, with high finger drop, was around 19 newtons at the first day. When 'Hom Thong' bananas ripened at 25°C under high RH, they lost their firmness rapidly to 3 newtons at the end of the experiment. The firmness of 'Namwa' bananas declined slightly and they were firmer than 'Hom Thong' bananas at 4-5 days after peel colour change to yellow (Figure 19).

'Namwa' bananas had more pedicel rupture force than 'Hom Thong' bananas during all stages ripening, while then pedicel rupture force of both banana cultivars decreased in parallel fashion during ripening (Figure 20).

At the first day, resistance to finger drop of 'Namwa' and 'Hom Thong' bananas was at 5 kg (Figure 21). Resistance to finger drop of 'Hom Thong' bananas declined more sharply than that of 'Namwa' bananas. Resistance to finger drop of 'Namwa' bananas was much higher than that of 'Hom Thong' bananas at the end of experiment.

Water content of the peel in the middle of the fruit was the same at both cultivars levels. Water content was around 87% at the first day. Later on, water content of 'Hom Thong' bananas slightly decreased while 'Namwa' bananas decreased markedly. 'Hom Thong' bananas had water content greater than 'Namwa' bananas during fruit ripening (Figure 22).

In both of 'Hom Thong' and 'Namwa' bananas, PG activity in the peel at the pedicel adjacent to the rupture area and at the middle of the fruit rapidly increased during ripening. PG activity in the pedicel adjacent to the rupture area of 'Hom Thong' bananas was higher than that of 'Namwa' during ripening period, while PG activity in the middle of 'Namwa' bananas was comparable to that of 'Hom Thong' bananas, except on day 5 of ripening (Figure 23).

PME activities of 'Namwa' bananas in both peel at the middle of the fruit and the pedicel adjacent to the rupture area decreased rapidly and were higher than that of 'Hom Thong' bananas whose PME activities remained stable during ripening. PME activities of 'Namwa' bananas in both peel at the middle of the fruit and the pedicel adjacent to the rupture area were comparable. Similarly, PME activities of 'Hom Thong' bananas in both peel areas were comparable (Figure 24).

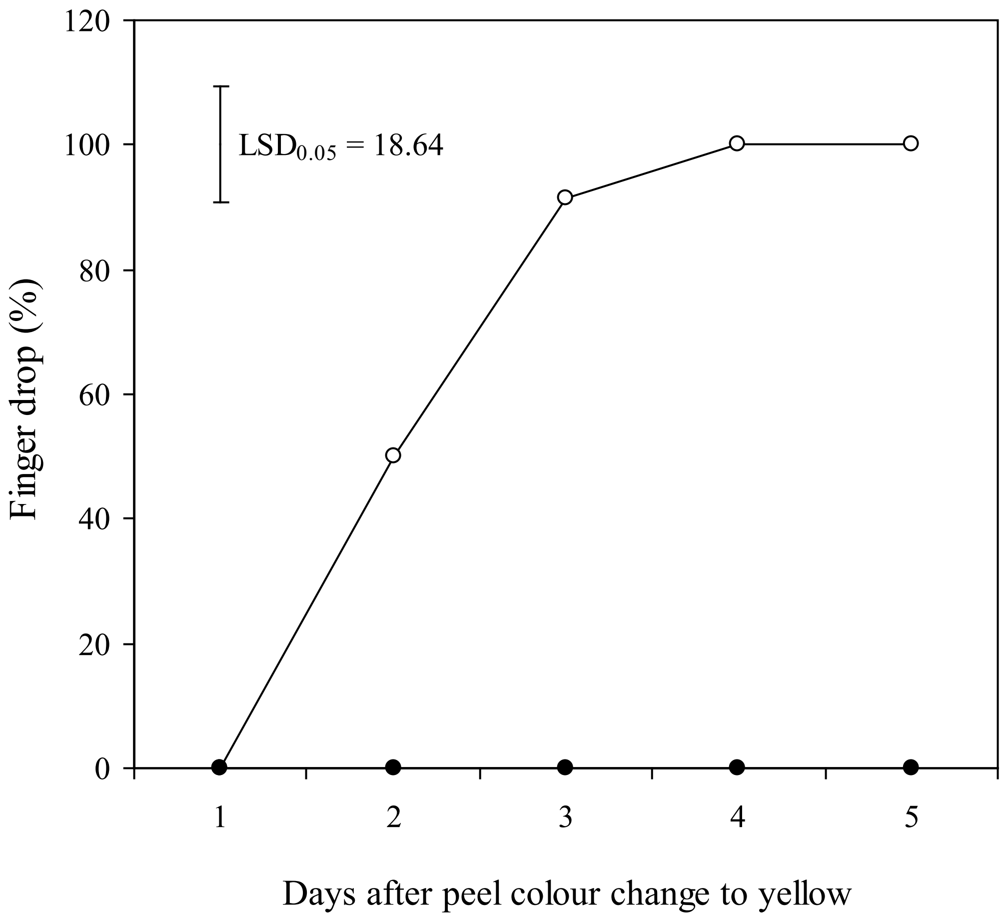

Figure 18 Finger drop of 'Hom Thong' (○) and 'Namwa' (●) bananas during ripening under high RH.

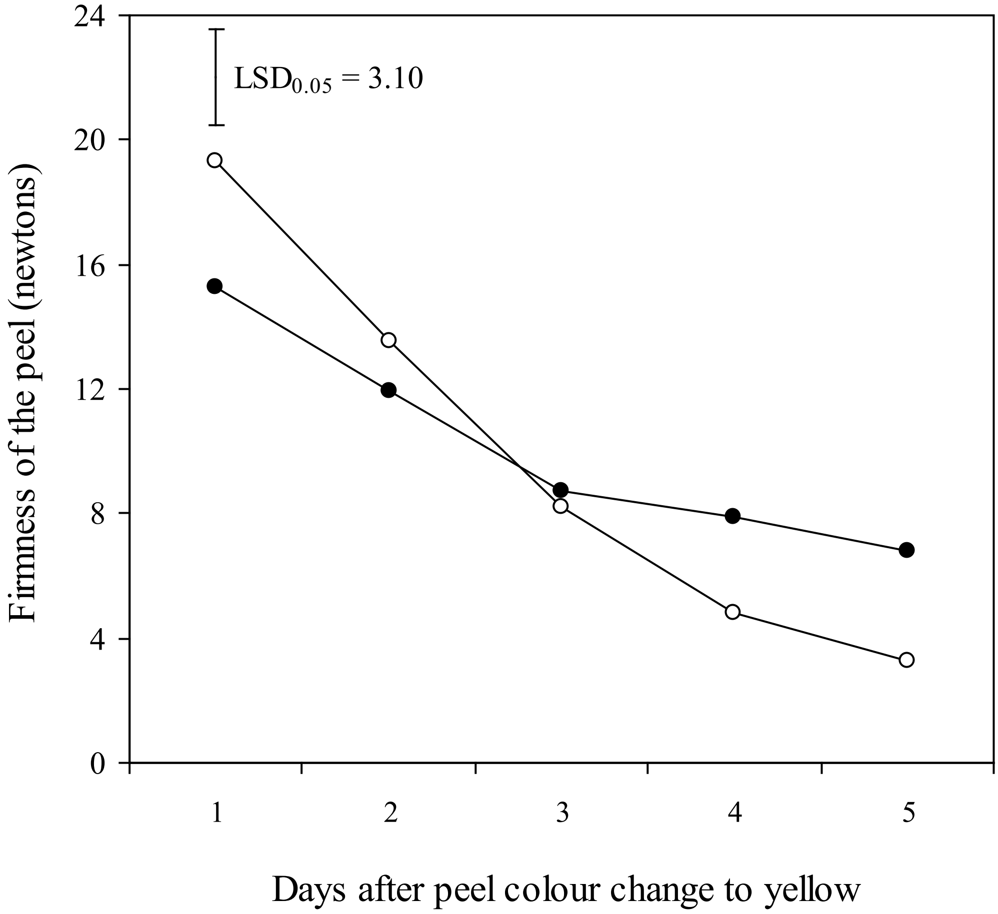

Figure 19 Firmness of the peel of 'Hom Thong' (○) and 'Namwa' (●) bananas during ripening under high RH.

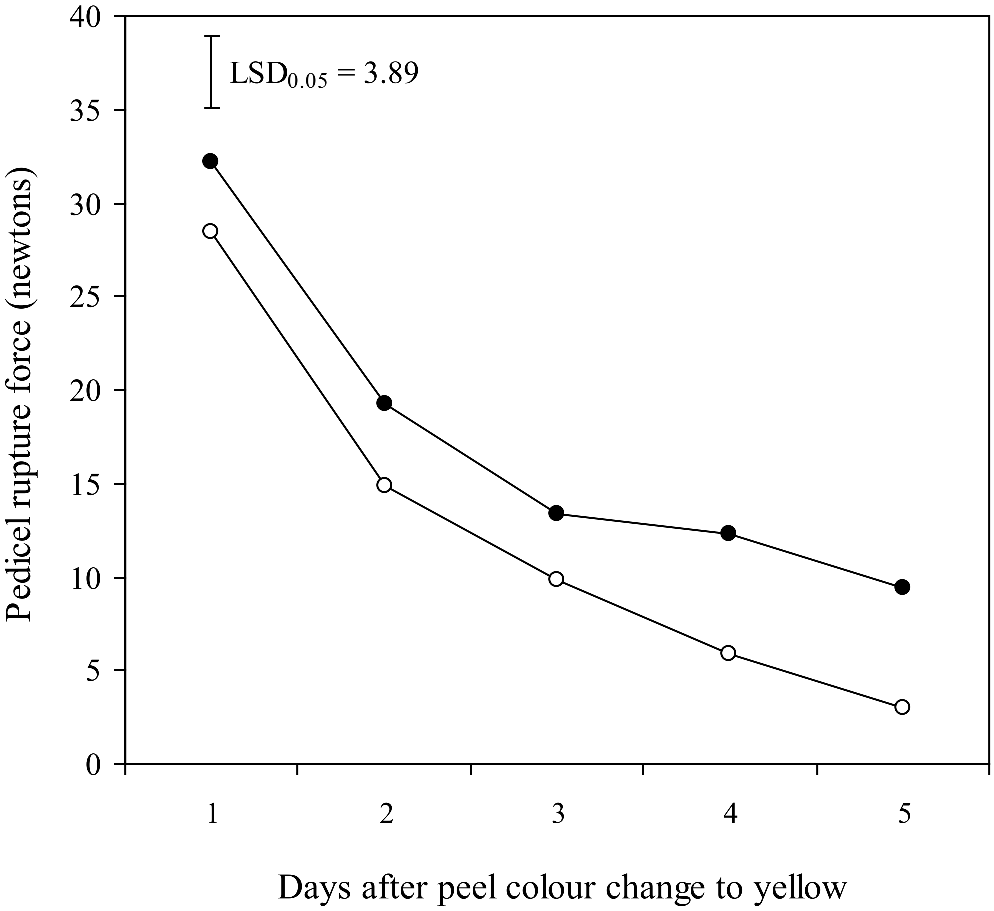

Figure 20 Pedicel rupture force of 'Hom Thong' (○) and 'Namwa' (●) bananas during ripening under high RH.

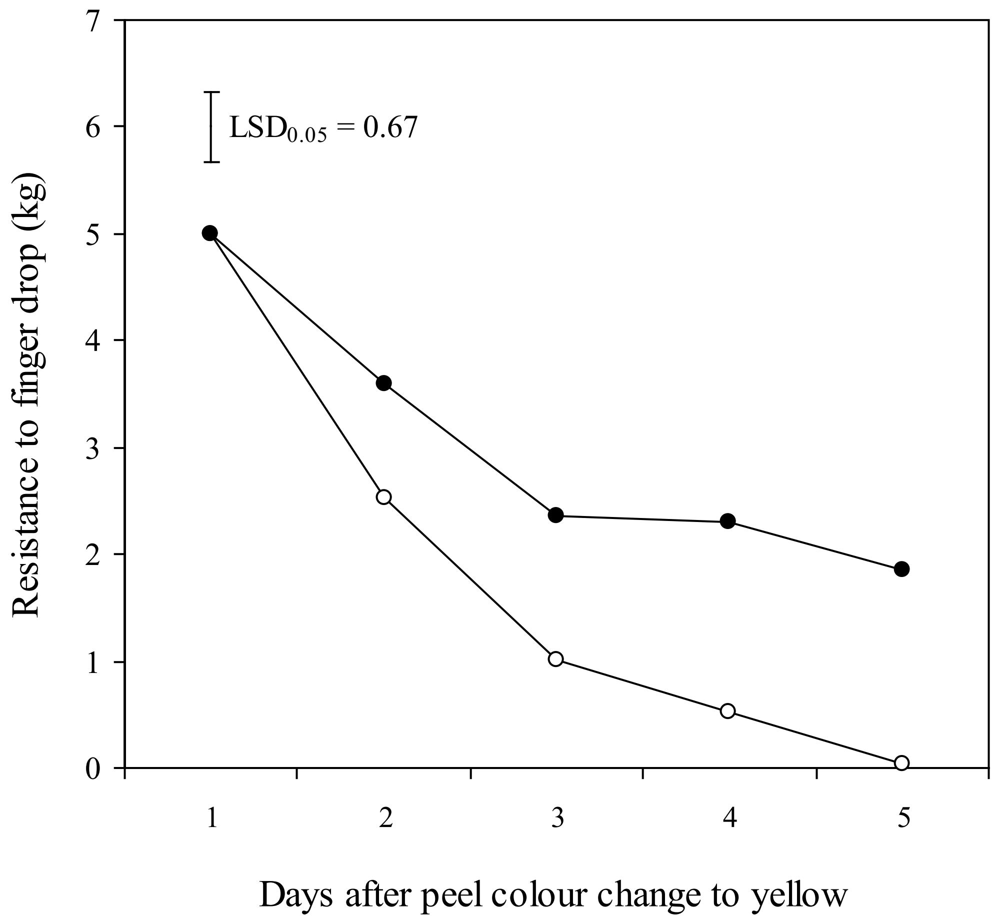

Figure 21 Resistance to finger drop of 'Hom Thong' (○) and 'Namwa' (●) bananas during ripening under high RH.

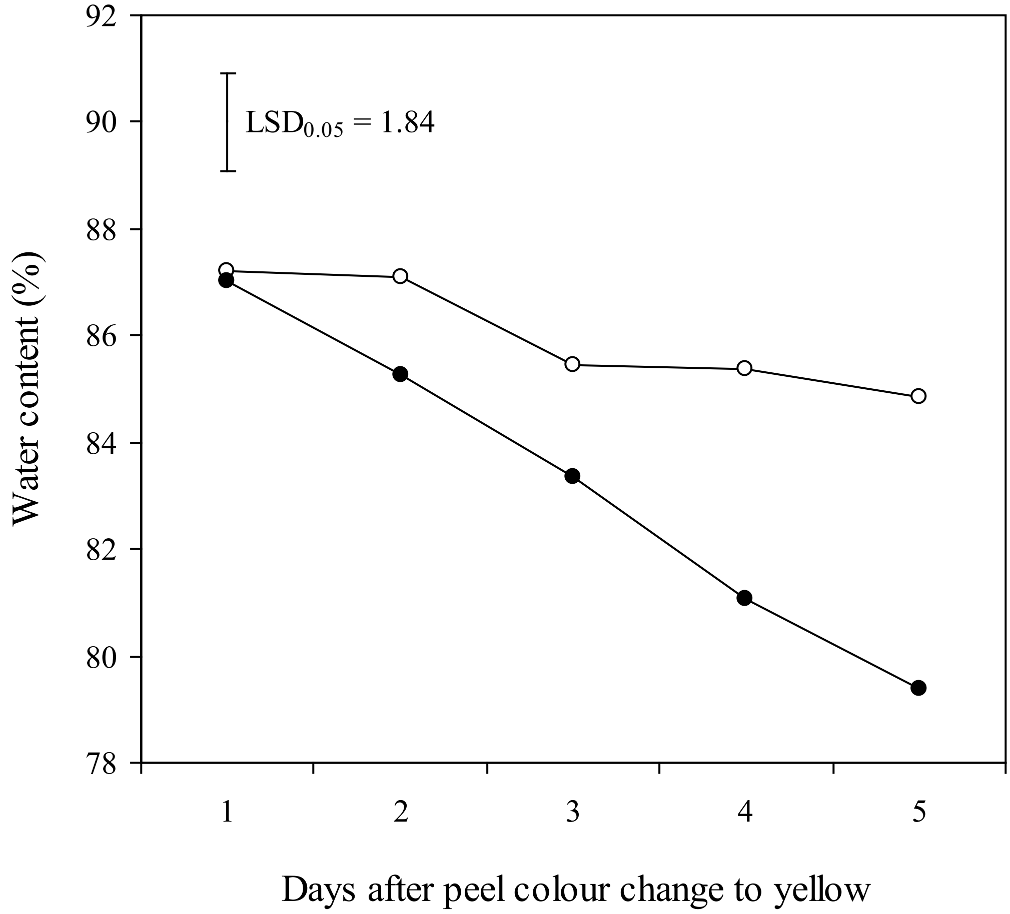

Figure 22 Water content in peel at the middle of the fruit of 'Hom Thong' (○) and 'Namwa' (●) bananas during ripening under high RH.

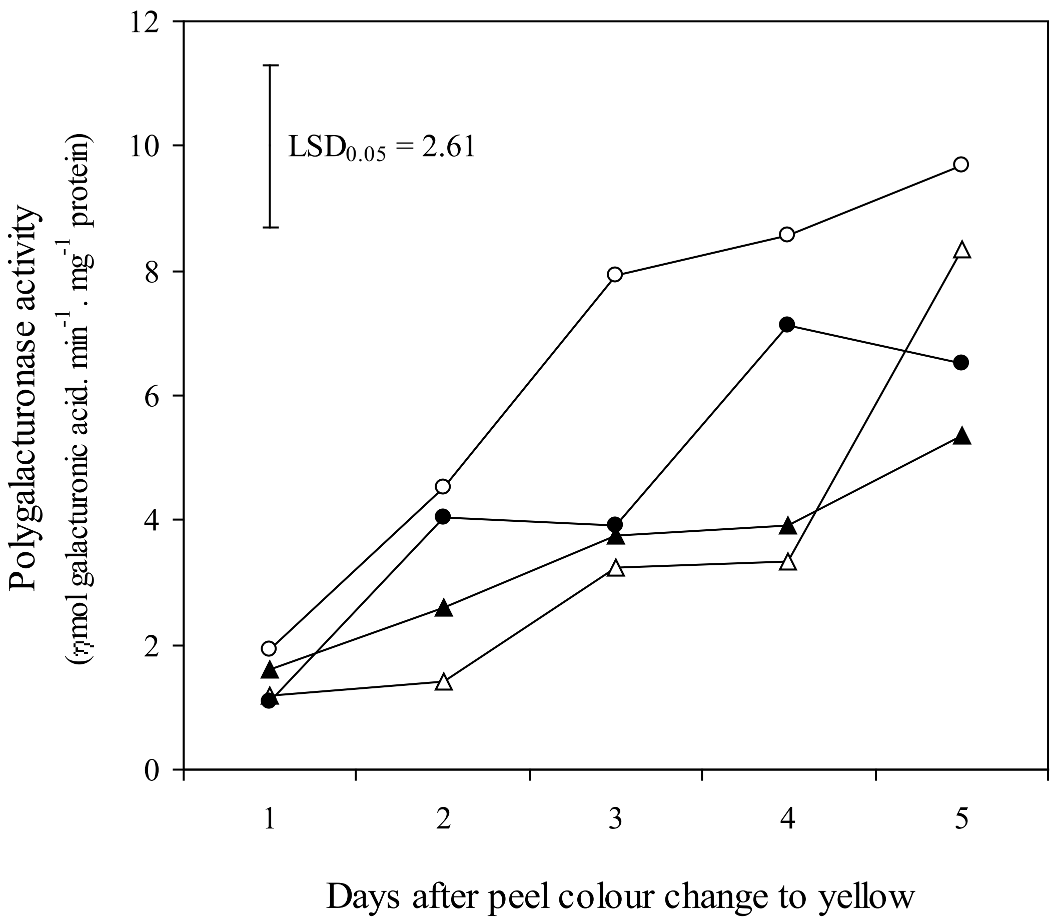

Figure 23 PG activity in peel at the middle (△, ▲) and at the pedicel (○, ●) of 'Hom Thong' (○ , △) and 'Namwa' (● , ▲) bananas during ripening under high RH.

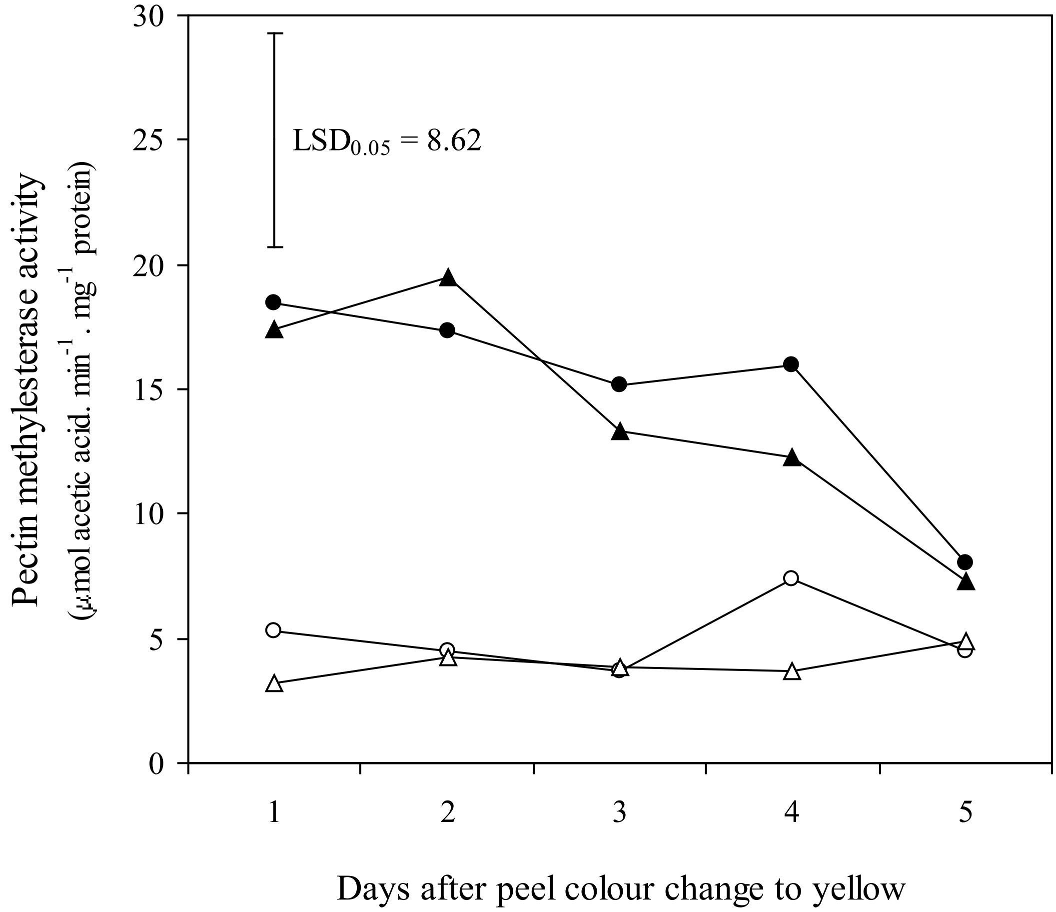

Figure 24 PME activity in peel at the middle (△, ▲) and at the pedicel (○, ●) of 'Hom Thong' (○, △) and 'Namwa' (●, ▲) bananas during ripening under high RH.

Experiment IV: Histochemical study of the formation of an abscission zone in relation to finger drop of 'Hom Thong' and 'Namwa' bananas

Sections of the peel at the pedicel adjacent to rupture area on day 1 and day 4 of 'Hom Thong' and 'Namwa' bananas were stained with safranin-O and fast green for gross an abscission layer examination, the cells of the peel at the rupture area did not form the abscission layer (Figure 25 and 26). When stained with ruthenium red to visualize pectic substances, staining was quite stronger and higher of the parenchyma cell wall of the peel at the rupture area of 'Hom Thong' bananas ripening on day 1 (Figure 27A) than that of 'Hom Thong' bananas ripening on day 4 (Figure 27B). In 'Namwa' banana was slightly different ruthenium red staining at the rupture area of bananas ripening on day 1 and day 4 (Figure 28).

Experiment V: Gene expression of cell wall hydrolase(s) involved in finger drop in 'Hom Thong' and 'Namwa' bananas

In the pedicel at adjacent to the rupture area, the level of *MAPG* transcript in 'Namwa' bananas was lower than that in 'Hom Thong' bananas (Figure 29A). The level of *MAPG* transcript in the middle of 'Namwa' and 'Hom Thong' bananas decreased upon ripening. *MAPG* mRNA accumulation in the middle of 'Hom Thong' bananas was a little bit higher than that in of 'Namwa' banana, at all stages (Figure 29B). The abundance of *MAPG* transcript in the pedicel adjacent to the rupture area was higher than that in the middle, in both of bananas (Figure 30A). The abundance of *MAPG* mRNA was involved in the activity of PG in peel at the middle of the fruit and at the pedicel adjacent to the rupture area in both banana cultivars. PG activity and *MAPG* transcript in the pedicel adjacent to the rupture area of 'Hom Thong' bananas were higher than those of 'Namwa' bananas (Figure 30).

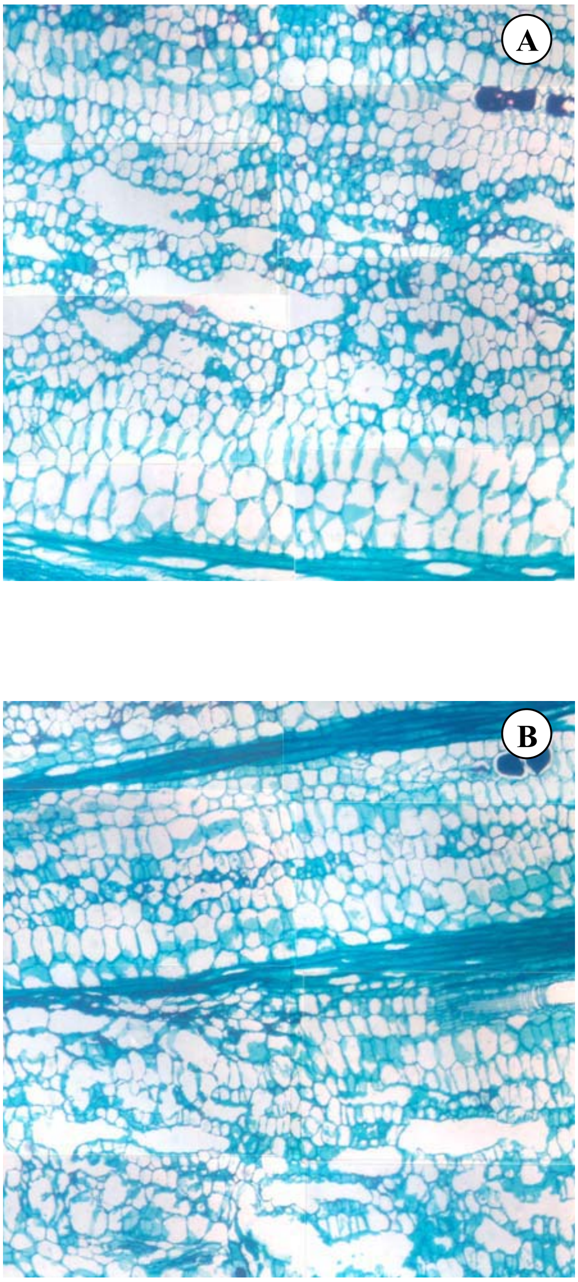

Figure 25 Peel at the pedicel adjacent to the rupture area of 'Hom Thong' banana ripening on day 1 (A) and day 4 (B) under high RH was stained with safranin-O and fast green. (4X)

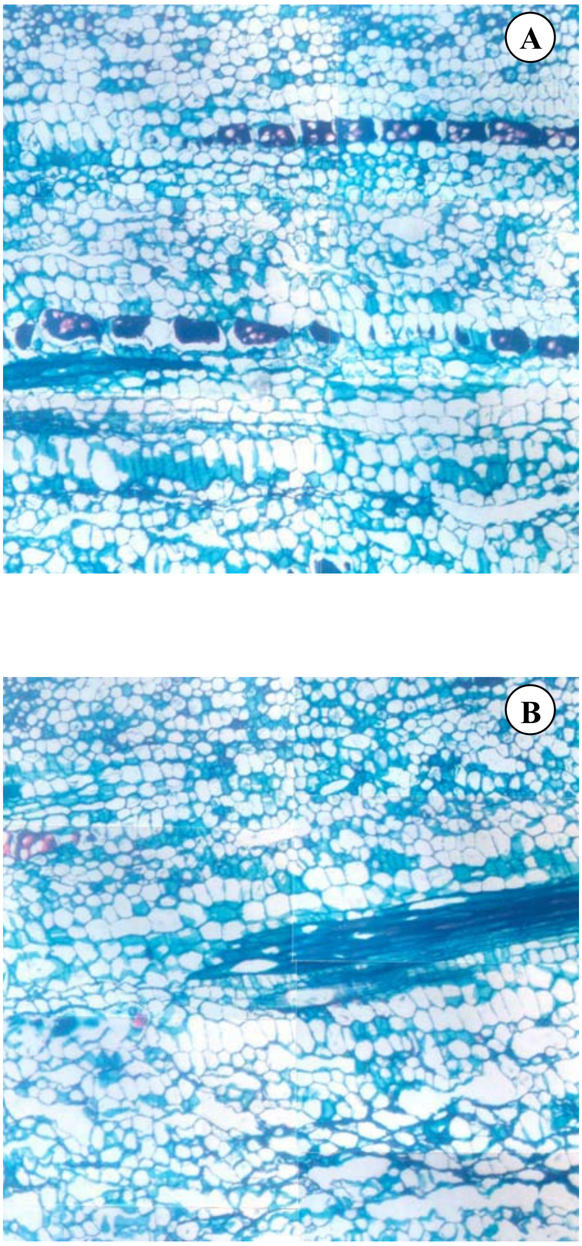

Figure 26 Peel at the pedicel adjacent to the rupture area of 'Namwa' banana ripening on day 1 (A) and day 4 (B) under high RH was stained with safranin-O and fast green. (4X)

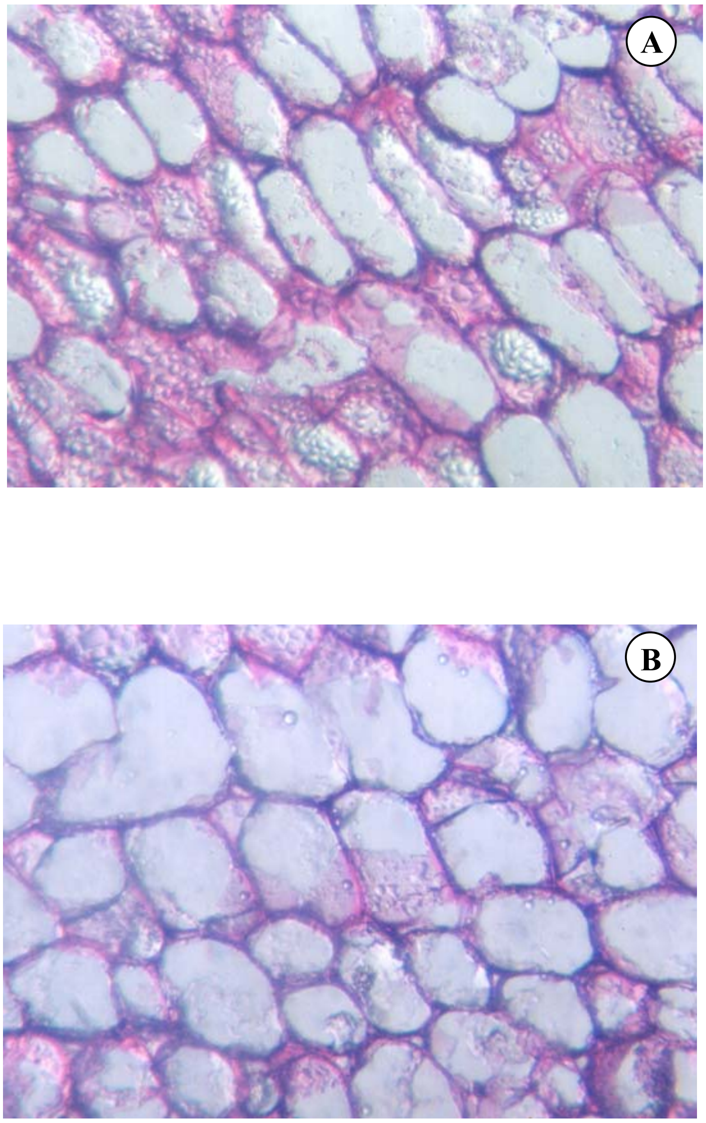

Figure 27 The localized of pectic substances at the pedicel adjacent to the rupture area of 'Hom Thong' banana ripening on day 1 (A) and day 4 (B) under high RH. (10X)

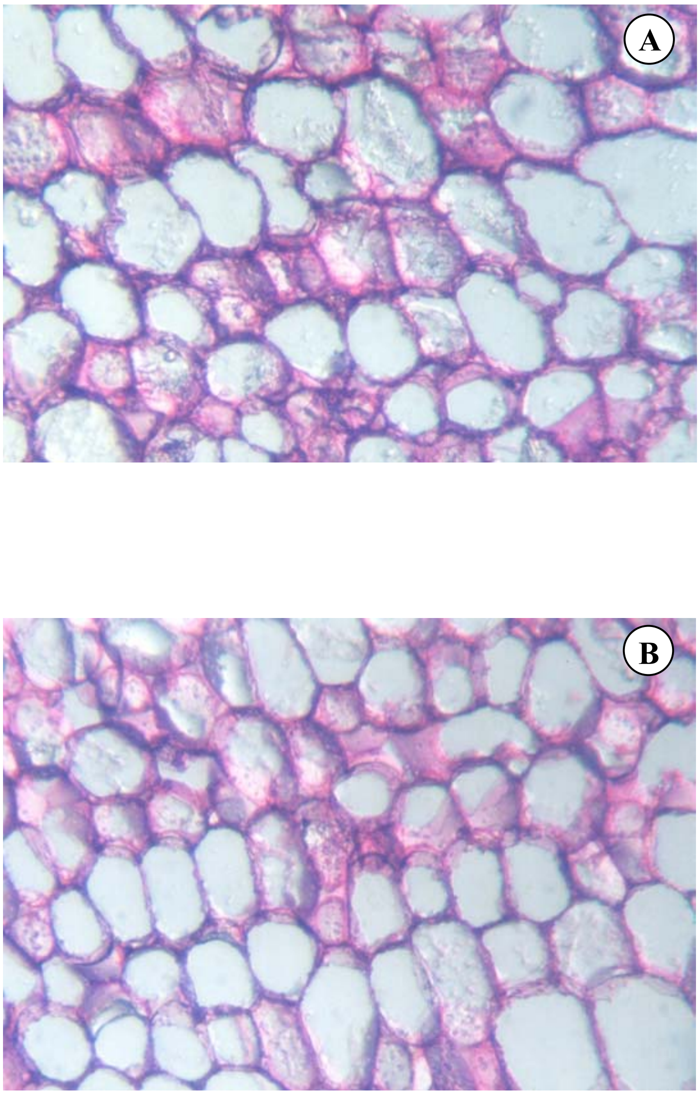

Figure 28 The localized of pectic substances at the pedicel adjacent to the rupture area of 'Namwa' banana ripening on day 1 (A) and day 4 (B) under high RH. (10X)

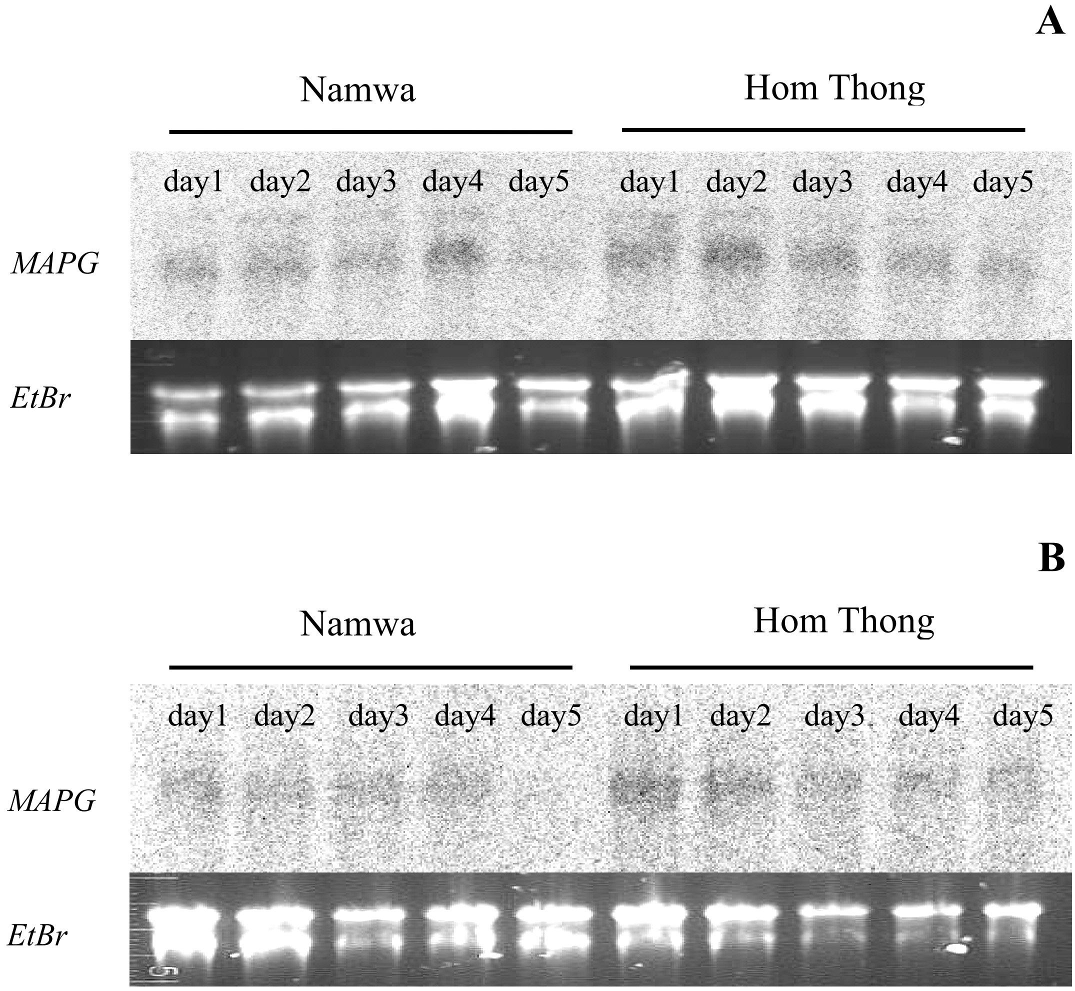

Figure 29 RNA gel blot-analysis of *MAPG* mRNA abundance in peel of bananas. Total RNA (10 μg) from the peel at the pedicel adjacent to the rupture area (A) and at the middle (B) of 'Namwa' and 'Hom Thong' bananas at day 1, day 2, day 3, day 4 and day 5 after peel colour change to yellow were electrophoresed and then hybridized with probe. Equal loading of RNA was confirmed by staining gels with ethidium bromide (EtBr).

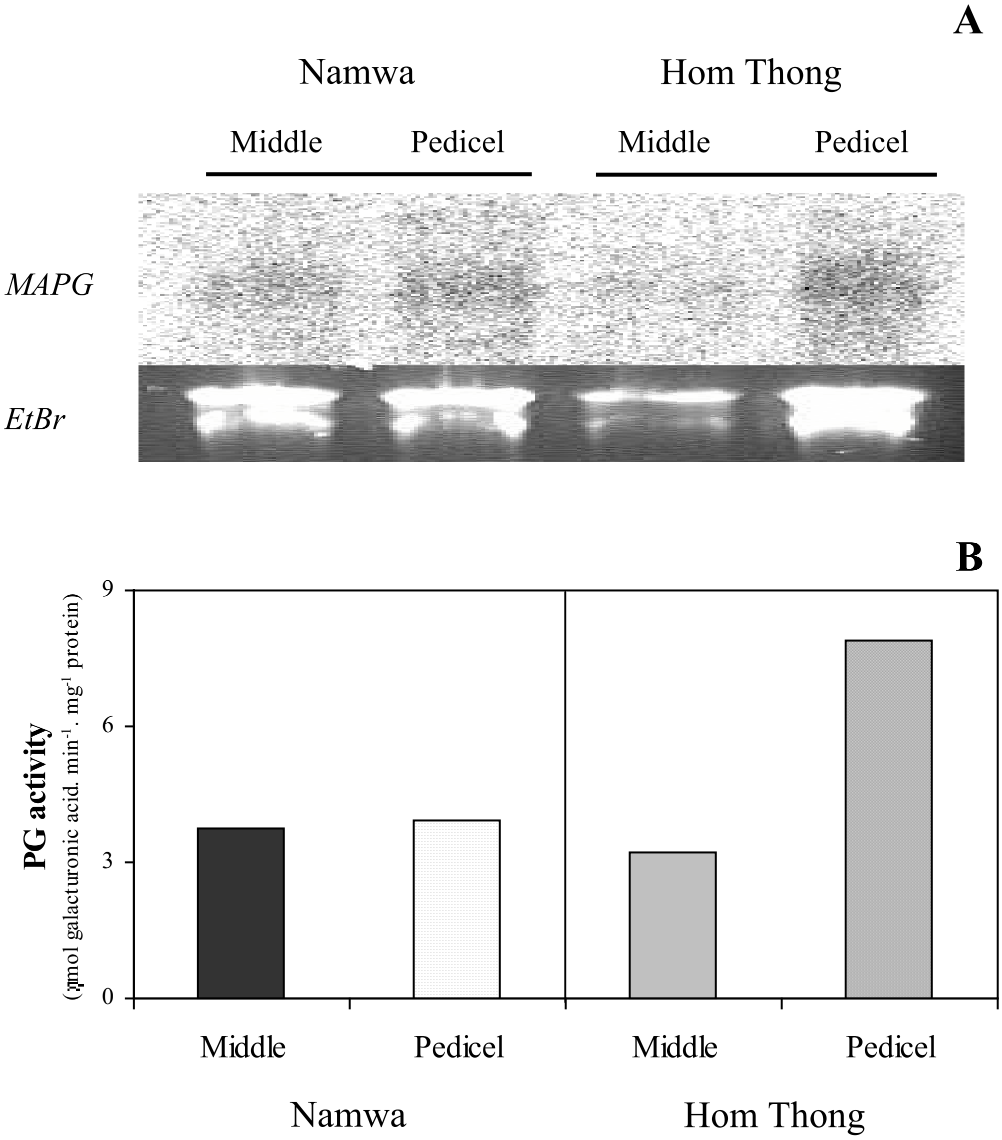

Figure 30 RNA gel blot-analysis of *MAPG* mRNA abundance in peel of bananas. Total RNA (10 μg) from the peel at the pedicel adjacent to the rupture area and at the middle of 'Namwa' (left) and 'Hom Thong' (right) bananas at 3 days after peel colour change to yellow. Equal loading of RNA was confirmed by staining gels with ethidium bromide (EtBr) (A). PG activity in peel at the middle and at the pedicel of 'Namwa' (left) and 'Hom Thong' (right) bananas at 3 days after peel colour change to yellow (B).