Discussion

An increase in soluble solids content of the pulp occurred concomitant with loss of firmness (Figure 2B), as reported in other banana cultivars (Seymour, 1993). In the pulp, Water-soluble pectin and ammonium oxalate-soluble pectin increased sharply and alkali-soluble pectin increased steadily (Figure 3A), while in the peel water-soluble pectin increased slightly, alkalin-soluble pectin decreased slowly and alkalin-soluble pectin remained unchanged (Figure 3B). This indicates that cell wall solubilization occurs to a greater extent in the pulp than in the peel (Smith et al., 1989).

The decrease in pulp firmness (Figure 2B) was accompanied by an increase in the activities of polygalacturonase (Figure 4A), pectinesterase (Figure 4B) and β-galactosidase (Figure 5A). However, the increase in polygalacturonase activity was more prominent than the increase in pectinesterase and β-galactosidase activities. This was similar to results obtained in durian fruits (Ketsa and Daengkanit, 1999), and in mango (Ketsa et al., 1998). The difference in polygalacturonase activity in the peel and pulp reflected the increases in water-soluble pectin in the peel and pulp. polygalacturonase activity in the peel was greater during the early phase of ripening. This resulted in more water-soluble pectin in the peel than in the pulp. Polygacturonase activity in the pulp increased more than that in the peel during the latter phase of ripening, resulting in higher pulp water-soluble pectin (Figure 4). This result suggests that polygalacturonase is mainly responsible for solubilization of cell-wall pectin, thus for the softening of bananas (Seymour and Gross, 1996). In tomato, polygacturonase activity was highly correlated with softening in normal fruits, whereas polygacturonase activity was virtually absent from non-softening transgenic plants. However, fruit softening was not affected in these plants, despite greatly reduced polygalacturonase levels (1% of the wild type; Smith et al., 1988). In other experiments, over-expression of polygacturonase in a non-softening mutant failed to induce softening, although pectic depolymerization and solubilization did occur (Giovannoni *et al*., 1989). Thus, despite the strong relationship between polygacturonase activity and water-soluble pectin polygacturonase alone may not be sufficient to induce pulp softening of banana.

Pectinesterase activity in the peel decreased sharply, while pectinesterase activity in the pulp increased markedly (Figure 4B). This is consistent with the decrease in ammonium axalate-soluble pectin in the peel (Figure 3A) and with the increase in ammonium oxalate-soluble pectin in the pulp (Figure 3B). Removal of methyl groups from the galacturonic acid chain by pectinesterase (Fischer and Bennett, 1991) would allow more pectin to be extracted by ammonium oxalate (Ketsa et al., 1998). pectinesterase activity has also been found to increase during softening of other fruit, such as cherry (Andrew and Li, 1995) and African mango (Aina and Oladunjoye, 1993). In contrasty, pectinesterase activity has been found to decrease during softening of avocado (Awad and Young, 1980) and mango (Abu-Sarra and Abu-Goukh, 1992).

β-Galactosidase activity in the peel increased more rapidly than in the pulp and β-galactosidase activity in the peel, at its maximum, was 10 fold higher than that in the pulp (Figure 4A). This suggested that β-galactosidase may play a major role in the loss of peel firmness. β-galactosidase activity has also been found to increase during softening of cherry (Barrett and Gonzales, 1994) and mango (Ali et al., 1995; Ketsa et al., 1998). The difference in polygalacturonase and β-galactosidase activities in the peel and pulp of banana supports the view that cell wall changes in peel and pulp are quite different. Cellulase activity was very high in the peel of unripe banana and very low during softening, while cellulsae activity in the pulp was low throughout softening period (Figure 5B). This suggested that cellulase may not be involved in banana softening.

Recently banana pulp ripening-specific transcript encoding putative pectate lyases like polygalacturonase, catalyses the cleavage of α(1-4) galacturonan mechanism has been reported (Dominguez-Puigjaner et al., 1997; Medina-Suarez et al., 1997). Their results suggest that polygalacturonase may play a role in the loss of firmness in ripening banana.

In conclusion, the present study indicates that cell wall changes and activities of cell wall hydrolases are quite different in the peel and pulp, during softening of 'Khai' banana.

Bananas ripened at 25°C/90% RH for 24 h showed low finger drop than those ripened at 25°C/90% RH for 72 h. The longer they exposed to 25°C/90% RH, the more they developed finger drop. This indicated that bananas ripened under high RH too long were prone to develop finger drop more seriously than those ripened with a shorter exposure to high RH (Semple and Thompson, 1988).

Softening of ripening fruits is associated with alternation in cell wall and middle lamella structure (Seymour and Gross, 1996). These changes include solubilization of cell wall pectin involving the action of cell wall hydrolytic enzymes PG, PME and GAL. Bananas with high finger drop had greater activities of PG (Figure 6) and PME (Figure 8) in peel at the pedicel during ripening, resulting in more water-soluble pectin in peel at the pedicel than that in peel at the middle fruit (Figure 9) This may lead to localize a weakening peel at the pedicel, which in turn produces finger drop. It is not know how bananas exposed to long period of high RH induced greater activities of PG and PME in peel at the pedicel than those exposed to short period of high RH during ripening.

GAL activities in peel at the middle fruit and at the pedicel of bananas with low finger drop and exposed to a short period of high RH were greater than those with high finger drop and exposed to a long period of high RH (Figure 10). This suggested that GAL may not have relationship with finger drop.

Finger drop of 'Hom Thong' bananas ripened at low RH (65-70%) occurred more slowly than those ripened at high RH (90-95%) (Figure 11). This result was similar to the report of Semple and Thompson (1988) that low RH at the later stage of

bananas ripening reduced finger drop. Prayurawong (1999) reported that 'Khai' bananas ripened at high RH (90%, 25°C) for 24 hours showed less finger drop than those ripened for 72 hours. However, on day 5 after peel colour change, bananas ripening under both RHs was not significantly different. They were 100% finger drop (Figure 11).

Firmness of the peel (Figure 12) and pedicel rupture force (Figure 13) of 'Hom Thong' bananas ripening under low and high RHs decreased and had no significant difference during ripening. Although, pedicel rupture force is related to finger drop of Cavendish banana (New and Marriott, 1983; Semple and Thompson, 1988), it is not related to finger drop in 'Hom Thong' banana. Therefore, pedicel rupture force is not a good indicator of finger drop in 'Hom Thong' bananas. Peel puncture force at the full ripe stage has been shown not to be a good indicator of finger drop in Dwarf Brazilian banana (Paull, 1996). This is the same case as the peel firmness of 'Hom Thong' bananas. Bananas ripening at low RH had water content in the peel less than bananas ripening at high RH (Figure 14). The results suggest that the high RH condition reduce water loss from the peel of banana. The water content at the fully ripe stage of bananas ripened under both RHs was not significantly different and correlated with finger drop (day 5).

The activities of PG (Figure 15) and PME (Figure 16) in the peel at the middle of the fruit and the pedicel adjacent to the rupture area of 'Hom Thong' bananas was not influenced by ripening under low and high RH. However, PG activity in the peel of bananas ripened under high RH was higher and correlated with the development of finger drop. Moreover, the activity of PG activity in peel at the pedicel adjacent to the rupture area was higher than in the middle of the fruit (Figure 15). The result was similar to the report of Prayurawong (1999) found the peel at the stem end of ripened 'Khai' bananas contained higher PG activity than the peel in the middle of the fruit. This result suggested that PG degrade cell wall pectic substance in the peel at the pedicel adjacent to the rupture area more than at the middle of the fruit. Peel softening has been associated with changes in the cell wall pectic components (Seymour, 1993). Therefore, increasing PG activity in the pedicel adjacent to rupture area may result in decreased pedicel rupture force and firmness of the peel, resulting in finger drop of 'Hom Thong' bananas during ripening. These results support that the development of finger drop during 'Hom Thong' bananas ripening involved in the weakening of the pedicel connecting the finger to the banana crown of a hand (Baldry et al., 1981).

PME activity in peel at the pedicel adjacent to the rupture area was not significantly different from peel at the middle of the fruit although the peel at pedicel adjacent to the rupture area had greater PME activity than the peel at the middle (Figure 16). Chalardkid (1992) reported that the activity of PE (PME) in the pedicel of 'Hom Thong' bananas slowly increased and became stable after peel colour change. PME activity may not be involved in finger drop during banana ripening. Additionally, the activity of GAL in the peel at the middle of the fruit was higher than at the pedicel adjacent to the rupture area and PME activity was not significantly

different in peel of bananas ripened under both relative humidity conditions (Figure 17). This suggested that GAL may not be involved in the development of finger drop.

'Hom Thong' (*Musa* AAA Group) and 'Namwa' (*Musa* ABB Group) bananas ripening at 25°C, 85-90% RH showed 0% finger drop in 'Namwa' bananas as 100% finger drop in 'Hom Thong' bananas at the last day of ripening (Figure 18). Some cultivars, particularly triploid (Semple and Thompson, 1988) and tetraploid, are rather susceptible to finger drop (Marriott, 1980). Finger drop It has been reported in banana in triploid cultivars but it there are no studies comparing different groups. This study compared finger drop between 'Hom Thong' and 'Namwa' bananas. The result showed that 'Namwa' bananas did not show finger drop although 'Namwa' banana is in the triploid group as it is 'Hom Thong' banana. However, both of bananas are different in the genome types. This result suggests that banana with B genome type developed less finger drop than banana with A genome type. Therefore, finger drop of bananas may be correlated with the number and type of genome.

The decrease in firmness of the peel (Figure 19), pedicel rupture force (Figure 20) and resistance to finger drop (Figure 21) of 'Hom Thong' and 'Namwa' bananas during ripening occurred concomitantly with an increase of PG activity. Firmness of the peel at the later stage of ripening of 'Hom Thong' bananas was lower than that of 'Namwa' banana but it was not significantly different. This supported the conclusion that the peel puncture force at the fully ripe stage is not a good indicator of finger drop (Paull, 1996). Pedicel rupture force and resistance to finger drop of 'Namwa' bananas were greater than those of 'Hom Thong' bananas during developmental finger drop. As the result suggested that both of parameters are good indicators for finger drop but resistance to finger drop may be a better indicator than pedicel rupture force.

The activity of PG in the peel at the pedicel adjacent to the rupture area of 'Hom Thong' bananas was higher than that of 'Namwa' bananas (Figure 23) and the increase of PG activity was accompanied with an increase finger drop and the decrease of resistance to finger drop. Finger drop of 'Namwa' bananas did not occur but 'Namwa' bananas still had high PG activity in the peel during fruit ripening. Moreover, PG activity in 'Hom Thong' bananas was completely different from 'Namwa' bananas only at day 3 and day 5. PG activity may be related to finger drop. Consequently, PG may partially degrade pectic components in the middle lamella and PL may collaborate with PG to degrade pectic substances. PL activity in banana pulp showed a substantial increase during ripening. The enhanced levels of PL activity corresponded with an increase in soluble polyuronides (Marin-Rodrigez et al., 2003). As a result, the peel at the pedicel adjacent to the rupture area softens and weakens because PG degrades cell wall components in the pedicel connecting to the banana hand during ripening.

In contrast, PME activity in the peel of 'Namwa' bananas was highest at the first day and decreased later, and was higher than that of 'Hom Thong' bananas (Figure 24). In addition, the activity of PME in the peel at the pedicel adjacent to the rupture area was not significantly different at the middle of the fruit in both bananas.

This suggests that PME activity may not be associated with the development of finger drop.

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, 1974; Semple and Thompson, 1988). The histochemical study of the pedicel adjacent to the rupture area did not find the abscission zone at this area (Figures 25 and 26). This suggested that the development of finger drop did not require abscission zone. It confirms that the softening and weakening at the pedicel adjacent to the rupture area were the main cause of finger drop in 'Hom Thong' bananas. The softening and weakening of the pedicel adjacent to the rupture area were correlated with the decrease of pedicel rupture force and resistance to finger drop of bananas.

MAPG is expressed in the peel of bananas throughout the ripening. *MAPG* mRNA abundance at day 5 was very low in peel at the middle of the fruit and at the pedicel adjacent to the rupture area of 'Namwa' bananas (Figure 29). The abundance of *MAPG* mRNA was correlated with PG activity at the pedicel adjacent to the rupture area of 'Namwa' bananas during ripening (Figure 30). 'Hom Thong' bananas had more expression of *MAPG* mRNA in peel of the middle of the fruit at the early stage and declined at the later ripening stage, and it was not correlated with PG activity in both areas of the peel. The appearance of *MAPG* mRNA and PG activity were not tightly coupled in peel of the middle of the fruit and the pedicel adjacent to the rupture area, suggesting that increase in PG activity may also be regulated by translational or posttranslational mechanisms (Sirit and Bennett, 1998).

MAPG mRNA in the pedicel adjacent to the rupture area of 'Hom Thong' bananas had more abundance than that of 'Namwa' banana, at all stages of ripening (Figure 29A). This suggests that *MAPG* mRNA of 'Hom Thong' bananas (greater finger drop) express more than *MAPG* mRNA of 'Namwa' (no finger drop). The increase of *MAPG* mRNA in the pedicel adjacent to the rupture area is involved in the activity of PG in both of banana cultivars during ripening. *MAPG* mRNA expression corresponds to the activity of PG and the development of finger drop. Hence, PG partially degrades of pectic components in the middle lamella, *MAPG* mRNA may be related to the development of finger drop in 'Hom Thong' bananas during ripening.

Summary and Recommendation

The study on finger drop of ripening bananas can be summarized and recommended as following :

1. Cell wall degradation in the peel and pulp during softening required different mechanisms

2. Finger drop of 'Khai' and 'Hom Thong' bananas ripened under high RH increased more rapidly and greatly than that those ripened under low RH.

3. Polygalacturonase and pectin methylesterase activities were high while β-galactosidase activity was low in peel at the pedicel adjacent to the rupture area of 'Hom Thong' bananas ripened under both low and high RH.

4. Finger drop of 'Hom Thong' bananas was high while finger drop was absent in 'Namwa' bananas.

5. Polygalacturonase activity in the pedicel adjacent to the rupture area of 'Hom Thong' bananas was higher than that of 'Namwa' bananas. In contrast, pectin methylesterase activity in the pedicel adjacent to the rupture area of 'Hom Thong' bananas was lower than that of 'Namwa' bananas.

6. Finger drop was correlated with polygalacturonase activity in peel at the pedicel adjacent to the rupture area, but it was inversely correlated with pectin methylesterase and GAL activities in the pedicel adjacent to the rupture area.

7. Finger drop in 'Hom Thong' bananas did not require the abscission zone at the pedicel adjacent to the rupture area.

8. The abundance of *MAPG* transcript in the pedicel adjacent to the rupture area was higher than that in the middle of the fruit in 'Namwa' and 'Hom Thong' bananas. It is suggested that *MAPG* transcript is related to polygalacturonase activity and it may be involved in finger drop of ripening banana.

9. Study on finger drop should be also extended to other banana cultivars.

10. Cell wall hydrolases other than polygalacturonase, pectin methylesterase and β-galactosidase should be included for further study.

11. Factors affecting finger drop other than relative humidity such as temperature, ethylene, calcium content and physical properties of the pedicel should be studied intensively.

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Output จากโครงการวิจัยที่ได้รับทุนจาก สกว.

1. ผลงานตีพิมพ์ในวารสารวิชาการนานาชาติ

Ketsa, S. 2003. Cell wall degrading enzymes and softening of ripening banana. Thai J. Agric. Sci. 36(3):233-242. (ภาคผนวกหมายเลข 1)

2. การนำผลงานวิจัยไปใช้ประโยชน์

2.1 เชิงพาณิชย์ โดยมีการให้คำแนะนำกับเกษตรกร ผู้ค้าและผู้ส่งออกที่ติดต่อขอทราบคำแนะนำในเรื่องการบ่มผลกล้วยให้สุก โดยมาพบด้วยตนเองหรือทางโทรศัพท์ และบรรยายในหัวข้อการบ่มผลไม้ให้สุกรวมผลกล้วย ในหลักสูตรการฝึกอบรมเรื่องเทคโนโลยีหลังการเก็บเกี่ยว ให้กับบุคคลทั่วไปและบุคลากรระดับปฏิบัติการและผู้บริหารของบริษัทที่มีซูเปอร์มาร์เก็ต

2.2 เชิงสาธารณะ มีการร่วมมือกับ Prof.Dr.J.R. Botella, Department of Botany, University of Queensland Australia นักศึกษา คปก. (น.ส. วชิรญา อิ่มสบาย) ได้ไปฝึกอบรมเทคนิคด้านชีวโมเลกุลที่ห้องปฏิบัติการของอาจารย์นี้ 6 เดือน และมีการร่วมมือในการวิจัยต่อโดยจะส่งนักศึกษา คปก. ไปฝึกอบรมในห้องปฏิบัติการนี้อีกในอนาคต

2.3 เชิงวิชาการ จากข้อมูลที่ได้จากวิจัยของโครงการนี้ นำไปใช้ในการบรรยายในเรื่องสรีรวิทยาของผลกล้วยหลังการเก็บเกี่ยว วิชาไม้ผลเศรษฐกิจ 1 (007541, Industrial Fruit Crop I) เทคโนโลยีหลังการเก็บเกี่ยวของพืชสวน (007482, Postharvest Technology of Horticultural Crops) สรีรวิทยาหลังการเก็บเกี่ยว (007582, Postharvest Physiology of Horticultural Crops) และสรีรวิทยาหลังการเก็บเกี่ยวขั้นสูง (007682, Advanced Postharvest Physiology 0f Horticultural Crops) ซึ่งเปิดสอนสำหรับนิสิตปริญญาตรีและบัณฑิตระดับปริญญาโทและเอก ของภาควิชาพืชสวน คณะเกษตร มหาวิทยาลัยเกษตรศาสตร์

นักวิจัยในโครงการ 2 คน คือ น.ส. อภิวรา ประยูรวงศ์ และ น.ส. วชิรญา อิ่มสบาย โดยคนแรกได้จบการศึกษาระดับปริญญาโท สาขาพืชสวน และขณะนี้ได้เป็นนักวิชาการฝ่ายตรวจสอบคุณภาพของผลิตผลพืชสวนที่จัดซื้อเพื่อส่งเข้าบริษัทที่มีธุรกิจซูปเปอร์มาร์เก็ต ส่วนคนที่ 2 นั้น ขณะนี้เป็นอาจารย์สังกัดภาควิชาพืชสวน คณะเกษตร กำแพงแสน มหาวิทยาลัยเกษตรศาสตร์

3. **อื่น ๆ**

- ผลงานกำลังรอตีพิมพ์ ตีพิมพ์ในวารสารนานาชาติ คือมี 2 เรื่องคือ Changes in cell wall hydrolase activities in relation to finger drop of 'Kluai Khai' during ripening (ภาคผนวกหมายเลข 2) และ Are cell wall hydrolases involved in banana finger drop? (ภาคผนวกหมายเลข 3) นอกจากนี้ยังมีการนำเสนอผลงานวิจัยในที่ประชุมวิชาการนานาชาติ 2 ครั้ง คือ Australasian Postharvest Horticulture Conference, วันที่ 23-27 กันยายน 2544 ณ ประเทศออสเตรเลีย โดยเสนอโปสเตอร์เรื่อง Finger drop of Kluai Khai (*Musa* AA Group) (ภาคผนวกหมายเลข 4) และ The 5th International Postharvest Symposium ระหว่างวันที่ 6-11 มิถุนายน 2547 ณ ประเทศอิตาลี โดยเสนอโปสเตอร์เรื่อง Changes of cell wall hydrolases in relation to finger drop in 'Hom Thong' and 'Nam Wa' bananas (ภาคผนวกหมายเลข 5)

เอกสาร

ภาคผนวกหมายเลข 1

Cell Wall Degrading Enzymes and Softening of Ripening Banana

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Abstract

Three pectin fractions (water-soluble, alkali-soluble, and ammonium oxalate soluble) and activities of polygalacturonase (EC 3.2.1.15), pectinesterase (EC 3.2.1.11), β-galactosidase (EC 3.2.1.23) and cellulase (EC 3.2.1.4) were studied during ripening of banana ('Kluai Khai', *Musa* AA Group), both in the peel and pulp. Soluble pectin increased in the pulp, not in the peel. Pectinesterase activity decreased in the peel and increased in the pulp, whereas polygalacturonase activity increased in both parts. β-Galactosidase activity increased much more in the peel than in the pulp. Cellulase activities in both peel and pulp did not change. The results indicate that cell wall degradation in the peel and pulp is quite different.

Key words : β-galactosidase, cellulase, firmness, Musa AA Group, pectinesterase, peel, polygalacturonase, pulp

Introduction

Softening in many ripening fruits is accompanied by a decrease in the amount of insoluble pectin substances (protopectin) and a concomitant increase in soluble pectin (Huber, 1983). Various enzymes may be involved in cell wall degradation in ripening fruits (Fischer and Bennet, 1991). Polygalacturonase for example, catalyzes the hydrolysis of ∝-1, 4-glycosidic linkages of pectic substances and results in an increase in water-soluble pectin (Seymour and Gross,1996). Pectinesterase is responsible for the deesterification of pectin, a step required before polygalacturonase action (Huber, 1983). β-Galactosidase hydrolyses galactans, but its role in softening is not yet clear (Gross, 1990).

Although the presence of polygalacturonase, pectinesterase and β-galactosidase and cellulase in various fruits have been shown to be correlated with softening, only a few reports refer to banana fruit (Huber, 1983; Fischer and Bennet, 1991). Markovic *et al*. (1975) reported the presence of endo- and exo-polygalacturonase in ripening bananas. Pectinesterase activity has also been identified in banana tissue (Hultin and Levine, 1965; De Swardt and Maxie, 1967; Brady, 1976). Increased polygalacturonase activity has been found, while results on pectinesterase activity are conflicting (Smith *et al.,* 1989). There are apparently no reports on β-galactosidase and cellulase activities in ripening banana. In this study we report the changes in pectic substances and cell wall hydrolase activities both in the peel and pulp of banana fruit, during ripening.

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Materials and Methods

Fruit material

Bananas 'Kluai Khai' (*Musa* AA Group) used in this study were purchased from a banana plantation in Petchaburi Province, western Thailand. Bananas were harvested at mature green stage with the full three-quarter. Bunches were placed in corrugated cardboard boxes and transported by truck to the laboratory within 2 h of harvest. Hands from the middle 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 (29°C and 72% RH). For analysis, peel and pulp with 3 cm wide of individual fingers at the middle from 15 fingers per 3 hands were separated intervally, pooled and frozen at –80°C until use.

Peel colour, and firmness soluble solids content

Every other day, 15 bananas were randomly sampled for determination of peel colour, firmness and soluble solids content. Peel colour was determined using a colorimeter (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). Each analysis had 5 replications for each treatment.

Results

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. Peel colour of bananas for the first 4 days remained more green than yellow and the peel turned more yellow than green and full yellow on day 6 (Figure 1A). 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 1A).

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). Based on statistical analysis by *t*-test, peel firmness was significantly greater than pulp firmness throughout the study period (unpublished data).

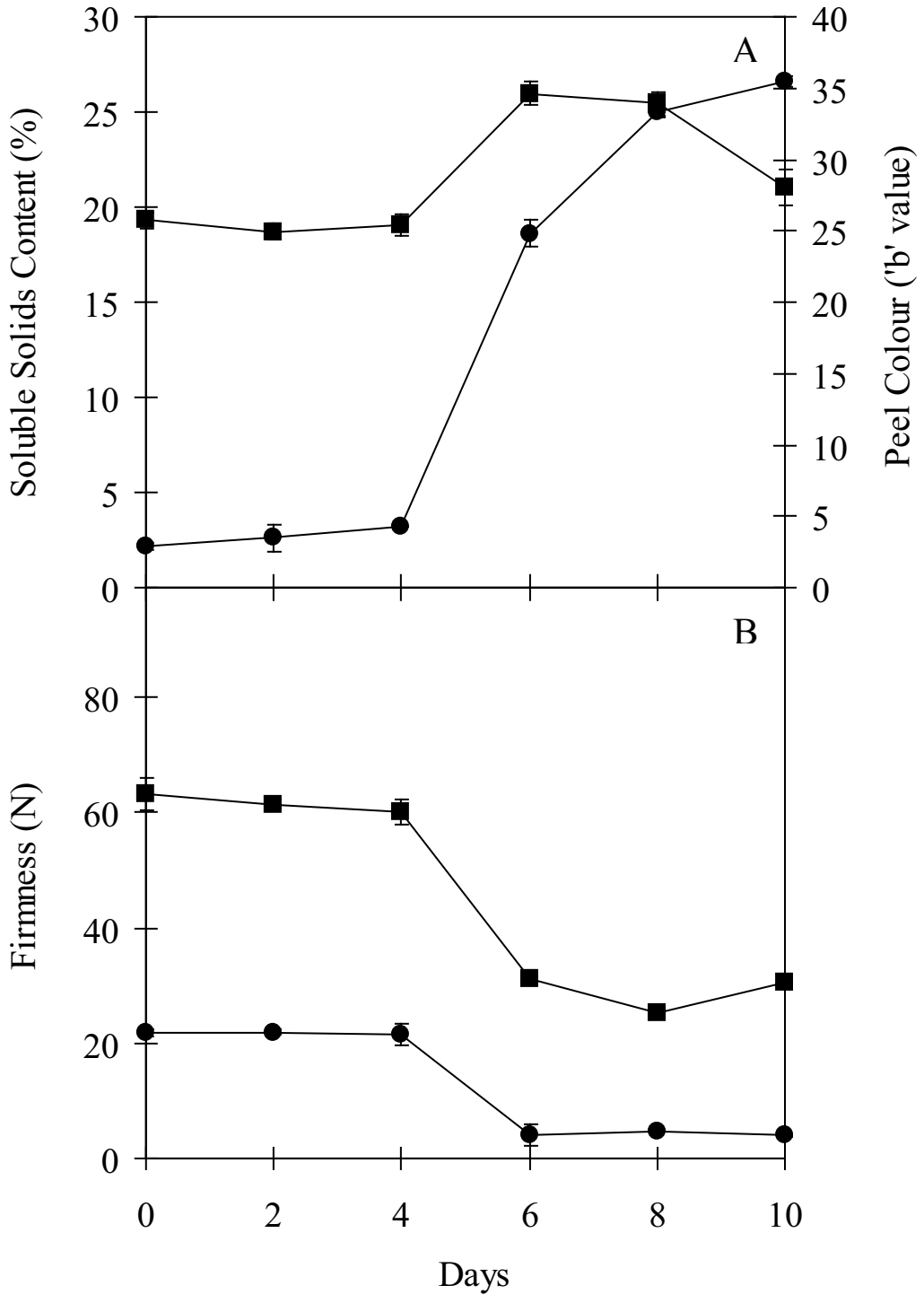

Figure 1 Changes in (A) soluble solids content (●) in the pulp, peel colour (■) and (B) firmness of 'Kluai Khai' banana with (■) and without (●) the peel during ripening. Bars indicate standard errors of means.

Pectic substances

Water-soluble pectin content of the peel decreased slightly from day 0 to day 4, then increased to a maximum on day 6 and remained steady thereafter (Figure 2A). Water-soluble pectin content of the pulp was stable from day 0 to day 6, then increased rapidly to a maximum at day 8. There was more water-soluble pectin in the pulp than in the peel (Figure 2B).The peel ammonium oxalate-soluble pectin content decreased during the 10 days of ripening (Figure 2A), while ammonium oxalate-soluble pectin content of the pulp increased throughout the ripening period (Figure 2B). Alkali-soluble pectin content of the peel was stable during the ripening period (Figure 2A), while alkali-soluble pectin content of the pulp increased progressively (Figure 2B).

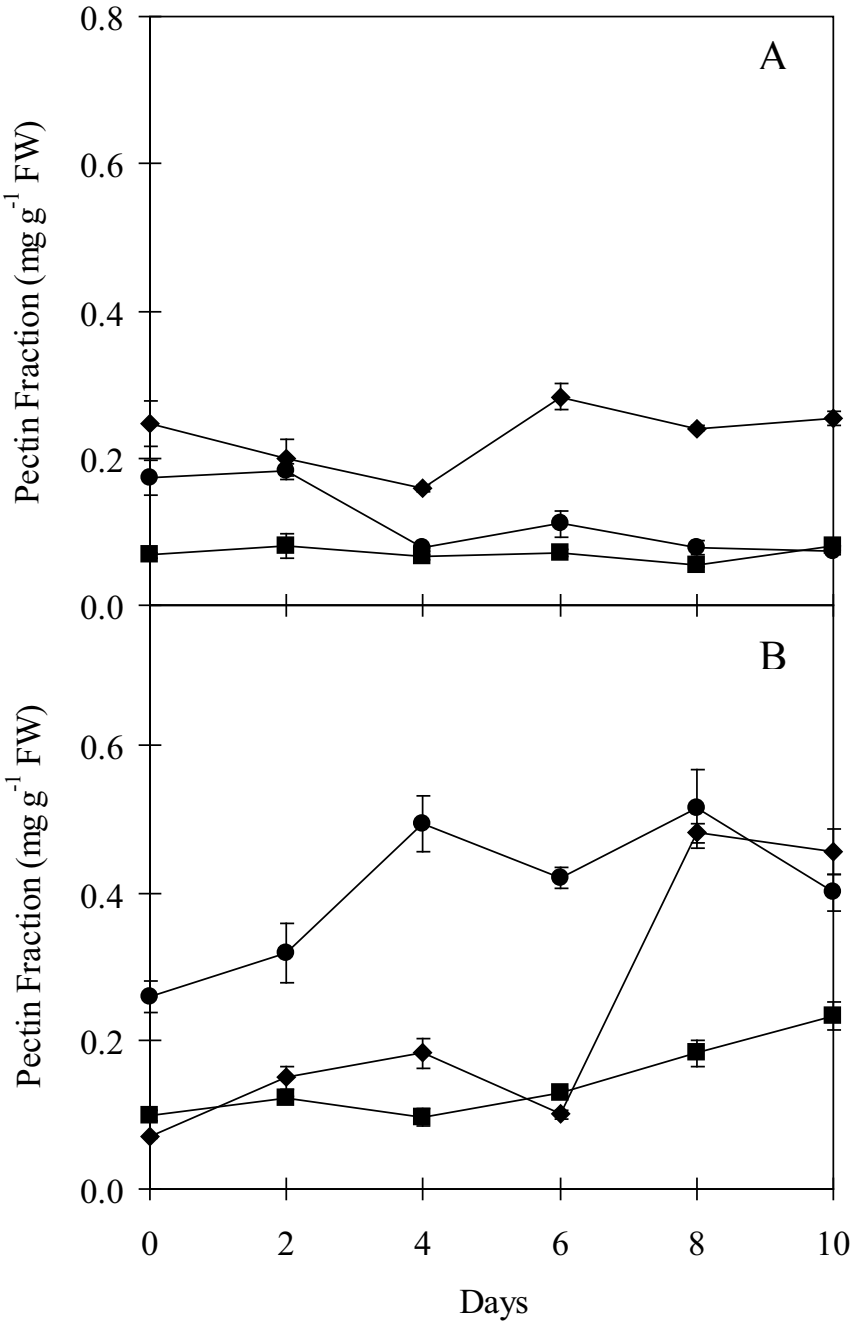

Figure 2 Changes in water-soluble pectin (◆) ammonium oxalate-soluble pectin (●) and alkali-soluble pectin (■) in the peel (A) and pulp (B) of 'Khuai Khai' banana during ripening. Bars indicate standard errors of means.

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 to a lower level than at harvest (Figure 3A). Polygalacturonase activity of the peel of unripe banana increased to a maximum on day 6, then decreased. Polygalacturonase activity of the pulp of unripe banana was lower and increased progressively to a maximum on day 8, and decreased thereafter (Figure 3B).

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 decreased (Figure 4B). 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 4A).

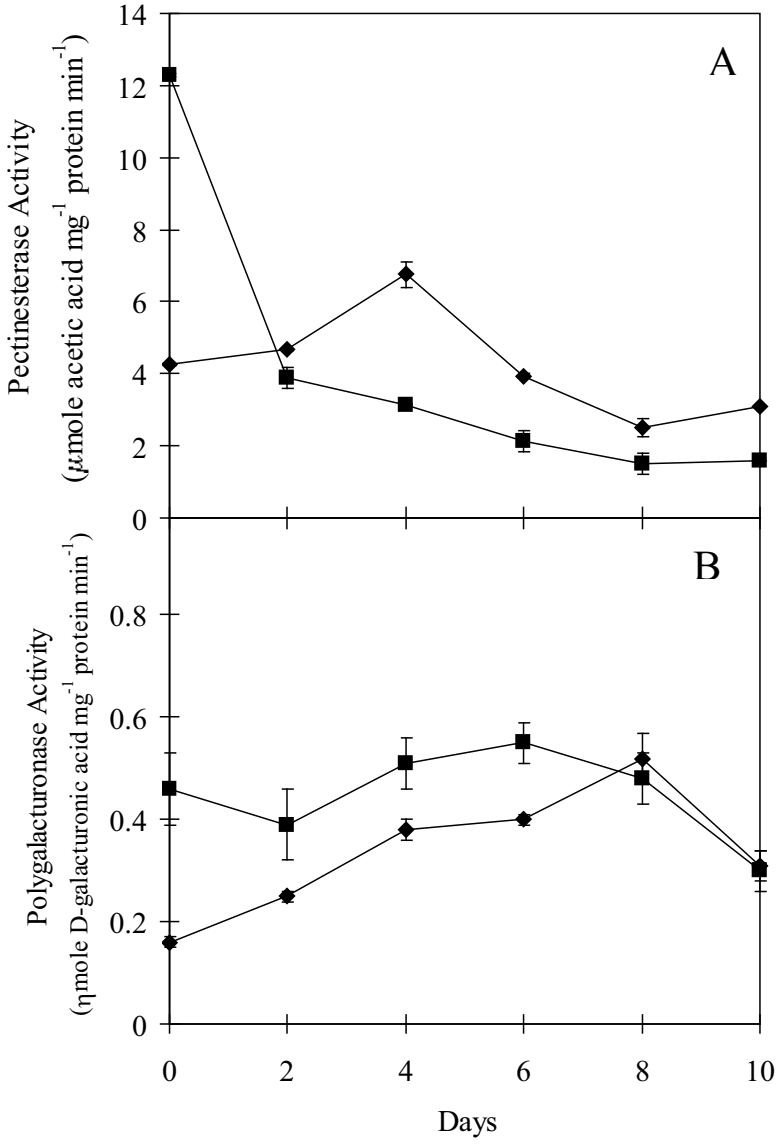

Figure 3 Changes in pectinesterase (A) and polgalacturonase (B) activities in the peel (■) and pulp (◆) of 'Kluai Khai' banana during ripening. Bars indicate standard errors of means.

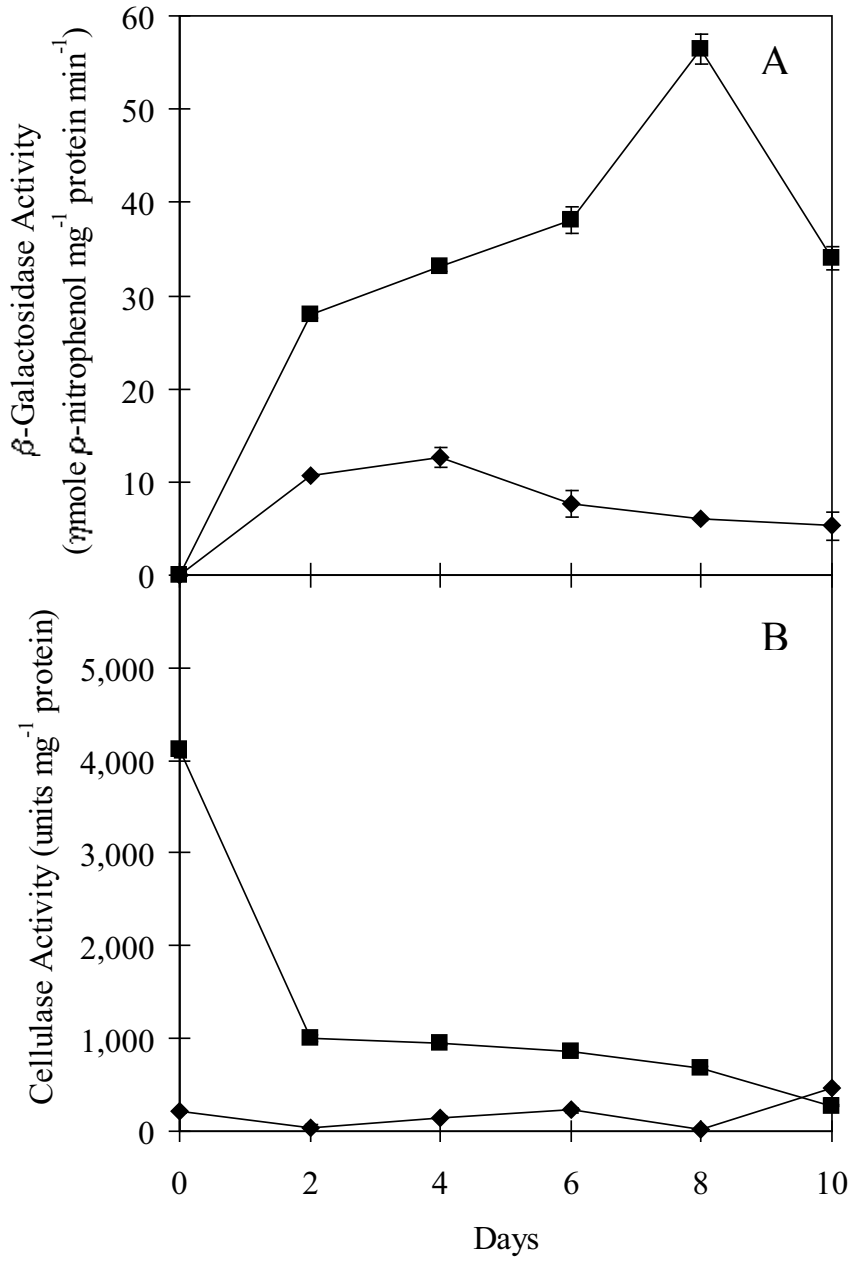

Figure 4 Changes in β-galactosidase (A) and cellulase (B) activities in the peel (■) and pulp (◆) of 'Kluai Khai' banana during ripening. Bars indicate standard errors of means.

Discussion

An increase in soluble solids content of the pulp occurred concomitantly with the loss of firmness (Figure 1B) and climacteric rise in ethylene production and respiration (unpublished data), as reported in other banana cultivars during ripening (Seymour, 1993). Water-soluble pectin, ammonium oxalate-soluble pectin and alkaline-soluble pectin, all increased in the pulp during ripening (Figure 2A), while in the peel water-soluble pectin increased slightly, alkaline-soluble pectin decreased slowly and alkalin-soluble pectin remained unchanged (Figure 2B). This indicates that cell wall solubilization occurs to a greater extent in the pulp than in the peel (Smith *et al*., 1989). However, the changes in the pulp occurred steadily

over the 10 days of ripening, while firmness, soluble solids content and peel colour were stable for the first 4 days, and then showed a sharp change from day 4 to day 6. There was no concomitant sharp changes in cell wall pectin fractions between day 4 and day 6. This suggests that change in cell wall solubilization may not be solely responsible for pulp softening during ripening. Pulp and peel of unripe bananas contain about 20-25% and 3% of the fresh weight, respectively. Starch which imparts cellular rigidity is broken down to sugars during ripening (Seymour, 1993). It can be speculated that softening in ripening bananas may be partially due to the breakdown of starch and other non-pectic polysaccharides in the peel and pulp (Lizada *et al*.,1990). Thus stable firmness of the peel and pulp for the first 4 days may be due to a lower cell wall solubilization and high starch content of unripe bananas.

The decrease in pulp firmness (Figure 1B) was accompanied by an increase in the activities of polygalacturonase (Figure 3A), pectinesterase (Figure 3B) and β-galactosidase (Figure 4A). However, the increase in polygalacturonase activity in the pulp was more prominent than the increase in pectinesterase and β-galactosidase activities. This was similar to the results obtained in durian fruits (Ketsa and Daengkanit, 1999), and in mango (Ketsa *et al*., 1998). The difference in polygalacturonase activity in the peel and pulp was reflected by the increases in water-soluble pectin in the peel and pulp. Polygalacturonase activity in the peel was greater than in the pulp during the early phase of ripening. This resulted in more water-soluble pectin in the peel than in the pulp. Polygacturonase activity in the pulp increased more than that in the peel during the latter phase of ripening (Figure 3B), resulting in higher pulp water-soluble pectin (Figure 2A). This result suggests that polygalacturonase is mainly responsible for solubilization of cell-wall pectin, thus for the softening of bananas (Seymour and Gross, 1996). In tomato, polygacturonase activity was highly correlated with softening in normal fruits, whereas polygacturonase activity was virtually absent from non-softening transgenic plants. However, fruit softening was not affected in these plants, despite greatly reduced polygalacturonase levels (1% of the wild type; Smith *et al*., 1988). In other experiments, over-expression of polygacturonase in a non-softening mutant failed to induce softening, although pectic depolymerization and solubilization did occur (Giovannoni *et al*., 1989). Thus, despite the strong relationship between polygacturonase activity and water-soluble pectin polygacturonase alone may not be sufficient to induce pulp softening of banana.

Pectinesterase activity in the peel decreased sharply, while pectinesterase activity in the pulp increased markedly (Figure 3B). This is consistent with the decrease in ammonium axalate-soluble pectin in the peel (Figure 2A) and with the increase in ammonium oxalate-soluble pectin in the pulp (Figure 2B). Removal of methyl groups from the galacturonic acid chain by pectinesterase (Fischer and Bennett, 1991) would allow more pectin to be extracted by ammonium oxalate (Ketsa *et al*., 1998). Pectinesterase activity in the pulp was found to show high correlation to softening, while softening in the peel appeared to occur independently of this enzyme. This conflicting finding was similar to the report by Smith *et al.* (1989). Pectinesterase activity has also been found to increase during softening of other fruits, such as cherry (Andrew and Li, 1995) and African mango (Aina and Oladunjoye, 1993). In contrast, the pectinesterase activity has been found to decrease during softening of avocado (Awad and Young, 1980) and mango (Abu-Sarra and Abu-Goukh, 1992).

The β-galactosidase activity in the peel increased more rapidly than in the

pulp and β-galactosidase activity in the peel, at its maximum, was 10 fold higher than that in the pulp (Figure 3A). This suggests that β-galactosidase may play a major role in the loss of peel firmness. β-galactosidase activity has also been found to increase during softening of cherry (Barrett and Gonzales, 1994) and mango (Ali *et al*., 1995; Ketsa *et al*., 1998). The difference in polygalacturonase and β-galactosidase activities in the peel and pulp of banana supports the view that cell wall changes in peel and pulp are quite different. Cellulase activity was very high in the peel of unripe banana and very low during softening, while cellulase activity in the pulp was low throughout softening period (Figure 4B). This suggested that cellulase may not be involved in banana softening.

Recently banana pulp ripening-specific transcript encoding a putative pectate lyases such as polygalacturonase which catalyses the cleavage of α(1-4) galacturonan chains has been reported (Dominguez-Puigjaner *et al.,* 1997; Medina-Suarez *et al.,* 1997). Their results suggest that enzymes that cleave pectins other than polygalacturonase may play a role in the loss of pulp firmness in ripening banana. Cell wall solubilization occurred to a greater extent in the pulp than peel during rapid softening, while β-galactosidase activity was more prominent in the peel than pulp and peel softening occurred independently of pectinesterase. These results suggest softening in banana fruit to be a complex process, the mechanisms of which may differ between the peel and pulp (Smith *et al.*, 1989).

In conclusion, the present study indicates that cell wall changes and activities of cell wall hydrolases are quite different in the peel and pulp, during softening of 'Kluai Khai' banana.

Acknowledgement

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เอกสาร
ภาคผนวกหมายเลข 2

Changes in and Cell Wall Hydrolase Activities In relation to Finger Drop of 'Kluai Khai' during Ripening

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ABSTRACT

Bananas 'Kluai Khai' (*Musa* AA Group) kept at 25°C (90% RH) for 24 h had low finger drop, while those kept 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 pectinemethylesterase and polygalacturonase and water-soluble pectin than the peel at the middle fruit of ripened bananas. Bananas with high finger drop had greater activities of pectinemethylesterase and polygalacturonase than those with low finger drop. β-Galactosidase apparenthy had no relationship with finger drop in bananas 'Kluai Khai'.

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INTRODUCTION

Banana fruits are marketed as groups of fingers attached together to form a hand. If individual fingers are dislodged from the hand, they have a lower market value. This effect is called 'finger drop' and this is defined as physiological softening and weakening of the pedicel which causes the individual fruit of a hand to separate very easily from the crown. Finger drop increases during banana ripening and some cultivars are more susceptible to it than other (Baldry et al., 1981). Relative humidity (RH) during the later phase of banana ripening has been implicated in finger drop; low RH reducing finger drop (Semple and Thompson, 1988). Changes in the pectic components of the primary cell walls and middle lamella are probably the main cause of the changes in texture of banana peel during ripening (Seymour, 1993). Enzymes implicated in degradation of pectic polysaccharides have been found in banana (Smith et al., 1989). This study was undertaken to determine the relative contributions of pectics substances and cell wall hydrolase enzymes to the development of finger drop in 'Kluai Khai' (*Musa* AA Group) during ripening.

MATERIALS AND METHODS

Plant material

Bananas 'Kluai Khai' (*Musa* AA Group) used in thes 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 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. Peel tissue at the middle fruit and at the pedicel of bananas was pooled and frozen at -80°C until use.

Finger drop test

At intervals, finger drop of bananas were determined by gently lifting the hands to 20 cm above the floor. The number of fingers with fuly broken pedicels was recorded.

Pectic substances and cell wall hydrolases

Water –soluble pectin was analysed using the procedure described by Robertson (1979). Extract and assay of pectinemethylesterase (PME), polygalacturonase (PG) and β-galactosidase (GAL) were described in Hagerman and Austin (1986), Yoshida et al. (1984) and Ross et al. (1993), respectively. Protein content was determined according to Bradford (1976).

RESULTS

Bananas 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 1).

PME 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 PME activities at a maximum of peel at the middle fruit was greater than that of peel at the pedicel. PME 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 2).

Similary PG activities in peel at the middle fruit and at pedicel of bananas increased to a maximum at day 6 and decreased thereafter and PG activities in peel at the pedicel were greater than in peel at the middle fruit. PG 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 PG activities at maximum in peel at the pedicel of bananas ripened at 25°C/90% RH for 24 h (Figure 3).

GAL activities in peel at the middle fruit and at the pedicel of bananas increased throughout the study period. But GAL 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 4).

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 5).

DISCUSSION

Bananas ripened at 25°C/90% RH for 24 h showed low finger drop than those ripened at 25°C/90% RH for 72 h. The longer they exposed to 25°C/90% RH, the more they developed finger drop. This indicated that bananas ripened under high RH too long were prone to develop finger drop more seriously than those ripened with a shorter exposure to high RH (Semple and Thompson, 1988).

Softening of ripening fruits is associated with alternation in cell wall and middle lamella structure (Seymour and Gross, 1996). These changes include solubilization of cell wall pectin involving the action of cell wall hydrolytic enzymes PG, PME and GAL. Bananas with high finger drop had greater activities of PG (Figure 2) and PME (Fingure 3) in peel at the pedicel during ripening, resulting in more water-soluble pectin in peel at the pedicel than that in peel at the middle fruit (Figure 4) This may lead to localize a weakening peel at the pedicel, which in turn produces finger drop. It is not know how bananas exposed to long period of high RH induced greater activities of PG and PME in peel at the pedicel than those exposed to short period of high RH during ripening.

GAL activities in peel at the middle fruit and at the pedicel of bananas with low finger drop and exposed to a short period of high RH were greater than those with high finger drop and exposed to a long period of high RH (Figure 5). This suggested that GAL may not have relationship with finger drop.

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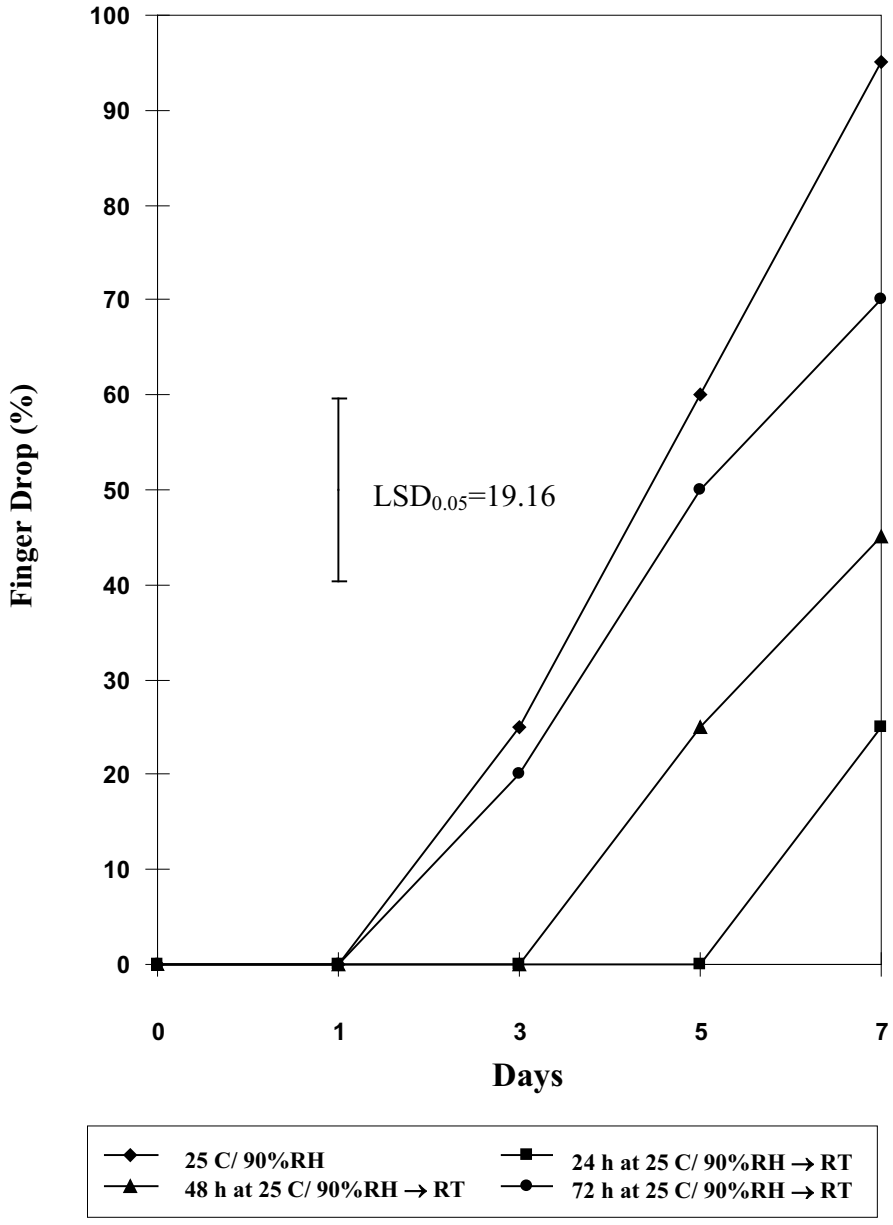

Figure 1 Finger drop of 'Khai' bananas ripened at 25 °C (90%RH) for 24, 48 or 72 h and transferred to room temperature (RT).

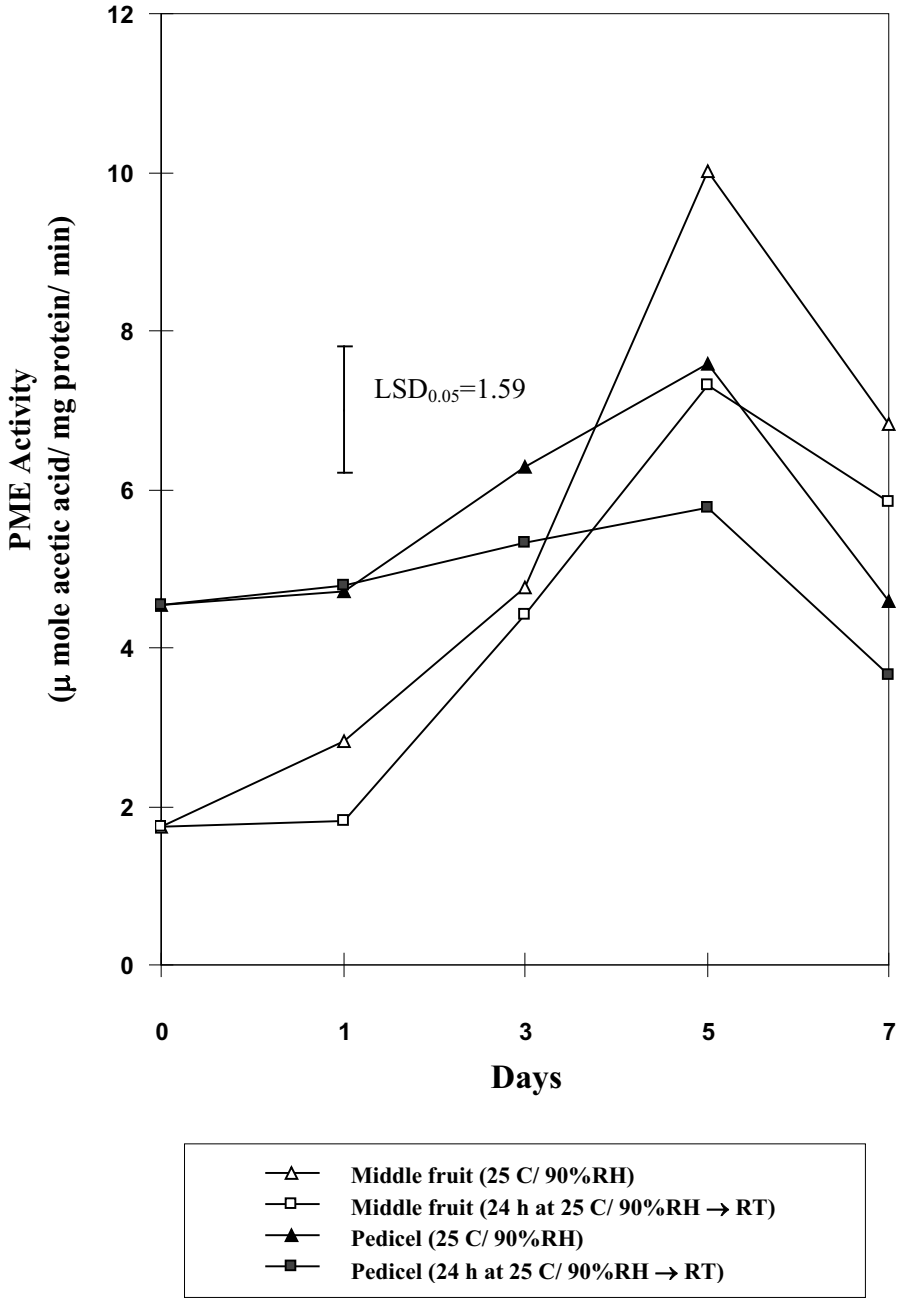

Figure 2 Changes in activity of pectinemethylesterase in peel of 'Khai' bananas at the middle fruit (△ , □) and at the pedicel (▲ , ■).

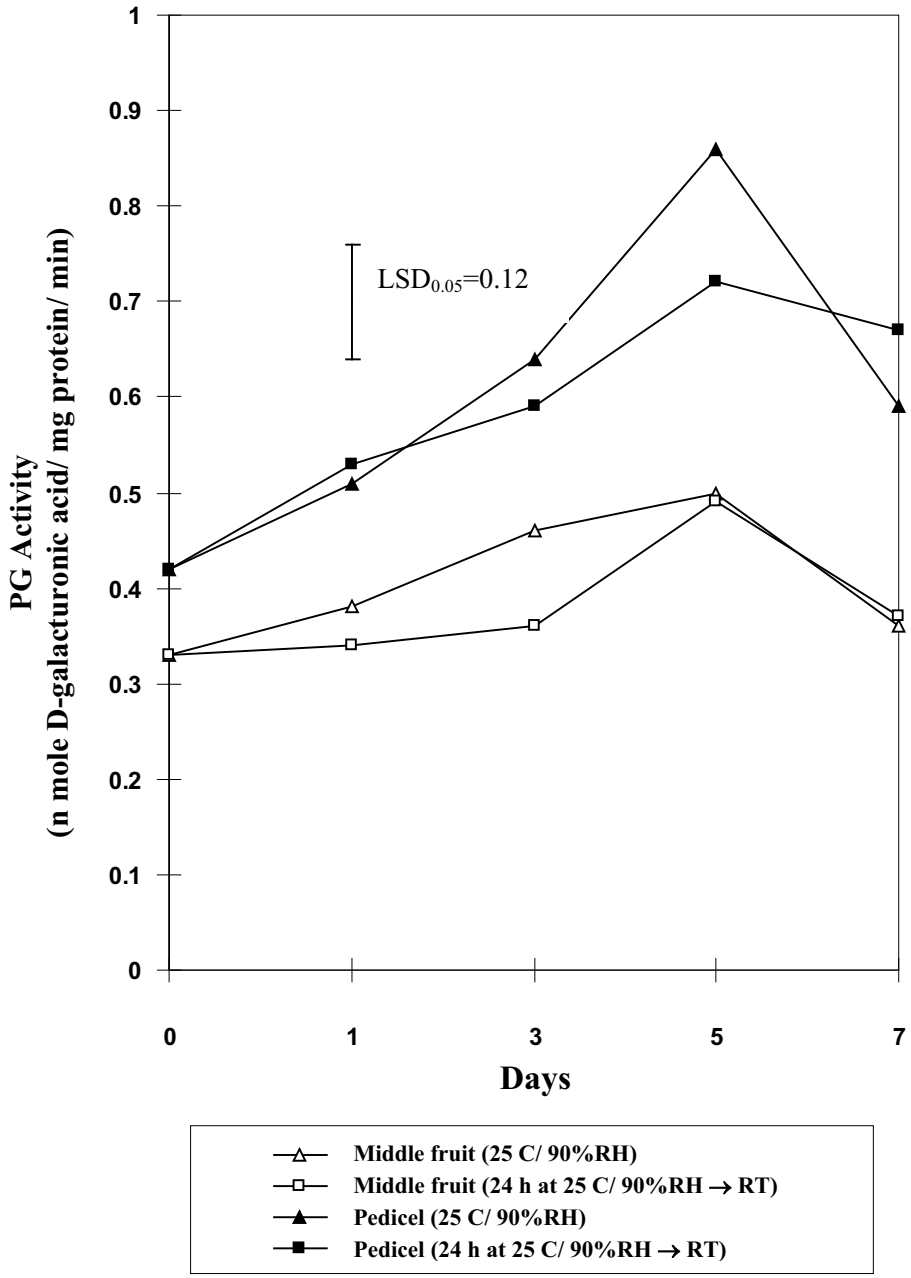

Figure 3 Changes in activity of polygalacturonase in peel of 'Khai' bananas at the middle fruit (△ ,□) and at the pedicel (▲ ,■).

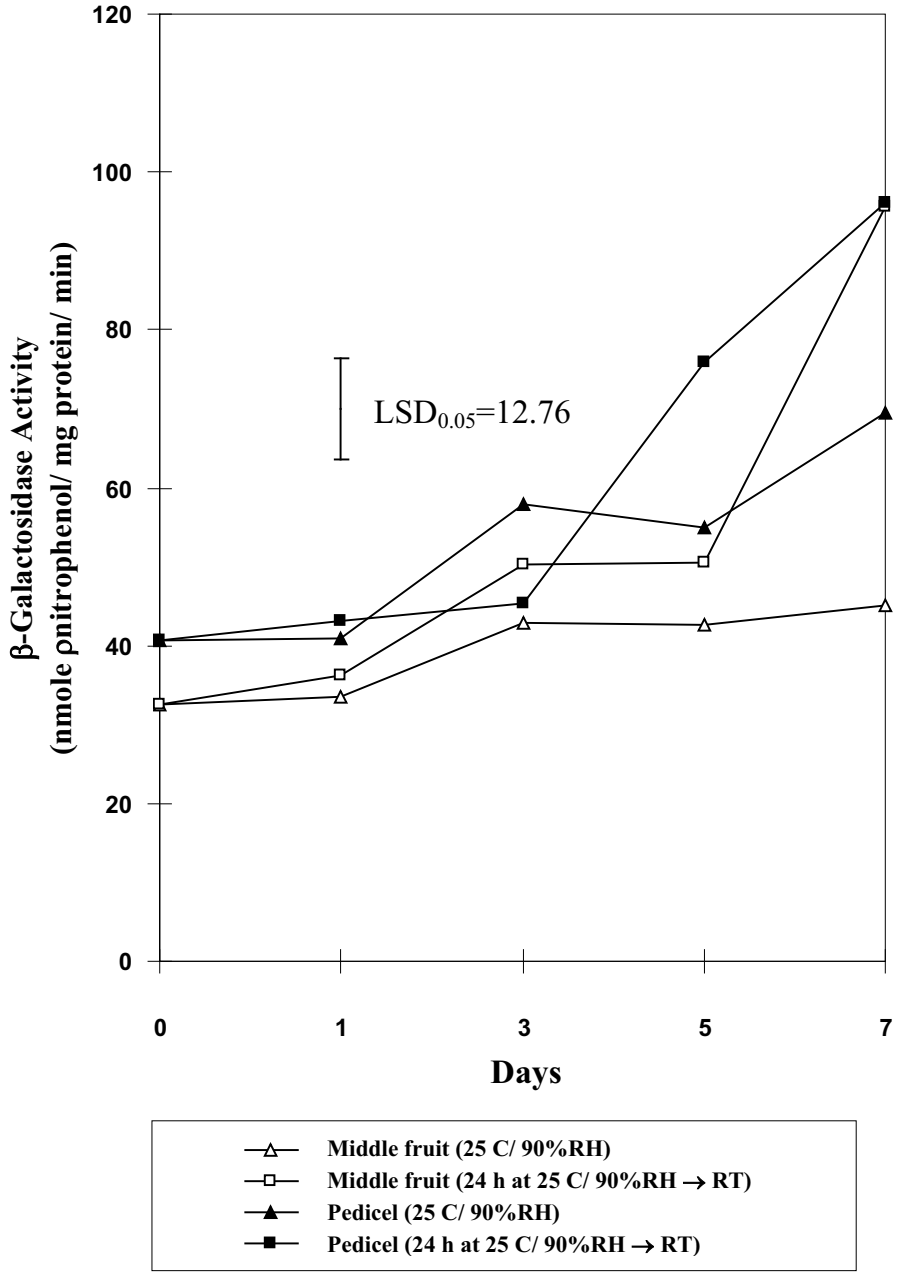

Figure 4 Changes in activity of β–galactosidase in peel of 'Khai' bananas at the middle fruit (△ ,□) and at the pedicel (▲ ,■).

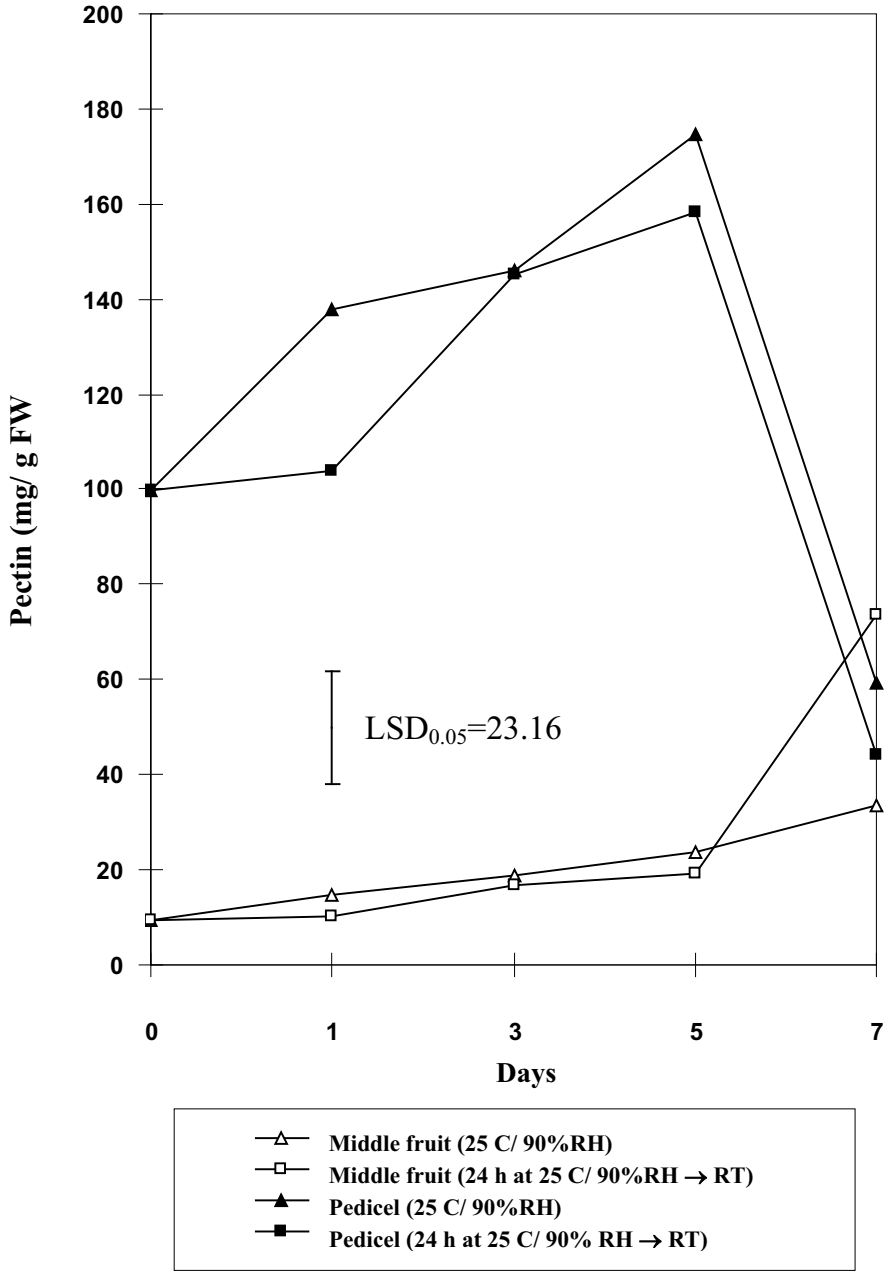

Figure 5 Changes water-soluble pectin in peel of 'Khai' bananas at the middle fruit (▲, ■) and at the pedicel (△, □).

เอกสาร

ภาคผนวก 3

Are cell wall hydrolases involved in banana finger drop?

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Abstract

Fruit drop from hands of 'Hom Thong' bananas (*Musa cavendishii*) is not due to abscission but to breaking at the junction of the pedicel and pulp, which seems due to weakening of the peel at this point. We investigated the possible role of pectinesterase (PE) and polygalacturonase (PG) in the peel. Hands of freshly harvested 'Hom Thong' (AAA Group) and 'Namwa' (ABB Group) bananas were briefly treated with ethephon and allowed to ripen at 25°C (~85% RH). All 'Hom Thong' bananas dropped from the hands within four days after peel yellowing, whereas 'Namwa' fruit did not drop. PE activity in the peel at the rupture area of 'Hom Thong' was much lower than that of 'Namwa' bananas. PG activity in the peel at the rupture area of 'Hom Thong' bananas rapidly increased, but not clearly more than in 'Namwa' bananas. In 'Hom Thong' bananas the PG activity in the peel at the rupture area was consistently higher than in the peel at the middle of the fruit, and no

such difference was found in ‘Namwa’ bananas. Northern blotting showed that the expression of a *PG* transcript in the peel at the rupture area in ‘Namwa’ bananas was lower than in ‘Hom Thong’ bananas. We conclude that there is as yet inconclusive evidence for a role of PG, and that PE seems not involved.

Keyword: Banana; Finger drop; Polygalacturonase; Pectinesterase; *PG* transcript

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1. Introduction

Drop of individual fruit (fingers) is a disorder during banana ripening. It was defined by Baldry *et al.* (1981) as the physiological softening and weakening which causes the individual fruit in a hand to separate from the crown. Finger drop limits the marketability of many banana varieties. Hands with fingers missing cannot be sold to consumers and individual fruit that have dropped have no pedicel and can therefore also not be sold. Finger drop has been reported in several groups: in a diploid cultivar (Prayurawong, 1999), in the triploid Cavendish AAA Group (Semple and Thompsom, 1988), and in tetraploid cultivars (Marriott, 1980). Susceptibility varies widely. For example, among the triploids ‘Valery’ is considerably more prone to finger drop than ‘Gros Michel’ (New and Marriott, 1983).

Finger drop is stimulated by high relative humidity (RH; Semple and Thompson, 1988), ethylene (Paull, 1996), and by higher ripening temperature (Semple and Thompson, 1988). In addition, more mature hands were more susceptible (Paull, 1996).

Finger drop seems to be due to localized weakening of peel at the pedicel (New and Marriott, 1983; Semple and Thompson, 1988). Banana peel softening has been suggested to be due to depolymerisation of pectic substances in the primary cell wall and the middle lamella (Seymour, 1993). These changes involve action of cell wall hydrolases such as polygalacturonase (PG), pectinesterase (PE), β-galactosidase and cellulase (Huber, 1983). It is not known if any of these hydrolytic enzymes is

associated with banana finger drop. We therefore attempted to determine the possible role of PG and PE.

2. Materials and methods

2.1. Plant material

Fruit of 'Hom Thong' ((*Musa cavendishii*; AAA Group) and 'Namwa' (ABB Group) bananas were harvested and kept at commercial maturity in the morning. De-handed bananas were placed in corrugated cardboard boxes and transported by refrigerated truck (25°C) to the laboratory within 3 h of harvest. In the laboratory, hands, after selection for the uniformity of size and colour, were cleaned in a solution of 100 μl l^{-1} chlorine (Clorox). 'Hom Thong' and 'Namwa' bananas were then dipped for 2-3 min in 500 mg l^{-1} ethephon for uniform ripening. They dried at ambient temperature (29-30°C). Ripening occurred at 25°C and 85-90% RH. The hands were monitored daily for finger drop, pedicel rupture force, resistance to finger drop and enzyme activity. The peel at the middle of the fruit and at the pedicel as to the rupture area was sampled. Peel from five hands of each treatment was pooled and frozen at -80°C until further use.

2.2. Finger drop

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

2.3. Pedicel rupture force

Pedicel rupture force was measure by pressing down a wedge probe at the pedicel until it separated from the fruit. The required force was expressed in Newtons (N). Twenty fruit were measured in each treatment.

2.4. Resistance to finger drop

The method was modified from Cerqueira *et al.* (2003). Banana fruit was inserted in a hole and held by a big clip, connected to a spring weight. As the pedicel of banana was pulled, the piston of the spring weight and a marker moved together. The marker on the spring weight stopped when the pedicel broke. The force at the moment of rupture was indicated on the marker. The resistance to finger drop was expressed in kg.

2.5. Enzyme assays

Extraction and assay methods for PG and PE were as described in Yoshida *et al.* (1984) and in Hagerman and Austin (1986), respectively. Protein content in the enzyme extracts was estimated using the Bradford (1976) method. Specific activity of the enzyme was expressed as units per mg protein.

2.6. Total RNA

The protocol was modified from Chang *et al.* (1993). 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, vortexed for 10 min, and centrifuged at 4,500 rpm for 30 min (4°C). The supernatant was collected

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, then extracted once more using chloroform:isoamyl alcohol. The aqueous phase was collected and two volumes of ethanol were added, then allowed to precipitate at -20°C for 4 h. The mixture was centrifuged at 12,000 rpm at 4°C. Ethanol was discarded and the remainder was vacuumed dry for 10 min; the pellet was then re-suspended in cool DEPC-water.

2.7. RT-PCR

cDNA was synthesized from 1 mg of total RNA using the 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.. 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 sec, 56°C for 30 sec and 72°C for 30 sec.

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*.

2.8. RNA blotting and hybridization

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 h 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).

2.9. Statistical analysis

All experiments were repeated twice. Using a SAS package, the data were treated by analysis of variance, calculating the least significant difference (LSD) between means determined at the 5% level.

3. Results

3.1. Finger drop, rupture force, resistance, and anatomy

Finger drop of 'Hom Thong' bananas started on day 2 after peel yellowing and increased to 100% within 4 days. 'Namwa' bananas did not show finger drop during this ripening period (Fig. 1A).

'Namwa' bananas had a higher pedicel rupture force than 'Hom Thong' bananas, during the five days of the experiment. The pedicel rupture force of both banana cultivars decreased in parallel fashion (Fig. 1B).

On the first day of the experiment, the resistance to finger drop of 'Namwa' and 'Hom Thong' bananas was both 5 kg (Fig. 1C). The resistance in 'Hom Thong' declined more than in 'Namwa' bananas. It was statistically different at 5% level from day 2, and this difference increased during the subsequent three days (Fig. 1C).

A detailed histochemical study of the peel and the interior of the pedicel at the rupture area did not reveal an abscission zone (data not shown).

3.2. PG and PE activity

Both in 'Hom Thong' and 'Namwa' bananas, PG activity in the peel increased during ripening. This increase was observed both in peel taken from the middle of the fruit and in peel from the rupture area. In one experiment no statistical difference was found in PG activity at the rupture area, between 'Hom Thong' and 'Namwa' bananas, except on day 3 and day 5 (Fig. 2A). In a repeat experiment, in contrast, the difference was statistically significant from day 2, the day of first finger drop (Fig. 2B).

Initially, the PE activity in the peel of 'Namwa' was much higher than in 'Hom Thong' bananas, both in peel taken from the middle of the fruit and peel from the rupture area. In 'Namwa' the PE activity decreased and had reached similar levels as those in 'Hom Thong' by day 5 of the experiment (Fig. 3).

3.3. Expression of a PG transcript

On day 1 and 2 the expression of a *PG* transcript, called *Musa acuminata PG (MaPG),* isolated from the peel at the rupture area, was lower in 'Namwa' than in 'Hom Thong' bananas (Fig. 4A). The level of *MaPG* in the peel at the middle of 'Namwa' bananas was also lower (Fig. 4B). The abundance of *MaPG* transcript in the peel at the rupture area was higher than that in the middle, in both cultivars (Fig. 4C).

4. Discussion

The results show a large difference in finger drop between the two triploid cultivars studied. The data on peel firmness indicate that the cultivar which is susceptible to finger drop has a weaker peel at the area where the break occurs. This difference may be due to cell wall hydrolase activity. We found no conclusive evidence for a role of PG in the process, as the data seem conflicting. On the one

hand, the PG activity in the peel of ‘Hom Thong’ bananas - at the place where the rupture occurred - was consistently higher than in the middle of the peel, which did not rupture (no difference was observed on day 1, when rupture was not yet found). This may suggest a role of PG in finger drop. The molecular data may also be interpreted as positive evidence for a role of PG. There were higher levels of a PG transcript in the peel of ‘Hom Thong’ bananas, close to the rupture area, as compared to the expression in the same area in ‘Namwa’ bananas, where no rupture occurred. But, on the other hand, the evidence became inconclusive because the PG activity, as determined in our assay, was not consistently higher. In the peel at the rupture area of ‘Hom Thong’, compared with that in ‘Namwa’ bananas (Fig. 2A and 2B).

Although the data on PG seem inconclusive, those on PE are rather straightforward. They indicate that PE is not involved in cell wall weakening that leads to banana fruit drop.

The resistance to finger drop was much higher in ‘Namwa’ than in ‘Hom Thong’ bananas. The results indicate that this parameter is highly correlated with finger drop. Pedicel rupture force also tended to be higher in ‘Namwa’ than in ‘Hom Thong’ bananas, but the difference was just statistically significant on day 2 and not significant on day 3. Resistance to finger drop therefore seems a better indicator than pedicel rupture force.

We did not observe an abscission zone at the area of rupture in ‘Hom Thong’ bananas. This indicates that the rupture occurs due to breakage, which in turn is due to weakening of the peel. As the breakage often occurs at the junction of the pedicel and the fruit flesh, the internal forces that hold the fruit together may be weak at this point. The junction represents the sudden transition between a rather lignified tissue (the pedicel) and a rather unlignified one (the fruit pulp). At this point the fruit seems to be held together mainly by the peel. If the peel is too weak to hold the weight of the fruit it will break. Softening of the peel may be an important feature that determines finger drop. Apart from the cell wall hydrolases that were presently studied, other such hydrolases may be involved, or the softening may be a purely physical phenomenon. Along with others (Semple and Thompson, 1988), we found that finger drop was consistently promoted by high RH. This factor may be sufficient to weaken the peel.

It is concluded that fruit drop in banana is not due to true abscission but to breaking of the tissue, and that peel strength seems the limiting factor in this breakage. The evidence for a role of PG in the peel at the area where the break occurs is as yet unconvincing, whereas the evidence seems to indicate that PE is not involved.

Acknowledgements

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Legends to Figures

Fig. 1. Finger drop (A), pedicel rupture force (B) and resistance to finger drop (C) of 'Hom Thong' (○) and 'Namwa' (●) bananas held at high RH. Time is days after the peel had became mostly yellow.

Fig. 2. PG activity in peel at the middle (△ ,▲) and at the pedicel (○ , ●) of 'Hom Thong' (○ , △) and 'Namwa' (● , ▲) bananas held at high RH. Time is days after the peel had became mostly yellow. A: data of the same experiment as the data shown in Figs 1, 3 as A. B: data from a repeat experiment was with similar finger drop as in Fig. 1.

Fig. 3. PE activity in peel at the middle (△ ,▲) and at the pedicel (○ , ●) of 'Hom Thong' (○ , △) and 'Namwa' (● , ▲) bananas held at high RH. Time is days after the peel had became mostly yellow.

Fig. 4. 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 the peel had become. In C a direct comparision is given between the pedicel at the rupture area and at the middle of the fruit, on day 3. Equal loading of RNA was confirmed by staining gels with ethidium bromide (EtBr).

Figure 1

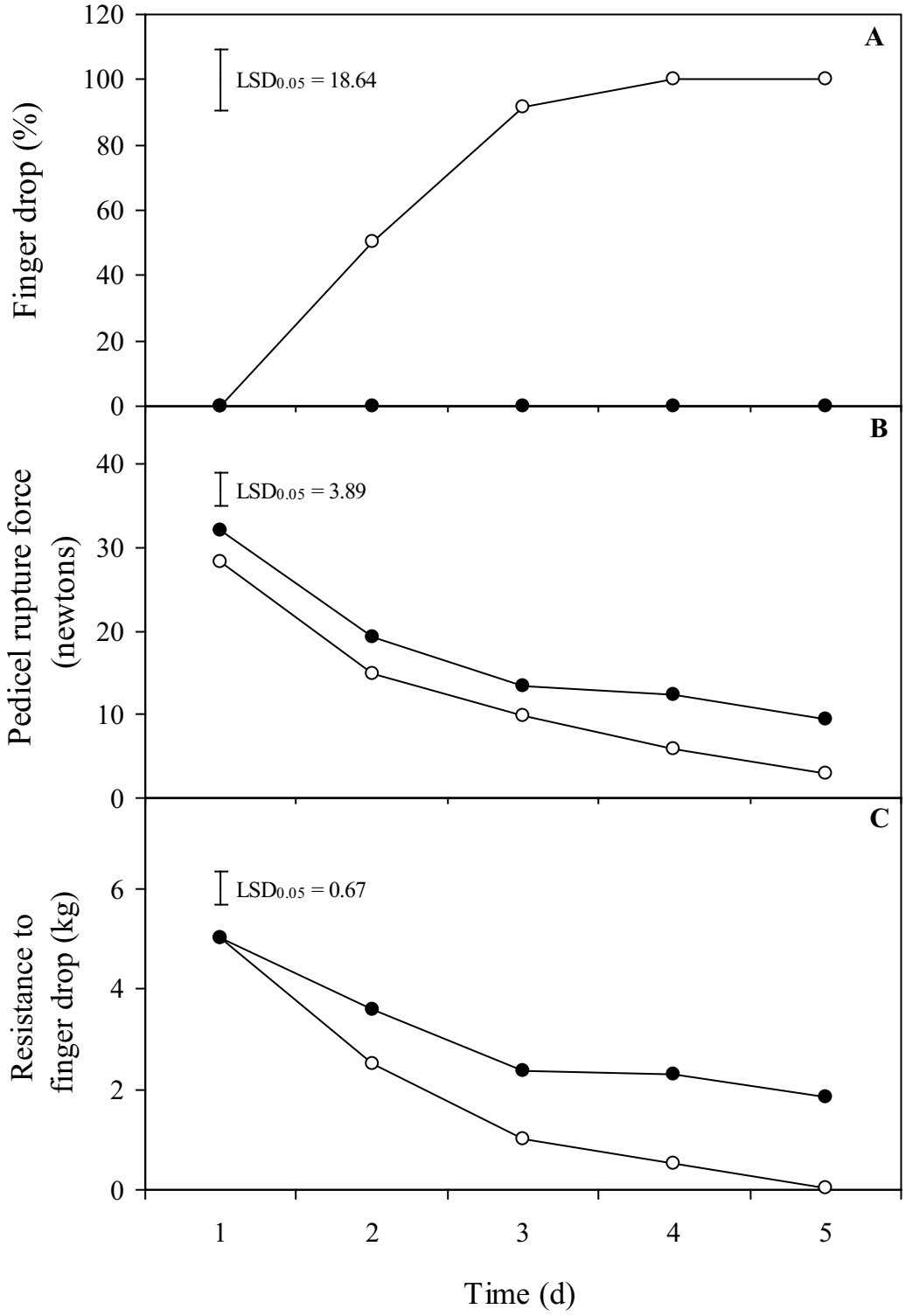

A
B
C
Finger drop (%)
Pedicel rupture force (newtons)
Resistance to finger drop (kg)
LSD0.05 = 18.64
LSD0.05 = 3.89
LSD0.05 = 0.67
120
100
80
60
40
20
0
40
30
20
10
0
6
4
2
0
1
2
3
4
5
Time (d)

Figure 2

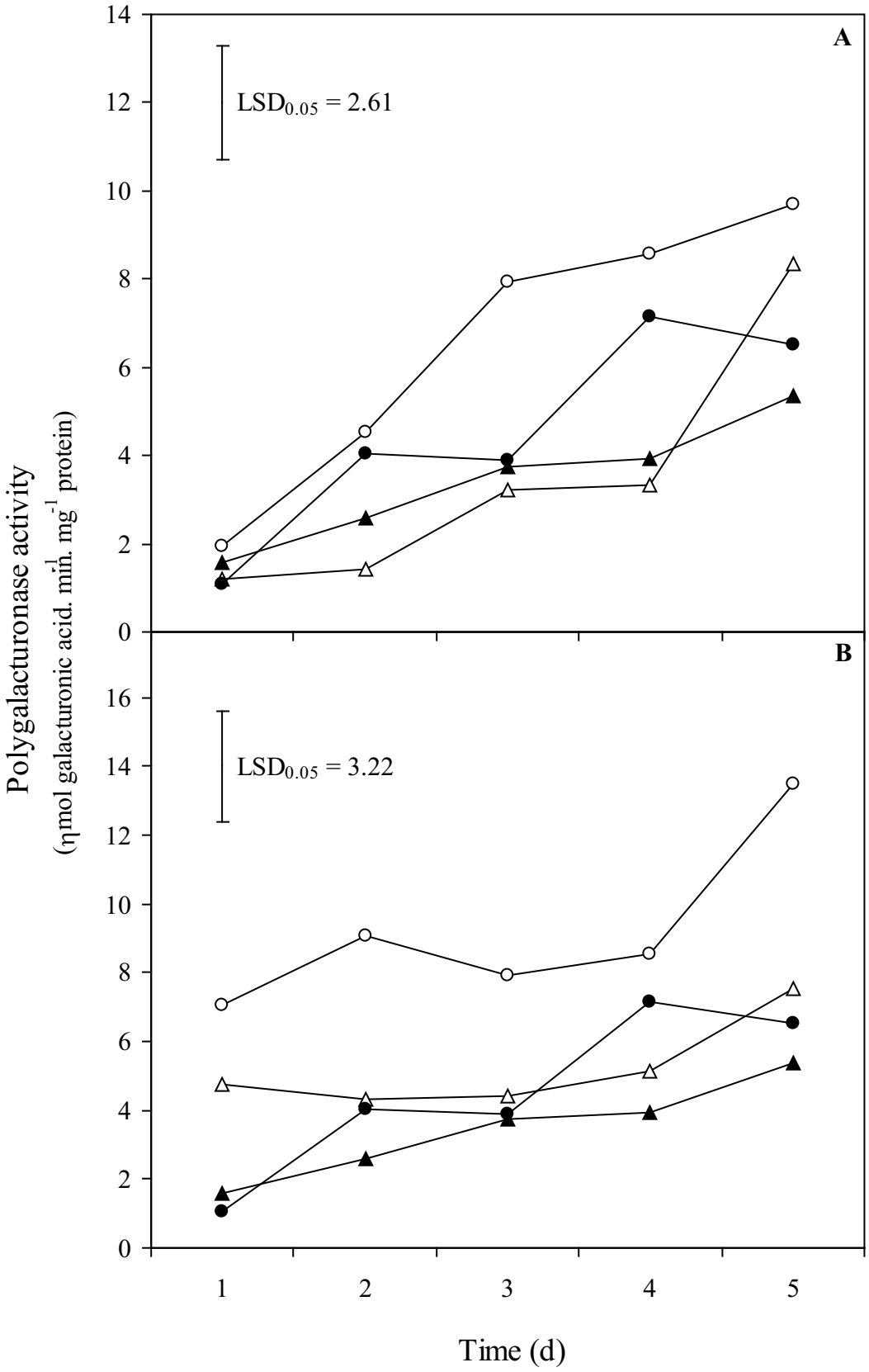

A
B
LSD0.05 = 2.61
LSD0.05 = 3.22
Polygalacturonase activity
(ηmol galacturonic acid. min. mg-1 protein)
Time (d)
0
2
4
6
8
10
12
14
16
1
2
3
4
5

Figure 3

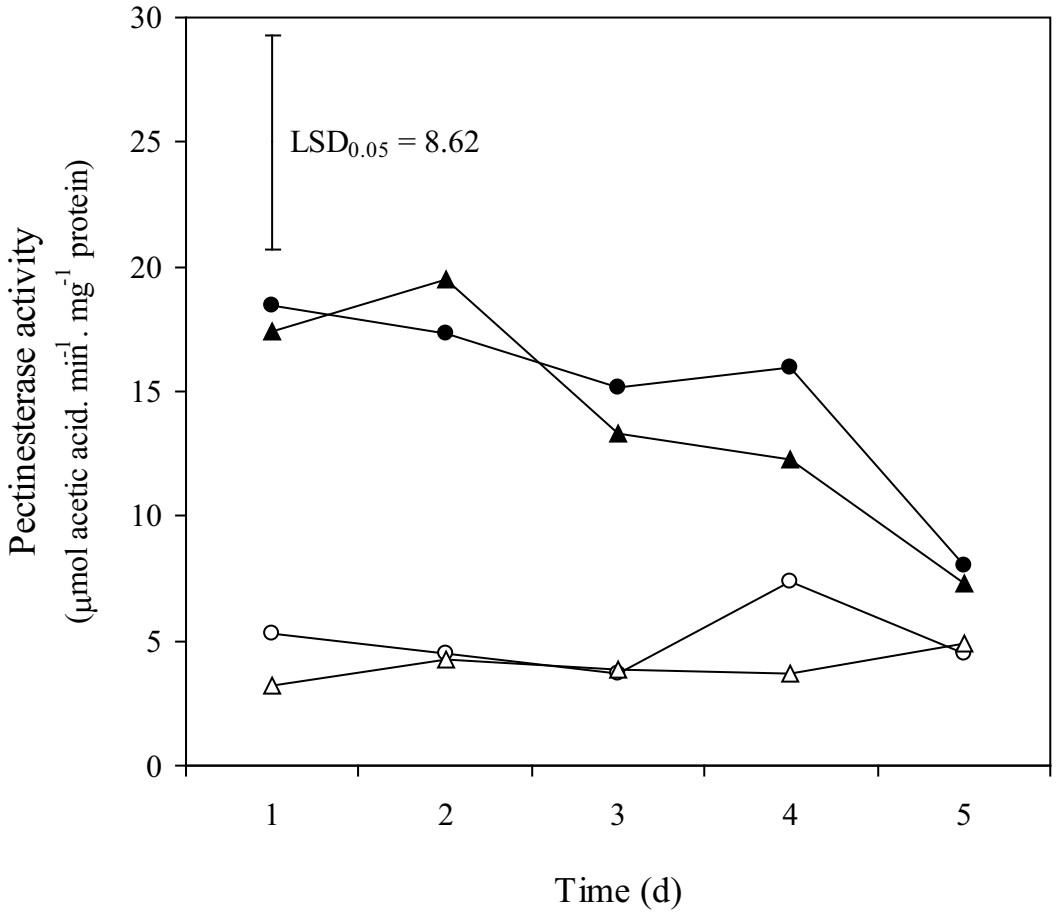
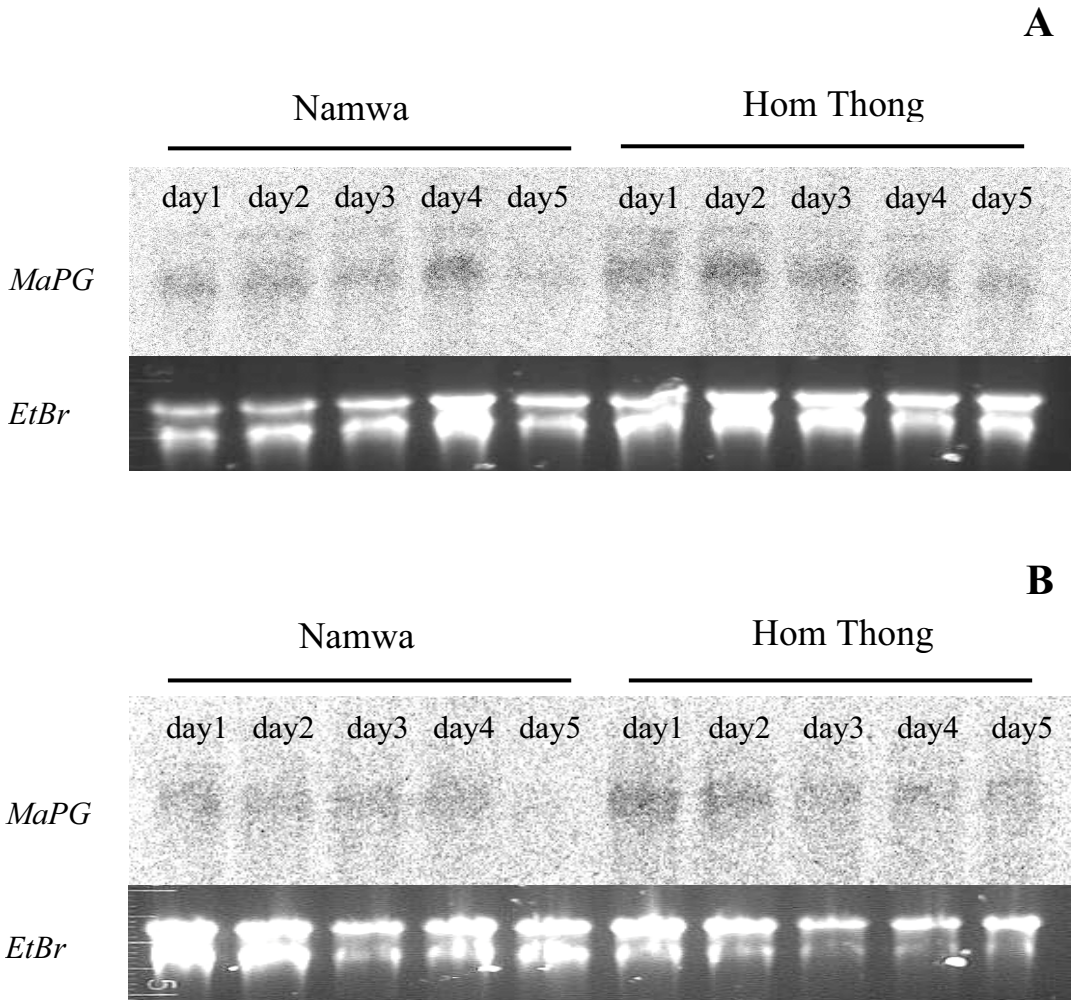

30
25
20
15
10
5
0
LSD0.05 = 8.62
Pectinesterase activity
(µmol acetic acid. min-1. mg-1 protein)
1
2
3
4
5
Time (d)

Figure 4


A
Namwa
Hom Thong
day1 day2 day3 day4 day5 day1 day2 day3 day4 day5
MaPG
EtBr
B
Namwa
Hom Thong
day1 day2 day3 day4 day5 day1 day2 day3 day4 day5
MaPG
EtBr
C
Namwa
Hom Thong
Middle Pedicel Middle Pedicel
MaPG
EtBr

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ภาคผนวกหมายเลข 4

CHANGES IN PECTIC SUBSTANCES AND CELL WALL HYDROLASE ACTIVITIES DURING THE DEVELOPMENT OF FINGER DROP IN 'KLUAI KHAI'

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ABSTRACT

Bananas 'Kluai Khai' (*Musa* AA Group) kept at 25°C (90% RH) for 24 h had low finger drop, while those kept 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 pectinemethylesterase and polygalacturonase and water-soluble pectin than peel at the middle fruit of ripened bananas. Bananas with high finger drop had greater activities of pectinemethylesterase and polygalacturonase than those with low finger drop. β-Galactosidase apparently had no relationship with finger drop in bananas 'Kluai Khai'.

INTRODUCTION

Banana fruits are marketed as groups of fingers attached together to form a hand. If individual fingers are dislodged from the hand, they have a lower market value. This effect is called 'finger drop' and this is defined as physiological softening and weakening of the pedicel which causes the individual fruit of a hand to separate very easily from the crown. Finger drop increases during banana ripening and some cultivars are more susceptible to it than other (Baldry et al., 1981). Relative humidity (RH) during the later phase of banana ripening has been implicated in finger drop; low RH reducing finger drop (Semple and Thompson, 1988). Changes in the pectic components of the primary cell walls and middle lamella are probably the main cause of the changes in texture of banana peel during ripening (Seymour, 1993). Enzymes implicated in degradation of pectic polysaccharides have been found in banana (Smith et al., 1989). This study was undertaken to determine the relative contributions of pectics substances and cell wall hydrolase enzymes to the development of finger drop in 'Kluai Khai' (*Musa* AA Group) during ripening.

RESULTS

Bananas 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 1).

PME 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 PME activities at a maximum of peel at the middle fruit was greater than that of peel at the pedicel. PME 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 2).

Similarly PG activities in peel at the middle fruit and at pedicel of bananas increased to a maximum at day 6 and decreased thereafter and PG activities in peel at the pedicel were greater than in peel at the middle fruit. PG activities in peel at the middle fruit of bananas ripened at 25°C / 90%RH for 24 and 72 h were not significantly different while PG activities at the maximum in peel at the pedicel of bananas ripened at 25°C / 90% RH for 24 and 72 h were significantly different (Figure 3).

GAL activities in peel at the middle fruit and at the pedicel of bananas increased throughout the study period. But GAL 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 4).

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 significantly different (Figure 5).

'Kluai Khai'
(*Musa* AA Group)

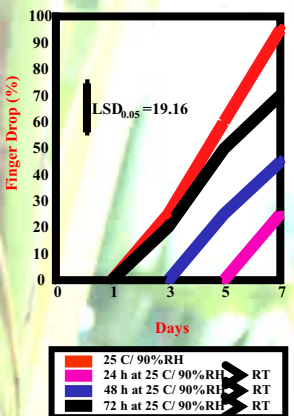

Figure 1 Finger drop of 'Kluai Khai' ripened at 25°C (90%RH) for 24, 48 or 72 h and transferred to room temperature (RT).

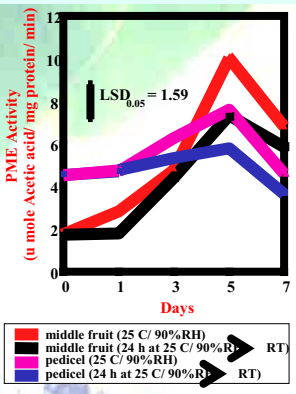

Figure 2 Changes in activity of pectinemethylesterase in peel of 'Kluai Khai' at the middle fruit (,) and at the pedicel (,).

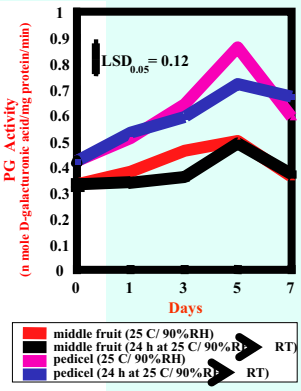

Figure 3 Changes in activity of polygalacturonase in peel of 'Kluai Khai' at the middle fruit (,) and at the pedicel (,).

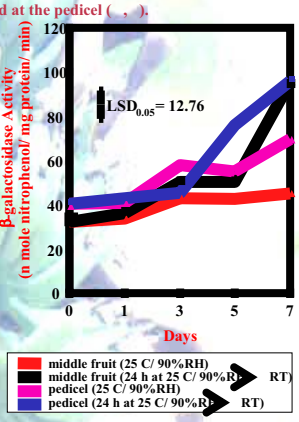

Figure 4 Changes in activity of β-galactosidase in peel of 'Kluai Khai' at the middle fruit (,) and at the pedicel (,).

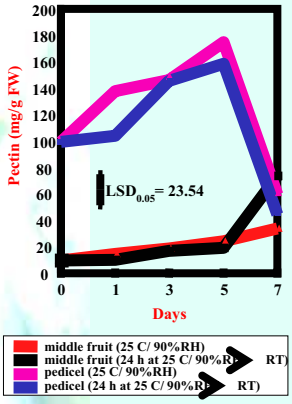

Figure 5 Changes in water-soluble pectin in peel of 'Kluai Khai' at the middle fruit (,) and at the pedicel (,).

DISCUSSION

Bananas ripened at 25°C / 90%RH for 24 h showed low finger drop than those ripened at 25°C / 90%RH for 72 h. The longer they exposed to 25°C / 90%RH, the more they developed finger drop. This indicated that bananas ripened under high RH too long were prone to develop finger drop more seriously than those ripened with a shorter exposure to high RH (Semple and Thompson, 1988).

Softening of ripening fruits is associated with alternation in cell wall and middle lamella structure (Seymour and Gross, 1996). These changes include solubilization of cell wall pectin involving the action of cell wall hydrolytic enzymes PG, PME and GAL. Bananas with high finger drop had greater activities of PG (Figure 3) and PME (Figure 2) in peel at the pedicel during ripening, resulting in more water-soluble pectin in peel at the pedicel than that in peel at the middle fruit (Figure 5). This may lead to localize a weakening peel at the pedicel, which in turn produces finger drop. It is not know how a long exposure of bananas to high RH induced greater activities of PG and PME in peel at the pedicel than those exposed to a short period of high RH during ripening.

GAL activities in peel at the middle fruit and at the pedicel of bananas with low finger drop and exposed to a short period of high RH were greater than those with high finger drop and exposed to a long period of high RH (Figure 4). This suggested that the development of finger drop in bananas 'Kluai Khai' may not be related to GAL.

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ภาคผนวกหมายเลข 5

CHANGES OF CELL WALL HYDROLASES IN RELATION TO FINGER DROP OF 'HOM THONG' AND 'NAM WA' BANANAS

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Introduction

Drop of individual fruit (fingers) is a disorder during ripening of 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. Finger drop limits the marketability of many banana varieties. Hands with finger missing cannot be sold to consumers and individual fruit that have dropped have no pedicel and therefore also not be sold to consumers. Finger drop has been reported in the triploid Cavendish AAA group of cultivars (Semple and Thompson, 1988), in tetraploid cultivars (Marriott, 1980), and in a diploid cultivar (Prayurawong, 1999).

The drop seems to be due to localized weakening of peel at the pedicel (New and Marriott, 1983; Semple and Thompson, 1988). In general, peel softening has been suggested to be due to depolymerisation of pectic substances in the primary cell wall and the middle lamell (Seymour, 1993). These changes involve action of cell wall hydrolases such as polygalacturonase (PG), pectin methylesterase (PME), β-galactosidase and cellulase (Huber, 1993).

Whether cell wall hydrolase activity is associated with banana finger drop is unknown. In the present study we attempted to determine the possible role of PG and PME.

Materials & Methods

Harvested 80% Maturity of 'Hom Thong' and 'Nam Wa' bananas

↓

Washed in 100 ppm NaOCl

↓

Dipped in 500 ppm benomyl for 2 min

↓

Dipped in 500 ppm ethephon for 2 min

↓

Bananas ripened at 25°C (85-90%)

Evaluations

- **Finger drop**
- **Resistance to finger drop**
- **PG activity**
- **PME activity**
- **Northern blotting of PG**

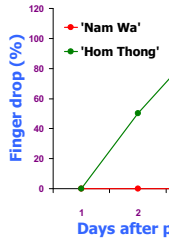
Banana fruit were monitored daily. The peel at the middle of the fruit and the pedicel adjacent to the rupture area was sampled. Peel from five hands of each treatment was pooled and frozen at -80°C until use for PG and PME assays and total RNA extraction.

Results

Finger drop and resistance to finger drop

Finger drop of 'Hom Thong' bananas started on day 2 after peel yellowing and increased to 100% within 4 days. 'Nam Wa' bananas did not show finger drop during ripening period (Fig. 1).

On the first day of the experiment, the resistance to finger drop of 'Nam Wa' and 'Hom Thong' bananas was both 5 kg. The resistance in 'Hom Thong' bananas declined more rapidly than in 'Nam Wa' bananas (Fig. 2).



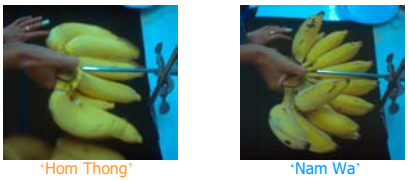


Fig.1 Finger drop of 'Hom Thong' and 'Nam Wa' bananas.

PG and PME activity

Both in 'Hom Thong' and 'Nam Wa' bananas, PG activity in the peel increased during ripening. This increase was observed in peel taken from the middle of the fruit and in peel from the area of rupture. No statistical difference was found in PG activity in area of rupture, between 'Hom Thong' and 'Nam Wa' bananas, except on day 3 and day 5 (Fig. 3).

Initially, the PME activity in the peel of 'Nam Wa' bananas was much higher than in 'Hom Thong' bananas, both in peel taken from the middle of the fruit and from the rupture area. In 'Nam Wa' bananas the PME activity decreased and had reached similar levels as in 'Hom Thong' banana by day 5 of the experiment (Fig. 4).

Expression of a PG transcript

On day 1 and 2 the expression of a *PG* transcript, called *Musa acuminata PG (MAPG),* in the peel at the rupture area, was lower in 'Nam Wa' bananas than in 'Hom Thong' bananas (Fig. 5A). The level of *MAPG* in the peel at the middle of 'Nam Wa' bananas was also lower (Fig. 5B). The abundance of *MAPG* transcript in the peel at the rupture area was higher than that in the middle, in both cultivars (Fig. 5C).

Conclusion

It is concluded that fruit drop in banana is due to breaking of the tissue at the pedicel adjacent to the rupture area. The evidence for a role of PG in the peel at the area where the break occurs is as yet unconvincing, whereas the evidence seems to indicate that PME is not involved.

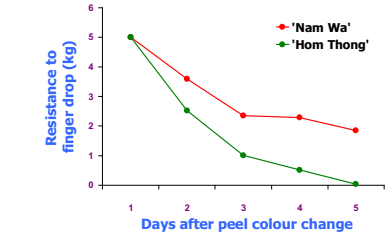


Fig.2 Resistance to finger drop of 'Hom Thong' and 'Nam Wa' bananas.

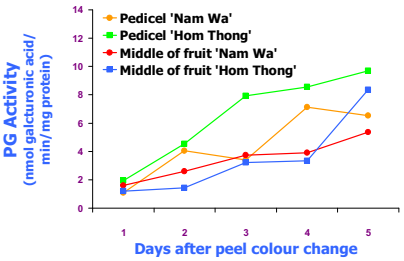


Fig.3 PG activity in peel of 'Hom Thong' and 'Nam Wa' bananas.

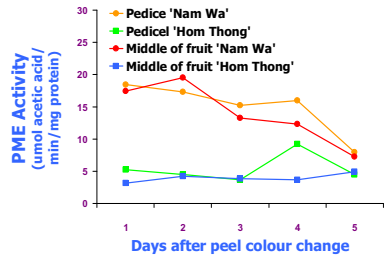


Fig.4 PME activity in peel of 'Hom Thong' and 'Nam Wa' bananas.

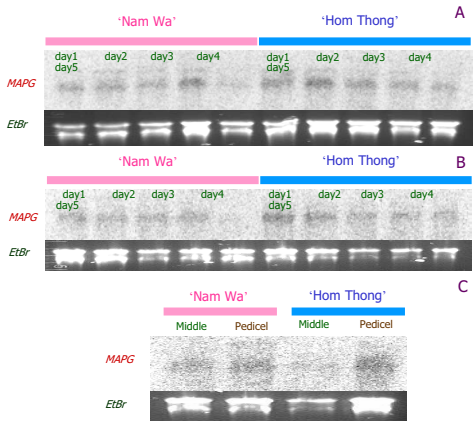


Fig.5 RNA gel blot-analysis of *MAPG* mRNA abundance in peel of bananas. Total RNA (10 μg) from the peel at the pedicel adjacent ot the rupture area (A) and ath the middle (B) of 'Nam Wa' and 'Hom Thong' bananas at day 1, 2, 3, 4 and 5 after the peel had become. A direct comparison is given between the pedicel at the rupture area and at the middle of the fruit, on day 3 (C). Equal loading of RNA was confirmed by staining gels with ethidium bromide (EtBr).

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Acknowledgements

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