DISCUSSION

PART I: Cell Wall Metabolism during Fruit Dehiscence

1. Change in Solubility of Cell Wall Compositions

At the beginning of the experiment it was found that the amount of chelating-soluble pectin in the DZ was about 25 % higher than that in the husk, where as the other two fractions were about the same or slightly higher in the DZ. However, the actual difference in the amount of pectin in the two tissues should be much higher than that shown in the graphs due to the fact that the DZ samples were taken at a thickness of 5 mm, whereas the DZ width varied between 0.5 mm to 2 mm, or 1.25 mm on average, according to the report by Koksungnoen (2005). Hence the chelator-soluble pectin in the DZ should be 2 times higher than that in the husk. This information indicated that the DZ was already formed with high amount of pectin to facilitate the dehiscence, even through the amount of pectin in the DZ and the husk was only one-tenth of that found in the pulp as reported by Imsabai (2002).

During durian fruit ripening, pectin fractions in both the husk and the DZ were modified similarly, particularly during the first 6 days. Later, when the dehiscence was became more apparent, increased levels of water- and chelator-soluble pectin were found in the DZ. The changes in these pectin fractions were similar to trends found during softening in pears (Ahmed and Labavitch, 1980), tomatoes (Brummell and Labavitch, 1997), melon (Rose *et al*., 1998) and grapes (Nunan *et al*., 1998), but the degree of change here was 25 and 50% of that in tomato and melon, respectively. The amount of pectin in the two tissues was also much less than that found in other fruits including the pulp of durian, where it was found that pectin content was about 10 times higher (Imsabai *et al*., 2002). Na_2CO_3-soluble pectin, however, was not different between the two tissues, but also increased at during later dehiscence.

Since all pectin fractions increased in both the husk and the DZ, it could be stated that pectin did not change from the less soluble to the more soluble form as in fruit softening. Rather, pectin might be synthesized in durian pericarp, particularly in the DZ. This finding was in line with a report by Mitchem *et al*. (1991) and Mitchem and Gross (1989), which showed that during tomato softening pectin synthesis was still active. The synthesis of pectin during durian dehiscence might be needed for cell enlargement that had been reported during floral organ abscission in arabidopsis (Patterson, 2001). However, a microscopic study of the durian DZ by Koksungnoen, (2005) could not confirm cell enlargement during durian dehiscence. Pectin polysaccharide in the chelator-soluble fraction showed a more pronounced molecular size shift downward than the water-and Na_2CO_3-soluble fraction. Together with the observation that the largest amount of pectin was in chelator-soluble fraction, it could be suggested that this pectin fraction may play a major role in durian dehiscence.

Gel filtration profiles of polyuronides extracted sequentially in water, chelating agents and Na_2CO_3 solutions, revealed that all classes of the wall pectin were depolymerized to some extent, as shown by a downshift in the position of the eluted peaks as dehiscence continued. Hence during durian dehiscence pectin in the DZ was depolymerized similarly to that found in fruit softening. It could also be observed from the data that the total amount of pectin of all sizes in both water- and chelator- soluble fractions also increased as dehiscence progressed in agreement with the data in the solubility study. However, the total amount of the Na_2CO_3-soluble fraction only increased during the first 6 days, and then declined. The change in this fraction was different from that found in the solubility study, but similar to those reported in softening of many fruits. However, the decline of this pectin fraction could not account for the large increase in the other two fractions. It was therefore confirmed that during durian dehiscence not only pectin was being degraded, but was also being synthesized both in the DZ as well as in the husk itself.

Hemicelluloses fractions clearly decreased during storage. The loosely-bound glycan (1 M KOH-soluble fraction) decreased by half at the end of storage, but there was no clear difference between the DZ and the husk tissues. The tightly-bound glycan (4 M KOH-soluble fraction) also decreased, slowly in both tissues during the first 4 days, but more in the DZ during the last 6 days when dehiscence was well underway. The difference in these wall fractions between the husk and the DZ during the later part of storage should be more apparent if the contamination of the husk tissues in the DZ samples were to be taken into account. Although the solubility of hemicellulose changed very little during dehiscence and the data indicated only small differences, the actual difference is likely to be higher due to the fact that the collected DZ always contained an appreciable amount of husk tissue sometimes more than 70% of the tissue area collected. If the husk tissue was changing less than the DZ tissue, its presence would dilute the changes measured for DZ tissue.

The molecular size distribution of hemicellulose did not differ greatly between the husk and the DZ (Figures 12 and 13). There was only a slightly changed in the molecular weight profile of the loosely-bound glycan during durian fruit dehiscence (Figure 12). Changes in the tightly-bound glycan were more apparent, exhibiting a decrease in molecular size both in the husk and the DZ. A more obvious change in molecular size was observed in the DZ. With this finding, it can be stated that the change in hemicellulose was also involved in the dehiscence process of durian, but might be only a minor factor of the process. The involvement of hemicellulose degradation in abscission and dehiscence had not been demonstrated before in other plant tissues. The changes in these two fractions were much smaller less (by about 3 times) than those found in kiwifruit (Redgwell *et al.*, 1991), tomato (Huber and O'Donoghue, 1993; Rose *et al.*, 1998) and peach (Brummell *et al.*, 2004), which softened significantly during ripening.

The changes observed in pectin and hemicellulose components in durian pericarp shown above indicated also that the husk and the DZ tissues became soften during durian ripening, although this softening was not obvious to the consumer as compared to the pulp.

2. The Activities of Cell Wall Degrading Enzymes

At the beginning of the experiment PME, β-gal and EGase activities in both the DZ and the husk were about the same, whereas PG activity in the DZ, however, was two times higher than that in the husk. This indicated that, right from the time of harvesting, wall materials in the DZ had already been processed differentially from those in the husk.

During the following two days, all four enzymes activities in the DZ increased dramatically. These changes in enzymes activities allowed for the degradation of cell wall material in the DZ to proceed smoothly. Since PME was known to remove methyl groups from pectin, a change which may make possible the cross-linking of free carboxyl groups with calcium ions, pectin became susceptible to degradation by PG (Brummell and Harpster, 2001). Moreover, β-gal degraded the galactan side chain of pectin, resulting in increased pectin solubility and decreased molecular size (de Veau *et al*., 1993). The degradation by β-gal also created a porous structure of the wall polymer and allowed other enzymes better access to wall materials including pectin (Pressey, 1983). On the other hand, β-gal activity in the husk remained stable during the first two days. This relatively low β-gal activity would limit the degradation of pectin and other wall materials in the husk, even though PME, PG and EGase activities with the capacity to degrade them had already increased.

Beyond day 2, PME, PG and EGase activities in the DZ continued to increase toward the end of storage, except β-gal activity, reached a peak on day 4, then decreased to a level close to that at the start of the experiment. This change in β-gal activity implied that this enzyme was only needed early in the wall degradation process, as had been previously demonstrated in the course of softening of grape fruit (Barnavon *et al*., 2000). During the later period of durian storage the higher activities of PME, PG and EGase would degrade wall materials substantially, allowing the dehiscence to occur smoothly. In the husk tissue, on the other hand, β-gal activities began to increase after day 2, reaching a peak on day 6 and then declining. The change of this enzyme was very similar to that in the DZ, but occurred 2 days later. In contrast, the activities of PME and EGase in the husk became stable after day 2 towards the end of storage. Only PG activities continued to increase in parallel to those in the DZ. With the limitation of PME and EGase activities, wall materials in the husk tissue would not be degraded to the same extent as that in the DZ. The evidence of this limited degradation in the husk could be observed in molecular size distribution data in Figures 7-9, 12 and 13. Nevertheless, the changes in all 4 enzymes activities together with the depolymerization of wall materials indicated that the husk would soften to some extent, though this was not clearly observed.

Although there was a marked relation between the increased EGase activity and the decreased in hemicellulose content, especially during the last 6 days in storage, it should be noted that the higher EGase activity in the DZ did not specifically explain the smaller molecular size of glycan in the DZ when compared to that of the husk. It might have been possible that EGase digested the glycan to

very small molecules which were subsequently washed away during the AIS preparation process. Hence, the change in molecular size of glycan left in the KOH fractions could not be observed. The result indicated also that this enzyme might be involved in the degradation of other pectin side chains during durian dehiscence as well. An increase in EGase activity was also reported during the dehiscence of oilseed rape (Meakin and Roberts, 1990).

Dehiscence studies in the past were primary carried out in *Brassica napus* and Arabidopsis. All studies were done on enzymes. No works on the wall composition changes were found. In *B. napus*, it was shown that EGase was the responsible enzyme for pod dehiscence (Chauvaux *et al*., 1997; Meakin and Roberts, 1990). This process is also controlled by auxin. When the synthetic auxin, 4-CPA was applied, the enhance activity of EGase and dehiscence was delayed (Chauvaux *et al*., 1997). PG gene expression was also detected during the dehiscence process in *B. napus* (Petersen *et al*., 1996; Jenkin *et al*., 1996). In addition XET gene was up-regulated in the dehiscence zone at the final stage of the dehiscence. In Arabidopsis, EGase was recognized in the dehiscence zone (Henrissat *et al*., 2001). PG expression was also detected during the separation process (Jenkins *et a*l., 1999; Roberts *et al*., 2002). The study in durian reveals that for dehiscence to precede not only EGase and PG were necessary but PME and β-gal activities were also needed. Other enzymes such as PL, expansin and XET known to be involved in other cell separation processes (Roberts *et al*., 2002) and not determined in this study, might probably coordinate the degradation of the wall composition, and subsequently allow dehiscence to proceed as well.

PART II: Effect of Gibberellic Acid (GA_3) on Durian Fruit Dehiscence

1. Effect on Fruit Ripening

The results from this experiment showed that GA_3 treatment delayed fruit dehiscence for 2 days compared to the control (Figure 18). This treatment also clearly delayed the change in peel color (Figure 20), but only slightly reduced fruit weight loss (Figure 19). These results were similar to that found in 'Chanee' durian where 50 and 100 ppm GA_3 sprays could delay color change and fruit dehiscence for 2 and 3 days, respectively (Siriatiwat, 1988; Nampradit, 1991). The retarding effect of GA_3 treatment on fruit ripening and senescence is widely recognized. GA_3 tended to reduce the peel firmness and caused an increase in soluble solids (SS) content during fruits ripening in prickly and cactus pear (Mejia and Cantwell, 2003; Schirra *et al*., 1999), sweet cherry (Usenik *et al.*, 2005) and persimmon (Ben-Arie *et al*., 1989). This treatment also reduced fruit cracking by enhancing the thickness of cuticles, and increasing dimensions of epidermal cells (Usenik *et al.*, 2005).

On the effect of GA_3 in delaying durian color development, similar results had been reported in cactus pear (Schirra *et al*., 1999), apples (Saure, 1990; Awad and de Jager, 2002), citrus (Rasmussen, 1973; Davies *et al*., 2001) and persimmon (Ben-Arie *et al*., 1996). The general action of GA_3 was believed to delay the degradation of chlorophyll as well as to reduce the formation of anthocyanin and carotenoid pigments (Schirra *et al*., 1999). The efficiency of GA_3 depended on the concentration used and varied among species, tissues and organs. The effect of this plant growth regulator, which delays the change in peel color without effecting changes in pulp softening during durian storage, might be because GA_3's inability to penetrate the pulp's thick peel. Therefore, the efficiently of GA_3 may not have a controlling effect on the process of pulp softening.

The slight reduction in weight loss by the GA_3 treatment was similar to that report by Siriatiwat (1988). This effect was likely to be due to the reduction in ethylene production and respiration of the GA_3 treated fruits. Other reports have shown that GA_3 treatment influenced the changes in the plant's cuticle and surface wax, which plays a central role in regulating water loss (Riederer and Schreiber, 1995). In cactus pears, the epicuticular wax of mature fruit has a number of irregular plate-like units, but after applied GA_3, the platelets were less pronounced than those of untreated fruits (El-Otmani and Coggins, 1995).

2. Effect on Solubility of Cell Wall Composition and Its Degrading Enzymes

Fractionation of pectin indicated that treatments with GA_3 had a different impact on the solubilization of all three pectin fractions. In GA_3 treated fruit, the Chelator-soluble pectin (CSP) content was lower than the control. The increase in CSP on the later days after harvest was also reduced (Figure 22). The effect of GA_3 on the other two fractions, water- and Na_2CO_3-soluble pectin fractions (Figures 21 and 22), was less clear, but showed similar trends. A study in tomato (Mignani *et al*., 1995) also showed that GA_3 reduced the accumulation of water-soluble pectin. The

results suggested that GA_3 altered the pattern of pectin solubility changes, which may be due to the reduction in the activity of pectic enzymes.

The changes in both loosely bound and tightly bound matrix glycans were not clearly affected by GA_3 treatments. However, the change in tightly bound glycan seemed to be slower than the control (Figure 24 and 25). Ben-Arie *et al*. (1995) showed that GA_3 either delayed or inhibited all cell wall composition changes found to accompany tomato fruit softening, but that the extractability of matrix glycans (both loosely and tightly bound) did not differ between control and GA_3-treated fruits.

The results shown above indicated that GA_3 had more influence on pectic enzymes than on hemicellulosic enzymes. However, GA_3 treatments were found to reduce the activities of all four cell wall degrading enzymes (Figures 26-29) during the process of durian dehiscence. EGase activity was reduced by approximately 50% when applying a GA_3 treatment, while the effect of this treatment resulted in about 20% reduction in other enzymes. Thus, the lower amount of water- and chelator-soluble pectins in GA_3 treated DZ (Figures 21 and 22), could be explained by the lower level of PME, PG, β-gal and EGase activities (Figures 26-29). The results here also confirmed the role of these four enzymes during durian fruit dehiscence as shown in the first part of this study.

The results supported earlier findings of reducing the activity of abscission zone EGase in pea internodes by about 30% when compared to the control (Broughton and McComb, 1971). In abscission zones, a 25% reduction in PME specific activities was noted in GA_3 treated dwarf pea internodes (Broughton and McComb, 1971). In cherry, GA_3 decreased the activities of PG and PME (Andrew and Shulin, 1995), and this was correlated with the maintenance of the fruit firmness (Kondo and Danjo, 2001). Another enzyme that was known to be affected by GA_3 was PL in banana, where the increase in climacteric peaks was delayed by 3 days (Payasi *et al*., 2004).

The observed effects of GA_3 on delayed softening or abscission along with a delays in ripening, suggested that GA_3 might influence those cell wall enzymes via its effect on ethylene synthesis. A preliminary study of ethylene production in durian showed that GA_3 treatments reduced ethylene synthesis and delayed the rate of increase in the climacteric peak for about 12 hours when compared to the control. It is possible that the inhibition of ethylene production by GA_3 was simply a directly effect to the rate of 1-aminocyclopropane-1-carboxylic acid (ACC) converted to ethylene (Ben-Arie and Ferguson, 1991). Similar results were found in carnation flowers, where endogenous levels of ACC were reduced with GA_3 treatment. The potential of the flowers to convert applied ACC to ethylene was not diminished by GA_3 during senescence (Saks and Van Staden, 1993).

A recent microarray study aimed at identifying the GA-responsive gene in arabidopsis, demonstrated that GA may affect ethylene biosynthesis and ethylene responses (Ogawa *et al*., 2003). Since it was known that in the absence of ethylene, the families of ethylene receptors (ETR1, ETR2, ERS1, ERS2 and EIN4) activate the

CTR1, which in turn represses the positive membrane regulator EIN2, additions of ethylene deactivates ethylene receptors resulting in deactivation of CTR1 and thereby releasing EIN2 to activate EIN3. The EIN3 transcription factor binds the regulatory sequence in the promoter of ethylene-regulated genes, thereby inducing transcription (Gazzarrini and McCourt, 2003).

The results from this two-part research of the dehiscence process in durian fruit provided some useful information for modifying or controlling the rate of fruit dehiscence. Farmers and consumers now have the option of using both, individual ethylene and GA_3, or a combination with varying ratios of these two plant hormones. This in turn should allow for optimizing durian fruit dehiscence in response to consumer demands.

CONCLUSION

The changes in cell wall compositions and enzyme activities during durian fruit dehiscence are summarized as the following:

1. Pectin became soluble in all fractions especially at later dehiscence and markedly in chelating reagents. Hemicelluloses became less soluble, and decreased further during durian fruit dehiscence in both loosely-and tightly bound glycans.

2. The molecular size of cell wall pectin decreased primarily in water-and chelator-soluble pectins, while the size distribution of molecular weight in hemicellulose did not change significantly during durian fruit dehiscence.

3. PME and PG moderately increased in their activities, and seemed to increase with this process at the final stage, while β-gal seemed to predominate at the early stages. EGase may play a more important role during durian fruit dehiscence**.**

4. After applying GA_3, durian fruit could delay fruit dehiscence for 2 days. The treatment delayed the changes in the peel's green coloration**.**

5. The effect of GA_3 reduced the solubilization of cell wall materials mainly in chelator-soluble pectin, while the treatment did not significantly reduce such solubilization in water-and Na_2CO_3-soluble pectin, including the hemicellulose fractions.

6. GA_3 treatments significantly reduced the activities of PME, PG, β-gal and EGase during the process of durian fruit dehiscence.