

RESULTS

PART I: Cell Wall Metabolism during Fruit Dehiscence

1. Pulp Firmness

Pulp firmness decreased sharply during the first 2 days in storage (from 15 to 13 Newton/cm²), then leveled off until day 6 and fell sharply afterward to 3 Newton/cm² on day 10 (Figure 1).

2. Dehiscence Score

Dehiscence started at around day 3 after harvest. The dehiscence score was about 1 and involved only 40% of the fruit samples on day 4. On day 8, all fruit samples showed some dehiscence and the score was about 2 (Figure 2).

3. Weight Loss

Weight loss of durian expressed as a percentage of initial fruit fresh weight, increased steadily during storage; from about 3% on day 2 to 25% at the end of storage (Figure 3).

4. Yield and Composition of Pectin Fractions

Water-soluble pectin (WSP) in the husk and the DZ remained constant during the first 6 days in storage at about 100 µg galacturonic acid/g fresh weight. The content of this fraction increased toward the end of storage to about 155 and 171 µg galacturonic acid/g fresh weight in the husk and the DZ, respectively (Figure 4).

Chelator-soluble pectin (CSP) content increased during storage in both the husk and the DZ, but was always higher in the DZ than in the husk, especially at the end of storage. The CSP content increased from 198 µg to 307 µg galacturonic acid/g fresh weight in the husk while the content of this fraction increased from 254 µg to 427 µg galacturonic acid/g fresh weight in the DZ (Figure 5).

Na₂CO₃-soluble pectin content was not significantly different between the husk and the DZ, slowly increasing during 8 days in storage (from about 60 µg to 90 µg galacturonic acid/g fresh weight) and then sharply increasing to 130 µg galacturonic acid/g fresh weight on day 10 (Figure 6).

5. Molecular Size Distribution of Pectin Fractions

5.1 Water-soluble fraction

The water-soluble pectin during durian fruit dehiscence was always slightly higher in the DZ as compared to the husk. The molecular weight profile gave a symmetrical peak at about fraction number 27-28 at harvest and early in storage, both in the husk and the DZ (Figure 7). On day 4, the polyuronide molecule began to

shift downward to smaller molecular weights, especially in the DZ. The profile revealed an increase in the relative amount of smaller polymers through the late dehiscence. In samples stored for 8-10 days, the profile of molecular weight exhibited a number of peaks in both husk and DZ tissues, with the molecular size distribution continuing to decrease to the end of storage duration.

5.2 Chelator-soluble fraction

Size fractionation of CSP at the beginning gave a trace amount of uronic acid closed to the column void volume and a main peak at fraction number 31. On day 2, two peaks were identified in both DZ and husk tissues, the first one appeared at fraction 23 for DZ and 27 for husk while the second peak appeared at fraction 33 in both tissues. The difference in the molecular size distribution between husk and DZ samples was clearest 6-10 days into the storage period where the DZ had higher peaks of smaller molecular sizes than in the husk (Figure 8).

5.3 Na₂CO₃-soluble fraction

Only relatively small peaks were seen when the fractions for the Na₂CO₃-soluble fraction were subjected to size exclusion analysis. The size exclusion chromatography profiles in both the husk and DZ tissues were much smaller than that found in the WSP and CSP. The total amount of Na₂CO₃-soluble pectin seemed to increase slightly during and from day 2 to day 6, and then declined in both tissues. The size of pectin in this fraction also changed to smaller sizes, as can be seen in the shift of peak toward the later fractions (Figure 9).

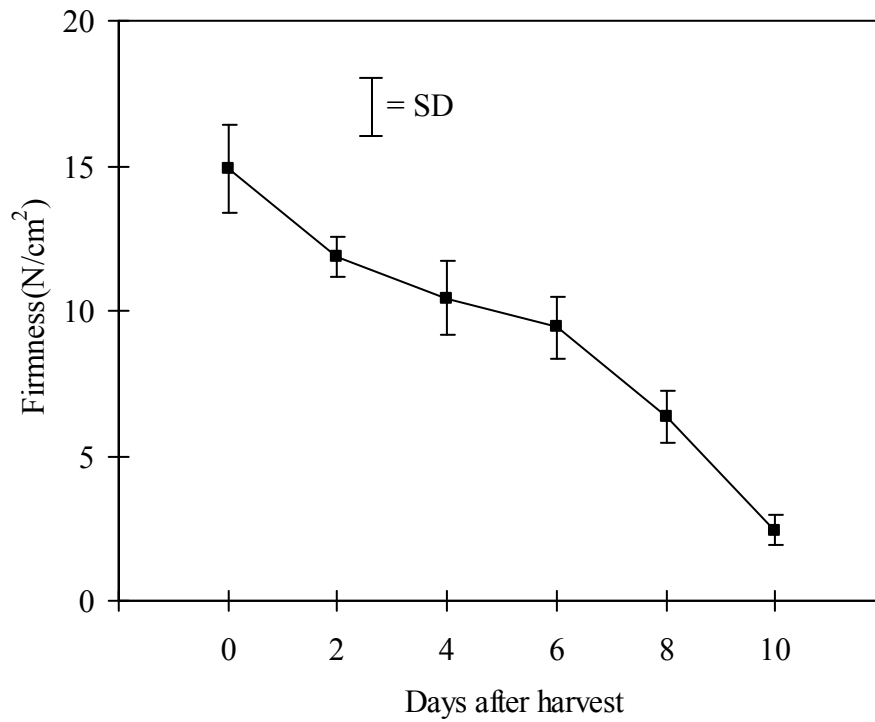


Figure 1 Pulp firmness of ‘Monthong’ durian harvested at 106 days after anthesis and then stored at room temperature ($28\pm 3^{\circ}\text{C}$) for 10 days.

$\bar{\text{I}}$ = standard deviation of data for each examination date

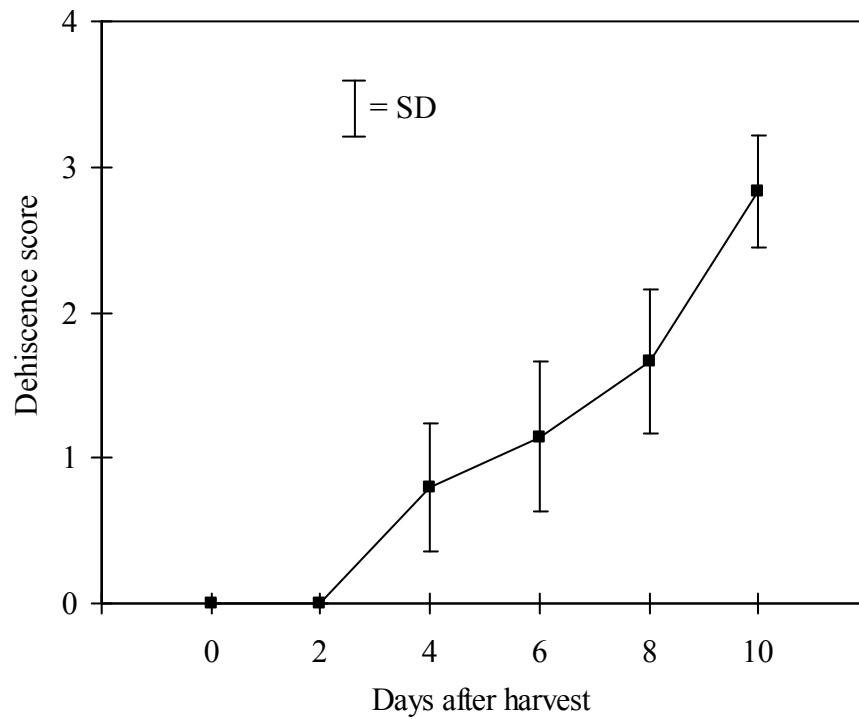


Figure 2 Average of dehiscence score per locules of 'Monthong' durian harvested at 106 days after anthesis and then stored at room temperature ($28\pm 3^{\circ}\text{C}$) for 10 days.

I = standard deviation of data for each examination date

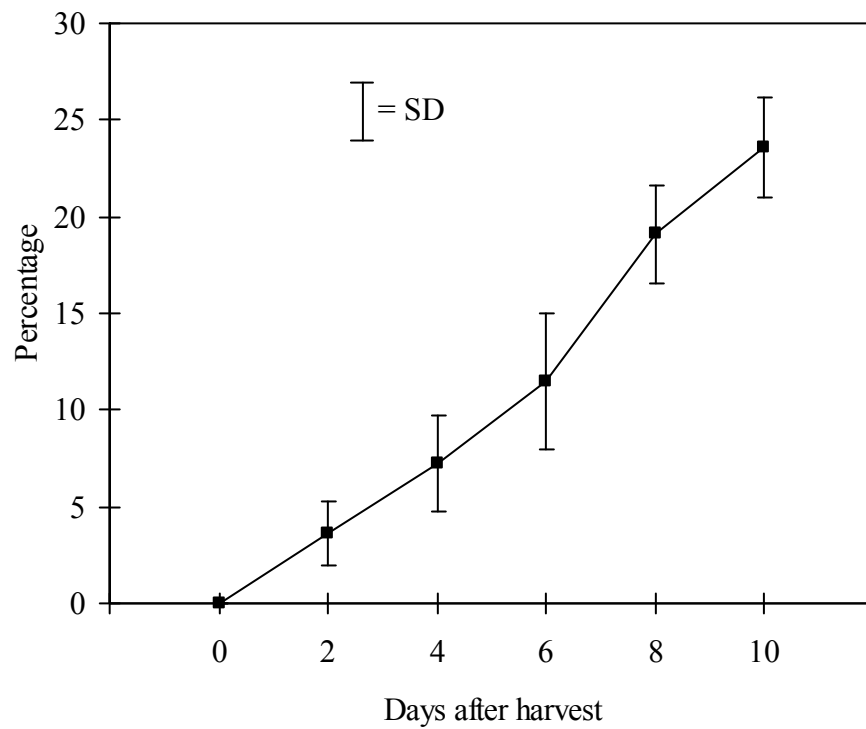


Figure 3 Percentage of weight loss of 'Monthong' durian harvested at 106 days after anthesis and then stored at room temperature ($28\pm 3^{\circ}\text{C}$) for 10 days.

$\bar{\quad}$ = standard deviation of data for each examination date

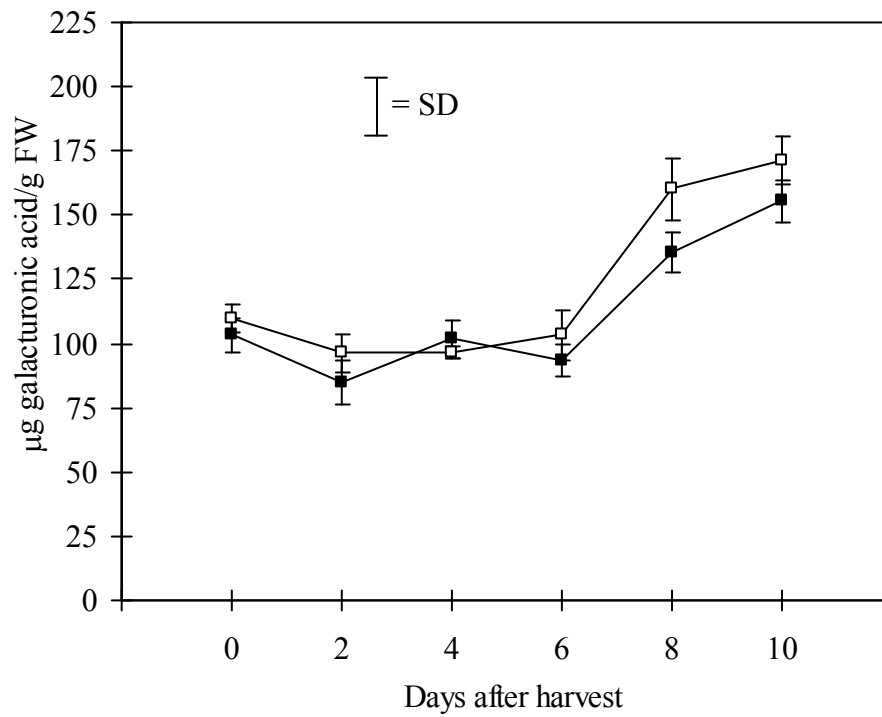


Figure 4 Water-soluble pectin in the husk (■) and the DZ (□) of ‘Monthong’ durian harvested at 106 days after anthesis and then stored at room temperature ($28\pm 3^\circ\text{C}$) for 10 days.

┆ = standard deviation of mean for each treatment

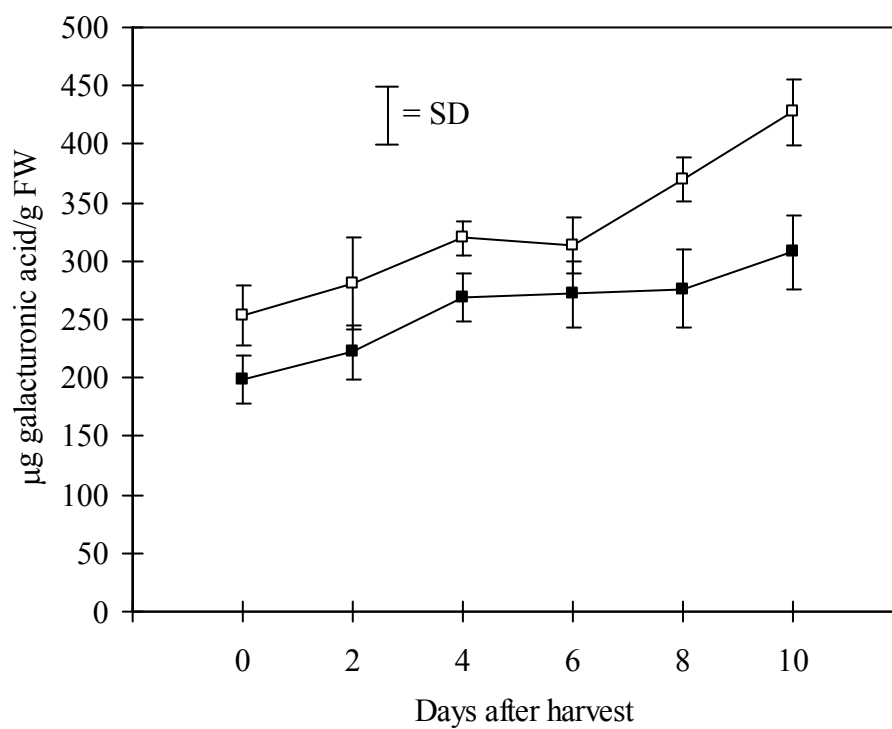


Figure 5 Chelator-soluble pectin in husk (■) and the DZ (□) of 'Monthong' durian harvested at 106 days after anthesis and then stored at room temperature ($28\pm 3^{\circ}\text{C}$) for 10 days.

┆ = standard deviation of mean for each treatment

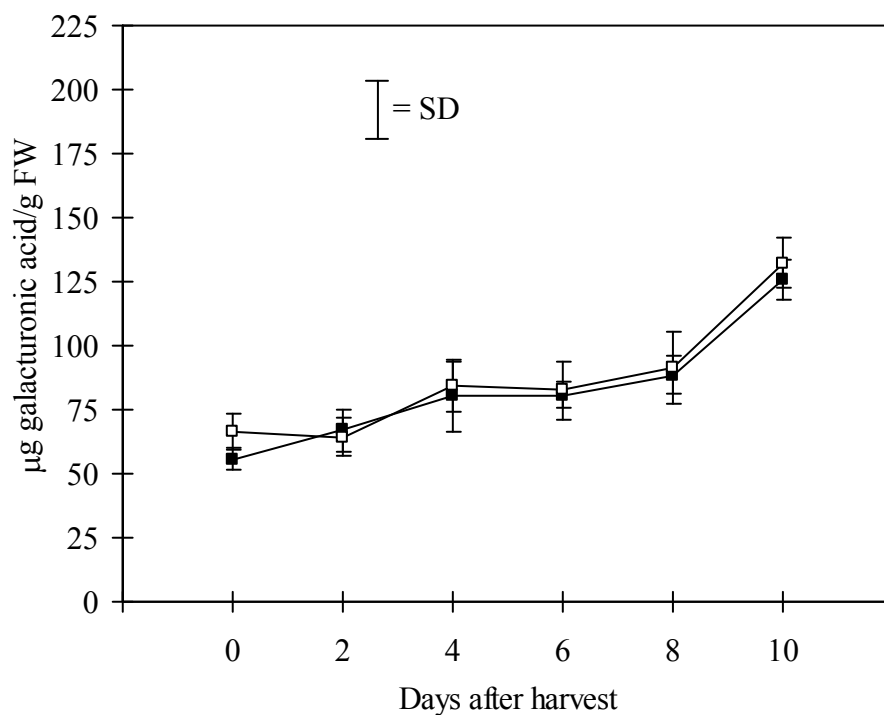


Figure 6 Na_2CO_3 -soluble pectin in the husk (■) and the DZ (□) of 'Monthong' durian harvest at 106 days after anthesis and then stored at room temperature ($28\pm 3^\circ\text{C}$) for 10 days.

┆ = standard deviation of mean for each treatment

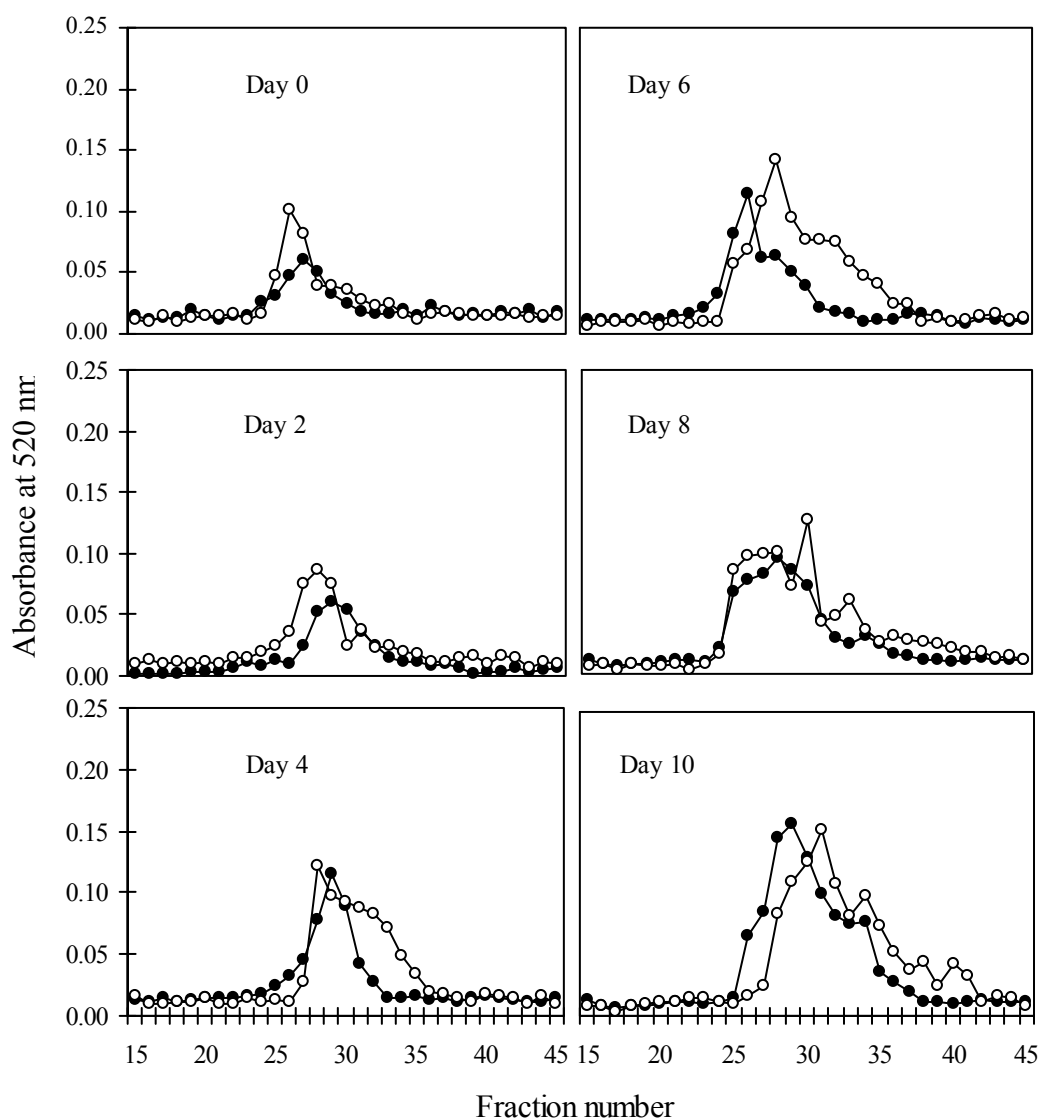


Figure 7 Molecular size distribution of water-soluble pectin in the husk (●) and the DZ (○) of 'Monthong' durian harvest at 106 days after anthesis and sampled at 2 day intervals as fruits were stored at room temperature ($28\pm 3^{\circ}\text{C}$) for 10 days.

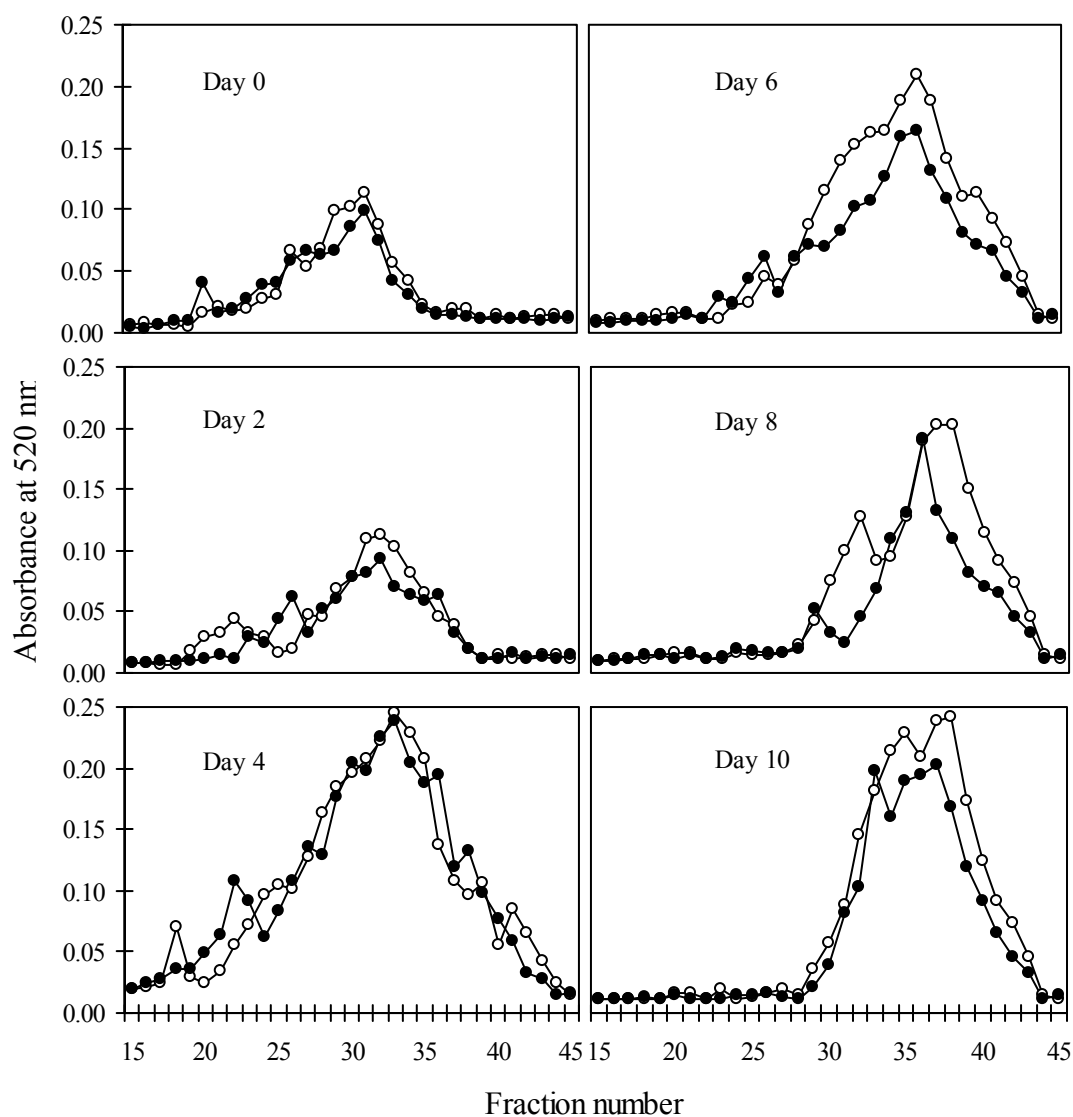


Figure 8 Molecular size distribution of chelator-soluble pectin in the husk (●) and the DZ (○) of 'Monthong' durian harvest at 106 days after anthesis and sampled at 2 day intervals as fruits were stored at room temperature ($28\pm 3^{\circ}\text{C}$) for 10 days.

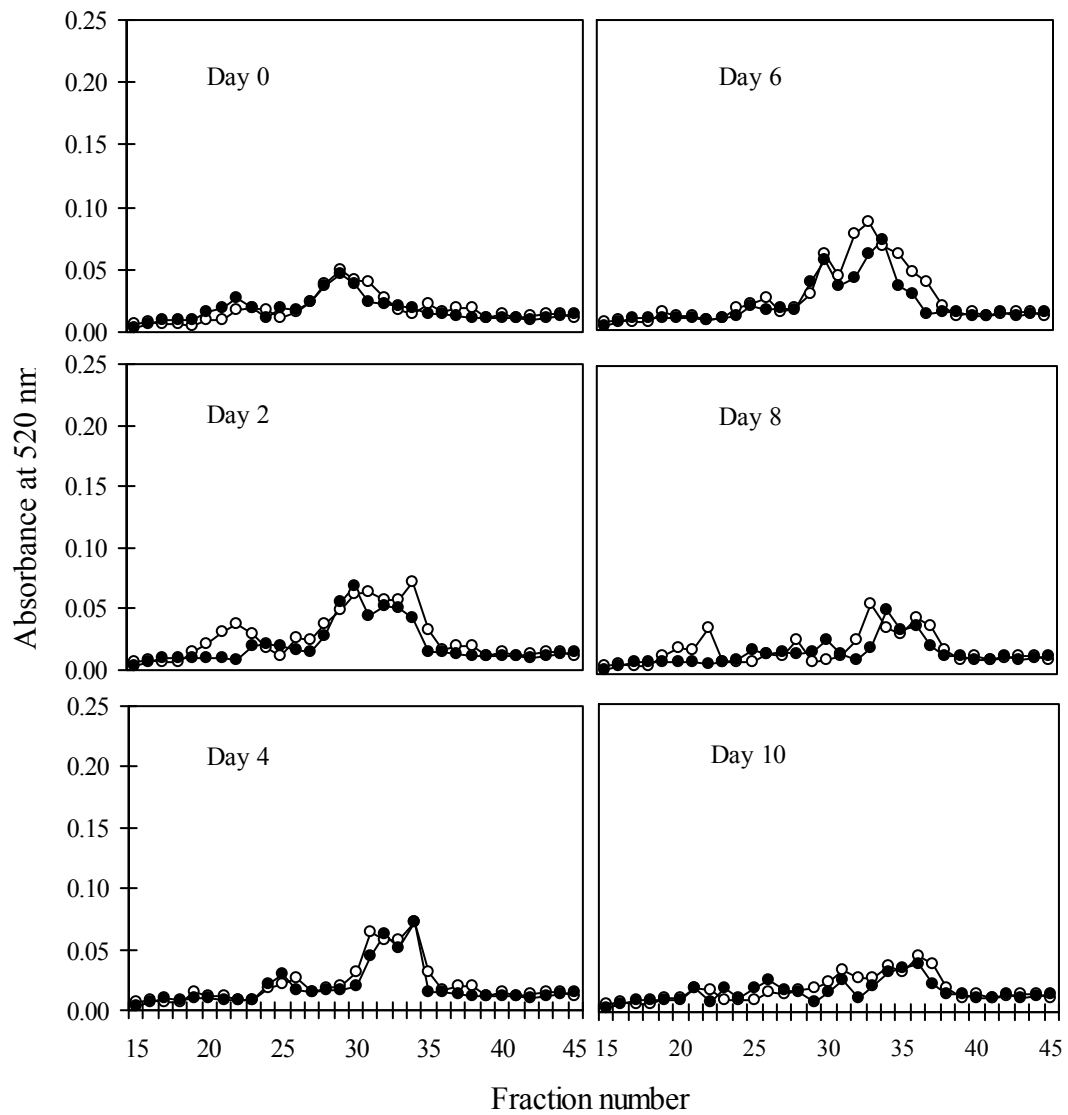


Figure 9 Molecular size distribution of Na_2CO_3 -soluble pectin in the husk (●) and the DZ (○) of 'Monthong' durian harvest at 106 days after anthesis and sampled at 2 day intervals as fruits were stored at room temperature ($28\pm 3^\circ\text{C}$) for 10 days.

6. Yield and Composition of Hemicellulose Fractions

Hemicellulose (1 and 4 M KOH-soluble fractions); i.e., loosely- and tightly bound matrix glycans, respectively and the residues left after the series of extraction, slowly decreased as sampling was done over the period of storage. There were no differences in the concentration of these two fractions in both the husk and the DZ. The amount of loosely-bound matrix glycans decreased from 40 to 25 μg glucose equivalents/g fresh weight and showed no differences between the husk and the DZ over the 10 day storage period (Figure 10). The content of tightly bound matrix glycans decreased slowly and steadily in the husk cell wall preparations over the 10 day storage period. The pattern of changes in this fraction from the DZ tissue was the same for the first 4 days and then abruptly dropped at day 6. Thereafter, the pattern of changes again paralleled that of the husk tissue (Figure 11).

7. Molecular Size Distribution of Hemicellulose Fractions

The change in molecular size distribution of hemicellulose fractions were, at most, small and did not differ between the husk and the DZ (Figure 12 and 13). There was no change in the molecular weight profile of the hemicellulose content in 1 M KOH-soluble fraction during durian fruit dehiscence (Figure 12). However, the amount of this fraction decreased continuously during storage duration.

The molecular weight profile of the 4 M KOH-soluble fraction showed a single peak at fraction number 24. The hemicellulose on this fraction decreased in molecular weight with dehiscence, indicated by the shifting of the peak downward (to fraction number 28) in samples that had been stored longer (Figure 13).

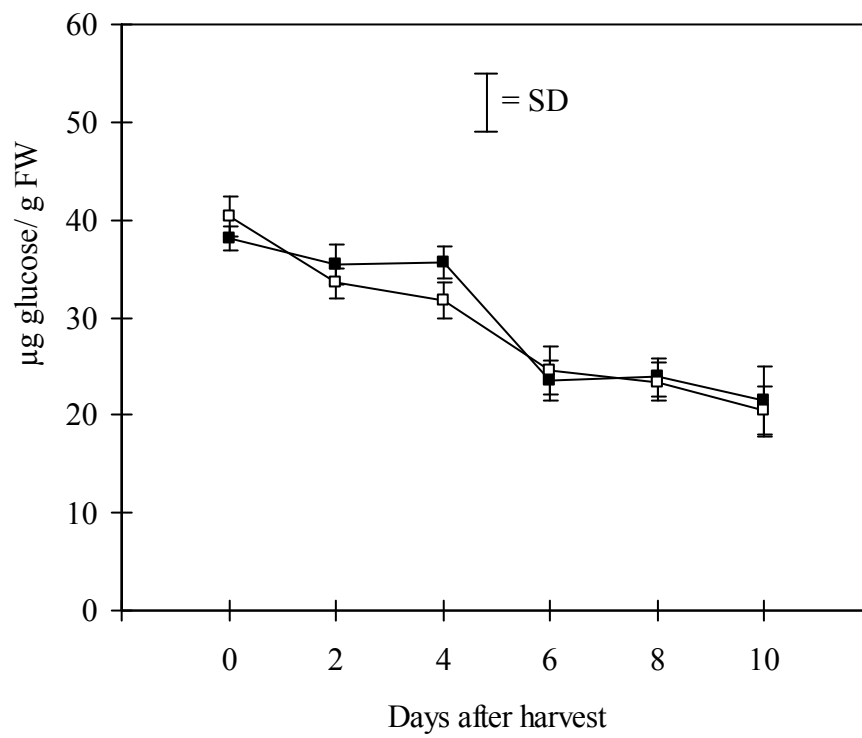


Figure 10 1 M KOH-soluble fraction in the husk (■) and the DZ (□) of 'Monthong' durian harvested at 106 days after anthesis and then stored at room temperature ($28\pm 3^{\circ}\text{C}$) for 10 days.

┃ = standard deviation of mean for each treatment

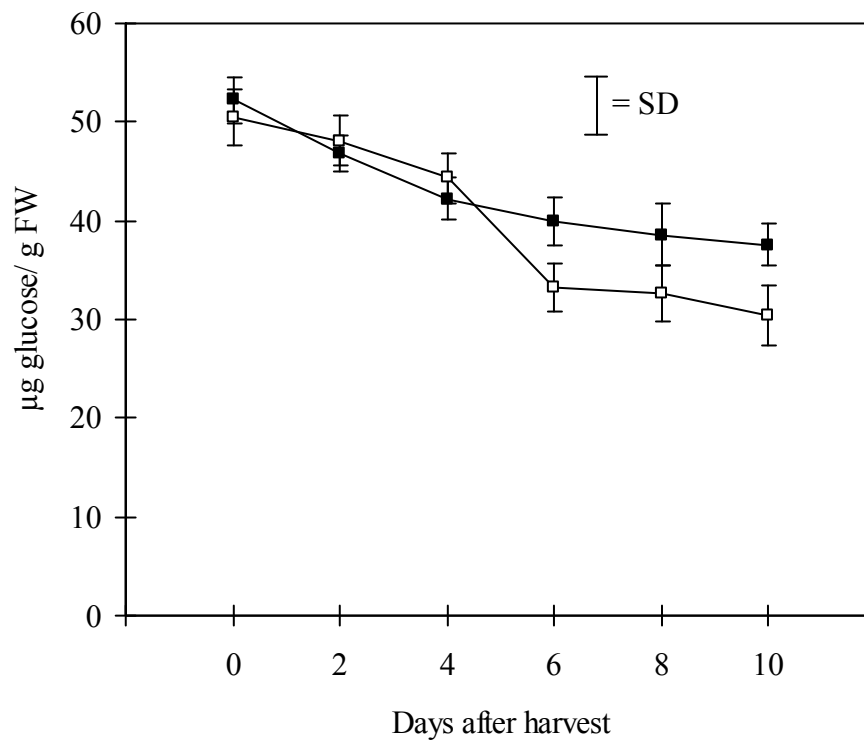


Figure 11 4 M KOH-soluble fraction in the husk (■) and the DZ (□) of 'Monthong' durian harvested at 106 days after anthesis and then stored at room temperature ($28\pm 3^{\circ}\text{C}$) for 10 days.

┆ = standard deviation of mean for each treatment

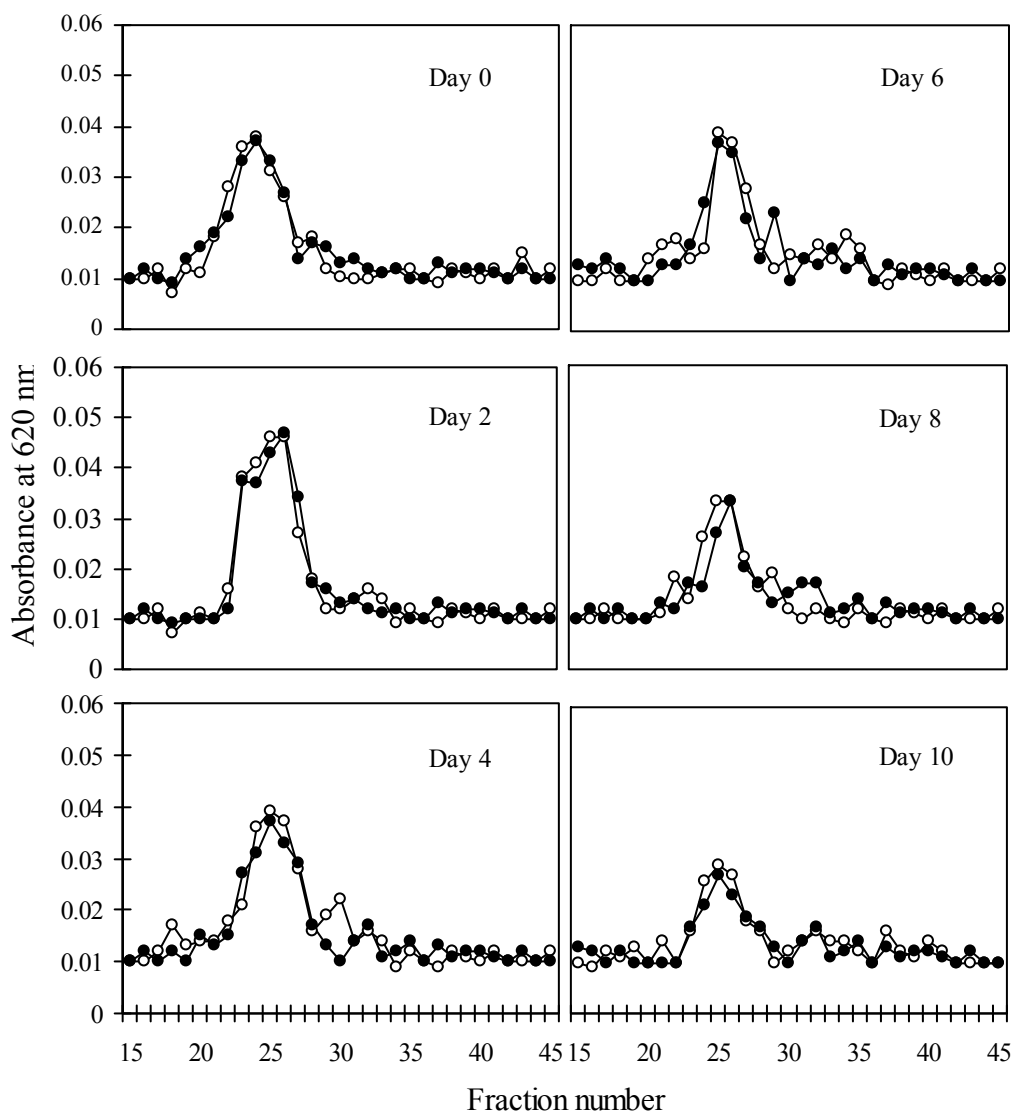


Figure 12 Molecular size distribution of 1 M KOH-soluble hemicellulose in the husk (■) and the DZ (□) of 'Monthong' durian harvested at 106 days after anthesis and then stored at room temperature ($28\pm 3^{\circ}\text{C}$) for 10 days.

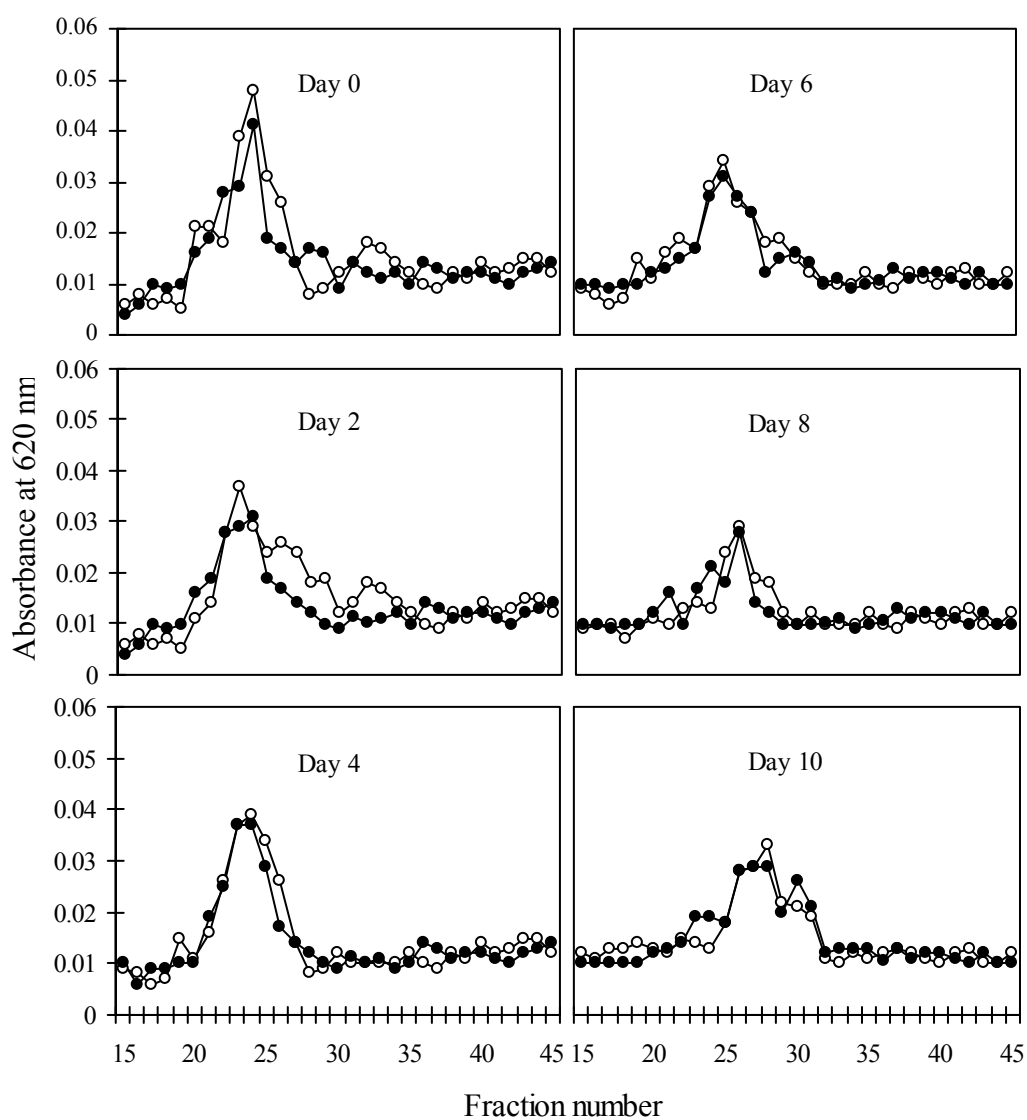


Figure 13 Molecular size distribution of 4 M KOH-soluble hemicellulose in the husk (■) and the DZ (□) of 'Monthong' durian harvested at 106 days after anthesis and then stored at room temperature ($28\pm 3^{\circ}\text{C}$) for 10 days.

8. Activities of Cell Wall Hydrolytic Enzyme

8.1 Pectinmethylesterase (PME) activity

PME activity in the husk was relatively low at the beginning (at about 17 η mol acetic acid/mg protein), then increased to a level that remained stable from day 6 to 10 (at about 27 η mol acetic acid/mg protein). PME activity in the DZ paralleled that in the husk until day 6 but then continued to increase to day 10 to about 37 η mol acetic acid/mg protein, at the end of sampling (Figure 14).

8.2 Polygalacturonase (PG) activity

In the husk, the PG activity increased from 13 to 40 η mol galacturonic acid/mg protein during ripening. Whereas in the DZ the activity started at 24 η mol galacturonic acid/mg protein, then increased in parallel with that in the husk until day 8 at about 38 η mol galacturonic acid/mg protein. The activity of this enzyme in the DZ sharply increased to about 62 η mol galacturonic acid/mg protein on day 10. The activity of this enzyme was always slightly higher in the DZ than in the husk. However, dramatically higher PG activity was found in the DZ on day 10 (Figure 15).

8.3 β -galactosidase (β -gal) activity

β -gal in both the husk and the DZ, had similar activity at the beginning at about 37 η mol p -nitrophenol/mg protein. On days 2, the activity in the husk remained constant, while that in the DZ sharply doubled on day 4 to a maximum of about 84 η mol p -nitrophenol/mg protein. The activity of this enzyme in the DZ then sharply decreased by 30% 2 days later, and leveled off towards the end of storage. In the husk, the activity increased two days later, and reached a maximum on day 6 then sharply declined in a similar pattern as observed in the DZ (Figure 16).

8.4 Endo-(1,4) β -D-glucanase (EGase) activity

The activity of EGase at the beginning was similar in both tissues, ca. 1,400 unit/mg proteins. After that, the activity of this enzyme increased to about 2,500 unit/mg protein on day 2 and remained stable for 2 days. After that the activity in the DZ continued to increase, reaching ca. 4,000 unit/mg protein on day 10, while the activity in the husk remained unchanged (Figure 17).

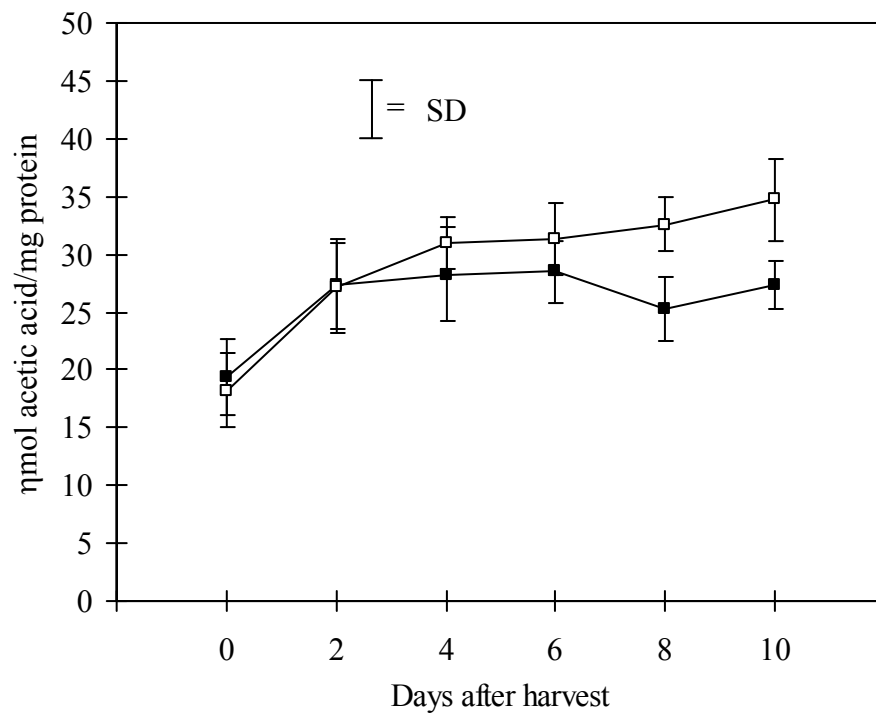


Figure 14 Pectinmethylesterase (PME) activity in the husk (■) and the DZ (□) of 'Monthong' durian harvested at 106 days after anthesis and then stored at room temperature ($28\pm 3^{\circ}\text{C}$) for 10 days.

┆ = standard deviation of mean for each treatment

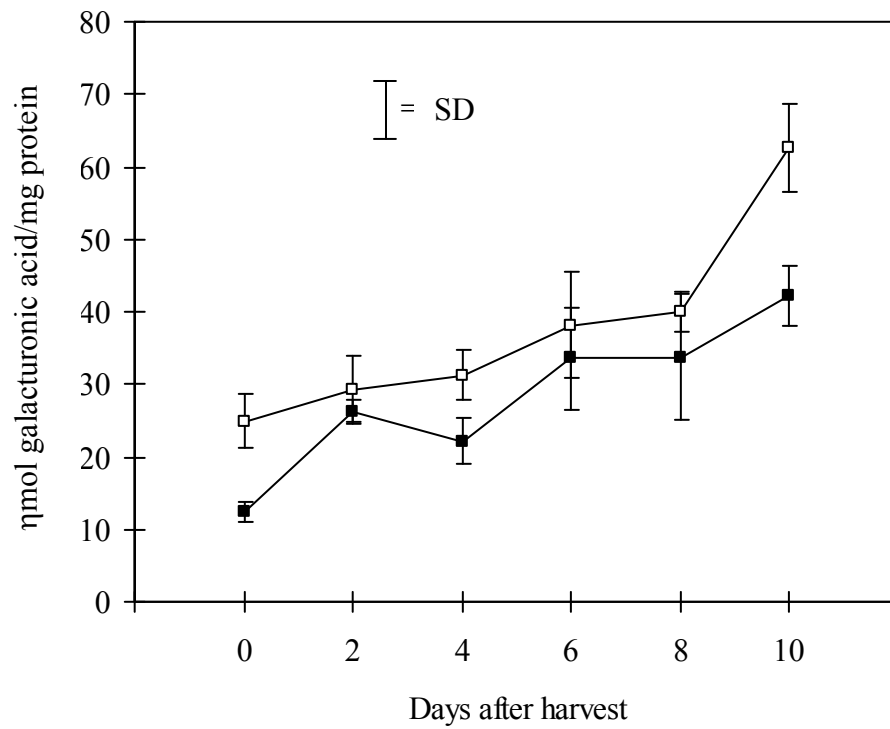


Figure 15 Polygalacturonase (PG) activity in the husk (■) and the DZ (□) of 'Monthong' durian harvested at 106 days after anthesis and then stored at room temperature ($28\pm 3^\circ\text{C}$) for 10 days.

┆ = standard deviation of mean for each treatment

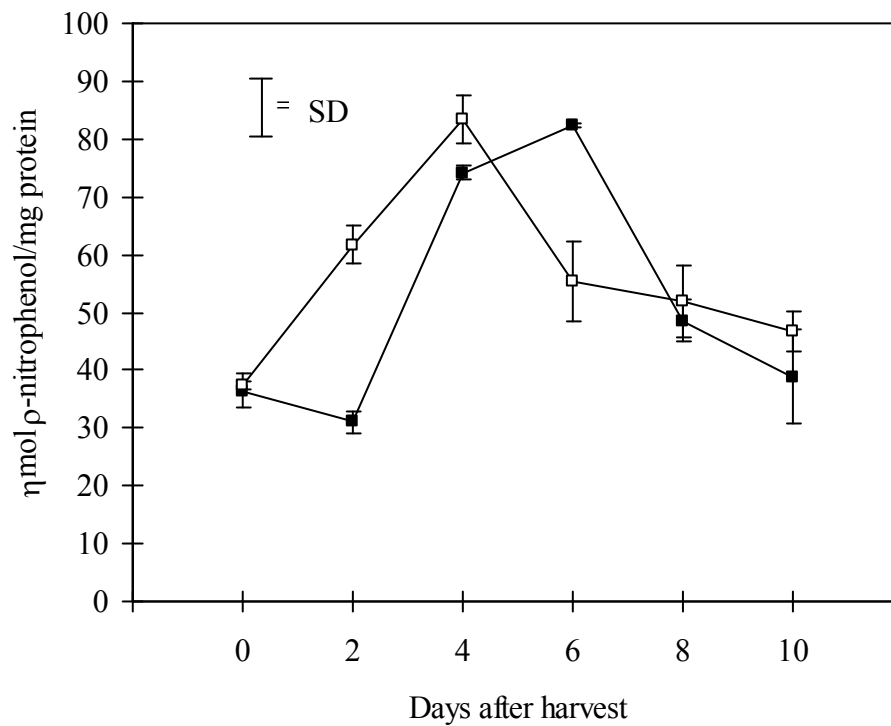


Figure 16 β -galactosidase activity in the husk (■) and the DZ (□) of 'Monthong' durian harvested at 106 days after anthesis and then stored at room temperature ($28\pm 3^\circ\text{C}$) for 10 days.

┃ = standard deviation of mean for each treatment

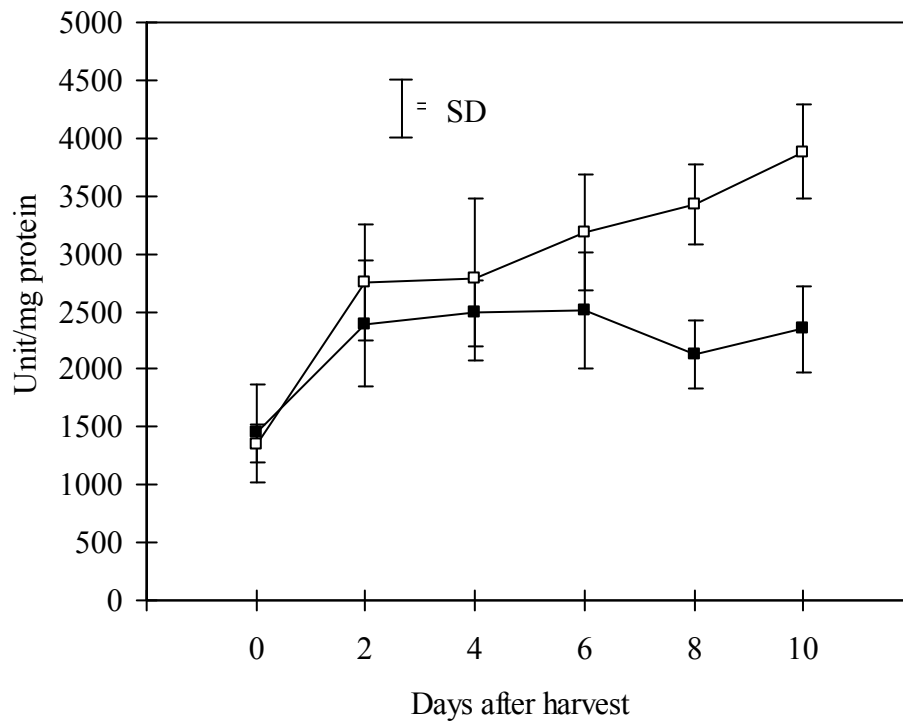


Figure 17 Endo (1,4)β-D-glucanase activity in the husk (■) and the DZ (□) of ‘Monthong’ durian harvested at 106 days after anthesis and then stored at room temperature ($28\pm 3^{\circ}\text{C}$) for 10 days.

┆ = standard deviation of mean for each treatment

PART II

Effect of Gibberellic Acid (GA₃) on Durian Fruit Dehiscence

1. Dehiscence Scores

Dehiscence in durian fruits stored at room temperature started on day 2 in the control while it started on day 4 for the GA₃ treated fruits. The dehiscence increased steadily in a similar manner in both treatments. At the end of storage, the percentage of dehisced fruit was about 50% in control and only 10-30% in GA₃ treated ones. The dehiscence score was about 2.5 and 3.5, respectively (Figure 18).

2. Percentage of Weight Loss

During 8 days in storage, the percentage of weight loss in the control fruit was rather steady at about 2% per day. There was a slightly lower weight loss in the GA₃ treated fruit (Figure 19).

3. Change in Peel Color

The treatment with GA₃ preserved the green color of the husk as could be seen from the L value scale that increased only slightly. In control, the L value scale markedly increased after day 2 to a maximum on day 6, and then declined (Figure 20).

4. Fractionation of Cell Wall Polysaccharides

4.1 Change in pectin fraction

Water-soluble pectin (WSP) increased from about 100 µg at harvest to about 200 galacturonic acid/g fresh weight on day 6 in the control and GA₃ treated fruits, respectively. There was a significantly higher amount of WSP in the control than in the GA₃ treated fruits (Figure 21). CSP content in the control was slightly higher than in the GA₃ treated fruit. The content remained rather stable in both treatments during the first 6 days in storage and then increased on the last day (Figure 22). Na₂CO₃-soluble pectin remained relatively unchanged during 6 days in storage, but then on the last day, increased to about 175 µg and 150 µg galacturonic acid/g fresh weight in the control and GA₃ treatments, respectively. The pattern of changes in this fraction in the two treatments roughly paralleled one another (Figure 23).

4.2 Change in Hemicellulose fractions

Changes in hemicellulose fractions (1 and 4 M KOH) were not significantly different between control and GA₃ treatments (Figure 24 and 25). The amount of 1 M KOH-soluble fraction decreased from 75 µg galacturonic acid/g fresh weight at the beginning to 30 µg galacturonic acid/g fresh weight on day 8 (Figure 24). The amount of 4 M KOH-soluble fraction decreased more than the 1 M KOH-

soluble fraction from 100 μg galacturonic acid/g fresh weight at the beginning of storage to 60 μg galacturonic acid/g fresh weight on day 8 (Figure 25).

5. Enzyme Activities

PME activity at the beginning was about 20 ηmol acetic acid/mg protein. The activity increased more in the control than in GA_3 treatment, reaching its maximum level on day 8. The activity in GA_3 treated fruits increased slightly, reaching a peak on day 6 then declined (Figure 26).

PG showed activity at the beginning with about 40 μg galacturonic acid/g fresh weights and remained stable during the first 2 days. The activity in both, control and GA_3 treated fruits, markedly increased during day 4 to day 8, but was higher in the control fruits than in the GA_3 treated fruits (Figure 27).

β -gal activity increased sharply in the control from about 40 ηmol ρ -nitrophenl/mg protein at the beginning, to 70 ηmol ρ -nitrophenl/mg protein on day 2, and remained unchanged thereafter. In GA_3 treated fruits, the activity slowly increased on day 4 to 55 ηmol ρ -nitrophenl/mg protein, and remained stable thereafter (Figure 28).

EGase activity at the beginning was about 38 μg glucose reducing group equivalents/g fresh weight. The activity of this enzyme increased in the controls from day 2 and later in the GA_3 -treated fruits. By the end of storage the activity of this enzyme was around 100 μg glucose/ g fresh weight in both tissues (Figure 29).

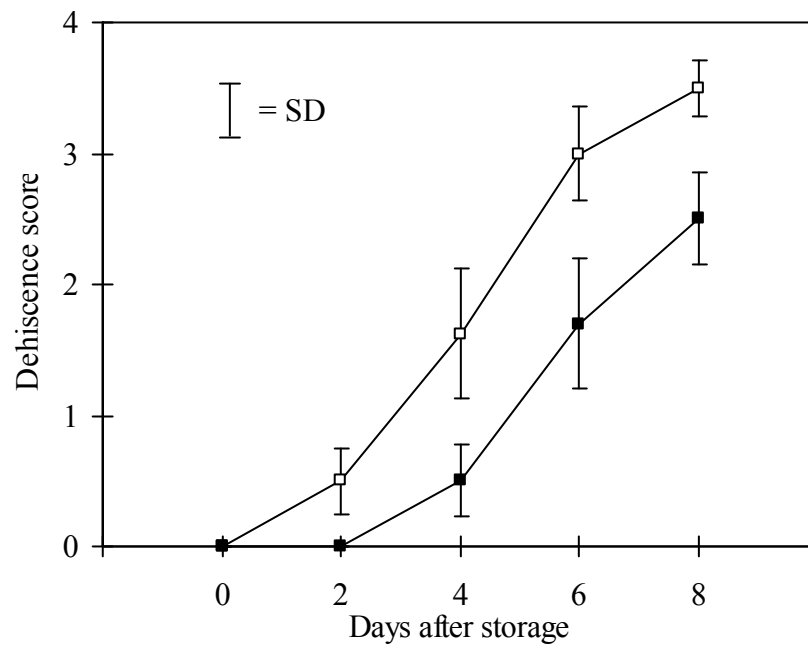


Figure 18 Average dehiscence score in the control (□) and GA₃ treated (■) 'Monthong' durian harvested at 106 days after anthesis and then stored at room temperature (28±3°C) for 8 days.

┆ = standard deviation of mean for each treatment

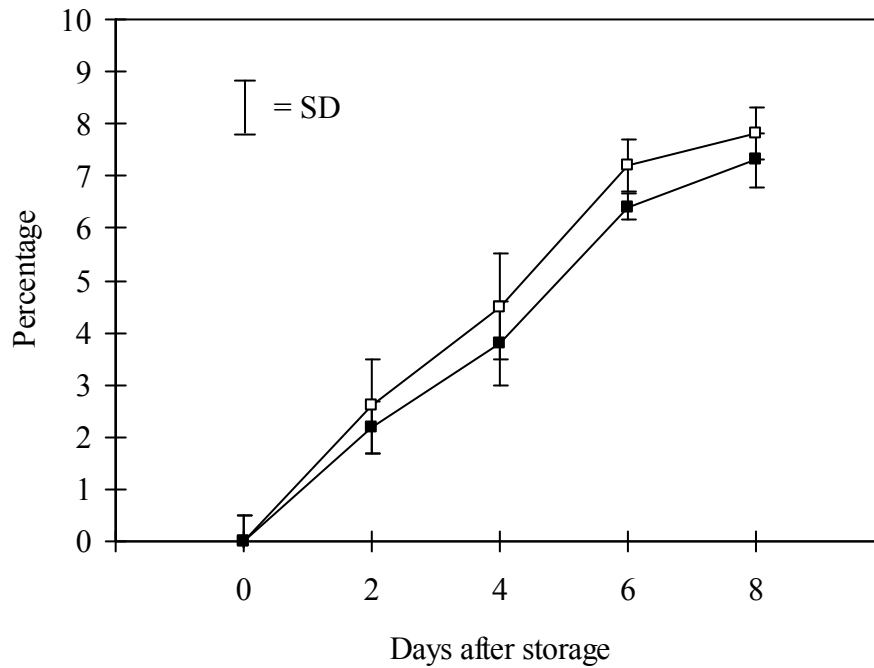


Figure 19 Percentage of weight loss in the control (□) and GA₃ treated (■) 'Monthong' durian harvested at 106 days after anthesis and then stored at room temperature ($28\pm 3^{\circ}\text{C}$) for 8 days.

┆ = standard deviation of mean for each treatment

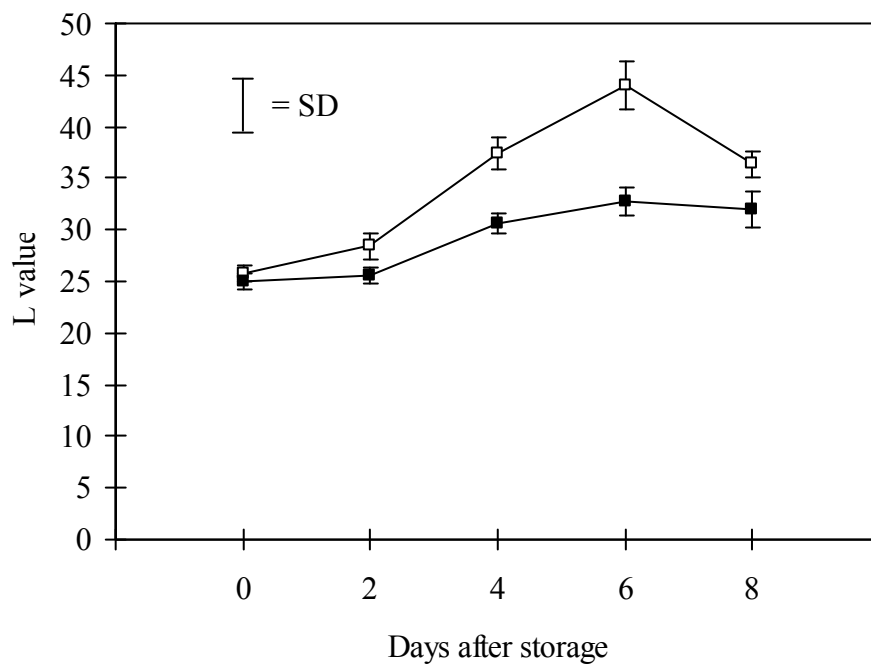


Figure 20 L-value scale in the control (□) and GA₃ treated (■) 'Monthong' durian harvested at 106 days after anthesis and then stored at room temperature (28±3°C) for 8 days.

┆ = standard deviation of mean for each treatment

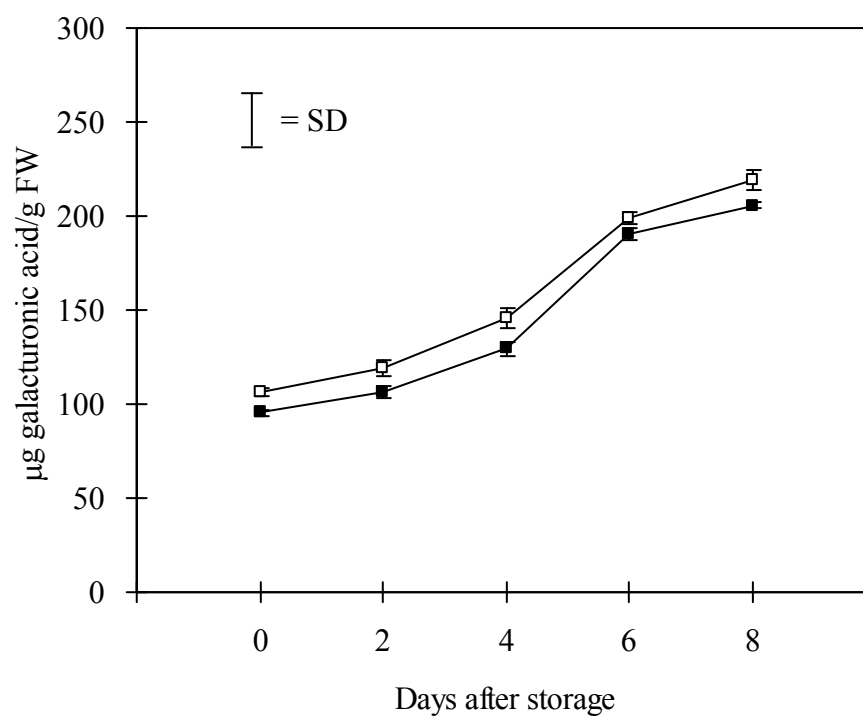


Figure 21 Water-soluble pectin from the DZ of the control (□) and GA₃ treated (■) of 'Monthong' durian harvested at 106 days after anthesis and then stored at room temperature (28±3°C) for 8 days.

┃ = standard deviation of mean for each treatment

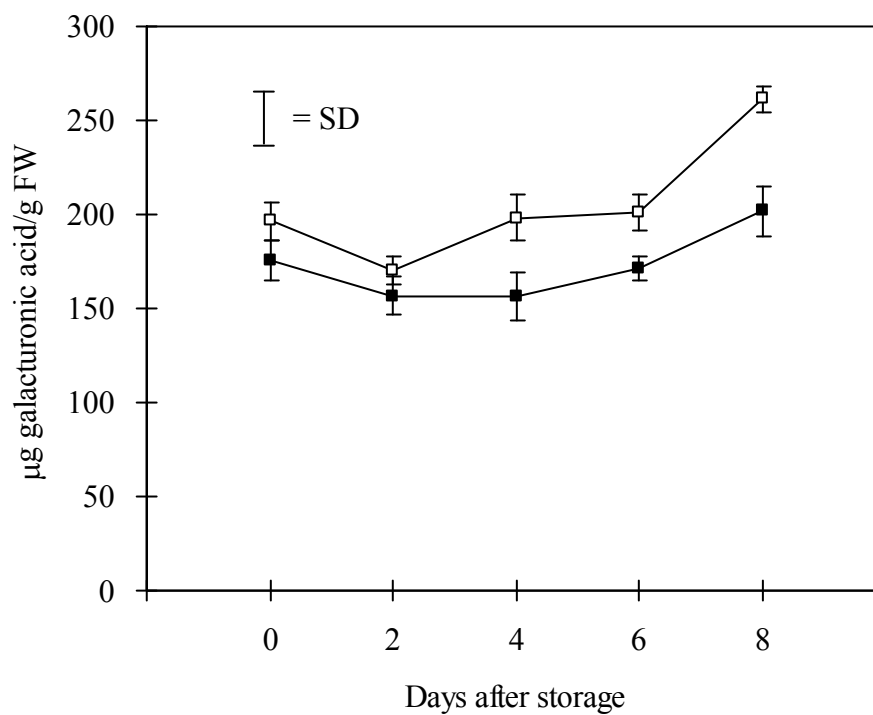


Figure 22 Chelator-soluble pectin from the DZ of the control (□) and GA₃ treated (■) 'Monthong' durian harvested at 106 days after anthesis and then stored at room temperature (28±3°C) for 8 days.

┆ = standard deviation of mean for each treatment

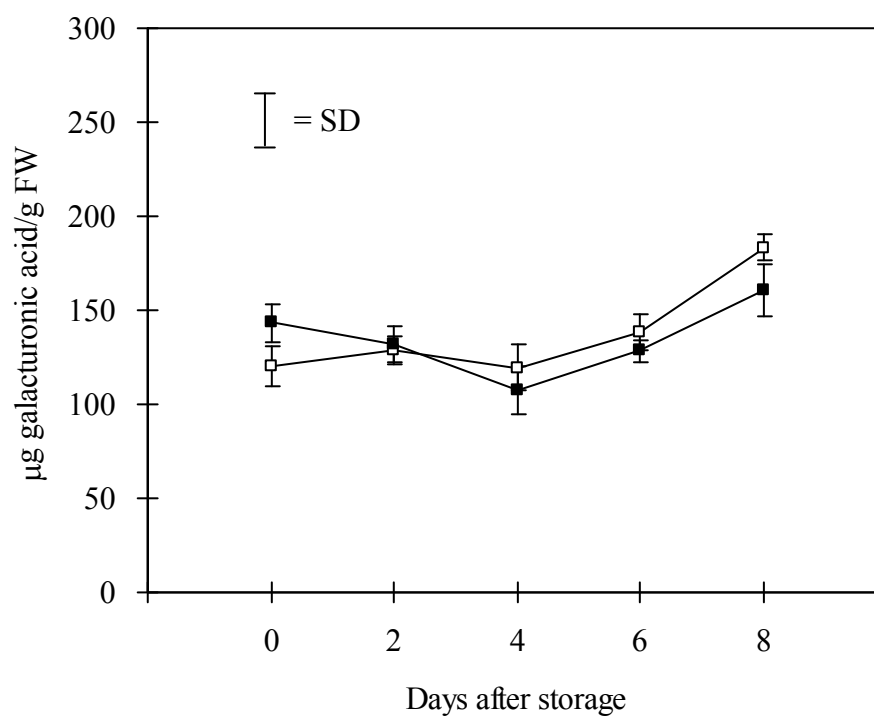


Figure 23 Na_2CO_3 -soluble pectin from the DZ of the control (□) and GA_3 treated (■) 'Monthong' durian harvested at 106 days after anthesis and then stored at room temperature ($28\pm 3^\circ\text{C}$) for 8 days.

┆ = standard deviation of mean for each treatment

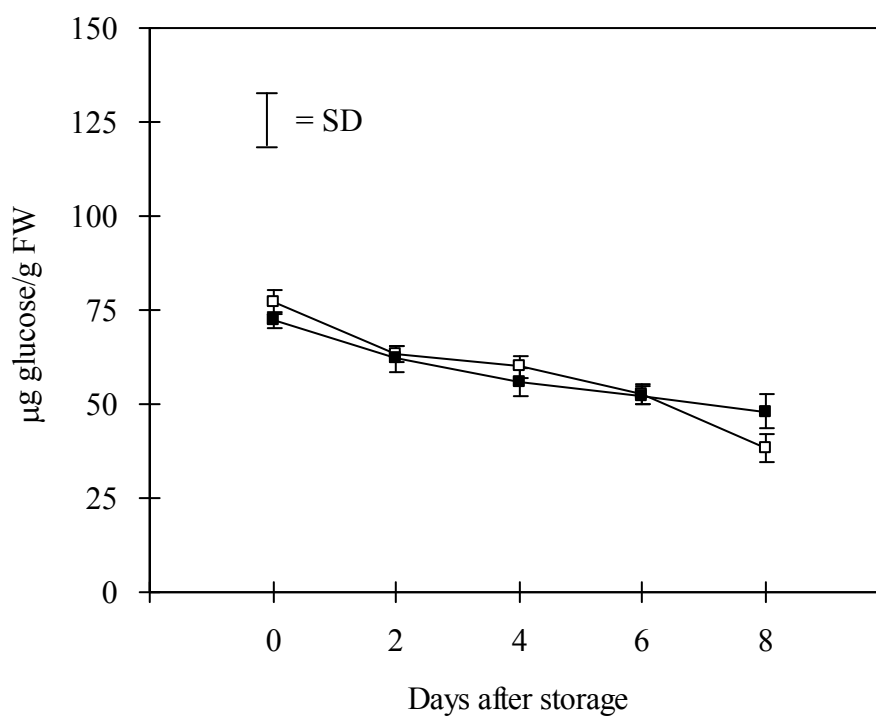


Figure 24 1 M KOH-soluble fraction from the DZ of the control (□) and GA₃ treated (■) 'Monthong' durian harvested at 106 days after anthesis and then stored at room temperature (28±3°C) for 8 days.

┆ = standard deviation of mean for each treatment

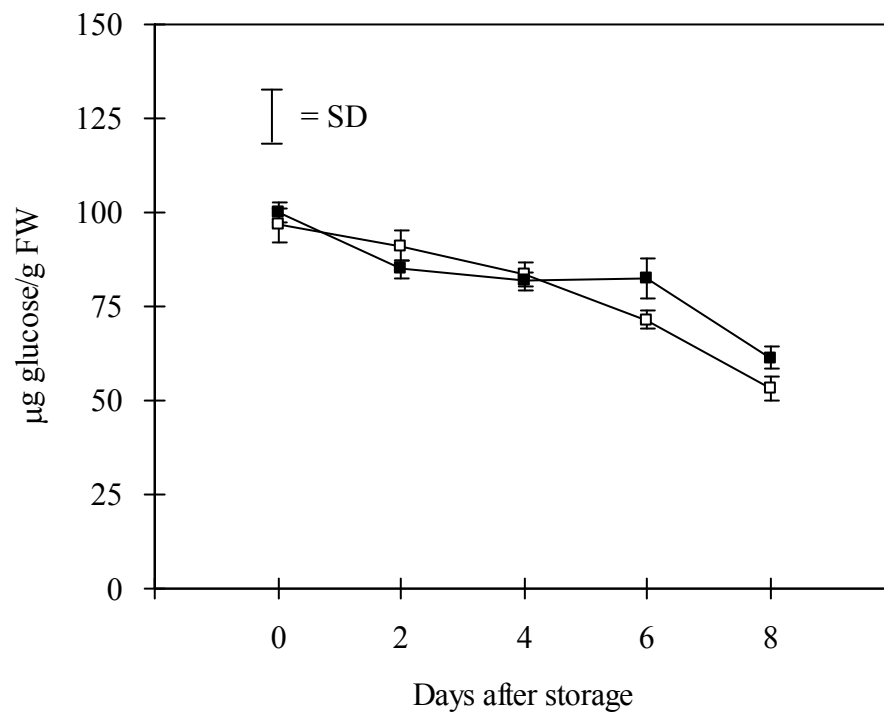


Figure 25 4 M KOH-soluble fraction from the DZ of the control (□) and GA₃ treated (■) 'Monthong' durian harvested at 106 days after anthesis and then stored at room temperature (28±3°C) for 8 days.

┆ = standard deviation of mean for each treatment

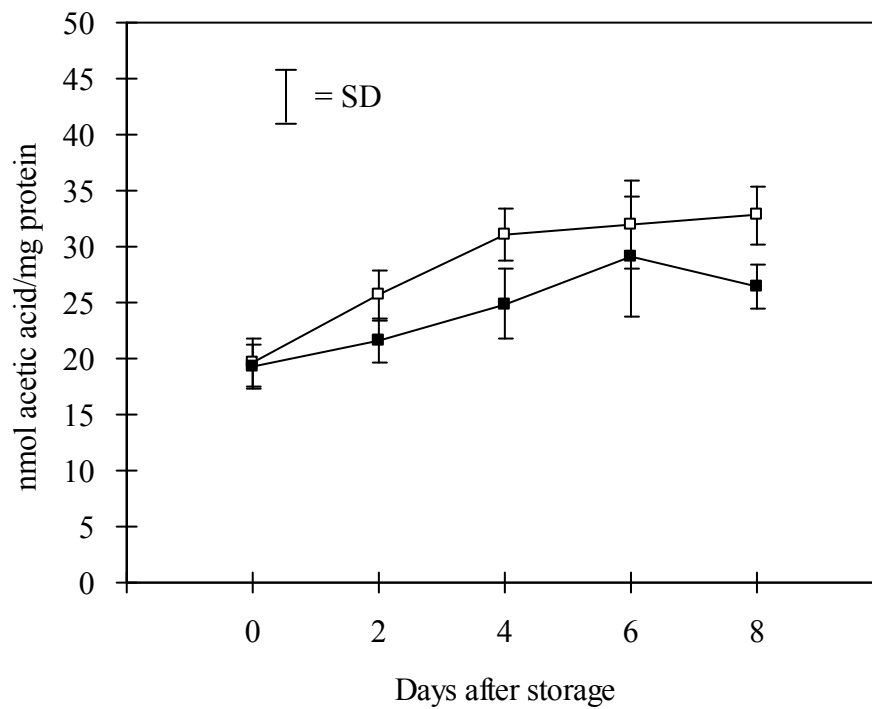


Figure 26 Pectinmethylesterase activity control (□) and GA₃ treated (■) 'Monthong' durian harvested at 106 days after anthesis and then stored at room temperature (28±3°C) for 8 days.

┃ = standard deviation of mean for each treatment

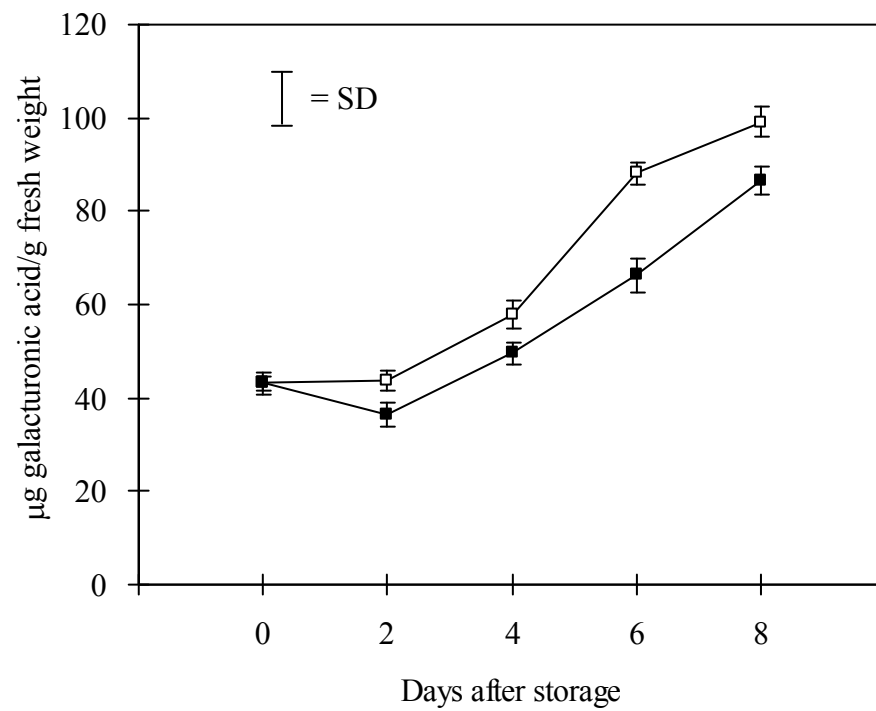


Figure 27 Polygalacturonase activity from the DZ of the control (\square) and GA_3 treated (\blacksquare) 'Monthong' durian harvested at 106 days after anthesis and then stored at room temperature ($28\pm 3^\circ\text{C}$) for 8 days.

$\bar{\text{I}}$ = standard deviation of mean for each treatment

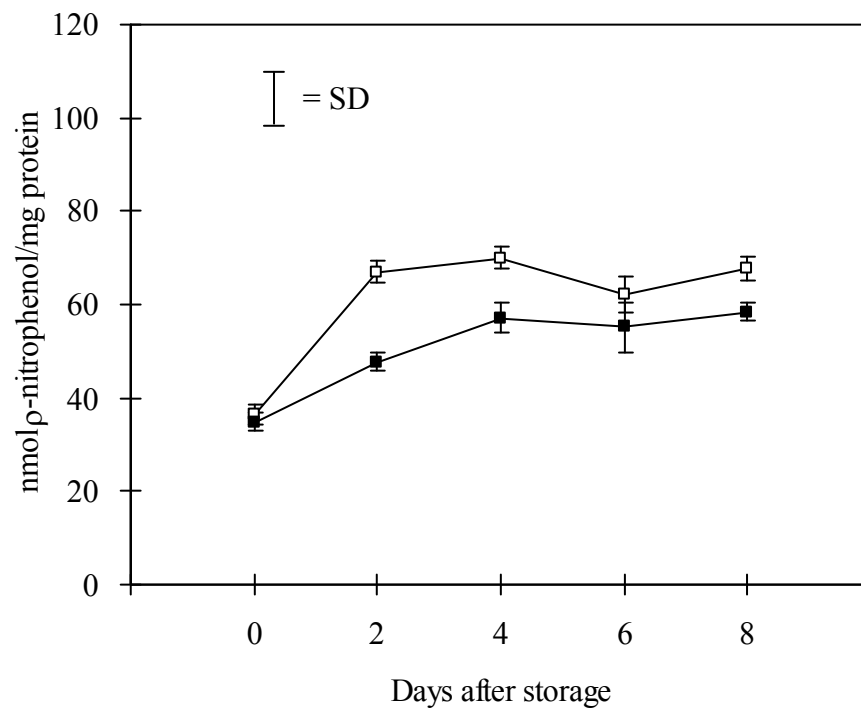


Figure 28 β -galactosidase activity from the DZ of control (□) and GA₃ treated (■) 'Monthong' durian harvested at 106 days after anthesis and then stored at room temperature ($28\pm 3^\circ\text{C}$) for 8 days.

┆ = standard deviation of mean for each treatment

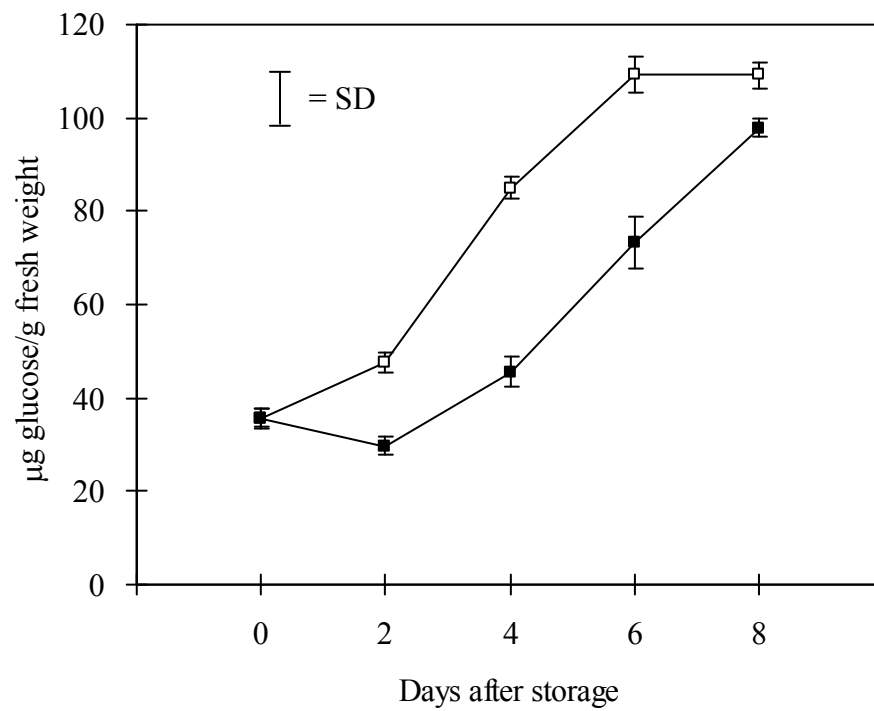


Figure 29 Endo-(1,4)β-D-glucanase activity from the DZ of the control (□) and GA₃ treated (■) 'Monthong' durian harvested at 106 days after anthesis and then stored at room temperature (28±3°C) for 8 days.

┆ = standard deviation of mean for each treatment