

Polygalacturonase and Pectin Methylesterase Activities of CaCl₂ Treated Red-Fleshed Dragon Fruit (*Hylocereus polyrhizus*) Harvested at Different Maturity

¹Awang, Y.B., ¹S.H. Chuni, ¹M.T.M. Mohamed, ²Y. Hafiza and ¹R.B. Mohamad

¹Faculty of Agriculture, Universitiy Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia

²Faculty of Science and Technology, Universitiy Sains Islam Malaysia,
Bandar Baru Nilai, 71800, Nilai, Negeri Sembilan Malaysia

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ABSTRACT

Fruits harvested at different maturity possess different biochemical constituents and physiological properties that make the fruits may react somewhat differently to the postharvest treatment. A study to examine the activity of Polygalacturonase (PG) and Pectin Methylesterase (PME) enzymes during storage in dragon fruit (*Hylocereus polyrhizus*) harvested at 28 days (Index 3) and 34 days (Index 5) after anthesis and postharvest treated with 0, 2.5, 5.0 and 7.5 g L⁻¹ CaCl₂ was performed. The PG activity was lower in younger fruit and vice-versa for PME activity. Increasing concentration of CaCl₂ effectively reduced the activity of both enzymes. PG activity for fruit treated with 0, 5 and 7.5 g L⁻¹ CaCl₂ increased linearly with the time of storage while its activity for the fruit treated with 2.5 g L⁻¹ CaCl₂ was lower at the beginning of storage. PG activity of Index 5 fruits increased almost linearly during storage while its activity in Index 3 fruits was low at the early days of storage and later continued to increase until day seven. At both maturity indices, the PME activity was low at the early days of storage and later continued to increase until day seven. Overall, results obtained indicated that CaCl₂ postharvest treatment reduced both PME and PG activities thus slowing down the softening process giving an evident that calcium possess a distinguishable role in the reducing softening of fruit, regardless of maturity index.

Keywords: Dragon Fruit, Cell Wall Degrading Enzymes, Postharvest CaCl₂ Treatment

1. INTRODUCTION

Red-fleshed dragon fruit (*Hylocereus polyrhizus*) is a climbing cacti and has gained popularity due to its highly nutritious and delicious fruits. The fruit is normally harvested at its full maturity stage (33-35 days after anthesis) and at this stage, the fruit is characterized by its bright red skin with dark red flesh. Fruits harvested at full maturity may have a short storage life, which is partially associated with the disintegration of cell wall that bring about changes in fruit firmness which is largely linked with the activity of cell wall degrading enzymes, including Polygalacturonase (PG), Pectin Methylesterase (PME) and β -galactosidase.

Dragon fruit is a fast developing fruit. Its maturation and ripening are coupled with the increasing concentration of soluble solids and ascorbic acid contents, alongside with decreasing in fruit firmness. The fruit pH is generally decrease with the advancement of fruit ripening, followed by an increase in the pH as the ripening progressed (Novita, 2008) and this make the fruit more palatable. Therefore, harvesting the fruit at varying maturity would give fruits of different physico-chemical and organoleptic quality. Younger fruits would be firmer, contains higher concentration of acids but generally having a lower sugar content. More mature fruit would contain relatively lower acids with higher sugar concentration but it could have a soft texture. The

Corresponding Author: Awang, Y.B., Faculty of Agriculture, Universitiy Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia

fruit therefore is expected to have different activity of cell wall enzymes. This study dealt with two cell wall enzymes; polygalacturonase and pectin methylesterase. Changes in cell wall integration and fruit softening is brought about by a coordinated action of hydrolytic enzymes on the cell wall that target to the pectic matrix which occurs together with other biochemical and physiological activities within the cell which convert the fruit become more palatable.

Previous research has shown that calcium plays a significant role in reducing mechanical damage of both climacteric and non-climacteric fruits (Lamikanra and Watson, 2004). If the activities of PG and PME can be reduced in the whole fruit via application of calcium in dragon fruit as observed in the fresh-cut dragon fruit (Chuni *et al.*, 2010), the longevity of the fruit can be extended. By treating the fruit with calcium, the rate of firmness loss is expected to be reduced as calcium would reduce the cell wall degrading enzymes activity. In this study, changes in the activities of polygalacturonase and pectin methylesterase were investigated on dragon fruit harvested at two different maturity indices (Index 3 and 5) treated with varying levels of CaCl₂ during 7 days of storage.

2. MATERIALS AND METHODS

2.1. Plant Material and Post-Harvest CaCl₂ Treatment

Dragon fruits (*Hylocereus polyrhizus*) of uniform size with maturity indices 3 and 5 (corresponding to 28 and 34 days after anthesis, respectively) were harvested from a commercial farm in Nilai, Negeri Sembilan, Malaysia. The fruits were then rinsed with tap water, air-dried and soaked in Tween-20 for 5 min and left to dry at room temperature (25°C). Once dried, the fruits were dipped into four levels of CaCl₂ concentration (0, 2.5, 5.0 and 7.5 g L⁻¹) for two hours and air-dried. Zero (0 min) duration of dipping is referred to as a very quick dip of less than 5 sec. The flesh of the fruit was cut into small cubes (~1 cm³) for analysis.

2.2. Enzyme Extraction

Extraction of PG and PME enzymes was performed as described in Chuni *et al.* (2010). Ten g of tissues were homogenized using a domestic blender in 20 mL of a buffer solution containing 0.1 M sodium citrate, 1 M NaCl, 13 mM EDTA, 10 mM β-mercaptoethanol and 2% (w/v) polyvinylpyrrolidone (PVP-40) at pH 4.6. The extracts were left for 30 min with occasional stirring. The supernatants were recovered by centrifugation at

29000×g for 30 min and kept at 4°C in order to extend the shelf life of the enzymes (up to one month) and prevent significant loss in their activity.

2.3. Assay of Enzymes

Polygalacturonase (PG, EC 3.2.1.67) activity was measured by the 2-cyanoacetamide method based on the spectrophotometric determination of reducing groups released (Gross, 1982). The enzyme was assayed in a solution made using 0.75 mL of 1.5% (w/v) polygalacturonic acid, 0.1 ml sodium acetate (0.1 M) and 1.0 mL supernatant at pH 5.2, adjusted using HCl. The mixture was incubated for 1 hour at 37°C. The absorbance was measured by using a spectrophotometer (Model PRIM Light 230V) at 276 nm. Monogalacturonic acid was used as the standard to establish the calibration. PG activity was expressed as nkatal/g Fresh Weight (FW). One nkatal was defined as the amount of enzyme that releases in mol of reducing group (monogalacturonic acid) per one hour.

Pectin Methylesterase (PME, EC 3.1.1.11) was assayed using 0.5 mL of crude extract added to a buffer solution containing 25 mL 1% (w/v) pectin, 0.3 M NaCl and 0.1 ml acetate buffer (0.1 M, pH 4.5) and titrated with 0.01 N NaOH until the reading stable at pH 7.3 using a glass electrode pH meter (CRISON GLP 21) at 30°C. Enzyme activity was expressed as nequivalent carboxyl group.g⁻¹.s⁻¹ Fresh Weight (FW) using the following equation:

$$\text{Vol. NaOH} \times \frac{\text{Vol. crude extract}}{\text{Assay vol. (0.5 ml)}} \times \frac{1 \times 310^{-3} \text{ L}}{10 \text{ g sample}} \times \frac{0.01 \times 10^9 \text{ neqv}}{10 \text{ min} \times 60 \text{ sec}}$$

2.4. Data Analysis

The experiment was conducted in a Complete Randomized Design (CRD) with three replications. Data obtained were subjected to Analysis of Variance (ANOVA) and means comparisons were performed by using Least Significance Difference (LSD) at p≤0.05 level with SAS package (version 9.0, Cary, NC, USA). Regression analysis was also carried out to examine the trend of the response of the enzymes vs. time of storage for different concentrations of CaCl₂.

3. RESULTS AND DISCUSSION

3.1. Activity of Polygalacturonase

Changes in the PG activity in fruit at two different stages of maturity as affected by post-harvest calcium treatment were shown in **Table 1** and **Fig. 1**.

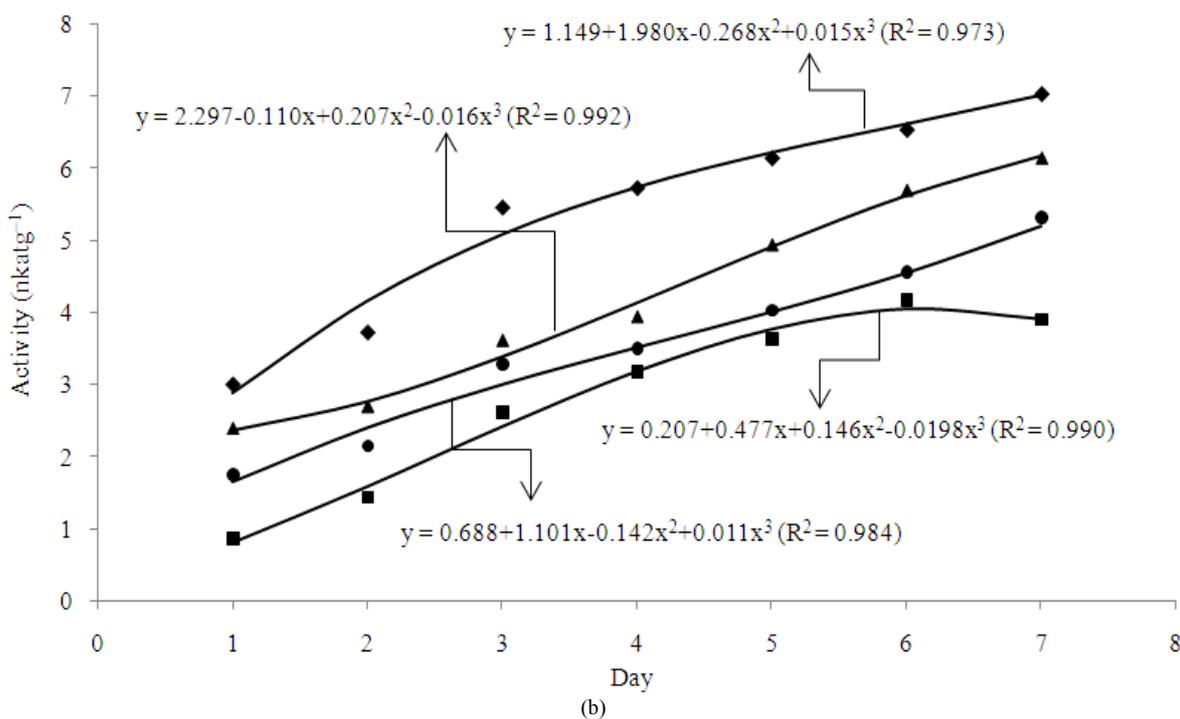
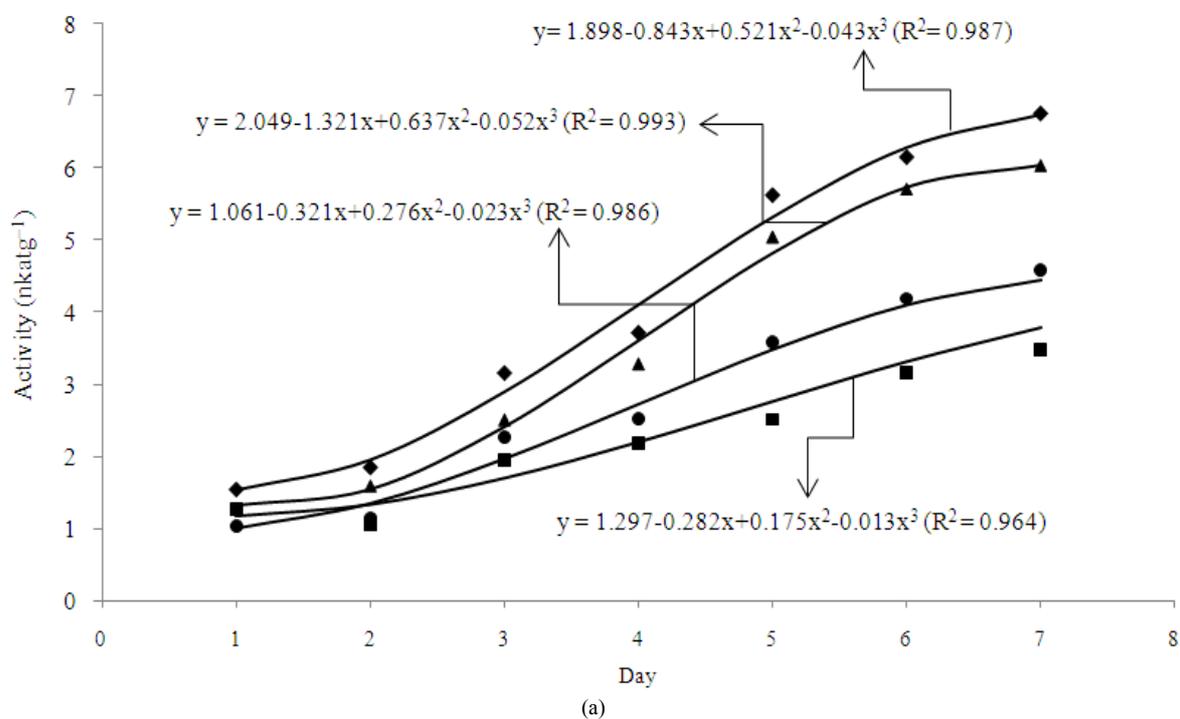


Fig. 1. Changes in the activity of Polygalacturonase (PG) as affected by CaCl₂ concentration; (a) PG in fruit maturity index 3, (b) PG in fruit maturity index 5, treated with four concentration of CaCl₂

Table 1. Effects of different CaCl₂ concentration and maturity indices on the activities of PG and PME enzymes of *Hylocereus polyrhizus*

Maturity index	CaCl ₂ concentration (g/L)	PG activity (nkat/g FW)	PME activity (neqv g ⁻¹ s ⁻¹)
Index 3	0.0	4.16	51.18
	2.5	3.63	44.16
	5.0	2.77	33.91
	7.5	2.22	27.95
Index 5	0.0	5.38	37.86
	2.5	4.20	31.46
	5.0	3.50	26.44
	7.5	2.82	21.05
F-test (Significant level)			
Maturity Index		***	***
CaCl ₂ Concentration		***	***
Interaction		**	***
LSD _{0.05}		0.19	1.81

Note: ** and *** denote significant at p<0.01 and p<0.001, respectively

Analysis of variance shows that PG activity differed significantly (p<0.05) between maturity indices. PG activity was higher in more ripen fruit (Index 5) compared to Index 3 fruit (p<0.05), a result paralleled to the one obtained in previous study (result not shown). Result in **Table 1** also indicated that different CaCl₂ concentration significantly affected the PG activity for both maturity indices (p<0.05). Fruits treated with 7.5 g L⁻¹ CaCl₂ gave the lowest PG activity, followed by fruit treated with 5, 2.5 and 0 g L⁻¹ CaCl₂ for both maturity indices. There was also an interaction found between maturity indices and different calcium concentration (p<0.01). It can be stated that dragon fruit with different maturity and treated with varying levels of CaCl₂ concentration acted together to altered the PG activity.

Figure 1 shows that activity of PG for fruit treated with varying levels of CaCl₂ concentration differed significantly and behave differently over time as indicated by significant interaction between CaCl₂ concentration and storage time (p<0.001). The lowest PG activity for fruit with maturity index 3 (**Fig. 1a**) after seven days occurred in fruit treated with 7.5 g L⁻¹ CaCl₂ followed by fruit treated with 5 g L⁻¹ CaCl₂ with their respective values of 2.22 and 2.77 nkat g⁻¹ FW. Similar result was obtained for fruit with maturity index 5 (**Fig. 1b**). For both maturities, the trend of the response curves was almost similar. The PG activity increased with the storage time but the value was decreased as the CaCl₂ concentration increased. Low PG activity obtained from these two treatments reflected the depressing effect of varying CaCl₂ concentration and maturity indices on PG activity of dragon fruit.

Post-harvest application of CaCl₂ has been proven to reduce the enzymes levels and increase the neutral

sugar in fruits (Manganaris *et al.*, 2005). Exogenously applied calcium binds the negative charges of de-esterified uranic acid residues generated by PME during ripening and therefore enhancing the tissue's mechanical strength (Magee *et al.*, 2003). Previous research done on freshly cut cantaloupes has shown that CaCl₂ treatment improves the fruit firmness and quality (Luna-Guzman *et al.*, 1999). These results might indicate that CaCl₂ has reduced the PG activity thus slowing down the softening process. It is therefore evident that calcium possess a distinguishable role in the reducing softening of fruit.

3.2. Activity of Pectin Methylesterase

Table 1 and **Fig. 2** show the changes in PME activity of fruit with two maturity indices that has been given a post-harvest CaCl₂ treatment. Like PG, there was a significant difference (p<0.05) in PME activity between maturity indices in PME. Contrary from PG activity, PME activity was higher in unripe fruit (Index 3) compared to more mature fruit (Index 5). Beside maturity index, varying levels of CaCl₂ concentration also contributed to the significant effect of PME activity for both maturity indices (p<0.05). High CaCl₂ concentration reduced the PME activity. At 0 g L⁻¹ CaCl₂, the activity of PME was the highest, followed by fruit treated with 2.5, 5 and 7.5 g L⁻¹ CaCl₂. Maturity index and calcium concentration itself altered the PME activity markedly (p<0.001). The effect of CaCl₂ on PME activity was different for fruits of varying maturity stages as shown by a significant interaction between maturity index and calcium concentration (p<0.001).

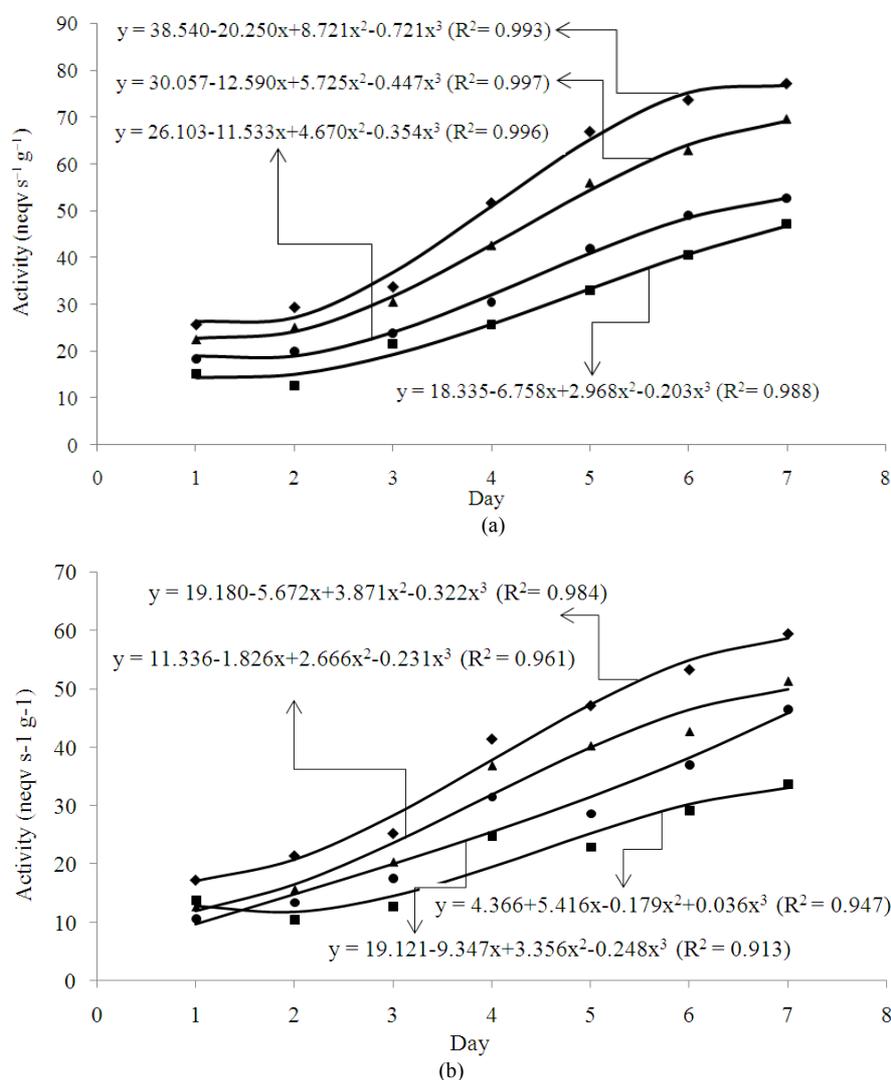


Fig. 2. Changes in the activity of Pectin Methylsterase (PME) as affected by CaCl₂ concentration; (a) PME in fruit maturity index 3, (b) PME in fruit maturity index 5, treated with four different concentration of CaCl₂ concentration. (◆ 0 g L⁻¹, ▲ 2.5 g L⁻¹, ● 5.0 g L⁻¹, ■ 7.5 g L⁻¹)

Response curve for PME (**Fig. 2**) show that over storage time, there was a significant difference ($p < 0.001$) for PME activity of fruit treated with different level of CaCl₂ for both maturity indices. The trends of the curve were similar for both maturity indices. There was a sharp increase in PME activity after four days of storage for fruit treated with 0 g L⁻¹ CaCl₂. Like PG, PME activity for all treatments increased over time but when the Ca concentration increased, the PME activity was decreased.

Post-harvest dips in CaCl₂ solutions allows the formation of COO⁻ groups from the pectin content of the fruits with which Ca²⁺ ions can form salt-bridge cross-

links (Saftner *et al.*, 2003). This makes the cell wall less accessible to the softening enzymes. Since PG only hydrolyses homogalacturonan regions whose uranic acid residues have been previously demethylated by PME (De Assis *et al.*, 2001) and since pectins are synthesized and deposited on the cell wall (Staehelin and Moore, 1995), the negative charges generated by PME are necessary for calcium binding to the cell wall and to bring out calcium's firming effects. Thus, the calcium application to the fruit can significantly contribute to the texture retention in fruit. Furthermore, increasing Ca concentration in dragon fruit has been beneficial in

reducing incidence of postharvest diseases (Awang *et al.*, 2011; 2013).

4. CONCLUSION

PG and PME acted differently depend on the maturity index of the fruit. PG activity was higher in more mature fruit (Index 5) while PME activity was higher in more unripe fruit (Index 3). Clear evidence on the depressing effect of CaCl₂ on PG and PME activities suggests that the salt could be used as a post-harvest treatment on dragon fruit to prolong the shelf-life of the fruit. Higher concentration of CaCl₂ (7.5 g L⁻¹) can effectively reduce the PG and PME activities compared to 0, 2.5 and 5 g L⁻¹ CaCl₂. By giving the pre-harvest calcium treatment in early stage (Index 3), perhaps the PME activity can be reduced further and this can lead to the reduction of PG activity as well.

5. ACKNOWLEDGEMENT

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