



Full Length Article

Cell Wall Enzymes Activities and Quality of Calcium Treated Fresh-cut Red Flesh Dragon Fruit (*Hylocereus polyrhizus*)

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ABSTRACT

This study was aimed at evaluating the effect of post-cut application of CaCl₂ on activity of polygalacturonase (PG) and pectin methylesterase (PME) and quality of fresh-cut dragon fruit (*Hylocereus polyrhizus*). Fruit slices were prepared from fully matured fruits before being dipped into three levels of calcium concentration (CaCl₂: 0, 2.5 & 7.5 g L⁻¹) at four durations of dipping (0, 4, 8 & 12 min). The activities of PG and PME enzymes of fruits extract were lower when treated with high concentration of CaCl₂ for a longer duration of dipping. The Ca treatment did not cause any marked effects on colour, pH, titratable acidity and ascorbic acid content. Soluble solids content and Ca content in cut fruit were affected by duration of dipping. The firmness of fruit slices treated at the highest CaCl₂ concentration (7.5 g L⁻¹) increased at the beginning of the treatment but reduced as the durations of dipping were extended to 8 and 12 min. Lack of a linear increase in tissue firmness of fresh-cut dragon fruit in response to high concentrations of CaCl₂ post-cut application showed that treatment should be administered with a great care to appropriate concentration of CaCl₂ duration of exposure are applied. © 2010 Friends Science Publishers

Key Words: Pitaya; Fruit quality; Polygalacturonase; Pectin methylesterase; Minimally processed fruit

INTRODUCTION

Softening of ripening fruits is associated with the alterations in cell wall and middle lamella structure, whereby the pectin wall is increasingly depolymerized and this process is accompanied by the increase in the level of water and chelator-extractable pectins (Cosgrove, 2001). Beside pectins, hemicellulose and cellulose also undergo a significant structural modification during ripening. The sequential disassembly of wall polysaccharides during fruit ripening may involve both covalent and non-covalent interactions mainly as the result of action of cell wall degrading enzymes (Chin *et al.*, 1999).

Cell wall degrading enzymes, also known as softening enzymes are a group of enzymes such as polygalacturanase (PG), β -galactosidase, pectin methylesterase (PME) and cellulase (Tavarini *et al.*, 2008). Activities of these enzymes lead to solubilization of cell wall pectin. Beside enzymes, there are many other factors that can contribute to the softening of fruits. This include differences in the architecture of the primary walls of different type of fruits, which may compose of different proportion of polysaccharides and glycoproteins (hydroxyproline-rich extensins), phenolic esters (ferulic & coumaric acids) and ionic and covalently bound minerals e.g., calcium and boron (Cosgrove, 2001).

Previous studies have shown that calcium plays a

significant role in reducing mechanical damage of fruit by retardation of firmness loss (Karakurt & Huber, 2003; Saftner *et al.*, 2003; Omaira *et al.*, 2007; Shafiee *et al.*, 2010; Oms-Oliu *et al.*, 2010). Calcium (Ca) ameliorates fruit firmness by binding to carboxyl groups of the pectic homogalacturonan backbone, as hypothesized by the egg-box model (Grant *et al.*, 1973) and may protect the pectic backbone PG-mediated depolymerization (Wehr *et al.*, 2004). Ca content in fruit dipped with Ca was found to increase in peach, when the fruits were immersed in two Ca concentrations (62.5 & 187.5 mM) and its positive effects were reported in term of reduced symptoms of chilling injury and activity of PG and PME (Manganaris *et al.*, 2007).

The increased in Ca was also found to be effective in quality retention of fresh-cut fruit. Wounding that occur during preparation of fresh cut fruit, among others cause disassembly of the pectin matrix, which is also mediated by the action of pectic enzymes (Brummell & Harpster, 2001). This wounding may increase the level of pectic enzymes as observed by Karakurt and Huber (2003) for PG and β -galactosidase in cut-papaya. Due to possible role of Ca in protecting the pectic backbone from the enzymes, Ca was found to maintain the freshness of various types of cut fruits e.g., cantaloupe (Luna-Guzmán *et al.*, 1999), honeydew (Saftner *et al.*, 2003), mango (Souza *et al.*, 2006) and strawberries (Aguayo *et al.*, 2006).

Despite many research reports on the effects of Ca concentration and its sources, not much work has been done to establish the combined effects of Ca concentration and duration of exposure on activity of cell wall enzymes and fruit quality. Among the limited reports, Luna-Guzmán *et al.* (1999) reported that firmness of cantaloupe cylinders dipped in CaCl₂ for 1 min showed the same effect as after 5 min dips. In this study, the effects of varying concentrations of CaCl₂ and dipping duration on activity of polygalacturonase (PG) and pectin methylesterase (PME) enzymes, as well as on various aspects of quality parameters were examined.

MATERIALS AND METHODS

Plant materials and Ca treatment: Fully ripened dragon fruits (33-35 days after anthesis) of uniform size were harvested from a commercial farm in Nilai, Negeri Sembilan, Malaysia. The fruits were peeled, quartered and dipped into three levels of Ca concentration (CaCl₂: 0, 2.5 & 7.5 g L⁻¹) at four durations of dipping (0, 4, 8 & 12 min). Zero (0 min) duration of dipping is referred to as a very quick dip of less than 5 seconds. After dipping, the fruit samples were kept in a cold room (12 ± 1°C) for five days and removed. The fruit qualities were analysed and crude extraction of PG and PME enzymes was performed.

Enzyme extraction and assay: Extractions of PG and PME enzymes were performed at 4°C as described by Zainon *et al.* (2004). 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 x g for 30 minutes and kept at 4°C.

The activity of the enzymes was assayed every day after extraction for seven consecutive days. The PG enzyme was assayed in a solution made using 0.75 mL of 1.5% (w/v) polygalacturonic acid, 0.1 mL 0.6 M NaCl and 1.0 mL supernatant at pH 5.2, adjusted using HCl. The mixture was incubated for 1 h at 37°C. Reducing sugars released was estimated by the cyanocetamide method (Gross, 1982) using monogalacturonic acid as the standard. PG activity obtained was expressed as ηkatal/g fresh weight (FW). On the other hand, PME was assayed using 0.5 mL of crude extract, added to a solution containing 25 mL of 1% (w/v) pectin and 0.3 M NaCl. The mixtures were titrated with 0.01 N NaOH to pH 7.3, which were stabilized for a minimum of 10 min. Enzyme activity was calculated and expressed as ηequivalent carboxyl group g⁻¹ s⁻¹ fresh weight (Zainon *et al.*, 2004).

Fruit analysis: Variations in firmness, color, soluble solids content (SSC), titratable acidity (TA), pH and ascorbic acid contents of flesh of the fruits following the treatments were analysed. The firmness was determined using a texture analyzer (Instron Universal Testing Machine, Model 5543,

Instron Corp, Canton, MA) by measuring the maximum penetration force (N) required during tissue breakage using a 5 mm diameter flat probe. The measurement of firmness was done at three locations for each sample. The color was determined using a chroma meter (Minolta CR-200 Chroma meter, Osaka, Japan) and was expressed as lightness (L), hue angle (h°) and chroma (C*). Color measurement was also done at three locations for each sample and white tile was used as standard for calibration.

Soluble solids content (SSC) of the fruits was determined using a digital refractometer meter (Model PR-32, Atago, Japan) by squeezing the juice onto prism of the refractometer, while titratable acidity (TA) was determined using diluted fruit juice (1 juice: 4 distilled water) prepared using the same fruits used for SSC measurement. Ten mL of the diluted juice was titrated with 0.1 N NaOH to pH 8.1 (Model CRISON GLP 21, Barcelona, Spain). The TA was calculated and expressed as percentage of citric acid. Ascorbic acid determination was carried out by using 2, 6-dichlorophenol-indophenol dye method (Ranggana, 1977). The reading was measured by using a spectrophotometer (Model PRIM Light 230V, Mainz, Germany) at 518 nm.

For determination of Ca content, the flesh of the fruits were further cut into smaller pieces and dried at 60°C in an air-circulating oven. A 0.25 g of the dried, finely ground fruit samples were digested in 5 mL of sulphuric acid (H₂SO₄) on hot plate at 450°C in a fume chamber for seven min. A 10 mL of hydrogen peroxide (H₂O₂) was added into the mixtures and the heating continued for another four min. The solution mixtures were made-up to 100 mL with distilled water. Ca content was measured using an atomic absorption spectrophotometer (Perkin Elmer, Model AAS 3110, Palo Alto, California).

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). 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 and dipping durations.

RESULTS

Polygalacturonase (PG) and Pectin methylesterase (PME) activities: Increasing Ca concentrations significantly reduced the activity of PG enzyme (*p* < 0.001) with average values decreasing from 5.21 to 4.87 and 3.29 nkatg⁻¹ FW as the concentration of CaCl₂ increasing from 0 to 2.5 and 7.5 g L⁻¹, respectively (Fig. 1). The effects of CaCl₂ concentration however, varied according to dipping duration (*p* < 0.001). The rate of reduction in PG activity in fruits treated with higher CaCl₂ concentration was faster than that treated with a lower CaCl₂ concentration. The average rate of reduction in PG activity for fruit treated with

2.5 g L⁻¹ CaCl₂ was 0.097 nkatg⁻¹ FW for every min increase in dipping time, while the corresponding value for fruit treated with 7.5 g L⁻¹ CaCl₂ was 0.138 nkatg⁻¹ FW (Fig. 1).

The activity of PG in crude extract of the Ca-treated fruit increased with time (Fig. 1) indicating that this enzyme continued to degrade cell wall at a higher rate as the storage time prolonged. Based on the increasing trend of PG activity, its activity can be expected to continue beyond day seven. At day seven, among the treated fruits at both CaCl₂ concentrations, the activity of PG of fruits dipped at a longer duration was lower (Figs. 1a & 1b).

The effects of Ca on PME activities of the fresh-cut red flesh dragon fruits were similar to the effects on the PG activities (Fig. 2). The activity of PME of the crude extract obtained from control fruits was 47.06 neqv g⁻¹ s⁻¹ and the corresponding values for fruits treated with 2.5 and 7.5 g L⁻¹ CaCl₂ were 42.06 and 38.50 neqv g⁻¹ s⁻¹, respectively. As for PG, the activity of PME continued to increase with time (days after crude extraction).

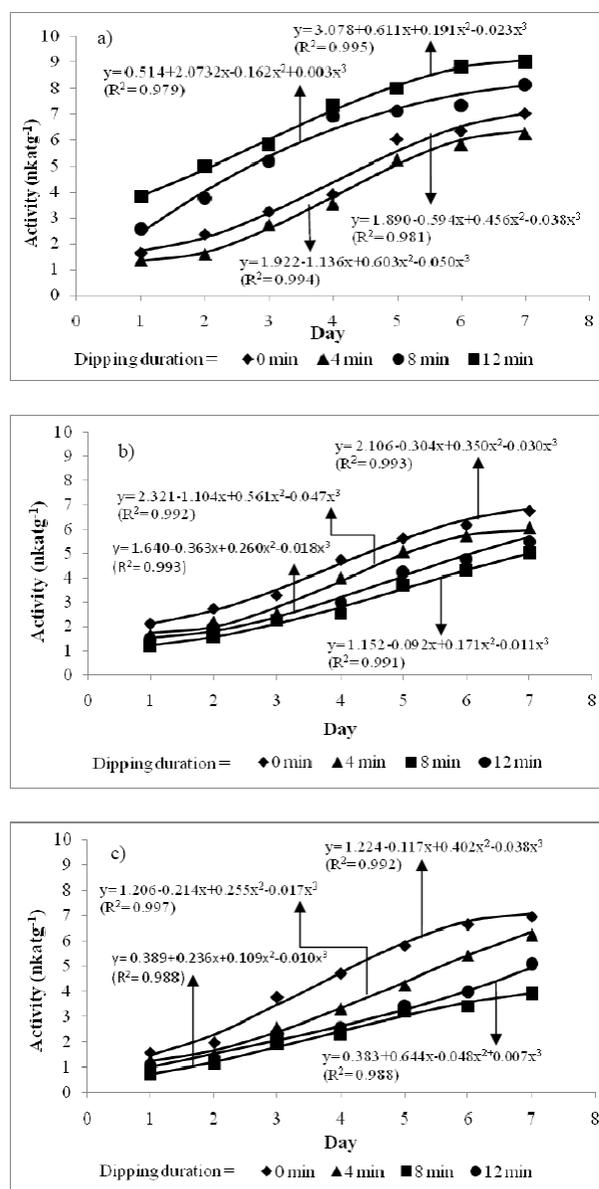
Fruit quality: The treated fruits were analysed for its firmness, soluble solids content (SSC), titratable acidity (TA), pH, Ca content, ascorbic acid content and colour on the same day as the samples were removed from the cold room. CaCl₂ concentration and dipping duration did not alter the concentration of titratable acids, pH and ascorbic acid of the fresh-cut dragon fruit (Table I). While Ca concentration did not affect the soluble solid content, it was significantly higher ($p < 0.05$) in fruits experiencing a longer duration exposure to CaCl₂. There was a significant interaction between Ca concentration and duration of dipping on fruit firmness (Fig. 3). However, the firmness of fruit slices treated at the highest CaCl₂ concentration (7.5 g L⁻¹) increased at the beginning of the treatment but decreased as the duration increased to 8 and 12 min. Increasing concentration of Ca in the dipping solution did not affect the Ca content in fresh-cut fruit, but the concentration increased when a longer dipping duration was administrated ($p < 0.05$). Variations in Ca concentrations and dipping durations did not affect all the colour components measured (Table I).

Table II presents correlation between parameters measured in this study. Apparently the activity of both cell wall enzymes were negatively correlated with the fruit Ca content (r for PG vs. Ca = -0.371, $p < 0.05$; r for PME vs. Ca = -0.381, $p < 0.05$). Results in the table also show that fruits with high Ca concentration contained low ascorbic acid ($r = -0.358$, $p < 0.05$).

DISCUSSION

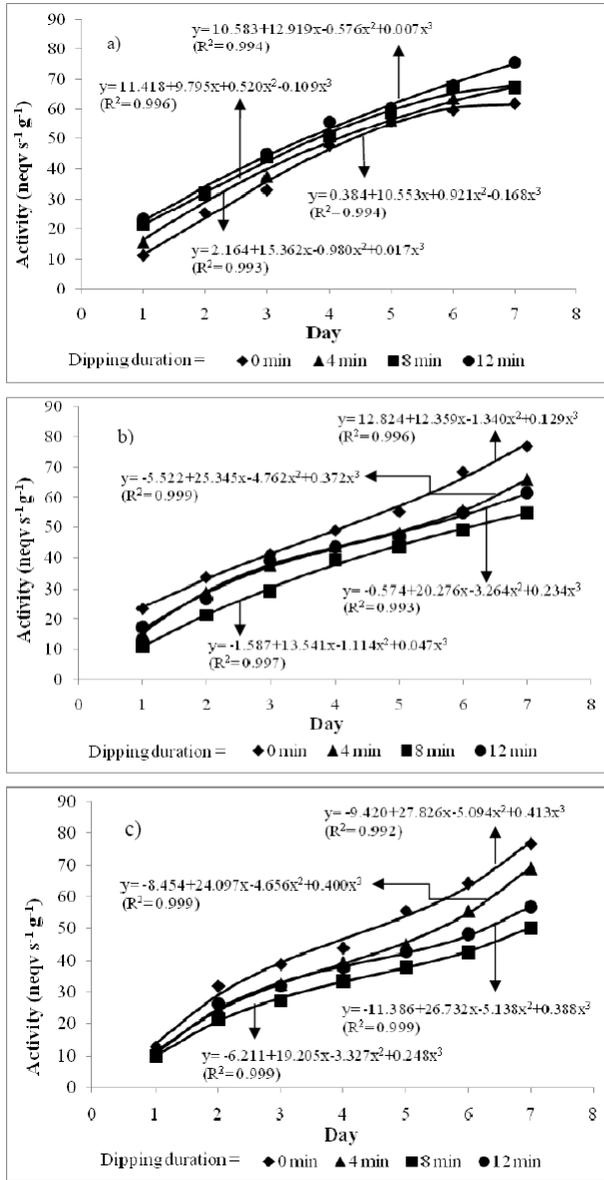
Softening of dragon fruit is paralleled with a gradual increase in activities of the major cell wall degrading enzymes such as PG, PME, β -galactosidase and cellulase and modification of the various pectic and hemicellulosic components of the cell wall. These enzymes may probably

Fig. 1: Changes in activity of polygalacturonase (PG) as affected by different concentration of CaCl₂ and duration of dipping (a) 0 g/L CaCl₂, (b) 2.5 g/L CaCl₂ and (c) 7.5 g/L CaCl₂



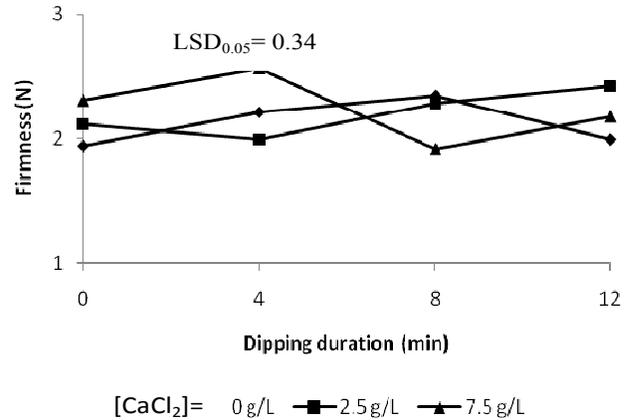
be significant to cell wall modifications at any stage of ripening (Almeida & Huber, 2007). However, there are several factors or treatments that can affect these enzymes activities. Several authors have suggested that factors such as pH and mineral composition may modify the catalytic activities of cell wall degrading enzymes (Huber & O'Donoghue, 1993; Giovane *et al.*, 1994; Chun & Huber, 1998; Almeida & Huber, 2007). Among the mineral composition, Ca perhaps is one of the most effective treatment to reduce cell wall degrading enzymes activities (Wehr *et al.*, 2004). Results suggested that PG and PME activities in fresh-cut dragon fruits can be altered by post-cut

Fig. 2: Changes in activity of pectin methylesterase (PME) as affected by different concentration of CaCl₂ and duration of dipping (a) 0 g/L CaCl₂, (b) 2.5 g/L CaCl₂ and (c) 7.5 g/L CaCl₂



Ca dips treatment. Increase Ca concentrations reduced the activities of PG and PME (Figs. 1 & 2). This is further substantiated by a significant negative correlation between Ca content and PG and PME activities (Table II). It is therefore evident that Ca possesses a distinguishable role in reducing the PG and PME activities. Post-harvest dips in Ca chloride solutions allows the formation of COO⁻ groups from the pectin content of the fruits with which Ca²⁺ ions can form salt-bridge cross-links. This makes cell wall to become less accessible to the enzymes that cause softening. Since PG only hydrolyses homogalacturonan regions whose uranic acid residues have been previously demethylated by

Fig. 3: Effect of CaCl₂ and duration of dipping on firmness of slices of red flesh dragon fruits



PME (de Assis *et al.*, 2001) and since pectins are synthesized and deposited on the cell wall largely esterified (Staehelin & Moore, 1995), the negative charges generated by PME are necessary for Ca to bind onto the cell wall and to bring out firming effects of Ca. Thus, the Ca application to the fruit can significantly contribute to the texture retention in fruit, which was measured as firmness in this study. However, to obtain the beneficial effect of Ca, the sources of firming agents need to be chosen carefully. In this study, where CaCl₂ was used as the source, positive effects of Ca on firmness at the highest concentration disappeared with increased exposure time. This could be attributed to the toxicity effect of Cl⁻, which would lead to cell injury and leakage (Munns & Tester, 2008) and hence turgor loss that leads to reduced tissue firmness.

Role of Ca in fruit quality is well established (Luna-Guzmán *et al.*, 1999; Chardonnet *et al.*, 2002; Beirão-da-costa *et al.*, 2007). However, results obtained in this study showed that most of the quality parameters measured were not influenced by post-cut Ca dips treatment and duration of dipping (Table I) except for fruit firmness, Ca content and SSC. Tissue firmness is an important quality attribute and the rate of firmness loss during storage, if occurred, may influence the shelf-life of fruit and their marketability (Tavarini *et al.*, 2008). The possible role of Ca²⁺ in retardation of ripening has been discussed by Mignani *et al.* (1995) in terms of PG expression or activity and production of pectic oligomers, which induce ripening and fruit softening. Ca treatment increased Ca content in tissue and the longer the fruits were dipped into CaCl₂, the higher the Ca content would be, perhaps due to more Ca has been mobilized and integrated into the cell wall. However, dipping duration and concentration of Ca chloride solution should be carefully administered on individual fruits, as longer dipping time especially when high Ca concentration is used which could lead to fruit damage.

Together with acidity, soluble solids content (SSC) are useful indicators of the flavour quality of fruits (Teixeira *et al.*, 2006). Fruit slices exposed to a longer duration of Ca

Table I: Effects of calcium concentration and duration of dipping on titratable acidity, pH, soluble solids content, firmness, colour, Ca content and ascorbic acid content of fresh-cut dragon fruit

Variables	Titratable acidity (%)	pH	Soluble solids content (%)	Colour			Ca (mg/100 g)	Ascorbic acid (mg/100g)	
				L*	C	h°			
Ca concentration (g/L)	Duration of dipping (Min)								
0	0	0.85	5.3	9.70	31.79	37.16	6.48	16.27	6.53
	4	0.60	5.3	10.57	31.17	36.21	6.76	19.47	6.53
	8	0.51	5.4	11.23	31.86	36.83	6.87	15.20	6.54
	12	0.68	5.3	10.83	30.94	37.29	8.77	21.60	6.53
2.5	0	0.85	5.4	11.27	31.51	36.56	7.33	14.00	6.54
	4	0.51	5.4	10.27	31.16	37.47	8.79	22.40	6.53
	8	0.60	5.4	10.80	30.45	33.53	9.33	19.33	6.53
	12	0.77	5.3	11.87	30.13	34.99	7.37	18.53	6.53
7.5	0	0.60	5.3	8.30	29.28	34.33	7.86	14.13	6.54
	4	0.60	5.4	12.77	31.07	35.86	6.56	21.73	6.53
	8	0.77	5.4	13.37	30.38	35.29	7.99	20.27	6.55
	12	0.77	5.3	13.03	31.33	36.36	7.70	29.07	6.52
F-test									
Ca concentration (C)	ns	ns	ns	ns	ns	ns	ns	ns	ns
Duration of dipping (D)	ns	ns	*	ns	ns	ns	*	ns	ns
Interaction (C X D)	ns	ns	ns	ns	ns	ns	ns	ns	ns
LSD _{pooled} (0.05)	-	-	2.62	-	-	-	8.74	-	-

Notes: *and ns denote significant at $p < 0.05$ and not significant, respectively

Table II: Pearson correlation coefficients of fresh-cut dragon fruits for parameters measured in this study (n=36)

Characteristics	Firmness	PG activity	PME activity	TA	pH	SSC	Ca	Ascorbic acid
Firmness	1.000	0.161 ^{ns}	0.281 ^{ns}	-0.165 ^{ns}	0.060 ^{ns}	0.219 ^{ns}	-0.142 ^{ns}	-0.076 ^{ns}
PG activity		1.000	0.821 ^{***}	-0.003 ^{ns}	-0.139 ^{ns}	-0.198 ^{ns}	-0.371*	0.072 ^{ns}
PME activity			1.000	0.029 ^{ns}	-0.059 ^{ns}	-0.160 ^{ns}	-0.381*	0.126 ^{ns}
TA				1.000	0.081 ^{ns}	0.241 ^{ns}	-0.021 ^{ns}	-0.122 ^{ns}
pH					1.000	-0.091 ^{ns}	0.220 ^{ns}	-0.197 ^{ns}
SSC						1.000	0.075 ^{ns}	0.153 ^{ns}
Ca							1.000	-0.358*
Ascorbic acid								1.000

Notes: *, *** and ns denote significant at $p < 0.05$, $p < 0.001$ and not significant, respectively

had a higher SSC. Working with cut cantaloupe, Lamikanra and Watson (2004) found that Ca treated samples had a lower respiration rate compared to untreated fruit and hence the utilization of sugar for respiration could have been reduced, thus maintaining its SSC level. Reduction in respiration of post-cut Ca treated fruit was also reported in honeydew by Saftner *et al.* (2003).

In conclusion, increasing Ca concentrations reduced the activity of polygalacturonase and pectin methylesterase enzymes in fresh-cut red dragon fruits and its effect was more apparent under a longer duration of dipping. Reduction in the activity of these enzymes occurred concurrently with the increase in fruit Ca as indicated by their negative correlations. However, the application of higher Ca concentration and duration of dipping do not seem to contribute to any other quality improvement of fresh-cut red dragon fruit.

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