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## The Roles of Calcium Lactate on Bruised Damage in Papaya Fruit

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Khurnpoon, L. and Siriphanich, J. (2018). The roles of calcium lactate on bruised damage in papaya fruit. *International Journal of Agricultural Technology* 14(6):871-880.

**Abstract** Bruise damage reduces postharvest quality and it was rejected by consumer. Lack detail about bruise mechanism reported in papaya fruit, the prevention and reduction of postharvest loss were limited. The tissue softening as a result in the change of cell wall solubility and depolymerization including changes in physical properties were following bruise damaged. The maintaining cell wall integrity by calcium lactate application before bruising was tested. Papaya fruits were non-dipped or dipped in 2% calcium lactate solution at 55°C for 5 minutes, then air dried at room temperature for 1 hour. Two groups of fruit sample were separated; one group was impacted from height level at 75 cm while the other was non-impacted. The result showed that bruise treatment reduced skin lightness, chroma and hue angle especially in 'Khak dam' papaya, but could maintain by calcium lactate application. This treatment also preserved the reduction of fruit firmness by 4 days concomitant with the solubilization of pectin during bruising was reduced by calcium lactate treatment. Water-, Na<sub>2</sub>CO<sub>3</sub> - and potassium hydroxide-soluble fraction were low in calcium lactate treatment both with and without bruise damage. Non-significant different between non-dipped and dipped in calcium lactate in TSS, skin color development and fresh weight loss in bruise treatment. However, calcium lactate treatment reduced the increase in bruise area of 20 and 10% in 'Khak dam' and 'Holland' papaya, respectively. Fruit decay incidence was 5% low in fruit treated with calcium lactate following bruise damage than non-dipped.

**Keywords:** calcium lactate, bruise, pectin, cell wall, softening

### Introduction

Bruise damage had been report to reduce fruit quality in many fruits (Ahmadi, 2010). The symptom mainly skin browning and pulp softening increase within 2 hours after impact in tomato, apple and pear (Van linden *et al.*, 2008; Garcia *et al.*, 1995; Blahovec and Paprstein, 2005). In papaya, bruise severity differ among cultivars generally more severe in 'Khak dum' than

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'Holland' papaya (Khurnpoon and Siriphanich, 2011) as a results of different in cell wall pectin component and molecular size distribution of pectin where changed to smaller size during storage of bruised fruit (Goulao *et al.*, 2008). Calcium solution mostly as calcium chloride and calcium lactate play a role to preserve the integrity of cell wall resulted in delay fruit softening. The firming preservation by calcium solution may build a complex of calcium ions with cell wall and middle lamella pectin (Morris, 1980) that helps to stabilize of cell wall and effect of cell turgor pressure (Mignani, 1995). However, the unfavorable was affect by using calcium chloride for texture preservation (Monsalve-Gonzalez *et al.*, 1993) and appeared the residual amount of calcium chloride remained on the surface of the products that was not accepted by the consumer. Calcium lactate was represents an alternative calcium source without reported flavor difference. Calcium lactate at concentration between 0.5-2.0% has been used as a firming agent for processed strawberry (Morris *et al.*, 1985), and grapefruit (Baker, 1993). In addition, the application of calcium lactate has been reported in some fresh cut produces. Application of 2.0% of calcium lactate helps to preserve pulp firmness and delayed the changes in fruit quality during storage at ambient temperature for 70 days (Rehman *et al.*, 2017). Many reports showed that the combined use of calcium lactate with heat treatment gave the better results of firming preservation in fresh cut apple (Alandes *et al.*, 2006), carrot (Rico, 2007) and lettuce (Martin-Diana, 2006). Limited information exists on the effect of bruise damage on cell wall metabolism particularly pectic component of the primary cell wall. Increased pectin degradation was found in wounded tissue of fresh cut tomato which causes by pectin hydrolysis as a resulted in wound-induced enzyme synthesis (Huber *et al.*, 2001). In contrast with the compared study between intact and fresh cut tomato by Chung *et al.* (2006) found limited activities in the cut fruit. However, the associated change in the cell wall pectin was not examined. Beside the change in cell wall pectin, the change in xyloglucan network was likely to be associated with bruising (Hadfield and Bennett, 1998). The results in tomato showed no substantial change in PME and PG activity with bruising, although PG activity increased significantly with ripening. The degree of demethoxylation was slightly reduced in wounded tissue 3 h after impact-bruising. Bruising did not lead to significant changes in pectin solubility or degree of polymerization within 3 h of impact (Van linden *et al.*, 2008). Although calcium solution acts as firming agent in many kinds of fruits, the effect of calcium lactate application before mechanical impact bruising in papaya has not been studied yet. Our study aims to understand the effect of calcium lactate on bruise damage of papaya fruit. The work focused on the change in physiological and biological of cell wall pectin.

## **Materials and methods**

'Khak dum' and 'Holland' papaya were harvested at maturity stage (25 and 50% of yellow skin color developed) with uniform shape and size. Fruit samples were washed with chlorinated water then air dried at room temperature. A total of 72 fruits for each cultivar were obtained for 6 treatments. Fruit samples were dipped in water (control treatment) or 2% (w/w) calcium lactate solution for 5 min in 55°C then placed on plastic tray until dried and stored at room temperature for 24 h before bruising. Mechanical impact bruising for bruise treatments were obtained by dropped from height level at 75 cm. Impact site was marked with permanent-ink and started to evaluated physiological changes after 2 h of impact that was represented for day 0. The experiment was conducted using a completely randomized design (CRD) and comparison of treatment means was analyzed using Duncan's multiple range test (DMRT) at 5% level. Fruit physiological parameters and Cell wall extraction and analysis were recorded as followed.

### ***Change in fruit skin color***

Skin color development was determined by using the Color flex spectrophotometer. Data were reported as L value (lightness) ranging from 0 (brightness) -100 (darkness), chroma (C) and hue angle (H) values were represented to the saturated and the purity of color, respectively.

### ***Change in fruit quality***

The position of damage areas for each fruit sample after dropped from height level at 75 cm was labelled with permanent ink. The area of damage was evaluated by drawing an area on tracing paper then transferred to a graph paper in the area size of about 1 square centimeter. The damage area was reported as square centimeter (cm<sup>2</sup>). Change in fruit firmness was measured by fruit firmness tester with a 5 mm diameter of plunger head. Fruit samples were cut in cross section for 2 pieces each then press the plunger head depth approximately 0.5 cm. The data was reported in Newton (N). The total soluble solids (TSS) content was determined from blended 10g of pulp tissue to get about 5 ml of same juice. Two drop of fruit juice was dropped on prism of hand refractometer (Effigi<sup>®</sup>) then read through an eye piece and reportrf in the unit of %brix. After determined all quality parameters, the tissue samples were collected from the damage area. The sample was cut into small pieces then kept in freezers at -20°C for cell wall extraction and analysis.

### ***Cell wall extraction and analysis***

An alcohol insoluble residue (AIR) was prepared from frozen tissue by thawing and left 1 h prior to extraction. Approximately 20 g of tissue was boiled in 100 ml ethanol at 70°C for 5 minutes then blended and filtered through whatman#1 filter paper. The residues were washed with excess alcohol and acetone, respectively. The residues were then air dried with remained constant in weight. Dry samples as represented AIR were ground in mortar and stored in desiccators until analysis. AIR of 50 mg was weight and extracted with 20 ml distilled water in 50 ml Erlenmeyer flash at room temperature for 2 hours with shaking. The suspension was then centrifuged at 6,000g for 15 minutes and the supernatant was collected and kept in plastic conical tube at 15°C for analyze. The remained residues were re-extracted with the same amount of distilled water as described previous above. The supernatant were pooled with the earlier collected and represented as water soluble fraction (WSF). The residues from WSF was then sequentially extracted with 50 mM CDTA containing 100 mM potassium acetate pH 4.0, 50 mM Na<sub>2</sub>CO<sub>3</sub> containing 20 mM NaBH<sub>4</sub> and 4 N KOH, respectively. Each fraction was done twice in the same amount of extractant and same steps as descript for WSF. The supernatants were represented as CDTA-soluble fraction (CSF), Na<sub>2</sub>CO<sub>3</sub>-soluble fraction (NSF) and KOH-soluble fraction (KSF), respectively. The uronic acid content (µg galacturonic acid/mg AIR) was analyzed in triplicate by following the method of Blumenkrantz and Asboe-Hansen (1973) with slightly modified.

### ***Statistical analysis***

This experiment was designed as completely randomized designed (CRD). All treatments were run with at six replicates. Data was comparisons by analysis of variance (ANOVA) followed by Duncan's multiple range test with significance level at p<0.05.

### **Results**

Fruit skin lightness in 'Khak dam' and 'Holland' papaya increased slowly during the first 2 days. The reduction of skin lightness was found in the fruit impacted from height level at 75 cm whereas continue increased in control of 'Khak dam' papaya. In 'Holland' papaya, the skin lightness for control and bruised treatment slightly increased until days 4 then declined until days 6. Skin lightness and chroma value showed significantly different between bruised

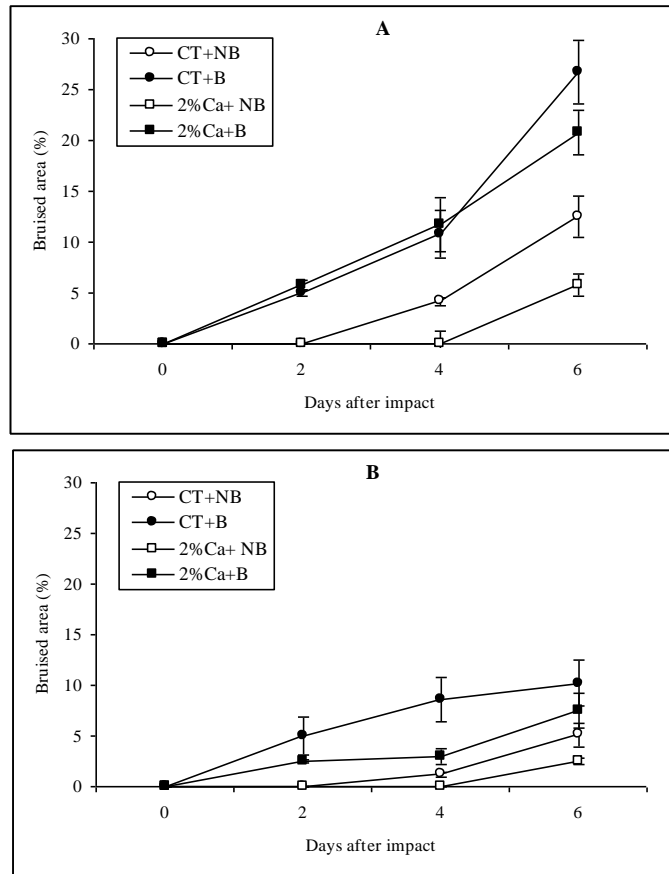
treatment and other but only in ‘Khak dam’ papaya. No significantly different had found in all color indices; lightness, chroma and hue angle values after 6 days in storage for ‘Holland’ papaya (Table 1).

Percentage of bruised area incidence appeared in fruit skin of ‘Khak dam’ papaya 2 days earlier than ‘Holland’ papaya. Bruise severity as showed by bruise area on the last day in storage, approximately 25 and 12% in ‘Khak dam’ and ‘Holland’ papaya, respectively (Figure 1A and 1B).

Pulp firmness in ‘Khak dam’ papaya rapidly decreased in pulp firmness by 40-120 N from the beginning. Calcium lactate at 2% reduced the softening of fruit pulp by 25 and 15 N as compared to bruised and non-bruised treatment without calcium lactate application. However, the pulp firmness continuously decreased through the end of storage, ranged from 4.89-12.62 N. In ‘Holland’ papaya, fruit firmness decreased rapidly on days 2, about 20-120 N from the beginning. Bruise treatment enhanced the reduction of pulp firmness by 40 N as compared to non-bruised while treatment of 2% calcium lactate could preserved the pulp firmness by 43 when compared to calcium treated with and without bruised damage. On days 6, the highest in pulp firmness was found in control and 2% calcium lactate treatments, approximately 15 N higher than bruised treatment without calcium lactate application (Figure 2A and 2B).

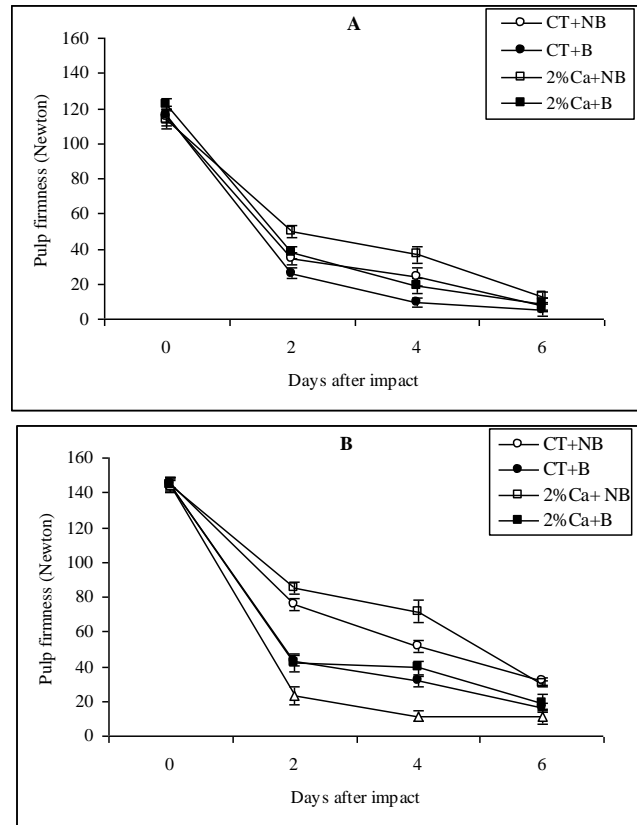
**Table 1.** Skin lightness, chroma and hue angle values change in control (non-bruised) and bruised papaya with and without calcium lactate application after 6 days of impact.

Cultivars	Treatments	Skin color parameters		
		Lightness	Chroma	Hue
Khak dam	Control	44.77±3.12a	32.27±3.12a	1.49±0.26
	Bruised	37.89±3.39b	18.70±1.77c	1.27±0.41
	2%Ca+Non-bruised	46.24±2.31a	32.96±2.39a	1.40±0.38
	2%Ca+Bruised	45.49±2.54a	29.20±2.55b	1.39±0.33
	<i>F-test</i>	*	*	ns
Holland	Control	58.72±3.91	52.31±3.22	1.10±0.43
	Bruised	57.50±2.77	55.24±2.95	1.11±0.27
	2%Ca+Non-bruised	56.12±2.85	50.40±3.07	1.07±0.33
	2%Ca+Bruised	56.61±3.04	48.66±4.65	1.08±0.32
	<i>F-test</i>	ns	ns	ns



**Figure 1.** Percentage of bruised area in ‘Khak dam’ (A) and ‘Holland’ (B) papaya fruit after dipped and non-dipped in 2% calcium lactate solution then impacted from height level at 75 cm during 6 days in storage at room temperature.

No significantly different among treatment in total soluble solids content, slightly increased from 8 to 12 % brix on day 2 then declined to the same content of the beginning in ‘Khak dam’ papaya. Otherwise, TSS content remained constant at 12 % brix during 4 days after impacted with slightly increased in 75 cm of impacted height without calcium lactate application from 11.8 to 12.8 % brix then declined. Calcium application could reduce the increase in fresh weight loss during storage duration by 5 and 10% in ‘Khak dam’ and ‘Holland’ papaya, respectively (Table 2).



**Figure 2.** Pulp firmness in ‘Khak dam’ (A) and ‘Holland’ (B) papaya fruit after dipped and non-dipped in 2% calcium lactate solution then impacted from height level at 75 cm during 6 days in storage at room temperature.

Uronic acid content in ‘Khak dam’ and ‘Holland’ papaya had more solubilized in WSF than other fractions. On 6 days after impacted, bruise treatment showed high WSF content by 470.17 and 489.40  $\mu\text{g}$  galacturonic acid/mg AIR in ‘Khak dam’ and ‘Holland’ papaya, respectively. The lowest WSF content was found in fruit treated with 2% calcium lactate without bruised impact by 281.83 and 371  $\mu\text{g}$  galacturonic acid/mg AIR in ‘Khak dam’ and ‘Holland’ papaya, respectively. CSF content appeared to be high in bruised treatment in ‘Khak dam’ while it was lower in fruit treated with 2% calcium lactate without bruised damage treatment. Fruit treated with 2% calcium lactate then impacted from height level of 75 cm had higher in uronic acid content, at about 326.46 and 260.70  $\mu\text{g}$  galacturonic acid/mg AIR in ‘Khak dam’ and ‘Holland’ papaya, respectively. NSF and KSF showed lowest content in fruit treated with 2% calcium lactate then impact from height level of 75 cm. ‘Khak

dam' papaya had more solubilized of cell wall pectin in NSF and KSF approximately 4 and 1.5 times than 'Holland' papaya (Table 3).

**Table 3.** Uronic acid content ( $\mu\text{g}$  galacturonic acid/mg AIR) in the different pectin fractions of control (non-bruised) and bruised papaya tissue with and without calcium lactate application after 6 days of impact.

Cultivars	Treatments	Uronic acid content ( $\mu\text{g}$ galacturonic acid/mg AIR)			
		WSF	CSF	NSF	KSF
Khak dam	Control	409.94 $\pm$ 14.21b	160.09 $\pm$ 15.06c	144.72 $\pm$ 12.05a	160.09 $\pm$ 15.62ab
	Bruised	470.17 $\pm$ 19.27a	199.22 $\pm$ 14.33b	148.20 $\pm$ 20.77a	170.52 $\pm$ 19.88a
	2%Ca+Non-bruised	281.83 $\pm$ 26.33d	229.65 $\pm$ 11.31a	133.13 $\pm$ 15.64b	152.26 $\pm$ 13.36b
	2%Ca+Bruised	326.99 $\pm$ 31.72c	216.46 $\pm$ 10.09a	94.58 $\pm$ 10.89c	129.65 $\pm$ 12.42c
	<i>F-test</i>	*	*	*	*
Holland	Control	439.80 $\pm$ 20.11b	140.10 $\pm$ 15.39c	169.90 $\pm$ 10.92a	195.40 $\pm$ 26.14a
	Bruised	489.40 $\pm$ 15.63a	126.20 $\pm$ 17.16c	152.60 $\pm$ 15.38 b	153.40 $\pm$ 18.33b
	2%Ca+Non-bruised	371.10 $\pm$ 17.58d	386.40 $\pm$ 16.03a	32.80 $\pm$ 7.81c	53.40 $\pm$ 16.59d
	2%Ca+Bruised	385.90 $\pm$ 20.52c	260.70 $\pm$ 20.48 b	22.10 $\pm$ 4.39c	86.50 $\pm$ 10.31c
	<i>F-test</i>	*	*	*	*

Water soluble fraction (WSF), chelator soluble fraction (CSF), carbonate soluble fraction (NSF) and potassium hydroxide fraction (KSF)

## Discussion

Fruit damage became softer with 2 days, about 5 times softer than the beginning. This change was parallel to the decrease in the skin lightness especially at the impact side. The bruise area appeared not much change to the lesser skin lightness as report in apple (Mitsuhashi-Gonzalez et al., 2010). It could explain by the different in major chemical composition between papaya and apple. High phenolic content in apple much appeared the skin or flesh browning after impact. The high calcium concentration resulted in decrease of fresh browning symptom. This symptoms have been directly associated with calcium content in many kinds of fresh. Therefore, calcium dips raise the possibility of producing fruits less susceptible to fresh browning symptoms. The same action of calcium salts has also been reported for fresh-cut fruit (Luna-Guzman and Barrett, 2000), where the enzymatic browning of fresh is result of different metabolic pathways. However, bruise damage affected the development of skin color (yellowness) by having non uniformity in 'Khak dam' papaya but not in 'Holland' papaya. This mechanical damage also caused an abnormal or uneven ripening in papaya. The mind effect of calcium lactate was found from this study by maintaining the reduction of skin lightness in 'Khak dam' papaya. The fruit quality as report by TSS content was not



influenced by the postharvest calcium dips, and had no affect by bruise impacted. The effect of calcium lactate on fruit quality was found only on fruit softening. Calcium lactate application at 2% reduces the decrease in pulp firmness both in 'Khak dam' and 'Holland' papaya. The results of cell wall solubilization markedly in WSF showed the positive correlation with the reduction in pulp softening. This could explain by the change in pectin properties from protopectin to be a soluble form. Postharvest calcium application could maintain cell turgor, membrane integrity, tissue firmness and delays membrane lipid catabolism, extending storage life of fresh fruits (Garcia et al., 1996). Chardonnet et al. (2003) reported that 2% calcium chloride was enough to maximum calcium accumulation in the cell wall. Calcium links with pectin to form of 'egg box' model leading to cell wall network that increase wall strength (White and Broadley, 2003).

### **Acknowledgement**

The author would like to thanks the Thailand Research Fund for financial support.

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(Received: 23 July 2018, accepted: 30 October 2018)