Effect of Hot Water Treatment Combined with Calcium Chloride on Postharvest Disease and Quality in Red Pitaya (*Hylocereus polyrhizus*)

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Key words: Red pitaya, *Hylocereus polyrhizus*, Hot water treatment, Calcium chloride

Summary

The objective of this study was to investigate the effect of hot water treatment in combination with calcium chloride on postharvest diseases and the fruit quality of red pitaya (*Hylocereus polyrhizus*). The effect of hot water plus calcium chloride treatment (HW+$CaCl_2$) on postharvest diseases and fruit quality was investigated by immersing pitaya fruit in hot water at 55 ℃ containing $CaCl_2$ at 0, 0.5, 1.0, 1.5, and 2.0% for 5 min. Results found that HW+$CaCl_2$ at concentrations over 1.5% significantly reduced the severity of the postharvest disease without affecting fruit quality. Furthermore, HW+$CaCl_2$ at 2% had the highest calcium content in the peel. In addition, HW+$CaCl_2$ at 2% resulted in higher catalase and ascorbate peroxidase activities in pitaya fruit during storage. These results demonstrated that hot water treatment at 55 ℃ combination with calcium chloride treatment could reduce disease development and thus maintain pitaya fruit quality during postharvest storage.

Introduction

Pitaya known as dragon fruit or pitahaya, belongs to the vine cacti of the genera *Hylocereus* of the botanical family Cactaceae. The plantation areas of pitaya have

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increased enormously and distributed all over the world in tropical and subtropical regions due to their richness in health properties, nutritional values, and being a source of polyphenols, vitamin C, organic acids, and pigments (especially betalains and flavonoids) (Suh *et al*., 2014). However, the pitaya production faced with disease problems, especially postharvest decays, led to yield and quality reduction.

Among various non-chemical approaches to control of postharvest decay in the fresh pitaya, hot water treatment (HWT) appear to be one of the most effective and promising methods to control postharvest decays in environmentally friendly ways. The use of high temperature in heat treatment could prevent fungi germination and growth. Despite the beneficial effect of heat treatment, the complete control of decay and disease is rarely accomplished by heat treatment alone. So, the combined treatments may provide an effective control system and enhance heat treatment efficiency (Schirra *et al*., 2011).

It has been reported that calcium strengthened cell wall structure, which could maintain fruit firmness and reduce the accessibility of pathogen in papaya and pitaya. (Ayon-Reyna *et al*., 2017a; Awang *et al*., 2011). The combination of hot water treatment and calcium chloride (HW+$CaCl_2$) resulted in a better profile of bioactive compounds and induced higher antioxidant activity especially total phenolic compounds in papaya and kiwi fruit (Ayon-Reyna *et al*., 2018 and Shahkoomahally and Ramezanian, 2015). However, heat tolerance is influenced by many factors, for instance, cultivar, harvest maturity, and growing condition. Therefore, the response of fruits to heat treatment is different. Thus, the aim of this study were to investigate the effect of hot water treatment in combination with calcium chloride on postharvest diseases and the fruit quality of red pitaya (*Hylocereus polyrhizus*).

Materials and Methods

1. Plans materials

Red pitaya fruit cv. 'Da Hong' at maturity stage were obtained from a commercial orchard farm in Bagui, Sanyi Township, Miaoli, Taiwan. After harvested, pitaya fruits were transported to National Chung Hsing university immediately. The fruits were sorted according to shape, size, and without physical damage or disease. The fruits were sanitized with hypochlorous acid water (HOCl) followed by tap water and air-dried at ambient temperature.

2. Experiment

Pitaya fruits were randomly divided into 5 groups. The first group without any treatment was

served as a control. The other three lots were immersed in distilled water containing calcium chloride ($CaCl_2 \cdot 2H_2O$) at different concentrations including 0.5, 1, 1.5, and 2% in a hot water bath at 55℃ for 5 min. After application, pitaya fruit was air-dried and placed into an airtight acrylic chamber, which connected with an air pump to increase relative humidity (RH) for 7 days at ambient temperature

3. Disease development

Disease incidence data were expressed as the percentage of fruit having disease symptoms per total number of fruits observed in each treatment on day 0, 3, 5, and 7. The data were expressed as the percentage of fruits with disease symptoms by using the formula: (total number of infected fruits/ total number of fruits) × 100.

Disease severity was assessed as the extent of the total disease symptom areas on each fruit surface by using a 5-point scale including, 0 = no disease symptom, 1 = 1 - 20% of disease symptom areas, 2 = 21 - 40% of disease symptom areas, 3 = 41 - 60% of disease symptom areas, 4 = 61 - 80% of disease symptom areas, and 5 = 81 - 100% of disease symptom areas on day 0, 3, 5, and 7. The data were expressed as a percentage of fruit surface with disease symptoms by using the formula: (sum of numerical disease rating/ total number of fruits × 5) × 100.

4. Fruit physiochemical quality and peel color

4.1 Weight loss

The weight of fruit in grams was measured using an electronic scale METTER/PB3002. During storage, pitaya fruits were sampled and weighed on day 0, 3, 5, and 7. The data were expressed as the percentage of weight loss by using the equation: weight loss (%) = [(initial fruit weight (g) – fruit weight at indicated day of storage (g))/ initial fruit weight (g)] × 100.

4.2 Firmness

Sampling pitaya fruits were cut in half horizontally. Fruit firmness was measured using a fruit penetrometer (Effegi, FT 327, Wilson, Italy) with an 11 mm diameter probe on both sides at the center of the fruit. The data were expressed in N/cm^2 by using the formula: reading value × $9.8/0.55 \times 0.55 \times \pi$.

4.3 Total soluble solids (TSS)

TSS were determined by using a digital refractometer (Palette, PR- 32, Atago, Japan) and expressed as °Brix. 2-3 drops of juice from pitaya flesh which was cut from the center of fruit were dropped on the refractometer's prism to measure TSS content.

4.4 Titratable acidity (TA)

TA was evaluated by titrating to pH 8.2 with 0.1 N NaOH. 1 ml of pitaya juice was mixed with 29 ml of pure water then 1 drop of phenolphthalein (indicator) was added. The mixed solution was titrated with 0.1 N NaOH until it started to change color to pink. The results were

calculated by using malic acid as the main acid component and were expressed as a percentage of TA in pitaya juice.

4.5 Vitamin C

Vitamin C was measured using a reflectometer, RQ-flex plus 10 (Merck KGaA, S4271, Darmstadt, Germany). 1 g of pitaya flesh was homogenized with 4 ml of metaphosphoric acid. Test paper was dipped into an extraction solution then measured with a reflectometer. The vitamin C content was calculated as mg/ 100 g of fresh fruit.

4.6 Peel color

The peel color of pitaya fruit was determined by using a MiniScan EZ spectrophotometer (MSEZ-4000S, Hunter Associates laboratory. Inc., USA). Two different sites of each fruit were measured. For peel color determination were expressed in chromaticity values of L*, a*, b*, C* and h°.

5. Calcium content

Pitaya flesh from each treatment was collected and separated to two parts, including peel and pulp. The samples were dried to constant weight by freeze-dried machine at -50 ℃, 100 Kpa (KINGMECH, model no. FD 24-6PL). Dried samples were milling into a fine powder with an electric blender and stored in a bag for further analysis. A 0.5 g of each milled sample was weighed out into porcelain crucibles. Then they were put in the muffle furnace and ignited at 200 ℃ for 2 h and increased temperature to 400 ℃ for 1 h then finally increased temperature to 550 ℃ for 2 h. The ash was dissolved with 5 ml of 2 N HCl and the resulting solution was filtered through Whatman No. 42 filter paper to 25 ml volumetric flask diluted to volume with deionized water. The ash filtrate solutions were used to determine calcium content. The ash filtrate solutions were pipetted 1ml into a tube. 3 ml of deionized water and 1 ml of 5 % lanthanum oxide were added then vortex. The elements were detected by an atomic absorption spectrophotometer (Hitachi Model Z-2300). The calcium was calculated as: (Conc. (ppm) × ashing liquid capacity (ml) × dilution factor × 10^{-4}/ dry weight (g) and express unit as %.

6. Defense-related enzymes activity

Extraction for catalase (CAT) and ascorbate peroxidase (APX) activities were evaluated according to Wang *et al*., 2008. Then 2 grams of fresh pitaya pulp were homogenized on ice with 0.1 M phosphate buffer pH 7.8 (1 mM DTT, 1 mM PEG 6000, and 0.1 mM EDTA) containing 1 mM PVPP. The homogenates were then centrifuged at 13,000×g for 20 min at 4 ℃. The supernatants were filtered through miracloth and used to analyze enzyme activity.

6.1 Assay of CAT activity

The supernatants were used for analysis according to Kato and Shimizu (1987). The reaction mixture used supernatant for 20 µL with 100 µL of 100 mM phosphate buffer pH 7.0 and 20 µL

of 50 mM H_2O_2. CAT activity was determined by using ELISA Reader (BMG LABTECH, FLUO star Omega, Germany) and read absorbance at 240 nm. Blank was prepared with 0.1 M phosphate buffer pH 7.8 (1 mM DTT, 1 mM PEG 6000, and 0.1 mM EDTA) for 20 L. CAT activity was calculated as: ΔA240/ 40 ($mM^{-1}·cm^{-1}$) × 0.3 (reaction volume, mL)/ 0.082 (cm) × (0.5/0.01) (dilute factor)/ g (fresh weight)/ 1 (min) and expressed in units as unit·g^{-1}·FW.

6.2 Assay of APX activity

The supernatants were used for analysis according to Nakano and Asada (1981). The reaction mixture used supernatant for 10 µL with 100 µL of 150 mM phosphate buffer pH 7.0, 100 µL of 1.5 mM ascorbate, 40 µL of 0.75 mM EDTA, and 50 µL of 60 mM of H_2O_2. APX activity was determined by using ELISA Reader (BMG LABTECH, FLUO star Omega, Germany) and read absorbance at 290 nm. Blank was used 0.1 M phosphate buffer pH 7.8 (1 mM DTT, 1 mM PEG 6000 and 0.1 mM EDTA) for 10 µL. APX activity was calculated as: ΔA290/ 2.8 ($mM^{-1}·cm^{-1}$) × 0.3 (reaction volume, mL)/ 0.082 (cm) × (0.5/0.01) (dilute factor)/ g (fresh weight)/ 1 (min) and express unit as unit·g^{-1}·FW.

7. Statistical analysis

The data of the experiment were performed by statistical analysis by using COSTAT 6.4 statistical software (CoHort Software, USA) and subject to one-way analysis of variance (ANOVA) for a completely randomized design (CRD) statistical model. The experimental data was analyzed with the least significant difference (LSD) test at $p \leq 0.05$.

Results

During the storage period, disease incidence was not significantly different among treatments. However, fruits treated with hot water treatment with calcium chloride (HW+$CaCl_2$) at 1.0, 1.5, and 2% did not show disease symptoms until day 5 of the storage (Fig. 1A). Similarly, the disease severity of treated fruits was significantly lower than the control group at the end of storage, which was 25, 25, 20, and 20% of disease symptom areas of $CaCl_2$ at 0.5, 1.0, 1.5, and 2.0% with HWT, respectively (Fig. 1B).

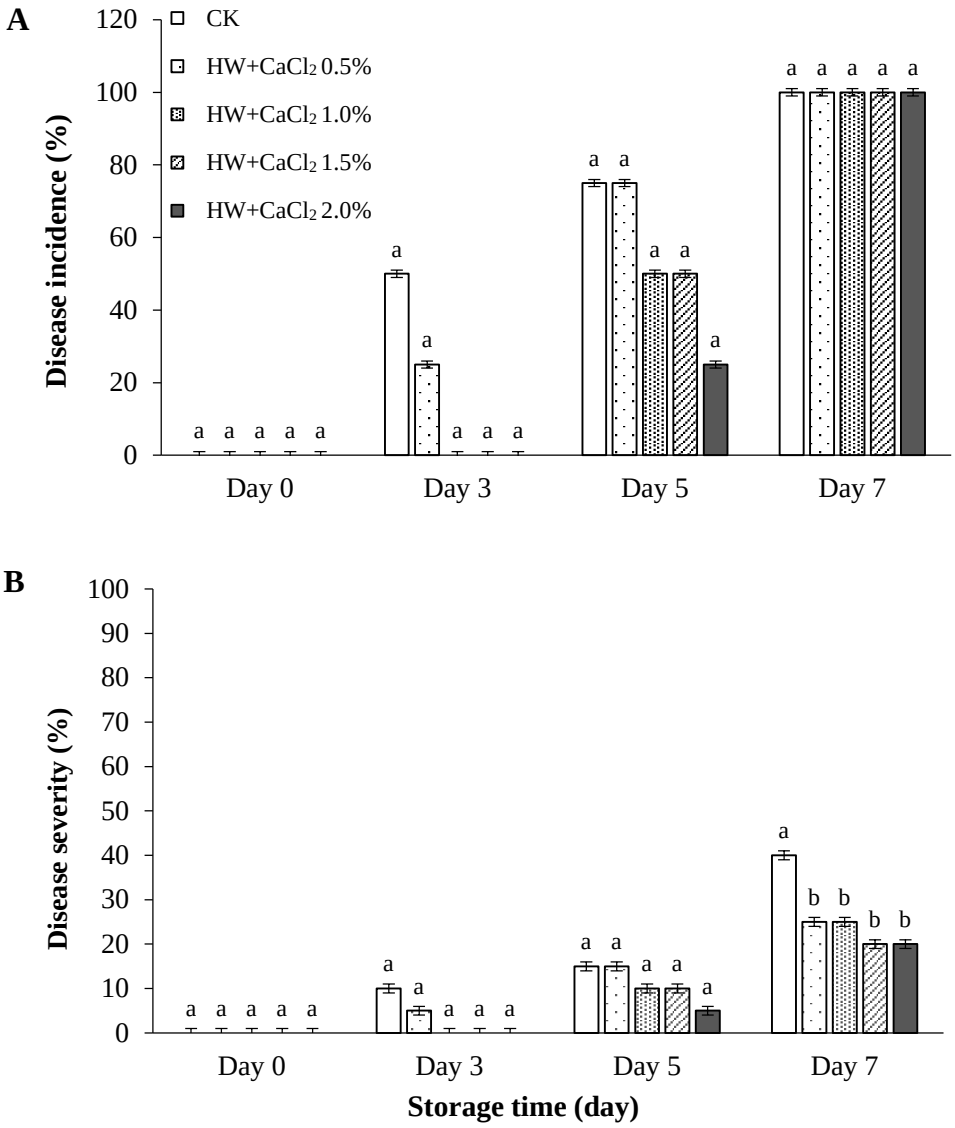

Fig. 1. Effect of hot water treatment at 55 ℃ for 5 min combined with calcium chloride on A.) disease incidence and B.) disease severity of pitaya fruit during 7 days of storage at ambient temperature. Vertical bars represent the standard error of mean. Values having different letters at the same time are significantly ($p < 0.05$) different according to ANOVA test.

These results indicated the combination treatment (HW+CaCl2) delayed the onset of diseases on pitaya fruit (Fig. 2). Thus, the flesh of pitaya fruit from the control group and HWT with 2.0% of CaCl2 were collected to investigate defense-related enzyme activity.

HWT with 1.5% of $CaCl_2$ exhibited the highest percentage of weight loss and HWT with 2.0% of $CaCl_2$ showed the lowest percentage of weight loss, respectively (Fig. 3A). There were no significant differences in firmness on days 0, 3, and 5 of storage in all treatments. At the end of storage, fruits treated with HWT combined with CaCl2 at a concentration over 1.0% revealed higher fruit firmness than the control group (Fig. 3B). The TSS, TA, and vitamin C content were not significantly different in all treatments throughout storage (Table 1). Therefore, these results indicate that HWT in combination with $CaCl_2$ did not have any effect on the pitaya fruit physiochemical properties during storage at ambient temperature.

The L*, A*, and C* values declined slightly during the storage period. However, the a* and C* values were not significantly different in all treatments during the 3rd, 5^{th}, and 7^{th} days of storage. At the end of the storage, HWT with 1.5% of $CaCl_2$ significantly exhibited higher L* value, b* value, and h° than other treatments (Table 2). It suggested that the pitaya peel color in HWT with 1.5% of $CaCl_2$ has darker red than other treatments.

Fig. 2. Appearance of pitaya fruit after treated with hot water treatment at 55 ℃ for 5 min combination with calcium chloride during 7 days of storage at ambient temperature. Scale bar is 5 cm.

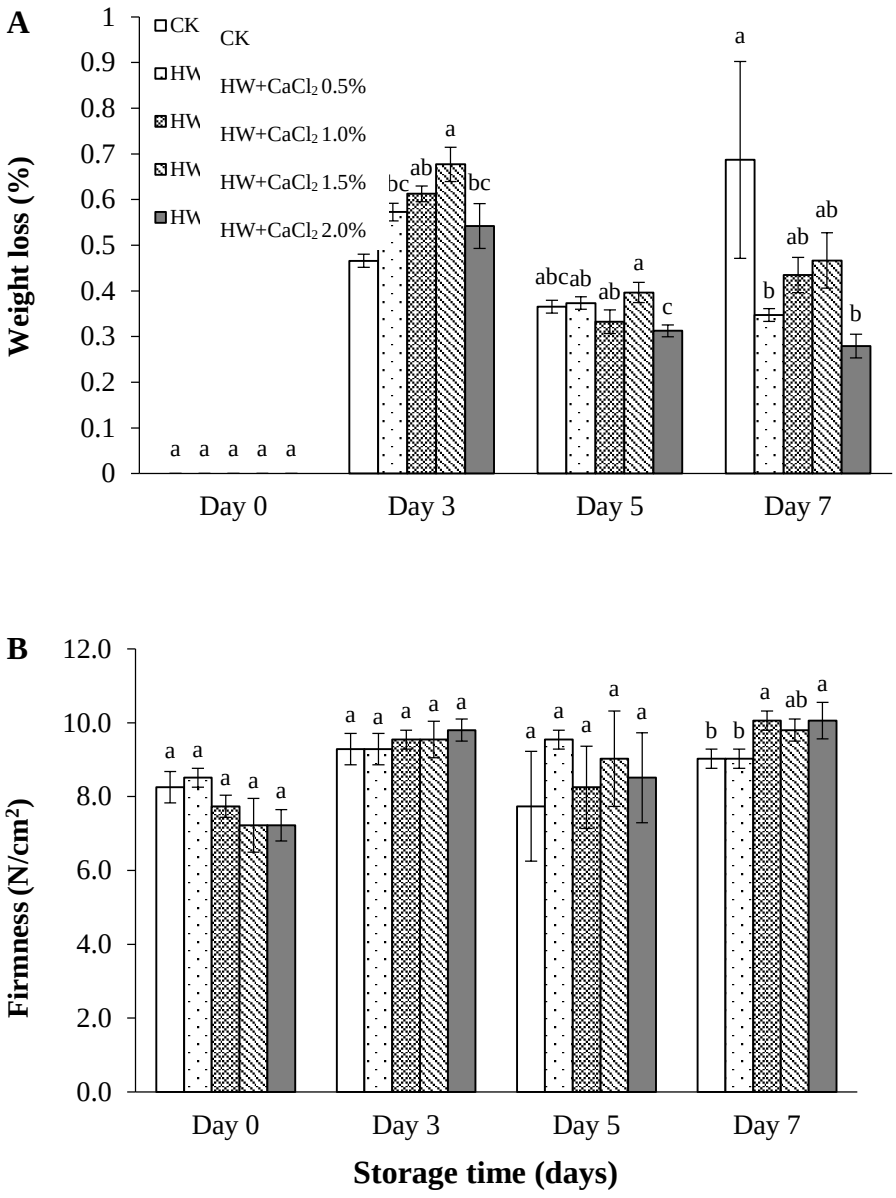

Fig. 3. Effect of hot water treatment at 55 ℃ for 5 min combined with calcium chloride on A.) weight loss and B.) firmness on pitaya fruit during 7 days of storage at ambient temperature. Vertical bars represent the standard error of mean. Values having different letters at the same time are significantly ($p < 0.05$) different according to ANOVA test.

Table 1. Effect of hot water treatment at 55 ℃ for 5 min combined with calcium chloride on the fruit quality of pitaya during 7 days of storage at ambient temperature.

Day	Treatment	Total soluble solids (°Brix)	Titratable acidity (%)	Vitamin C (mg/100 g)
0	CK	13.03[a]	0.10[a]	23.50[a]
	HW+$CaCl_2$ 0.5%	13.15[a]	0.12[a]	25.50[a]
	HW+$CaCl_2$ 1.0%	11.93[a]	0.10[a]	22.38[a]
	HW+$CaCl_2$ 1.5%	11.50[a]	0.10[a]	25.88[a]
	HW+$CaCl_2$ 2.0%	11.85[a]	0.12[a]	25.50[a]
3	CK	13.05[a]	0.11[a]	23.25[a]
	HW+$CaCl_2$ 0.5%	13.05[a]	0.12[a]	23.88[a]
	HW+$CaCl_2$ 1.0%	13.10[a]	0.12[a]	22.0[a]
	HW+$CaCl_2$ 1.5%	12.08[a]	0.10[a]	21.88[a]
	HW+$CaCl_2$ 2.0%	11.40[a]	0.11[a]	23.63[a]
5	CK	13.15[a]	0.13[a]	31.25[a]
	HW+$CaCl_2$ 0.5%	13.00[a]	0.13[a]	34.00[a]
	HW+$CaCl_2$ 1.0%	12.65[a]	0.13[a]	31.00[a]
	HW+$CaCl_2$ 1.5%	13.35[a]	0.13[a]	33.38[a]
	HW+$CaCl_2$ 2.0%	13.55[a]	0.13[a]	31.13[a]
7	CK	13.10[a]	0.11[a]	31.88[a]
	HW+$CaCl_2$ 0.5%	11.75[a]	0.11[a]	31.63[a]
	HW+$CaCl_2$ 1.0%	9.35[a]	0.12[a]	35.38[a]
	HW+$CaCl_2$ 1.5%	11.98[a]	0.13[a]	33.25[a]
	HW+$CaCl_2$ 2.0%	11.95[a]	0.12[a]	32.00[a]

Values having different letters at the same column are significantly ($p < 0.05$) different according to ANOVA test.

Table 2. Effect of hot water treatment at 55 ℃ for 5 min combined with calcium chloride on peel color of pitaya during 7 days of storage at ambient temperature.

Day	Treatment	L^*	a^*	b^*	C^*	h°
0	CK	38.40^{a}	39.98^{ab}	6.90^{a}	40.65^{ab}	9.77^{a}
	HW+$CaCl_2$ 0.5%	38.94^{a}	41.75^{ab}	7.22^{a}	42.42^{ab}	9.70^{a}
	HW+$CaCl_2$ 1.0%	37.64^{a}	39.48^{b}	6.53^{a}	40.08^{b}	9.52^{a}
	HW+$CaCl_2$ 1.5%	40.88^{a}	42.12^{a}	8.74^{a}	43.06^{a}	11.68^{a}
	HW+$CaCl_2$ 2.0%	39.31^{a}	41.42^{ab}	8.96^{a}	42.42^{ab}	12.21^{a}
3	CK	37.89^{a}	38.86^{a}	7.21^{a}	39.61^{a}	10.28^{a}
	HW+$CaCl_2$ 0.5%	39.03^{a}	40.63^{a}	6.15^{a}	41.13^{a}	8.53^{a}
	HW+$CaCl_2$ 1.0%	38.32^{a}	40.34^{a}	7.00^{a}	41.00^{a}	9.81^{a}
	HW+$CaCl_2$ 1.5%	41.05^{a}	40.46^{a}	7.88^{a}	41.25^{a}	11.01^{a}
	HW+$CaCl_2$ 2.0%	38.39^{a}	39.49^{a}	7.73^{a}	40.26^{a}	11.00^{a}
5	CK	37.23^{b}	37.00^{a}	6.08^{a}	37.60^{a}	8.90^{ab}
	HW+$CaCl_2$ 0.5%	38.61^{ab}	38.34^{a}	5.01^{a}	38.68^{a}	7.39^{b}
	HW+$CaCl_2$ 1.0%	39.50^{ab}	38.92^{a}	7.21^{a}	39.63^{a}	10.30^{ab}
	HW+$CaCl_2$ 1.5%	40.46^{a}	39.95^{a}	8.21^{a}	40.82^{a}	11.46^{ab}
	HW+$CaCl_2$ 2.0%	37.10^{b}	36.88^{a}	6.69^{a}	37.49^{a}	10.27^{a}
7	CK	36.41^{b}	35.52^{a}	5.81^{ab}	36.05^{a}	8.99^{b}
	HW+$CaCl_2$ 0.5%	37.69^{b}	36.89^{a}	6.06^{ab}	37.42^{a}	9.10^{b}
	HW+$CaCl_2$ 1.0%	36.98^{b}	36.83^{a}	5.26^{b}	37.24^{a}	8.04^{b}
	HW+$CaCl_2$ 1.5%	40.16^{a}	39.87^{a}	9.04^{a}	40.92^{a}	12.68^{a}
	HW+$CaCl_2$ 2.0%	37.43^{b}	37.31^{a}	7.52^{ab}	38.09^{a}	11.25^{ab}

Values having different letters at the same column are significantly ($p < 0.05$) different according to ANOVA test.

Calcium (Ca) content in pitaya flesh treated with HWT at 55 ℃ in combination with different concentrations of $CaCl_2$ was not significantly different among treatments and when compared to the control group. As shown in Fig. 4A, Ca content in pitaya flesh ranging from 0.32 - 0.41%. However, the Ca content in the peel of pitaya after treatment increased with the increase of $CaCl_2$ concentration, which was not significantly different among treatments. In the pitaya peel, the significantly highest Ca content was found in HWT with 2.0% of $CaCl_2$ with a value of 7.24%. While control treatment showed only 5.43 % of Ca content in peel (Fig. 4B).

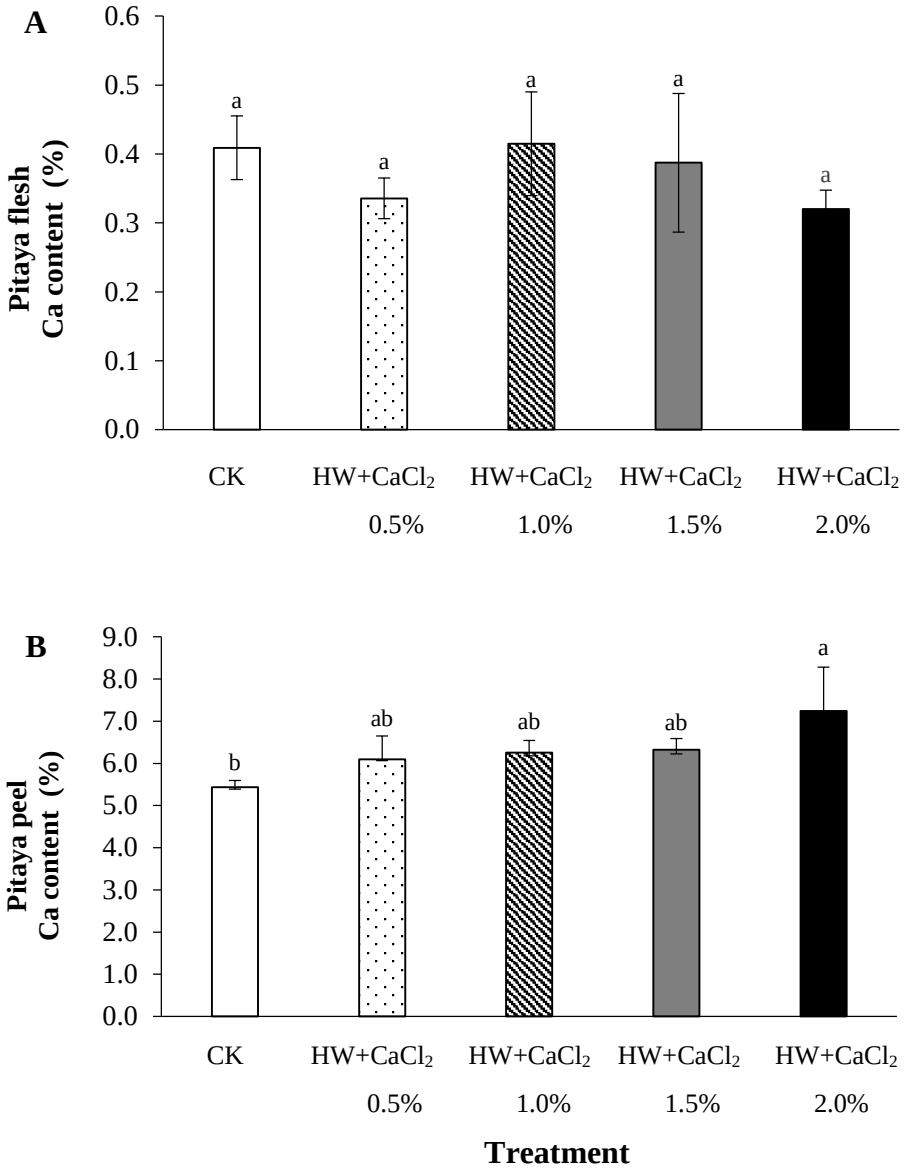

Fig. 4. Calcium content in A.) pitaya flesh and B.) pitaya peel after treated with hot water treatment in combination with different concentrations of calcium chloride. Vertical bars represent the standard error of mean. Values having different letters at the same time are significantly ($p < 0.05$) different according to ANOVA test.

CAT activity in control treatment was constant throughout storage period. Whereas CAT activity in pitaya fruit treated with HWT at 55 ℃ in combined with 2.0 % of $CaCl_2$ was elevated on day 3 of storage and then declined and was lower than the control group (Fig. 5A). APX activity in combination treatment was increased continuously throughout the storage at ambient temperature. While APX in control treatment was increased on day 5 of storage then declined (Fog. 5B). However, there was not significant difference between treated and untreated fruit.

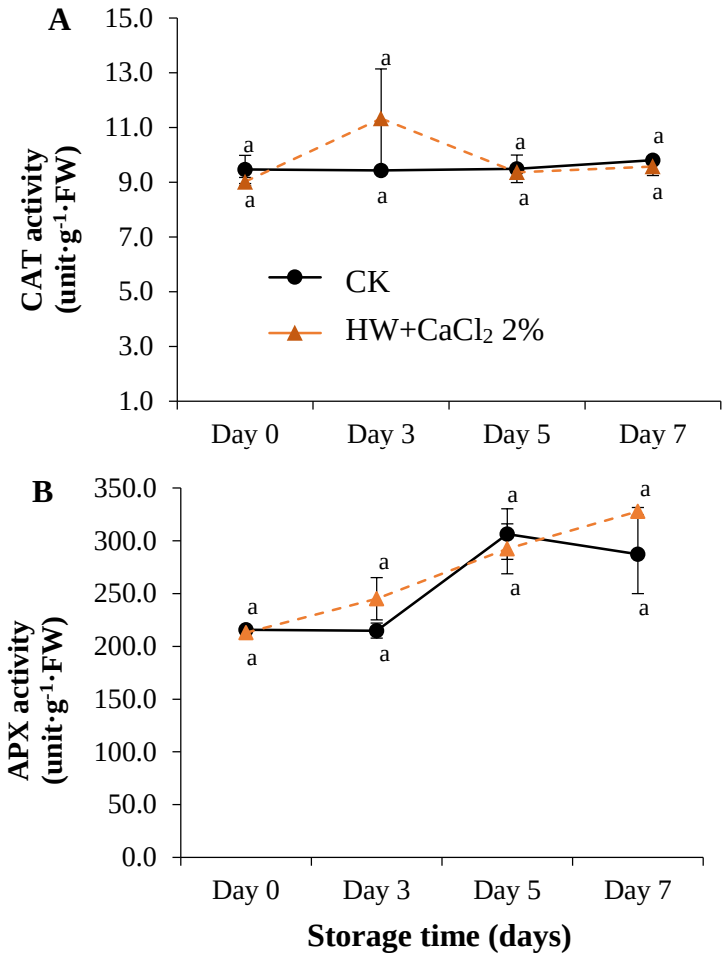

Fig. 5. Effect of hot water treatment at 55 ℃ for 5 min combined with calcium chloride 2% on A.) CAT activity and B.) APX activity during 7 days of storage at ambient temperature. Vertical bars represent the standard error of mean. Values having different letters at the same time are significantly ($p < 0.05$) different according to ANOVA test.

Discussion

This study found that weight loss in combined treatment was significantly lower than control at the end of storage. HWT combined with $CaCl_2$ at concentrations over 1.0% showed significantly higher in fruit firmness. Ayon-Reyna *et al*. (2017a) reported that the combined treatment of HWT with $CaCl_2$ melted the epicuticular wax, which covered most of the stomata thus reducing water loss in papaya fruit during storage. A recent study reported that postharvest treatment of $CaCl_2$ significantly delayed loss of fruit firmness in papaya fruit due to the inhibition of the cell wall degrading enzymes activity and the expression of cell wall softening-related genes (Gao *et al*., 2020).

Furthermore, the combined treatment maintained the physiochemical quality (TSS, TA, and vitamin C) of red pitaya fruit throughout the storage period. Similarly, calcium treatment had no significant effect on TSS and TA of red pitaya (Ghani *et al*., 2011). Pitaya peel in combined treatment showed a higher a* value than untreated fruit but not a significant difference, indicating that treated fruit resulted in red color in pitaya peel more than control. In addition, calcium treatment did not cause any significant effect on color, pH, TA, and ascorbic acid content of fresh-cut red flesh pitaya (Chuni *et al*., 2010).

The calcium content in pitaya peel in this study was significantly higher in the combined treatment of HWT and 2% of $CaCl_2$ (Fig. 4B). The synergistic effect between HWT and $CaCl_2$ suggested that the high temperature increased cell wall permeability and Ca diffusion through porous apoplasms and more calcium enters the cytosol. In a similar study, Aguayo *et al*. (2008) showed that $CaCl_2$ treatment at 60 ℃ significantly increased the concentration of bound calcium in fresh-cut 'Amarillo' melon.

This study demonstrated that hot water treatment combined with calcium chloride (HW+$CaCl_2$) delayed the onset of postharvest diseases on red pitaya fruit, which was $CaCl_2$ at concentration over 1.0% resulted more effectively (Fig. 2). According to the synergistic effect between HW+$CaCl_2$, which strengthens the cell wall, reducing the fruit susceptibility to pathogen attack. Additionally, heat can directly inhibit fungal germination and growth, or even kill fungus on the fruit surface. Further, HWT can induce fruit defense mechanisms. Similarly, HWT with $CaCl_2$ at 1% resulted in wax-covered natural opening thus forming a barrier that reduced the disease incidence in papaya fruit (Ayon-Reyna *et al*., 2017a).

This study also found that HW+$CaCl_2$ elevated CAT activity on day 3 and increased APX activity throughout storage, but not significantly different with untreated fruit. In agreement with Jin *et al*. (2016) indicated that heat treatment prevents the reduction of

CAT and APX activity during the storage of strawberry fruit. A recent study revealed that HWT followed by chitosan and k-carrageenan coating reduced the accumulation of H_2O_2 and regulated CAT and APX activities in pitaya peel thus enhanced the resistance against postharvest during storage (Nguyen *et al*., 2020). Subsequently, the combined treatment of HWT with $CaCl_2$ can be used to control postharvest disease and maintain fruit quality and peel color of pitaya during storage at ambient temperature.

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熱水合併氯化鈣處理對紅龍果 (*Hylocereus polyrhizus*) 果實採收後病害及品質的影響

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關鍵字：紅龍果、*Hylocereus polyrhizus*, 熱水處理、氯化鈣

摘要：本研究調查熱水處理、氯化鈣處理對紅龍果(*Hylocereus polyrhizus*)果實品質及病害的影響。將紅龍果浸泡在含有氯化鈣濃度為 0%、0.5%、1.0%、1.5%及2.0%之 55℃熱水 5 分鐘，調查合併熱水及氯化鈣處理(HW+$CaCl_2$)對紅龍果果實採後病害及果實品質之影響。結果顯示，當氯化鈣濃度大於 1.5%會顯著降低採收後病害的嚴重程度，且不影響果實品質。而氯化鈣濃度在 2%時，果皮有最高的鈣含量，且貯藏期間果實有較高的過氧化氫酶及抗壞血酸過氧化物酶活性。綜合上述結果，以 55℃ 或熱水合併氯化鈣處理皆可有效降低貯藏病害之發生，並維持採收後紅龍果果實之貯藏品質。

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