



Effects of hot water and calcium lactate treatments on fresh-cut quality of papaya

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ABSTRACT

Purpose: Papaya is one of the most common fruits which has been produced in southern part of Iran for fresh consumption. Demand for fresh-cut products has increased due to the changes in consumer attitudes. However, fresh-cut papaya is prone to softening during storage. This study carried out to determine effects of calcium lactate and hot water treatments (HWT) for maintaining fresh-cut papaya quality. **Research method:** After preparing fresh cut cubes of papayas, the pieces were dipped in calcium lactate (CaL) or combined with HWT. Physico-chemical parameters including calcium concentration (Ca), firmness and soluble solids concentration, catalase and peroxidase activities, pectin methyl esterase (PME) and polygalacturonase activities (PG), total phenolic content and radical scavenging activities, and microbial growth were measured during 9 days of storage at 5 °C. **Findings:** At the end of storage, CaL treatment combined with HWT increased calcium (Ca) concentration, catalase activity, total phenolic content and radical scavenging activities (DPPH) of papaya slices, but decreased peroxidase, PG and PME activities compared to the control. In addition, these treatments reduced microbial growth compared to the control. **Limitations:** No limitations were encountered. **Originality/Value:** Both HWT with CaL showed potential for increasing markeability on fresh cut papaya with their generally recognized as safe status for consumers.

INTRODUCTION

Papaya (*Carica papaya* L.) is one of the world's main fruits and the ripe fruit is an excellent source of carotenoids, vitamins, and polysaccharides (Yeoh et al., 2014).

The demand for fresh-cut products has increased due to consumer preferences in relation to freshness and textural quality characteristics (Madani et al., 2015). Calcium is generally recognized as safe (GRAS) and can be used for decreasing physiological disorders of fruits and in increasing postharvest quality of fruits and vegetables (Madani et al., 2015).

Calcium makes fruits firmer by binding to the pectin carboxyl groups (Madani et al., 2014). Fresh-cut papaya has become progressively popular among consumers but, the main problems related to the fresh-cut papaya are firmness loss, reduction of nutritional value and microbial loads development (Tabassum & Khan, 2020).

As an effective physical treatment, Hot water treatment (HWT) is free from chemical residues and easily applicable during the washing process (Kim et al., 1993; Aguayo et al., 2015). HWT maintained fresh-cut quality of eggplants (Barbagallo et al., 2012), and onions (Siddiq et al., 2013). Moreover, it has been confirmed that calcium ascorbate could increase the quality of apple fruit (Aguayo et al., 2015). Also, postharvest application of calcium increased firmness and delayed ethylene production (Gao et al., 2020). However, based on our knowledge combined effects of calcium lactate and hot water treatments have not been studied for fresh-cut papaya. Therefore, this study was carried out to determine effects of calcium lactate and HWT for maintaining fresh-cut papaya quality.

MATERIALS AND METHODS

Plant material

Papaya fruit at ripeness stage 4 (ie peel more yellow than green) maturity and uniform size and free from noticeable physical and fungal infection were obtained from a commercial orchard in Sistan & Balouchestan, Iran on the same day of harvest. The papaya fruits were transported to the postharvest laboratory. Fruits were washed with chlorinated water (0.01%) and air dried at (25±2 °C) and 75-80% RH in the laboratory before treatment.

Preparation of chemicals and treatments

Calcium lactate (CaL) (Sigma-Aldrich, St. Louis, MO. USA) was used as the source material in the experiment. Calcium lactate was used at 1.5% with 0.3% Tween 20 as surfactant agent. The papayas were peeled, de-seeded and sliced in to approximately 2.5 cm³ cubes with a sharp stainless steel sterilized knife. Six treatments were used for this experiment: (1) dipping slices in 1.5% calcium lactate (CaL) for 3 min (T2), (2) HWT (50 °C) for 1 min (HWT1) (T3), (3) HWT (55 °C) for 1 min (HWT2) (T4), (4) dipping in 1.5% calcium lactate (CaL) +HWT (50 °C) for 1 min (HWT₁) (T5), (5) dipping slices in in 1.5% calcium lactate (CaL)+ HWT (55 °C) for 1 min (HWT₂) (T6). Fruit slices that were dipped in distilled water acted as control (T1). After dipping, the papaya slices were then drained and allowed to air dry for 10 min. An electric water bath (Stuart, SBS40, OSA, UK) with digital temperature regulator profile was used to maintain the relevant temperature. Then, fresh-cut slices were placed into foam trays (175x80x45mm) over-wrapped with plastic films (0.02 mm thick PVC) and kept in an ice bath after bagging. All treatments stored at 5± 1°C for 9 days. Quality and biochemical analyses were measured during 9 days of storage.

Calcium concentration, firmness and soluble solids concentration measurement

Approximately 20g were taken from fresh cut samples for each replicate and calcium concentration was determined according to Madani et al. (2014) using an atomic absorption spectrophotometer (Perkin Elmer, Model AAS 3110, Palo Alto, California, USA) and results were expressed as mg g^{-1} DW. Flesh firmness was measured by a digital fruit hardness tester (STEP Systems GmbH, Germany) with a 5 mm diameter probe and results expressed in Newton. Soluble solids concentration (SSC) was measured by the methods of Ali et al. (2010) and results were expressed as % SSC.

Microbial activation analysis

Petrifilm plates (3M Center, Minn., USA) method used for yeast and mold. Also, aerobic bacteria measured according to the method described by Santhirasegaram et al. (2013) and calculated as colony forming units (CFU). The results were expressed as ($\log \text{CFU g}^{-1}$) of fresh fruit.

Enzyme activities

Catalase (CAT), Peroxidase (POD), Pectin methyl esterase activity (PME) and Polygalacturonase (PG) extraction and assay

The procedure for extraction and assay of CAT was performed using methods described by Shadmani et al. (2015). The absorbance was measured at 240 nm using a spectrophotometer (UV-Visible Double Beam, U-2800, Hitachi, Japan). One unit of enzyme activity was defined as one micro mole of hydrogen peroxide oxidised $\text{mL}^{-1}\text{min}^{-1}$ at 25°C . The results were expressed as enzyme units per gram fresh weight (U g^{-1} FW).

Peroxidase (POD) activity was determined using the method described by Rivera-Pastrana et al. (2014). POD activity was reported as the decomposition of 1 mmol of guaiacol min^{-1}mg of protein. Total protein concentration was determined according to (Bradford, 1976) using bovine serum albumin as standard.

The procedure for extraction of pectin methyl esterase activity (PME) and polygalacturonase (PG) activities was performed using methods described by Madani et al. (2014). For PME activity one unit was defined as the amount of the enzyme required to release 1 μmol of methyl ester per min per g of original fresh weight of flesh. For PG activity one unit of enzyme activity was defined as amount of enzyme required to liberate 1 nM of galacturonic acid per min per g of original fresh weight.

Total phenolic content (TPC) and radical scavenging activities (DPPH)

Extraction and measurement of the total phenolic content of the papaya slices was conducted using method described by Ong et al. (2013) where papaya slices (approximately 2.0 g) from each tray were homogenized with 10 ml of methanol (80%) as an extraction solvent. The extract solution was then incubated in the dark at 45°C . The phenolic content in papaya fresh-cut fruit was determined using Folin-Ciocalteu total phenolic method (Singleton & Rossi, 1965; Ong et al., 2013) and expressed as mg of gallic acid equivalents (GAE) per gram of fresh weight (mg GAE g^{-1} FW).

The DPPH assay was measured based on the method described by George et al. (2015). The radical scavenging activity was calculated accordingly (1):

$$\% \text{ DPPH inhibition} = (A_{\text{control}} - A_{\text{sample}}/A_{\text{control}}) \times 100 \quad (1)$$

where A_{control} is absorbance reading of control and A_{sample} is absorbance reading of the sample.

Experimental design and statistical analysis

All experiments were carried out using completely randomized design (CRD). Four replicates per treatment were used for all experiments. Data were subjected to Analysis of Variance (ANOVA) using the Statistical Analysis System (SAS) version 8.2 (SAS Institute Inc., Cary, NC, USA). The means were compared with the Duncan's Multiple Range Test (DMRT) at significance level of 0.05.

RESULTS AND DISCUSSION

Calcium concentration, firmness and SSC of fresh cut fruit

In this study, initially no differences were found among treatments for calcium concentration in the pulp, but after 3, 6 and 9 days the highest (27-28.7%) found for CaL (T2), CaL+HWT₁ (T5) and CaL+HWT₂ (T6) compared to the control. However, no differences were obtained among these treatments (Table 1). Moreover, Silveira et al. (2011) reported increased in calcium in 'Galia' melon slices treated with calcium sources for 1min when combined with heat treatment (60 °C).

Table 1. Calcium concentration (Ca), flesh firmness and soluble solids concentration (SSC) in fresh-cut papaya fruit slices following postharvest applications of hot water (HWT₁=50 °C, HWT₂=55 °C) and calcium lactate (CaL) treatments after storage at 5 °C for 3, 6 and 9 days

Quality parameter	Treatment	Storage period (day)			
		0	3	6	9
Ca (mg g ⁻¹ DW)	Control (T1)	6.20a ²	6.95c	6.90b	7.10b
	CaL (T2)	6.25a	7.32a	7.50a	7.57a
	HWT ₁ (T3)	6.25a	7.15b	7.02b	7.20b
	HWT ₂ (T4)	6.28a	7.14b	7.00b	7.23b
	CaL HWT ₁ (T5)	6.30a	7.35a	7.54a	7.61a
	CaL +HWT ₂ (T6)	6.35a	7.37a	7.57a	7.67a
Firmness (Newton)	Control (T1)	17.55a	10.20c	6.02b	3.12b
	CaL (T2)	19.9a	14.35b	9.87a	6.40a
	HWT ₁ (T3)	19.67a	13.32bc	12.55a	7.27a
	HWT ₂ (T4)	18.70a	14.17b	13.77a	7.87a
	CaL+HWT ₁ (T5)	18.32a	17.90a	13.12a	7.70a
	CaL+HWT ₂ (T6)	19.9a	17.82a	12.45a	7.72a
SSC (%)	Control (T1)	6.15a	7.47a	8.27a	9.57a
	CaL (T2)	6.28a	6.60b	6.72b	7.27b
	HWT ₁ (T3)	6.24a	6.66b	6.70b	7.17b
	HWT ₂ (T4)	6.25a	6.68b	6.75b	7.15b
	CaL+HWT ₁ (T5)	6.21a	6.62b	6.58b	6.57b
	CaL+HWT ₂ (T6)	6.18a	6.61b	6.64b	6.75b

²Small letters in columns for each parameter show the mean comparison among treatments. Means with the same letter are not significantly different by Duncan's multiple range test at $P \leq 0.05$.

Results showed that firmness decreased during storage for all treatments. However, after 6 and 9 days at storage all treatments had the least firmness loss compared with the control (Table 1). The positive effects of Ca treatment when combined with high temperature on firmness of fresh-cut products have been confirmed. Rico et al. (2007) indicated synergic effect of Ca and heat treatment for firmness. Soluble solids concentration of fresh cut papaya fruit increased during storage. However, after 9 days of storage all treatments had the lowest SSC compared with the control (Table 1). The effects of CaL dip and heat treatments caused a delay of SSC increase, likely because of inhibitory effects on enzymes' activities which are involved in the hydrolysis (Shen et al., 2013).

Microbial activation

Calcium and HWT (T2, T3, T4, T5, T6) induced a reduction in yeast and mold and aerobic plate after 9 days of storage (Table 2). At the beginning of the experiment, counts were 2.78 log CFU g⁻¹, for control without any differences with treatments for yeast and mold. After 9 days of storage, the control resulted in maximum yeast and mold loads (3.9 log CFU g⁻¹). However, T2, T3, T4, T5, T6 had lowest load compared with the control, while HWT+ calcium (T5, T6) did not differ statistically (3.47 and 3.44 log CFU g⁻¹). Aerobic plate count for papaya slices for control showed the lowest reductions on day 9 (Table 2), while the (T2, T3, T4, T5, T6) resulted in the lowest counts. Aguayo et al. (2015) showed that calcium treatments decreased microbial load of fresh-cut apple. It seems that calcium can increase cell wall rigidity and resistance to decay (Madani et al., 2014). Heat treatments control decay directly by inhibiting spore germination and mycelia growth. Therefore, treatments inhibit pathogen development (Sivakumar & Fallik, 2013). Values of aerobic microorganisms observed in this study are within those limits of by the standard of the European Union (4.7 log₁₀ CFU g⁻¹) and NOM-093 (5.2 log₁₀ CFU g⁻¹) for fresh-cut fruit (Ayón-Reyna et al., 2015). Also, yeast and mold counts were lower than the critical limits (5 log CFU g⁻¹) for yeasts (Jacxsens et al., 2003) after 9 days of storage, of fresh cut papaya.

Table 2. Yeast and mold count and aerobic plate count of fresh-cut papaya fruit slices following postharvest applications of hot water (HWT₁=50 °C, HWT₂=55 °C) and calcium lactate (CaL) treatments after storage at 5 °C for 3, 6 and 9 days

Quality parameter	Treatment	Storage period (day)			
		0	3	6	9
Yeast and mold count (log CFU g ⁻¹)	Control (T1)	2.78a ^z	3.64a	3.85a	3.93a
	CaL (T2)	2.78a	3.45ab	3.49b	3.60b
	HWT ₁ (T3)	2.75a	3.46ab	3.31b	3.57b
	HWT ₂ (T4)	2.74a	3.40b	3.33b	3.55b
	CaL+HWT ₁ (T5)	2.76a	3.28b	3.40b	3.47b
	CaL+HWT ₂ (T6)	2.77a	3.31b	3.41b	3.44b
Aerobic plate count (log CFU g ⁻¹)	Control (T1)	3.86a	4.47a	4.48a	4.62a
	CaL (T2)	3.81a	4.43a	4.50a	4.51b
	HWT ₁ (T3)	3.80a	4.22b	4.33b	4.47b
	HWT ₂ (T4)	3.79a	4.21bc	4.34b	4.45b
	CaL+HWT ₁ (T5)	3.82a	4.20bc	4.30b	4.43b
	CaL+HWT ₂ (T6)	3.84a	4.15c	4.32b	4.42b

^zSmall letters in columns for each parameter show the mean comparison among treatments. Means with the same letter are not significantly different by Duncan's multiple range test at P ≤ 0.05.

Calcium ions (Ca^{2+}) are involved in the function of many enzyme actions and physiological processes (Madani et al., 2014). Moreover, Kou et al. (2014) indicated that dipping pear slices with calcium chloride increased activity of CAT and decreased POX activity during storage. In addition, Sala and Lafuente, (1999) revealed that heat treatment increased activity of CAT in mandarin fruit. Also, Yahia et al. (2007) indicated that heat treatment decreased POD activity of tomato, while increased CAT activity. Based on these results, it can be supposed that Ca and HWT might delay the oxidation of fresh-cut papaya by means of regulating the antioxidant enzyme system. PME and PG activities increased during storage (Table 3). However, all treatments decreased PME and PG activities when compared with the control. It has been suggested that heat treatments interrupt cell wall hydrolytic enzymes activity as the reason of delay or poor softening in fruit (Paull & Chen, 2000). The role of Ca in decreasing activities of PME and PG observed in tomato pericarp discs (Mignani et al., 1995; Madani et al., 2015). Temperature also plays a significant role for decreasing PME and PG activities on heat-treated strawberries (Vicente et al., 2005). The results of this work also show a synergistic effect of temperature and Ca on PME and PG activities.

Table 3. Catalase (CAT), peroxidase (POX), pectin methyl esterase activity (PME) and polygalacturonase (PG) activity of fresh-cut papaya fruit slices following postharvest applications of hot water ($\text{HWT}_1=50\text{ }^\circ\text{C}$, $\text{HWT}_2=55\text{ }^\circ\text{C}$) and calcium lactate (CaL) treatments after storage at $5\text{ }^\circ\text{C}$ for 3, 6 and 9 days

Quality parameter	Treatment	Storage period (day)			
		0	3	6	9
CAT ($\text{Ug}^{-1}\text{FW}^{-1}$)	Control (T1)	2.22a ^z	1.55b	1.37b	1.10b
	CaL (T2)	2.16a	2.07a	1.80a	1.57a
	HWT ₁ (T3)	2.19a	2.12a	1.90a	1.74a
	HWT ₂ (T4)	2.17a	2.15a	1.92a	1.75a
	CaL+HWT ₁ (T5)	2.20a	2.14a	1.95a	1.74a
	CaL+HWT ₂ (T6)	2.21a	2.17a	1.94a	1.77a
POD ($\text{mM guaicol}^* \text{min}^{-1} \text{mg protein}^{-1}$)	Control (T1)	13.09a	12.26a	11.70a	11.85a
	CaL (T2)	13.05a	9.87b	9.06b	8.46b
	HWT ₁ (T3)	13.06a	9.02c	8.50b	7.85b
	HWT ₂ (T4)	13.29a	9.13c	8.65b	7.57b
	CaL+HWT ₁ (T5)	13.26a	10.31b	8.78b	8.55b
	CaL+HWT ₂ (T6)	13.04a	10.41b	9.04b	8.30b
PME ($\text{U g}^{-1}\text{FW}^{-1}$)	Control (T1)	0.12a	0.35a	0.52a	0.76a
	CaL (T2)	0.11a	0.24bc	0.37b	0.53b
	HWT ₁ (T3)	0.14a	0.21cd	0.35bc	0.55b
	HWT ₂ (T4)	0.10a	0.22d	0.36bc	0.56b
	CaL+HWT ₁ (T5)	0.13a	0.26bc	0.32bc	0.45c
	CaL+HWT ₂ (T6)	0.11a	0.27b	0.31c	0.45c
PG ($\text{U g}^{-1}\text{FW}^{-1}$)	Control (T1)	0.90a	1.82a	2.13a	2.44a
	CaL (T2)	0.95a	1.31bc	1.58b	1.85b
	HWT ₁ (T3)	0.85a	1.27c	1.57b	1.78b
	HWT ₂ (T4)	0.92a	1.31bc	1.50bc	1.77b
	CaL+HWT ₁ (T5)	0.87a	1.34bc	1.40c	1.57c
	CaL+HWT ₂ (T6)	0.91a	1.34b	1.38c	1.54c

^zSmall letters in columns for each parameter show the mean comparison among treatments. Means with the same letter are not significantly different by Duncan's multiple range test at $P \leq 0.05$.

Table 4. Total phenolic content (TPC) and Radical scavenging activities (DPPH) of fresh-cut papaya fruit slices following postharvest applications of hot water (HWT₁=50 °C, HWT₂=55 °C) and calcium lactate (CaL) treatments after storage at 5 °C for 3, 6 and 9 days

Quality parameter	Treatment	Storage period (day)			
		0	3	6	9
TPC (mg GAE g ⁻¹ FW)	Control (T1)	0.37a ^z	0.24b	0.21b	0.17b
	CaL (T2)	0.38a	0.32a	0.31a	0.25a
	HWT ₁ (T3)	0.36a	0.24b	0.23b	0.19b
	HWT ₂ (T4)	0.32a	0.24b	0.23b	0.18b
	CaL+HWT ₁ (T5)	0.33a	0.33a	0.29a	0.26a
	CaL+HWT ₂ (T6)	0.35a	0.32a	0.30a	0.24a
DPPH (%)	Control (T1)	56.4a	67.94c	60.9b	55.9b
	CaL (T2)	57.00a	76.8a	72.74a	70.64a
	HWT ₁ (T3)	68.64a	68.64bc	62.74b	57.1b
	HWT ₂ (T4)	57.44a	69.44bc	62.44b	55.0b
	CaL+HWT ₁ (T5)	56.3a	75.6ab	71.3a	70.5a
	CaL+HWT ₂ (T6)	57.24a	76.5a	72.0a	70.8a

^zSmall letters in columns for each parameter show the mean comparison among treatments. Means with the same letter are not significantly different by Duncan's multiple range test at $P \leq 0.05$.

Total phenolic content (TPC) and radical scavenging activities (DPPH)

Effects of calcium treatment and HWT treatments on total phenolic content (TPC) and radical scavenging activities (DPPH) are presented in Table 4 and showed that T2, T5 and T6 maintained higher TPC and DPPH compared to the control after six and nine days in storage. Supapvanich et al. (2012) stated that CaCl₂ treatment maintained nutritional quality of fresh-cut sweet leaf bush, particularly total phenols and DPPH scavenging activities. Moreover, George et al. (2015) indicated that heat treatment could increase antioxidant capacity and total phenols of pineapple and mango. We recommend that combination of Ca and HWT might be an effective strategy for maintaining antioxidant activity in fresh cut papaya during cold storage.

CONCLUSION

The results of this study indicate that CaL or HWT alone maintained quality of fresh cut papaya. However, combination of CaL and HWT had additive effects to preserve quality of fresh-cut papaya which included increased calcium content, CAT activity, antioxidant activity and decreased in microbial loads and POD, PG and PME activities at 5 °C after 9 days of cold storage.

Conflict of Interest

The authors have no conflict of interest to report.

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