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Research article

The combined effect of calcium, pectin methylesterase and mild heat on frozen mango quality

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Abstract: Both single and mixed solutions of calcium and pectin methylesterase were studied to examine how they improve the quality of frozen mango. The result showed that calcium and pectin methylesterase had a synergistic effect on the mango, helping to improve firmness and decrease the drip loss of the frozen fruit. To increase the efficiency of the mixed solution of pectin methylesterase and calcium, this study examined three immersion temperatures (25, 40 and 50 °C). Pectin methylesterase activity, calcium content, β-carotene content, firmness, drip loss and color of the samples were evaluated. The treatment at 40 °C caused high pectin methylesterase activity and calcium content. The firmness of the frozen-thawed mango increased two-fold from 1.25 to 2.50 N, and the drip loss decreased from 15.19 to 9.87 g/100 g when compared with the control samples. Moreover, the frozen-thawed mango that was immersed in pectin methylesterase and calcium at 40 °C had the highest lightness and hue value and the lowest browning index.

Keywords: mango; freezing; calcium; pectin methylesterase; temperature

1. Introduction

Mango (*Mangifera indica* L.) is an important and popular tropical fruit. Thailand's Office of Agricultural Economics reported that 59,139 tons of mangoes were exported in 2017, with a value of approximately USD 10.17 million [1]. However, mangoes are perishable and quite susceptible to

bruising. Freezing is one process that has been used to prolong the shelf life and the availability of the fruit. Decreases in temperature during freezing usually slow the rate of the chemical reactions and microbial growth that are responsible for the deterioration of food quality. Nevertheless, the formation of ice during the freezing process can destroy cell compartments and cause textural changes, especially for frozen fruits [2]. Sriwimon and Boonsupthip [3] and Charoenrein and Owcharoen [4] reported that after freeze-thawing, the firmness of ripe mango decreased by around 50–85% when compared with that of the unfrozen fruit

Calcium (Ca) and pectin methylesterase (PME) have been reported to maintain fruit quality. Ca preserves the firmness of fruits, by cross-linking with pectin in the middle lamella and the cell wall of plants, which stabilizes the cell walls [5]. The PME enzyme catalyzes the de-esterification of pectin, exposing free carboxylic groups that form Ca bridges between contiguous pectin chains, to give an "egg-box" structure [6]. Consequently, the degradation of bioactive compounds within the cell should be minimized. However, the individual and combined effects of adding Ca and PME on the quality of frozen fruits and stability of β-carotene, a bioactive compound in mango, have not yet been well observed. Moreover, the pre-freezing immersion conditions should be studied to ascertain the most appropriate treatment option for Ca absorption and PME activity. The process temperature has a significant impact on many reactions. Therefore, this research assessed the influence of different pre-freezing protocols (calcium chloride ($CaCl_2$) and PME solution) combined with mild temperatures on the quality of frozen mango.

2. Materials and method

2.1. Sample preparation

The ripe mangoes (cv. Nam Dok Mai) were purchased from the market in Udon Thani, Thailand. The mango samples were selected for similar maturity, based on size, the color of both peel and flesh, total soluble solids content and titratable acidity. The moisture content, the total soluble solids content and the titratable acidity of the mango samples were shown in Table 1. For the uniformity of the samples, 20% from both the respective blossom and stem ends of the mango samples were excluded. Then, the mangoes were washed, peeled and cut. The sample size was 1.5 × 1.5 × 1.5 cm.

Table 1. Composition of the mango samples.

Composition	Mean ± SD
Moisture (g/100 g)	80.29 ± 0.45
Total soluble solids (Brix)	15.00 ± 1.02
Titratable acidity (g citric acid/100 g)	0.48 ± 0.05

2.2. Treatments

The first experiment studied the influence of $CaCl_2$ and PME on the texture and drip loss of the frozen mango. Approximately 160 mango cubes from 8 mango fruits were investigated for each replication. Mango cubes were immersed in 0.01 g/mL $CaCl_2$ solution, 0.001 U/mL PME (according the manufacturer, the optimum temperature of the PME was 40–50 °C; Novoshape, Novozymes, Bagsvaerd, Denmark) solution and the mixed solution of 0.001 U/mL PME and 0.01 g/mL $CaCl_2$ for

2 min at 25 °C, respectively. The selected immersion time was from our preliminary study. The preliminary result showed that the firmness of mangoes after immersion in 0.01 g/mL $CaCl_2$ from 2 to 30 min were not significant differences (Figure 1). Moreover, the firmness of the mangoes started to decrease after immersion for 60 min. Therefore, the immersion time for 2 min was chosen in this study. Immersion in distilled water at 25 °C for 2 min, was used as the control sample. The ratio between the mango cubes and the solution was 1:3 w/w. Following the immersion process, the mango cubes were packed as eight pieces per plastic bag. The thermocouples, which were connected to the data logger, were inserted into the center of the mango samples, to record the temperature during the freezing and thawing process. The samples were frozen at −30 °C until the temperature at the midpoint of the samples attained −25 °C. The freezing rate of the mango samples was 1.1 °C/min. The mangoes were then kept at −18 °C until analysis. Before analysis, all frozen samples were thawed at 4 °C until the temperature at the midpoint of the samples reached 4 °C.

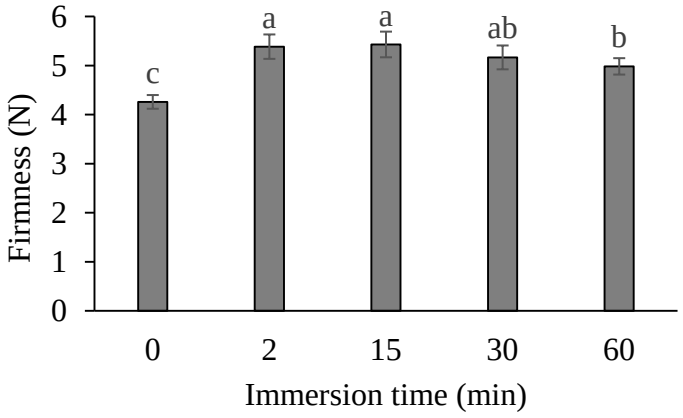

Figure 1. Firmness of mangoes after treated with 0.01 g/mL $CaCl_2$ solution for different immersion times. Error bars represent standard deviation. The bars with different letters denote significant differences ($p < 0.05$).

The subsequent experiment investigated the impact of solution temperatures on the quality of the frozen mango. As for the second experiment, 240 mango cubes from 12 mango fruits were investigated for each replication. The mango cubes were immersed in the mixed solution of 0.001 U/mL PME and 0.01 g/mL $CaCl_2$ at 25, 40 and 50 °C, respectively, for 2 min. Control samples were immersed in distilled water at 25, 40 and 50 °C, respectively, for 2 min, to represent only the effect of temperature. After immersion, all treatments were frozen, using the same methods as those described above in the first experiment.

2.3. Pectin methylesterase (PME) activity

The PME activity of the samples was determined according to the method outlined by Anthon and Barrett [7]. Immediately after immersion, the sample cubes were sliced to 1 mm thickness. Two grams of mango slices were placed in a glass tube containing 18 mL of distilled water. Once closed with screw caps, the tubes were incubated at 25 °C for 60 min in a shaking water bath. The liquid (150 µL) of

each sample was collected immediately after placing the mango slices in the distilled water and after incubation for 60 min, respectively. The methanol content was determined by added 50 μL of the immersion liquid and 50 μL of 1 U/mL alcohol oxidase (from *Pichia pastori*; Sigma, St Louis, MO, USA) to a microplate. The microplate was kept at room temperature for 20 min, and then 100 μL of acetylacetone reagent (24 μL of acetylacetone, 36 μL of glacial acetic acid and 12 mL of 2 mol/L ammonium acetate) was added. After reaction at room temperature for 60 min, the absorbance of each sample was recorded on a microplate photometer (Synergy™ HT Multi-Mode, BioTek Instruments, Inc., USA) at 405 nm.

2.4. Calcium content

The unfrozen samples were ground, and 5 g was then oven-dried at 105 °C, followed by incineration at 550 °C in a muffle furnace. The ash was dissolved with 1 N HNO_3 and 10,000 ppm $LaCl_3$, brought to 50 mL and filtered (Whatman No. 4 filter paper). An atomic absorption spectrophotometer (AA-6300, Shimadzu, Japan) with an air–acetylene flame was used to determine the Ca content at a wavelength of 422.7 nm according to the method of Alonso et al. [8].

2.5. β-carotene content

Both unfrozen and frozen samples (5 g) were weighed, mixed with tetrahydrofuran, and passed through a nylon filter membrane with 0.45 μm pore size. The β-carotene content was analyzed using a high-performance liquid chromatography instrument (LC 1200 Series, Agilent Technologies, USA) equipped with an Eclipse XDB-C18 column (5 μm, 4.6 × 150 mm; Agilent Technologies, USA). The mobile phase composed of acetonitrile, methanol and tetrahydrofuran at the ratio of 58:35:7 v/v/v. The flow rate was fixed at 1.5 mL/min. β-carotene was detected at 460 nm by a UV-Vis detector according to the method of Robles-Sánchez et al. [9]. Standard β-carotene was used to construct the standard curve.

2.6. Texture

The texture of both unfrozen and frozen-thawed samples was measured using a texture analyzer (TA.XTplus, Stable Micro Systems, UK) with a 36 mm cylindrical flat head probe (P36). Ten mango cubes (1.5 cm^3) were used per treatment. The compression rate was set at 1 mm/s. The sample was compressed for 50% strain, and the firmness of the sample (N) was recorded from the maximum peak force [10].

2.7. Drip loss

The drip loss was determined by the method of Lowithun and Charoenrein [11]. Four pieces of frozen mangoes were weighed and placed on an absorbent paper. To eliminate evaporation during thawing, the samples were packed in double-layered zip-lock plastic bags. The thawing was conducted at 4 °C. The absorbent paper was periodically weighed until reaching a constant value.

$$\text{Drip loss (g/100 g)} = (W_t - W_0)/W_s \times 100\% \quad (1)$$

where W_0 and W_t are the weight of the absorbent paper before and after thawing, respectively, and W_s is the weight of the sample.

2.8. Color

The color (CIE $L^*a^*b^*$ system) of all frozen-thawed samples was recorded using a HunterLab colorimeter (Mini Scan XE Plus, Hunter Associates Laboratory, Inc., USA). The hue value ($h°$) and browning index were calculated [12].

$$h° = \text{arctangent}\ (b^*/a^*) \tag{2}$$

$$\text{Browning index} = 100 \times (x - 0.31)/0.1 \tag{3}$$

$$\text{where } x = (a^* + 1.75\ L^*)/(5.645\ L^* + a^* - 3.012\ b^*) \tag{4}$$

2.9. Statistical analysis

Triplicate experiments were done. The obtained data were analyzed using SPSS for Windows, according to the randomized complete block design for both parts of the study. The means were compared by Duncan's multiple range test ($p < 0.05$).

3. Results and discussion

3.1. Effect of calcium and PME on quality of mangoes

Firmness is an important quality of fruits. The firmness of the mangoes treated with different solutions at 25 °C is shown in Table 2. In a comparison of the unfrozen and frozen-thawed mangoes, the frozen-thawed mangoes had lower levels of firmness because the ice crystals that developed during the freezing process damaged the cells. After thawing, there was some liquid loss from the damaged cells, and this resulted in a change in texture.

Table 2. Firmness and drip loss of mangoes treated with different solutions at 25 °C.

Treatment	Firmness of unfrozen samples (N)	Firmness of frozen-thawed samples (N)	Drip loss (g/100 g)
Control	4.58 ± 0.06^b	1.28 ± 0.06^b	21.06 ± 0.18^b
Ca	6.09 ± 0.04^a	2.79 ± 0.18^a	15.11 ± 0.11^c
PME	4.18 ± 0.25^b	1.39 ± 0.04^b	36.16 ± 0.01^a
PME + Ca	6.30 ± 0.40^a	2.87 ± 0.34^a	12.69 ± 0.45^d

*Note: Data are the mean ($n = 3$) ± standard deviation. Different superscript letters in the same column are significantly different ($p < 0.05$). Ca, calcium; PME, pectin methylesterase.

For both the unfrozen and frozen-thawed mangoes, immersion in the Ca solution and the mixed PME + Ca solution, respectively, increased their firmness ($p < 0.05$) compared to the control sample. Nevertheless, the PME solution did not improve the texture of the unfrozen and frozen-thawed mangoes. The reason for this is because Ca is essential for forming the cross-links between adjacent

pectin molecules, which are constituents of the middle lamella, and the cell wall creates Ca pectate, allowing a greater cell to cell adhesion and increased firmness of the product's texture [13]. Gonzalez-Aguilar et al. [14] reported that a mixture of Ca and antioxidants decreased the loss of firmness and color deterioration of fresh-cut mango, without impacting on its sensory characteristics. Sirijariyawat and Charoenrein [10] improved the texture of mango cubes both pre- and post-freezing by immersing the fresh fruit in 0.01 g/mL $CaCl_2$ solution. Sohail et al. [15] noted that Ca maintained the firmness of peach during cold storage. Moreover, Guillemin et al. [16] found the use of PME + Ca under vacuum conditions (0.05 bar) before pasteurization improved the firmness of apple pieces. Similarly, our results showed that the PME solution alone did not improve the texture of mangoes before and after freezing, as the Ca content in mango was probably low and so insufficient cross-links were formed. In turn, the firmness of the PME-treated mangoes did not improve. Likewise, Fraeye et al. [17] observed that prior infusion with PME could not increase the firmness of strawberries exposed to thermal treatments.

When the frozen mangoes were thawed, some liquid was lost since ice crystals damaged the cells of the frozen fruit. The mangoes treated with PME had the highest drip loss, followed by the control sample, the mangoes treated with Ca, and finally, the mangoes treated with PME + Ca, which presented the lowest drip loss ($p < 0.05$) (Table 2).

PME plays a role in the de-esterification reaction of pectin, which eliminates the methyl group and creates the free carboxyl group. In insufficient Ca conditions, the pectin molecule with the free carboxyl group is the substrate for pectin depolymerizing enzymes, which are associated with texture loss. The depolymerizing enzymes include polygalacturonases and pectate lyase [18]. Therefore, the PME-treated mangoes had the highest drip loss.

The mango flesh treated with Ca showed lower drip loss than the control group and the PME-treated sample. This result is justified given that the presence of methyl groups in pectin molecules inhibits the formation of Ca bridges between pectin molecules [19] and, therefore, the ice crystals damage the mango cells. PME catalyzes the specific demethoxylation of homogalacturonan within the plant cell wall, releasing methanol and protons and creating the negatively charged carboxyl groups [18]. The demethoxylated homogalacturonan can then bond with Ca and strengthen the cell wall. Consequently, the frozen mangoes treated with the PME + Ca had the least drip loss. Sirijariyawat et al. [20] found that the microstructure of a sample infused with PME + Ca was similar to that of its fresh counterpart.

3.2. Effect of solution temperatures on quality of mangoes

Since the concentration of Ca in this study was low (0.01 g/mL) and the molecular weight of PME was high, the diffusion of the Ca and PME into the samples were difficult. In order to enhance the efficiency of the PME and Ca, the immersion temperatures of 25, 40 and 50 °C were studied.

3.2.1. Pectin methylesterase (PME) activity

Irrespective of the temperature, immersion in PME + Ca increased PME activity (Table 3). The highest PME activity occurred after immersion in PME + Ca at 40 ºC ($p < 0.05$). For the controls, immersion in water at 40 and 50 ºC also increased the activity of the PME naturally present in the mangoes (Table 3). From our research, the optimum temperature for PME activity of mangoes treated with exogenous PME was 40 ºC. The optimal temperature for PME activity is known to vary

between 45 and 70 °C, and is inactivated at around 65–80 °C, depending on the enzyme source and the surrounding environment [21–23]. Sila et al. [24] demonstrated the activity of carrot PME remained high at 30–50 °C but decreased almost 50% at 55 °C, and 95% of the enzyme was inactive at 60 °C. Rico et al. [25] reported a higher activity of PME in carrots exposed to Ca solution at 50 °C compared with 25 °C. Beirão-da-Costa et al. [26] recorded a 2–3-fold increase in the PME activity of kiwifruit after increasing the temperature from 25 to 45 °C. Accordingly, the treatment temperature is an important factor in PME activation, and the optimum temperature depends on the source of the enzyme. For the purpose of increasing the effectiveness of the enzyme, other methods such as a vacuum impregnation were reported to apply instead of the simple immersion in order to improve the diffusion of the enzyme into the fruit samples [16,17,20].

Table 3. Pectin methylesterase (PME) activity, calcium (Ca) content and β-carotene content of mangoes treated at different solution temperatures.

Treatment	PME activity (μmol/g/hour)	Ca (mg/100 g)	β-carotene (mg/100 g)	
			Unfrozen	Frozen-thawed
Water25 °C	0.22 ± 0.07^{d}	14.70 ± 0.66^{c}	0.25 ± 0.01^{a}	0.30 ± 0.01^{a}
Water40 °C	0.39 ± 0.08^{c}	22.39 ± 0.39^{c}	0.22 ± 0.02^{a}	0.23 ± 0.04^{a}
Water50 °C	0.41 ± 0.00^{c}	20.65 ± 0.62^{c}	0.22 ± 0.01^{a}	0.24 ± 0.01^{a}
PME + Ca25 °C	0.78 ± 0.06^{b}	51.29 ± 9.77^{b}	0.25 ± 0.04^{a}	0.26 ± 0.02^{a}
PME + Ca40 °C	1.01 ± 0.06^{a}	65.41 ± 2.59^{a}	0.31 ± 0.00^{a}	0.32 ± 0.06^{a}
PME + Ca50 °C	0.78 ± 0.02^{b}	68.78 ± 6.95^{a}	0.27 ± 0.04^{a}	0.26 ± 0.02^{a}

*Note: Data are the mean (n = 3) ± standard deviation. Different superscript letters in the same column are significantly different ($p < 0.05$).

3.2.2. Calcium content

The mangoes treated with PME + Ca at all temperatures displayed higher Ca contents than the controls, which were immersed in distilled water ($p < 0.05$) (Table 3). Moreover, the mangoes treated with PME + Ca at 40 and 50 °C had higher Ca contents than those treated at 25 °C ($p < 0.05$). The cell wall and cell membrane of fruits soften at high temperatures. Consequently, Ca easily diffused into the mango samples. The same phenomenon was described by García et al. [27], whereby the Ca content in strawberries increased after immersion in $CaCl_2$ at 45 °C relative to immersion at 25 °C.

3.2.3. β-carotene content

β-carotene is a natural antioxidant and can be converted to vitamin A. Although the PME activity, Ca content and firmness of mangoes increased after treatment with PME + Ca, the β-carotene content of all treatments and the control samples were not significantly different ($p > 0.05$) either before or after the freezing process (Table 3). It indicated that the immersion solution and temperature did not affect the β-carotene content of the mangoes. Consistent with this result, Robles-Sánchez et al. [9] noticed that immersion in a combined solution of ascorbic acid, citric acid and $CaCl_2$ did not affect the β-carotene content of the fresh-cut mangoes. Additionally, the freezing process was also reported not to have affected the β-carotene content of some fruits and vegetables [28,29].

3.2.4. Firmness

Immersion in the PME + Ca solution at all three temperatures, respectively, improved the firmness of unfrozen (Figure 2a) ($p < 0.05$) and freeze-thawed mangoes (Figure 2b). Although immersion of the unfrozen fruit in water at a high temperature (40 and 50 °C) resulted in a soft texture, dipping the fruits in the PME + Ca solution at 40 °C was the most proficient condition for increasing the firmness of the frozen-thawed fruit. The control unfrozen sample (water 25 °C in Figure 2a) had a firmness value of 4.29 N, which decreased to 1.25 N in the frozen-thawed control (water 25 °C in Figure 2b) while the firmness of the PME + Ca-treated sample at 40 °C was 2.50 N, subsequent to the freezing and thawing process. It was evident that prior immersion in PME + Ca at 40 °C was the optimum condition for improving the firmness of mango flesh when exposed to the freezing and thawing process. This firmness result is related to the optimum temperature of PME found in this study (Table 2). Comparable immersion temperature results have been documented by Trindade et al. [30] for fresh-cut mangoes. Their study concluded that firmness was well preserved by treatments using low levels of Ca and temperatures from 35 to 40 °C. Alonso et al. [31] studied the effects of Ca, thermal treatment and Ca combined with the thermal treatment on pectin content and degree of esterification in frozen cherry. They suggested that mild temperatures (50 °C) had a synergic effect with Ca and enhanced the demethoxylation of pectin. Therefore, the interaction between Ca and adjacent pectin molecules was encouraged, and the depolymerization of pectin was prevented.

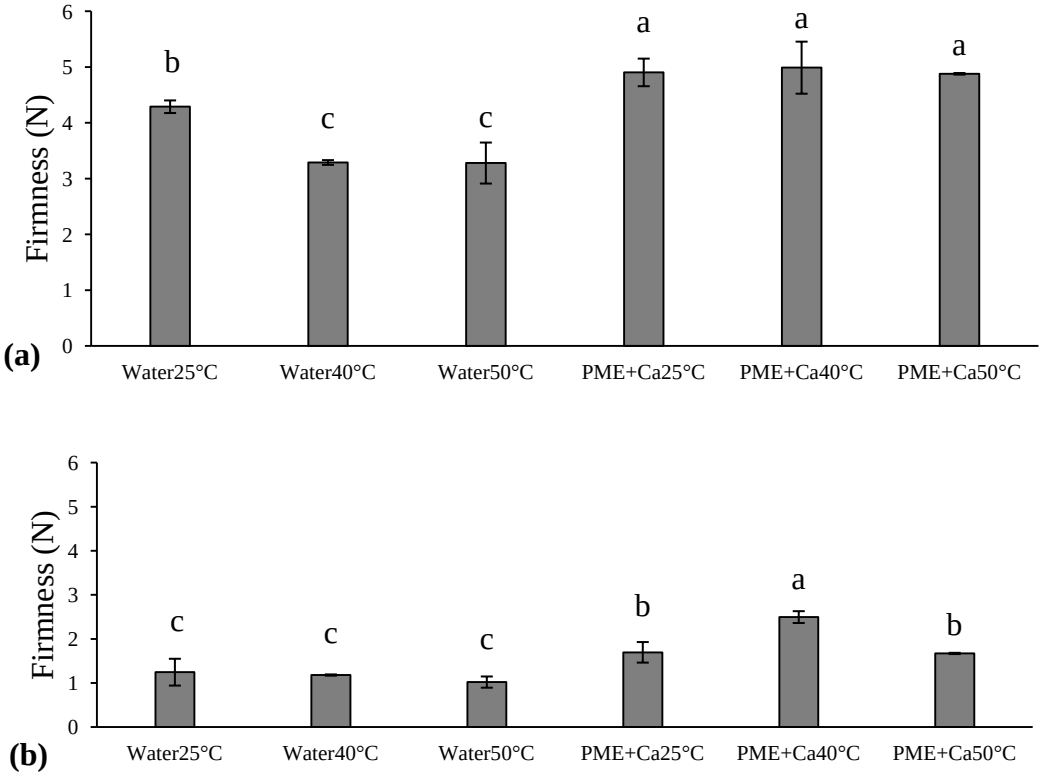

Figure 2. Firmness (N) of unfrozen (a) and frozen-thawed mangoes (b) treated with different solution temperatures. Error bars represent standard deviation. The bars with different letters denote significant differences ($p < 0.05$).

3.2.5. Drip loss

Immersion in PME + Ca at all three temperatures decreased the drip loss of mango flesh when compared with the samples immersed in distilled water. The PME + Ca-treated sample at 40 °C had a greater efficiency in reducing drip loss than the treatment at 25 °C (Figure 3). Conversely, mild heat immersion (40 and 50 °C) alone caused high drip loss. Immersion in the PME + Ca solution at 40 °C was the most effective condition for diminishing the drip loss of the frozen mangoes because, at this temperature, the Ca easily diffused into the sample. It was also the optimum temperature for PME to catalyze the demethoxylation reaction and create free carboxyl groups. In the sufficient Ca condition, the demethoxylated pectin could cross-link with the Ca ions, reinforcing the middle lamella and plant cell wall [18,19]. Thus, the fruit cells were less damaged by the ice crystals and could retain the water on thawing. In comparison to the control samples at 40 and 50 °C, the PME displayed high levels of activity (when compared to the control sample at 25 °C) under these conditions, but there was insufficient Ca to form extensive bridging with the pectin chain. Thus, the pectin chains were digested by polygalacturonase, which resulted in a high drip loss when compared with the other treatments.

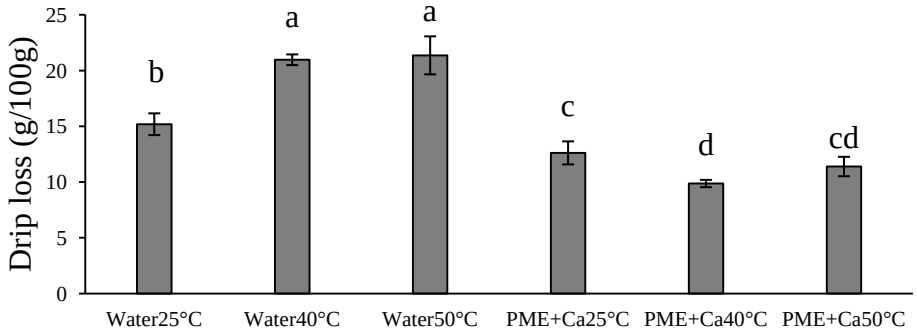

Figure 3. Drip loss (g/100 g) of mangoes treated with different solution temperature. Error bars represent standard deviation. The bars with different letters denote significant differences ($p < 0.05$).

3.2.6. Color

Color is a physical property that impacts consumers' desire to purchase produce. The increases in the immersion temperature of the PME + Ca solution improved the lightness (L^*) of the frozen mangoes (Table 3). The mangoes treated with PME + Ca at 40 °C had the highest L^* and $h°$ and the lowest browning index (Table 3). It demonstrated that the color of this frozen–thawed mango flesh was in the yellow range and that it had less redness and brownness when compared with the results of the other treatments. By exposing the fruit to this pre-treatment condition, the mango cells were probably least damaged, so the browning reaction occurred less extensively than when using the other treatments.

Table 3. Color of frozen–thawed mangoes treated with different solution temperatures.

Treatment	*L**	Hue angle (*h*°)	Browning index
Water25 °C	60.60 ± 1.53[b]	81.40 ± 0.45[b]	181.21 ± 1.91[a]
Water40 °C	62.11 ± 0.78[b]	82.99 ± 1.68[b]	186.94 ± 1.49[a]
Water50 °C	59.76 ± 0.20[b]	80.98 ± 0.57[b]	199.89 ± 1.79[a]
PME + Ca25 °C	61.80 ± 1.58[b]	82.15 ± 1.57[b]	185.87 ± 16.79[a]
PME + Ca40 °C	66.87 ± 1.04[a]	85.94 ± 0.19[a]	129.52 ± 11.93[b]
PME + Ca50 °C	63.04 ± 2.41[ab]	82.52 ± 0.26[b]	176.83 ± 13.82[a]

*Note: Data are the mean (n = 3) ± standard deviation .Different superscript letters in the same column are significantly different ($p < 0.05$).

4. Conclusion

Only PME could not improve the texture of both unfrozen and frozen–thawed mangoes. The PME + Ca mixture was more beneficial than the Ca treatment alone, in decreasing the drip loss of the frozen mango. Increasing the immersion temperature could improve the efficiency of the PME + Ca solution. The effect of the immersion temperature on improving firmness and decreasing the drip loss of the frozen mangoes was evident at the immersion temperature of 40 °C. After freezing and thawing, the firmness loss of the sample pre-treated with PME + Ca at 40 °C was decreased from 70.9% to 41.7% when compared with the untreated frozen–thawed sample. Furthermore, the immersion in PME + Ca at 40 °C showed good color mango flesh. This frozen-thawed mango had a higher lightness and hue value while the browning index was lower than that of the frozen-thawed control.

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Conflict of interest

All authors declare no conflicts of interest in this paper.

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