

Texture improvement of fresh and frozen mangoes with pectin methylesterase and calcium infusion

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Abstract

BACKGROUND: A major problem of mango products is texture loss. The effect of commercial pectin methylesterase (PME) and calcium infusion on improvement of the texture of both fresh and frozen-thawed mango cubes was investigated in the present study.

RESULTS: The weight gain and moisture content of mango samples were greater at relatively high vacuum level (10 kPa). The PME activity of samples infused with PME and calcium at 10 kPa increased fourfold in comparison with that of control and water-infused samples. The combined effect of PME and calcium was found to improve the texture and microstructure of both fresh and frozen-thawed mangoes. Fresh mangoes infused with PME and calcium at 10 kPa showed significantly higher firmness than control fresh samples. Frozen-thawed mangoes infused with PME and calcium at 50 kPa and atmospheric pressure had superior texture and microstructure in comparison with control frozen-thawed samples.

CONCLUSION: The results of the present analysis allow for a better appreciation of the role of PME, calcium and appropriate infusion conditions in improving the texture of both fresh and frozen-thawed mangoes.

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Keywords: mango; pectin methylesterase; calcium; infusion; frozen

INTRODUCTION

Mango (*Mangifera indica* L.) is one of the most important and popular tropical fruits. Data from the Food and Agriculture Organization (FAO) of the United Nations indicate an import of 3 million tons of mangoes with a value of approximately US\$210 million into the USA in 2008 (FAO <http://faostat.fao.org>). However, a major problem of mango products is texture loss, which is apparent in both fresh-cut produce and frozen products. The firmness of fresh-cut fruit is subject to significant reduction during cold storage. Gonzalez-Aguilar *et al.*¹ reported a decrease in the firmness of 'Keitt', 'Kent' and 'Ataulfo' mango cultivars during storage at 5 °C for 21 days. Dea *et al.*² also showed a decrease in the firmness of fresh-cut 'Kent' mango slices during storage at 5 °C for 7 days. Conversely, the firmness of frozen mango products is reduced because of ice crystals that form during the freezing process. These ice crystals initiate rupture of cellular membranes and cell walls, leading to texture losses. Chassagne-Berces *et al.*³ studied the effects of three different freezing protocols (at -20 °C in a cold chamber, at -80 °C in a nitrogen gas chamber and by immersion in liquid nitrogen) on the firmness of 'Kent' mangoes. Their results showed that the firmness of frozen mango cylinders decreased to around 60% of the initial firmness, regardless of the freezing protocol applied. Sriwimon and Boonsupthip⁴ also reported a decline in the firmness of 'Nam Dok Mai' mango cubes to approximately 50–60% of the initial firmness after being frozen in a -40 °C air blast freezer or a -80 °C cryogenic freezer.

Addition of the enzyme pectin methylesterase (PME) and calcium has been reported to have beneficial effects on maintaining

fruit firmness.^{5–8} Enzyme infusions potentially alter the texture, flavour and other sensory attributes of food items. The process of de-esterification of pectin by PME and subsequent chelation of calcium by ionised carboxylic acid groups on adjacent pectic acid chains is typically depicted in the form of an 'egg-box' structure.⁹ Since infusion into fruits is limited by the molecular size of enzymes and the rate of passive calcium diffusion, vacuum infusion can be used to infiltrate the exogenous enzyme into fruit pieces more rapidly and homogeneously in comparison with merely soaking.¹⁰

Banjongsinsiri *et al.*⁶ used a vacuum to infuse PME into strawberry halves, which resulted in a twofold increase in firmness in comparison with water-infused controls; however, the firmness value reported was not significantly different from that of non-infused fruit. Degraeve *et al.*⁵ reported the synergistic action of PME and calcium in the vacuum infusion solution on strawberry fruit texture. The vacuum-assisted procedure was observed to accomplish an uptake of infusion solution (PME and calcium) and hence was capable of improving the firmness of strawberries.^{7,8}

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However, a large pressure difference caused by a rapid vacuum release may reduce the effectiveness of this process, as some tissue is subject to sudden compression and cell rupture. Moreover, the fruit tissue may be subject to waterlogging after vacuum infusion.⁹ Banjongsinsiri *et al.*¹¹ studied the effect of infusion with Valencia orange PME and calcium under vacuum (85 kPa or 16.1% vacuum) for 10 min at room temperature on the texture of fresh-cut mangoes. They found that this infusion process did not improve the firmness of fresh-cut mango cubes. However, they did not study the effect of varying the vacuum level during the infusion process. To date, there has been no report on the effect of PME and calcium infusion on the texture of frozen mangoes.

Therefore the objective of this study was to investigate the combined effects of mixtures of PME and calcium and vacuum levels during the infusion process on the texture and microstructure of both fresh and frozen-thawed mangoes.

MATERIALS AND METHODS

Materials

Ripe mangoes (*M. indica* L.) of 'Kent' variety were purchased from a local supermarket in Davis, CA, USA. Fruit were selected for uniformity in size and similar maturity based on flesh colour, texture and total soluble solids content. In this study, mangoes with total soluble solids in the range 16–19 °Brix and firmness in the range of 23–30 N were chosen. The firmness of mango flesh, measured after the skin had been removed, was evaluated using a texture analyser (TA.XT2, Texture Technologies, 18 Fairview Road, Scarsdale, NY) with a 25 kg load cell and an 8 mm cylindrical flat head probe (P8). A compression depth of 8 mm and a compression rate of 1 mm s⁻¹ were used. The maximum peak force was expressed as firmness (N). Approximately 20% of the total length from both the stem end and the blossom end of each fruit was discarded, as those parts are highly diverse in terms of fruit properties. Only the central part of each fruit was used to minimise any variation within samples. Eighteen mangoes per replication were washed, peeled and cut into cubes of 1.5 cm. Fourteen mango cubes were cut from each fruit. To reduce any potential variation in the initial sample between treatments, two cubes from each fruit were segregated for each individual treatment. Therefore 18 mangoes were represented in each treatment.

Treatments

Untreated mango cubes and mango cubes infused with water were used as control samples. The infusion solution under evaluation was composed of 0.001 U mL⁻¹ PME (commercial PME from *Aspergillus aculeatus*, Novoshape, Novozymes, Bagsvaerd, Denmark) and 10 g l⁻¹ calcium chloride. The optimal PME concentration was selected based on a preliminary study. A sample/infusion solution ratio of 1:3 (w/w) was used to ensure complete immersion of all samples in the infusion solution. Three levels of vacuum pressure, ranging from no vacuum (atmospheric pressure, 101.325 kPa) to a medium level of vacuum (50 kPa or 50.6% vacuum) and the highest level of vacuum (10 kPa or 90.1% vacuum), were used.

For infusion at atmospheric pressure, 35 cubes of mango (105 g) were immersed in 315 g of infusion solution for 10 min at atmospheric pressure and room temperature (25 °C). For infusion under vacuum conditions, 35 cubes of mango (105 g) and 315 g of infusion solution were placed in a plastic container. An aluminium mesh was used to immerse the sample in the infusion solution.

The plastic containers were placed in a vacuum oven (Isotemp Vacuum Oven Model 280A, Thermo Fisher Scientific, Pittsburgh, PA, USA) at controlled pressure levels as indicated by the pressure gauge of the instrument.

The samples were submerged in the infusion solution for 10 min at both 50 and 10 kPa. After 10 min the vacuum was released to attain atmospheric pressure within 1 min, with subsequent disposal of the solution. Infusion of liquid solution occurred during the vacuum release. The samples were then placed on paper towels for 5 min, packed in plastic bags and kept at 25 °C for 2 h prior to analysis or freezing. This ensured adequate time for the reaction of the enzyme and calcium with the pectin present in the mango samples. Twenty-five cubes of mango from each treatment were analysed for the properties of fresh samples. Ten cubes of mango from each treatment were frozen in an air blast freezer (Conrad freezer, Barber Colman Company, Rockford, IL, USA) at -50 °C until the central temperature of the samples reached -25 °C. These frozen samples were subsequently kept in a -20 °C chest freezer for 14 days. The frozen samples were thawed at 4 °C for 2 h and kept at 25 °C for 30 min prior to analysis. The experiments were repeated in triplicate.

Weight gain

The weight gain of samples after infusion was calculated as

$$\text{weight gain (\%)} = [(W_t - W_i)/W_i] \times 100$$

where W_i is the initial sample weight and W_t is the sample weight after infusion. The measurements were done in triplicate.

Moisture content

The moisture content of samples was analysed by dehydration of the samples in a vacuum oven (the same vacuum oven as used for immersion) at 70 °C until attainment of a constant weight. The measurements were done in triplicate.

PME activity

The PME activity of unfrozen samples was determined by the method outlined by Anthon and Barrett,¹² with analysis of the methanol released from the samples with alcohol oxidase (from *Pichia pastoris*, Sigma, St Louis, MO, USA) and acetyl acetone (Sigma). To allow for the determination of the release of methanol, both control samples and samples immediately after infusion were cut into slices approximately 1 mm thick. Then 18 mL of water and 2 g of mango slices were placed in a glass bottle with a screw cap. The bottles were sealed and incubated in a shaking water bath at 25 °C for 60 min. Samples (200 µL) of immersion liquid were collected immediately after immersion and after incubation for 60 min. To determine the methanol content, 50 µL of sample was added by pipette to a microplate. As a standard, 50 µL of 0–2 mmol L⁻¹ methanol was added by pipette to the microplate. The assay was started by adding 50 µL of 1 U mL⁻¹ alcohol oxidase. The alcohol oxidase was prepared by diluting the enzyme with 0.2 mol L⁻¹ phosphate buffer (pH 7) until a final concentration of 1 U mL⁻¹ was attained. After 20 min at room temperature, 100 µL of acetyl acetone reagent was added. The acetyl acetone reagent was composed of 12 mL of 2 mol L⁻¹ ammonium acetate, 36 µL of glacial acetic acid and 24 µL of acetyl acetone. After an additional 60 min at room temperature, the absorbance at 405 nm was read on a microplate photometer (Multiskan FC, Thermo Fisher Scientific). The measurements were done in triplicate. PME activity was expressed as µmol methanol g⁻¹ sample h⁻¹.

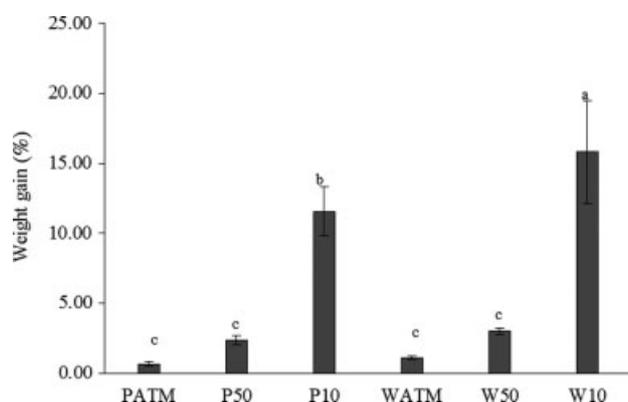


Figure 1. Weight gain of mango cubes infused with PME and calcium at atmospheric pressure (PATM), 50 kPa (P50) and 10 kPa (P10) and infused with water at atmospheric pressure (WATM), 50 kPa (W50) and 10 kPa (W10). Bars indicate standard deviation, with different letters denoting significant differences ($P < 0.05$).

Texture

The texture of both fresh and frozen-thawed samples was determined by means of a texture analyser (TA.XT2, Stable Micro Systems) with a 25 kg load cell and a 50 mm cylindrical flat head probe (P50). Firmness was measured by compression at 50% strain and a compression rate of 1 mm s^{-1} . The maximum peak force was expressed as firmness (N). Ten replicate pieces of fruit were used for each treatment.

Microstructure

Samples were sectioned with a razor-blade to a thickness of approximately 1 mm. Their microstructure was observed using a confocal laser scanning microscope (Olympus IX 71, Olympus Inc., Center Valley, PA, USA) with a $10\times$ objective lens and an exposure time of 5000 ms. The microscope was controlled by Metamorph software (Molecular Devices Inc., Sunnyvale, CA, USA). The cell walls of the samples were stained with calcofluor white stain (Sigma-Aldrich, Buchs, Switzerland). The maximum emission wavelength of this fluorescent dye is 433 nm and excitation occurs at about 355 nm.

Statistical analysis

The experiments were done in triplicate. The obtained data were subjected to one-way analysis of variance. Duncan's multiple range test was used to compare means ($P < 0.05$).

RESULTS AND DISCUSSION

Weight gain

Figure 1 shows the weight gain of samples following infusion under various conditions. The weight gain was greater at relatively high vacuum level (10 kPa). The weight gain of mango cubes infused with either PME and calcium solution or water at the highest level of vacuum (10 kPa) was significantly different ($P < 0.05$) in comparison with the other samples. For the medium level of vacuum (50 kPa), a smaller amount of tissue gases was drawn out, so, when the vacuum was released, less liquid solution was infused into the sample in comparison with infusion at 10 kPa. At 10 kPa, samples infused with water showed a significantly higher ($P < 0.05$) weight gain than those infused with PME and calcium.

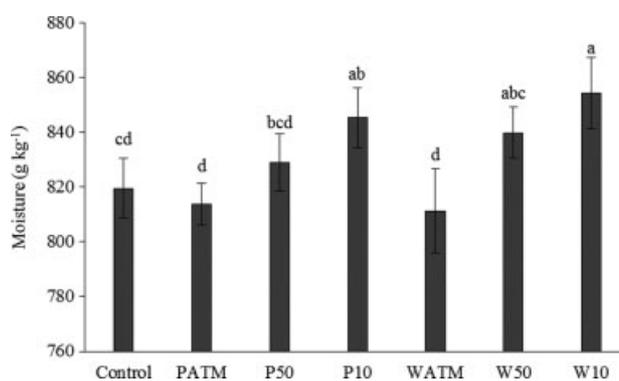


Figure 2. Moisture content of mango cubes untreated (control), infused with PME and calcium at atmospheric pressure (PATM), 50 kPa (P50) and 10 kPa (P10) and infused with water at atmospheric pressure (WATM), 50 kPa (W50) and 10 kPa (W10). Bars indicate standard deviation, with different letters denoting significant differences ($P < 0.05$).

During vacuum application, tissue gases were drawn out of the samples, and infusion of the liquid solution occurred when the vacuum was released.⁹ Therefore weight gain was caused by the movement of water, calcium and PME from the infusion solution into the fruits after vacuum release. Similar findings to ours were reported for eggplant cylinders infused at 68 kPa (32.9% vacuum) for 15 min at 30°C .¹³ The authors reported the percentage yield (percentage of weight after process divided by initial weight) of samples after infusion in water and in a combined solution of PME and calcium as 180 and 165% respectively. Fraeye *et al.*⁸ infused strawberry halves with fungal PME and calcium chloride solution at 0 kPa and reported the mass of infused strawberries as a percentage of their original mass. The mass of infused strawberry halves was approximately 130%. This weight gain was relatively higher than that found in our study. However, the comparison of weight gains from different investigators is difficult because of differences in fruit sample properties and infusion conditions (i.e. vacuum level, infusion temperature and time).

Moisture content

The moisture content of all samples is shown in Fig. 2. The moisture results correlated well with the weight gain of samples. Immersion at atmospheric pressure did not affect the moisture content of samples, but the moisture content of samples infused with either water or PME and calcium solution was highest at the highest vacuum level (10 kPa). This most likely resulted from the movement of water into the fruit pieces during the time when the vacuum was released. The moisture content of mango cubes infused at 10 kPa was significantly different ($P < 0.05$) from that of control samples but was not significantly different ($P > 0.05$) from that of samples infused at the medium level of vacuum (50 kPa). Comparable results were found in both samples infused with water and those infused with PME and calcium solution. There is no comparative published information on moisture content changes in other fruits after vacuum infusion.

Waterlogging of the tissues was apparent in mango cubes after vacuum release and was most readily observed after infusion at 10 kPa. This finding was in agreement with the results of Banjongsinsiri *et al.*¹¹ Waterlogging likely has an effect on the translucent appearance of fresh-cut samples. Nevertheless, no difference was found in the visual appearance of all frozen-thawed samples.

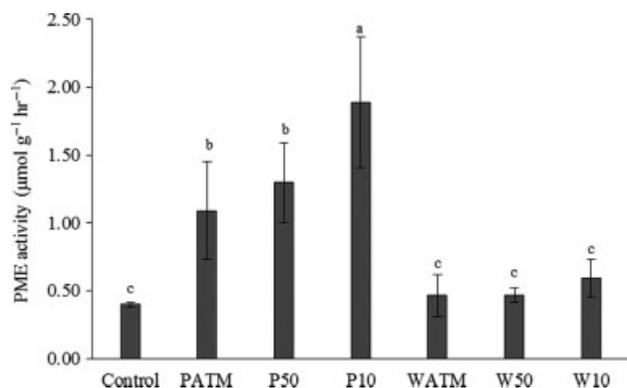


Figure 3. PME activity of mango cubes untreated (control), infused with PME and calcium at atmospheric pressure (PATM), 50 kPa (P50) and 10 kPa (P10) and infused with water at atmospheric pressure (WATM), 50 kPa (W50) and 10 kPa (W10). Bars indicate standard deviation, with different letters denoting significant differences ($P < 0.05$).

PME activity

Infusion with water did not affect the endogenous PME activity of mango cubes at any pressure level (Fig. 3). However, the PME activity of mango cubes was significantly higher ($P < 0.05$) after infusion with calcium and PME solution at all pressure levels. The specific pressure level had a strong effect on the resulting PME activity of fruits. Samples infused with PME and calcium at the highest vacuum (10 kPa) showed the highest PME activity ($P < 0.05$). This may have been due to the fact that gases present in the fruit cubes were drawn out of the tissues more effectively at this high level of vacuum (10 kPa) than at the medium level of vacuum (50 kPa).

Vacuum infusion, as compared with atmospheric immersion, has the advantage of removing almost all interior gases that exist in the apoplastic spaces between cells; thus the enzyme solution has the ability to occupy these spaces and remains within the tissue after infusion.⁹ An increase in PME activity after vacuum infusion was also reported in previous research on strawberries and apples.^{6,10,14} The enzyme penetration is due to hydrodynamic phenomena allowing the external solution to fill the sample pores.¹⁰ Guillemin *et al.*¹⁰ studied the importance of vacuum level on the PME distribution in apple cubes after vacuum infusion with PME and calcium solution at different vacuum levels (50, 30 and 5 kPa or 50.6, 70.4 and 95.1% vacuum respectively). They similarly reported better penetration of PME into apple cubes at higher vacuum level, resulting in a homogeneous distribution of PME in apple cubes after vacuum impregnation at 5 kPa.

Firmness

Fresh mangoes

The firmness of all samples was defined by the maximum peak force and is given in Table 1. Infusion of fresh samples with water was found to decrease their firmness, and a significant effect ($P < 0.05$) was found at the highest vacuum (10 kPa). This result was likely due to waterlogging of the cells after the vacuum was released. Conversely, samples that were vacuum infused with PME and calcium at 10 kPa attained a significantly higher ($P < 0.05$) level of firmness. This was probably due to the accumulation of calcium and PME in the mango cubes after infusion at the high vacuum level of 10 kPa (Figs 1 and 3).

The de-esterification of pectin by PME and the chelation of calcium by ionised carboxylic acid groups on adjacent pectic acid

Table 1. Firmness of mango cubes before and after freezing

Treatment ^a	Firmness (N) ^b	
	Fresh	Frozen
Control	52.53 ± 4.68b	19.81 ± 1.76b
PATM	59.70 ± 8.52ab	23.71 ± 2.28a
P50	56.83 ± 5.88b	23.83 ± 2.05a
P10	70.39 ± 11.45a	17.62 ± 2.14b
WATM	46.85 ± 6.33bc	19.50 ± 1.77b
W50	49.32 ± 4.65bc	18.57 ± 0.35b
W10	39.49 ± 3.31c	12.21 ± 1.43c

^a Untreated (control), infused with PME and calcium at atmospheric pressure (PATM), 50 kPa (P50) and 10 kPa (P10) and infused with water at atmospheric pressure (WATM), 50 kPa (W50) and 10 kPa (W10).

^b Data are mean ± standard deviation. Means in the same column followed by different letters are significantly different ($P < 0.05$).

chains may result in chain association in an 'egg-box' structure. Such a phenomenon results in an overall increase in firmness.⁹ Vacuum infusion with PME and calcium has also been reported to increase the firmness of peaches and strawberries.^{6,7,15} Conversely, Banjonginsiri *et al.*¹¹ reported that infusion with Valencia PME and calcium under vacuum (85 kPa or 16.1% vacuum) for 10 min at room temperature did not improve the firmness of mango cubes. They also found a heat-sensitive PME inhibitor in mango that could inhibit the activity of Valencia PME. PME inhibitors have been reported to be active against several plant PMEs (e.g. those from kiwi, orange, apple, tomato, apricot, carrot, potato and banana), whereas they do not inhibit fungal PMEs.¹⁶ Consequently, our study, which used a fungal PME, demonstrated the efficiency of PME and calcium in improving the texture of fresh mango cubes.

Frozen mangoes

After freezing and thawing, the firmness of all samples decreased in comparison with that of fresh, unfrozen samples (Table 1). Frozen-thawed samples infused with PME and calcium at 50 kPa and atmospheric pressure had significantly higher ($P < 0.05$) firmness than control frozen-thawed samples, while samples infused with water at a pressure of 10 kPa attained the lowest level of firmness ($P < 0.05$).

The pressure differential during vacuum application at 10 kPa and subsequent vacuum release resulted in the rupture of cell integrity and a subsequent decline in firmness. Moreover, the high level of vacuum also resulted in the movement of a large amount of water from the infusion solution into the fruits. Therefore the lowest level of firmness was found in samples infused with water at a pressure of 10 kPa.

Fresh samples infused with PME and calcium at 10 kPa had a superior level of firmness in comparison with control fresh samples (Table 1). However, after freezing and thawing, the firmness of these samples was not significantly different ($P > 0.05$) from that of control frozen-thawed samples. During freezing, the water present in the samples partially formed ice crystals, which negatively affected the integrity of the cellular compartments, leading to a decreased level of fruit firmness after freezing and thawing.

Samples that were vacuum infused at 10 kPa had a higher moisture content than control samples (Fig. 2). Accordingly, during freezing, a larger amount of ice crystals was formed, which were responsible for extensive microstructural changes in the fruit

tissues, resulting in a decrease in firmness. Thus PME and calcium addition may be ineffective in maintaining the cell integrity of these samples after freezing and thawing.

Rincon and Kerr¹⁷ studied the combined process of partial osmotic dehydration with 30 °Brix sucrose solution followed by freezing to improve the quality of frozen mangoes. They found that after osmotic dehydration the moisture content decreased from 0.870 to 0.793 g g⁻¹ pulp. The hardness of control samples decreased by 51.21% after freezing and frozen storage for 7 weeks, whereas the hardness of osmotically dehydrated samples decreased by only 16.26%. They suggested that pretreatment by osmotic drying can remove some of the available water, thus leaving less water to form ice crystals, which may damage the tissues and reduce hardness, during freezing.

Van Buggenhout *et al.*¹⁸ reported a marked improvement in the texture of strawberries following their infusion with PME and calcium at 1 kPa for 5 min, in particular when combined with rapid (−25 °C) or cryogenic (−80 °C) freezing, which was absent when typical slow freezing (−18 °C) was used.

Microstructure

The cell walls of all samples were stained with calcofluor and observed with a confocal laser scanning microscope. The micrographs of control fresh mangoes and all frozen-thawed mangoes are shown in Fig. 4. Control fresh mangoes were composed of well-defined circular to elliptical and regular cells (Fig. 4a). Although the texture results confirmed the dissimilarity of treatments, neither infusion with PME and calcium nor infusion with water altered the appearance of the cells. The microstructure of these samples was still composed of intact cells of rough and regular shapes comparable to those of fresh mangoes. The effect of vacuum infusion on the microstructure of mangoes was found to be in line with its effect of strawberries.^{8,19,20}

The freezing and thawing process was found to cause tissue damage in all samples (Figs 4b–4h). The cells of frozen-thawed mangoes were of irregular shape, with visible loss of cell wall integrity. This observation resulted from the formation of ice crystals during freezing. In comparison with control frozen-thawed samples, the microstructure of samples infused with PME and calcium at 50 kPa (Fig. 4d) or atmospheric pressure (Fig. 4c) was most similar to that of fresh mangoes (Fig. 4a), while samples infused with PME and calcium at 10 kPa (Fig. 4e) showed some damage. However, samples infused with water at 10 kPa (Fig. 4h) were found to be subject to severe tissue damage and considerable loss of cell integrity as a result of their high moisture content (Fig. 2). This water would be converted to a lot of ice crystals during freezing and thus cause tissue damage after thawing.

The presence of such differences in microstructure confirmed that infusion of PME and calcium at either 50 kPa or atmospheric pressure was an effective process to decrease the extent of tissue damage during freezing. These microstructure observations correlated well with the texture results. Vacuum infusion with PME and calcium was also reported to be effective in preserving the structural integrity of strawberry tissues.¹⁹

CONCLUSION

The combined effects of PME and calcium were confirmed to improve the texture and microstructure of both fresh and frozen-thawed mangoes. The vacuum level during infusion was found to be a significant factor affecting the properties of infused mango cubes. Vacuum infusion with PME and calcium at 10 kPa resulted in the highest PME activity in mangoes, and this condition was established to be the most suitable for preserving the texture of fresh mangoes. PME activity and moisture content were the most significant factors affecting the quality of frozen-thawed mangoes. Samples infused with PME and calcium at either 50 kPa

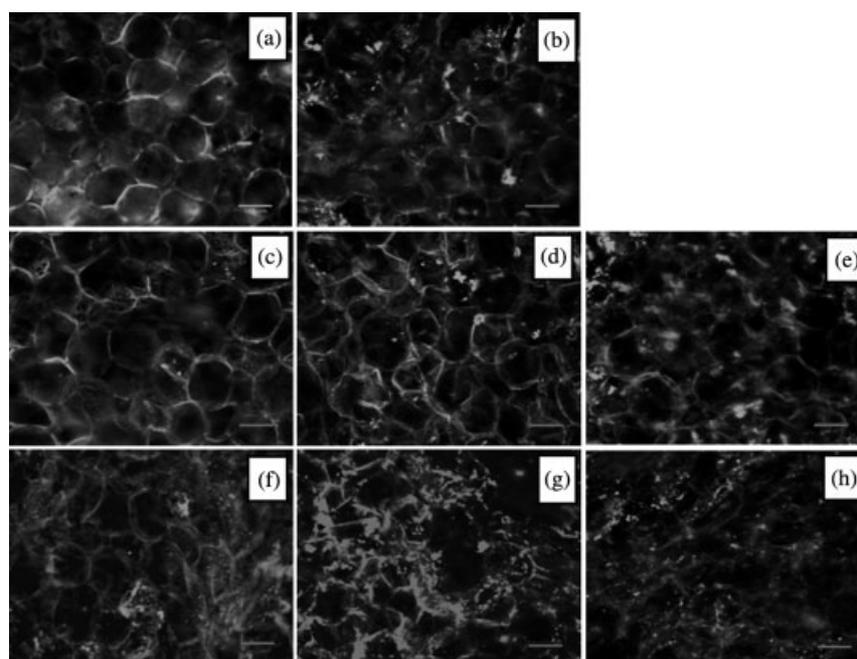


Figure 4. Micrographs of (a) control fresh and (b–h) frozen-thawed mangoes observed by confocal laser scanning microscope: (b) control; (c) PATM, infusion with PME and calcium at atmospheric pressure; (d) P50, infusion with PME and calcium at 50 kPa; (e) P10, infusion with PME and calcium at 10 kPa; (f) WATM, infusion with water at atmospheric pressure; (g) W50, infusion with water at 50 kPa; (h) W10, infusion with water at 10 kPa. Bars = 100 μm.

or atmospheric pressure had higher PME activity than control samples, while the moisture content of these infused samples was not affected by the infusion process. These two pressure levels used for infusion of PME and calcium resulted in superior texture and microstructure in comparison with control frozen-thawed samples.

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