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ORIGINAL PAPER

Effects of Vacuum Impregnation with Calcium Lactate and Pectin Methylesterase on Quality Attributes and Chelate-Soluble Pectin Morphology of Fresh-Cut Papayas

Hongshun Yang[1,2] · **Qiongying Wu**[3] · **Li Ying Ng**[1] · **Shifei Wang**[4]

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Abstract Vacuum impregnation was used to improve the quality attributes of fresh-cut papayas. Vacuum pressure of 5 kPa was applied for 5 min, then calcium lactate (1%, *w*/*w*) and pectin methylesterase (PME) (15 U/ml), alone and in combinations (calcium lactate plus PME), were vacuum impregnated into fresh-cut papaya cubes. Papaya cubes were stored at 4 °C, and the quality of fresh-cut papaya was studied at intervals for 8 days. The hardness and chewiness levels of fresh-cut papayas that were treated with calcium lactate and PME were 8.02 and 7.83 times of untreated fresh-cut papayas at day 8, respectively. After vacuum impregnation, colour of fresh-cut papayas changed significantly ($P < 0.05$) and an overall weight loss was observed as well. Chelate-soluble pectin (CSP) was extracted and its content correlated well with texture properties of fresh-cut papayas. Qualitative and quantitative analyses of CSP were conducted using atomic force microscopy. The proportion of chain widths greater than 45 nm had increased 35.0% in fresh-cut papayas vacuum impregnated with calcium lactate and PME at the end of storage. The results indicate that a combination of calcium ions and PME was able to maximally preserve the quality attributes of fresh-cut papayas and extend the shelf life.

Keywords Vacuum impregnation · Fresh-cut papaya · Calcium lactate · Pectin methylesterase · Chelate-soluble pectin · Atomic force microscopy

✉ Hongshun Yang
chmynghs@nus.edu.sg

[1] Food Science and Technology Programme, c/o Department of Chemistry, National University of Singapore, Singapore 117543, Republic of Singapore

[2] National University of Singapore (Suzhou) Research Institute, 377 Lin Quan Street, Suzhou Industrial Park, Suzhou, Jiangsu 215123, People's Republic of China

[3] School of Biotechnology, Jiangsu University of Science and Technology, Zhenjiang, Jiangsu 212018, People's Republic of China

[4] Changzhou Qihui Management and Consulting Co., Ltd., Changzhou, Jiangsu 213000, People's Republic of China

Introduction

Papaya (*Carica papaya* L.) is a popular inexpensive fruit, largely produced in several tropical and subtropical countries (Canizares and Mauro 2015). It is highly favoured for its sweet mellow flavour, a good source of nutrients and full of beneficial bioactive compounds that protect the body from oxidative stress, reducing the risk of cardiovascular diseases and some types of cancer (Sancho et al. 2014; Gayosso-García Sancho et al. 2011; Udomkun et al. 2014). Fresh-cut papayas are convenient, but the accelerated catabolism of cell wall components (Karakurt and Huber 2003) and the loss of cellular fluid (Hodges et al. 2000) result in a rapid loss of firmness and were deemed to be of intolerable quality. Therefore, an effective preservation method is needed to delay the deterioration of these fruits.

Vacuum impregnation (VI) techniques are based on the exploitation of void phase of vegetable tissues, where the exchange of gas and native liquids with an external solution is promoted under the action of hydrodynamic mechanism (Derossi et al. 2013; Mao et al. 2016). The product of interest is immersed in an impregnation solution containing the solutes to be infused into the fruits or vegetables. Together, they are exposed to vacuum pressure (5–10 kPa) that caused the expansion and diffusion of the internal gas down a pressure gradient. When atmospheric pressure is restored, an influx of

the impregnation solution will occur as a result of a reduced pore volume from compression (Fito et al. 2001).

Calcium applications have been widely used to produce beneficial effects on fresh fruit qualities such as delaying membrane lipid catabolism and improving firmness by cross-linking with both cell wall and middle lamella pectins (Udomkun et al. 2014). The degree of deesterification can be further enhanced by pectin methylesterase (PME) as this enzyme deesterifies pectin, thus releasing more free carboxylic acid groups to react (Guillemin et al. 2008; Sirijariyawat et al. 2012).

Changes in pectin morphology can be visualised by atomic force microscopy (AFM), which is able to capture nanostructural changes of pectins (Chong et al. 2015; Liu et al. 2017; Zhang et al. 2017). The degradation mode of structural polysaccharides was correlated with physicochemical properties, further to elucidate the roles of these structural polysaccharides (Feng et al. 2014; Yang et al. 2007b).

The objective of this experiment was to investigate the effects of VI with calcium ions and PME on the quality attributes and polysaccharide morphology of fresh-cut papayas. Screening steps for suitable vacuum pressure and time exposed to vacuum pressure were performed. The quality attributes were assessed based on appearance and texture. Polysaccharide morphology was visualised by AFM.

Materials and Methods

Fruit Material

Papayas of the cultivar 'Sekaki' were imported from Malaysia and bought from a local supermarket (Fairprice Finest, Singapore). Defect-free papayas of similar size (length 24–28 cm and weight 1.2–1.6 kg) with 70–75% of yellow skin and soluble solid contents of 9.0–10.5 ^{0}Brix were selected. The peel colour of papayas was assessed visually, whilst the soluble solid content was determined from the extracted juice with an Atago refractometer (RX-5000α, Tokyo, Japan). The extracted juice was centrifuged at 10,500*g* at 15 °C for 8 min to separate the juice from fruit pulps. Five papayas that met the above-mentioned criteria were isolated for test individually on a single set of manipulative variable (vacuum pressure or time exposed to vacuum pressure) in screening steps, whilst 15 papayas were isolated and run individually for the test of effectiveness of VI with calcium ions and PME in improving quality attributes of fresh-cut papayas. Papayas were bought and used on the same day. The fruits were gently washed with tap water before cutting.

Preparation of Impregnation Solution

An isotonic impregnation solution was prepared with commercial sugar based on the average ^{0}Brix values of the papayas. In the screening step, 1% (*w*/*w*) calcium lactate pentahydrate (Sigma-Aldrich Co., Ltd., St. Louis, MO, USA) on a wet basis was dissolved into the solution. The control group for the screening step was fresh untreated samples. After the screening steps, 15 U/g of fungal PME (Creative BioMart, Shirley, NY, USA) was incorporated. The volume ratio of sample to solution was kept at 1:3.

Vacuum Impregnation Treatment

Papaya cubes of 1.5 cm were immersed in the impregnation solution at room temperature, and a wire mesh was used to keep the cubes completely submerged in the solution. The beaker of fruits ready for VI was then placed in a dessicator connected to a vacuum pump which was controlled by a vacuum controller (CVC 2, Vacuubrand, Germany). In the screening steps, vacuum pressure (absolute pressure, 101 kPa in atmosphere pressure group) was first varied at 5, 10 and 20 kPa with time exposed to vacuum pressure fixed at 10 min. After which, atmospheric pressure was gradually restored back within 30 s and the fruits continued submerging in the impregnation solution for 10 min more. When VI was completed, the VI-treated samples were drained and blotted dry. Part of the papaya cubes were set aside for texture analysis immediately after VI, whilst the remainder was stored in Ziplock® bags according to number of storage days and kept at 4 °C for analysis at days 1, 2 and 3. The control used in the screening steps was untreated fresh-cut samples (non-VI), which acted as a benchmark for VI performance. Vacuum pressure with the best performance was then selected to be used in time screening at 5, 10 and 15 min, following the same procedure.

The selected vacuum pressure and time exposed to vacuum pressure were then set to study the effectiveness of VI with calcium lactate and PME. The investigation consisted of three experimental groups, which were samples separately treated with 1% (*w*/*w*) calcium lactate in isotonic sucrose solution (Ca), 15-U/ml fungal PME in isotonic sucrose solution (PME), 15-U/ml fungal PME and 1% (*w*/*w*) calcium lactate in isotonic sucrose solution (PME + Ca) as well as two control groups, of which one group contained isotonic sucrose solution only (VI control) and the latter was left untreated (non-VI). Samples in the first four categories were subjected to VI at the conditions determined from the screening steps.

After VI, papaya cubes had to be kept at room temperature for 2 h for incubation of PME (Fraeye et al. 2010). The papaya cubes were subject to colour measurement and texture profile analysis (TPA) for a period of 8 days.

TPA

TPA tests were conducted with the TA-XT2i Texture Analyser (Stable Micro Systems Ltd., Godalming, Surrey, UK). The

operating parameters settings were load cell 25 kg, probe 35-mm-diameter aluminium cylinder, pre-test speed 1 mm/s, test speed 0.5 mm/s, post-test speed 0.5 mm/s, compression degree 40%, time 12 s and trigger force 3.0 g. Papayas were allowed to incubate at room temperature after removal from the refrigerator for 10 min before measurement (Yang et al. 2007a).

Weight Loss

Papaya cubes were weighed before and after VI to determine the change in weight after processing. After storing papayas into Ziplock® bags according to storage day, the initial and final weights of papaya cubes in each bag were recorded to monitor the change in weight with time (Sirijariyawat et al. 2012).

Colour Measurement

Colour measurements of the papaya cubes were carried out on 15 samples using a bench-top spectrophotometer (CM-5, Konica Minolta Sensing Inc., Osaka, Japan). The colour of papayas was expressed as CIE colour space values, L^*, a^* and b^*. Luminosity L^*represents the brightness of the sample, whilst a^* and b^* represent the colour direction from green (−) to red (+) horizontal axis and blue (−) to yellow (+) vertical axis, respectively. In order to quantify the true colour of the papayas, psychometric coordinates, chroma (C; Eq. 1) and colour difference (ΔE; Eq. 2) based on fresh untreated sample were calculated from these values (Perez-Cabrera et al. 2011).

$$C = \sqrt{(a^*)^2 + (b^*)^2} \tag{1}$$

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \tag{2}$$

where $\triangle E$ is the colour difference, $\triangle L^*$ is the difference of L^* between VI-treated sample and fresh untreated sample, $\triangle a^*$ is the difference of a^* between VI-treated sample and fresh untreated sample and $\triangle b^*$ is the difference of b^* between VI-treated sample and fresh untreated sample.

Chelate-Soluble Pectin Extraction and Determination

All chemicals used as stated from here henceforth were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO) unless otherwise stated. Cell wall materials were fractionated according to Chen et al. (2009). About 10 g of papaya was ground in a cold mortar and boiled in 200 ml of 80% (*v*/*v*) ethanol for 20 min to inactivate endogenous enzymes. The mixture was then left to cool and filtered using vacuum pump, and the residue was collected. Boiling of the residue was repeated twice. Subsequently, the residue would be incubated overnight with 50 ml of dimethyl sulphoxide (DMSO):water (9:1, *v*/*v*) at 4 °C to remove starch. Filtration of the residue was done to separate it from the solvent, and it was washed with deionised water and transferred to 200 ml of chloroform:ethanol (2:1, *v*/*v*) for 10 min. The cell wall material was obtained after filtration and washing with 200 ml of acetone twice until total whitening. Chelate-soluble pectin (CSP) was extracted from the cell wall material by suspending it in 10 ml of 50 mM sodium acetate buffer (pH 6.5) containing 50 mM 1,2-cyclohexanediaminetetraacetic acid (CDTA). After shaking it for 4 h, the supernatant was collected by centrifugation at 13,500*g* for 15 min at 4 °C. This extraction procedure was further repeated twice, and the supernatants collected were combined. The CSP fractions were then stored below −18 °C after extraction to preserve it for analysis.

CSP content of papaya was assayed with the carbazole colourimetry method with galacturonic acid as the standard (Liu et al. 2009) with slight modifications. CSP solution of 2 ml was added to 12 ml of sulphuric acid (98%, *w*/*w*) in an ice bath. It was then boiled for 10 min in a 70 ± 5 °C water bath and cooled under running tap water. After which, the samples were incubated and mixed with 0.5 ml of carbazole ethanol solution for 30 min. Absorbance readings were then taken at 530 nm using a UV-Vis spectrophotometer (UVmini-1240, Shimadzu, Japan) at room temperature. Pectin solutions were diluted to within the range of the standard, and results were expressed as milligram of galacturonic acid per 100 g fresh weight.

AFM Image Analysis

AFM of CSP of fresh untreated sample at day 0 and all treatment groups at day 8 were conducted according to previous research (Liu et al. 2009; Zhang et al. 2008) using a version 2.1 TT-AFM (AFM Workshop, Signal Hill, CA, USA) in tapping mode. The CSP solution was diluted to a suitable concentration to obtain a clear image. About 0.9 μl of the diluted solution was pipette onto a freshly cleaved mica sheet (Muscovite Mica; Electron Microscopy Sciences, Hatfield, PA, USA) mounted onto an AFM specimen disc (TED Pella Inc., Redding, CA, USA) with double-sided adhesive tape and blow-dried. The samples were scanned in air using a TM 190-A-15 tip (Sensa Probes, USA) at a scan rate of 4.0 Hz. The force constant and resonance frequency of the tip were 25–95 N/m and 145–230 kHz, respectively.

The AFM images were examined offline with an AFM software (Gwyddion, AFM Workshop, Signal Hill, CA, USA). Flattening correction was done to reduce the noise of the sample, and the height and peak width at half chain height of the pectin chains were obtained by section analysis. At least 50 measurements from 5 images were taken for each sample to provide reliable statistical results.

Modelling for the Vacuum Impregnation Process

To explain the pectin interaction with Ca^{2+} and PME clearly, a model of interaction was drawn with ChemDraw (version 14.0.0.117; Waltham, MA, USA).

Statistical Analysis

Screening and final processing experiments were run 5 and 15 independent times, respectively. Results were statistically analysed using analysis of variance (ANOVA) at a 5% significance level, and Duncan's multiple-range tests for differences of different groups were carried out using SPSS (version 19.0) statistical software to determine the effects of VI with calcium lactate and PME treatment together with storage time on the physicochemical properties of papayas. Values of parameters from TPA, colour coordinates and AFM results were expressed as means ± standard deviation.

Results and Discussion

Screening Steps for Vacuum Pressure and the Time Exposed to Vacuum Pressure

Hardness can be defined as the force required for a given deformation of the sample. In screening steps, the hardness of papaya cubes was measured as the indicator.

As seen from Fig. 1a, the hardness of all papayas that were VI treated decreased significantly after VI. This could be due to the sudden large-pressure difference experienced by the papaya cubes when vacuum pressure was applied and during restoration of atmospheric pressure. This sudden expansion and compression weakened the cell structures (Guillemin et al. 2008), thus resulting in a loss of firmness after VI. The level of vacuum pressure applied had no effect on the extent of firmness loss in vacuum-impregnated papayas after VI as all three pressure groups showed a similar level of hardness (Fig. 1a).

The level of hardness in control samples decreased with storage days, whereas the calcium-treated samples managed to maintain their level of hardness attained after VI with time. In some cases, hardness of 5- and 10-kPa treated papayas increased after 3 days of storage to similar level as before VI. This softening of control sample is consistent with other research findings (Karakurt and Huber 2003), which showed that polygalacturonase activity was elevated in fresh-cut papayas as compared to the intact fruit due to increased ethylene production from wound-induced damage. This enzyme is responsible for the depolymerisation of polyuronide and rapid softening of the fruit, which caused fresh-cut papayas to be of unacceptable quality within 2 days (Karakurt and Huber 2003). The delayed softening in pressure-treated groups could be due to the effect of calcium ions which formed cross-linkages amongst pectin chains as they bind to free carboxyl groups, thereby strengthening the cell wall (Udomkun et al. 2014). This indicated that VI was effective in infusing calcium ions into the fruit core.

Papaya cubes exposed to higher vacuum pressure were better at maintaining their hardness with storage time as the hardness of papaya treated under 5 kPa was significantly harder than the control (Fig. 1a). The larger-pressure gradient initiated at 5 kPa could have led to a more effective removal of gases from the apoplastic spaces between cells, which would be replaced with relatively more calcium ions during pressure restoration. Guillemin et al. (2008) also reported that at higher vacuum pressure of 5 kPa, a more homogeneous and better penetration of PME was achieved in apple cubes. Therefore, 5 kPa was selected for the next screening step for the amount of time exposed to vacuum pressure.

The effects of VI at different immersion time on the hardness of papayas displayed a similar trend as mentioned previously, whereby the hardness of all treated samples were reduced and the samples treated with 5 and 15 min significantly reduced after VI. However, a gradual rise in hardness with storage time with 5- and 15-min samples was either superior to or at equivalent with control by day 3 of storage (Fig. 1b). Based on the results obtained, 5 min of immersion under vacuum pressure was better at retaining the same level of hardness as day 0 fresh samples, and the 5-min sample at day 3 had a hardness level which was not significantly different from before it was being VI treated. Therefore, the parameters screened for VI were determined to be a vacuum pressure (VP) of 5 kPa and an exposure time to VP of 5 min.

Effects of Vacuum Impregnation on Quality Attributes of Papaya Cubes with Calcium Lactate and PME

Fungal PME was used instead of plant PME as PME inhibitors in fruits were found to inhibit plant PME but not fungal PME (Sirijariyawat et al. 2012). Moreover, the use of calcium lactate can overcome the issues of bitterness and residual flavour due to calcium chloride (Udomkun et al. 2014). In addition, it also prevents carcinogenic compounds from being formed, which had been related to the use of chlorine (Martin-Diana et al. 2007). These steps were taken to enhance the future marketability of the product.

Figure 2 shows the weight loss of all treatment groups after VI and over a period of 8 days. There was a net weight loss after VI in all treatment groups. This could be due to the more effective efflux of native liquid together with the gas phase in the fruit when the pressure was lowered (Zhao and Xie 2004). This loss in mass was not replaced by the impregnation solution when vacuum was released. The higher molecular mass of components in the impregnation solution such as PME

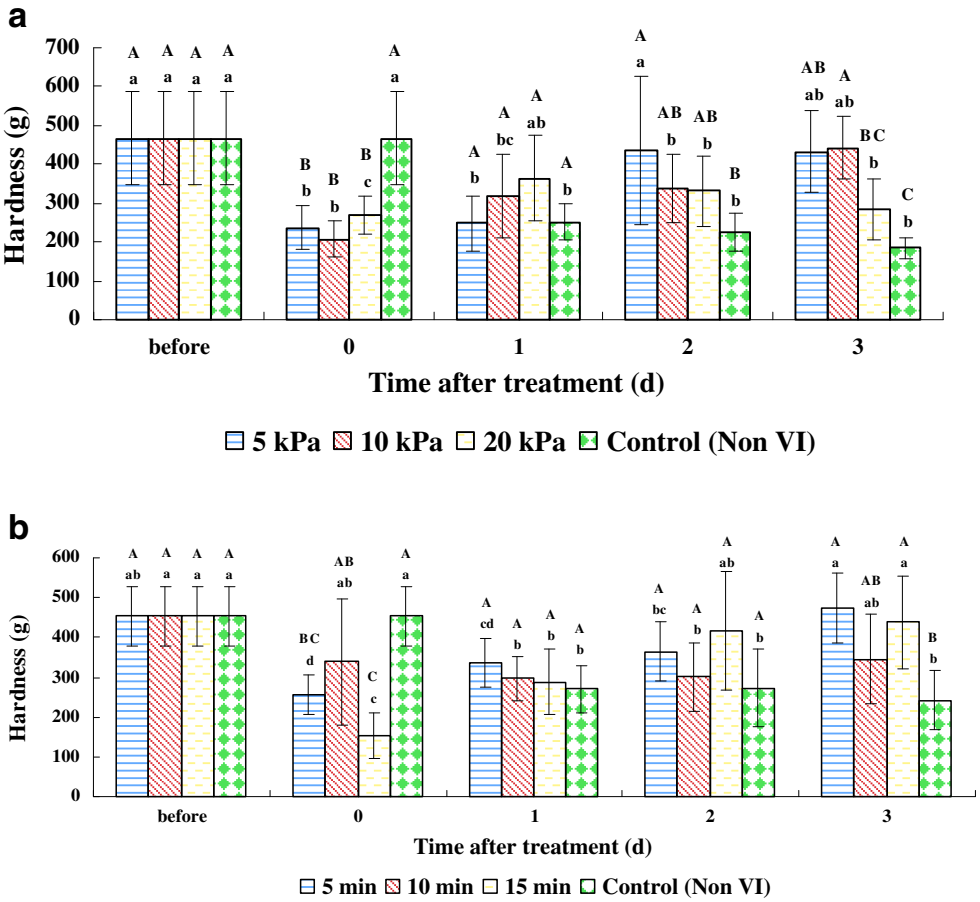


Fig. 1 Effects of vacuum impregnation on papaya hardness during storage. **a** Different vacuum pressure for 10 min. **b** Different exposure time to 5 kPa. Results are expressed as mean value (*bar*) with standard deviation (*error bar*), $n = 5$. *Different capital letters* indicate significant differences ($P < 0.05$) amongst different vacuum pressure treatments, and *lowercase letters* indicate significant differences ($P < 0.05$) with time

could have impeded with movement of solutes into the empty porous structure.

A gradual rise in weight loss with storage time was also observed in all groups except for VI control (Fig. 2). PME + Ca group showed a lower-percentage mass loss than all other groups during 8 days of storage. This was likely due to the better preserved pectin structures in PME + Ca group which retarded moisture and juice losses.

TPA was used instead of a single hardness parameter only for providing a better representation of the actual fruit texture as TPA attempts to replicate the mastication action upon the fruit. Rapid softening of fresh-cut papayas is a major limiting factor of their shelf life as the inevitable rupture of cell wall when cutting the fruit releases pectinolytic enzymes, which diffuse to interior of tissue and catalyse the breakdown of pectins (Toivonen and Brummell 2008). This compromised

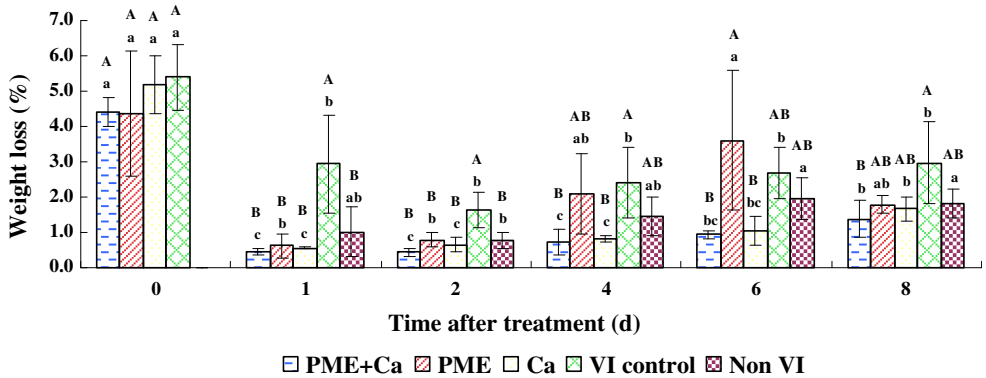


Fig. 2 Average percentage weight loss of papayas under different VI treatment with storage time. d_0 weight of samples taken 2 h after VI, *PME + Ca* VI with PME and calcium lactate, *PME* VI with PME only, *Ca* VI with Ca only, *VI control* VI without added solutes and *non-VI* untreated samples. All VI was carried out in an isotonic sucrose solution at 5 kPa for 5 min. Results are expressed as mean value (*bar*) with standard deviation (*error bar*), $n = 3$. *Different capital letters* indicate significant differences ($P < 0.05$) amongst treatments, and *lowercase letters* indicate significant differences ($P < 0.05$) with storage time

the structural integrity of fresh-cut papayas. In addition, the increase in wound-induced ethylene production promotes polygalacturonase (PG) activity, which catalyses the depolymerisation of pectin, further weakening cell wall structures (Karakurt and Huber 2003). Therefore, these factors caused the decline in hardness for PME, VI control and non-VI groups (Fig. 3a). Softening of papayas in PME group was likely due to insufficient endogenous calcium ions to bind to free carboxyl groups released by the added PME to form a strong network of cross-linked pectin chains (Lara et al. 2004). In addition, the catalytic action of PME increased pectin solubility and thus increasing pectin susceptibility to degradation by other pectinolytic enzymes, such as PG (Chávez-Sánchez et al. 2013).

It was also consistent that PME + Ca managed to sustain the highest level of hardness with storage time followed by Ca-treated group (Fig. 3a). The former was more effective due to the availability of more deesterified carboxyl groups from the enzymatic action of PME for calcium binding.

Springiness measures the ability of the sample to recover after deformation. As seen from Fig. 3b, there was not a single treatment group that consistently displayed significant difference from the rest, indicating that springiness of the fruit was not influenced by the respective treatment of VI. The loss of springiness with time was due more likely to the softening of fruit with storage time. Cohesiveness is a measure of the extent to which a product is able to withstand a second deformation relative to its performance under the first deformation. It can be used as an estimate of the ability and rate at which a material breaks down. Thus, product with high cohesiveness tends to break easily. Figure 3c displays that a general increasing trend in cohesiveness with storage time for all treatment groups was noted, which could be due to the progressive weakening of cell wall structure with time. The last parameter, chewiness, is defined as the work done during chewing to reach a steady state for swallowing. As it is a function of the other three parameters, chewiness is, therefore, highly influenced by a large change in any one of these parameters. The significantly more chewy papayas from PME + Ca groups (Fig. 3d) were to a large extent, dependent on hardness which was shown to have the most significant impact on textural differences amongst the different VI treatment groups. The chewiness of tofu was also reported by Shih et al. (2002) to be influenced by hardness. Hence, chewiness of papayas displayed a similar trend as that of hardness (Fig. 3a, d).

The change in luminosity, L^*, and chroma, C, with time shared a similar trend. A drastic loss in both parameters was observed after VI treatment (Fig. 4a, b). This was also reported in other reports involving VI-treated pear (Perez-Cabrera et al. 2011) and kiwi fruit (Talens et al. 2002). The change in these parameters could be attributed to a modification of the gas-liquid composition in the porous structure of fruits as the gas spaces present in the fruit core was removed and replaced with an impregnation solution by VI. This resulted in a rise of the tissue refractive index, thereby enhancing light absorption over scattering (Chiralt and Talens 2005). As a result, the papaya cubes gained translucency which was reflected as a drop in C values and L^* values. This would be observed as a decrease in intensity and brightness of the colour of papaya flesh, respectively.

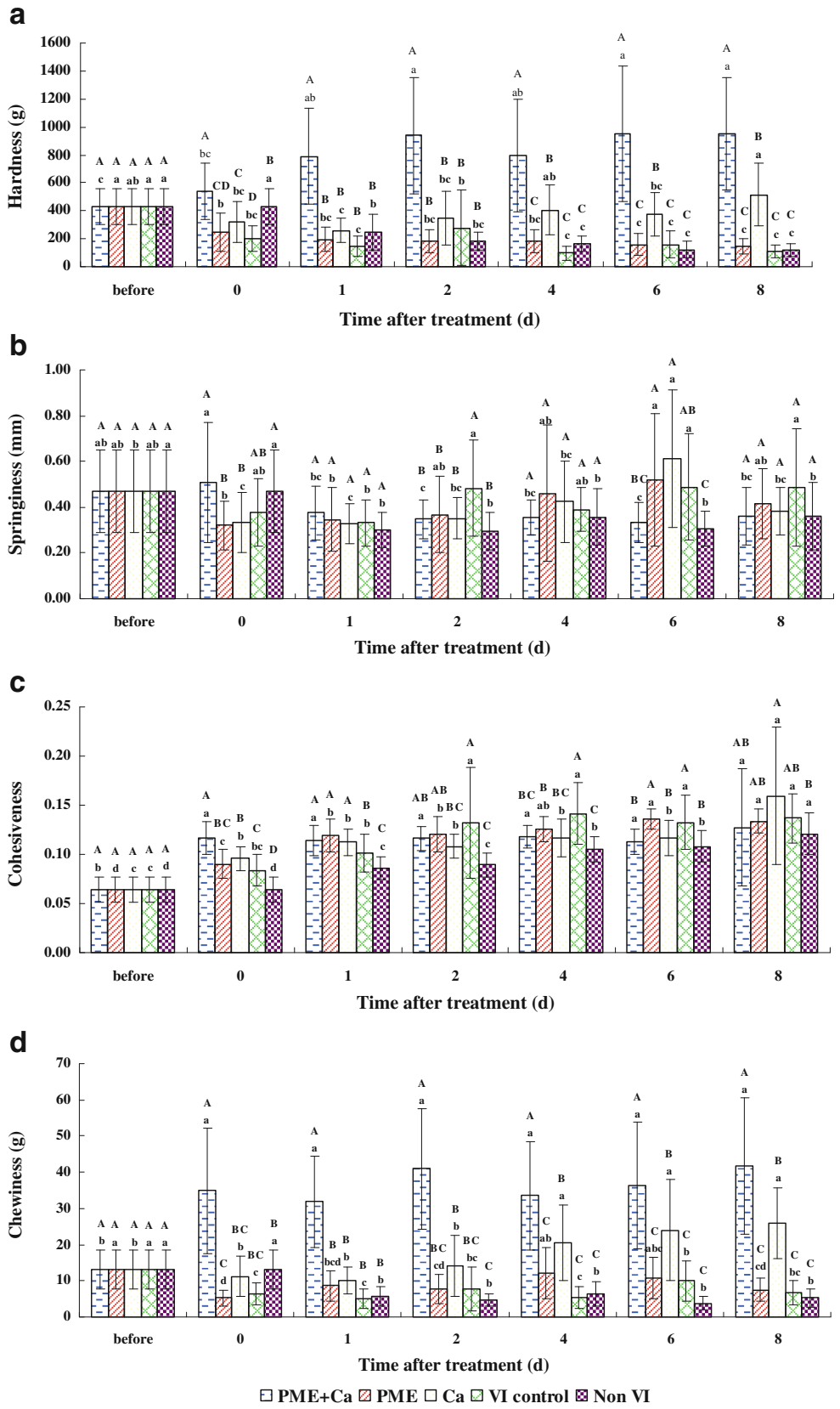
Fig. 3 TPA of papaya cubes before and after VI with different solutes impregnated for a period of 8 days. **a** Hardness. **b** Springiness. **c** Cohesiveness. **d** Chewiness. *PME + Ca* VI with PME and calcium lactate, *PME* VI with PME only, *Ca* VI with Ca only, *VI control* VI without added solutes and *non-VI* untreated samples. All VI was carried out in an isotonic sucrose solution at 5 kPa for 5 min. Results are expressed as mean value (*bar*) with standard deviation (*error bar*), $n = 15$. *Different capital letters* indicate significant differences ($P < 0.05$) amongst treatments, and *lowercase letters* indicate significant differences ($P < 0.05$) with storage time

For papayas that were not treated with VI, there was a progressive decline in L^* and C values with storage time (Fig. 4a, b) that was accompanied with a rise in ΔE values (Fig. 4c). This was likely due to the degradation of pigment molecules in papayas such as carotenoids that are prone to oxidation. A correlation between L^* and C values with carotenoid content in fruit mesocarp was also determined in 'Manila' mango (Ornelas-Paz et al. 2008).

Papayas that were vacuum impregnated showed a slower rate of decline in L^* and C values with time (Fig. 4a, b). The removal of gases from the fruit when vacuum pressure was applied could have played a significant role in slowing down the rate of carotenoid oxidation as the air spaces in papayas were replaced with the impregnation solution. Low-oxygen atmosphere had been proven to help preserve carotenoids (Boon et al. 2010). Thus, vacuum impregnation might have protective effect against bioactive compounds or nutrients in fruits that would otherwise deteriorate quickly under normal conditions. This beneficial effect of VI would have to be confirmed by further testing of carotenoid content in VI-treated and untreated papayas.

Effects of Vacuum Impregnation on the CSP of Papaya Cubes with Calcium Lactate and PME

Out of the various pectin fractions, CSP was thought to be most closely linked to the effects of calcium treatment (Lara et al. 2004). CSP binds to the cell wall ionically and interacts with calcium ions to slow down the dissolution of the middle lamella, thereby preserving the structural integrity and firmness of fruits. Besides that, added calcium ions were reported to initiate mobilisation of uronic acid from sodium carbonate soluble pectin (SSP) to CSP (Yang et al. 2009). SSP is the fraction of pectin that is covalently bounded to cell wall (Brummell 2006). This allows for the binding of calcium ions to the negatively charged free carboxyl groups of pectins to



strengthen the cell wall structure. Hence, CSP was extracted to analyse its physical morphology under AFM as it was supposed that physical morphology plays a more determining role in the protective effects of cell wall materials instead of

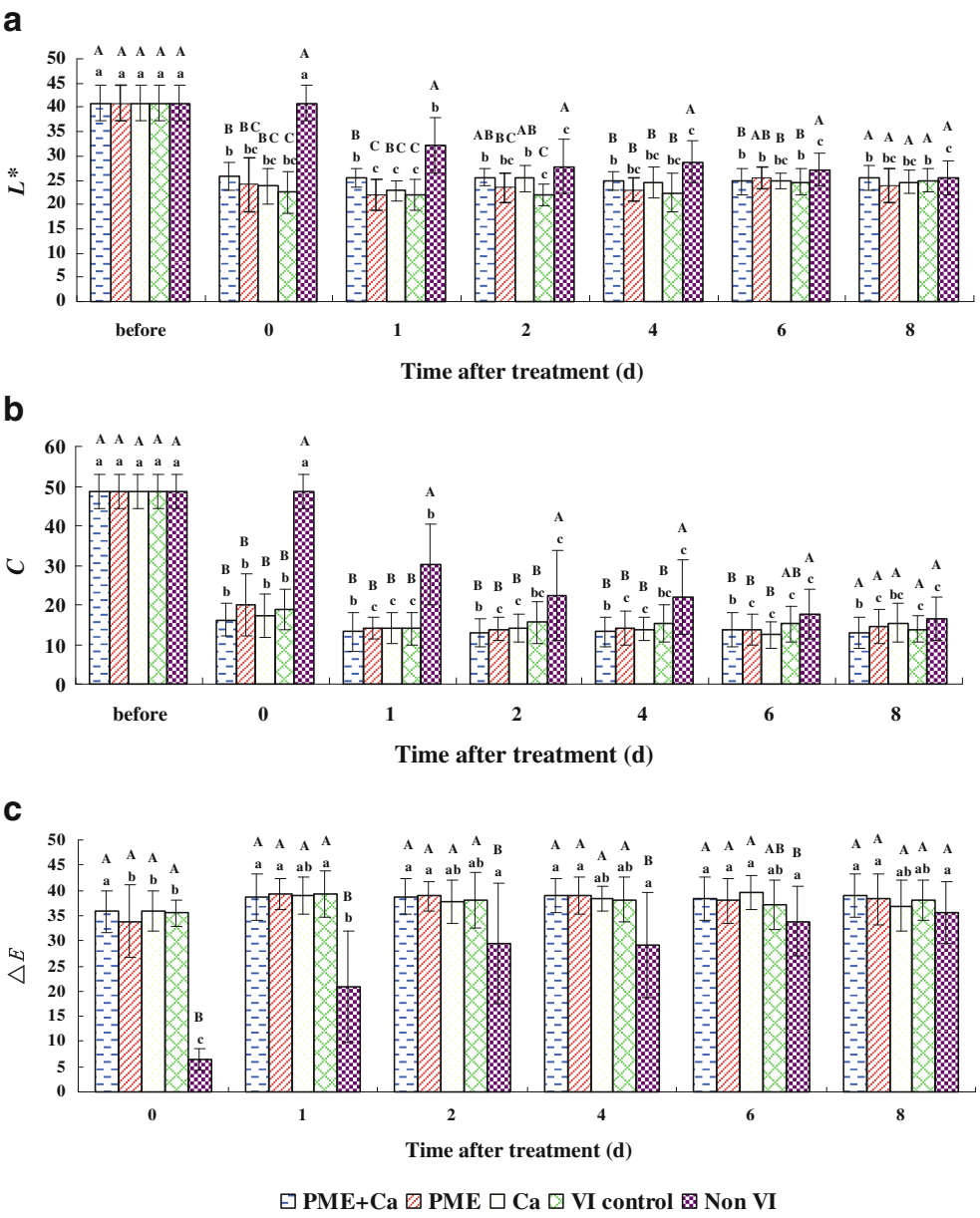


Fig. 4 Colour of papaya cubes before and after VI with different solutes impregnated for a period of 8 days. **a** Luminosity, L^*. **b** Chroma, C. **c** Overall colour change, ΔE. *PME + Ca* VI with PME and calcium lactate, *PME* VI with PME only, *Ca* VI with Ca only, *VI control* VI without added solutes and *non-VI* untreated samples. All VI was carried out in an isotonic sucrose solution at 5 kPa for 5 min. Results are expressed as mean value (*bar*) with standard deviation (*error bar*), $n = 15$. *Different capital letters* indicate significant differences ($P < 0.05$) amongst treatments, and *lowercase letters* indicate significant differences ($P < 0.05$) with storage time

the chemical composition of pectins (Liu et al. 2009). Therefore, only fresh untreated sample at day 0 and all samples at final day of storage were imaged in order to characterise distinct changes in pectin morphology.

AFM was successfully used for the characterisation of the heterogeneous pectin structures. Structures such as linear single fractions (ls), polymers (p) and branching (br) could be imaged, thus allowing for the qualitative analysis of the pectin structures under different VI treatments. Figure 5a presents an AFM image of CSP from freshly cut, untreated papaya. The pectins consisted of mostly long and branched chains, which indicated that pectinolytic enzymes had not depolymerised pectins. The presence of polymers that entangled together could also be seen from Fig. 5a, which appeared as a conglomerate (cg). This was characteristic of large CSP polymers (Xin et al. 2010). In contrast, AFM images of the other treatment groups at day 8 were mostly made up of short and fragmented pectin chains as the polymers might have been depolymerised and solubilised by enzymes such as PG and galactosidase at day 8 of storage (Fig. 5b–f).

Papaya cubes that were impregnated with PME and calcium lactate revealed highly branched pectin structures (Fig. 5b), which resembled a network of cross-linked pectin chains that helped to maintain the hardness of papaya cubes. This formation was due to the interaction of calcium ions with free carboxyl groups of pectin chains and may have a protective effect from pectinolytic enzyme by making it less accessible to enzymes. In contrast, branching was less common in other treatment groups, which were also softer than papayas from PME + Ca (Fig. 5c–f). Therefore, the qualitative analysis

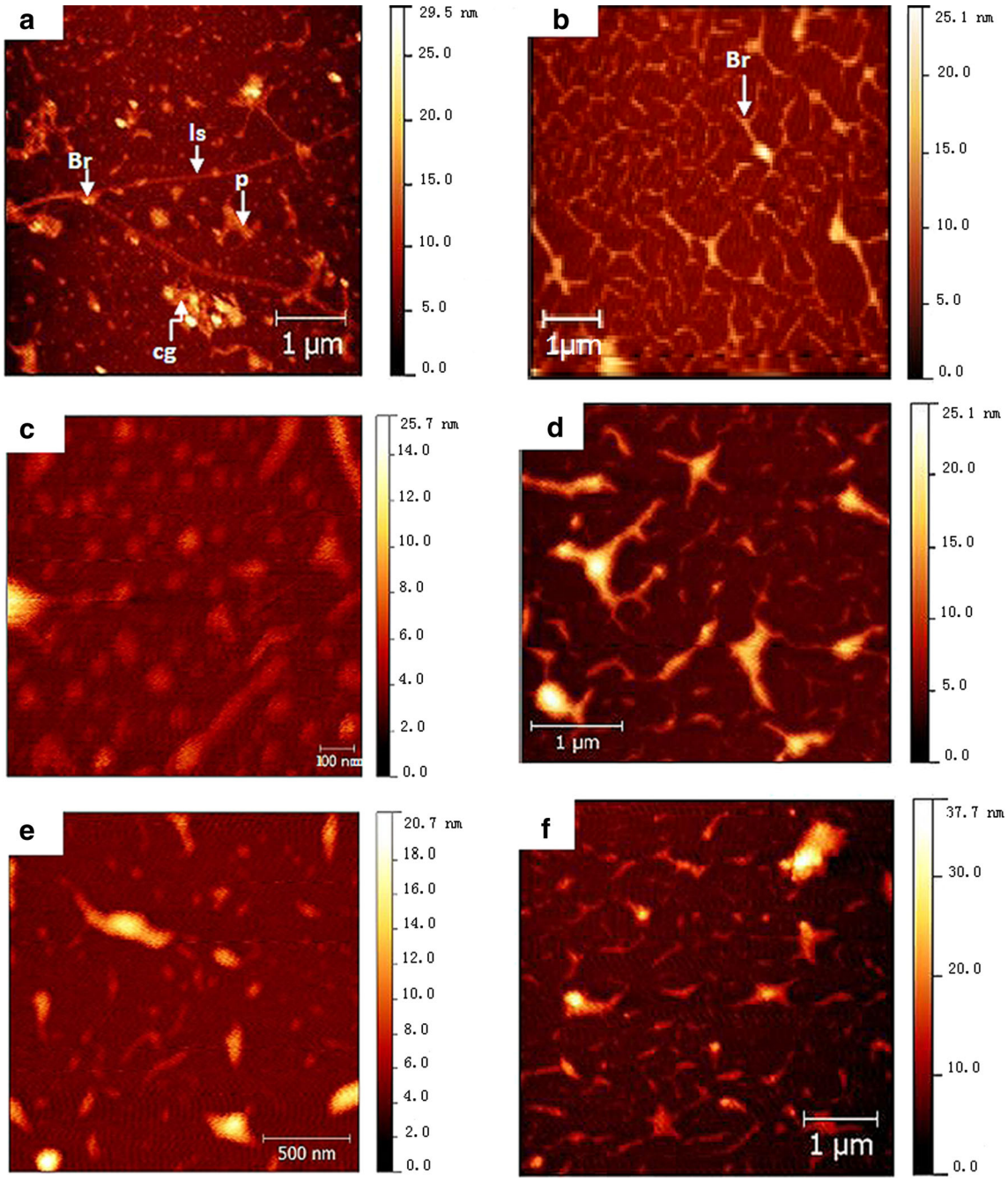


Fig. 5 AFM images of CSP from fresh-cut papayas. **a** Fresh untreated (non-VI) papayas at day 0, image size 5 × 5 μm. **b** PME + Ca-impregnated papayas at day 8, image size 5 × 5 μm. **c** PME-impregnated papayas at day 8, image size 1 × 1 μm. **d** Ca-impregnated papayas at day 8, image size 4 × 4 μm. **e** VI control papayas at day 8, image size 2 × 2 μm. **f** Fresh untreated (non-VI) papayas at day 8, image size 5 × 5 μm. *ls* linear single fractions, *p* polymers, *br* branching and *cg* conglomerate

of the physical morphology of pectin paralleled with that of hardness (Fig. 3a) and that the length of pectin and branching was related to firmness of fruit tissues.

Individual pectin chain widths and corresponding height are shown in Table 1. Effects of pectin chain widths across treatment and with time could then be deduced. The length of pectin was not quantified as molecular combing technique was not applied to stretch the pectin chains which naturally tend to tangle up. This effect is especially significant for CSP (Chen et al. 2013). Manipulating and stretching of pectin was not performed as the natural arrangement of CSP was the focus in current study.

As shown in Table 1, pectin chain widths were affected by the solutes impregnated during VI. VI treatment which gave rise to firmer fruits, such as in PME + Ca and Ca groups, had higher proportion of pectin with larger chain widths. At least

Table 1 Effects of vacuum impregnation on chain widths of chelate soluble pectin of papayas at days 0 and 8

W (nm)	Day 0		Day 8									
	Non-VI		PME + Ca		PME		Ca		VI control		Non-VI	
	N (%)	*H* (nm)	*N* (%)	*H* (nm)	*N* (%)	*H* (nm)	*N* (%)	*H* (nm)	*N* (%)	*H* (nm)	*N* (%)	*H* (nm)
15–24	2.6	3.00 ± 0.00	–	–	31.1	2.42 ± 0.48	3.0	3.13 ± 0.00	6.1	2.47 ± 0.02	–	–
25–34	–	–	–	–	15.6	2.54 ± 0.43	12.1	2.28 ± 0.34	9.1	2.63 ± 0.48	11.8	2.61 ± 1.25
35–44	12.8	3.02 ± 0.48	18.0	2.96 ± 0.85	24.4	2.47 ± 0.83	24.2	2.47 ± 0.38	24.2	2.68 ± 0.18	41.2	2.86 ± 0.55
45–54	25.6	2.76 ± 0.65	32.7	3.36 ± 0.79	17.8	2.46 ± 0.61	42.5	2.65 ± 0.53	51.5	2.77 ± 0.49	20.6	2.87 ± 0.44
55–64	33.4	3.89 ± 0.59	20.4	4.54 ± 1.52	6.7	3.56 ± 0.52	18.2	2.69 ± 0.37	9.1	2.90 ± 0.26	23.5	2.73 ± 0.43
65–74	17.9	3.61 ± 0.79	22.4	5.12 ± 1.08	4.4	3.11 ± 0.41	–	–	–	–	–	–
75–84	5.1	4.67 ± 0.33	1.6	7.35 ± 0.00	–	–	–	–	–	–	2.9	2.60 ± 0.00
85–94	–	–	4.9	3.72 ± 1.69	–	–	–	–	–	–	–	–
95–104	2.6	2.50 ± 0.00	–	–	–	–	–	–	–	–	–	–

Values were expressed as means ± standard deviation, $n = 5$. AFM images have at least five pectin measurements for each image

W the peak width of chain half height, *N* percentage of a particular range of chain widths in that sample, *H* the height of pectin chains, – not detected or below detection level

82.0 and 60.6% of pectin chain widths were longer than 45 nm in PME + Ca and Ca groups, respectively. In contrast, PME and non-VI only had 28.9 and 47.0% of pectin chain widths larger than 45 nm, respectively. Although VI control had the same percentage as Ca, it should be noted that the distribution of pectin chain widths in VI control group was skewed towards lower pectin chain widths. Degradation of pectin polysaccharides can cause the collapse of cell cohesiveness (Wakabayashi 2000). Therefore, pectins with well-intact structure, made up of higher percentage of larger *W* values, had higher firmness (Fig. 3a).

Furthermore, degradation of pectin chain widths occurred with storage time. This was indicated by the increase in percentage of smaller *W* values for non-VI group from day 0 to day 8 (Table 1). This was a natural senescence process of fruits as pectinolytic enzymes in fruits continued to degrade pectin. Decrease in pectin chain widths was linked to the degradation of pectin side chains (Xin et al. 2010). This was in good agreement with AFM qualitative analysis as the absence or scarce presence of branched pectin in both of the AFM image from PME and VI groups was observed (Fig. 5c, e). The higher percentage of large chain widths in PME + Ca was thus due to the increased capability of cross-linking between homogalacturonans of pectin as a result of the combined effects of PME and calcium ions.

The height parameter obtained from the software analysis served as a guide to locate pectin in the AFM images and from the height of the pectin chains determined; information such as whether the chain was branched or a crossed over of two single chains could be gathered (Adams et al. 2003). Usually, the height of pectin is in the range of 1–3 nm with wider range of 0.16–5.3 nm reported for tomato pectin (Xin et al. 2010). Pectin height of the range 1–5 nm was also reported in 'Jinxiu' and 'Milu' peaches (Yang et al. 2009).

In post-harvest studies of fruits, peel colour of fruits is invariably a selection criterion to evaluate fruit ripeness, but

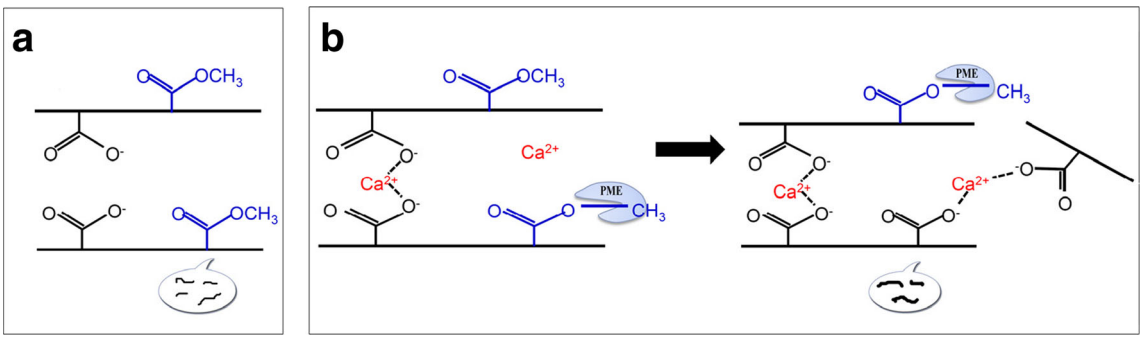


Fig. 6 Schematic diagram of pectin interaction with Ca^{2+} and PME and their corresponding pectin widths (in *speech bubble*). **a** Two pectin chains with carboxy (*black*) and carboxymethyl (*blue*) side chains; pectin chain widths are smaller. **b** Cross-linkage between pectins by Ca^{2+} and deesterification of carboxymethyl (*blue*) group by PME (*blue circle*)—free carboxyl group released by PME is available for interaction with Ca^{2+} and a new pectin chain; pectin chain widths are larger

this is often a matter of subjective judgement which can influence repeatability of experiment. Manrique and Lajolo (2004) found that skin colour of papayas did not necessarily correlate with fruit texture. Therefore, in order to get consistent results in the future, flesh firmness ought to be part of the selection criterion. In addition, various vitamins and antioxidant contents present in papaya after VI and with time should be determined to establish the protective effect VI may have over them. Of great importance is the determination of carotenoid content which can be used to relate to colour change in papaya with storage time.

According to Anjongsinsiri et al. (2004), large molecules such as PME are likely to be non-uniformly distributed in the fruit flesh. Hence, in order to better evaluate the effectiveness of VI or to improve the infusion process, location of enzymes within the fruit tissue should be visualised. This can be done through various techniques such as by labelling enzymes with fluorescent (Culver et al. 2000). With the determination of a successful impregnation of PME into papayas, it is also essential to test for enzyme activity. This is because enzymes infused into fruit may be inactivated or exhibit low activity due to the ionic conditions of the fruit. A rapid method known as PME print technique is available to detect localisation of PME after VI and to give a qualitative analysis of enzyme activity (Anjongsinsiri et al. 2004).

An obligatory and essential future work will be to match instrumental data with human subjects as instrumental measurements tend to be more sensitive than humans and may detect changes which from the consumers' point of view may be insignificant. The converse is true also when human subjects can identify qualities such as off-flavour in food that cannot be detected by machineries. Therefore, it is necessary to conduct sensory evaluation to evaluate if the improved hardness and change in colour of VI-treated papayas will be deemed as acceptable or well liked amongst consumers. Further actions based on sensory results can then be taken to improve the properties of VI-treated fresh-cut papayas. Microbiological testing should accompany sensory evaluation too. This is to ensure the safety of food before we consider any sensory evaluations of fresh-cut papayas.

A schematic diagram of pectin interaction with Ca^{2+} and PME and their corresponding pectin widths was proposed (Fig. 6). Pectin chains contain carboxy and carboxymethyl side chains. After Ca^{2+} and PME were added, cross-linkages were formed between pectins by Ca^{2+} and carboxymethyl groups were deesterified by PME. The free carboxyl group released by PME would be available for interaction with Ca^{2+} and a new pectin chain; thus, due to the increased capability of cross-linking between homogalacturonans of pectin, a strong network of cross-linked pectin chains was formed, and the percentage of large chain widths in PME and Ca^{2+} was raised. Therefore, the hardness and chewiness levels of fresh-cut papayas were improved. Since the components used are compatible with organic foods in principle, the elucidation of interaction is helpful for developing organic compatible processing technologies (Li et al. 2015; Liu et al. 2016; Yu and Yang 2017; Zhang and Yang 2017; Zhao et al. 2017).

Conclusion

A vacuum pressure of 5 kPa with exposure to vacuum pressure set for 5 min was determined to be favourable for VI of papayas. Firmness of fresh-cut papayas was shown to deteriorate rapidly, but VI of papayas with calcium lactate and PME was successful in being able to improve the hardness of the fruit when stored for 8 days. The combination of PME and calcium lactate was more effective in improving fruit firmness than infusing calcium alone. This might be due to the release of freer carboxyl groups from pectin by PME and thus enabling more cross-linkages between calcium ions and pectin chains to be formed.

Colour of papayas, in terms of L^* and C, was shown to decrease with storage time as a result of degradation of pigment molecules. VI was able to slow down the rate of degradation as the gaseous phase in the fruit, which was responsible for oxidising the pigment molecules was removed during VI.

Nanostructural changes of CSP correlated well with the hardness of papaya. Increasing percentage of shorter pectin width was evident of degradation of pectin branches which resulted in firmness loss. Therefore, PME + Ca group with the highest proportion of great pectin width at day 8 of storage had the hardest texture. Hence, VI is a technique with much potential in improving quality attributes of fresh-cut papayas.

Compliance with Ethical Standards

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Conflict of Interest The authors declare that they have no conflict of interest.

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