Food Chemistry 289 (2019) 40–48

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Research Article

Effects of calcium and pectin methylesterase on quality attributes and pectin morphology of jujube fruit under vacuum impregnation during storage

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ARTICLE INFO

Keywords:
Nanostructure
Calcium
Hardness
Quality
Polysaccharide
Atomic force microscopy
Jujube
Pectin methylesterase

ABSTRACT

Calcium chloride (1% w/w, $CaCl_2$) and pectin methylesterase (PME) (15 U/mL) were vacuum impregnated (VI) into jujubes to preserve their quality. The nanostructure of jujube pectin was investigated using atomic force microscopy (AFM) to determine the degradation mechanism of pectin. $CaCl_2$ with PME under VI treatment (VI + Ca + PME) maintained jujubes' quality. Weight loss in VI + Ca + PME group at day 56 was only 60.36% of that in control group (CK). Firmness, soluble solids content, and ascorbic acid content of jujubes in VI + Ca + PME group were higher than those in CK. Firmness was highly positively correlated with sodium carbonate-soluble pectin (SSP) content. According to AFM results, frequencies of molecules with a width ≥60 nm of water-soluble pectin (WSP), chelate-soluble pectin (CSP), and SSP were the highest in VI + Ca + PME group at the end of storage. WSP, CSP, and SSP degradation was delayed by VI + Ca + PME treatment. The quality of jujubes was effectively maintained by VI + Ca + PME treatment.

1. Introduction

As a native fruit, Chinese jujube (*Zizyphus jujube Miller*) has been used as a food and in traditional Chinese medicine for its abundant nutritional qualities. However, it can be stored for no more than ten days at room temperature. To extend its shelf life, the effect of packaging and preservation methods on the physicochemical properties of jujubes has been studied (Kou et al., 2019; Ozturk, Bektas, Aglar, Karakaya, & Gun, 2018; Wang et al., 2012; Yang, Yang, Chen, Hua, & Jiang, 2013). Nevertheless, novel strategies to extend jujubes' shelf life should be explored.

Calcium, as an edible fruit preservative, has been widely used as a firming agent to extend the shelf life of, for example, jujubes, strawberry, and papaya (Ayón-Reyna et al., 2017; Li, Ban, Li, & Xue, 2014; Zhang, Zhao, Lai, Chen, & Yang, 2018). As an essential mineral in plant cell walls, calcium can effectively suppress quality decline, preserve integrity, and reduce the permeability of the cell membrane in fruit during storage (Aguayo, Requejo-Jackman, Stanley, & Woolf, 2015). The firmness, pectin content, and nutrient substances in jujubes treated with calcium were maintained effectively (Zeraatgar, Davarynejad, Moradinezhad, & Abedi, 2018; Zhi et al., 2017). In addition, calcium inhibited the activities of pathogens in jujubes (Guo et al., 2016). Calcium has been widely used to extend the shelf life of apricots because of the cross-linking between Ca^{2+} and the carboxyl in pectin, which can inhibit the decomposition of the cell wall (Liu, Chen, et al., 2017).

Pectin is the main component of the plant cell wall. The functional properties of pectin in apricots, at both harvest and postharvest time, are closely related to fruit qualities, especially firmness (Liu, Chen, et al., 2017; Liu, Tan, Yang, & Wang, 2017). Tissue softening of Chinese red bayberry is caused by degradation of the fruit cell wall structure (Li, Zhang, Chen, Lai, & Yang, 2018), such as pectin solubilisation and depolymerisation. Cell wall enzymes are mainly responsible for the changes in pectin properties, especially polygalacturonase (PG), and pectin methylesterase (PME). The esterification group on the galacturonic acid carboxyl group in the pectin molecular chain is removed by PME, which demethylates pectin, providing the necessary conditions for PG catalysis (Gwanpua et al., 2017). Fungal PME is usually obtained from *Aspergillus niger* strains, and can inhibit plant PME activity (Sirijariyawat, Charoenrein, & Barrett, 2012). Low-methoxy pectin generated under the action of PME can interact with calcium to maintain the stability of pectin and the cell wall structure (Guillemin et al.,

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https://doi.org/10.1016/j.foodchem.2019.03.008
Received 11 October 2018; Received in revised form 3 March 2019; Accepted 5 March 2019
Available online 06 March 2019
0308-8146/

2008). Thus, there is great interest in methods to enhance the permeation of calcium and PME using physical technologies to preserve fruit and vegetables.

Vacuum impregnation (VI) is achieved by the exchange of the gas and native liquids with an impregnation solution, resulting in a pressure change under mechanical operation (Mao et al., 2017; Yang, Wu, Ng, & Wang, 2017). The quality of fresh-cut papayas treated with vacuum impregnation with calcium lactate and PME was preserved effectively, and their shelf life was extended (Yang et al., 2017). The firmness of mangoes, pineapple, and diced apple treated with calcium combined with PME under vacuum impregnation was significantly enhanced (Guillemin et al., 2008; Pan et al., 2014; Sirijariyawat et al., 2012). Demethylation by PME generates low methoxy pectin, which when linked with Ca^{2+}, forms a strong pectin crosslink structure. The pectin crosslink structure could maintain and improve the firmness of Japanese radish (Ando, Hagiwara, & Nabetani, 2017). It is necessary to determine the mechanism of the inhibition of fruit softening by calcium and PME under VI treatment.

Atomic force microscopy (AFM), a useful tool to analyze the microscopic structures of macromolecules, can be used to examine structures and molecules at the nanostructure level, and the morphologies of polysaccharides in their natural state. Therefore, AFM technology can capture individual pectin molecules and polymers directly (Li et al., 2018). Nanostructural information (qualitative and quantitative) of polysaccharide molecules, including direct imaging of pectin from cherries and the branching structure of pectin, were also characterized successfully using AFM (Xin, Chen, Lai, & Yang, 2017). Determining polysaccharide nanostructures is useful to reveal the underlying mechanism of physicochemical and textural changes of food materials.

The aim of the present study was to determine the effects of VI with calcium chloride and PME on the postharvest quality attributes of jujubes. The nanostructure of jujube pectin was characterized using AFM to determine the correlations between the morphological characteristics of pectin molecules and the physicochemical properties of jujubes. The results will reveal the effects of VI with calcium chloride and PME on the quality of jujubes during storage, which would support the application of vacuum impregnation technology in the preservation of fruits and vegetables.

2. Materials and methods

2.1. Fruit material

Jujubes (*Zizyphus jujube Miller*, cv '*Nongke I*'), selected on the basis of uniform size (15 g ± 1 g), shape, skin colour (about 50% red), ripening stage (maturity 7–8), and soluble solids content (22–27 °Brix) were harvested in October from an orchard in Yuanyang town, Xinxiang, Henan, China. Samples were transported to the laboratory within 2 h. Approximately 30 jujubes were washed with distilled water and used for fresh analysis. The other fruits (1600 jujubes) were randomly divided into four groups: VI with an isotonic sucrose solution (CK); VI with calcium chloride ($CaCl_2$, 1%, w/w, Zhengzhou Ruipu Biological Engineering Co., Ltd., Zhengzhou, China) in isotonic sucrose solution (VI + Ca); VI with 15 U/mL pectin methylesterase (PME, Creative Biomart, New York, US) in isotonic sucrose solution (VI + PME); VI with 1% $CaCl_2$ and 15 U/mL PME in isotonic sucrose solution (VI + Ca + PME).

2.2. VI treatment

The VI treatment of jujubes was performed according to the methods of Yang et al. (2017), Liu, Chen, et al. (2017), Liu, Tan, et al. (2017), and Li et al. (2018). A vacuum chamber (10 L) that included a vacuum pump (FY-1H-N, Zhejiang Feiyue electromechanical Inc., Zhejiang, China) and a thermostatic water bath (DC-2006, Ningbo Xinzhi Biological Polytron Technologies Inc., Ningbo, Zhejiang, China) was used. $CaCl_2$ and PME were dissolved in an isotonic impregnation solution (about 24 °Brix) at concentration of 1% and 15 U/mL, respectively. The mass ratio of the sample (500 g) to the impregnation solution (1500 g) was kept at 1:3. A vacuum pressure of 5 kPa was applied to the system for 10 min at 20 °C. Atmospheric pressure was then restored for 10 s, and the sample was maintained in the impregnation solution for 5 min. After treatment, jujubes were drained, rinsed with distilled water to remove the attached solution, and gently blotted with tissue paper. All treated fruit were stored in a polyethylene terephthalate box in a refrigerator (temperature, 1 ± 1 °C; relative humidity, 75%). Every 7 days, 30 jujubes were randomly selected from each group and analyzed.

2.3. Firmness, weight loss, soluble solids content, and ascorbic acid measurement

The firmness of the jujubes was evaluated using a TA-XT2i Texture Analyser (Stable Micro Systems Ltd., Godalming, Surrey, UK) according to the method detailed by Liu, Chen, et al. (2017) and Liu, Tan, et al. (2017). Peeled fruit were cut into cylindrical samples of $7 \times 7 \times 5\ mm^3$. The operating parameters were as follows: Aluminum cylinder probe of 35 mm in diameter, pre-test speed of 5 mm/s, test-speed of 0.5 mm/s, post-test speed of 0.5 mm/s, compression degree of 30%, and trigger force of 5.0 g. Ten jujubes from each treatment group were measured individually to obtain a representative result.

Twenty jujube fruit were used to measure weight loss, and all experiments were conducted in triplicate. Weight loss was calculated using Eq. (1) (Yang et al., 2017).

$$\text{Weight loss}\,(\%) = \frac{m_0 - m}{m_0} \times 100\% \quad (1)$$

where m is the weight of jujubes at different storage times; and m_0 is the weight of fresh jujubes.

The soluble solids content (SSC) was measured according to the method of Liu, Chen, et al. (2017) and Liu, Tan, et al. (2017). A bench top Digital Refractometer (Nova-Tech International, Inc., Houston, TX, USA) was used to assess the juice extracted from about 50 g samples of four jujube fruit and the results were expressed as °Brix. All experiments were conducted in triplicate.

2.4. Ascorbic acid content analysis

The ascorbic acid (Vit C) content was measured using the 2,4-dinitrophenylhydrazine colorimetry method according to Han et al. (2015). Jujube flesh samples (10 g) from eight jujube fruit were homogenised with 10 mL of oxalic acid (2%, w/v) in a blender. The mixture was then centrifuged at 12,000 × *g* for 15 min at 4 °C, and the procedure was repeated twice with oxalic acid (1%, w/v). The obtained supernatant was distilled to 200 mL with oxalic acid (1%, w/v). The diluted supernatant (4 mL) and 2 mL 2% (w/v) 2,4-dinitrophenylhydrazine solution were reacted in conical flask for 3 h at 37 °C. The mixture then was cooled in an ice-water bath, and 5 mL sulfuric acid (85%, w/w) was added. After incubation for 30 min, the absorbance was assayed at 500 nm using a UV-2000 spectrophotometer (Shanghai Instrument Analysis Instrument Co., Ltd., Shanghai, China). Standard Vit C solution was used to construct a standard curve. The following formula was used to calculate X (Vit C content of the jujubes, g/kg):

$$X = \frac{c \times V}{m} \times F \times 10^{-3} \quad (2)$$

where m is the weight of the jujubes; c is the Vit C content obtained from the standard curve; V is the distilled volume; and F is the dilution multiplier.

2.5. Pectin determination

Cell wall materials (CWM) were fractionated according to methods detailed in previous reports (Chen et al., 2018; Liu, Tan, et al., 2017). Jujube flesh samples (10 g) from eight jujube fruit were boiled with 200 mL of 80% (v/v) ethanol for 20 min. After cooling to room temperature, the mixture was filtered, and the residue was boiled with ethanol twice more. The residue was placed in 50 mL of dimethylsulphoxide (DMSO): water (9:1, v/v) for 24 h at 4 °C. The mixture was then filtered and transferred to 200 mL of chloroform and ethanol (2:1, v/v) for 10 min. After filtration, the final sample was washed with acetone until totally white. The residue comprised the cell wall material.

Water-soluble pectin (WSP) was extracted by suspending the CWM in 10 mL of deionised water, then shaking for 4 h. The supernatant was collected by centrifugation at 13,500 × *g*, at 4 °C for 15 min. This procedure was repeated twice and the supernatants were pooled as the WSP. Then, 50 mmol/L cyclohexanetrans-1,2-diamine tetra-acetate (CDTA, Sinopharm Chemical Reagent, China) and 10 mL 50 mmol/L sodium carbonate solution containing 2 mmol/L CDTA was used to replace the deionised water to obtain chelate-soluble pectin (CSP) and sodium carbonate-soluble pectin (SSP), respectively.

The pectin content was determined using the carbazole colorimetry method (Zhang, Chen, Zhang, Lai, & Yang, 2017), with galacturonic acid as the standard. Pectin solution (2 mL) was blended with 12 mL of sulfuric acid (98%, w/w) and boiled for 10 min. The mixture was then cooled using tap water, and incubated at room temperature for 30 min after mixing with 0.5 mL of carbazole ethanol solution. The absorbance at 530 nm was then determined using a UV-2000 spectrophotometer (Shanghai Instrument Analysis Instrument Co., Ltd., Shanghai, China), The results were expressed as g/kg. All experiments were conducted in triplicate.

2.6. Atomic force microscopy analysis

Atomic force microscopy (AFM) analysis was conducted using a D-5A atomic force microscope in tapping mode (Zhuolun MicroNano Equipment Co., Ltd, Shanghai, China) (Mao et al., 2017). Pectin solutions were mixed using a vortex mixer (Fisher Scientific, Pittsburgh, PA, USA) and diluted to a suitable concentration (about 0.5–30 μg/mL). Then, 10 μL of sample solution was pipetted rapidly onto the surface of a freshly cleaved mica slice and dried naturally before scanning. The resonance frequency and the scanning frequency of the probe were 330 kHz and 0.5–2 Hz, respectively.

AFM images were analyzed offline using AFM software (SPMQuickView Microsoft, Redmond, WA, USA). Qualitative information (width) was obtained by section analysis. The frequency (F_q) was defined as the percentage of pectin chains with a particular width among all the chains observed (Yang, 2014). At least twenty images from each sample were analyzed to obtain statistically meaningful results.

2.7. Statistical analysis

The results were analyzed using Excel 2010 (Microsoft), SPSS 20 (IBM Corp, Armonk, NY, USA), and GraphPad Prism 5 (GraphPad Software Co., Ltd., San Diego, CA, USA). Data are expressed as the mean ± standard deviation of three replicated determinations. One way analysis of variance was used to determine significant differences at $P < 0.05$.

3. Results and discussion

3.1. Quality properties of jujubes

The firmness of VI treated jujube fruit is shown in Fig. 1A. The

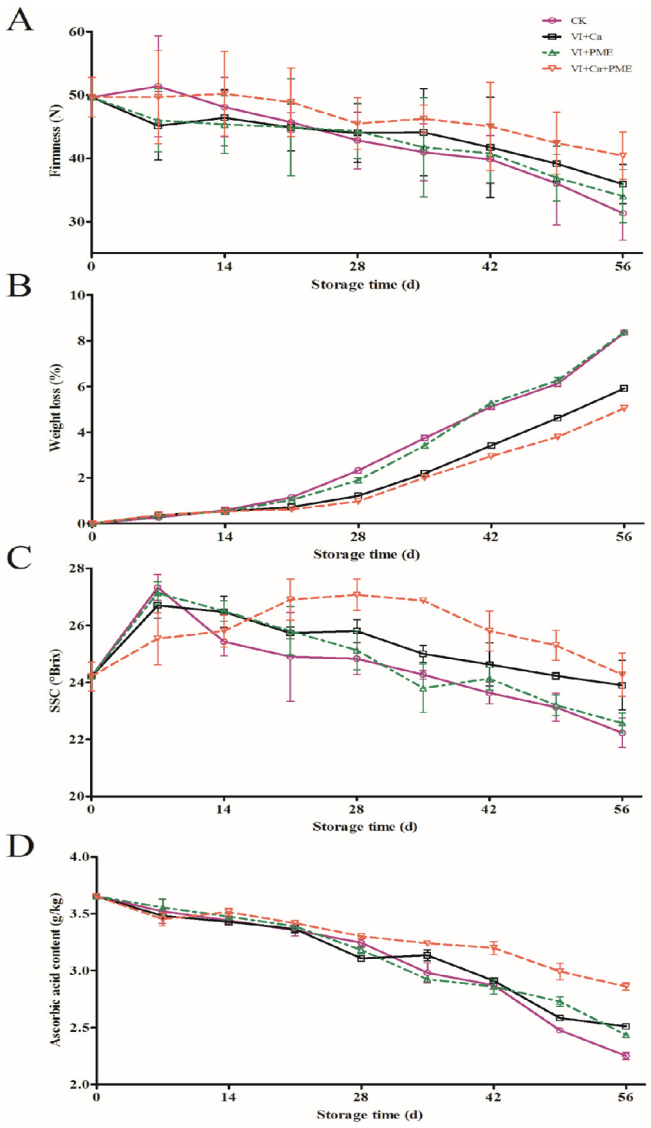

Fig. 1. The quality properties of jujubes during storage. (A) Firmness, (B) Weight loss, (C) Soluble solids content (SSC), and (D) Ascorbic acid content. Note: CK, jujubes treated with vacuum impregnation (VI) with an isotonic sucrose solution; VI + Ca, jujubes treated with vacuum impregnation (VI) combined with calcium chloride; VI + PME, jujubes treated with VI combined with pectin methylesterase; VI + Ca + PME, jujubes treated with VI combined with calcium chloride and pectin methylesterase. Error bars in firmness data represent the standard deviation of the mean of ten replicates. Error bars in Weight loss, SSC, and Ascorbic acid content data represent the standard deviation of the mean of three replicates.

firmness of the jujubes of all groups decreased during storage. Li et al. (2018) reported that the change in quality of Chinese red bayberry was usually accompanied with a loss of firmness because of the disassembly of the cell wall. After storage for 14 days, the firmness of the jujubes in the VI + Ca + PME group was the highest among the four groups. At the 56th day, the firmness of the VI + Ca + PME group (40.44 N) was greater than that of the CK group (31.31 N), VI + Ca (35.92 N) group, and VI + PME group (34.01 N).

PME catalyses the de-esterification of methyl esters of pectin, and thus promotes Ca^{2+} in fruit tissue to combine with non-esterified C-6 in galacturonic acid residues to form a stable composite structure (Ando et al., 2017), which can lead to an increase in pectin stability. The increase of exogenous calcium and PME strengthened the interaction between Ca^{2+} and carboxyl groups in the VI + Ca + PME group, which

could inhibit the softening of jujubes. The firmness of VI + Ca + PME treated samples was most effectively maintained. This was caused by the improvement of the integrity and mechanical properties of the cell wall (Saba & Sogvar, 2016).

Postharvest fruit are susceptible to weight loss; therefore, it is very important to control the rate of weight loss to prolong the shelf life of fruit. Fig. 1B shows that all groups of jujubes experienced weight loss during storage. However, the weight losses in the VI + Ca and VI + Ca + PME groups were lower than those in the CK and VI + PME groups during storage. On the 56th day, the weight losses in the CK, VI + Ca, VI + PME, and VI + Ca + PME groups were 8.35%, 5.92%, 8.38%, and 5.04%, respectively. The weight loss in the VI + Ca + PME was only 60.36% of that of the CK group. Evaporation and respiration processes lead to water loss, which results in the weight loss of fruit during postharvest storage (Chong, Lai, & Yang, 2015). The moisture and juice in jujubes were markedly maintained by VI + Ca and VI + Ca + PME treatment, which were closely related to their greater firmness.

The soluble solid content (SSC) is an important quality index to examine fruit quality during postharvest storage. SSC is related to fruit ripening, and a higher amount of soluble solids can also contribute to the flavor of fruit, especially sweetness. Fig. 1C shows that the SSC increased at the beginning of stage, which might have been caused by conversion of the starch material and synthesis of carbohydrates (Comabella & Lara, 2013). With prolonged storage, the SSC showed a declining trend, which was possibly because the soluble sugar in the fruit was gradually consumed as the substrate of respiration (Petriccione et al., 2015). On the 56th day, the VI + Ca + PME group had the highest SSC level (24.27 °Brix), followed by the VI + Ca group (23.90 °Brix), which were both much higher than the level in the CK (22.23 °Brix) and VI + PME (22.57 °Brix) groups. The greater the decline in SSC, the greater the degradation of fruit quality (Li et al., 2015). This result showed that the VI + Ca and VI + Ca + PME treatments could preserve the quality of the jujubes, and was similar to the report of El-Motty, El-Shiekh, Mohamed, and Shahin (2007) in apricots. A similar SSC change trend was also reported in Chinese cherries during storage (Xin et al., 2017).

Ascorbic acid (Vit C) is a nutrient and important antioxidant component in jujubes. It can act also as a reducing and a chelating agent to scavenge free radicals (Zeraatgar et al., 2018). Vit C in jujubes decomposes during storage, leading to a decreased content (Fig. 1D). The respiration of fruit can increase the concentration of CO_2, which induces loss of Vit C (Li et al., 2015; Zhang, Hao, Li, & Wang, 2016). In fresh jujubes, the Vit C content was 3.65 g/kg (day 0). After storage, the Vit C content in the CK group decreased to 2.25 g/kg (day 56), which was only 61% of that in the fresh fruit. However, the Vit C contents in the VI + Ca + PME, VI + PME, and VI + Ca groups were 2.86 g/kg, 2.43 g/kg, and 2.51 g/kg, respectively. This result indicated that VI + Ca + PME treatment could reduce oxidation and inhibit the decomposition of Vit C more effectively than VI + Ca and VI + PME treatment.

3.2. Pectin content

The WSP, CSP, and SSP contents in jujubes are shown in Fig. 2. The maximal WSP contents in the CK, VI + Ca, VI + PME, and VI + Ca + PME groups were obtained at 21, 28, 14, and 28 days, respectively (Fig. 2A). The WSP content then decreased with further storage. At the beginning of apricot storage, pectin enzymes promoted the change of insoluble protopectin to soluble pectin and the disassembly of pectin-cellulose-hemicelluloses (Liu, Chen, et al., 2017; Liu, Tan, et al. 2017). Thus, the WSP content showed an initial increasing trend. The WSP then aggregates to form CSP, leading to a decline in WSP levels (Liu et al., 2009). The WSP content in the VI + Ca + PME group (5.43 g/kg) was higher than that in the CK (5.06 g/kg), VI + Ca (5.24 g/kg), and VI + PME (4.99 g/kg) groups at day 56, which showed

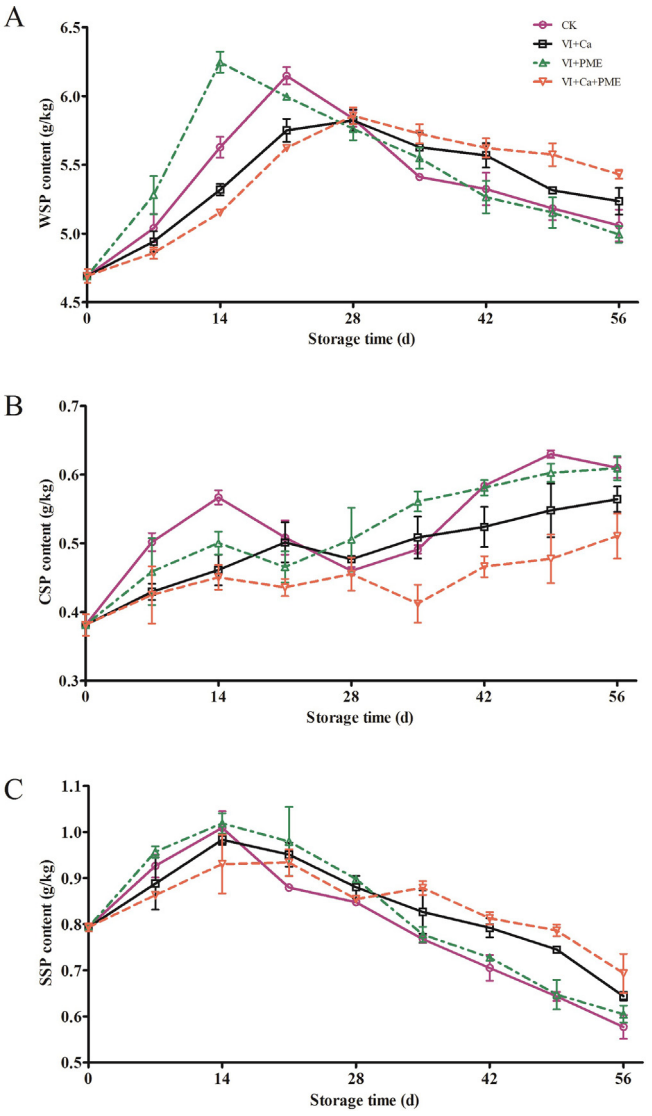

Fig. 2. The content of (A) water-soluble pectin (WSP), (B) chelate-soluble pectin (CSP), and (C) sodium carbonate-soluble pectin (SSP) in jujubes. *Note:* CK, jujubes treated with vacuum impregnation (VI) with an isotonic sucrose solution; VI + Ca, jujubes treated with vacuum impregnation combined with calcium chloride; VI + PME, jujubes treated with VI combined with pectin methylesterase; VI + Ca + PME, jujubes treated with VI combined with calcium chloride and pectin methylesterase. Error bars represent the standard deviation of the mean of three replicates.

that the hydrolysis of pectin was inhibited by the VI + Ca + PME treatment. This may be because the cross-linking between calcium and pectin decreased the membrane permeability, making pectin less accessible to enzymes, similar to that reported in papaya (Ayón-Reyna et al., 2017).

The CSP content (Fig. 2B) in jujubes showed an upward trend during storage, from 0.38 g/kg (0 day) to 0.61 (CK), 0.56 (VI + Ca), 0.61 (VI + PME), and 0.51 g/kg (VI + Ca + PME) at the end of storage. The increasing CSP content was observed at the late storage times, which resulted from the SSP side chains being broken by beta galactosidase (β-Gal), α-L-arabinofuranosidase (α-AF) (Gwanpua et al., 2017) and water loss (Zhang, Chen, Lai, Wang, & Yang, 2018). Meantime, the WSP and SSP were demethylated by PME, and crossed bonded with calcium, forming CSP (Yang et al., 2017). However, a significant decline in the CSP content was observed in CK group at 14 day, from 0.57 g/kg (day 14) to 0.42 g/kg (day 28). In the fruit softening progress, cell wall degradation is accompanied by a decrease in CSP (Chen, Hung,

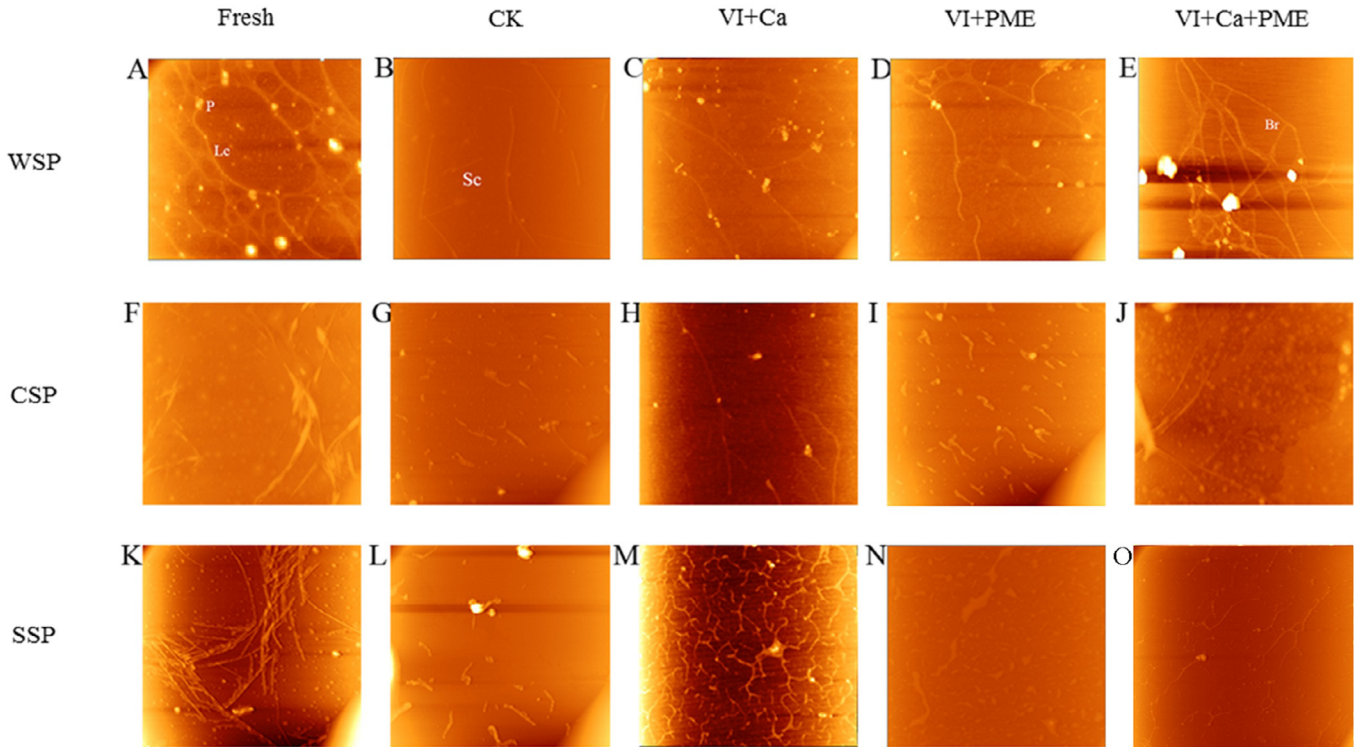

Fig. 3. Atomic force microscopy (AFM) images of water-soluble pectin (WSP) (images A–E), chelate-soluble pectin (CSP) (images F–J), and sodium carbonate-soluble pectin (SSP) (images K–O) chains in jujubes. A, F, K: images from jujubes made from fresh fruit (0 day); B, G, L: images from jujubes with vacuum impregnation (VI) with an isotonic sucrose solution treated group (56 days); C, H, M: images from jujubes of the vacuum impregnation combined with calcium chloride treated group (56 day); D, I, N: images from jujubes of the vacuum impregnation combined with pectin methylesterase treated group (56 days); E, J, O: images from jujubes of the vacuum impregnation combined with calcium chloride and pectin methylesterase treated group (56 days); Scan area: 5.000 μm × 5.000 μm. Lc: long straight chains; Sc: short chains; Br: branched chains; P: polymer structure.

Chen, & Lin, 2017). The CSP content in the VI + Ca and VI + Ca + PME groups was lower than that in CK and VI + PME groups during storage, which resulted from inhibition of the transformation between SSP and CSP. In addition, molecular bonding between the constituents of the cell wall was strengthened because of the calcium pectate formed between calcium and pectic acid, and the pectin-cellulose-hemicellulose crosslinked structure was more stable (Saba & Sogvar, 2016), leading to a lower CSP yield in the VI + Ca + PME group. The lower water loss in the VI + Ca + PME group could also result in a lower CSP content.

Fig. 2C shows the changes in the SSP content. The SSP content in the CK, VI + Ca, VI + PME, and VI + Ca + PME groups reached the highest value at day 14. Thereafter, the SSP content declined with prolonged storage time. However, the SSP content in the VI + Ca + PME group was higher than that in the CK, VI + Ca, and VI + PME groups after day 35. On the 56th day, the SSP content in the CK group was the lowest (0.58 g/kg); however, in the VI + Ca + PME group, it was 0.69 g/kg. Thus, VI + Ca + PME treatment is an effective method to inhibit the degradation of SSP. The degradation of SSP is related to the decrease in the firmness of fruit. Thus, the reduced degradation of SSP contributed to the firmness of jujube fruit of VI + Ca + PME group being the highest among all the groups at the end of storage.

3.3. AFM analysis of pectin

3.3.1. WSP

The textural breakdown of fruit is closely correlated with the changes in cell wall structures. The degradation of cell wall polysaccharides was investigated at the molecular level to understand the mechanism of tissue softening of fruit during postharvest storage. According to Zhang, Zhao, et al. (2018), degradation of cell wall polysaccharides in strawberry is associated with modification of pectin chains, especially the chain width, height, and length.

Fig. 3A–E shows the AFM images of WSP molecules of jujubes. For fresh jujubes, the WSP molecules were aggregated, and most of them formed large polymers or blocks. Only a few single linear chains were observed (Fig. 3A). During storage, more short chains were observed in the CK group (Fig. 3B). Long linear chains with branched structures were observed in the VI + Ca, VI + PME, and VI + Ca + PME groups (Fig. 3C–E). Moreover, a crosslinked structure was also observed in the VI + Ca + PME group. These changes indicated that the pectin substances depolymerised during ripening. However, the VI + Ca + PME group showed less pectin depolymerisation.

As shown in Fig. 4A, the F_q of WSP of small chains increased with storage time, which was similar to the result obtained in apricots (Zhang, Chen, et al., 2018). It is clear that the F_q of the smaller chain width was higher in the CK group than in the other groups on day 56. For fresh fruit, the F_q for width between 60 and 80 nm was 24.33% (0 day), while for the CK, VI + Ca, VI + PME, and VI + Ca + PME groups at day 56, it was 15.39%, 22.73%, 10.71%, and 34.47%, respectively. VI + Ca and VI + Ca + PME treatment could both delay the degradation of pectin, possibly because the increase in the calcium content in the cell walls could enhance the crosslinking between the pectin molecular chains. Manganaris, Vasilakakis, Diamantidis, and Mignani (2007) reported that calcium application in peach fruit could lead to an increase in the tissue calcium content. The changes in calcium content under VI will be investigated in our future research.

3.3.2. CSP

According to Fig. 3F–J, CSP molecules in fresh jujubes mainly comprised complex branches and long chains, and some CSP molecules were linked with CDTA (Fig. 3F) (Li et al., 2018). After storage, more

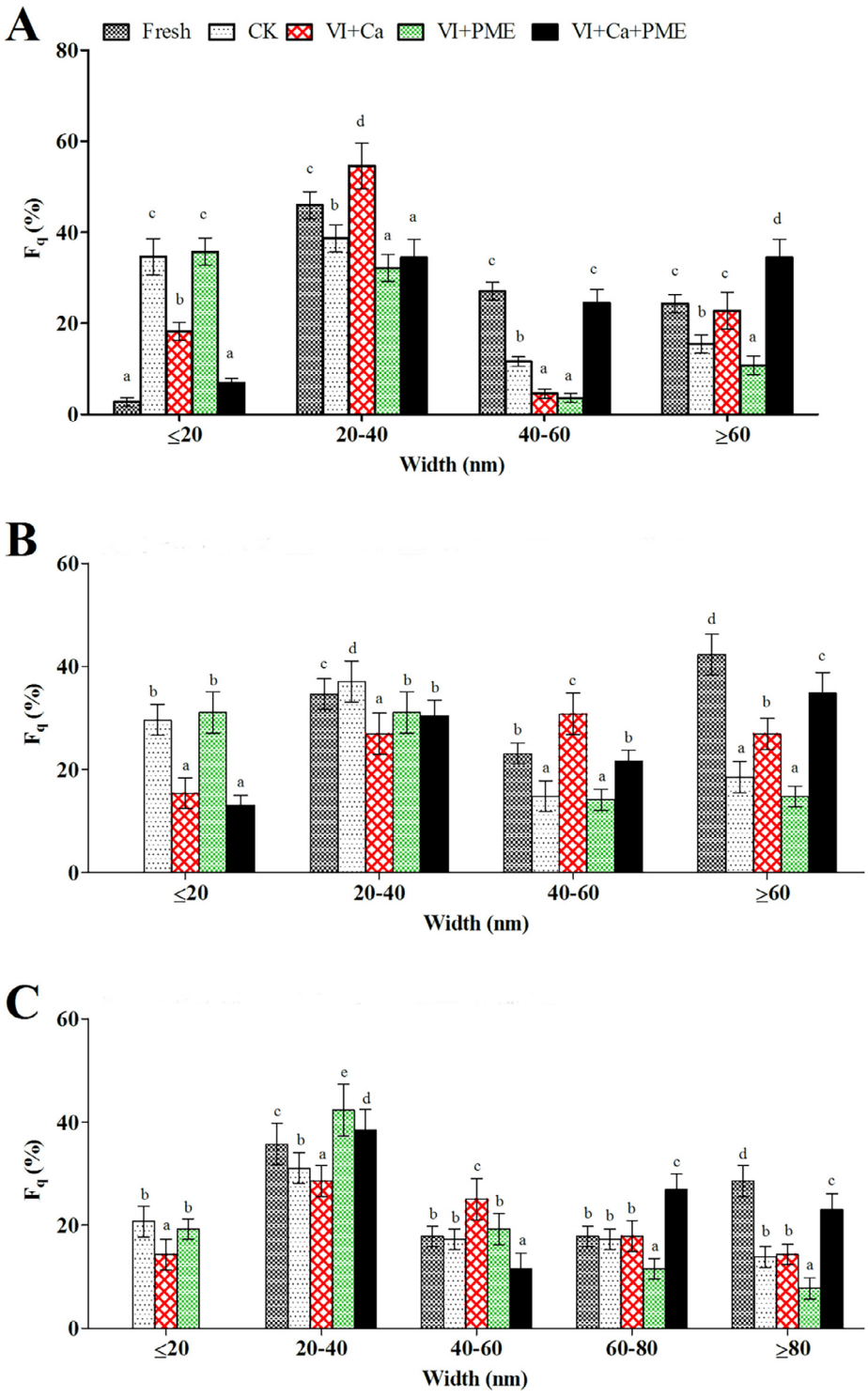

Fig. 4. Width of (A) water-soluble pectin (WSP), (B) chelate-soluble pectin (CSP), and (C) sodium carbonate-soluble pectin (SSP) chains in jujubes. Fresh, untreated fresh jujubes (0 day); CK, jujubes treated with vacuum impregnation (VI) with an isotonic sucrose solution (56 days); VI + Ca, jujubes treated with vacuum impregnation (VI) combined with calcium chloride (56 days); VI + PME, jujubes treated with VI combined with pectin methylesterase (56 days); VI + Ca + PME, jujubes treated with VI combined with calcium chloride and pectin methylesterase (56 days); F_q, the percentage of pectin chains of particular width among all the chains observed. Error bars represent the standard deviation of the mean of three replicates. Different small case letters indicate a significant difference at $P < 0.05$ among different treatment methods.

short chains, and fewer branches and polymer structures were observed (Fig. 3G–J). In Fig. 3H and J, long linear pectin chains still existed. The results showed that CSP was degraded during storage, especially in the CK group. However, in the VI + Ca and VI + Ca + PME groups, the degradation progress was delayed.

The quantitative analysis of CSP molecules is shown in Fig. 4B. Compared with fresh fruits (0%), chains with a smaller width (≤20 nm) were found in the CK (29.62%), VI + Ca (15.38%), VI + PME (31.03%), and VI + Ca + PME (13.05%) groups at day 56. Meanwhile, the F_q of chain width ≤20 nm in the VI + Ca + PME group was the lowest in all treatment groups. About 34.78% of the chain width of CSP in the VI + Ca + PME group was greater than 60 nm, which was a higher proportion than that observed in the CK (18.52%), VI + Ca (26.92%), and VI + PME (14.80%) groups. VI + Ca + PME treatment promotes the cross-linking between Ca^{2+} and pectin, inhibiting pectin degradation and maintaining the quality of jujube fruits (Li et al., 2018; Mao et al., 2017). The effect of VI + Ca + PME treatment was significantly better than that of Ca^{2+} or PME alone. These results confirmed the findings of a previous study, in which diced pineapple was treated with calcium and PME (Pan et al., 2014).

Table 1
Correlation matrix of physicochemical properties and firmness of jujubes during storage.

	Sample	Firmness	WSP	CSP	SSP
Firmness	CK	1.00			
	VI + Ca	1.00			
	VI + PME	1.00			
	VI + Ca + PME	1.00			
WSP	CK	−0.18	1.00		
	VI + Ca	−0.37	1.00		
	VI + PME	0.14	1.00		
	VI + Ca + PME	−0.16	1.00		
CSP	CK	−0.98**	0.03	1.00	
	VI + Ca	−0.96**	0.45	1.00	
	VI + PME	−0.95**	−0.03	1.00	
	VI + Ca + PME	−0.92**	0.49	1.00	
SSP	CK	0.76*	0.47	−0.82**	1.00
	VI + Ca	0.69*	0.22	−0.70*	1.00
	VI + PME	0.81**	0.60	−0.76*	1.00
	VI + Ca + PME	0.87**	0.27	−0.67*	1.00

Note: *Correlation is significant at $P < 0.05$. **Correlation is significant at $P < 0.01$. CK, jujubes treated with vacuum impregnation (VI) with an isotonic sucrose solution; VI + Ca, jujubes treated with vacuum impregnation (VI) combined with calcium chloride; VI + PME, jujubes treated with VI combined with pectin methylesterase; VI + Ca + PME, jujubes treated with VI combined with calcium chloride and pectin methylesterase.

3.3.3. SSP

The AFM images of SSP chains from jujubes are shown in Fig. 3K–O. In fresh jujubes, polymer structures and entangled long pectin chains were observed. No unimolecular linear molecules were observed. However, at the end of storage, the polymer structures had disintegrated, and short chains were the main form of pectin. Meanwhile, some unimolecular linear molecules were also observed in the AFM images (Fig. 3K–O). Long pectin chains still existed in the VI + Ca and VI + Ca + PME groups (Fig. 3M and O). Thus, VI + Ca and VI + Ca + PME treatment could delay the progress of pectin degradation.

The quantitative analysis of SSP molecules is shown in Fig. 4C. For the fresh jujubes, 28.56% of them had a width ≥80 nm. After storage, the F_q values of large chains with width > 80 nm in the CK, VI + Ca, VI + PME, and VI + Ca + PME groups were 13.80%, 14.28%, 7.70%, and 23.08%, respectively. Compared with the CK, VI + Ca, and VI + PME groups, small chains with a width ≤20 nm were not observed in the VI + Ca + PME group.

3.4. Correlation analysis between firmness and pectin

The correlation matrix of firmness and pectin content of the jujubes is shown in Table 1. The firmness of jujubes correlated positively with the SSP content, but correlated negatively with the CSP content. No significant correlation was found between firmness and the WSP content.

According to Zhang, Chen, et al. (2018), modification and degradation of the apricot cell wall structure could contribute to tissue softening and turgor loss. In addition, pectin is the major component of cell wall materials. Studies have reported the close relationship between the pectin content and tissue softening, in which fruit pectin can undergo solubilization and depolymerization during cherry tomato ripening, resulting in tissue softening (Zhang et al., 2017). In apricots, the pectin-cellulose-hemicellulose net structure was altered, leading to a loss of fruit firmness (Liu, Chen, et al., 2017; Liu, Tan, et al., 2017). Moreover, Figueroa et al. (2010) stated that this degradation can be attributed to enzymatic actions. For example, PME catalyses the de-esterification of methyl esters and provides substrates for pectin in Japanese radish hydrolysis by PG (Ando et al., 2017; Gwanpua et al.,

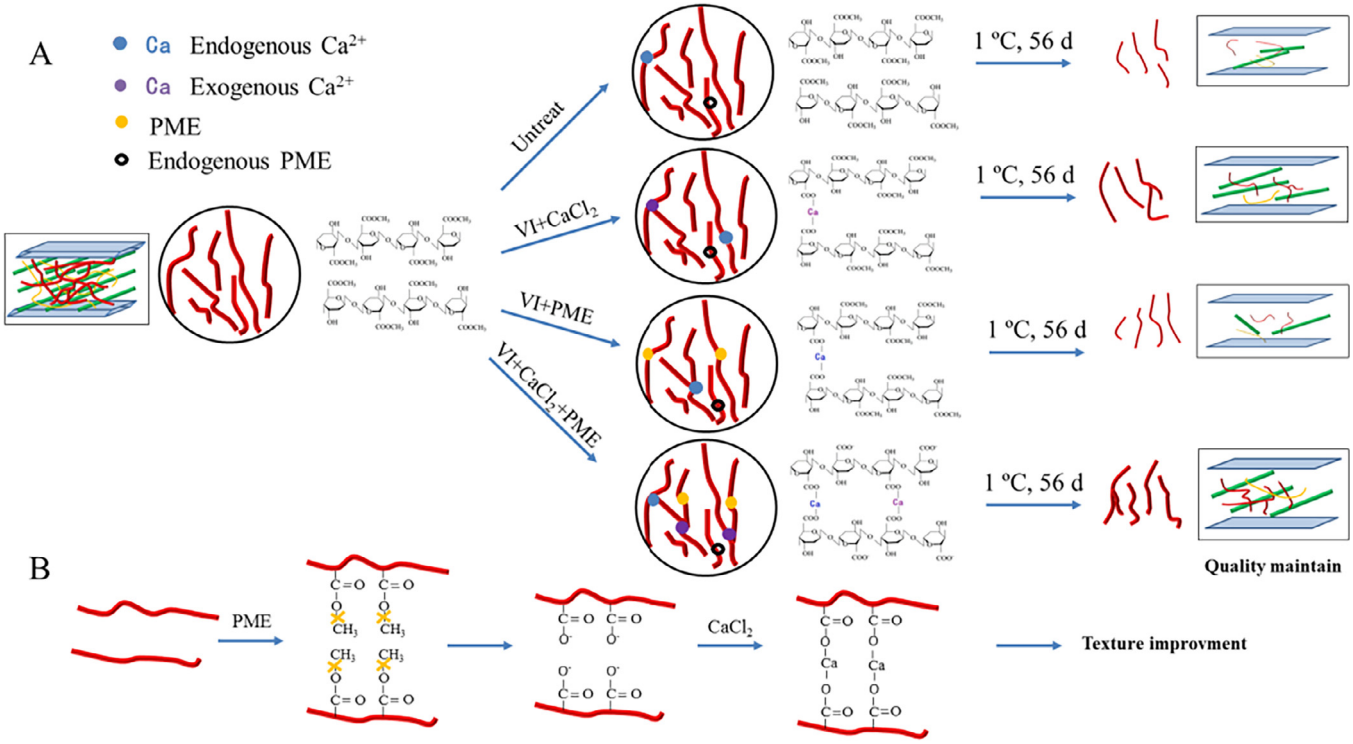

Fig. 5. Schematic image of pectin chains changed in jujubes treated by vacuum impregnation (VI) combined with calcium chloride and pectin methylesterase. Note: CK, jujubes treated with vacuum impregnation (VI) with an isotonic sucrose solution; VI + Ca, jujubes treated with VI combined with calcium chloride; VI + PME: jujubes treated with VI combined with pectin methylesterase; VI + Ca + PME: jujubes treated with VI combined with calcium chloride and pectin methylesterase. The red lines represent pectin. The yellow and green lines represent hemicellulose and cellulose, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2017).

A schematic image of the changes to the pectin chains during storage in the CK, VI + Ca, VI + PME, and VI + Ca + PME groups is shown in Fig. 5. The esterification group on the galacturonic acid carboxyl group in the pectin molecular chain was removed by PME, generating pectin demethylation and crosslinking with Ca^{2+}, which could maintain the stability of pectin in papaya (Fig. 5B) (Yang et al., 2017). The exogenous calcium and PME supplement promoted further calcium crossed-bonding with pectin under VI (Fig. 5A). According to the AFM results, at the end of storage, the pectin chains (WSP, CSP, and SSP) in the VI + Ca + PME group was obviously wider than those in the other groups, and this result corresponded with the higher fruit quality (firmness, weight loss, SSC, and Vit C) (Fig. 1). The pectin chains hydrolyzed by PME formed low-methoxy pectin, which can easily interact with calcium (Fig. 5B), maintaining the stability of pectin and the cell wall structure (Guillemin et al., 2008). In addition, the cross-linked structure formed between Ca^{2+} and pectin has a positive effect on apricot firmness during storage (Liu, Chen, et al., 2017; Liu, Tan, et al., 2017). The softening of jujubes was delayed by VI + Ca and VI + Ca + PME treatment, which revealed that these treatments had positive effects on jujube firmness and maintained higher SSC and Vit C contents. In conclusion, VI + Ca + PME treatment was a promising method to improve the texture and maintain the quality of jujubes during storage.

4. Conclusions

VI + Ca + PME treatment had a positive effect on firmness, weight loss, SSC, and Vit C content of jujubes. Compared with that of the CK, VI + Ca, and VI + PME groups, the firmness of jujubes in the VI + Ca + PME group was the firmest (40.44 N) at the end of storage. Meanwhile, the firmness of jujubes was highly positively correlated with the SSP content. According to the AFM results, the F_q of WSP (34.47%), CSP (34.78%), and SSP (50.01%) molecules of width ≥60 nm were the highest in the VI + Ca + PME group. Together with the pectin contents analysis, these results indicated that the degradation of WSP, SSP, and CSP was delayed by VI + Ca + PME treatment. The higher quality of the VI + Ca + PME-treated fruit was probably caused by the permeation of PME and Ca via VI. VI + Ca + PME treatment is a promising technique to maintain the quality of jujubes.

Acknowledgements

Projects 31501535 and 31371851 supported by NSFC, Foundation for Young Key Teachers of Henan University of Technology (21420051), and Natural Science Foundation of Jiangsu Province (BK20181184) are acknowledged. An industry project supported by Funlife Food Industry (Chongqing) Co., Ltd. (R-143-000-673-597) also contributed to this research.

Conflict of interest

We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with this manuscript. We have no financial and personal relationships with other people or organisations that can inappropriately influence our work.

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