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Effect of calcium treatment on nanostructure of chelate-soluble pectin and physicochemical and textural properties of apricot fruits

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ABSTRACT

The effects of calcium and storage time on physicochemical properties and nanostructure of chelate-soluble pectin (CSP) of apricots (*Prunus armeniaca* L.) at 0 °C were investigated. During the storage, the firmness did not change with the contents but consistent with the morphology changes of CSP, which were characterized by atomic force microscopy (AFM). The branching structures of CSP decreased, meanwhile, the frequencies of chains with small width (<35 nm) and lengths (<500 nm) of CSP chains increased, which showed more in the control group than in the 1% calcium treated group. Compared to the control and 3% calcium treated groups, treatment with 1% calcium delayed the changes of physicochemical properties and degradation of the depolymerization of CSP during the fruit softening. The results provided us with a way to investigate the quality indexes from structural studies of nanoscale.

1. Introduction

Fruit firmness is a major factor that influences the appreciation of consumer and storage characteristics of fruits. It is very important to maintain the fruit texture in order to prolong the shelf-life and maintain good flavor. It was indicated that the softening of fruit texture has a close relationship with the depolymerisation and solubility of flesh cell wall polysaccharides (Brummell, Cin, Cristo, & Labavitch, 2004; Vicente, Costa, Martínez, Chaves, & Civello, 2005). Reasonable contents of calcium can retain high levels of ionically-bound pectins and retard the textural degradation (Chira, Chira, & Balan, 2006; Manganris, Vasilakakis, Diamantidis, & Mignani, 2007; Souty et al., 1995; Vicente, Saladié, Rose, & Labavitch, 2007). The increased firmness of fruits after calcium treatment could be due to several effects of calcium. One of the most important effects is that calcium could increase the binding of the cell wall polysaccharides.

Fruit cell wall polysaccharides extracted sequentially with water, chelator, and alkaline were good resources to study the modifications of pectin polysaccharides of fruits (Femenia, Sánchez, Simal, & Rosselló, 1998; Kirby, Ng, Waldron, & Morris, 2006; Lara, García, & Vendrell, 2004; Yang, An, Feng, Li, & Lai, 2005; Yang, Feng, An, & Li, 2006; Yang, Lai, An, & Li, 2006; Zhang et al., 2008). Chelate-soluble pectin (CSP) is considered ionically-bound pectin. It contains the middle lamella pectin which is responsible for cell cohesion. Lara et al. (2004) reported that calcium treatment retards the loss of CSP and dissolution of the middle lamella, and preserved the structure of the middle lamella of fruit cortex.

Although calcium treatment is widely used in the preservation of postharvest fruits preservation and its effects on the CSP content and chemical composition have been investigated (Ali, Chin, & Lazan, 2004; Lara et al., 2004; Missang, Renard, Baron, & Drilleau, 2001), it is believed that physical morphology rather than chemical composition of the plant cell wall pectin determines the protective effects of the cell wall materials. Unfortunately, the effects of calcium treatment on the morphology of fruit CSP are not clear due to its high heterogeneity. Most of the reported methods provide sample-wide average for pectin samples and do not show the variable structures of CSP (Yang et al., 2007).

Atomic force microscopy (AFM) is powerful in characterizing heterogeneous macromolecules as a nanotechnology tool (Yang et al., 2007). It has been applied on characterizing cell wall polysaccharides (Kirby et al., 2006), pectins of unripe tomato and beet (Kirby, MacDougall, & Morris, 2008), pectins from peaches (Yang, Feng, et al., 2006; Yang, Lai, et al., 2006; Yang et al., 2005), sodium carbonate-soluble pectin (SSP) and hemicellulose from cherries (Chen et al., 2009; Zhang et al., 2008). These AFM results have revealed the depolymerization of fruit polysaccharides extracted from cell wall materials during fruit ripening. However, to our best knowledge, there was no report about the effects of calcium on the detailed morphology changes of fruit pectins. Using AFM would provide an approach to directly study the effects of calcium treatment on the morphology of fruit polysaccharides.

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Apricots (*Prunus armeniaca* L.) are climacteric, highly perishable stone fruits. Apricots soften rapidly after harvest, which limits their marketing time (Antunes, Correia, Miguel, Martins, & Neves, 2003). The application of calcium for extending the shelf-life of apricots has been reported (Antunes et al., 2003; Chira et al., 2006; Souty et al., 1995). However, the fundamental of the effects of calcium treatment on fruit texture was not clear, and researches only rely on try and error to decide the usage of calcium for apricots (El-Motty, El-Shiekh, Mohamed, & Shahin, 2007).

The aim of this study was to investigate the effects of calcium on physicochemical properties, firmness and nanostructure of CSP of apricots. The morphological characteristics of CSP were examined and qualitative and quantitative information of CSP chains was characterized. The results would help to illustrate the effects of calcium on the fruit texture and physicochemical properties of apricots during storage.

2. Materials and methods

2.1. Fruit material and treatment

'Jinhong' apricots (*P. armeniaca* L.), harvested early in the morning in May from an orchard in Xinyang, Zhengzhou, Henan province, China, were transported to the laboratory within 2 h after harvest. The fruits were selected according to their size (medium), ripening stage (firm-ripe) and presence of stalks. Ten apricots were washed and then used for initial analysis. The others were randomly classified into three lots for the three treatments: control (distilled water), $CaCl_2$ (1%) and $CaCl_2$ (3%). The treatments were performed by placing the fruits in $CaCl_2$ solution or distilled water for 2 min (Antunes et al., 2003). After allowing the fruits to dry at ambient temperature (25 °C) for 1 h, fruits were stored at 0 °C in a temperature-controlled chamber. Ten fruits of each lot were randomly removed from storage and used for analyses every 6 days. The fruits were placed at 25 °C for 3 h before the test.

2.2. Determination of quality parameters

2.2.1. Firmness measurement

The fruits were peeled and cut into $10 * 5$ mm ($D * H$) pieces. Texture profile analysis (TPA) was performed with the fruit pieces by a TA-XT2i texture analyzer (Stable Micro Systems Ltd., Godalming, Surrey, UK). The operating parameters were as follows: load cell = 25 kg, probe = 35 mm diameter aluminum cylinder, pre-test speed = 5 mm/s, test-speed = 0.5 mm/s, post-test speed = 0.5 mm/s, compression degree = 30%, time = 10 s, and trigger force = 3.0 g (Shao, Tu, Zhao, Chen, & Zhao, 2006). The firmness is defined as the peakforce of the first compression of the sample. Ten apricots were measured individually for each treatment group.

2.2.2. Titratable acidity, soluble solids content and pH

Titratable acidity (TA), soluble solids content (SSC) and pH were determined with juice obtained from 10 fruits from each group. TA, expressed as percent malic acid was determined with well mixed juice by titration against NaOH (0.1 M) using a titrometer. SSC was measured with a refractometer (WYT-J, Sichuan, China). pH of the solution was determined by a potentiometer with electrodes using 5 mL fruit juice diluted in 50 mL distilled water (El-Motty et al., 2007; Lara et al., 2004).

2.3. Cell wall materials preparation and CSP extraction and content analysis

Cell wall material (CWM) was extracted with 10 fruits each time per group according to the method of Deng, Wu, and Li (2005) with slight modifications. Peeled fresh apricots (10 g) were boiled in ethanol (80%, v/v) for 20 min, then the ethanol was decanted by filtration. This procedure was repeated three times, after which the solid residue was transferred to 50 mL DMSO/H_2O (9:1, v/v) and incubated at 4 °C for 12 h. After filtration, the residue was transferred to chloroform–ethanol solution (2:1, v/v) for 10 min and subsequently washed with acetone. The residue materials recovered were CWM. The CWM from each group was dissolved with 10 mL ultra purified water for 4 h at 25 °C for three times, and then it was centrifugated at 10,000*g* at 4 °C for 10 min (Shanghai Anting Scientific Instrument Factory, Shanghai, China). The pellet was then extracted with 10 mL 50 mM cyclohexane-trans-1,2-diamine tetra-acetate (CDTA) on a shaker for 4 h at 25 °C to fractionate CSP. After centrifugation (10,000*g*, 4 °C for 10 min), the supernatant was collected. The remained precipitates were subjected to reiteration extraction for two more times. Then all the above three supernatants were pooled as CSP. The solution was stored in freezer below −18 °C and thawed before analysis (Yang, Lai, et al., 2006).

The CSP fractions were quantified by the Carbazole colorimetry method with slight modifications, using galacturonic acid as standard (Zhang et al., 2008). CSP solution (2 mL) was blended with 12 mL of sulfuric acid (98%, w/w) in a test tube and cooled using tap water, then the mixture solution was boiled for 10 min and cooled again. Carbazole ethanol solution (0.5 mL) was added into the solution and incubated at room temperature for 30 min. The absorbance at 540 nm was determined using a UV-2000 spectrophotometer (Unico Instrument Co., Ltd., Shanghai, China). Galacturonic acid was from Sigma–Aldrich Co., Ltd., St. Louis, MO, USA. The results were expressed as g·100 g^{-1} fruit weight (FM) (Keutgen & Pawelzik, 2007).

2.4. AFM operation and image analysis of CSP fractions

The nanostructure of CSP was conducted using a multimode NanoScope IIIa AFM (Vecco Metrology Group, Digital Instruments, CA, USA) equipped with E (J) scanner according to the previous methods (Kirby et al., 2008; Yang, Lai, et al., 2006). About 10 μL of diluted solution at reasonable concentration (about 10 μg mL^{-1}) was dropped onto the surface of freshly cleaved mica sheets for obtaining optimal images. A slight molecular combing technique with minimum force exerted was applied on the solution by a glass cover slip to comb the solutions for pulling the pectin out without taking the chelator away (Yang, An, & Li, 2006). The mica with solutions was dried in air at room temperature. Tapping mode was carried out. The AFM method is according to Yang, Chen, An, and Lai (2009). The imaging was conducted in air and NSC 11/no Al tip (MikroMasch, Wilsonville, OR, USA) was used. The resonance frequency and force constant of the tip were 330 kHz and 48 N/m, respectively (Yang, Lai, et al., 2006). The scan rate was about 0.5–2 Hz.

The AFM images were analyzed offline with AFM software (Version 5.30r3sr3). Section analysis was performed to determine the width and height of sample. The surface profiles of the sections were then plotted. The horizontal and vertical distances of the chains were recorded as width (W) and height (V), respectively. Number of particular chain widths or lengths observed was recorded as frequency (Fq). The length (L) of single chain was determined by plotting the chain with the software (Yang, Lai, et al., 2006). At least 10 images were analyzed for each sample to obtain reliable results.

2.5. Statistical Analysis

The data were analyzed with SPSS software version 13.0 for Windows (SPSS Inc., Chicago, IL, US). Analysis of variance (ANOVA)

was performed to determine the effects of calcium treatments and storage time on firmness, SSC, TA, pH, CSP content and nanostructure of CSP from AFM images. Pearson's correlation analysis between the parameters was performed using SAS 9.1.3 software (SAS Institute Inc., Cary, NC, US). Mean comparisons were performed using Duncan's test to examine differences between treatments and storage time. Comparisons that yielded $P < 0.05$ were considered significant.

3. Results and discussion

3.1. Effect of calcium treatment and storage time on fruit firmness

A close relationship exists between fruit texture and tissue calcium levels. The role of calcium in maintaining the firmness of fruit is based on the calcium bridges between pectin molecules in the middle lamella, which is responsible for fruit cell cohesion (Cosgrove, 2005; El-Motty et al., 2007). The loss of calcium in either pectin molecules or middle lamella can result in fruit softening. It was reported that fruits softened coinstantaneously with the increase of fruit flesh free calcium (Chira et al., 2006; Manganris, Vasilakakis, Diamantidis, & Mignani, 2007). Cold storage with 0 °C were chosen because that the changes of the fruit physicochemical properties and nanostructural changes were less affected by microorganisms and the changes could be viewed by its natural changes in some degree.

For apricot fruits, Fig. 1A presents the changes of firmness from three groups (control, 1% $CaCl_2$ treatment, and 3% $CaCl_2$ treatment). The firmness of apricots decreased steadily in all three groups with storage. No significant differences among treatments were found at the first 12 days of storage. However, control and 1% $CaCl_2$ treated groups were significantly firmer than 3% $CaCl_2$ treated fruits starting from 18 to 30 days of storage, and firmness was almost constant thereafter until at the end of storage. The reason might be that the phytotoxicity of high concentration $CaCl_2$ can result in hydrolyzation of cell wall materials (Antunes et al., 2003; Souty et al., 1995). In the last stage of storage, 1% $CaCl_2$ treated fruit was firmer than that of control group, indicating that 1% $CaCl_2$ treatment could maintain the firmness of apricots for long period of time. The results were in accordance with those obtained by Antunes, Correia, Miguel, Martins, and Neves (2003). The reason was that at this reasonable concentration, Ca^{2+} bridges were formed between pectin uronic acid carboxyl functions, creating pectin egg-box and borate diesters of two rhamnogalacturona II, which could integrate the pectin polysaccharides in the cell wall and increase the firmness (Vicente et al., 2007).

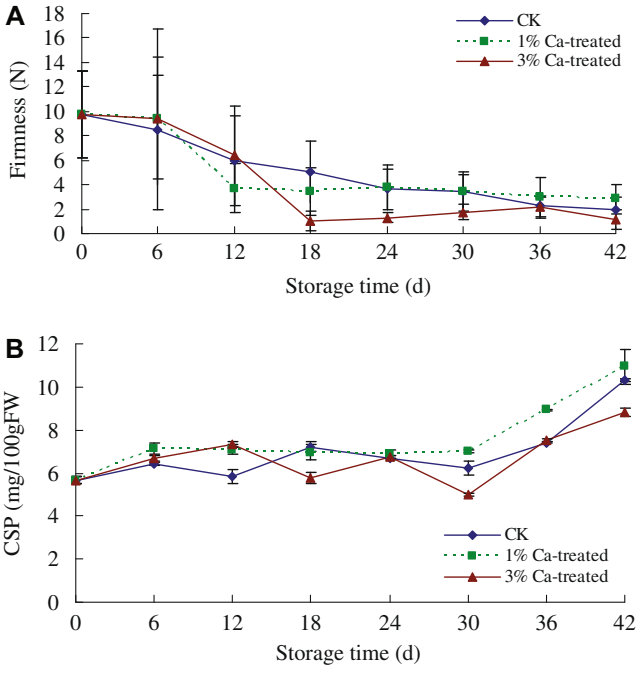

Fig. 1. Firmness (A) and CSP content (B) of apricot fruits treated by calcium and then stored at 0 °C. *Note*. CK means control group.

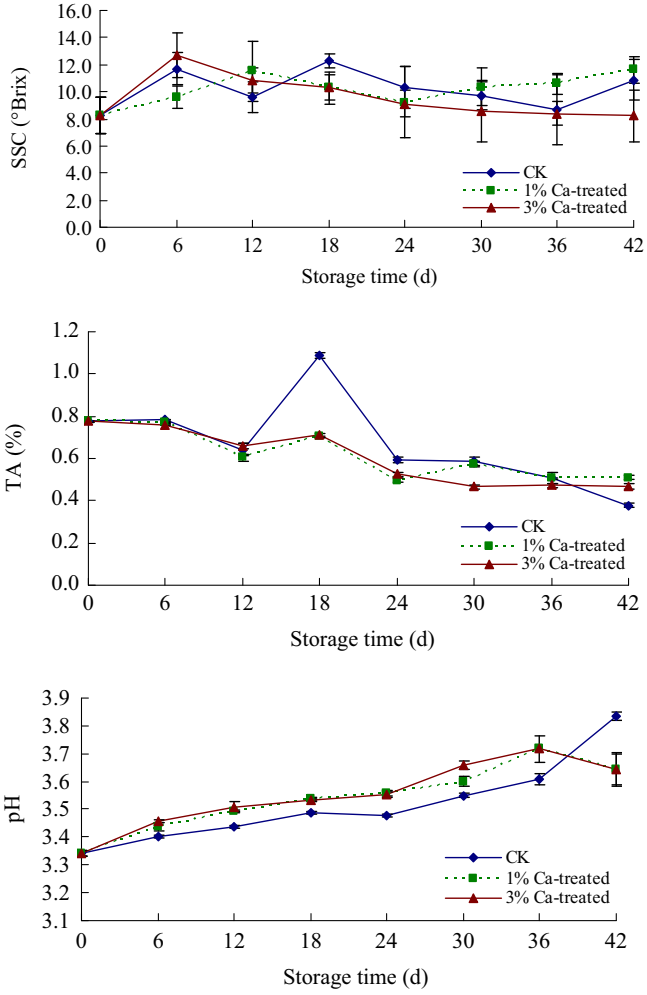

Fig. 2. Effect of calcium treatment on SSC, TA, pH of apricot fruits stored at 0 °C. *Note*. TA and pH were triplicate measured. SSC were from 10 repeated results. *Note*. CK means control group.

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

	Firmness	CSP	TA	SSC	pH
Firmness	1.00				
CSP	−0.26	1.00			
TA	0.63**	−0.35	1.00		
ssc	0.24	0.36	0.38	1.00	
pH	−0.74***	0.56**	−0.73***	−0.05	1.00

Note. *, **, *** represent significance at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

3.2. Effect of calcium treatment and storage time on physicochemical properties

CSP is ionically-bound pectin in fruit cell walls and maintains the structural integrity of cell walls (Lara et al., 2004). CSP plays an important role in the texture of fruits. Fig. 1B shows CSP content of three groups of apricots during storage. CSP content increased from 5.65 at harvest to 10.33, 10.84 and 8.89 mg/100 g^{-1} FM for control, 1% $CaCl_2$ group, and 3% $CaCl_2$ group, respectively, on day 42 of storage (Fig. 1B). The result was similar to the report by Femenia, Sánchez, Simal, and Rosselló (1998). CSP content of 1% $CaCl_2$ treated fruit was higher at late stage (days 24–42). Mobilization of uronic acid from the SSP (results were not showed here) to CSP fraction took place in response to applied calcium, indicating an increase in ionically-bound pectins in $CaCl_2$ treated fruit (Yang et al., 2009). The effect was especially obvious in 1% $CaCl_2$-treated apricots. Correspondingly, firmness of 1% calcium treated fruit was higher than that of the other two groups, which demonstrated that

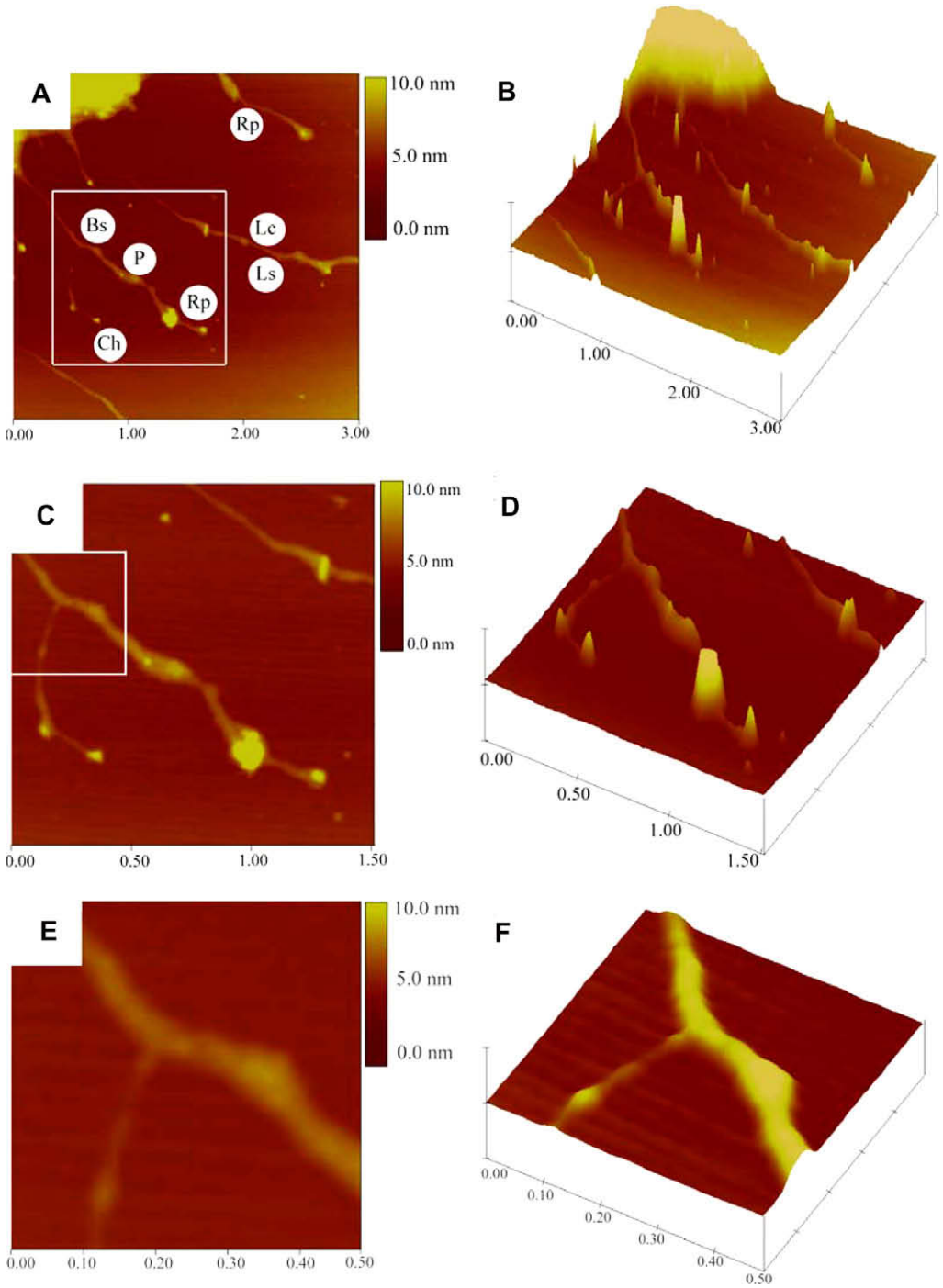

Fig. 3. AFM images of chelate-soluble pectin from apricot at harvest. Height bar = 10 nm. (A) Typical image, scan area 3.00 × 3.00 μm; (B) 3-dimensional image of image A, scan area: 3.00 × 3.00 μm; (C, D) zoom plane image and 3-dimensional image of the marked region of A, scan area: 1.5 × 1.5 μm; (E, F) zoom plane image and 3-dimensional image of the marked region of C, scan area: 0.5 × 0.5 μm. *Note*. Bs: branching structures; Ls: linear single fraction; Lc: long chain; Ch: chelator, CDTA; P: polymers; Rp: releasing point of pectin releasing from the CDTA.

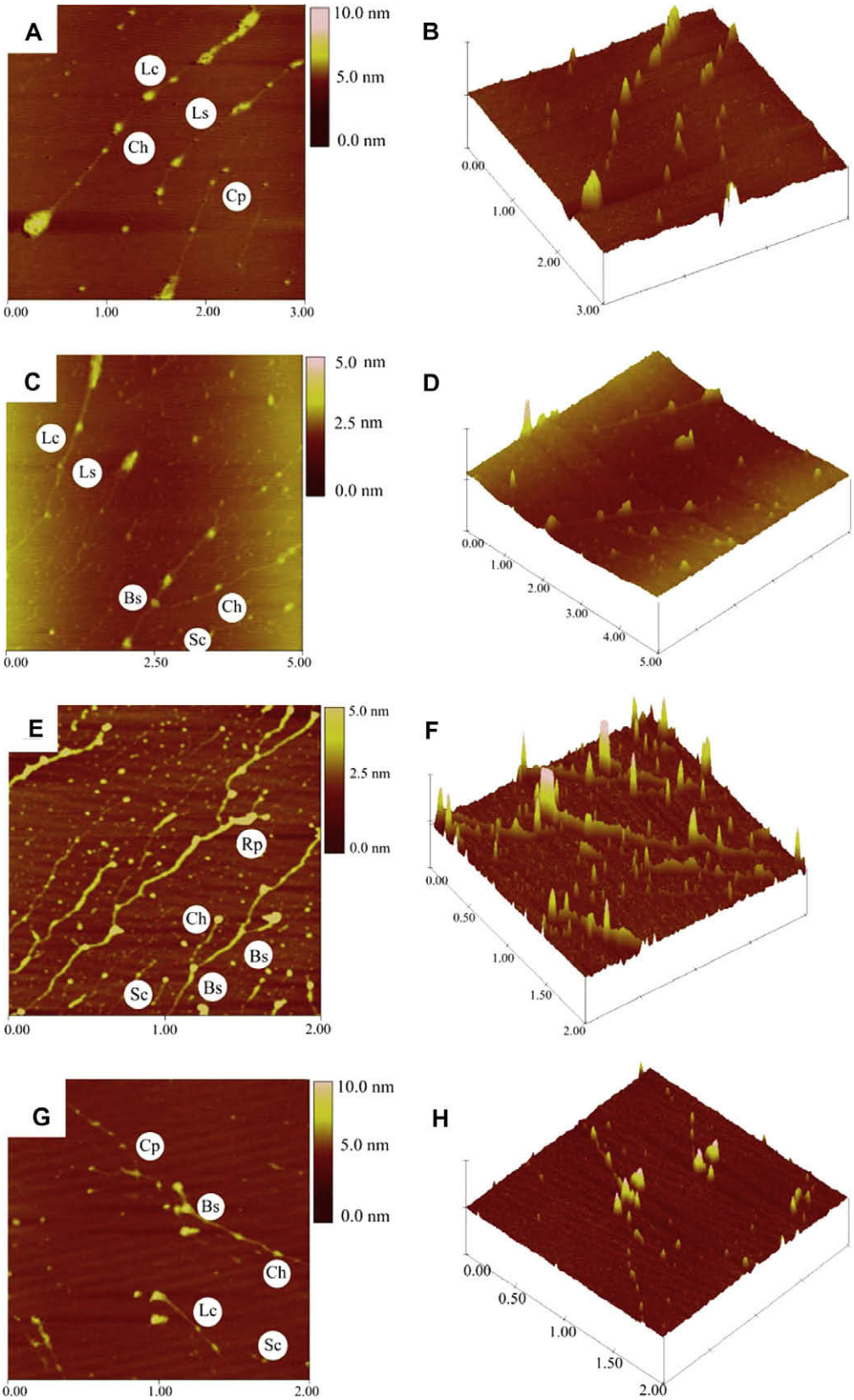

Fig. 4. AFM images of chelate-soluble pectin from apricot on days 18 (images A–F) and 42 (images G–L) of storage. Height bar: 5.0 nm for A–F; 3.6 nm for G-H; 10.0 nm for I–L. (A, B) typical plane and 3-dimensional images of CSP from apricots in control group on day 18, scan area 3.00 × 3.00 μm; (C, D) typical plane and 3-dimensional images of CSP from 1% $CaCl_2$-treated apricots on day 18, scan area 5.00 × 5.00 μm; (E, F) typical plane and 3-dimensional images of CSP from 3% $CaCl_2$-treated apricots on day 18, scan area 2.00 × 2.00 μm; (G, H) typical plane and 3-dimensional images of CSP from apricots in control group on day 42, scan area 3.00 × 3.00 μm; (I, J) typical plane and 3-dimensional images of CSP from 1% $CaCl_2$-treated apricots on day 42, scan area 5.00 × 5.00 μm; (K, L) typical plane and 3-dimensional images of CSP from 3% $CaCl_2$-treated apricots on day 42, scan area 3.00 × 3.00 μm; *Note*. Bs: branching structures; Ls: linear single fraction; Lc: long chain; Sc: short chain; Ch: chelator, CDTA; P: polymers; Rp: releasing point of pectin releasing from the CDTA; Cp: cleavage point of CSP chains. CK means control group.

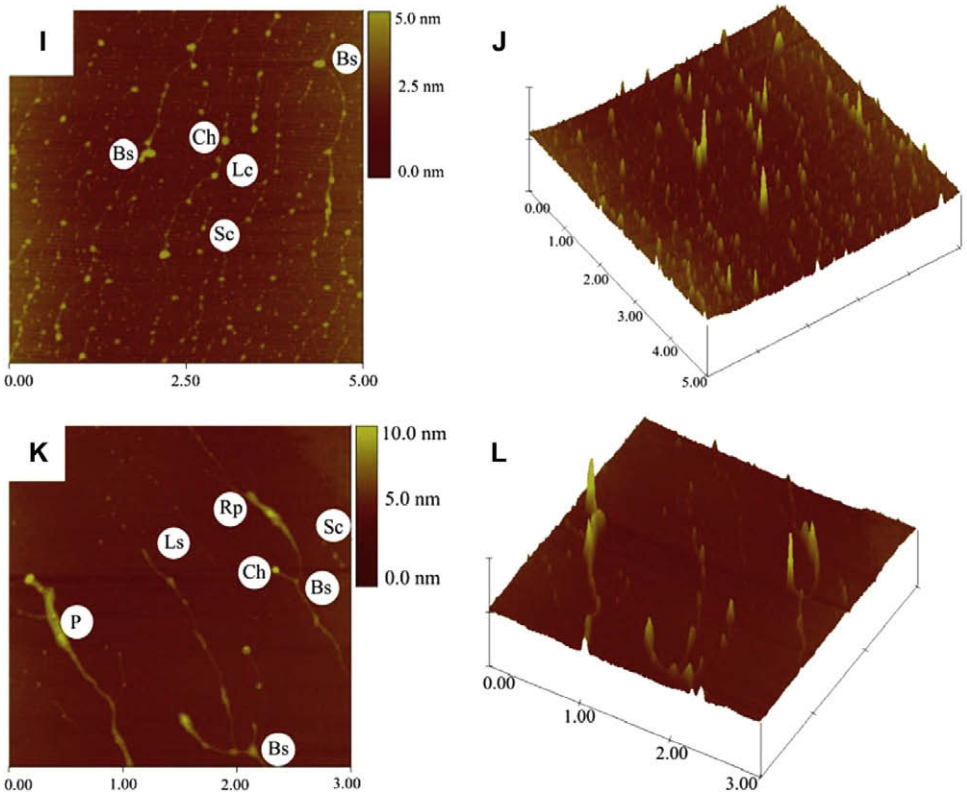

Fig. 4 *(continued)*

the content of CSP might have close relationship with firmness of apricots. And 1% $CaCl_2$ treatment might improve the resistance of apricots to ripening, which had been reported by Lara et al. (2004).

The effects of calcium treatment and storage on SSC, TA, pH are shown in Fig. 2. SSC increased at the beginning stage of cold storage, which indicated the solublization and synthesis of carbohydrates. SSC of 1% calcium treated fruits increased more slowly than that of the other two groups during storage, but they had highest level of SSC at the end of storage. SSC was closely related with the ripening of fruits (Antunes et al., 2003). The result showed that 1% $CaCl_2$ treatment could delay the ripening of apricot fruits, which partially agreed with the report of El-Motty, El-Shiekh, Mohamed, and Shahin (2007). Lowest SSC was found in 3% $CaCl_2$ treated group.

TA is commonly used to demonstrate the ripening stage of the fruits as well as to evaluate the fruit taste which is represented mainly by the balance between sweetness and acidity (Cordenunsi, Nascimento, & Lajolo, 2003; Souza, Scalon, Chitarra, & Chitarra, 1999). The results showed that TA of apricots generally had a trend of reduction with storage time except on day 18, especially for control group. High levels of TA appeared for all the three groups on day 18. This might be partially resulted from the elevation of CO_2 concentration and the reduction of O_2 during the climacteric stage, which affected the glycolytic enzyme systems, leading to the build-up of acids (Cordenunsi et al., 2003). Application of both concentrations of $CaCl_2$ reduced the climacteric significantly. At the end of storage, treatment of calcium slightly slowed the decrease of TA, which might be due to the acid oxidation. The results were similar with those of peach fruits (Manganris, Vasilakakis, Diamantidis, & Mignani, 2007) and strawberry (Souty et al., 1995). pH increased during the storage and it was significantly higher in calcium treated fruits than control.

Table 1 shows the correlation matrix of physicochemical properties and firmness of apricots during 0 °C storage. pH had significant correlation with firmness, CSP contents and TA of apricot fruits. No significant correlation was found between firmness and CSP contents. However, it did not mean that firmness was non related to the changes of CSP. We examined the morphology changes of CSP for further illustrate the relationship between firmness and CSP.

3.3. Effect of calcium treatment and storage time on CSP nanostructures

3.3.1. Qualitative nanostructure

AFM is a powerful technique for characterizing the molecular structures of polysaccharides from fruit cell walls (Kirby et al., 2006), and the results expand our knowledge on detailed structures. Branching structures of polysaccharides, for instance, could be characterized by AFM (Ovodova et al., 2006; Round, MacDougall, Ring, & Morris, 1997; Round, Rigby, MacDougall, Ring, & Morris, 2001). Furthermore, the degradation modes of fruit pectin during storage could also be deduced through the statistical analysis of pectin chain widths that were determined by AFM (Yang, Feng, et al., 2006; Yang, Lai, et al., 2006; Yang et al., 2005).

Fig. 3 shows typical AFM images of CSP that were extracted at harvest. Both plane (Fig. 3A, C and E) and 3-dimentional height (Fig. 3B, D and F) images are shown in Fig. 3. The results showed that CSP chains extracted from fresh apricots at harvest were long (around 2–3 μm) and branched (Fig. 3), and a few cleavage points resulting from enzyme actions were observed, which was similar with CSP of yellow peaches (Yang, Lai, et al., 2006). Fig. 4 presents AFM images of CSP from apricot fruits that were stored for 18 and 42 days. With the increase of storage time, number of short chains (<1000 nm) increased which were accompanied by the appearance of cleavage points, and the polymers decreased, as shown in Fig. 4A.

CDTA is commonly used as a chelating agent to extract relatively undegraded and calcium-cross-linked pectins from the primary plant cell wall (Kirby et al., 2008; Missang et al., 2001). In

Table 2
Height (*V*) and frequency (Fq) of particular chain width (*W*) of CSP chains during 0 °C storage.

W (nm)			Day 18						Day 42					
			CK		1%		3%		CK		1%		3%	
	Fq (*N* (%))	*V* (nm)	Fq (*N* (%))	*V* (nm)	Fq (*N* (%))	*V* (nm)	Fq ((%))	*V* (nm)	Fq (*N* (%))	*V* (nm)	Fq (*N* (%))	*V* (nm)	Fq (*N* (%))	*V* (nm)
At harvest														
11.719	0 (0)	0 ± 0	0 (0)	0 ± 0	0 (0)	0 ± 0	0 (0)	0 ± 0	0 (0)	0 ± 0	0 (0)	0 ± 0	1 (1.3)	0.72 ± 0
15.625	0 (0)	0 ± 0	0 (0)	0 ± 0	0 (0)	0 ± 0	0 (0)	0 ± 0	1 (2.1)	0.92 ± 0	0 (0)	0 ± 0	0 (0)	0 ± 0
19.531	0 (0)	0 ± 0	0 (0)	0 ± 0	0 (0)	0 ± 0	0 (0)	0 ± 0	1 (2.1)	0.97 ± 0	0 (0)	0 ± 0	1 (1.3)	1.61 ± 0
23.438	0 (0)	0 ± 0	2 (8.0)	0.27 ± 0.03	0 (0)	0 ± 0	0 (0)	0 ± 0	7 (14.6)	1.14 ± 0.54	0 (0)	0 ± 0	14 (17.5)	0.79 ± 0.39
27.344	0 (0)	0 ± 0	0 (0)	0 ± 0	0 (0)	0 ± 0	0 (0)	0 ± 0	1 (2.1)	1.24 ± 0	0 (0)	0 ± 0	0 (0)	0 ± 0
31.250	0 (0)	0 ± 0	0 (0)	0 ± 0	0 (0)	0 ± 0	0 (0)	0 ± 0	4 (8.3)	1.37 ± 0.86	0 (0)	0 ± 0	0 (0)	0 ± 0
35.156	2 (4.9)	1.21 ± 0.81	1 (4.0)	0.47 ± 0	2 (5.6)	1.45 ± 0	1 (4.2)	1.73 ± 0	0 (0)	0 ± 0	4 (9.5)	0.94 ± 0.481	25 (31.3)	0.98 ± 0.44
39.063	1 (2.4)	0.46 ± 0	3 (12.0)	0.60 ± 0.07	1 (2.8)	0.17 ± 0	1 (4.2)	0.39 ± 0	4 (8.3)	1.09 ± 0.62	10 (23.8)	1.34 ± 0.86	10 (12.5)	1.15 ± 0.47
42.969	0 (0)	0 ± 0	0 (0)	0 ± 0	0 (0)	0 ± 0	0 (0)	0 ± 0	1 (2.1)	1.19 ± 0	0 (0)	0 ± 0	0 (0)	0 ± 0
46.875	4 (9.8)	1.87 ± 0.75	2 (8.0)	0.54 ± 0.07	2 (5.6)	0.50 ± 0.08	5 (20.8)	2.21 ± 0.95	2 (4.2)	0.90 ± 0.19	2 (4.8)	1.45 ± 1.25	8 (10.0)	1.12 ± 0.87
58.594	8 (19.5)	0.86 ± 0.69	8 (32.0)	0.67 ± 0.78	18 (50.0)	0.62 ± 0.41	10 (41.7)	1.82 ± 0.79	10 (20.8)	1.55 ± 0.623	9 (21.4)	1.88 ± 1.27	18 (22.5)	1.19 ± 0.85
70.313	1 (2.4)	2.35 ± 0	0 (0)	0 ± 0	1 (2.8)	0.61 ± 0	2 (8.3)	2.05 ± 0.71	10 (20.8)	1.381 ± 1.08	1 (2.4)	0.51 ± 0	2 (2.5)	2.00 ± 0.14
78.125	10 (24.4)	1.02 ± 0.58	4 (16.0)	1.31 ± 0.27	6 (16.7)	0.71 ± 0.22	1 (4.2)	1.78 ± 0	2 (4.2)	2.28 ± 1.22	10 (23.8)	1.91 ± 0.71	1 (1.3)	0.62 ± 0
82.031	2 (4.9)	2.35 ± 0.89	0 (0)	0 ± 0	0 (0)	0 ± 0	3 (12.5)	3.44 ± 1.44	0 (0)	0 ± 0	0 (0)	0 ± 0	0 (0)	0 ± 0
93.750	0 (0)	0 ± 0	0 (0)	0 ± 0	1 (2.8)	3.29 ± 0	0 (0)	0 ± 0	2 (4.2)	1.94 ± 1.41	0 (0)	0 ± 0	0 (0)	0 ± 0
97.656	2 (4.9)	1.75 ± 0.85	0 (0)	0 ± 0	3 (8.3)	1.49 ± 0.95	1 (4.2)	1.45 ± 0	1 (2.1)	1.88 ± 0	3 (7.1)	2.49 ± 0.31	0 (0)	0 ± 0
117.19	4 (9.8)	1.90 ± 1.09	3 (12.0)	1.33 ± 0.42	1 (2.8)	3.92 ± 0	0 (0)	0 ± 0	2 (4.2)	2.43 ± 1.01	2 (4.8)	2.12 ± 1.26	0 (0)	0 ± 0
125.00	0 (0)	0 ± 0	0 (0)	0 ± 0	1 (2.8)	1.45 ± 0	0 (0)	0 ± 0	0 (0)	0 ± 0	0 (0)	0 ± 0	0 (0)	0 ± 0
175.78	5 (12.2)	3.22 ± 0.55	2 (8.0)	1.69 ± 0.40	0 (0)	0 ± 0	0 (0)	0 ± 0	0 (0)	0 ± 0	1 (2.4)	3.79 ± 0	0 (0)	0 ± 0
234.38	2 (4.9)	1.84 ± 0.37	0 (0)	0 ± 0	0 (0)	0 ± 0	0 (0)	0 ± 0	0 (0)	0 ± 0	0 (0)	0 ± 0	0 (0)	0 ± 0

Note. CSP: chelate-soluble pectin; *W*: the width of CSP chains; *V*: the height of CSP chains relative to mica surface; Fq: numbers of times particular chain widths were observed, and also expressed as the percent of each chain width of all the chains observed. CK means control group.

this research, the chelator was left along with the pectin chains (marked as 'Ch' in the images, Fig. 3A, for instance), mild molecular combing technique was applied for maximum maintaining the original status of pectin and chelator while characterizing their structures (Yang, An, et al., 2006). This provided much 'genuine' structure of CSP extracted in the mixed solutions since the sample was imaged without separating CDTA and CSP.

AFM can characterize the integral heterogeneous structures of pectin, such as the branching structure (Bs), linear single fraction (Ls), long chain (Lc), short chain (Sc), cleavage point (Cp), releasing point of pectin releasing from CDTA (Rp) and polymer (P) (Yang, Lai, et al., 2006), polymer means several pectin chains aggregating together which are present even at low dilution and after speed shake, which is different from Ch, the chelator, CDTA. The height of the chains could be used to judge whether the chain was a branch or crossed over of two single chains (Adams, Kroon, Williamson, & Morris, 2003). AFM also can scan a relatively large area and further scan the featured zone to obtain enlarged image of that area. It can be clearer to view the details of CSP nanostructures in enlarged images (Figs. 3C, E, 4C, G, K). The corresponding 3-dimensional images (Figs. 3B, D and 4B, D, F, H, J L) could better present of the featured structures.

3.3.2. Quantitative nanostructure

Quantitative characterization of the nanostructures of CSP chains includes two aspects: general and individual structure information of CSP. For general quantitative information of CSP, about 30% of the pectin chains were observed to be single or multiple branching structures. A few examples of typical branching pectin molecules are shown as 'Bs' in Figs. 3A and 4A, E, I. At the last period of storage, only about 18% of the pectin chains showed branching structures in all the three groups, which was supported by the result of Femenia, Sánchez, Simal, and Rosselló (1998). The reason was that, as the fruit softened, carboxyl-based crosslinking sites were destroyed gradually due to the action of pectin degrading enzymes, which resulted in the deceased branches (Cosgrove, 2005).

AFM can be used to investigate the quantitative characteristics of individual pectin chains including width, length and height as well. Fruit pectins on day 0, day 18 and day 42 were selected as initial, middle and final stages of the storage. Therefore, the nanostructural changes of CSP in different storage stages could be investigated and analyzed. Table 2 presents the effects of calcium treatment and storage time on the widths of CSP chains and the corresponding Fq of apricot fruits. The distribution of CSP widths

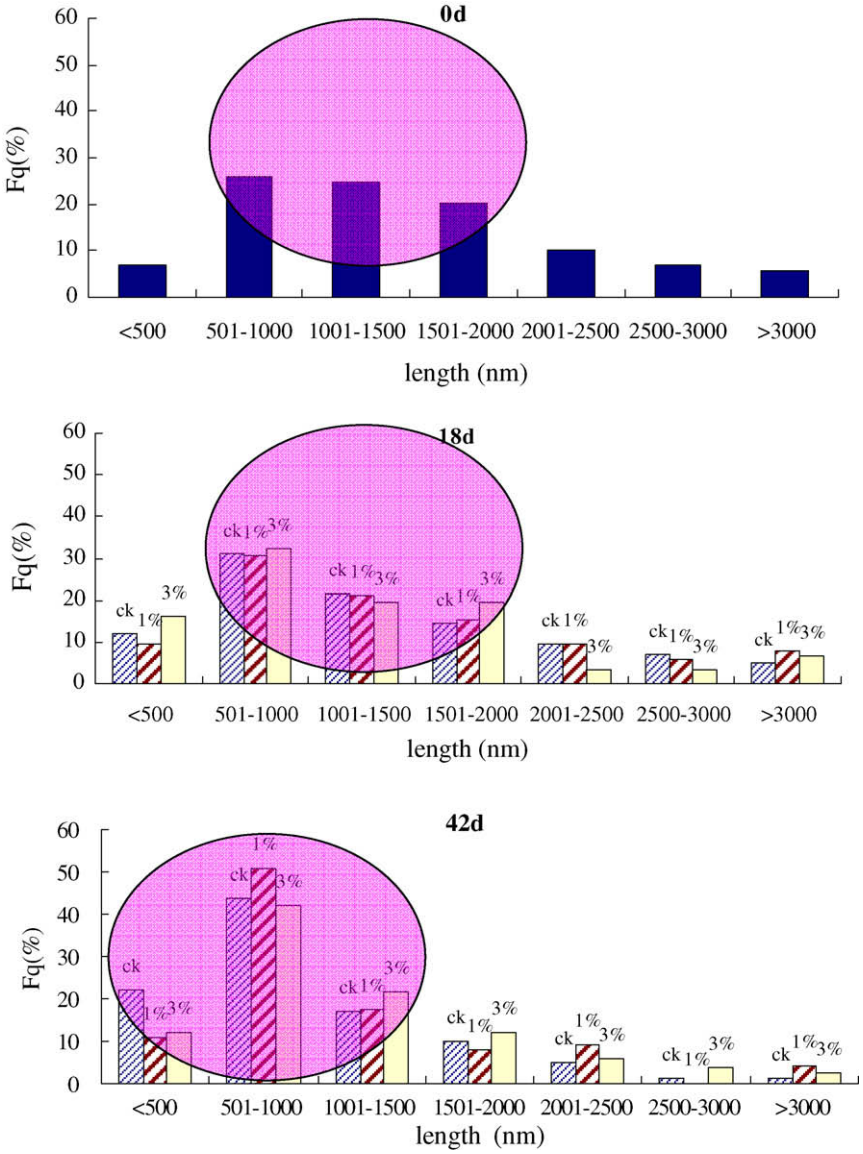

Fig. 5. Length distribution of chelate-soluble pectins at harvest, on days 18 and 42 during 0 °C storage. *Note*. CK means control group.

of apricot fruits was similar to that of 'Jinxiu' peaches (Yang, Feng, et al., 2006; Yang, Lai, et al., 2006), both CSPs of the two fruits shared some common values, and the values were all limited and intermittent. However, some values in 'Jinxiu' peaches did not appear in apricots which included 17.587, 29.297, 48.828, 52.734, 87.891, 136.72 and 156.25 nm. In addition, there were several new values (11.719, 15.625, 27.344, 31.250, 42.969, 46.875, 70.313, 82.031, 93.750, 125.00, 175.78 and 234.38 nm) that were not appeared in the CSP of 'Jinxiu' peaches (Yang, Lai, et al., 2006).

As shown in Table 2, the CSP widths were much influenced by the storage time, the Fq of chains of small width increased with storage time, which was similar to the result of peaches (Yang, Lai, et al., 2006). For different treated groups, 1% $CaCl_2$ treated group had less Fq of small width chains than control and 3% $CaCl_2$ groups during storage. On day 42, the Fq of widths smaller than 35 nm for control group was 29.2% and 20.1% for 3% $CaCl_2$ group, while Fq of 1% $CaCl_2$ group was zero. The reason 1% $CaCl_2$ treated group maintained the larger widths of CSP might be that homogalacturonans were ionically crosslinked by calcium between pectin molecules, and $CaCl_2$ treatment increased the content of calcium in cell wall which led to an increase in the capability of crosslinking between homogalacturonans. Souty et al. (1995) found low concentration of $CaCl_2$ treatment significantly reduced the breakdown of pectin chains of apricots from chemical results. However, CSP of 3% $CaCl_2$ treated fruits showed no significant difference from the control group from days 18 to 42, which was consistent with the physicochemical characteristics of low firmness and low content of CSP around the similar period of storage (Fig. 1). The decreased firmness was also found in 4% $CaCl_2$-treated apricots reported by Souty et al. (1995), that 4% $CaCl_2$ treatment accelerated the degradation of pectin chains. The reason was that high concentration calcium had toxicity for apricot cell in restraining the activity of pectin methylesterases, which hydrolyzed the methylesters from carboxyl groups to free carboxyl groups for Ca^{2+} crosslink formation between homogalacturonan domains (Cosgrove, 2005; Xisto, De Abreu, Corrêa, & Santos, 2004).

For chain heights (V), most of the apricot CSP had the values of V between 0.2 and 3.0 nm, mainly in the range of 1–2 nm (Table 2). There were no significant differences of V values among the three groups. The height of pectin from fresh albedo was about 0.43 nm, and it was 0.5–0.7 nm for tomato pectin, and 1–5 nm for peactin of 'Jinxiu' and 'Milu' peaches (Fishman, Cooke, Chau, Coffin, & Hotchkiss, 2007; Yang et al., 2009). The height of the apricot CSP was closer to that of peach pectin than that of tomato and albedo pectin (Yang, Lai, et al., 2006; Yang et al., 2006).

For the distribution of CSP lengths, the frequencies of length of single chains were shown in Fig. 5. The length of CSP was the length of single linear fractions (main chain) according to Yang, Lai, et al. (2006). It should be noted that only main chains of the CSP fraction were determined. And the linear molecules must not be entangling or overlapping each other and lay entirely within the AFM scanned area. The distribution of lengths was in the range of 400–3600 nm. It showed that the apricot CSP molecules were longer than the CDTA extracted pectin of tomato (20–460 nm) and alkali-treated sugar beet pectin (20–320 nm) and SSP of peaches (Kirby et al., 2008; Yang et al., 2009). This might be resulted from the differences among varieties of fruits. Another possible reason was that alkali treatment might result in some degree of degradation of pectin molecules through β-elimination reaction (Kirby et al., 2008).

It should be noted that textural differences among different ripening stages for the same cultivar are different from those among different cultivars. The former could be viewed as a result from enzymes and biochemical reactions, while the latter is largely determined by the cell wall skeleton of fruit flesh. Therefore, it is reasonable that our current result is consistent with that of Brummell et al. (2004), while it is not comparable with that of Yang et al. (2009).

Considering the largest three parts of length distributions of the CSP chains among the three groups, as shown in the oval areas in Fig. 5, most of the chain lengths of apricots at harvest and on day 18 were in the range of 500–2000 nm, while the largest parts of chain lengths were <1500 nm on day 42. The increase in short- and decrease in long chains indicated that $CaCl_2$ treatment could delay the degradation of pectin chains of apricots during storage. Compare the length distribution among the three groups during storage can bring further information of CSP chains. Generally, the length of CSP decreased with the storage, for example, on day 42, the Fq of chain with length longer than 3000 nm were 1.1%, 4.0%, and 2.4% for control, 1% $CaCl_2$ treated and 3% $CaCl_2$ treated groups, respectively, while the Fq of short chains (<500 nm) of control was 22.0%, 10.7%, and 12.0% for these three groups, respectively. The group of 1% $CaCl_2$ treatment had largest part of long chain (>3000 nm) and lowest part of short chain (<500 nm) among the three groups. It indicated that 1% $CaCl_2$ treatment could retard the degradation of chain, which was in accordance with the changes of widths and physicochemical properties.

4. Conclusions

During the storage, changes of physicochemical properties of apricot fruits were consistent with the nanostructural changes of CSP that determined by AFM. Treatment with 1% calcium retarded the changes of physicochemical properties and the depolymerization of CSP during storage. The results could help to illustrate the fundamental of changes of apricot fruits during storage.

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