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Effects of calcium treatment and low temperature storage on cell wall polysaccharide nanostructures and quality of postharvest apricot (*Prunus armeniaca*)

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ABSTRACT

Cell wall polysaccharides play an important role in postharvest fruit texture softening. Effects of calcium treatment combined with cold storage on the physical properties, polysaccharide content and nanostructure of apricots were investigated. Apricots were immersed in distilled water, 1% or 3% w/v calcium chloride, then stored at 5 °C or 10 °C. Storage at 5 °C significantly improved apricot quality and shelf life. Significant changes in the concentration and nanostructure of cell wall pectins and hemicelluloses revealed their disassembly and degradation during apricot storage. These modifications could be retarded by 1% w/v calcium chloride treatment. Meanwhile, the basic width units of apricot cell wall polysaccharide chains were 11.7, 31.2 and 39.1 nm for water-soluble pectin, 11.7, 17.6 and 19.5 nm for chelate-soluble pectin, and 15.6 and 23.4 nm for hemicellulose. The results suggest that texture of apricots can be effectively maintained by 1% calcium chloride treatment and storage at 5 °C.

1. Introduction

The texture of stone fruit is a key quality parameter that affects consumer preference. Texture affects postharvest handling, microbial safety, shelf life, consumer acceptability and suitability for further processing (Missang, Maingonnat, Renard, & Audergon, 2012). Many factors are known to influence texture; the composition, structure, and morphology of the fruit cell wall have been viewed as the most important (Brummell, 2006). The rigidity of cell walls is influenced by the composition and structure of the polysaccharide constituents (Peña & Carpita, 2004).

Many investigations have identified changes in texture during ripening leading to softening, loss of neutral sugars, and cell wall polysaccharide solubilisation and depolymerisation. At the microscopic scale, texture alterations are caused by cell wall degrading enzymes (Brummell, 2006; Goulao & Oliveira, 2008). However, though there are many studies about quantitative and qualitative biochemical changes during the physical changes in texture, the dismantling processes of fruit polysaccharides including pectins, hemicelluloses and cellulose have received little attention. The biochemical determinants related to these processes have not yet been fully elucidated (Salato, Ponce, Raffo, Vicente, & Stortz, 2013), which may be partially due to the apparent irregular behaviour of polysaccharide polymers in different fruit species (Manrique & Lajolo, 2004).

The atomic force microscope (AFM) has allowed for characterisation of complex and heterogeneous systems at molecular level, and can be used for studying polysaccharide morphology in conditions that simulate their natural state (Posé, Kirby, Mercado, Morris, & Quesada, 2012). These advantages have made AFM a preferred technology for investigating the ultrastructure of cell wall polysaccharides in different plant materials (Chen et al., 2011).

Apricot (*Prunus armeniaca*) is one of the most popular fruit with a yearly production of over 52,000 t in China according to FAOSTAT in 2013. Apricot is a climacteric fruit with a very short storage life, characterised by high heterogeneity in their tissue structure

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(Missang et al., 2012). In our previous report (Liu et al., 2009), we found that apricot texture was affected by the modification of chelate-soluble pectins within cell wall polysaccharides when treated with calcium and stored at 0 °C. In the present study, we have examined postharvest properties, content and microstructural changes in the cell wall pectins and hemicelluloses of apricots stored at 5 °C and 10 °C after calcium treatments. The aim of this study was to determine the effects of temperature and concentration of calcium chloride on postharvest properties, pectin and hemicellulose microstructure of apricot fruits.

2. Materials and methods

2.1. Fruit materials and postharvest treatment

Firm ripe, medium and uniformly sized "Jinhong" apricots (*Prunus armeniaca*) were hand-harvested with stalks still attached. In the morning, apricots were harvested from an orchard in Xingyang, Zhengzhou, China, and were transported to the laboratory within 2 h. A total of 900 fruits were randomly distributed among 60 trays lined with butter paper, and the trays were further divided into six groups. Two groups were immersed in 1% w/v of calcium chloride and named as '1% Ca' groups, another two groups were immersed in 3% w/v of calcium chloride and named as '3% Ca' groups, and the last two groups were immersed in distilled water and named as 'control' groups. Each immersion time was 2 min, and the trays were then fan dried. The fruits were then stored at 5 °C or 10 °C per Ca treatment. For each group, 15 fruits were randomly removed every 6 d during storage and placed at 25 °C for 3 h before analysis.

2.2. Determination of apricot fruit quality changes

2.2.1. Firmness of fruits

Apricot fruits were peeled and 3 cylinder pieces (10 mm × 5 mm, D × H) were cut along the equatorial circumference for each fruit used for firmness measurement. Texture profile analysis (TPA) was performed using a TA-XT2i texture analyser (Stable Micro Systems Ltd., Godalming, Surrey, UK). The operating parameters were as follows: load cell = 25 kg, probe = 35 mm diameter aluminium 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 (Liu et al., 2009). The firmness is defined as the peak force during the first compression of the sample.

2.2.2. Titratable acidity (TA) and soluble solids content (SSC)

Each group of peeled apricot fruit flesh was homogenised. SSC was determined for juice obtained from each group with a PAL-1 pocket refractometer (Atago Co. Ltd., Japan). TA, expressed as percent malic acid, was assayed by titrating the solution of 5 g of homogeneous apricot puree in 100 mL of deionised water against 0.1 M NaOH to pH 8.1 (Liu et al., 2009).

2.3. Separation and quantification of cell wall materials

Cell wall materials of apricot flesh were extracted according to methods described by Basanta, de Escalada Plá, Stortz, and Rojas (2013) with modifications. About 10 g of ground flesh was transferred into boiling ethanol for 30 min. The ethanol was filtered off and the alcohol insoluble residue (AIR) was collected and returned to boiling ethanol. The above process was repeated twice, and the final AIR was transferred into 50 mL of 9:1 v/v dimethyl sulphoxide (DMSO)-H_2O mixture and kept at 4 °C for 12 h. After filtration, the residue was soaked in 10 mL of 2:1 v/v chloroform-ethanol mixture for 10 min. Then the residue was washed with 50 mL acetone three times. The retentates were recovered as cell wall materials.

Different cell wall fractions were separated with different solvents. Ultra-purified H_2O (10 mL) was added to the cell wall material and the mixture was shaken at 25 °C for 4 h. The supernatant was then collected by centrifugation at 10 000*g* at 4 °C for 10 min, and the remaining cell wall materials were extracted twice more. All three supernatants were combined as water-soluble pectin (WSP). The residue was then extracted with 50 mM cyclohexane-*trans*-1,2-diamine tetra-acetate (CDTA) to obtain chelate-soluble pectin (CSP), with 50 mM/2 mM Na_2CO_3/CDTA for sodium carbonate-soluble pectin (SSP), and 4 M KOH (containing 100 mM $NaBH_4$) for hemicelluloses (HC). Remaining residue consisted mainly of cellulose (residue).

Samples of the WSP, CSP and SSP pectins were assayed for their uronic acid (UA) content by the carbazole-sulfuric acid method using galacturonic acid (GalA) as standard (Liu et al., 2009), and results were expressed as mg GalA/100 g FW (fresh weight). HC fractions were determined for total neutral sugar (NS) content by anthrone colorimetry method with glucose (Glc) as standard and expressed as mg Glc/100 g FW (Deng, Wu, & Li, 2005). Absorbance data were corrected based on a previous reported method to eliminate the interferences from neutral sugars and uronic acids, in the carbazole and anthrone methods, respectively (Posé et al., 2012).

2.4. AFM manufactured and image analysis

The nanostructure of cell wall polysaccharides was investigated using a multimode NanoScope IIIa AFM (Vecco Metrology Group, Digital Instruments, CA, USA) equipped with a Si_3N_4 cantilevered scanner (Chong, Lai, & Yang, 2015; Yang, 2014). The scan rate was 0.5–2 Hz and tapping mode was carried out. Approximately 10 μL of diluted cell wall polysaccharide extract solution at a concentration of approximately 10 μg/mL was agitated with a vortex mixer (Fisher Scientific, Pittsburgh, PA, USA), and dropped onto the surface of freshly cleaved mica sheets. The mica sheets were then dried in air at room temperature, and the images were flattened during processing.

The AFM images were analysed offline using AFM software (Version 5.30r3sr3). At least 20 images were examined for each sample for reliability. The images were sectioned to the direction at which the samples were analysed. Section analysis was carried out to obtain the height (H) and width (W, calculated by the peak width at half height) of sample. The length (L) of each single chain was obtained by plotting the chain using the software (Liu, Tan, Yang, & Wang, 2016; Yang, 2014).

2.5. Statistical analysis

The data were analysed using SAS 9.0 software (SAS Institute, Inc., Chicago, IL, U.S.A.). Analysis of variance (ANOVA) was conducted to determine significant differences, and mean comparisons were performed using Duncan's multiple range test. $P < 0.05$ was considered significant.

3. Results and discussion

3.1. Effects of calcium treatment and temperature on the properties of apricot fruits during postharvest storage

Changes in firmness, SSC and TA in the six groups are shown in Fig. 1. Shelf life of apricot fruits stored at 10 °C was just 18 d and the texture softening rate was faster than the 5 °C groups (Fig. 1A). For the apricots stored at 10 °C, 3% Ca resulted in lower

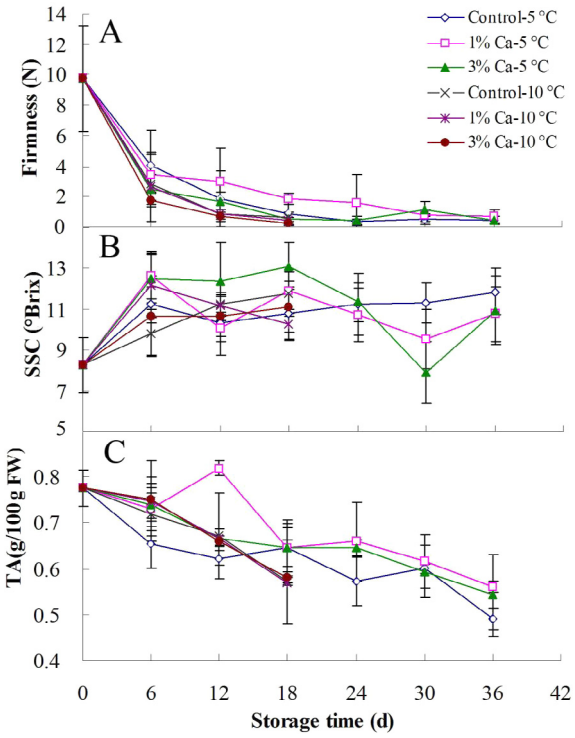

Fig. 1. Effect of calcium treatment (Control, 1% Ca or 3% Ca) and temperature (5 or 10 °C) on the firmness (N), SSC (soluble solids content; °Brix) and TA (titratable acidity; g/100 g FW) of postharvest apricots.

firmness compared with the control and those treated with 1% Ca at day 6. After day 6, no significant differences in firmness were found between the Ca groups and the control group. This suggests that calcium treatment is ineffective in slowing the rapid softening of apricots stored at 10 °C and consequently, storage temperature is more critical in delaying apricot softening than calcium treatment also reported by García, Ballesteros, and Albi (1995).

Compared to 10 °C, the colder storage temperature of 5 °C extended apricot shelf life to 36 d and slowed the softening rate of the fruits in the first 12 d. The maximum compression force required to break tissue was significantly higher when treated with 1% Ca (5 °C) compared with the other treatment groups between 12 and 24 d storage (Fig. 1A). Reduced apricot softening following 1% calcium treatment has also been confirmed in other reports (Hernández-Muñoz, Almenar, Ocio, & Gavara, 2006; Liu et al., 2009). It has been suggested that postharvest calcium treatment may increase free calcium and cell wall bound calcium (Manganaris, Vasilakakis, Diamantidis, & Mignani, 2007). This increase may result from the reaction of Ca^{2+} with pectic acid to form calcium pectate which enhances molecular bonding between components of cell wall and increases the firmness (Saba & Sogvar, 2016). However, the texture of apricot treated with 3% Ca at both 5 °C and 10 °C showed significantly softer texture than the control and 1% Ca groups at day 6 (Fig. 1A). This result was similar with our previous report which found high concentrations of $CaCl_2$ can induce phytotoxicity for cell wall hydrolysation and result in texture softening (Liu et al., 2009) as well as other reports (Antunes, Correia, Miguel, Martins, & Neves, 2003).

As shown in Fig. 1B, SSC in the control groups increased from 8.26 °Brix at harvest to 11.75 °Brix at 10 °C and 11.86 °Brix at 5 °C at the end of storage. However, the calcium treated groups showed more variation in SSC. Both the 1% and 3% Ca treatments resulted in higher SSC at day 6 than the control groups. Towards the end of storage, SSC of apricots treated with 1% Ca at 10 °C (18 d) and 5 °C (24–36 d) were significantly lower than the control. In other studies, calcium treatment also effectively retarded the increase of SSC in fig fruits (Irfan, Vanjakshi, Keshava Prakash, Ravi, & Kudachikar, 2013) and delayed the ripening of apricot fruits (El-Motty, El-Shiekh, Mohamed, & Shahin, 2007).

TA markedly decreased during the latter stage of storage (Fig. 1C). No significant differences in TA among 10 °C groups treated with different concentrations of calcium chlorides were observed. Different results were found at 5 °C; apricots treated with 1% Ca and 3% Ca had higher TA than the control and slowed the decrease in TA. Lower temperature combined with calcium treatment could reduce acid oxidation and slow the decrease of apricot TA, similar to previous reports (Liu et al., 2009; Manganaris et al., 2007).

3.2. Effects of calcium treatment and temperature on yields of apricot cell wall polysaccharides during postharvest storage

The chemical characterisations of WSP, CSP, SSP, and HC content throughout the storage period are shown in Fig. 2. In all six groups, pectins peaked on day 6 for WSP (Fig. 2A, ranging from

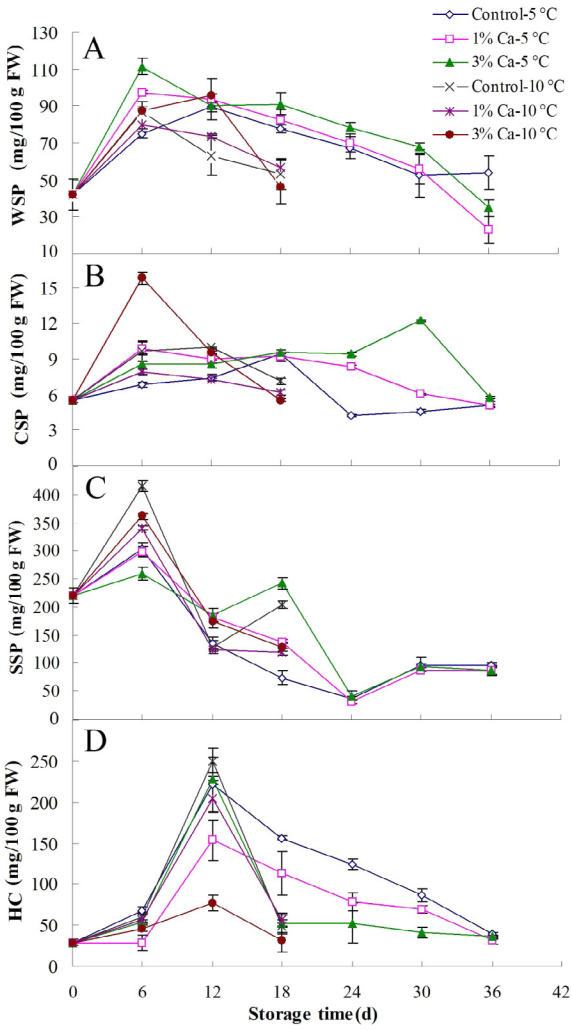

Fig. 2. Effect of calcium treatment (Control, 1% Ca or 3% Ca) and temperature (5 or 10 °C) on yields of apricot flesh cell wall polysaccharides during postharvest storage. *Note:* WSP: water-soluble pectin; CSP: chelate-soluble pectin; SSP: sodium carbonate-soluble pectin; HC: hemicellulose. WSP, CSP and SSP contents were expressed in mg GalA/100 g fresh weight. HC content was expressed in mg Glc/100 g fresh weight.

75.16 to 111.25 mg GalA/100 g FW), CSP (Fig. 2B, 6.84–15.79 mg GalA/100 g FW) and SSP (Fig. 2C, 260.11–414.81 mg GalA/100 g FW); concurrent with the sharp decrease in firmness observed on day 6 (Fig. 1A). Similar trends of UA in WSP in apricot storage were obtained by Stanley, Prakash, Marshall, and Schröder (2013). A peak in HC occurred on day 12 for all groups (Fig. 2D). Total UA content of the three pectins (WSP + CSP + SSP) decreased at the end of cold storage compared to at harvest, which was consistent with a previous report on apricots (Kovacs & Nemeth-Szerdahelyi, 2002).

The increase of pectin and HC from 0 d to 6 d and 12 d may be due to the disassembly of the "pectin-cellulose-hemicellulose" network over time, which increased the extractability of all cell wall polysaccharides. The earlier peak in pectins compared with HCs may be due to the early breakdown of pectins from the hemicellulose-cellose matrix compared with the depolymerisation of the whole matrix.

As shown in Fig. 2B, among the 10 °C groups, CSP of 3% Ca group had markedly higher UA content at 6 d (15.79 mg GalA/100 g FW) than control (9.68 mg GalA/100 g FW). For the 5 °C groups, CSP of both the 1% and 3% Ca groups showed higher UA content throughout the whole storage period except at 18 d and 36 d. The results suggest an increase in ionically-bound pectins in $CaCl_2$ treated apricots. Several other fruits treated with calcium also retained higher UA contents of CSP (Lara, García, & Vendrell, 2004; Ortiz, Graell, & Lara, 2011).

As shown in Fig. 2C, SSP was the most abundant (31.70–414.81 mg GalA/100 g FW) among the cell wall pectins in this study (Fig. 2A–C), and similar to other reports (Goulas, Minas, Kourdoulas, Vicent, & Manganaris, 2014; Salato et al., 2013). Furthermore, SSP significantly reduced from 220.83 mg GalA/100 g FW at harvest to 96.95, 86.61 and 86.91 mg GalA/100 g FW at the end of storage for the control, 1% Ca and 3% Ca groups at 5 °C, respectively. Higher yields of SSP have been linked with firmer texture during apple fruit storage (Ortiz et al., 2011). Compared to the control, treatments of 1% and 3% Ca retarded increasing SSP at day 6 at 10 °C storage and the decreasing of SSP at 12–18 d at 5 °C storage.

For groups stored at 10 °C, the HC neutral sugar content increased from 28.57 mg Glc/100 g FW to the highest value of 76.91 mg Glc/100 g FW for 3% Ca group and 250.34 mg Glc/100 g FW for control group (Fig. 2D) at 12 d. At the end of storage at 10 °C, the values dramatically decreased to 32.02, 56.51, 48.33 mg Glc/100 g FW for 3% Ca, 1% Ca and control group, respectively. A similar trend was also observed in HC content of other fruits (Chen et al., 2011; Vicente, Manganaris, Minas, Goulas, & Lafuente, 2013). Neutral sugar content of HC in apricots treated with 3% Ca was significantly lower than the control and those treated with 1% Ca in the latter stages of storage at 10 °C. For apricots stored at 5 °C, both Ca treatments resulted in lower levels of HC compared to the control group during 18–30 d of storage (Fig. 2D). Exogenously applied Ca could reduce softening related enzyme activity (Quiles, Hernando, Pérez-Munuera, & Lluch, 2007), which could prevent the degradation of cell wall and the extractability of HC in the final stages of storage.

3.3. Effects of calcium treatment and temperature on apricot cell wall polysaccharide nanostructure

3.3.1. Structural morphology of WSP, CSP, SSP and HC chains

Quantitative analyses of apricot cell wall polysaccharides were not sufficient to determine the effect of calcium treatment and cold storage. Therefore, surface morphology of cell wall polysaccharides was observed using AFM, and the plane topographical views for the characteristic morphologies of WSP (Fig. 3W-a – W-e), CSP (Fig. 3C-a – C-e), SSP (Fig. 3S-a – S-e) and HC (Fig. 3H-a – H-e) were generated. Morphologies in freshly harvested apricots (images a and b) and at the end of storage at 5 °C treated with water (image c), 1% Ca (image d) and 3% Ca (image e), are shown in Fig. 3. There were marked differences among the morphological properties of WSP, CSP, SSP and HC chains in apricot flesh as detected by AFM.

WSP were long straight chains without branches or aggregations (Fig. 3W-a – e, noted as 'Ls' in the figure), since the main component of the pectin domain may be homogalacturonan (HG). Water extracts the pectin loosely attached to the cell wall (Basanta, Ponce, Rojas, & Stortz, 2012). The results obtained in hazelnut skin (Montella et al., 2013) and leek (Kratchanova, Nikolova, Pavlova, Yanakieva, & Kussovski, 2010) also suggest that low amounts of neutral sugars and high GalA content are unusual in the water soluble fractions.

CSP had abundant and complex branches (Fig. 3C-a – b, noted as 'Br'). Similar to other findings, CDTA fractions contained more branched components (Prabasari, Pettolino, Liao, & Bacic, 2011). In our current research, markedly fewer branches were found at the end of storage (16%) than at harvest (34%), similar with our previous report (Liu et al., 2009). The results also agreed with other findings which correlated more pectin branching with higher firmness (Batisse, Buret, & Coulomb, 1996). As fruit softened, pectins became more soluble and the neutral sugar regions were gradually cleaved (Kondo & Danjo, 2001). Peña and Carpita (2004) also reported that a reduction in highly branched arabinans and debranching of component RG-I were accompanied by reduced firmness and cell separation during storage in apple.

The SSP chains (Fig. 3S-a – e) had relatively shorter fragments similar to that observed in other fruits (Chen et al., 2011; Chong et al., 2015; Yang, 2014). Previous studies suggested that Na_2CO_3 hydrolyses the diferulate bridges and releases the cross-linked pectin chains, resulting in a decrease in molecular weight for SSP (Basanta et al., 2013).

As shown in Fig. 3H-a and Fig. 3H-b, some parts of the HC chains exhibited net-like structures (noted as 'ns') at harvest. Chen et al. (2009) found that the HC structure of Chinese cherry was similar to a "broom" with many branches on one main chain. Net-like structures were no longer observed at the end of storage (images c, d and e), which indicated the depolymerisation of HC. Depolymerisation of HC was similarly observed in many fruits during storage (Brummell, Dal Cin, Crisosto, & Labavitch, 2004; Vicente et al., 2013).

3.3.2. Length and width changes of cell wall polysaccharide chains during apricot storage

In vitro measurements of compositions do not provide complete information about changes in cell wall polysaccharides. The length and width distributions of WSP, CSP and HC chains throughout the storage period, as shown in Fig. 4 and Table 1, were the most direct representations for the actual changes to the pectin chain polymers. WSP chains were found to be the longest in length, and the HC chains to be shortest (Fig. 4), which agreed with a previous finding that WSP had higher molecular weight compared to CSP and SSP (Basanta et al., 2013).

The frequency (Fq,%) of WSP chains (Fig. 4A) longer than 4.0 μm was 20.08% at harvest; however, this Fq value was significantly reduced in all groups at the end of storage, with a lowest value of 0.5% for the control stored at 5 °C, indicating the degradation of WSP chains during apricot softening. Additionally, WSP chains shorter than 500 nm were not detected at harvest, but at the end of storage the Fq values increased significantly. Apricots treated with 1% Ca and stored at 5 °C had lower Fq values of WSP chains <1.0 μm and higher Fq of 3.51–4.00 μm than the control at the end of storage, and coincided with a firmer texture (Fig. 1A).

The linear shaped chains in the AFM images for CSP fractions were used for the length determination according to Zhang,

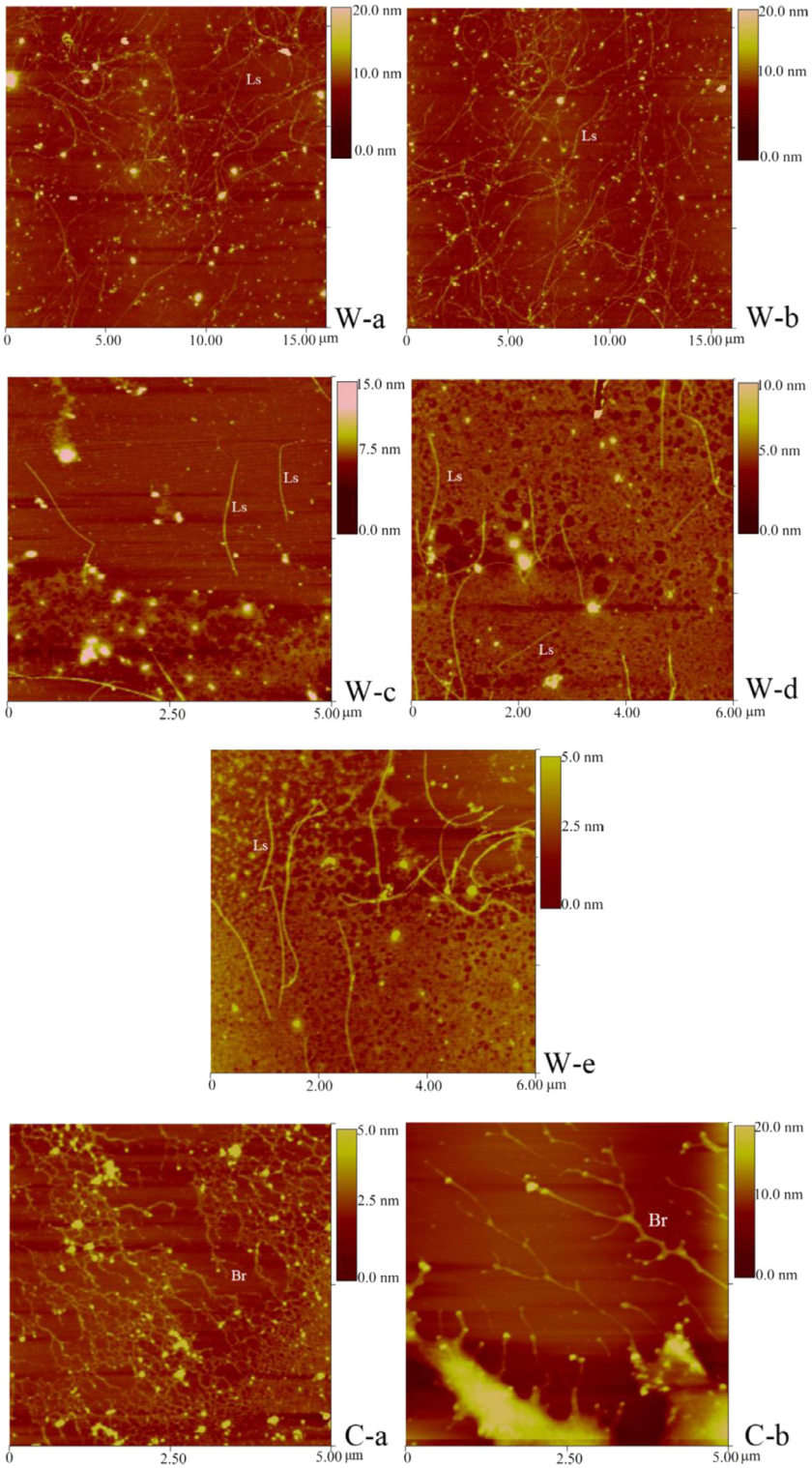

Fig. 3. Representative AFM topographical images of WSP (images W-a – W-e), CSP (images C-a – C-e), SSP (images S-a – S-e) and HC (images H-a – H-e) chains of apricots at beginning and end of storage after $CaCl_2$ treatment at 5 °C. *Note:* a, b: AFM images of apricot at harvest stage; c, d, e: AFM images at 36 d for Control (c), 1% $CaCl_2$ (d) and 3% $CaCl_2$ (e) treatment in 5 °C. Ls: long straight chain; Ss: short straight chain; Br: branched chain; Ns: net-like structure; Bm: broom-like structure; WSP: water-soluble pectin; CSP: chelate-soluble pectin; SSP: sodium carbonate-soluble pectin; HC: hemicellulose.

Chen, Zhang, Lai, and Yang (2016). The change in length of the CSP chains during storage was not as dramatic as in the length of the WSP chains (Fig. 4B). For instance, the Fq of long chains at the end of the storage was not significantly different from the Fq at harvest (1% Ca at 5 °C with lengths of 3.01–3.50 μm, 1% Ca at 10 °C with lengths of 2.01–3.50 μm, and 3% Ca at 10 °C with lengths of 2.01–3.00 μm). However, at the end of storage, the Fq of short chains with length <500 nm for all groups was significantly higher than at harvest (6.83%), and 1% Ca treatment reduced the fraction of the short CSP chains with length <500 nm after storage

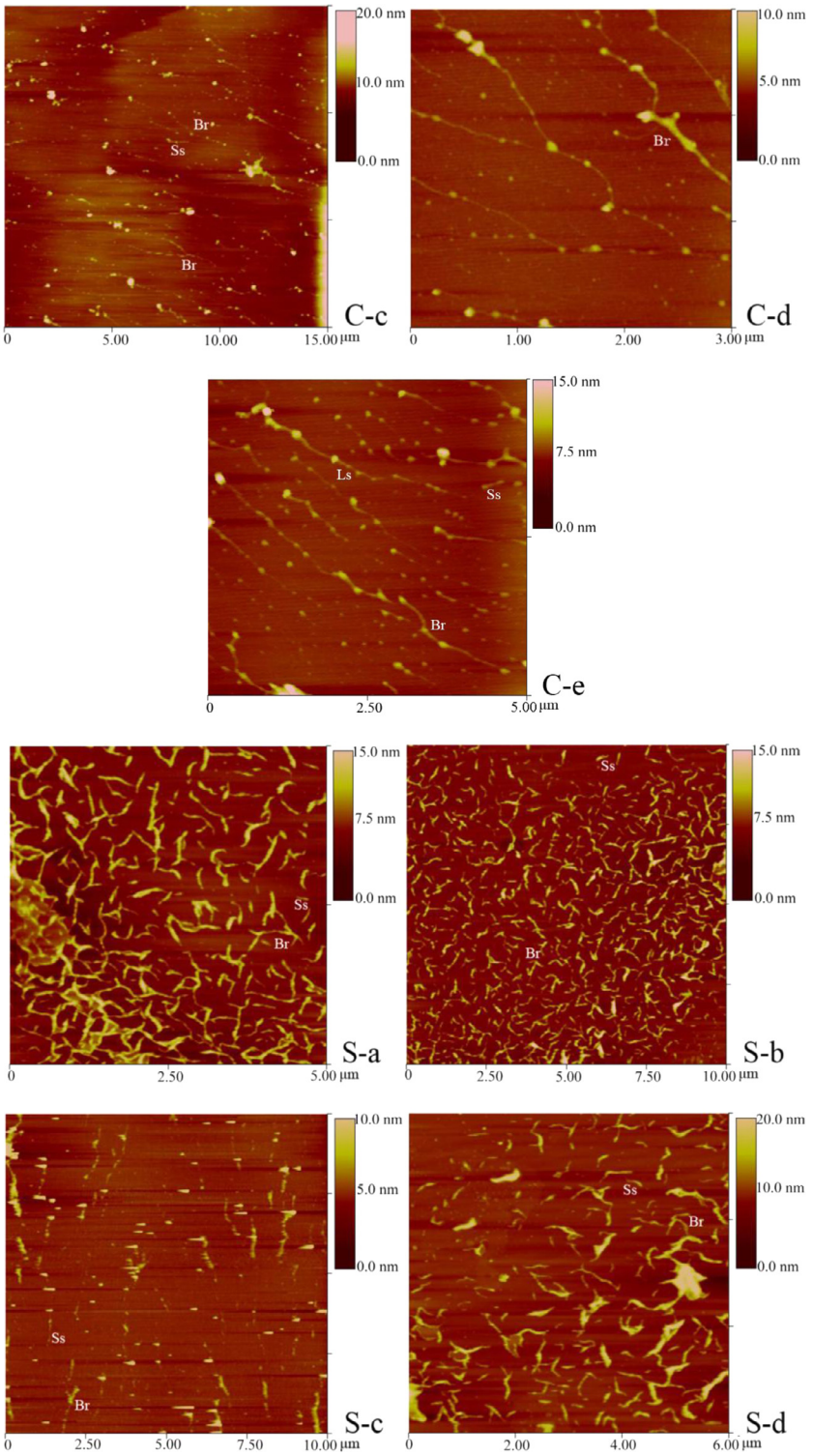

Fig. 3 (*continued*)

at both 5 °C and 10 °C. Solubilisation and depolymerisation of CSP takes place during fruit softening (Brummell et al., 2004). Calcium treatment increases the Ca^{2+} bridges between CSP chains and reduces their degradation (Liu et al., 2009).

It should be noted that some pectin length distributions in apricots treated with 3% Ca at 5 °C showed no differences to the control, such as WSP length in the range of 0.51–1.00, 3.01–3.50 and 3.51–4.00 μm, as well as the CSP <0.5, 2.51–3.00 and 3.01–3.50 μm. The results were consistent, with no differences in firmness between apricots treated with 3% Ca and the control at 5 °C, which suggest the concentration of $CaCl_2$ was not appropriate for apricot postharvest treatment and may be toxic to the apricot cell.

HC chain length values at harvest were mostly in the range of 101–250 nm (Fig. 4C), totalling an Fq of up to 73.07%, then becoming smaller (mainly <50–200 nm) at the end of storage. The Fq of HC chains with length of 201–250 nm significantly decreased from

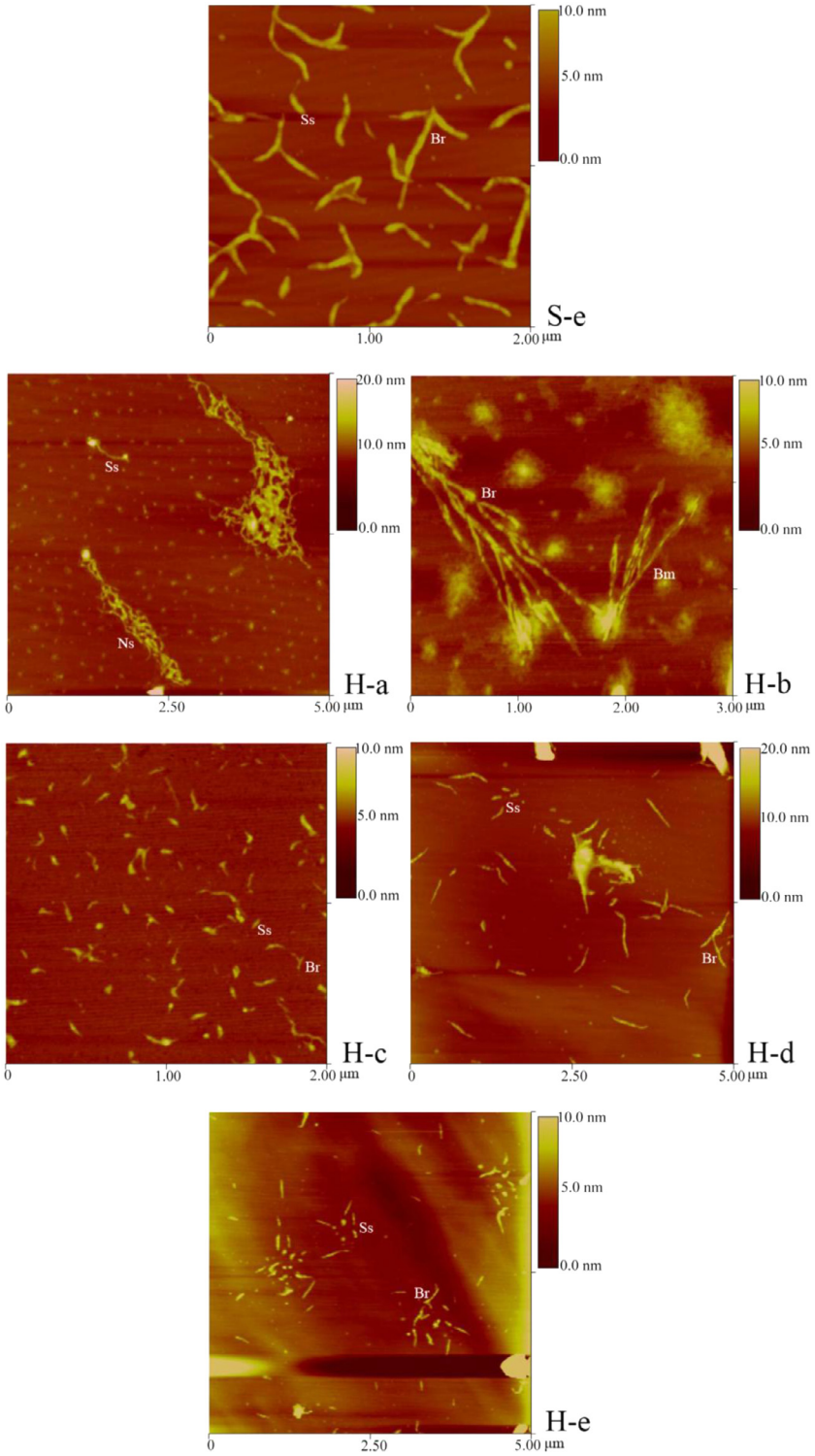

Fig. 3 (*continued*)

20.47% at harvest to 0–16.61% at the end of storage. Downward shifts in HC molecular mass distributions have also been found during the softening of several fruits (Salato et al., 2013). Fq of short HC chains <100 nm increased markedly as the fruits softened. Treatment with 1% $CaCl_2$ significantly decreased the Fq of HC chains length of 50–100 nm at the end of storage at both 10 °C and 5 °C. The above comparisons suggest that calcium treatment reduced the pectin-hemicellulose depolymerisation during apricot softening.

Studies have agreed that chain width of pectin polysaccharides in fruits significantly changes during postharvest ripening (Chen et al., 2011; Liu et al., 2009). Neutral sugars, coiled around or aligned along the RG regions and existing as short branches, may cause changes to the width of pectin chains (Chen et al., 2009; Chong et al., 2015; Liu et al., 2009; Yang, 2014).

The widths of WSP, CSP and HC chains measured by AFM in apricot fruits at harvest (0 d) and at the end of storage at 5 °C

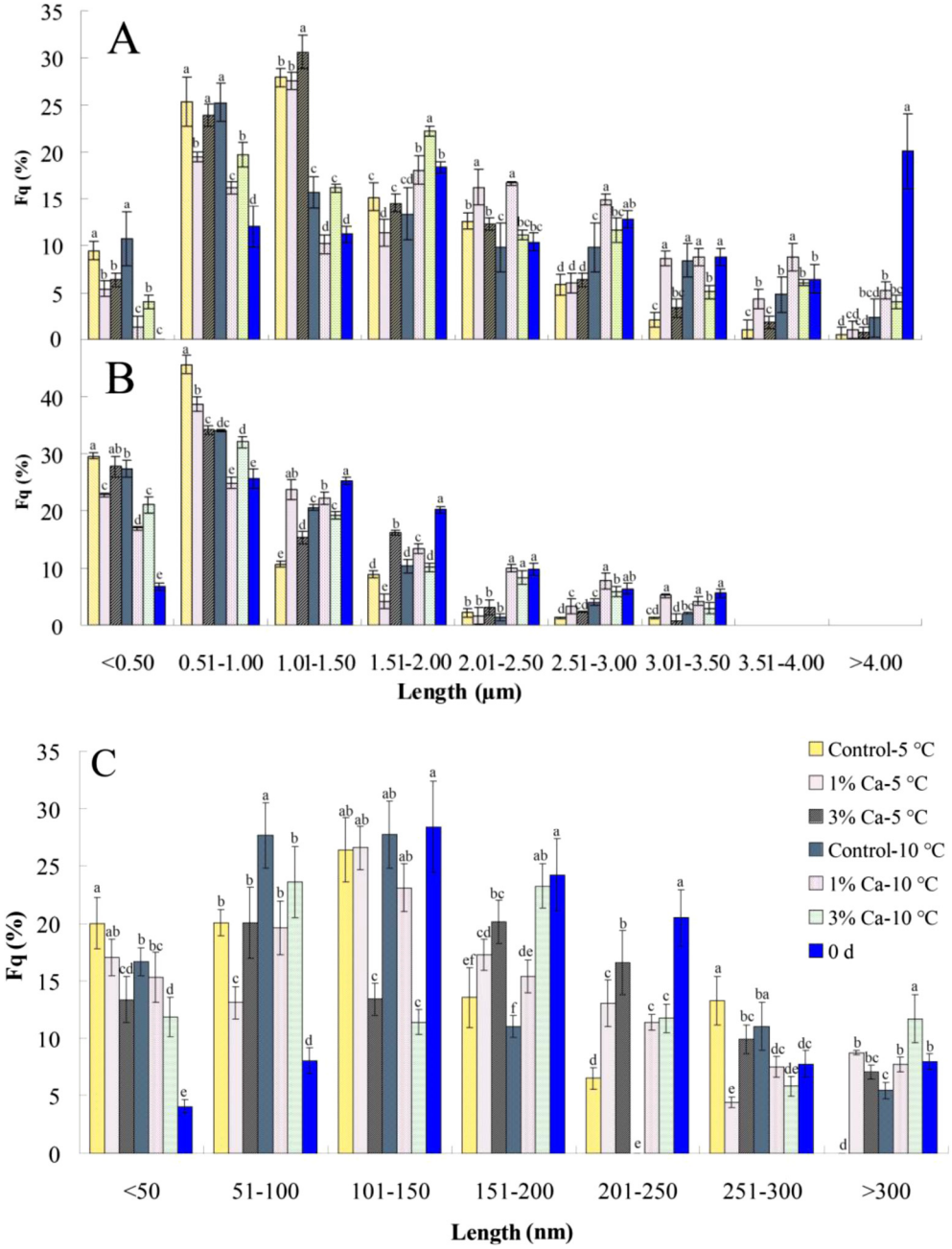

Fig. 4. Length of WSP (A), CSP (B) and HC (C) chains of postharvest apricots (0 d, 10 °C 18 d and 5 °C 36 d). *Note:* Fq (%): the frequency of length values in a particular range expressed as a percentage of total frequency. WSP: water-soluble pectin; CSP: chelate-soluble pectin; HC: hemicellulose.

(36 d) and 10 °C (18 d) are shown in Table 1. Sixteen values for WSP, 15 values for CSP and 4 values for HC chains were obtained for width distribution of different pectin polysaccharides. Similar to the changes in chain length distributions, the longer storage time at 5 °C (36 d) than at 10 °C (18 d) might have caused more thorough degradation, making the Fq of narrow chain at the end of storage at 5 °C greater than the Fq at 10 °C.

As shown in Table 1, the percentages of WSP chains with width <40 nm significantly increased from 7.14% at harvest to 24.66%, 30.50%, 42.59%, 57.89%, 61.12% and 66.66% at the end of storage for the control-10 °C, 1% Ca-10 °C, 3% Ca-10 °C, control-5 °C, 1% Ca-5 °C and 3% Ca-5 °C, respectively. The wide chains (117.2, 125.0 and 187.5 nm) disappeared after storage. The results indicate that WSP side chains of apricots were depolymerised and degraded during postharvest softening. Treatment of 1% and 3% calcium increased the Fq of WSP chains with narrower widths (<40 nm) compared to control groups at both 10 °C and 5 °C at the end of storage, which meant there were no positive effects of Ca treatments on WSP width degradation, similar with the results obtained in strawberries (Chen et al., 2011).

The wider CSP chains with width values of 82.0, 97.6, 117.2, 175.8 and 234.4 nm were not found at the end of storage, while narrower CSP chains appeared. The Fq of narrow chains with width <20 nm for apricots treated with 1% Ca was significantly lower than the control and those treated with 3% Ca at the end of storage at 10 °C and 5 °C. To summarise, CSP side chains were generally degraded during storage, and 1% Ca treatment delayed this process. The results were also confirmed in past literature (Liu et al., 2009).

The HC chain had 4 characteristic widths of 15.6, 23.4, 31.2 and 39.1 nm. In this study, the Fq of wider HC chains (>30 nm)

Table 1
Width changes of WSP, CSP and HC chains of apricots at harvest and after cold storage.

	Width (nm)	Fq (%)						
		0 d	10 °C 18 d			5 °C 36 d		
			Control	1% $CaCl_2$	3% $CaCl_2$	Control	1% $CaCl_2$	3% $CaCl_2$
WSP	11.7	0b	0b	0b	0b	5.26a	0b	0b
	23.4	0d	2.74c	0d	0d	18.42b	22.22a	23.33a
	31.2	3.57d	0e	0e	9.26a	7.89b	5.56c	0e
	35.2	0d	9.59c	18.64b	1.85d	0e	16.67b	30.00a
	39.1	3.57e	12.33d	11.86d	31.48a	26.32b	16.67c	13.33d
	46.9	17.86d	10.96e	27.12b	27.78b	21.05c	30.56a	3.33f
	58.6	14.29d	41.10a	30.51b	29.63b	10.53e	2.78f	20.00c
	62.5	0b	0b	0b	0b	2.63a	0b	0b
	70.3	14.29a	5.48c	0e	0e	7.89b	5.56c	3.33d
	78.1	14.29a	13.70a	11.86b	0d	0d	0d	6.67c
	93.8	3.57a	0b	0b	0b	0b	0b	0b
	97.6	0b	4.11a	0b	0b	0b	0b	0b
	117.2	10.71a	0b	0b	0b	0b	0b	0b
	125.0	3.57a	0b	0b	0b	0b	0b	0b
	187.5	10.71a	0b	0b	0b	0b	0b	0b
	250.0	3.57a	0b	0b	0b	0b	0b	0b
CSP	11.7	0e	6.90c	0e	3.85d	16.67a	9.90b	9.68b
	17.6	0c	0c	0c	0c	13.33b	13.64b	22.58a
	19.5	0b	0b	0b	0b	0b	4.55a	0b
	23.4	0d	6.90c	6.67c	0d	16.67a	9.09b	16.13a
	35.2	4.79d	24.14b	17.78c	19.23c	43.33a	18.18c	19.35c
	39.1	2.39f	27.59c	35.56b	38.46a	0g	22.73d	16.13e
	46.9	9.84b	10.34b	6.67c	7.69c	10.00b	4.55d	16.13a
	58.6	19.68c	20.69c	31.11a	26.92b	0e	13.64d	0e
	70.3	2.39b	0c	0c	3.85a	0c	0c	0c
	78.1	24.47a	3.45c	2.22d	0e	0e	4.55b	0e
	82.0	4.79a	0b	0b	0b	0b	0b	0b
	97.6	4.92a	0b	0b	0b	0b	0b	0b
	117.2	9.84a	0b	0b	0b	0b	0b	0b
	175.8	12.23a	0b	0b	0b	0b	0b	0b
	234.4	4.65a	0b	0b	0b	0b	0b	0b
HC	15.6	0e	21.05c	14.29e	19.05d	33.30a	26.67b	25.00b
	23.4	26.70d	31.58c	38.10b	33.33c	26.70d	40.00b	50.00a
	31.2	53.30a	42.11b	38.10c	38.10c	33.30d	33.33d	18.75e
	39.1	20.00a	5.26d	9.52b	9.52b	6.70c	0e	6.25c

Note: WSP: water-soluble pectin; CSP: chelate-soluble pectin; HC: hemicellulose. Fq (%): means the frequency of particular width value at harvest (0 d) and last storage period of storage at 10 °C (18 d) and 5 °C (36 d). Values in the same row with different letters mean significant differences by Duncan's multiple range test ($P < 0.05$).

decreased significantly after storage as the fruit softened. Fruits with crisper and harder textures have been reported to possessed thicker cellulose microfibers such as apples (Cybulska, Zdunek, Psonka-Antonczyk, & Stokke, 2013) and Chinese cherries (Chen et al., 2009). Furthermore, the width 15.6 nm was not observed at harvest, but the Fq values increased to 14.29–33.30% for all six treatment groups at the end of storage, among which the Fq value of 1% Ca stored at 10 °C was the lowest. Calcium treated groups possessed a higher percentage of HC with width of 15.6 nm compared with the control, which also indicated $CaCl_2$ treatments could slow down the HC side chain degradation during apricot storage.

3.3.3. Degradation schemes of apricot WSP, CSP and HC polysaccharides

The morphological and quantitative results of WSP, CSP and HC during apricot fruit softening showed that the pectin polysaccharides underwent degradation. As the fruit ripened during storage, the wider WSP, CSP and HC chains degraded gradually to shorter and narrower fragments. Furthermore, we deduced a schematic model for the degradation pathway of pectin polysaccharides from their characteristic width values as shown in Fig. 5. All of the wider chains are composed of 2–5 narrower chains. For instance, a WSP chain with width of 187.5 nm may be composed of shorter chains with widths of 70.3 and 117.2 nm, or 62.5 and 125.0 nm, and the 70.3 nm chain can be further decomposed into narrower chains with widths of 23.4 and 46.9, or 58.6 and 11.7 nm. From the degradation schemes, we found that all WSP chain width values can be broken down into basic unit widths of 11.7, 31.2 and 39.1 nm. Similarly, basic unit widths are 11.7, 17.6 and 19.5 nm for CSP, and 15.6 and 23.4 nm for HC. The current approach can incorporate other processing technologists for processing fruits, especially organic fruits (Mao et al., 2016; Zhao, Zhang, & Yang, 2017).

4. Conclusion

Fruit texture is largely dependent on cell wall composition. During apricot postharvest storage, firmness gradually decreases while pectin polysaccharides are gradually depolymerised and degraded, and the chain length and width values of WSP, CSP and HC gradually decrease as measured by AFM. Treatment with 1% Ca followed by cold storage at 5 °C can prolong apricot shelf life, maintain a firmer texture and reduce the degradation of cell wall polysaccharide main and side chains. Basic unit widths of apricot cell wall polysaccharides were found to be 11.7, 31.2 and 39.1 nm for WSP, 11.7, 17.6 and 19.5 nm for CSP, and 15.6 and 23.4 nm for HC, respectively. These results suggest that softening of apricots may follow a trend in polysaccharide degradation during storage, and the detailed mechanism may be of interest for further studies.

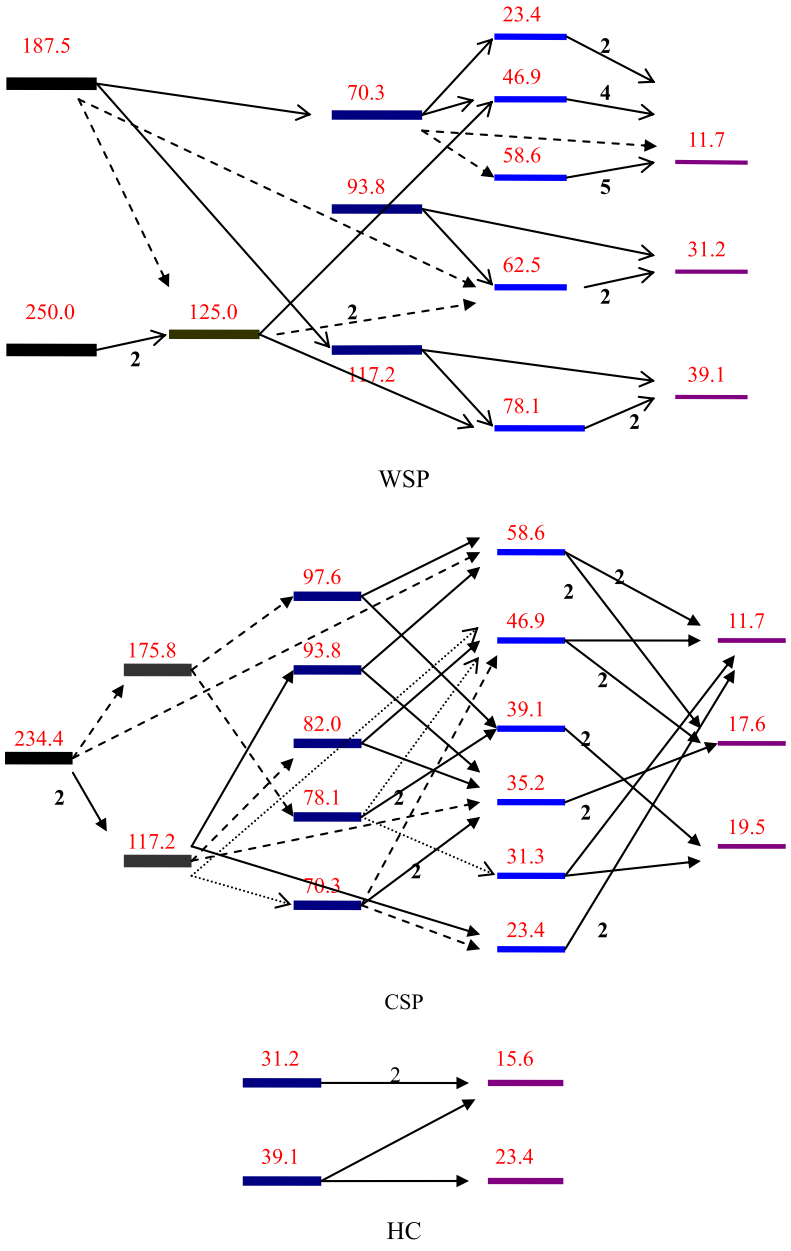

Fig. 5. Schematic image of degradation pathway of WSP, CSP and HC chain widths (nm) of postharvest apricots *Note:* WSP: water-soluble pectin; CSP: chelate-soluble pectin; HC: hemicellulose.

Conflict of interest statement

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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