



Effects of temperature and cultivar on nanostructural changes of water-soluble pectin and chelate-soluble pectin in peaches

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ABSTRACT

Two cultivars of peaches ('Cangfangzaosheng' and 'Songsenzaosheng') were stored at 2 °C, 8 °C and 15 °C for maximum of 41 days. The nanostructural changes of water-soluble pectin (WSP) and chelate-soluble pectin (CSP) of all cultivars were analyzed qualitatively and quantitatively by atomic force microscopy (AFM). The results show that long linear single pectin chains of peach were detached and the percentage of small width chains increased during storage. This phenomenon was also found with increased storage temperature. Moreover, the widths of pectin molecules of both peach cultivars were similar and very regular. Combined with previous reports, the results indicate that nanostructural degradations of pectin widths might be independent of their chemical content changes.

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1. Introduction

Pectin is a hetero-polysaccharide predominantly containing galacturonic acid residues. It mainly exists in the primary cell wall and intercellular space of higher plants. Pectin chain, composed of several components including homogalacturonan (HG), rhamnogalacturonan I (RG I), rhamnogalacturonan II (RG II) and xylogalacturonan (Pelloux, Rustérucchi, & Mellerowicz, 2007), contains many different monosaccharides.

Pectin is a vital component in middle lamellae of the cell wall for many plant tissues. It is the most abundant class of macromolecules within the cell-wall matrix (Billy et al., 2008). It plays an important role during ripening, handling and processing of fruits. Pectin, being an important element in regulating plant cell wall growth and maintaining integrity through non-covalent interactions with cellulose, is viewed to regulate the structure of the pores in the fruit cell wall. Pectin builds a matrix enclosing the load-bearing network of cellulose and cross binding glycans to maintain the skeleton of cell wall (Westereng, Michaelsen, Samuelsen, & Knutsen, 2008). During fruit softening, pectin undergoes solubilization and depoly-

merization, participating in cell wall loosening and disintegration (Billy et al., 2008).

Atomic force microscopy (AFM) can be used to investigate the detailed information of pectin changes during postharvest. It could characterize and examine the qualitative and quantitative information of macromolecules, as well as the interaction between different molecules (Liu & Wang, 2010; Yang et al., 2007). Different forms of pectin molecules were examined by AFM, including branches attached to pectin (Round, Rigby, MacDougall, Ring, & Morris, 2001), extracted fractions from fruit cell wall material (Kirby, MacDougall, & Morris, 2008; Liu et al., 2009; Yang, An, Feng, Li, & Lai, 2005), and hydrated sugar acid gels (Fishman, Cooke, & Coffin, 2004). In addition, structures influenced by extraction conditions (Fishman, Chau, Cooke, & Hotchkiss, 2008; Liu, Wei, Guo, & Kennedy, 2006) or storage conditions (Liu et al., 2009; Zhang et al., 2008) were also widely studied with AFM.

Peaches are favorite fruits due to their quality and nutrition value. However, they are perishable fruits due to rapid ripening and physiological deterioration after harvest. Cold storage is an effective way of controlling physiological decay and maintaining quality. However, under inappropriate conditions, textural or other indexes' changes during cold storage will limit the quality and storage life of peaches (Jin, Wang, Shang, Tong, & Zheng, 2009; Zhang et al., 2010). In previous reports, sodium carbonate-soluble pectin

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(SSP) was found closely related to its physicochemical properties, and its nanostructural changes were determined (Zhang et al., 2008, 2010). However, it is not clear whether the nanostructural changes of water-soluble pectin (WSP) and chelate-soluble pectin (CSP) were different from that of SSP, considering that the contents of WSP and CSP were insignificantly correlated with quality changes (Zhang et al., 2010).

The objective of this study was to elucidate the process of nanostructural changes of WSP and CSP of peach during cold storage. The results could illustrate the relationship between the nanostructure of pectin and fruit softening, which is useful for fruits during harvest and storage.

2. Materials and methods

2.1. Fruit materials

Two crisp peach (*Prunus persica* L.) cultivars ('Cangfangzaosheng' and 'Songsenzaosheng') were obtained directly from a private farm in Zhengzhou, Henan province, China. Both of the two peach cultivars were picked about two weeks before commercial maturity and had similar ripening stages. The fruits were harvested by hand and immediately transported to our laboratory in 2 h. Soon after the arrival at the laboratory, the fruits were selected according to uniform size, weight, color, disease free and no other defects. Then fruits were randomly divided into three groups and stored at 2 °C, 8 °C, and 15 °C, respectively. Each group had about 60 fruits (Zhang et al., 2010).

2.2. Cell wall preparation and pectin extraction

Procedures for the preparation of cell wall materials and extraction of pectin were as described by a previous publication with some modifications (Zhang et al., 2010). About 10 g flesh from 5 peeled peach fruits was ground rapidly in an ice-cold mortar, then added to a round bottom flask containing 200 mL 80% (v/v) ethanol (Tianjin Resent Chemicals Co., Ltd, China) and boiled for 20 min. The ethanol was decanted by filtration. This procedure was repeated two more times. After that, the residue was transferred to 50 mL dimethylsulphoxide (Tianjin Resent Chemicals Co., Ltd, China)/water (9/1, v/v) and incubated overnight at 4 °C. The suspensions were then filtrated with vacuum pump. The residue was water-washed and transferred to 200 mL chloroform (Suzhou Chemicals Co., Ltd, China): ethanol (2/1, v/v) for 10 min. Then the sample was filtrated and washed with 200 mL acetone (Luoyang Chemicals Co., Ltd, China) until total whitening, the residue was the fraction of cell wall material (CWM).

The CWM was extracted sequentially to obtain water-soluble pectin (WSP) and chelate-soluble pectin (CSP). It was stirred at 25 °C for 4 h in 10 mL distilled water. Then the mixture was centrifugated at 10,000 × g for 10 min at 4 °C. After centrifugation, the residue was subject to two additional distilled water extractions according to the above experimental procedure. The three supernatants were combined as WSP. The residue was further extracted for 4 h at 25 °C with 10 mL 50 mM trans-1,2-dia-minocyclohexane-N,N,N,N-tetraacetic acid (CDTA) (Tianjin Zinco Fine Chemical Institute, China). The remained pellet was reextracted with CDTA for twice more. After that, the mixture was centrifugated as above. The supernatants were collected as CSP.

2.3. AFM determination and image analysis

Peach pectin was characterized by a Nano-R2™ AFM (Pacific Nanotechnology Inc., Santa Clara, CA, USA) in 'noncontact' mode. The NSC 11/no Al tip (MikroMasch, Wilsonville, OR, USA) was used with scan rate of 0.5–2.0 Hz. The resonance frequency and force

constant of the tip were 330 kHz and 48 N/m, respectively. AFM determination was performed according to the methods which were used before (Yang et al., 2005; Yang, Lai, An, & Li, 2006). WSP and CSP solutions obtained above were diluted to a series of concentrations (about 0.5–30 µg/mL). The diluted solutions were agitated with a vortex mixer (Fisher Scientific, Pittsburgh, PA, USA). Samples (20 µL) were drop deposited onto freshly cleaved mica sheets (Muscovite Mica; Electron Microscopy Sciences, Hatfield, PA, USA). For investigating the original arrangement of pectins, manipulating and stretching of pectin molecules was not applied (Yang, An, & Li, 2006).

Then the solution was air-dried at room temperature before imaging. The mica with sample was attached to a 15-mm diameter AFM specimen disc (TED Pella Inc., Redding, CA, USA) using a double-sided adhesive tab. At least five samples of each group were observed using AFM, and for each group at least five to six fields were investigated. The widths of main chains of pectins were measured by the function of section analysis.

2.4. Statistical analysis

At least triplicate samples were included for each condition, and dozens of parallel samples were examined for each group under a certain condition in order to obtain reliable statistical results. The data obtained were statistically analyzed using SAS 9.1.3 software (SAS, Cary, NC, USA). Widths of pectin chains were determined and variance of the widths less than 1 nm was collected into the same groups.

3. Results and discussion

3.1. Effect of storage time and temperature on the nanostructure of WSP and CSP of peaches

AFM was successfully applied to characterize the heterogeneous structure of pectins, including linear, branching, polymers or blocks (Yang et al., 2005). Fig. 1 shows the AFM images of WSP of 'Cangfangzaosheng' and 'Songsenzaosheng' peaches.

Both of the two peach cultivars belong to crisp fruit. As observed in Fig. 1, the WSP molecules from fresh peaches of the two cultivars were aggregated, forming large polymers (p) or blocks (bl), with only a few of them forming single linear chains (ls). For 'Cangfangzaosheng' peaches, the size of the strands and polymers was found to gradually reduce during storage. The long single linear chains were detached and the small ones increased (compare Fig. 1a and b). Similar results were observed when storage temperatures increased (compare Fig. 1b–d). These changes may be due to the depolymerization or shortening of chain length of pectin substances, which occurs with increased pectin esterase and polygalacturonase activities during fruit ripening (Yaman & Bayoandurlu, 2002). Results of 'Songsenzaosheng' peaches were similar to those of 'Cangfangzaosheng' peaches. There was no significant difference between the two cultivars for WSP molecules. However, the aggregates of WSP molecules from fresh 'Songsenzaosheng' peaches were smaller than those from fresh 'Cangfangzaosheng' peaches. This may be attributed to the cultivar difference.

Nanostructural morphologies of CSP molecules were different from that of WSP, as can be seen from Fig. 2. The CSP molecules from peaches of both cultivars were mainly single linear chains with only a few small aggregates observed in fresh peaches (Fig. 2a). The size of single linear chains reduced with increased storage temperature during storage, which was similar to WSP molecules.

AFM images could also reveal the quantitative information of pectin molecules (Yang et al., 2005; Yang, Lai, et al., 2006). All the quantitative parameters of linear single fractions were analyzed

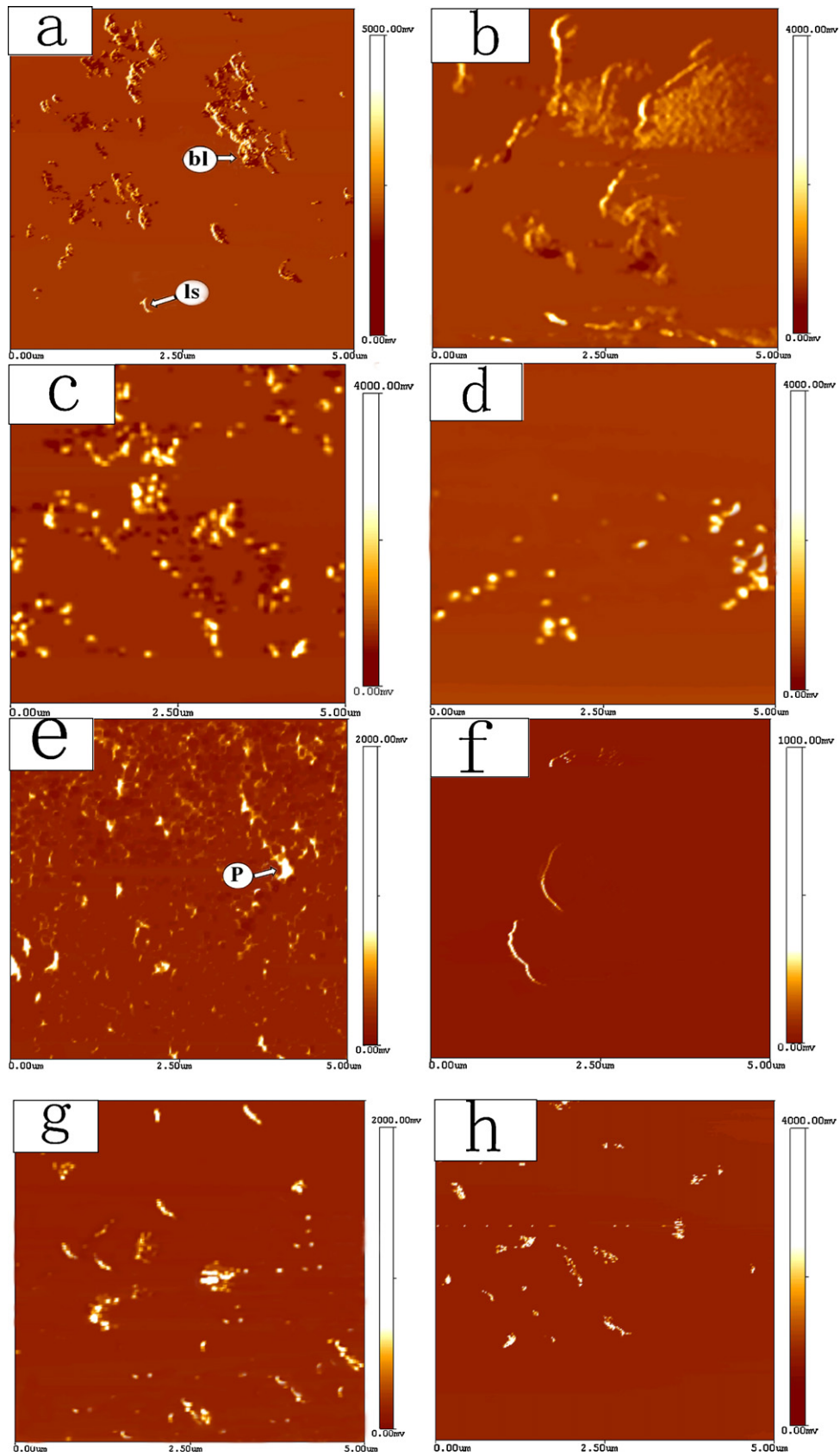


Fig. 1. AFM images of water-soluble pectin of peaches under cold storage. Scan area = $5 \mu\text{m} \times 5 \mu\text{m}$. (a) Fresh peaches; (b) day 26 at 2°C ; (c) day 26 at 8°C ; (d) day 26 at 15°C ; (e) fresh peaches; (f) day 26 at 2°C ; (g) day 26 at 8°C ; (h) day 26 at 15°C . Note: a, b, c, d 'Cangfangzaosheng' peach; e, f, g, h 'Songsenzaosheng' peach.

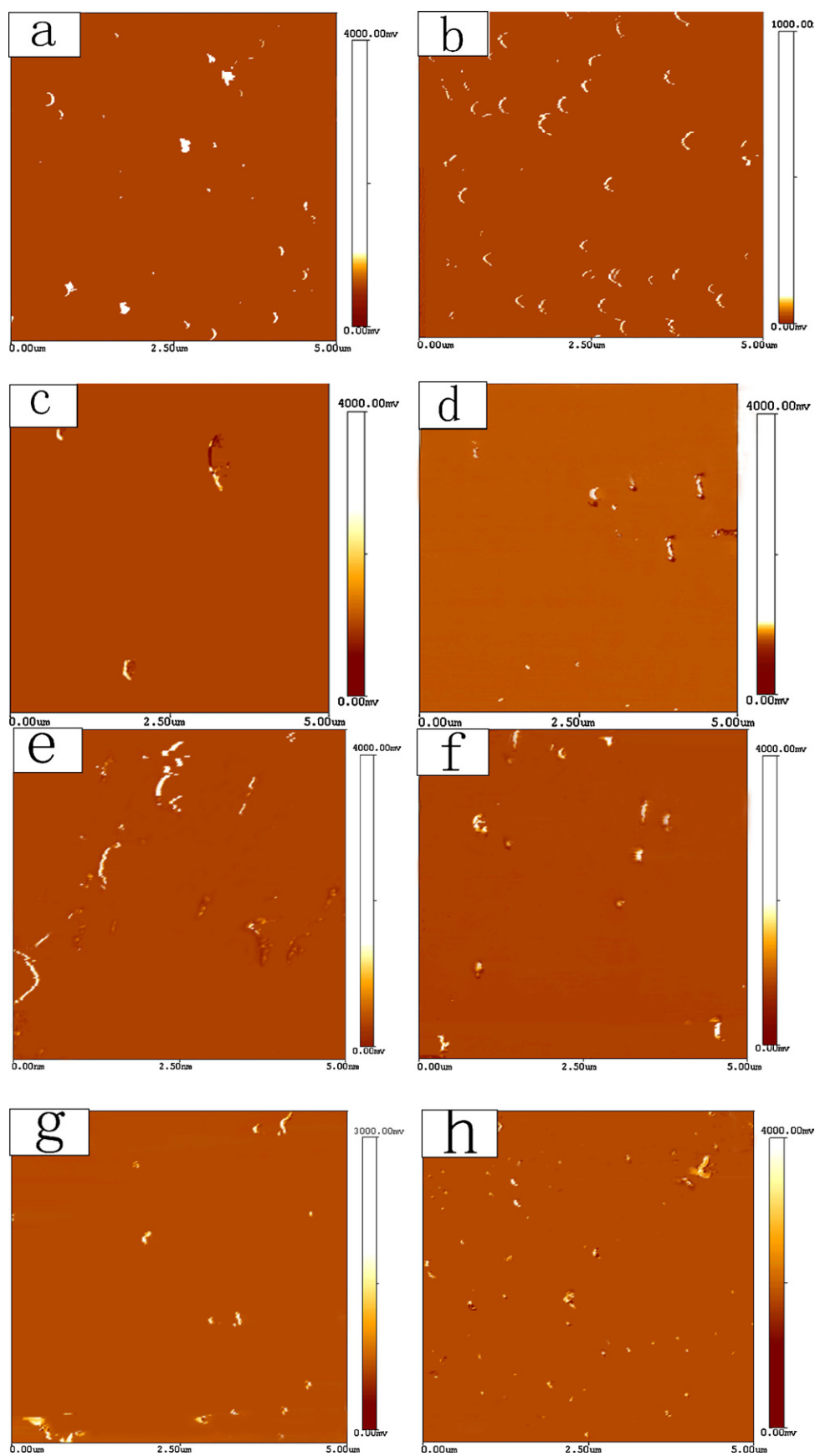


Fig. 2. AFM images of chelate-soluble pectin of peaches under cold storage. Scan area = $5\ \mu\text{m} \times 5\ \mu\text{m}$. (a) Fresh peaches; (b) day 18 at 2°C ; (c) day 18 at 8°C ; (d) day 18 at 15°C ; (e) fresh peaches; (f) day 18 at 2°C ; (g) day 18 at 8°C ; (h) day 18 at 15°C . Note: a, b, c, d 'Cangfangzaosheng' peach; e, f, g, h 'Songsenzaosheng' peach.

Table 1
The water-soluble pectin chain widths and the frequency of peaches under different storage temperature and time.

W (nm) ^a	Fq (N (%)) ^b									
	Cangfangzaosheng					Songsenzaosheng				
	2 °C			8 °C	15 °C	2 °C			8 °C	15 °C
	0 d	26 d	41 d	26 d	26 d	0 d	26 d	41 d	26 d	26 d
54	–	1 (2.5)	1 (2.3)	–	–	–	–	–	–	–
72	–	2 (5)	8 (18.2)	1 (14.3)	–	2 (5)	–	2 (5.9)	1 (5)	3 (11)
91	6 (14)	8 (20)	3 (6.8)	3 (42.9)	2 (11.8)	3 (7.5)	1 (14.3)	8 (23.5)	2 (10)	3 (11)
109	10 (23.3)	14 (35)	7 (15.9)	1 (14.3)	7 (41.2)	12 (30)	4 (57.1)	20 (58.8)	9 (45)	9 (33)
127	–	–	10 (22.7)	2 (28.6)	2 (11.8)	–	–	–	1 (5)	–
145	14 (32.6)	12 (30)	13 (29.5)	–	5 (29.4)	13 (32.5)	2 (28.6)	4 (11.8)	7 (35)	11 (40.7)
181	9 (20.9)	3 (7.5)	2 (4.5)	–	1 (5.9)	10 (25)	–	–	–	1 (3.7)
217	4 (9.3)	–	–	–	–	–	–	–	–	–

^a The widths of water-soluble pectin.

^b Number of times particular chain widths were observed.

by section analysis of the AFM software. In this study, W denoted the width of pectin molecular chains. The peak width of chain half height was applied to signify the chain width (Chen et al., 2009). The times that special chain width occurred was recorded as Fq. Aggregated polymers and linear chains, too small to be visualized precisely by the software, were not included for statistical analysis. The distributions of chain width of WSP and CSP molecules were shown in Tables 1 and 2, respectively. For both peach cultivars, frequency of WSP chains of smaller width was increased during storage. Higher temperature group had more WSP chains of smaller width than lower temperature group (Table 1). There was no significant difference of chain width distributions between the two cultivars. The trend of the width change of CSP molecules was similar to that of WSP molecules, while the change of CSP molecules was not as significant as WSP molecules (Table 2).

The chain widths of both WSP and CSP molecules from section analysis reflected a group of basic units (Tables 1 and 2). For WSP molecules, 54, 72 and 91 nm were basic units for 'Cangfangzaosheng' peach and 72, 91 and 109 nm were for 'Songsenzaosheng' peach. The basic units of CSP molecules of both 'Cangfangzaosheng' peach and 'Songsenzaosheng' peach were 54, 72 and 91 nm. The width of other types of chains can be composed by these basic units. For example, 109, 145 and 181 nm were approximately twice the size of 54, 72 and 91 nm, respectively. Number of 127 nm was approximately the sum of 54 and 72 nm, and 217 nm was the sum of 54, 72 and 91 nm. For WSP molecules, most of the wide values were the same in both peach cultivars. However, widths of 54 and 217 nm found in 'Cangfangzaosheng' did not appear in 'Songsenzaosheng'.

The obvious characteristics of the width of both pectin molecules under different cold storage conditions indicate that the linkage between different chains of pectin has certain regularity. In previous study, compared to SSP, the content of WSP and CSP

showed insignificant correlation with firmness (Zhang et al., 2010). However, the changes of the quantitative nanostructure of WSP and CSP were similar to SSP, which indicated that these changes might be independent of their chemical contents, indicating that changes of morphology and chemical contents might be two independent processes.

From results mentioned above, it was concluded that degradation of pectin molecules during cold storage may be attributed to the ripening and decay of fruits. It was obvious that lower temperature could extend the degradation of pectin. Thus, low-temperature storage might be a better and effective method of controlling texture and maintaining quality (Jin et al., 2009).

3.2. The relationship between the degradation of pectins (WSP and CSP) and softening of fruits

From the WSP and CSP chain widths described above, a schematic model of the degradation of pectin chains was proposed (Fig. 3). It suggests that WSP and CSP aggregates of peach gradually reduced during cold storage. The long single linear chains were detached and the small ones increased. The Fq of smaller W values of WSP and CSP chains increased during cold storage. For WSP and CSP molecules of the two cherry cultivars, conglomeration structures and polymers decreased while single linear chains increased along with the maturity of the fruits. This may be linked to the degradation of pectin associated with solubilization and depolymerization of middle lamella of fruits, which led to the softening of fruits. The degradation of pectin polysaccharides might cause the collapse of cell adhesiveness and consequently increased softening (Wakabayashi, 2000).

Pectin solubilization was accompanied with softening of fruit. Previous research showed that changes in water-soluble pectin

Table 2
The chelate-soluble pectin chain widths and the frequency of peaches under different storage temperature and time.

W (nm) ^a	Fq (N (%)) ^b									
	Cangfangzaosheng					Songsenzaosheng				
	2 °C			8 °C	15 °C	2 °C			8 °C	15 °C
	0 d	18 d	41 d	18 d	18 d	0 d	18 d	41 d	18 d	18 d
54	–	–	2 (10.5)	–	–	–	–	–	–	3 (5.2)
72	–	–	3 (15.8)	–	–	–	–	–	1 (4)	5 (8.6)
91	3 (17.6)	4 (6.6)	5 (26.3)	3 (20)	3 (12.5)	4 (12.5)	1 (5.6)	5 (25)	–	6 (10.3)
109	9 (52.9)	10 (16.4)	7 (36.8)	4 (26.7)	14 (58.3)	11 (34.4)	5 (27.8)	4 (20)	6 (24)	21 (36.2)
127	–	11 (18)	–	2 (13.3)	–	–	–	–	4 (16)	5 (8.6)
145	–	28 (45.9)	1 (5.3)	4 (26.7)	7 (29.2)	–	12 (66.7)	9 (45)	12 (50)	14 (24.1)
181	5 (29.4)	8 (13.1)	1 (5.3)	2 (13.3)	–	17 (53.1)	–	2 (10)	2 (8.3)	4 (6.9)

^a The widths of water-soluble pectin.

^b Number of times particular chain widths were observed.

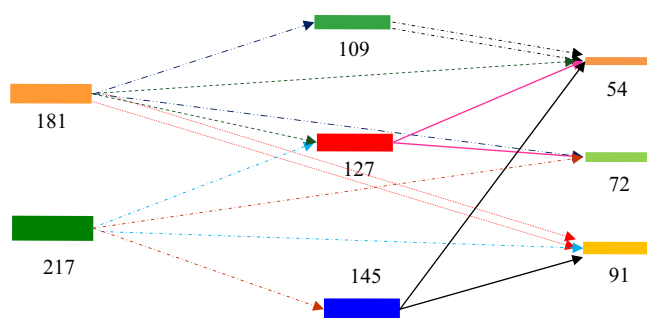


Fig. 3. A schematic model of the degradation of peach pectin chains in width during cold storage. *Note:* Numbers indicate the width of pectin chains.

were associated with papaya softening (Shiga et al., 2009). SSP was found closely related with firmness of peaches while WSP and CSP were not from correlation analysis (Zhang et al., 2010). During cold storage and maturity of fruit, the decrease of chain widths observed from current report might be the results of solubilization and degradation of WSP and CSP molecules, which might contribute to cell wall loosening and disaggregation. Pectin-breakdown and pectin-solubilization during fruit ripening and storage were simply the effects of pectin-degrading enzymes, and these pectin changes for WSP and CSP might be independent of their chemical changes, considering there was no significant correlation between them (Zhang et al., 2010).

From the results mentioned above, it was suggested that pectin enzymes should be targeted in order to illustrate the interaction between enzymes and cell wall polysaccharides on nanoscale. Together with the nanostructure of pectin, the pectin chemical structure should be further studied to explain the fundamental role of pectin in fruit softening at nano-level, which will offer an opportunity to delay fruit softening and to extend its shelf life. The results will be helpful for elucidating the effects of morphology changes of cell wall polysaccharides on fruit quality during postharvest.

4. Conclusion

The nanostructure of WSP and CSP molecules of peaches contains aggregates, single linear chains and branches. The aggregates of WSP and CSP molecules of peaches decreased during cold storage while linear chains increased. Similar phenomena were observed with increased storage temperature. Thus, a lower storage temperature could restrain the degradation of pectin. Quantitative analysis indicated that the widths of peach pectins (WSP and CSP) were composed of limited basic values. The nanostructural degradation of pectin widths might be independent of their chemical content changes.

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