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Changes in firmness, pectin content and nanostructure of two crisp peach cultivars after storage

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ABSTRACT

To investigate the fundamental of firmness changes of crisp peaches, firmness and pectin contents of two peach (*Prunus persica* L. Batsch) cultivars ('Cangfangzaosheng' and 'Songsenzaosheng') stored at 2 °C, 8 °C and 15 °C were investigated. Sodium carbonate-soluble pectin (SSP) extracted showed the highest correlation (positive) with firmness among the three kinds of pectins (water-soluble pectin, chelate-soluble pectin and SSP). The qualitative and quantitative information about SSP nanostructures were determined by atomic force microscopy (AFM). The widths of the peach SSPs were very consistent. The SSP chain widths of both peach cultivars were similar and were composed of several basic units. Schematic models of the changes of the chain widths were proposed. The results indicate that the firmness of peach was closely related with the contents and nanostructural characteristics of SSP, which might be hydrolyzed by enzymes in fruit flesh.

1. Introduction

Fruit texture is one of the most important quality properties that influence acceptability by consumers. It has been well established that texture changes are largely determined by the fruit cell wall and middle lamella polysaccharides (Manrique & Laiolo, 2004; Roeck, Sila, Duvetter, Loey, & Hendrickx, 2008). Cell wall polysaccharides mainly consist of pectin, hemicellulose and cellulose, while the middle lamella consists predominantly of pectin polysaccharides cross-linked with Ca^{2+}. Compared with hemicellulose and cellulose catabolism, fruit softening was more related to pectin solubilization and depolymerization (Rosli, Civello, & Martínez, 2004). Generally, softening of most fruit flesh is accompanied by changes in pectin structure (Ketsa, Chidtragool, Klein, & Lurie, 1999). Biochemical and/or chemical changes of fruit pectin were believed to result in textural changes of fruit flesh (Roeck et al., 2008). Pectin is a complex heteropolysaccharide, the chain of which contains many different monosaccharides that are composed of several components including homogalacturonan, rhamnogalacturonan I, rhamnogalacturonan II and xylogalacturonan (Pelloux, Rustérucci, & Mellerowicz, 2007). Investigating the structural changes of pectin chains will benefit to illustrating the fundamental of texture changes during cold storage.

Atomic force microscopy (AFM), as one of the nanotechnology tools, has been successfully applied in characterizing fruit and vegetable polysaccharides (Chen et al., 2009; Kirby, MacDougall, & Morris, 2008; Sriamornsak et al., 2008; Zhang et al., 2008), describing pectin degradation during storage (Yang, An, Feng, Li, & Lai, 2005; Yang, Feng, An, & Li, 2006; Yang, Lai, An, & Li, 2006) and pectin molecular manipulation (Yang, An, & Li, 2006). Except for providing the information of individual molecular chains and polymers (An, Yang, Liu, & Zhang, 2008), AFM also provides quantitative results at nanoscale without complex preparation of samples (Yang et al., 2007).

Peach is one of the favorite fruits due to its nutrition and quality value. However, peach easily develops chilling injury and corresponding physiochemical and textural changes during cold storage if under some inappropriate conditions, which limits its quality and storage life (Manganaris, Vasilakakis, Diamantidis, & Mignani, 2006). Many factors are involved in fruit softening during storage, and many measures including delayed storage, intermittent warming and calcium application have been used to reduce chilling injury and prolong cold storage life (Girardi et al., 2005; Manganaris, Vasilakakis, Diamantidis, & Mignani, 2007; Zhou et al., 2000). However, to our best knowledge, the fundamental of degradation of pectin under cold storage at nanostructure level has not been elucidated. Our previous research shows that firmness has

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close relationship with sodium carbonate-soluble pectin (SSP) (Zhang et al., 2008). Our work only focused on the changes of pectin chains in this paper.

The aim of this work was to investigate the fundamental of firmness changes including chilling injury of peaches under storage. The relationship among pectin contents, firmness, and SSP nanostructures were illustrated. Two crisp peach cultivars at commercial maturity were compared.

2. Materials and methods

2.1. Fruit material

Two crisp peach (*Prunus persica* L. Batsch) cultivars ('Cangfangzaosheng' and 'Songsenzaosheng' peaches) were harvested at commercial maturity according to skin background color of fruits. The fruits were harvested by hand at a farm in Zhengzhou, Henan province, China and transported to our laboratory within 2 h after harvest. Fruits with uniform size, weight, color, disease free and no other defects were selected, then each cultivar peaches were divided into three lots and stored at 2 °C, 8 °C, and 15 °C, respectively. Each group had about 60 fruits.

2.2. Firmness determination

Fruit firmness was measured using a TA-XT2i Texture Analyzer (Stable Micro Systems Ltd., Godalming, Surrey, UK). Two cylindrical slides (diameter 10 mm, height 5 mm) cut from peeled fruits were used for fruit firmness determination. Five fruits were measured for each condition. A cylindrical probe with a diameter of 35 mm was used. The operating parameters were: pre-test speed: 5.00 mm/s, test speed: 0.50 mm/s, post-test speed: 0.50 mm/s, period between cycles: 10 s, sample strain: 30%, trigger force: 3.0 g (Shao, Tu, Zhao, Chen, & Zhao, 2006).

2.3. Cell wall preparation and pectin extraction and determination

Cell wall material of peach flesh was extracted by methods described by Deng, Wu, and Li (2005), and Zhang et al. (2008) with slight modification. Ten gram peeled peach flesh from five peaches (same as the five that were used for firmness determination) was ground rapidly in an ice-cold mortar, then transferred to 200 ml 80% (v/v) boiling ethanol for 20 min. The sample was cooled to room temperature, and then filtrated with vacuum pump. The residue was re-extracted with 200 ml 80% ethanol two times as described above. After that, the residue was incubated overnight at 4 °C with 50 mL dimethysulphoxide (DMSO, Tianjin Resent Chemical Co., Ltd., China): water (9:1, v/v) to remove starch. Then it was water-washed and transferred to 200 ml chloroform: ethanol (2:1, v/v) for 10 min. The sample was filtrated and washed with 200 ml acetone until total whitening, the residue was cell wall material.

The cell wall material was suspended in 10 ml distilled water, agitated at 25 °C for 4 h. After centrifugation at 10,000*g* for 10 min at 4 °C, the residue was subject to two additional distilled water extractions according to the same experimental procedure. The three supernatants were collected as water-soluble pectin (WSP). For further extraction of the residue, 10 ml 50 mM trans-1,2-diaminocyclohexane-*N, N, N′, N′*-tetraacetic acid (CDTA) (Tianjin Zinco Fine Chemical Institute, China) was used, the solution was shaken for 4 h at 25 °C and centrifuged as above. The remaining pellet was further extracted twice with 10 ml 50 mM CDTA and spun. The three supernatants were collected as chelate-soluble pectin (CSP). The final extraction was performed with 10 ml 50 mM Na_2CO_3 containing 2 mM CDTA, shaken and spun as above. The procedure was repeated twice and the three supernatants were combined as SSP.

The content of peach pectin was assayed by the Carbazole colorimetry method through determining the concentration of pectin solution, using galacturonic acid (Sigma-Aldrich Co., Ltd., St. Louis, MO, USA) as standard (Zhang et al., 2008). Pectin solution (2 ml) was mixed with 12 ml sulfuric acid (98%,w/w) in a test-tube and cooled immediately with ice water, then boiled for 10 min and cooled using running tap water. Then carbazole ethanol solution (0.5 mL) was added to the mixture and the mixed solution was incubated at room temperature for 30 min. The absorbance at 530 nm (A_{530} nm) was then determined with a UV-2000 spectrophotometer (Unico(Shanghai) Instrument Co., Ltd.) at room temperature. The concentration of pectin solution can be modified to a reasonable range for determination of pectin content. All the experiments were performed in triplicate.

2.4. Determination of molecular weight

The molecular weight of peach pectin was determined from the viscosity of the pectin solution on the basis of the Mark–Houwink equation $\eta_i = K \cdot M^{\alpha}$, where η_i is the intrinsic viscosity, K and α are constants of the pectin solutions.

A series of concentrations of pectin solutions were prepared, the solutions were heated to 20 °C, 15 ml heated pectin solutions were pipetted into the Ubbelohde viscometer for viscosity measurement. The determination of the intrinsic viscosity is to extrapolate the reduced viscosity (C, η_{sp}/C) to its value at zero solute concentration ($C \rightarrow 0$).

$$\eta_i = \lim_{C \rightarrow 0} \frac{\eta_{sp}}{C}$$

where C is the concentration of pectin solution (kg/m^3), η_{sp} is the specific viscosity, η_i is the reduced viscosity (m^3/kg) (Kar & Arslan, 1999; Lai & Yang, 2007).

2.5. AFM determination

AFM determination was conducted according to the previous methods (Yang et al., 2005; Yang, Feng et al., 2006; Yang, Lai et al., 2006). SSP solutions were diluted to a series of concentrations (about 0.5–30 μg/mL) and the diluted solutions were agitated with a vortex mixer (Fisher Scientific, Pittsburgh, PA, USA). Then about 20 μL of the diluted solution was deposited onto a piece of freshly cleaved mica sheets (Muscovite Mica; Electron Microscopy Sciences, Hatfield, PA, USA), modified molecular combing technique was applied with a glass slide for straightening the pectin (Yang, An et al., 2006), then the solution was air-dried at room temperature. The mica with the sample was attached to a 15-mm diameter AFM specimen disc (TED Pella Inc., Redding, CA, USA) using double-sided adhesive tabs. The imaging was conducted in air using a Nano-R2™ AFM (Pacific Nanotechnology Inc., Santa Clara, CA, USA) in "noncontact" mode. The NSC 11/no A1 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. Three samples of each group were observed using AFM, and for each group five to six fields were investigated.

The integrity of the AFM imaging could be verified through scanning standard references with certain roughness (Yang, Feng et al., 2006) or with regular surface shape that is provided by the AFM company (Pacific Nanotechnology Inc., USA).

2.6. AFM image analysis

The AFM images were analyzed offline using NanoRule+™ AFM software provided by the AFM company. Images of error signal

mode were analyzed. In this mode, small variations in sample's surface topography were removed and the edges of the objects were highlighted. The widths of pectin chains were measured by the function of section analysis. Parallel images for each sample were analyzed for obtaining reliable results.

2.7. Statistical analysis

Analysis of variance (ANOVA) ($P < 0.05$) and Duncan's multiple range test for differences among different groups were applied using SAS 9.1.3 software (SAS, Cary, NC, USA). Widths of pectin chains were calculated and variance of the widths less than 1 nm was collected into the same groups.

3. Results and discussion

3.1. Effects of storage temperature and time on peach firmness and flesh pectin contents

Firmness is one of the most important quality indexes for fruits. Fig. 1 shows the firmness of two cultivar peaches under different cold storage conditions. It should be noted that the experiment was ended on the day 42 for both 2 °C and 8 °C groups, and day 26 for 15 °C group, respectively, because at that time some peach fruits in both cultivars developed textural breakdown and partial rot. Therefore, the storage was terminated correspondingly considering the loss of commercial value. In each cultivar, the firmness shows statistical difference ($P < 0.05$) among the three temperature groups.

The two peach cultivars had similar firmness (37.2 for 'Cangfangzaosheng' and 41.3 N for 'Songsenzaosheng'). The firmness of both peach cultivars decreased when stored at 8 °C and 15 °C. Took 'Cangfangzaosheng' fruit for example, the firmness of 37.2 N at the time of harvest decreased to 5.3 and 5.5 N after 41 d and 26 d storage under 8 °C and 15 °C, respectively. Peaches stored at 2 °C were found to have the least decrease of firmness, which indicates that storage at lower temperature could delay the decrease of fruit firmness. Another reason might be due to more chilling injury at low temperature which helped maintain the high firmness. During fruit ripening, pectins underwent solubilization and depolymersation, which were believed to contribute to middle lamella erosion and primary cell wall disintegration that result in softening of fruit flesh and decrease of fruit firmness (Billy et al., 2008). Storage at low temperature reduced the rate of solubilization and depolymersation of fruit cell wall materials. The firmness of 'Songsenzaosheng' peach under 2 °C increased between the storage of 30–40 d. This phenomenon was comparable to previous reports about chilling injury (Zheng & Li, 2006).

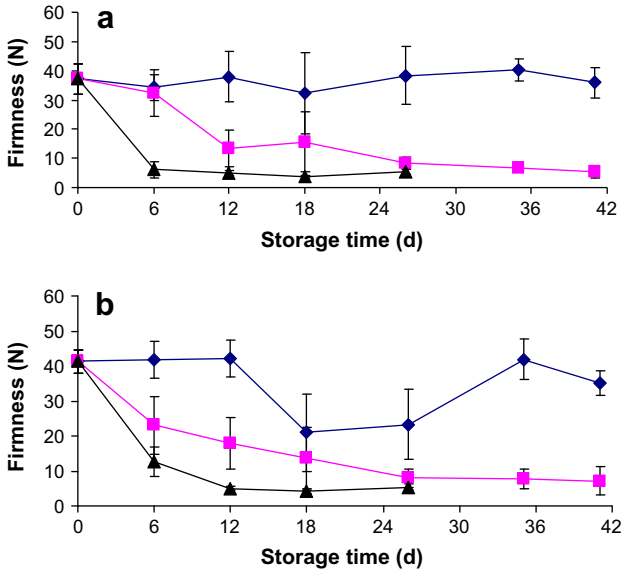

Fig. 1. Effect of storage time on the firmness of peaches at 2 °C (—◆—), 8 °C (—■—), and 15 °C (—▲—). (a) 'Cangfangzaosheng' peaches; (b) 'Songsenzaosheng' peaches. Error bars represent the standard deviation of the mean.

Pectin contents of two cultivar peaches under different cold storage temperatures were shown in Table 1. All the pectin contents showed statistical difference under different storage conditions with $P < 0.05$. Pectins in the two peach cultivars showed similar trend of change. The WSP contents of both peach cultivars increased during cold storage at each temperature. Changes of WSP contents of both cultivar peaches at 2 °C tended to be slower than those at 8 °C and 15 °C. For many fruits, protopectin solubilization during fruit ripening attributed to the increase of WSP contents. A decrease in insoluble, covalently bound pectins during fruit ripening also contributed to the increase of WSP contents (Rosli et al., 2004). The CSP contents of both peach cultivars increased during cold storage at each temperature. Compared to 8 °C and 15 °C, CSP contents of both peach cultivars at 2 °C increased more slowly. For SSP, the contents of both peach cultivars decreased during cold storage at each temperature. At 2 °C, 'Songsenzaosheng' peaches showed little variance while 'Cangfangzaosheng' showed increase.

The relationship between pectin contents and peach firmness at different storage conditions was shown in Table 2. All the pectins had some groups that showed significant correlation with firmness of flesh (8 °C 'Cangfangzaosheng' and 15 °C 'Songsenzaosheng' for WSP; 8 °C 'Songsenzaosheng' for CSP; and 8 °C 'Cangfangzaosheng' and 15 °C 'Songsenzaosheng' for SSP). However, in general, SSP had the highest correlation with firmness among all the pectins. And the correlation was positive. Therefore, the nanostructure of SSP was analyzed in the following to illustrate the fundamental mechanism of textural changes.

3.2. Effects of storage temperature and time on the nanostructure of SSP

AFM images of peach SSP in different groups were shown in Fig. 2. AFM can directly image the heterogeneous SSP structures, including cleavage point (cp), refers to a cleavage point between pectin molecules; releasing point, denotes a releasing point of pectin from the chelator, CDTA; Linear single fraction (ls), refers to linear chain without branches. Branching (Br) structure, determined by the heights of the chains. The genuine branch points had the same height as that of the main chain. Polymers (m), mean the association of SSP (Yang, Feng et al., 2006; Yang, Lai et al., 2006; Zhang et al., 2008).

The AFM images of SSP from fresh 'Cangfangzaosheng' peach indicated that most of the SSPs were aggregated, forming large aggregates, only a few SSPs formed single linear chains. After storage, SSPs were found to gradually reduce sizes of the strands and aggregates. As compared with Fig. 2a, Fig. 2b–d showed that the aggregates were reduced, the long single linear chains were detached and the small ones increased along with the rise of the storage temperature. Meanwhile, the small aggregates detaching from large aggregates could be viewed by AFM as well.

Compared to 'Cangfangzaosheng', the AFM images of SSP from fresh 'Songsenzaosheng' peaches indicated that most of the SSPs were aggregated, forming small aggregates (Fig. 2e). The effects of storage temperature (2 °C, 8 °C and 15 °C) could be seen by comparing the images in Fig. 2e–h. Similar phenomenon was found

Table 1
Effect of cold storage temperature and time on pectin contents of peaches (mg/100 g).

Pectin	No.	Storage time (d)						
		0	6	12	18	26	35	41
WSP	2C	$7.1^{a,DE}$	$4.5^{e,E}$	$8.6^{e,D}$	$14.5^{e,C}$	$55.0^{c,B}$	$56.5^{d,B}$	$77.0^{d,A}$
	2S	$4.6^{b,E}$	$8.2^{d,D}$	$8.1^{e,D}$	$18.7^{d,C}$	$9.0^{f,D}$	$198.3^{c,A}$	$169.2^{c,B}$
	8C	$7.1^{a,E}$	$9.0^{d,E}$	$30.3^{b,D}$	$71.0^{a,C}$	$78.9^{b,C}$	$215.2^{b,B}$	$257.1^{a,A}$
	8S	$4.6^{b,F}$	$14.3^{c,E}$	$17.3^{d,E}$	$28.2^{c,D}$	$86.0^{a,C}$	$285.2^{a,A}$	$240.6^{b,B}$
	15C	$7.1^{a,E}$	$29.9^{a,C}$	$26.4^{c,D}$	$47.6^{b,A}$	$37.0^{d,B}$	–	–
	15S	$4.6^{b,D}$	$20.6^{b,C}$	$34.6^{a,A}$	$30.4^{c,B}$	$21.3^{e,C}$	–	–
CSP	2C	$5.7^{b,E}$	$5.8^{f,E}$	$6.7^{e,D}$	$9.3^{f,B}$	$11.5^{e,A}$	$8.7^{d,C}$	$11.8^{d,A}$
	2S	$7.1^{a,E}$	$6.8^{e,E}$	$5.3^{f,F}$	$10.3^{e,D}$	$11.7^{e,C}$	$13.7^{c,B}$	$18.5^{c,A}$
	8C	$5.7^{b,G}$	$9.1^{d,F}$	$12.0^{d,D}$	$11.3^{d,E}$	$20.7^{d,C}$	$38.6^{a,B}$	$51.5^{a,A}$
	8S	$7.1^{a,G}$	$10.3^{c,F}$	$12.9^{c,E}$	$18.0^{c,D}$	$26.2^{c,C}$	$27.5^{b,B}$	$38.5^{b,A}$
	15C	$5.7^{b,D}$	$15.9^{a,C}$	$25.2^{a,B}$	$25.2^{b,B}$	$29.8^{b,A}$	–	–
	15S	$7.1^{a,E}$	$14.9^{b,D}$	$23.4^{b,C}$	$26.7^{a,B}$	$35.0^{a,A}$	–	–
SSP	2C	$373.2^{b,D}$	$524.8^{a,B}$	$391.7^{b,D}$	$390.7^{a,D}$	$342.4^{a,E}$	$645.3^{a,A}$	$633.5^{a,C}$
	2S	$470.1^{a,B}$	$445.2^{f,F}$	$466.8^{a,B}$	$198.8^{d,E}$	$348.3^{a,C}$	$496.9^{b,A}$	444.0c,D
	8C	$373.2^{b,B}$	$435.5^{b,A}$	$342.5^{c,C}$	$278.9^{b,D}$	$139.3^{c,G}$	$234.8^{d,F}$	$257.3^{c,E}$
	8S	$470.1^{a,A}$	$347.3^{c,B}$	$326.5^{d,CB}$	$233.5^{c,E}$	$67.4^{e,F}$	$295.1^{c,D}$	$320.0^{b,C}$
	15C	$373.2^{b,A}$	$225.5^{e,B}$	$188.0^{f,C}$	$159.3^{e,D}$	$81.7^{d,E}$	–	–
	15S	$470.1^{a,A}$	$301.2^{d,B}$	$212.3^{e,C}$	$68.8^{f,E}$	$147.5^{b,D}$	–	–

Note: Values with different small case superscript letters (a–f) in the same column within each pectin and capital superscript letters (A–G) in the same row indicate significant differences by the Duncan's multiple range test ($P < 0.05$), respectively. 2C (8C, 15C) and 2S (8S, 15S) denote 'Cangfangzaosheng' and 'Songsenzaosheng' peaches at 2 °C (8 °C, 15 °C), respectively.

in 'Cangfangzaosheng' peaches. The structure of SSPs was like 'crescent' when the 'Songsenzaosheng' peaches were stored under 2 °C and 8 °C for 18 d.

AFM images could reveal quantitative information of SSPs. The color bar legends at the right of images denoted the full height of the samples scanned. As shown in Fig. 2d, *W* denoted the width of SSP chains. The number of times that special chain width occurred was recorded as Fq. All the quantitative parameters of linear single fractions were analyzed by section analysis by the AFM software (Zhang et al., 2008).

The statistical results of SSP quantitative parameters were shown in Table 3. The Fq of smaller *W* values of SSP chains increased along with the increase of storage temperature and time. However, there was no obvious difference of *W* values and Fq between the two peach cultivars. The chain widths of SSP from section analysis reflected several basic units: 35 nm and 54 nm for 'Cangfangzaosheng', and 54 nm, 72 nm and 91 nm for 'Songsenzaosheng'. The width of other chains could be composed of these basic units in both cultivars. For example, 91 nm was the sum of 35 nm and 54 nm. 72 nm, 109 nm and 181 nm were approximately twice of the number of 35 nm, 54 nm and 91 nm, respectively. Number of 127 nm was approximately the sum of 54 nm and 72 nm, and 163 nm was the sum of 72 nm and 91 nm. Widths of SSP in both peach cultivars did have most of the same values, only 35 nm found in 'Cangfangzaosheng' did not appear in 'Songsenzaosheng'. The widths of SSP in the two peach cultivars here were comparable to the Chinese cherries (Zhang et al., 2008) and previous 'Jinxiu' yellow peaches (Yang, Feng et al., 2006). It indicates these fruits shared similar structural rules of pectin skeleton construction. Based on the results of chain widths, we proposed schematic models to show the degradation of SSP of the two peach cultivars (Fig. 3a and 3b).

It should be noted that the chain width determined by AFM had some deviations. Several factors including probe-broadening effect, surface characteristics of pectin chain and image environment could contribute to the deviations (Yang, Chen, An, & Lai, 2009). Another reason could be that some SSP chains or polymers were too small to be visualized precisely by the software and therefore not included in the statistics.

Even though the extraction methods of pectin fractions were very good, researchers were concerned that pectins imaged may be the cell wall materials. To make sure the objects of the AFM images were pectin rather than the fragment of cell wall material, the AFM images were compared with the published images of pectins and cell wall material (Sriamornsak et al., 2008). Another way was to determine the range of molecular weight of the samples, which could be determined by intrinsic viscosity of the samples. For pectins from fruits, according to the literature, $K = 0.00001$, and $\alpha = 1.22$ (Salomov, Kadyrov, & Sultanov, 1990). And according to the relationship between intrinsic viscosity and molecular weights, the molecular weight could be determined by the intrinsic viscosity. When the intrinsic values were 40.636 and 23.83 m^3/kg, the molecular weights were 2.61×10^5, and 1.69×10^5 $kg/kg\ mol^{-1}$, respectively. The results were comparable to the orange peel pectin (Kar & Arslan, 1999), which demonstrates that the AFM images were from pectin rather than the cell wall fragment.

Table 2
Correlation analysis between firmness and pectin contents of peaches.

Group		WSP	CSP	SSP
2C	Firmness	0.39	0.06	0.26
2S	Firmness	0.22	−0.28	0.80
8C	Firmness	−0.76*	−0.75	0.80*
8S	Firmness	−0.65	−0.83*	0.73
15C	Firmness	−0.86	−0.87	0.88
15S	Firmness	−0.91*	−0.83	0.91*

Note: *indicates significance at $P < 0.05$. 2C (8C, 15C) and 2S (8S, 15S) denote 'Cangfangzaosheng' and 'Songsenzaosheng' peaches at 2 °C (8 °C, 15 °C), respectively.

3.3. The relationship between pectin contents, fruit firmness and the nanostructure of SSP

Previous research showed correlation between firmness and different kinds of pectins of fruits, however, the pectins that were related to the firmness were found varied with different fruit and cultivars. Fishman, Levaj, and Gillespie (1993) observed a high correlation between changes of alkaline-soluble pectin (the extraction procedure and sample were similar to SSP of this manuscript) and the texture of two peach cultivars, while CSP was reported to have close relationship with texture under different

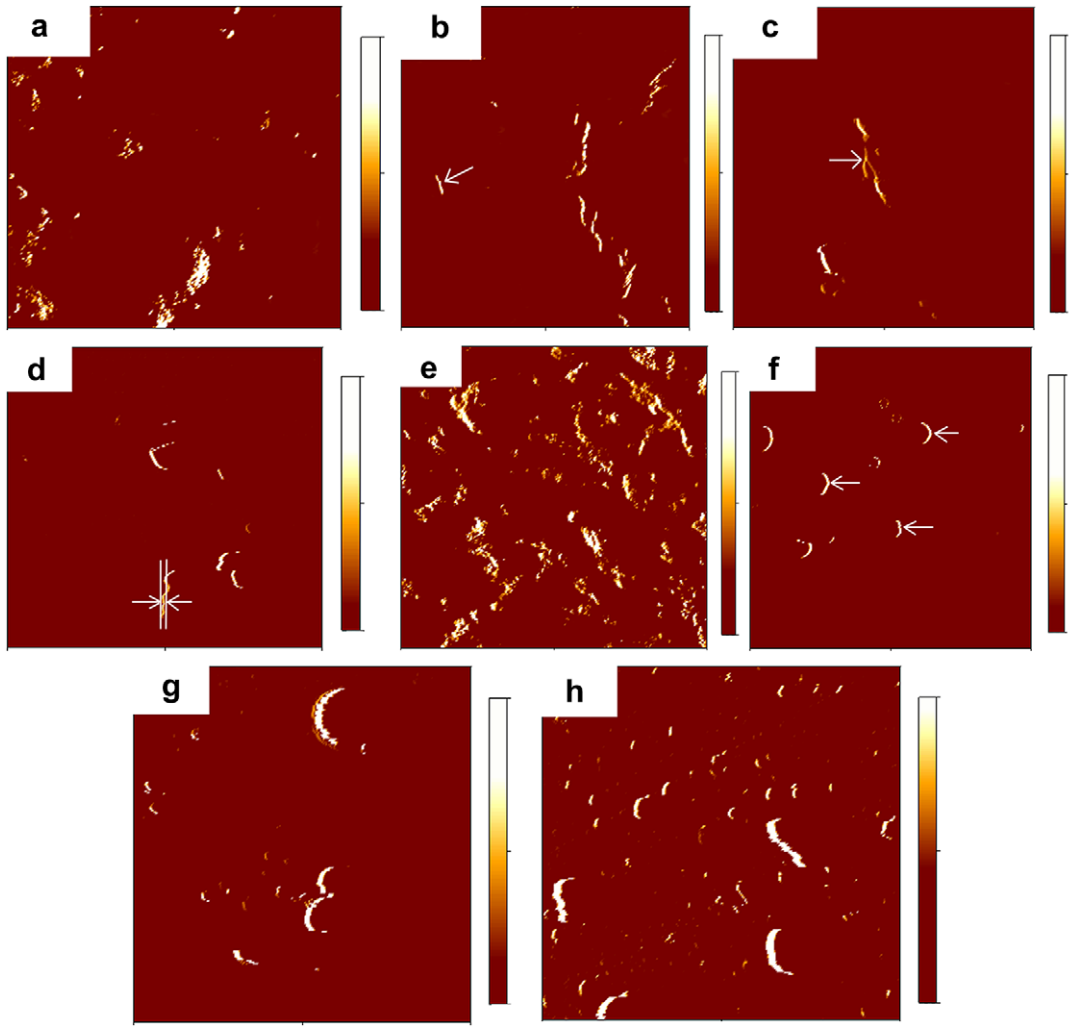

Fig. 2. AFM images of SSP of peaches under cold storage. Scan area = 5 μm × 5 μm, height bar = 500 mV: (a) fresh peach of 'Cangfangzaosheng', arrow points to the polymer; (b) day 41 at 2 °C of 'Cangfangzaosheng', arrow points to linear single fraction; (c) day 41 at 8 °C of 'Cangfangzaosheng', arrow points to branching; (d) day 18 at 15 °C of 'Cangfangzaosheng', the distance between the arrows is width of the chain; (e) fresh peach of 'Songsenzaosheng'; (f) day 18 at 2 °C of 'Songsenzaosheng', arrows point to linear single fraction; (g) day 18 at 8 °C of 'Songsenzaosheng'; (h) day 18 at 15 °C of 'Songsenzaosheng'.

storage stages of peach fruits (Brummell, Cin, Cristo, & Labavitch, 2004). Some researchers found WSP was closely related to the texture in peach and apple (Billy et al., 2008; Rosli et al., 2004).

Textural differences among different cultivars are different from those among different ripening stages for the same cultivar. While enzymes and biochemical processes contribute a lot to texture differences among different stages of the same cultivar, the evolution of cell wall skeletons of peaches have great effects on the textural differences among different cultivars, as illustrated in Yang et al. (2009).

Pectin structures were much different between melting and non-melting components, especially for sodium carbonate-soluble pectin (Yang et al., 2009). Furthermore, the shelf life of melting cultivar was limited, and the fruit firmness of melting cultivar decreased a lot and much mechanical loss happened during handling and distribution. Therefore, we focused the study on the fundamental differences of the SSP between two non-melting cultivars.

In this research, the SSP aggregates reduced, the single linear chains increased, and the Fq of smaller *W* values of SSP chains increased during 8 °C and 15 °C storage. The fruit softened along with the decrease of the fruit firmness in these groups. The decreased chain width observed from AFM might be associated with solubilization and depolymerization of middle lamella of fruits, which were found in ripening of papaya fruit (Manrique &

Table 3
Effects of storage temperature and time on the SSP chain widths and the frequency of peaches.

W (nm)	Fq									
	Cangfangzaosheng					Songshenzaosheng				
	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
35	–	–	2	–	2	–	–	–	–	–
54	–	–	11	1	2	1	–	–	–	–
72	1	1	4	–	4	2	–	1	1	3
91	5	6	11	2	6	4	3	3	7	14
109	9	6	–	2	–	7	12	7	9	17
127	4	4	8	2	10	–	–	1	–	11
145	7	13	9	5	9	8	16	–	14	9
163	–	2	–	–	–	–	–	–	–	6
181	3	2	2	3	–	7	4	–	4	10

Note: *W*: the widths of SSP; Fq: number of times particular chain widths were observed.

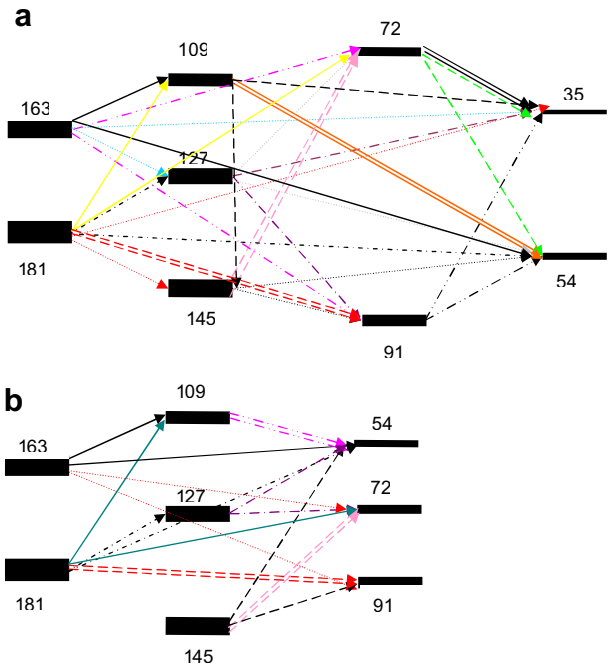

Fig. 3. Schematic images of the degradation of pectin chains in width. Note: The numbers indicate the width of pectin chains.

Laiolo, 2004). The solubilization and depolymerization might contribute to cell wall loosening and disaggregation (Billy et al., 2008). During fruit storage, the middle lamella and primary cell wall structures were disassembled, meanwhile, pectin side chains depolymerized due to enzyme effects. These changes led to the loss of firmness, softening of fruit, increase of fruit postharvest decay and decreased quality of fresh fruit (Rosli et al., 2004). However, for 2 °C group which developed chilling injury in the storage, even though the firmness did not change much, the SSP still showed the degradation trend from the results of chain widths, which indicates that the degradation of chain width was not the sole main reason for maintaining the firmness of fruits. One possible process involved was the hydrolysis of chains catalyzed by enzymes in fruit flesh and further hydrolyzed chains were covalently connected by cations in the fruit cells, which could maintain high firmness at low temperature storage (Billy et al., 2008; Girardi et al., 2005; Rosli et al., 2004). But the firmness was not only dependent on the nanostructure of SSP, many other chemical changes could be involved in this process. Further research could help to examine the model of pectin structure proposed by Vincken et al. (2003).

4. Conclusions

Firmness and pectin contents of two crisp peach cultivars stored under 2 °C, 8 °C and 15 °C were investigated. SSP showed the highest correlation with firmness among the three kinds of pectins. The SSP chain widths of the two peach cultivars shared many values which were composed of several basic units, respectively. Schematic models of the changes of SSP chains were proposed. The results show that the firmness of peach was closely related with the contents and nanostructure of SSP.

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