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Effects of ripening stage and cultivar on physicochemical properties and pectin nanostructures of jujubes

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ABSTRACT

Two Chinese jujube (*Zizyphus jujuba*) cultivars ('Huanghua' and 'Zhanhua') at unripe and ripe stages were investigated. During ripening, the weight, pH, hardness, and chewiness of both cultivars decreased while titratable acidity, total soluble solid content, and pectin contents increased. More than 75% pectins of the fruits were water-soluble pectin (WSP). Pectins shared the common major compositions of Gal, Rha and GalUA. For both cultivars, most of the chain widths were between 47 and 70 nm for unripe while less than 40 nm for ripe jujubes. Compared to unripe fruits, all pectins of ripe fruits had less percentage of wide and long pectin chains. All the pectin contents of cultivar 'Huanghua' changed to a degree greater than that of cultivar 'Zhanhua' during ripening. Changes of pectin nanostructure and neutral sugar composition might be responsible for the major physicochemical properties of jujubes.

1. Introduction

Quality of fruits and vegetables depends largely on the intercellular adhesion and mechanical strength of their cell wall compositions. Pectin, a complex polysaccharide, is one of the major components in the primary cell wall and middle lamella of fruits and vegetables. During fruit ripening, pectin polymers depolymerize and solubilize within cell walls, resulting in decreased molecular weight and increased fruit solubility. Solubilized pectin further decomposes, leading to reduced amount of total pectin.

Modification and depolymerization of pectin, hemicellulose and cellulose is related to fruits' textural properties, fruit softening and ripeness during postharvest. One example is, hemicellulose molecules of crisp Chinese cherry fruit have more thicker chains than soft cherry fruit, and the width and length of hemicellulose molecular chains are closely relevant to cherry's texture (Chen et al., 2009). Thus, understanding these cell wall polysaccharide structures and compositions is crucial for elucidating the textural properties of fruits. It is always challenging to restore the cell wall compositions and build a degradation model by studying the changes of saccharide composition, physicochemical properties and quality of fruits (Manganaris, Vasilakakis, Diamantidis, & Mignani, 2006), requiring research in different aspects, e.g., nanostructural analysis.

Fruit inner structures and texture were traditionally investigated by cellular chemistry, sensory evaluation and consumer feedback, as well as cellular structural changes using scanning electron microscope. However, these techniques did not provide direct evidence for the degradation of molecular aggregates (Allan-Wojtas, Sanford, McRae, & Carbyn, 2003). Since invention, atomic force microscopy (AFM) has made great contributions to investigating cells, plant cell wall ultrastructures and polysaccharides including single polysaccharide molecules, polysaccharide colloids and polymers (Gunning et al., 1998; Morris et al., 2001; Morris, Woodward, & Gunning, 2011; Liu & Wang, 2010). In addition, AFM was applied in both qualitative and quantitative analysis of structural changes of cell wall polysaccharides during fruit ripening and softening (Zhang et al., 2012). Moreover, AFM results were consistent with other techniques for the determination of neutral sugars including linkage analysis of pectin molecules (Round, Rigby, MacDougall, Ring, & Morris, 2001). For example, changes of the widths of pectins including water-soluble pectin (WSP), chelate-souble pectin (CSP) and sodium carbonate-soluble pectin (SSP) during controlled atmosphere and normal atmosphere were determined by AFM (Yang, An, Feng, Li, & Lai, 2005; Yang, Feng, An, & Li, 2006b; Yang, Lai, An, & Li, 2006c). Correlation between physico-chemical characterization and AFM results was also discussed (Fishman, Chau, Cooke, Yadav, & Hotchkiss, 2009).

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Chinese jujube (*Zizyphus jujube Miller*) is a native fruit that has been used as food and traditional Chinese medicine for thousands of years (Li, Fan, Ding, & Ding, 2007; Li et al., 2009; Wang et al., 2011). Nutritional quality and effects of packaging and preservative measures on physicochemical properties of jujubes have been studied for better extending their shelf life. However, the fundamental mechanism of jujubes' physicochemical changes is still unclear, in particular, changes of firmness during postharvest of jujube.

In the current research, two Chinese winter jujubes cultivars ('Huanghua' and 'Zhanhua') at two ripening stages, unripe and ripe, were chosen for a comparative study. The aim was to elucidate the mechanism of jujube ripening and help improve fruit postharvest preservation and transportation. Nanotructures of CSP and SSP in ripening jujubes were qualitatively and quantitatively characterized by AFM. In addition, pectin contents and neutral sugar compositions were assayed with the Carbazole colorimetry method and high-performance liquid chromatography (HPLC), respectively.

2. Materials and methods

2.1. Fruit materials

Two Chinese winter jujubes (*Zizyphus jujuba*) cultivars ('Huanghua' and 'Zhanhua') were included in the current study. Two different stages (unripe and ripe) of maturity were classified by color chart. The ripe jujube was selected at commercial maturity stage with 80–90% of the total peel turning red, while the unripe jujube was about 7 d before becoming ripe (Jiang, Sheng, Jiang, & Zhou, 2004). Both the cultivars were bought in Zhengzhou, Henan province, China. The fruits were transported to our laboratory within 4 h after harvest. Fruits with uniform size, disease free, and no other defects were selected.

2.2. Physicochemical analysis

The fruits were peeled and cut into 10 mm × 5 mm (diameter × height) pieces. Texture profile analysis (TPA) was performed on the fruit pieces by a TA-XT2i texture analyzer (Stable Micro Systems Ltd., Godalming, Surrey, UK). The operating parameters were set 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 (Zhang et al., 2010). Hardness was determined from the peak force during the first compression cycle. Cohesiveness was calculated as a ratio of the areas delimited by the second compression cycle and the first one. Springiness was obtained as a ratio of the time measured between the start of the second area and the second compression direction reversal divided by the time measured between the start of the first area generation and the first compression's direction reversal. Chewiness was calculated by multiplying hardness, cohesiveness, and springiness. Ten jujube fruits were measured individually for each treatment group.

The color of the jujubes was determined by a chromatic meter (CR-400, Konica Minolta Group, Japan). Unpeeled jujube fruit was cut into pieces of same size. The color spaces were expressed as L^*, a^* and b^*, which was known as the Cielab color space. L^* represented the brightness of the sample, a^* and b^* represented the color directions. The $+a^*$ was the red direction, while the $-a^*$ was green direction; the $+b^*$ was yellow direction, while the $-b^*$ was blue direction.

The titratable acidity (TA), as percent malic acid, was assayed by indicator titration of 50 mL diluted juice (50 mL of pressed jujube juice were diluted to 250 mL with distilled water) with 0.1 M NaOH. Phenolphthalein was used as indicator and titration was terminated when the color of the solution changed into pink without fading in 30 s. The results were expressed as g of citric acid equivalent per g of fresh weight (FW). Ten fruits were randomly chosen for measuring total soluble solids (TSS) content of each lot. TSS content was assayed using a digital refractometer (WYT-J, Sichuan, China) at 20 °C. The result of TSS was expressed as °Brix (Wang et al., 2011; Zhang et al., 2008).

2.3. Cell wall preparation and pectin determination

Cell wall material of jujube flesh was extracted with a previous method with slight modifications (Zhang et al., 2008). Peeled jujube flesh (10 g) was cut and boiled in 200 ml of 80% (v/v) ethanol for 20 min to inactivate potential cell wall modifying enzymes. The sample was filtrated using vacuum pump after being cooled to room temperature. Then the same procedure was repeated two more times. Next, the residue was incubated overnight at 4 °C with 50 mL dimethysulphoxide (DMSO, Tianjin Resent Chemical Co., Ltd, China):water (9:1, v/v) for removing starch. Subsequently, the sample was transferred to 200 mL of chloroform: ethanol (2:1, v/v) for 10 min, filtrated and washed using 200 mL acetone until total whitening. The residue was collected as cell wall material.

The cell wall material was then suspended in 10 mL double distilled water, shaken for 4 h at 25 °C, and centrifuged at 10,000 × *g* at 4 °C for 10 min (Shanghai Anting Scientific Instrument Factory, Shanghai, China). The same procedure was repeated for two more times. All the supernatants were collected and combined as the fraction of water-soluble pectin (WSP). The residue was resuspended in 10 mL of 50 mM cyclohexanetrans-1, 2-diamine tetra-acetate (CDTA, Tianjin Zinco Fine Chemical Institute, Tianjin, China), shaken for 4 h, and centrifuged as described above. The supernatant was collected and the residue was re-extracted twice with CDTA. All supernatants were collected together as the fraction of chelate-soluble pectin (CSP). Then the residue was resuspended in 10 mL of 50 mM Na_2CO_3 containing 2 mM CDTA (Na_2CO_3, Tianjin Zinco Fine Chemical Institute, Tianjin, China), shaken for 4 h, and centrifuged as described above. The supernatants were collected, and the procedure was repeated for two more times. All the three supernatants were combined as the fraction of sodium carbonate-soluble pectin (SSP).

The pectin content of jujubes was assayed with the carbazole colorimetry method (Zhang et al., 2008). Galacturonic acid (Sigma–Aldrich Co., Ltd., St. Louis, MO, USA) was used as standard. Pectin solution (2 mL) was combined with 12 mL of sulfuric acid (98%, w/w) in a test tube, mixed and cooled with ice water immediately, boiled for 10 min and then cooled with running tap water. Carbazole ethanol solution (0.5 mL) was added to the solution, mixed and incubated at room temperature for 30 min, then the absorbance at 530 nm was determined using a UV-2000 spectrophotometer (Unico Instrument Co. Ltd., Shanghai, China) at room temperature. The CSP solution could be diluted for matching the result of standard galacturonic acid. All experiments were conducted in triplicate and results were expressed as mg of galacturonic acid per 100 g of FW.

2.4. Pectin neutral sugar composition analysis by HPLC

The neutral sugar composition of pectin was analyzed by HPLC using a previous method with some modifications (Xin et al., 2010). Pectin solution was dissolved in 2 mL of 2 M trifluoroacetic acid and hydrolyzed at 120 °C for 2 h. After being cooled to room temperature, each reaction mixture was transferred to a micro-centrifuge tube and then freeze-dried. The dried sample was labeled directly with 1-phenyl-3-methyl-5-pyrazolone (PMP) by adding 100 μL of PMP solution (0.5 M in methanol) and 100 μl of sodium hydroxide solution (0.3 M), mixed and incubated at 70 °C for 2 h. PMP was used for quantitative determination of the neutral sugar composition.

Then the mixture was neutralized by adding 100 μL of hydrochloric acid solution (0.3 M). Chloroform (0.5 mL) was then added and mixed thoroughly by vortexing. After the organic phase was carefully removed and discarded, the extraction process was repeated two more times. The resulting aqueous phase was filled to 1 mL with distilled water and mixed for HPLC analysis.

Analysis of the PMP-labeled neutral sugar compositions was carried out on a HPLC system (Model 1100, Agilent Technologies Inc., Santa Clara, CA, USA). A C-18 column, 4.6 × 250 mm, 5 μm, optimized for the separation of PMP-labeled carbohydrates, was applied with the mobile phase of 0.1 mol/L phosphate buffer (pH 6.9)-acetonitrile (83:17, v/v). The flow rate was 1 mL/min and the wavelength of UV detection was 254 nm. Individual sugars were identified by comparing the retention time with those of standards.

2.5. Nanostructural characterization and analysis

The nanostructure of pectin was analyzed with a Multimode NanoScope IIIa AFM (Digital Instruments, Santa Barbara, CA, USA) equipped with an E(J) scanner (Liu et al., 2009). AFM was conducted with tapping mode in a glove box with 30–40% of relative humidity at about 25 °C. The relative humidity inside the glove box was adjusted and stabilized by silica gel before experiments.

Pectin solution was diluted to about 10 μg/mL and votexed with a XW-80A Vortex mixer (Shanghai Jinke Co., Ltd., Shanghai, China). Then it was pipetted rapidly onto a freshly cleaved mica surface. The mica surface was then dried by forced air using an ear syringe, which had a similar effect as molecular manipulation on pulling out the pectin molecules from the solution (Yang, An, & Li, 2006a). The imaging was conducted in air with a scan speed of 2 Hz. The scanner was adjusted to select and capture small areas within the region that was capable of scanning (Yang et al., 2006c). NSC 11/No Al tip was used with resonance and force constant of 330 KHz and 48 N/m, respectively.

AFM images were analyzed offline using an AFM software (BinOffline500-0609) provided by the company. After the noise of the samples was reduced through the function of flattening of the software, high quality images were then obtained. The bright and dark areas in the AFM images were corresponding to high and low parts in the observed pectin samples, respectively. It should be noted that different scales were applied in the vertical (about several microns) and horizontal axes (about several nanometers), and height mode images for each group were used for the analysis (Yang et al., 2006b).

Both qualitative and quantitative information was obtained from AFM analysis. The chain features of pectin were characterized, including cleavage points, long chains, linear single fractions, polymers and short chains. The dimensions (width and height) of the pectin molecules were obtained using section analysis of the AFM software before the images were flattened. The length of pectin chain was determined by plotting the main chain with the software. The width (W) and height (V) of a single strand were determined by measuring the horizontal distance and vertical distance, respectively (Yang et al., 2006a,b,c). The number of specific chain widths was recorded as frequency (Fq) (Liu et al., 2009).

2.6. Statistical analysis

TA, pectin contents and neutral sugar compositions were determined in triplicate. Ten replicates were performed for TSS and firmness. At least ten AFM images of three parallel samples were analyzed for each condition. The zoom images of marked square in large area images were not included in the statistics. Statistical analyses using analysis of variance (ANOVA) ($P < 0.05$) and Duncan's multiple range tests for differences in the quantitative dimensions of pectin chains were performed using SAS software (Version 9.1.3, SAS, Cary, NC, USA). Data of physicochemical properties and vertical distances of pectin chains were expressed as mean ± standard deviation. Comparisons that yielded $P < 0.05$ were considered significant.

3. Results and discussion

3.1. Effects of ripening stages and cultivars on physicochemical properties of jujube

During ripening, appearance of jujube fruits underwent a series of changes. Table 1 shows the effects of ripening stages and cultivars on the physicochemical properties including pectin contents (WSP, CSP and SSP), firmness and color of winter jujubes. The results indicated that ripening stages and cultivars had a significant effect on the changes of average weight, TA, TSS, pH and pectin content. During maturation, from unripe to ripe stages, the weight and pH of both cultivars decreased while the TA and TSS contents increased.

Although both cultivars had a similar trend of changes in TA, TSS and pH during ripening, they were much different. For 'Huanghua' cultivar, the TA of the unripe and ripe fruits was 0.13% and 0.18%, respectively; the TSS was 13.00 and 21.10 °Brix, respectively, and the pH was 4.95 and 4.78, respectively. In contrast, for 'Zhanhua' cultivar, the TA was 0.21% and 0.23%, the TSS was 14.22 and 23.77°Brix, and the pH was 4.93 and 4.63, respectively for unripe and ripe fruits. Both TA and TSS of 'Zhanhua' cultivar were higher than those of 'Huanghua' cultivar. Moreover, TSS was positively correlated to TA for both cultivars.

For both cultivars, most of the pectins were WSP, accounting for more than 75% of all pectins. This did not apply for many other fruits, for instance, 'Shijixiang' strawberries contained around 30% of WSP 'Cangfangzaosheng' and 'Songsenzaosheng' peaches contained less than 3% (Chen et al., 2011; Zhang et al., 2010). During ripening, WSP content increased from 154.72 to 581.44 mg·100 g^{-1} FW^{-1} for 'Huanghua' cultivar, while from 631.06 to 670.42 mg·100 g^{-1} FW^{-1} for 'Zhanhua' cultivar. Thus, both cultivars had a trend of increased WSP content, although the WSP content of 'Huanghua' cultivar increased more than that of 'Zhanhua' cultivar, indicating that the content of WSP in jujubes may be related with cultivar difference.

The differences of CSP between the two cultivars, and between the two ripening stages were significant. During ripening, CSP content increased from 43.8 to 68.91 mg 100 g^{-1} FW^{-1} for 'Huanghua' cultivar, while from 84.18 to 81.11 mg 100 g^{-1} FW^{-1} for 'Zhanhua' cultivar. CSP content of 'Huanghua' group was less than that of 'Zhanhua' group. In contrast, the SSP content of the two cultivars had a similar pattern of increase during ripening.

Firmness is one of the most important factors affecting the storage properties of fruits and vegetables. It is closely related to fruit sugar compositions, which can be investigated by high-performance liquid chromatography (HPLC) (Xin et al., 2010), or silylation (Li et al., 2007), and/or titration with Fehling's method (Li et al., 2009). Decreased firmness during ripening reflects cell enlargement during fruit growth. Different rates of firmness decrease, also known as softening, were characteristic of cultivars (Muskovics, Felföldi, Kovács, Perlaki, & Kállay, 2006). In both cultivars, firmness and color were significantly different between the two ripening stages. For 'Huanghua' cultivar, the hardness was 33.08 and 25.12 N for unripe and ripe stages, respectively. However, the values were 30.34 and 26.96 N, respectively, for unripe and ripe stages of 'Zhanhua' cultivar. The cohesiveness, springiness and chewiness were significantly different between the two cultivars at two ripening stages as well. During jujube ripening, the brightness decreased for both cultivars. At unripe stage the value of a^* was negative, suggesting the skin color was green for unripe

Table 1
Effects of ripening stages and cultivars on physicochemical properties of winter-jujubes.[a]

Group	Unripe 'Huanghua'	Ripe 'Huanghua'	Unripe 'Zhanhua'	Ripe 'Zhanhua'
Weight (g)	22.57 ± 2.05a	18.81 ± 1.63b	9.83 ± 1.36c	8.18 ± 1.30d
Hardness (kg)	3.38 ± 0.22a	2.56 ± 0.37c	3.10 ± 0.19ab	2.75 ± 0.22bc
Cohesiveness	0.68 ± 0.02c	0.74 ± 0.01ab	0.76 ± 0.01a	0.71 ± 0.01bc
Springiness	0.81 ± 0.01a	0.69 ± 0.02c	0.74 ± 0.02b	0.69 ± 0.03c
Chewiness (kg)	1.85 ± 0.11a	1.29 ± 0.17b	1.74 ± 0.14a	1.36 ± 0.13b
L^*	67.70 ± 0.93a	35.50 ± 1.17c	66.01 ± 2.03a	39.38 ± 2.76b
a^*	-11.31 ± 0.99c	16.75 ± 1.29b	-11.08 ± 1.32c	19.76 ± 2.08a
b^*	36.71 ± 0.83a	18.42 ± 2.04c	39.24 ± 0.93a	25.52 ± 6.55b
TA (%)	0.13 ± 0.00d	0.18 ± 0.00c	0.21 ± 0.01b	0.23 ± 0.00a
TSS (°Brix)	13.00 ± 0.95c	21.10 ± 2.74b	14.22 ± 1.88c	23.77 ± 2.39a
pH	4.95 ± 0.04a	4.78 ± 0.06b	4.93 ± 0.03a	4.63 ± 0.02c
WSP (mg/100 g, FW)	154.7 ± 4.6c	581.4 ± 13.1b	631.1 ± 41.8a	670.4 ± 27.6a
CSP (mg/100 g, FW)	43.8 ± 2.1c	68.9 ± 1.4b	84.2 ± 2.2a	81.1 ± 1.6a
SSP (mg/100 g, FW)	32.2 ± 3.5bc	66.4 ± 2.9a	29.0 ± 0.6c	34.7 ± 1.5b

[a] The values that have different letters in the same row are significantly ($P < 0.05$) different.

fruits. 'Zhanhua' fruits were redder than 'Huanghua' fruits. These results agreed well with previous studies indicating that the red rate, rot rate, and the browning index of fruits increased gradually while the firmness declined during ripening (Sun, Liu, Zhu, Zhou, & Wang, 2007).

3.2. Relationship among ripening stages, cultivars, and the neutral sugar composition of pectins

Table 2 shows the results of neutral sugar composition of jujube pectins (WSP, CSP and SSP) from different cultivars and ripening stages. The sugar content and composition in plants were known to differ greatly among cultivars according to their habitats, therefore the results can aid selection of cultivars for special food processing (Li et al., 2007). For WSP, Gal, Rha, and GalUA were the major compositions (>78%), which agreed well with others' findings (Li et al., 2007), while for CSP and SSP, except the same major compositions as WSP, Glc was another additional major composition. Compared to WSP and CSP, SSP did not contain Man, Xyl and Fuc.

Changes of CSP main chains were reflected by the ratio of GalUA to Rha, and the modification of CSP side chains was reflected by the changed ratios of Gal to Rha and Ara to Rha (Almeida & Huber, 2008). Glc and Gal were the most abundant noncellulosic neutral sugars in jujube CSP, followed by GalUA, Rha and Ara. Although the content of GalUA and Rha in CSP main chains increased during ripening for both cultivars, the ratio of GalUA to Rha decreased for 'Huanghua' group but increased for 'Zhanhua' group. Also during ripening, both the contents of Gal and Ara of CSP side chains increased while the ratios of Gal to Rha and Ara to Rha decreased in both cultivars. The loss of side chains could change the cell wall quality (rigidness or flexibility), affect the solubility and structure of pectin, and change firmness. These modifications of pectin were thought existed in tomatoes, strawberries and many other fruits including jujubes (Brummell, 2006).

3.3. Effects of ripening stages and cultivars on the nanostructures of CSP and SSP

The physicochemical properties of postharvest fruits were more closely related to CSP and SSP than WSP (Xin et al., 2010; Zhang et al., 2008). Therefore, only nanostructural information of CSP and SSP was included.

Fig. 1 shows AFM images of CSP from two jujube cultivars at two ripening stages. Fig. 2 shows AFM images of SSP. Quantitative results of the CSP and SSP chain widths were shown in Table 3. These results were consistent with previous reports (Yang et al., 2009). Pectin width difference in the two cultivars was larger than height difference. For example, the distribution of widths was 15–100 nm for CSP compared to 15–160 nm for SSP. In contrast, almost all the heights of the pectins (CSP and SSP) in the two cultivars were about 0.5–3 nm with most of the heights being 1–2 nm. The heights of the jujube pectin chains were smaller than those of peaches, and similar to that of tomatoes (Yang et al., 2009; Xin et al., 2010).

The widths of CSP chains from section analysis indicated that at unripe stage the widths of both cultivars were mainly in the range of 35–60 nm, compared to 15–35 nm for ripe stage (Table 3). The CSP chain widths of 39.06 and 46.88 nm accounted for the largest proportion of width for 'Huanghua' cultivar at unripe stage, while 35.16 nm for the unripe 'Zhanhua' cultivar. In ripe fruits of 'Huanghua' cultivar, the main CSP chain widths were 23.44 and 31.25 nm, while for 'Zhanhua' cultivar they were 19.53 nm. The difference in width may be one of the underlying reasons for the differences of texture and other physical properties in the cultivar. For both cultivars, the main vertical distances of CSP chains were greater than 1 nm for unripe jujubes, while less than 1 nm for ripe jujubes.

The quantitative width results of SSP chains were also shown in Table 3. The SSP chain widths of 58.59 and 78.13 nm accounted for the largest proportion for 'Huanghua' cultivar at unripe stage, while the values were 62.50 and 78.13 nm for the unripe 'Zhanhua' cultivar. For ripe fruits of 'Huanghua' cultivar, the main SSP chain width was 39.06 nm, compared to 19.53 nm for 'Zhanhua' cultivar. The vertical distances of 'Zhanhua' were significantly greater than 'Huanghua' for both ripening stages.

Fig. 3 shows the frequencies of pectin (Fig. 3(a) for CSP and Fig. 3(b) for SSP) chain lengths. The molecules for statistics were extended to be linear and were totally within the scanned zone of AFM. The length of pectin chains was defined as the length of single linear fractions (main chain) according to a previous report (Yang et al., 2006c). The chain lengths were mainly among 1500–2000 nm for unripe 'Huanghua' jujubes; however larger than 3000 nm for unripe 'Zhanhua' cultivars. The CSP chain lengths in ripe fruits were shorter than unripe fruits for both cultivars, with maximum frequency of chain lengths of 500–1000 nm for 'Huanghua' cultivar and less than 500 nm for 'Zhanhua' cultivar. In contrast, for SSP of unripe fruits, the chain length was greater than 3000 nm for 'Huanghua' jujubes while varying between 1500 and 2000 nm for 'Zhanhua'. At ripe stage, the maximum frequency of pectin length of 'Huanghua' jujubes was among 1000–1500 nm while less than 500 nm for 'Zhanhua' cultivar. Thus, during ripening, the chains of pectin with greater lengths decreased while the chains of smaller length increased, which may affect the changed properties of jujubes during ripening.

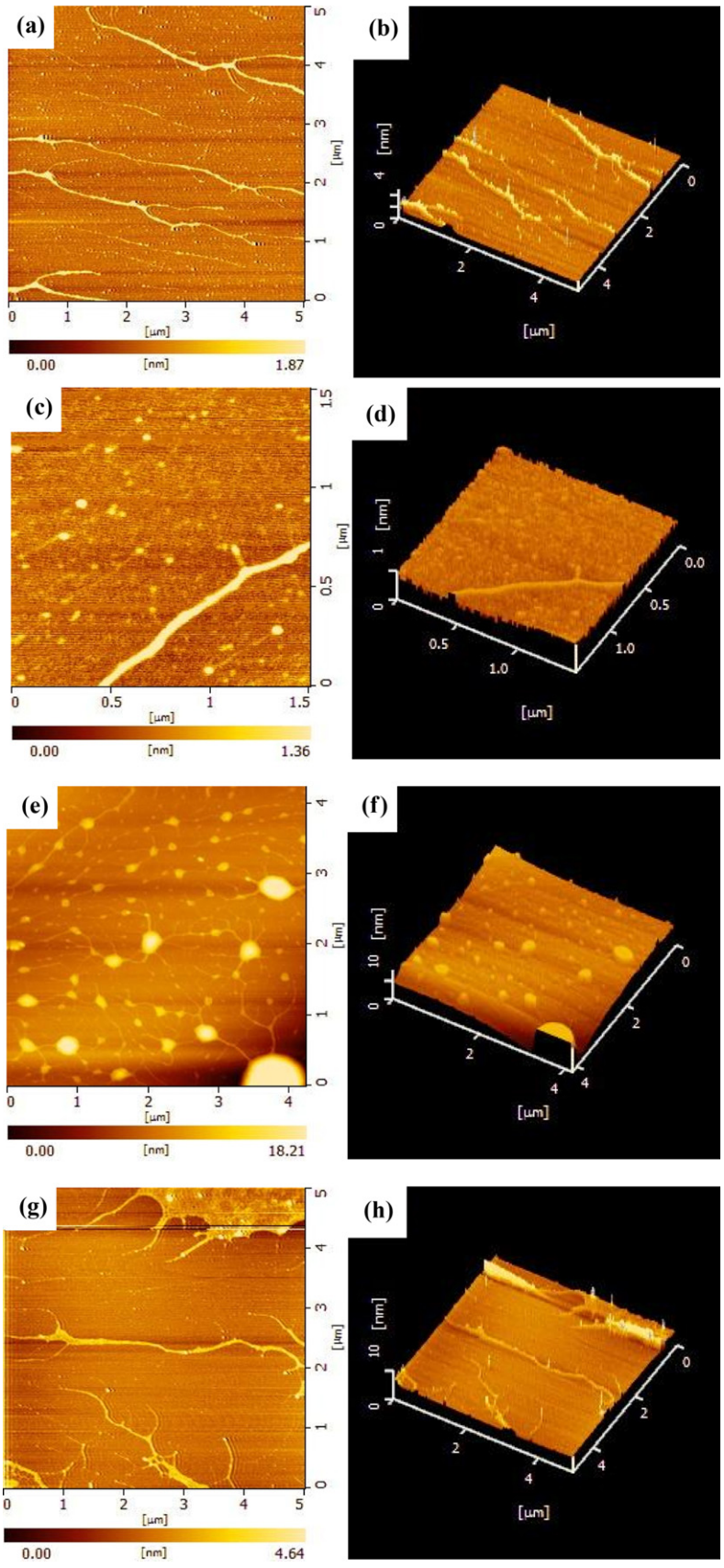

Fig. 1. AFM images of chelate-soluble pectin from two cultivar Chinese jujubes with two ripening stages. Typical plane (a) and 3-dimensional (3D) (b) images of unripe 'Huanghua' fruits, scan size: 5.0 μm × 5.0 μm; typical plane (c) and 3D (d) images of ripe 'Huanghua' group, scan size: 1.5 μm × 1.5 μm; typical plane (e) and 3D (f) images of unripe 'Zhanhua' fruits, scan size: 4.2 μm × 4.2 μm; typical plane (g) and 3D (h) images of ripe 'Zhanhua' fruits, scan size: 5.0 μm × 5.0 μm.

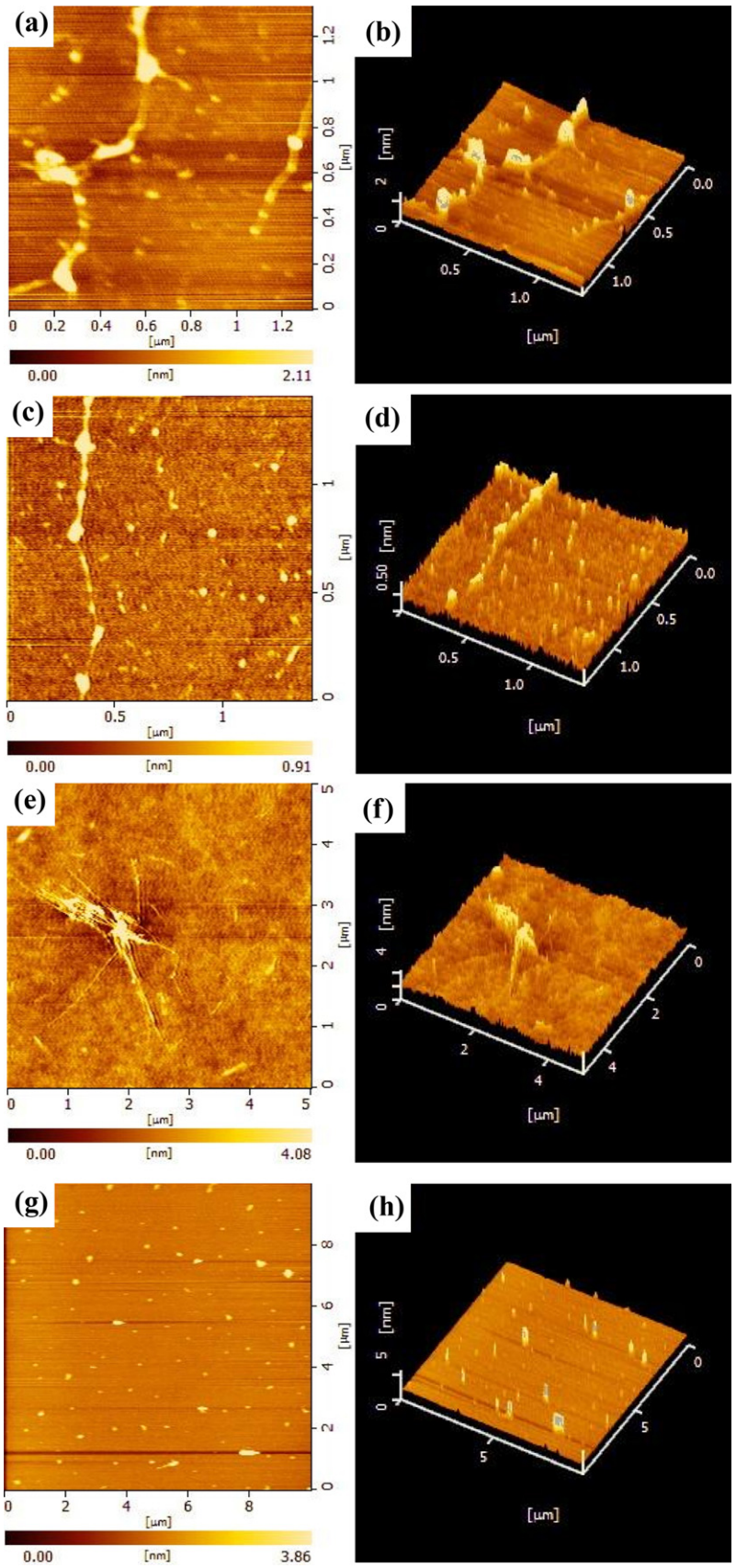

Fig. 2. AFM images of sodium carbonate-soluble pectin from two cultivar Chinese jujubes with two ripening stages. Plane (a) and 3D (b) images of unripe 'Huanghua' fruits, scan size: 1.3 μm × 1.3 μm. Plane (c) and 3D (d) images of ripe 'Huanghua' group, scan size: 1.4 μm × 1.4 μm. Plane (e) and 3D (f) images of unripe 'Zhanhua' fruits, scan size: 5.0 μm × 5.0 μm. Plane (g) and 3D (h) images of ripe 'Zhanhua' fruits, scan size: 10.0 μm × 10.0 μm.

Table 2
Neutral sugar compositions (mg/100 g) of jujube WSP, CSP and SSP in the two cultivars and two ripening stages.[a]

Monosaccharide		Unripe 'Huanghua'	Ripe 'Huanghua'	Unripe 'Zhanhua'	Ripe 'Zhanhua'
WSP	Man	1.12 ± 0.01c	1.91 ± 0.04b	2.11 ± 0.10a	2.01 ± 0.03b
	Rha	21.56 ± 0.51c	19.34 ± 0.30d	42.93 ± 1.06b	45.84 ± 1.57a
	GalUA	15.79 ± 0.15d	31.37 ± 1.24c	36.17 ± 0.80b	42.00 ± 1.24a
	Glc	3.12 ± 0.04c	3.03 ± 0.11c	5.89 ± 0.12a	4.13 ± 0.10b
	Gal	22.82 ± 2.76c	30.49 ± 2.18b	36.40 ± 2.40a	37.66 ± 2.12a
	Xyl	0.80 ± 0.05c	1.32 ± 0.04a	0.87 ± 0.06c	1.09 ± 0.07b
	Ara	2.51 ± 0.04c	2.80 ± 0.06c	8.36 ± 0.21b	9.68 ± 0.24a
	Fuc	8.91 ± 0.74ab	5.56 ± 0.66c	8.63 ± 0.51b	10.28 ± 1.18a
CSP	Man	1.50 ± 0.13c	2.70 ± 0.12a	0.53 ± 0.11d	1.81 ± 0.07b
	Rha	2.35 ± 0.10d	4.81 ± 0.13c	6.04 ± 0.09b	6.74 ± 0.08a
	GalUA	4.01 ± 0.17d	6.74 ± 0.08b	4.73 ± 0.21c	7.06 ± 0.16a
	Glc	12.18 ± 1.78a	8.58 ± 0.74bc	11.13 ± 2.15ab	6.07 ± 0.59c
	Gal	5.90 ± 1.26c	10.36 ± 1.31a	7.87 ± 0.13b	8.38 ± 0.14b
	Xyl	0.47 ± 0.12c	0.95 ± 0.17b	1.14 ± 0.05ab	1.31 ± 0.25a
	Ara	3.04 ± 0.15d	4.79 ± 0.21a	3.64 ± 0.05c	4.00 ± 0.19b
	Fuc	0.88 ± 0.11d	1.48 ± 0.33c	2.07 ± 0.08b	2.45 ± 0.17a
SSP	Man	–	–	–	–
	Rha	0.27 ± 0.12d	0.66 ± 0.05c	0.88 ± 0.08b	1.31 ± 0.11a
	GalUA	1.66 ± 0.19c	2.51 ± 0.08a	2.01 ± 0.18b	0.50 ± 0.16d
	Glc	2.04 ± 0.31bc	2.50 ± 0.09a	2.23 ± 0.11ab	1.77 ± 0.07c
	Gal	0.56 ± 0.09b	1.02 ± 0.13a	0.88 ± 0.05a	0.70 ± 0.08b
	Xyl	–	–	–	–
	Ara	0.51 ± 0.09b	1.10 ± 0.32a	0.64 ± 0.07b	–
	Fuc	–	–	–	–

[a] The values that have different letters in the same line are significantly ($P < 0.05$) different. Symbol '–' means no detection or below the detection level.

3.4. Relationship between the nanostructure of pectins and the corresponding jujube physicochemical properties

Table 1 shows that unripe fruits had a higher value of hardness than ripe fruit for both cultivars, the corresponding nanostructural chains of CSP and SSP demonstrated that for each cultivar, the unripe fruit had more frequency of wider chains of pectin than ripe fruit (Table 3). The results demonstrated that chain widths of pectins had a close relationship with the hardness of jujube fruits. Ripe jujube fruits contained more short pectin chains and lower values of hardness than unripe ones, indicating that the degradation of pectins in length was also related to decreased firmness. This type of relationship between nanostructure of pectins and hardness of jujube fruits was also found in Chinese cherries

Table 3
Frequency (*Fq*) and vertical distances (*V*) of chain widths (*W*) of CSP and SSP in the two cultivars and two ripening stages.[a]

Pectin	*W* (nm)	Unripe 'Huanghua'		Ripe 'Huanghua'		Unripe 'Zhanhua'		Ripe 'Zhanhua'	
		Fq (*N* (%))	*V* (nm)	*Fq* (*N* (%))	*V* (nm)	*Fq* (*N* (%))	*V* (nm)	*Fq* (*N* (%))	*V* (nm)
CSP	15.63	–	–	–	–	–	–	2 (8.7)	0.41 ± 0.35
	19.53	–	–	2 (12.5)	0.38 ± 0.17	–	–	9 (39.1)	0.59 ± 0.20
	23.44	–	–	5 (31.3)	0.57 ± 0.11	2 (11.1)	0.67 ± 0.29	4 (17.4)	0.58 ± 0.22
	31.25	2 (10.0)	0.91 ± 0.05	5 (31.3)	0.77 ± 0.29	2 (11.1)	0.97 ± 0.42	1 (4.3)	0.75 ± 0.00
	35.16	3 (15.0)	1.00 ± 0.54	2 (12.5)	1.4 ± 0.23	6 (33.3)	1.32 ± 0.63	–	–
	39.06	6 (30.0)	1.11 ± 0.69	1 (6.3)	1.23 ± 0.00	2 (11.1)	1.17 ± 0.35	4 (17.4)	0.85 ± 0.37
	46.88	6 (30)	1.34 ± 0.44	–	–	–	–	–	–
	54.69	–	–	–	–	3 (16.7)	1.11 ± 0.51	1 (4.3)	1.32 ± 0.00
	58.59	2 (10.0)	2.89 ± 1.07	1 (6.3)	1.66 ± 0.00	–	–	–	–
	62.50	1 (5.0)	3.42 ± 0.00			1 (5.6)	1.10 ± 0.00	1 (4.3)	1.58 ± 0.00
	78.13	–	–	–	–	1 (5.6)	2.83 ± 0.00	1 (4.3)	1.75 ± 0.00
	97.66	–	–	–	–	1 (5.6)	1.46 ± 0.00	–	–
SSP	15.63	–	–	–	–	–	–	2 (11.8)	1.33 ± 0.48
	19.53	–	–	–	–	–	–	6 (35.3)	1.41 ± 0.34
	23.44	–	–	–	–	–	–	2 (11.8)	1.56 ± 0.70
	31.25	–	–	4 (15.4)	0.51 ± 0.29	–	–	1 (5.9)	1.64 ± 0.00
	35.16	3 (14.3)	0.80 ± 0.39	3 (11.5)	0.75 ± 0.27	–	–	1 (5.9)	1.76 ± 0.00
	39.06	2 (9.5)	0.71 ± 0.05	9 (34.6)	0.24 ± 0.22	2 (8.3)	2.56 ± 1.10	2 (11.8)	1.93 ± 0.38
	46.88	2 (9.5)	0.24 ± 0.11	2 (7.7)	0.22 ± 0.11	2 (8.3)	2.88 ± 0.79	1 (5.9)	2.48 ± 0.00
	58.59	5 (23.8)	1.47 ± 1.31	5 (19.2)	0.37 ± 0.22	1 (4.2)	3.01 ± 0.00	1 (5.9)	2.56 ± 0.00
	62.50	1 (4.8)	1.18 ± 0.00	1 (3.8)	1.05 ± 0.00	5 (20.8)	3.07 ± 1.83	1 (5.9)	2.89 ± 0.00
	70.31	1 (4.8)	1.27 ± 0.00	1 (3.8)	1.10 ± 0.00	–	–	–	–
	78.13	5 (23.8)	2.35 ± 1.04	1 (3.8)	2.10 ± 0.00	5 (20.8)	3.22 ± 1.86	–	–
	82.03	1 (4.8)	2.86 ± 0.00	–	–	3 (12.5)	3.28 ± 0.85	–	–
	97.66	1 (4.8)	3.46 ± 0.00	–	–	1 (4.2)	3.80 ± 0.00	–	–
	108.61	–	–	–	–	1 (4.2)	3.92 ± 0.00	–	–
	135.76	–	–	–	–	2 (8.3)	3.20 ± 0.34	–	–
	156.25	–	–	–	–	2 (8.3)	3.02 ± 0.59	–	–

[a] *W*, the peak width of chain half height; *V*, the height of pectin chains; *Fq*, the numbers of times particular pectin chain widths were observed. Symbol '–' means no detection or below the detection level.

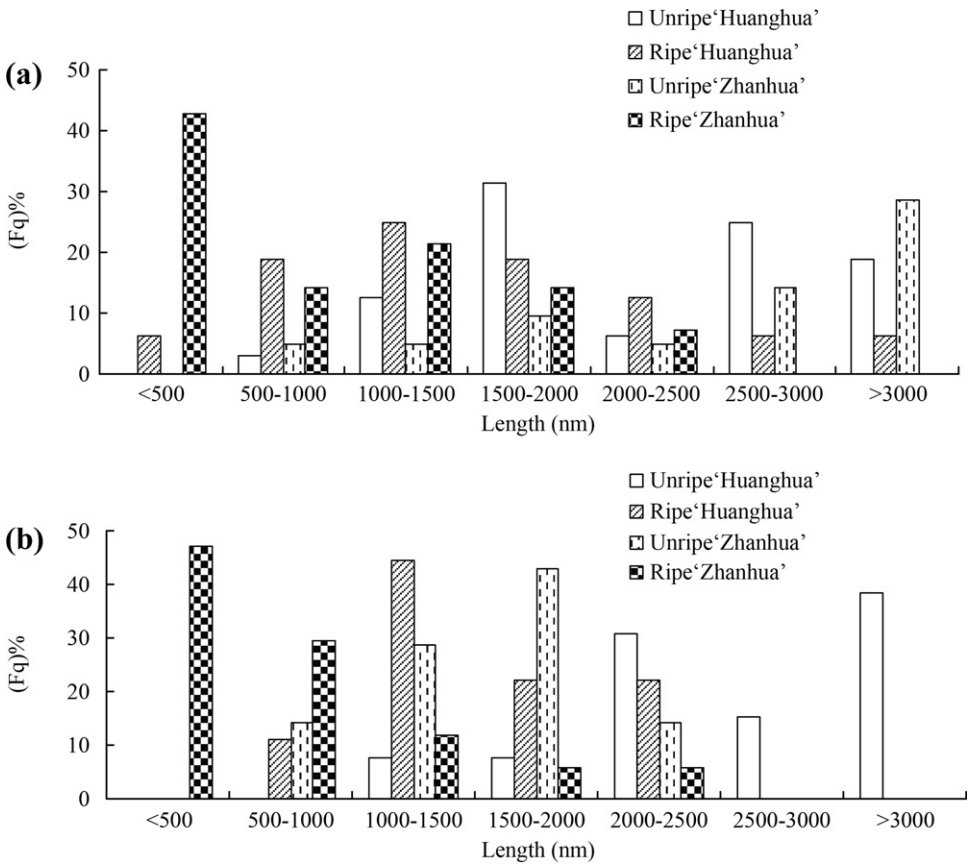

Fig. 3. Frequencies of pectin chain lengths of two cultivar jujubes with two different ripening stages. (a) Chelate-soluble pectin. (b) Sodium carbonate-soluble pectin.

(Zhang et al., 2008), suggesting some similar properties exist among fruits.

4. Conclusions

During ripening, the weight, pH, hardness, and chewiness of both jujube cultivars decreased while the TA, TSS, and pectin contents increased. For WSP, Gal, Rha, and GalUA were the major compositions while for CSP and SSP, except the same major compositions as WSP, Glc was additional major composition. In addition, SSP of the jujubes did not contain Man, Xyl and Fuc. Compared to unripe fruits, all pectins of ripe fruits had less percentage of wide and long pectin chains. Moreover, all the pectin contents of 'Huanghua' cultivar changed to a degree greater than that of cultivar 'Zhanhua' during ripening. Overall, pectin degradation at nanoscale level and the changes of neutral sugar composition may contribute to different physicochemical properties of jujubes.

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