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Nanostructural difference of water-soluble pectin and chelate-soluble pectin among ripening stages and cultivars of Chinese cherry[†]

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Nanostructure of water-soluble pectin (WSP) and chelate-soluble pectin (CSP) of two Chinese cherry (*Prunus pseudocerasus* L.) cultivars (soft cultivar 'Caode' and crisp cultivar 'Bende') with two different ripening stages were characterised using atomic force microscopy. Both cultivars shared some common values of chain widths for WSP or CSP, and both pectins shared several values of chain widths including 37, 55 and 61 nm. The results indicate that different cultivars shared similar components of pectin, and cultivar textural difference might be related to the interaction between pectin and other cherry components or the dissociation of pectin. During ripening, the wide WSP and CSP gradually dissociate in width. The results demonstrated that the changes of WSP and CSP of Chinese cherry in widths were a dissociation process.

Keywords: atomic force microscopy; pectin; nanostructure; ripening; cherry

1. Introduction

Cherry is a fruit which is widely consumed in many countries due to its nutrients as well as sensory quality (Martínez-Romero et al., 2006; Vursavus, Kelebek, & Selli, 2006). Chinese cherry (*Prunus pseudocerasus* L.) is a valuable fruit, the marketing value of which is largely dependent on its flesh texture. It is critical to understand the fundamental of the textural properties in order to better maintain and improve the postharvest properties of this fruit.

According to textural properties, Chinese cherry has two categories: soft cultivar and crisp cultivar (Chen et al., 2009; Zhang et al., 2008). Previous reports showed that nanostructural difference of sodium carbonate-soluble pectin (SSP) of two Chinese cherry cultivars at two ripening stages, SSP was the most-related pectin components to the physicochemical properties (Zhang et al., 2008). However, the differences of water-soluble pectin (WSP) and chelate-soluble pectin (CSP) among different Chinese cherries are still unclear. Recently, these two pectins in peaches were reported to have a degradation-mode

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independent of the changes of their physicochemical properties (Zhang et al., 2012). Therefore, it is very interesting to obtain a general idea of the differences of pectins among Chinese cherry with different cultivar and ripening stages.

Food nanotechnology provides a good tool to investigate the fundamental of food properties via characterising and manipulating food nanostructures (Yang, An, & Li, 2006; Yang et al., 2007). Atomic force microscopy (AFM) is a nanotechnology tool, which has successfully been applied to elucidate fruit pectins' morphological (Yang, Chen, An, & Lai, 2009), quantitative and quantitative changes (Fishman, Cooke, Chau, Coffin, & Hotchkiss, 2007; Zhang et al., 2008). The information from AFM could be used for elucidating the changes of quality attributes.

The objective of this study was to investigate the difference of WSP and CSP of Chinese cherry among different cultivars and ripening stages. The results could help understand the fundamental of texture difference of different Chinese cherries and maintain high quality of texture properties.

2. Results and discussion

Figure 1 shows the AFM images of WSP from different ripening stages and cultivars. It shows that ripening stages and cultivars influenced the qualitative morphology of WSP. Unripe fruit WSP was more conglomerated while ripe fruit WSP was much loose and dissociated. As shown in Figure 1, qualitative characteristics of WSP, such as branching

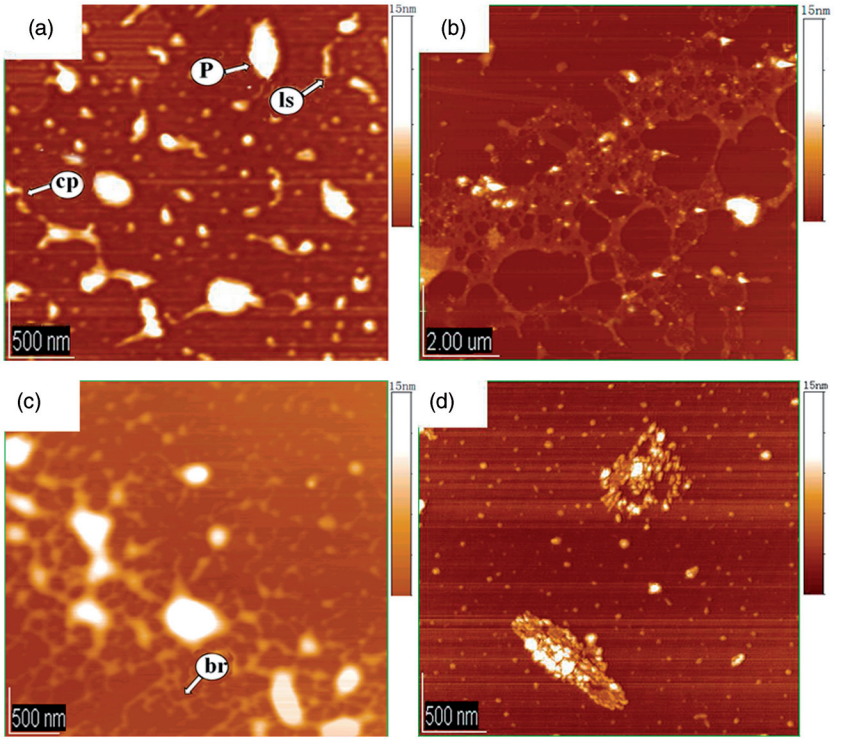

Figure 1. AFM images of WSP from Chinese cherry fruits. Height scale: 15 nm. (a) soft ripe fruit, image size: 2.95 μm × 2.95 μm; (b) soft unripe fruit, image size: 10.17 μm × 9.33 μm; (c) crisp ripe fruit, image size: 3.83 μm × 3.83 μm and (d) crisp unripe fruit, image size: 3.00 μm × 3.00 μm.
Note: P: polymers; ls: linear single fraction; cp: cleavage point; br: branching.

(br), cleavage point (cp), linear single fraction (ls) and polymers (P), can be clearly demonstrated by high resolution AFM images, these characteristics were similar to WSP from other fruits such as peaches or other pectins in Chinese cherry or other fruits (Yang, Lai, An, & Li, 2006; Zhang et al., 2008). Compared with ripe cherries, WSP in unripe cherries in both cultivars had more polymers that entangled or associated together, which was similar to SSP in Chinese cherries (Zhang et al., 2008).

Figure 2 shows the AFM images of CSP from different ripening stages and cultivars. The statistical results of CSP chain widths were shown in Table 2. The trend of CSP changes was similar to that of WSP discussed above.

As reported before, AFM was also powerful in determining the quantitative information of pectin chains. Quantitative parameters of pectin chains were determined using a section analysis of AFM. Detailed information about section analysis could be referred to Zhang et al. (2008). The quantitative information of pectin chains was recorded, calculated and reported with corresponding parameters. W denoted the peak width of half height of pectin chains; V represented the height of WSP chains, and Fq referred to the numbers of times particular chain widths were observed. The peak width of chain half height was used for determining the pectin chain widths (W). The way of width determination here was the same as SSP of Chinese cherries for maintaining consistence (Zhang et al., 2008). It should be noted that the colour bar legends (0–15 nm) at the right of images represented the scale of height of the samples scanned, which can be applied to calculate the height of the pectin chains (*z* scale). This z scale can be modified offline for

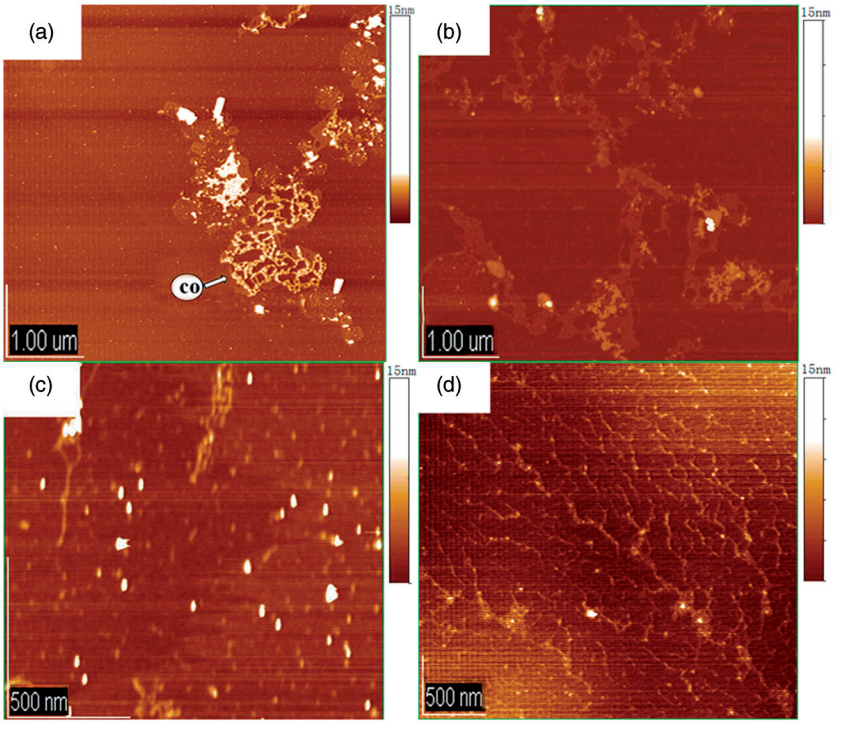

Figure 2. AFM images of CSP from Chinese cherry fruits. Height scale: 15 nm. (a) soft ripe fruit, image size: 4.96 μm × 4.96 μm; (b) soft unripe fruit, image size: 4.96 μm × 4.96 μm; (c) crisp ripe fruit, image size: 1.56 μm × 1.12 μm and (d) crisp unripe fruit, image size: 2.95 μm × 2.95 μm.
Note: co indicates conglomeration.

Table 1. Quantitative parameters of WSP of two Chinese cherry fruits.

W (nm)	Ripe soft fruit		Unripe soft fruit		Ripe crisp fruit		Unripe crisp fruit	
	Fq(N(%))	V (nm)	Fq(N(%))	V (nm)	Fq(N(%))	V (nm)	Fq(N(%))	V (nm)
37	1(20)	1.90 ± 0.00	–	–	3(37.5)	2.55 ± 0.01	–	–
55	3(60)	2.58 ± 0.27	–	–	–	–	–	–
61	1(20)	1.76 ± 0.00	–	–	4(50)	3.73 ± 0.45	–	–
76	–	–	1(12.5)	0.95 ± 0.00	–	–	–	–
82	–	–	–	–	1(12.5)	5.47 ± 0.00	–	–
85	–	–	2(25.0)	0.92 ± 0.00	–	–	–	–
91	–	–	3(37.5)	1.55 ± 0.88	–	–	1(16.7)	6.12 ± 0.00
110	–	–	1(12.5)	2.53 ± 0.00	–	–	2(33.3)	4.40 ± 0.85
140	–	–	–	–	–	–	2(33.3)	7.21 ± 0.28
176	–	–	1(12.5)	4.38 ± 0.00	–	–	1(16.7)	8.07 ± 0.00

Note: W: the peak width of half height of WSP chains; V: the height of WSP chains; Fq: the numbers of times particular chain widths were observed; N: Percent of particular chain widths to all the chain widths.

Table 2. Quantitative parameters of CSP of two Chinese cherry fruits.

W (nm)	Ripe soft fruit		Unripe soft fruit		Ripe crisp fruit		Unripe crisp fruit	
	Fq(N(%))	V (nm)	Fq(N(%))	V (nm)	Fq(N(%))	V (nm)	Fq(N(%))	V (nm)
17	–	–	–	–	9(75)	1.98 ± 0.32	–	–
27	1(14.3)	1.04 ± 0.00	–	–	3(25)	2.48 ± 1.07	–	–
37	5(71.4)	1.63 ± 0.50	3(50)	1.89 ± 0.83	–	–	3(60)	2.85 ± 1.64
47	–	–	1(16.7)	1.07 ± 0.00	–	–	1(20)	3.46 ± 0.00
55	1(14.3)	3.26 ± 0.00	1(16.7)	2.18 ± 0.00	–	–	1(20)	8.41 ± 0.00
61	–	–	1(16.7)	1.70 ± 0.00	–	–	–	–

Note: W: the peak width of half height of CSP chains; V: the height of CSP chains; Fq: refers to the numbers of times particular chain widths were observed; N: Percent of particular chain widths to all the chain widths.

better comparing different groups. In this manuscript, all the z ranges of AFM images were set 15 nm.

The quantitative results of pectin widths of WSP were shown in Table 1. The results clearly demonstrated that for the same cultivar fruits, ripe fruit had a higher frequency of small value widths of WSP, for instance, for soft cultivar ('Caode'), ripe fruit only had the WSP widths of 37, 55 and 61 nm, while unripe fruit had the WSP widths larger than 76 nm. Similar results were found in crisp cultivar ('Bende').

It should be noted that the quantitative value of WSP and CSP of Chinese cherries was not the absolute values of real pectins for at least two reasons as probe-broadening effects and side-by-side molecular associations could bring some inaccuracy of the data obtained by AFM (Yang, Lai, et al., 2006).

Tables 1 and 2 show that both WSP and CSP of Chinese cherries had discontinuous values of W. And, both cultivars shared some values of the widths, which indicated that the pectin components might be independent of the cultivar differences. Previous result

showed both crisp cultivars of peaches had this similar phenomenon. The results that soft and crisp cultivars of Chinese cherries had similar WSP and CSP chain widths would provide valuable knowledge for modifying the texture properties of Chinese cherries without many changes on the pectin components.

However, the current results did not show several basic values clearly for chain widths. SSP shared basic units of 37, 47, 55 and 61 nm of both cultivars and ripening stages. It was still applicable for the width of some values, for instance, 110 nm could be viewed as the sum of two 55 nm, 91 nm could be the sum of 37 and 55 nm and 176 nm could be composed by 37, 55 and 85 nm. However, for some values, 82 and 85 nm of WSP, for instance, cannot be summed from smaller values. Since the current results were much limited due to the limited number of the pectin chains, further study on other fruits is necessary to elucidate the fundamental of pectin chains among different cultivars and ripening stages.

Since we focused on the changes of widths, molecular manipulation of pectins for stretching the chains was not applied for better characterising the natural morphology of pectins. The length difference was then unavailable considering pectins were entangled in the direction of length. However, it could be studied when molecular manipulation is applied to stretch the chains (Yang, An, et al., 2006).

The height distribution of WSP and SSP chains was in the range of 1–8 nm, which was comparable to that of SSP of Chinese cherries (Zhang et al., 2008). The height values were much closer to that of peach (1–2 nm) than orange albedo and tomato (both were around 0.5 nm) (Fishman et al., 2007; Round, Rigby, MacDougall, Ring, & Morris, 2001; Yang, Lai, et al., 2006).

3. Experimental

3.1. *Fruits*

Two cultivars, 'Caode' (soft cultivar) and 'Bende' (crisp cultivar), of Chinese cherry (*Prunus pseudocerasus* L.) with two different ripening stages (ripe and unripe) for each cultivar was used. The ripening stages were determined by locally experienced farmers. The ripe fruit was chosen with fully developed commercial maturity, and the unripe fruit was defined around 7 days before being ripe. The fruits were manually harvested at a farm in Zhengzhou, Henan, China and transported to our laboratory in 2 h after harvest. Fruits of uniform size, no disease and other defects were used for further experiment.

3.2. *Extraction and preparation of cell wall material*

Cell wall material was prepared using the methods described by Zhang et al. (2008). Ten grams of pitted cherry flesh were pestled quickly in ice-cold mortar, then added in boiling ethanol (200 mL of 80% (v/v)) for 20 min. The sample was then cooled to room temperature and filtrated with vacuum pump. The residue was re-extracted using ethanol twice with the same procedure. The residue was then incubated at 4°C overnight, using 50 mL dimethysulphoxide : water (9 : 1, v/v). It was subsequently water washed and transferred to 200 mL of chloroform : ethanol (2 : 1, v/v) for 10 min, then the sample was filtrated and washed with 200 mL acetone until total whitening. The residue obtained was the fraction of cell wall material.

3.3. *WSP and CSP extraction and determination*

The cell wall material was suspended in pH 6.5 sodium acetate buffer (10 mL of 50 Mm) and agitated at 25°C for 4 h, then centrifuged at 4°C with $10{,}000 \times g$ for 10 min. The residue was subject to two additional 50 mM sodium acetate buffers. The soluble fractions were collected as WSP. The water-insoluble pellet was then resuspended in10 mL of 50 mM

sodium acetate buffer (pH 6.5) containing 50 mM CDTA for 4 h with shaking, and centrifuged as described above. Then, the residue was extracted twice with sodium acetate/CDTA, resuspended in 10 mL of 50 mM Na_2CO_3 containing 2 mM CDTA with shaking, and centrifuged as described above. The supernatant was collected as CSP. The experiments were conducted in triplicate.

3.4. *AFM imaging*

AFM imaging was performed according to the method described by Zhang et al. (2008). Pectin solutions were diluted to around 10 $\mu g\,mL^{-1}$ and disrupted with a vortex mixer (Fisher Scientific, Pittsburgh, PA, USA). A small volume of disrupted solutions was pipetted onto a mica surface and air-dried. Pectin morphology was carried out with an AFM (JSPM-5200, JEOL, Japan) in AC mode. NSC 11/no Al (MikroMasch, Wilsonville, Oreg., USA) tip (resonance frequency of 330 KHz and force constant of 48 $N\,m^{-1}$) was applied. All samples were imaged in air (Yang et al., 2007).

3.5. *AFM image offline analysis*

The AFM images obtained were analysed offline using a AFM software (Win-SPM System, Tokyo, Japan) provided by the company. In the height mode image, the bright and dark areas represent different heights with peaks and troughs, respectively. Height mode images were analysed and the quantitative results of pectin widths were measured by a section analysis (Yang et al., 2007).

3.6. Statistical analysis

SAS 9.1.3 software (SAS Inst. Inc., Cary, NC, USA) was used to analyse the analysis of variance (ANOVA; $p < 0.05$) and Duncan's multiple range test for differences among different groups. The quantitative results were represented as means ± standard deviations. Comparisons that yielded $p < 0.05$ were denoted significant. For each group, dozens of AFM images from different parallel samples and different scan areas were analysed for getting reliable and repeatable results.

4. Conclusions

AFM was used to determine the widths of WSP and CSP of soft cultivar 'Caode' and crisp cultivar 'Bende' Chinese cherries with ripe and unripe stages. Some of the chain widths existed in both cultivars and both pectins. Ripe fruits had higher frequency of small value chain widths for both WSP and CSP than unripe fruits, which indicated that pectin chains dissociate in widths during normal ripening process. The results could be used for directing measures to improve the texture properties of Chinese cherry in order to maintain the maximum commercial value during postharvest.

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