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Extraction temperature is a decisive factor for the properties of pectin

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ABSTRACT

Pectin is an important food thickener and gelling agent and also, based on its bioactivities, is becoming viewed as a healthy polysaccharide. The functional properties of pectin are related to its structure which, if raw materials are similar, is controlled by extraction conditions. In our previous study we found that pectin recovered from citrus canning processing water showed some unique structural features and properties compared to commercial citrus pectin. Thus, the mechanism of pectin extraction under different conditions including the canning process, an unusual condition for pectin extraction, was studied. The same raw material of citrus fruit segment membrane was used and the properties of the extracted pectin and the resulting residue were characterized. The unusual low extraction temperature, applied in canning, was identified as a key factor for the unique structural features of the recovered pectin. The low-temperature extraction resulted in reduced yields (~9%, half of the commercial process), meanwhile, it limited the hydrolysis of pectin side-chains. The proportion of arabinose (Ara), one major side-chain sugar, was much higher (~23%), than that of commercial citrus pectin, resulting in a lower proportion of galacturonic acid (GalA). Moreover, the low-temperature extracted pectin having these structural features exhibited greater apparent viscosity and higher galectin-3 binding affinity (3.87 μM). The current study suggests the mechanism of pectin extraction and supplies important information for the extraction of functionally tailored pectin.

1. Introduction

Pectin, a viscous polysaccharide, has been widely used in the food industry for hundreds of years as a thickening or gelling agent. Because of its flavor and natural origin from fruit, pectin became the most consumer-friendly hydrocolloid, commanding a global market over $1 billion, which is increasing annually by >5% (Ciriminna, Chavarria-Hernandez, Rodriguez Hernandez, & Pagliaro, 2015). A number of pectin bioactivities have been reported in recent decades (Maxwell, Belshaw, Waldron, & Morris, 2012) resulting in the development of health care pectin products. The functional properties of a pectin are related to pectin's structure (Wicker et al., 2014), which is largely impacted by the extraction method. However, there is still lack of clear information on the relation between structure/function and extraction. In a previous study, we recovered a unique pectic polysaccharide with a low galacturonic acid (GalA) content from citrus canning effluent in a project undertaken to reduce the chemical oxygen demand (COD) of this effluent, resulting in significant environmental and economic benefits (Chen et al., 2017). The mechanism by which this new pectin structure was obtained remains unclear. Citrus peel is the raw material of the commercial citrus pectin and citrus segment membrane is the raw material of the recovered pectin from canning effluent. Since citrus peel and citrus segment membrane were similar, and also, these two raw materials extracted by the same conditions would result in the same pectin (Zhang et al., 2018), we hypothesized that the extraction conditions probably resulted in the unique structural features of the recovered pectin.

Pectin is an old plant polymer which was first reported by Hennri

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Braconnot in 1825 (Willats, Knox, & Mikkelsen, 2006). It is a complex polysaccharide containing multiple different monosaccharide residues but particularly rich in galactoronic acid (GalA). While the precise structure of pectin remains unknown, it is commonly recognized that pectin consists of several different regions or domains, such as HG (homogalacturonan, a linear 1,4-linked α-D-GalA), RGI (rhamnogalacturonan I, a repeating disaccharide backbone of [→4)-α-D-GalA-(1 → 2)- α-L- rhamnose (Rha)-(1→] with different side chain of arabinose (Ara) and galactose (Gal)) and RGII (rhamnogalacturonan II, a HG backbone with rather complex side chain containing Rha) (Mohnen, 2008; Wu et al., 2019). Commercial pectins are mainly extracted from citrus peel and apple pomace and are a by-product of food production. Sugarbeet pectin and sunflower pectin are also of increasing commercial value (Ciriminna et al., 2015).

Most commercial pectins are extracted using hot acids, such as HCl or HNO_3, pH at ~1.5, at elevated temperature of ~85 °C (May, 1990; Mottern & Hills, 1946; Wang et al., 2017; Yapo, Robert, Etienne, Wathelet, & Paquot, 2007). There has been some research aimed at the optimization of extraction conditions including the influence of extraction pH value (Colodel, Vriesmann, Teofilo, & de Oliveira Petkowicz, 2018; Kalapathy & Proctor, 2001; Koubala et al., 2008) and acid type (Kaya, Sousa, Crepeau, Sorensen, & Ralet, 2014; Kliemann et al., 2009). Most extraction temperature optimization were conducted at the range of 60–90 °C (Andersen et al., 2017; Yapo et al., 2007), and a wider range beginning at 40 °C or 50 °C to a temperature of 80 °C has also been studied (Kurita, Fujiwara, & Yamazaki, 2008; Masmoudi et al., 2008). However, most of those extraction factors were optimized with the goal of improving extraction yield but not with the goals of controlling pectin structure and properties. Thus, information about how extraction impacts pectin structure and properties is very limited. In the process of citrus segment membrane removal during canning, using particular condition values, there is a complete lack of understanding of how extraction conditions impact pectin structure/function.

In the citrus segment canning process the hydrolysis temperature is significantly lower than those used in conventional pectin extraction processes so as to maintain citrus segment shape and flavor. The extraction time in the canning process is just half of the conventional time used to prepare citrus pectin. Moreover, the pH of the canning process is slightly lower than that used in the conventional pectin extraction process. The shape of segment membrane material extracted in the canning process is a sheet of the whole membrane surrounding a segment, while in a conventional citrus pectin process a powder is extracted.

This study examines all the factors in pectin extraction to understand the mechanism of pectin extraction under a wide range of conditions and to examine the relationship between extraction conditions and pectin structure and properties. The monosaccharide compositions of both the pectin extract and its residue were analyzed. By characterization of molecular weight, rheological property, and biological activity, the advantages of pectin prepared under canning conditions were identified.

2. Material and methods

2.1. Material and chemicals

Citrus unshiu fruits were collected from a citrus canning company, Ningbo, Zhejiang, China. The segment membranes were separated manually followed by blanching in boiling water for 3 s to thermally inactivate enzyme and air-dried in an oven at 45 °C for 48 h. The dried membranes were stored at room temperature in a desiccator until used. Monosaccharide standards and 1-phenyl-3-methyl-5-pyrazolone (PMP) were purchased from Sigma -Aldrich (Shanghai, China). Galectin-3 (Gal-3) protein was purchased from R&D Systems (USA). All other chemicals were analytical grade.

2.2. Experimental design and pectin extraction

In the canning process, citrus segments were hydrolyzed in an acidic chute for about 40 min. Extraction took place at room temperature and at a pH of ~1. The segment membranes being extracted were in the shape of a sheet. Thus, four conditions were identified to be different from the commercial method used to extract citrus pectin. For comparing the extraction impact between commercial pectin and canning process pectin, a series of experiments presented in Table 1 was designed by changing the condition one by one from canning process to commercial process. Sample 1 and sample 2 was the pectin extracted under commercial process and canning process, respectively. Sample 3 to 5 each experiment changing one condition level was to study the condition impact. Sample 6 was designed for increasing the GalA content based on the result of experiments 1 to 5. Dried citrus segment membrane as the raw material was used in all the experiments. The centrifugal mill (Model ZM 200, Retsch) with a mesh (0.25 mm aperture) was used for preparing the powdery sample. All the extractions used a solid–liquid ratio of 1:30 (w/v).

After extraction, the sample was centrifuged at 9300×*g* for 10 min. The resulting precipitate was washed once with deionized water followed by the re-centrifugation and a second washing. The two supernatants were collected together and after adjusting the pH to 3.5 were dispersed into two volumes of absolute ethanol. The precipitate was then separated by filtration through nylon cloth (38 μm), washed with ethanol, and squeezed dry before re-dissolving in water and freeze-drying.

2.3. Determination of monosaccharide composition

Monosaccharide composition was determined following PMP derivatization using reverse high-performance liquid chromatography (HPLC) (Strydom, 1994). In brief, dry samples (typically 2–3 mg) were dissolved in 2 M trifluoroacetic acid and hydrolyzed at 110 °C for 8 h, dried under a stream of nitrogen, and neutralized with 0.1 M sodium hydroxide. The derivatization was conducted by adding 450 μL PMP solution (0.5 M, in methanol) and 450 μL of 0.3 M sodium hydroxide to each hydrolyzed sample and incubating at 70 °C for 30 min. A 20 mM lactose solution was used as an internal standard. The reaction was stopped by using 0.3 M HCl to adjust the pH to neutral. Excess PMP reagent was removed by extracting with chloroform three-times. HPLC was equipped by Waters e2695 (Waters, US) with UV detection (2489 UV/Vis Detector, Waters, US) and used to monitor eluent at 250 nm and at 25 °C. The analysis used a Zorbax Eclipse XDB-C18 column (250 mm × 4.6 mm, 5 μm, Agilent, USA) with the mobile phases of solvent A (15% acetonitrile with 0.05 M potassium phosphate buffer (pH 6.9)) and solvent B (40% acetonitrile with the same buffer), eluted with gradient from 0% to 15% (solvent B) in the first 10 min, then from 15% to 25% (solvent B) in the next 20 min.

Table 1
Experiment was designed for comparing the commercial extraction process (sample 1) and canning process of pectin extraction (sample 2). Changing the condition one by one was conducted in experiment 3 to 5. In experiment 6 an attempt was made to increase GalA based on the result of experiments 1 to 5.

Sample names	Extraction conditions			
	Temperature/ °C	Time/ min	pH	Shape of raw material
1	**85**	**90**	pH=**1.5**	**powdery**
2	25	40	pH = 1	Sheet
3	**85**	40	pH = 1	Sheet
4	**85**	**90**	pH = 1	Sheet
5	**85**	**90**	pH=**1.5**	Sheet
6	*40*	40	pH = 1	Sheet

2.4. IR spectral analysis

The Fourier transform (FT) infrared (IR) analysis was performed using a Nicolet Avatar 370 instrument to obtain IR spectra. The degree of methoxylation (DM) was calculated by integration of the peaks area central at 1740 and 1630 cm^{-1} with applying the standard curve established by Monsoor et al. (Monsoor, Kalapathy, & Proctor, 2001). Samples (~1 mg) were dried (105 °C, 6 h), ground with 200 mg KBr, and then pressed into pellets. IR spectra were obtained using 32 scans over a range of wave numbers from 400 to 4000 cm^{-1}.

2.5. SEC-MALLS analysis

Samples were dissolved in 0.2 M NaCl solution to obtain a concentration of 1 mg/mL and filtered through a syringe-filter (membrane pore size, 0.45 μm) prior to injection using a 100 μL sample loop. The molar mass and root mean square (RMS) radius of gyration were determined. The detector used was a multi-angle laser-light scattering (MALLS) instrument (Wyatt Dawn Heleos-II, USA) combined with differential refractive index (RI) detector (Waters, USA). Isocratic elution on a series of columns (Shodex OH SB-G (pre-column), Shodex SB-806 HQ and Shodex SB-804 HQ (Showa Denko KK, Japan)) was performed (Mobile phase: 0.2 M NaCl solution, flow rate: 0.75 mL/min). Data were processed using ASTRA 6.1 software with the dn/dc value of 0.1355 mL/g.

2.6. Rheological measurements and visible viscosity test

The rheology properties were determined using a rheometer (Anton Paar MCR 302, Austria) with a cone (50 mm diameter, 1°) and plate geometry at 25 °C. All the samples were prepared in 2% (w/v) aqueous solution followed by centrifugation for degassing. Samples were stood for 3 h after degassing and stood for 20 min after the samples being gently loaded between the test plates.

The flow behaviors of the solution samples were presented by the viscosity curves with error bars of triplicate determinations and the data were fitted to the Herschel–Bulkley model: $\tau = \sigma_0 + K(\gamma)^n$, where τ is the shear stress, σ_0 is the yield stress, K is the consistency index, γ is shear rate and n is the flow behavior index.

The visible viscosity test was presented by images recorded by the camera (Canon EOS 550D). Samples were all dissolved by the same stirring in the same type of tube (10 mL) and stood for 1 h, then put down the tube on the same platform in the same direction. The timer was started immediately when the tubes were put down. After 5 s, the flowing state of the sample solution was recorded by the camera showing the flow distance reflecting the sample viscosity.

2.7. Surface plasmon resonance (SPR) assay

The affinity of pectin binding with Gal-3 was measured using a SPR instrument (BIAcore 3000 system, GE Healthcare). Gal-3 dissolved in a 200 μg/mL solution was injected into an activated CM-5 sensor chip for Gal-3 immobilization. Pectin samples were dissolved in HBS-EP buffer (0.01 M HEPES, 0.15 M NaCl, 3 mM EDTA, and 0.005% surfactant P20, pH 7.4) and diluted to a series of concentrations. Each sample was injected over the sensor chip for 180 s at a flow rate of 30 μL/min. The running buffer of HBS-EP was continually flowed for another 180 s as the dissociation period. The sensorgram was recorded and the data was analyzed using the BIAevaluation Software 4.1.1. The K_D (dissociation constant) was calculated by kd/ka, where kd is dissociation rate constant and ka is association rate constant.

2.8. Statistical analysis

Analysis of variance was carried out by SPSS 20.0 (IBM Corp., Armonk, NY, USA) with Tukey's test. Means were separated with labeling different letters as significantly different ($p < 0.01$).

3. Results and discussion

3.1. Monosaccharide composition

The extraction experiments were initially conducted the first five groups (labeled 1 through 5 in Table 1). The monosaccharide compositions were determined. Table 2 shows the monosaccharides were different between experiments 1 and 2, consistent with our former results (Chen et al., 2017). The canning process pectin (experiment 2) contained a significantly ($p < 0.01$) lower content of GalA, Rha and Gal, while higher content of Ara. This represents the unique compositional feature of the pectin recovered in our canning process. In experiment 3 as compared with experiment 2, the temperature as the only condition changed, adjusting it to the commercial extraction temperature. Surprisingly, sample 3 showed a similar monosaccharide composition as sample 1 (commercial process), with decreased Ara content and increased GalA content (Rha and Gal contents again show the same changing trends with GalA which, as the major and representative monosaccharide, will be discussed in the following). Thus, we find a key factor controlling pectin composition in comparing the first step. A change in temperature from 25 °C to 85 °C results in a major change in extract composition. This rule may be universally suitable for other raw materials, such as, citrus peel (Zhang et al., 2018), or even watermelon rind, an unusual material for pectin extraction, showing high GalA content with high-temperature extraction (Petkowicz, Vriesmann, & Williams, 2017).

The impact of other factors on the two extraction processes were next examined in experiments 4 and 5 to ascertain whether the temperature was the only important factor. Comparing the monosaccharide compositions between 3 and 4, 4 and 5, 5 and 1, most of them has no significant differences. These results suggest extraction time, pH, and material shape may not contribute the differences observed in the monosaccharide compositions of the pectin obtained in 1 and 2.

Based on the above results, showing that increased extraction temperature increased GalA content, an extraction temperature of 40 °C was

Table 2
Monosaccharide compositions (mol%) of samples from experiments 1 through 6.

	Sample names					
	1	2	3	4	5	6
Man	0.13 ± 0.03^{a}	0.15 ± 0.01^{a}	0.17 ± 0.19^{a}	0.32 ± 0.19^{a}	0.08 ± 0.05^{a}	0.08 ± 0.01^{a}
Rha	6.91 ± 0.43^{d}	3.57 ± 0.12^{a}	6.22 ± 1.01^{cd}	5.13 ± 0.83^{bc}	5.88 ± 0.23^{cd}	3.84 ± 0.03^{ab}
GlcA	0.25 ± 0.03^{a}	0.27 ± 0.02^{a}	0.2 ± 0.05^{a}	0.16 ± 0.11^{a}	0.17 ± 0.08^{a}	0.27 ± 0.03^{a}
GalA	74.85 ± 1.46^{b}	62.74 ± 0.47^{a}	74.91 ± 2.32^{b}	76.35 ± 0.85^{b}	73.42 ± 0.57^{b}	60.22 ± 0.65^{a}
Glc	2.16 ± 0.18^{b}	2.01 ± 0.23^{b}	2.18 ± 0.21^{b}	1.96 ± 0.39^{b}	1.9 ± 0.12^{ab}	1.31 ± 0.22^{a}
Gal	13.06 ± 0.8^{c}	7.57 ± 0.21^{a}	14.31 ± 0.81^{c}	14.4 ± 0.85^{c}	16.02 ± 0.17^{d}	9.95 ± 0.03^{b}
Xyl	0.76 ± 0.01^{bc}	0.49 ± 0.05^{ab}	0.83 ± 0.01^{cd}	1.05 ± 0.26^{d}	0.73 ± 0.03^{bc}	0.4 ± 0.01^{a}
Ara	1.86 ± 0.12^{b}	23.2 ± 0.37^{c}	1.16 ± 0.05^{ab}	0.63 ± 0.15^{a}	1.8 ± 0.05^{b}	23.92 ± 0.36^{c}

Means on the same row with different letters are significantly different ($p < 0.01$).

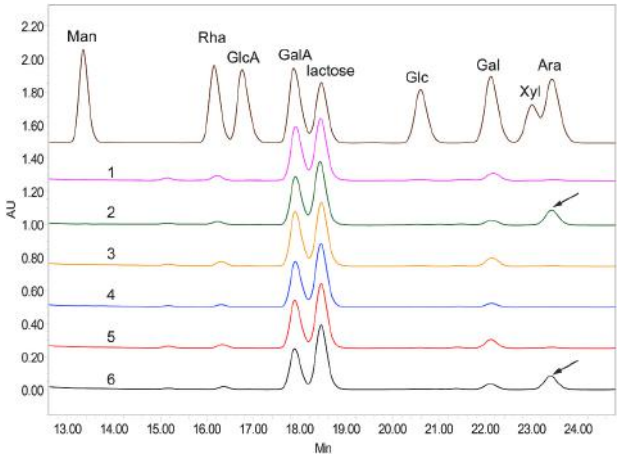

Fig. 1. Monosaccharide compositional analysis by HPLC of samples prepared from experiments 1 to 6 and the standards were separated and labeled on the top of each chromatogram. Differences in high Ara contents in samples from experiments 2 and 6 are marked with arrows.

conducted in experiment 6. On the other hand, since canning process pectin is a by-product from the segment membrane removal of citrus segment canning, 40 °C was the highest temperature limited to the requirement of protecting fruit's shape and flavor. Thus, the temperature possible for increasing GalA was only 40 °C. Unfortunately, GalA content did not increase under this condition. The monosaccharide composition of the pectin obtained in experiment 6 was similar to that observed in experiment 2. The HPLC chromatograms of the monosaccharide compositional analysis clearly showed that the experiments 2 and 6 contained much higher Ara content that correlated to a reduced GalA content (Fig. 1).

3.2. Extraction mechanism

Although the key factor impacting monosaccharide composition was identified, the extraction process is still important to understand. Table 3 shows the yields of the polysaccharides and the residues. Samples from experiments 2 and 6 showed a significantly ($p < 0.01$) lower yield (<10%) of polysaccharide. This can be explained by the reduced efficiency of low-temperature extraction and is consistent with higher residue amounts observed in these samples, suggesting that some of the polysaccharide still remained in the residue. This is probably the reason why most researchers have not used low-temperature extraction when optimizing polysaccharide yields (Andersen et al., 2017; Yapo et al., 2007). In the current study, the reduced recovery of polysaccharide using low-temperature acidic extraction does not pose a problem since the residue is typically further extracted with alkaline water in the canning industry (the recovered polysaccharides from this alkaline water extraction is not discussed in the present study). The reason for the increased content of GalA and the decreased content of Ara in the high-temperature extraction can be explained by side-chain hydrolysis by hot acid, rather than the failure of Ara to be extracted out, because for experiment 1, comparing with experiment 2, the reducing amount of residue was more than the increasing amount of polysaccharide yield. Ara is known to be the major component of pectin side-chains (Mao et al., 2019), and Ara is also more easily hydrolyzed by acid as reported (Morris, Belshaw, Waldron, & Maxwell, 2013). If hydrolysis could be avoided during the high-temperature extraction the yield of polysaccharide could be even higher and the Ara could be retained in the side-chain.

Table 4
The monosaccharide compositions (mol%) of the extraction residues from experiments 1, 2, and 3 are labeled residue 1, 2, and 3, respectively.

	Sample names		
	Residue 1	Residue 2	Residue 3
Man	10.23 ± 0.06^{b}	5.14 ± 0.35^{a}	9.96 ± 0.28^{b}
Rha	5.48 ± 0.06^{a}	6.7 ± 0.69^{b}	5.33 ± 0.36^{a}
GlcA	0.15 ± 0.01^{a}	0.2 ± 0.05^{a}	0.17 ± 0.01^{a}
GalA	1.59 ± 0.05^{a}	7.07 ± 1.77^{b}	2.79 ± 0.31^{a}
Glc	31.59 ± 0.04^{b}	21.33 ± 1.64^{a}	31.35 ± 0.3^{b}
Gal	12.41 ± 0.1^{a}	16.34 ± 0.06^{b}	12.71 ± 0.17^{a}
Xyl	33.54 ± 0.22^{c}	14.73 ± 0.11^{a}	30.38 ± 0.07^{b}
Ara	5.01 ± 0.15^{a}	28.49 ± 0.69^{c}	7.3 ± 0.14^{b}

Means on the same row with different letters are significantly different ($p < 0.01$).

The monosaccharide compositions of the residues generated in experiments 1, 2, and 3 are presented in Table 4. These data provide more information on the extraction. The residues from experiments 1 and 3 contain similar compositions and are very different from that of the residue from experiment 2, further confirming the importance of temperature on the extraction. The Ara and GalA contents in the residue from experiment 2 were significantly ($p < 0.01$) higher than the samples from experiments 1 and 3, even when considering that the residue from experiment 2 is double the amount of the residue from experiments 1 and 3 (Table 3). This suggests that the low-temperature extraction used in experiment 2 still leaves some of the pectin in the residue. Thus, the low-temperature extraction was not efficient. In contrast of the high-temperature extraction, the extraction of Ara from the cell wall and the hydrolysis of Ara from the extracted side-chains of the polysaccharide are both reduced by low-temperature extraction. In addition, all the residues contain high glucose and xylose indicating that most of the cellulose and hemicellulose remain in these residues (Coll-Almela et al., 2015). Fig. 2 presents the mechanism of the pectin extraction, illustrating the important impact of extraction temperature.

3.3. Physicochemical properties study

Monosaccharide compositional analyses in the extraction experiments show the importance of the extraction temperature. The increase of extraction temperature to 40 °C still keeps the monosaccharide composition looking like the one from room temperature extraction. However, 40 °C is the highest temperature that canning process allows. Thus, the composition of the polysaccharide recovered from citrus

Table 3
The polysaccharide yield and the residue amounts from the 6 extraction processes.

Yield/%	Sample names					
	1	2	3	4	5	6
Polysaccharides	21.66 ± 0.41^{bc}	8.81 ± 0.45^{a}	23.83 ± 0.98^{c}	20.07 ± 0.76^{b}	22.10 ± 1.04^{bc}	9.85 ± 0.34^{a}
Residue	25.29 ± 1.12^{a}	62.87 ± 1.56^{d}	31.13 ± 1.34^{c}	30.14 ± 0.86^{bc}	25.56 ± 0.92^{ab}	59.76 ± 2.02^{d}

The yield percentages were based on the raw material on a dry basis.
Means on the same row with different letters are significantly different ($p < 0.01$).

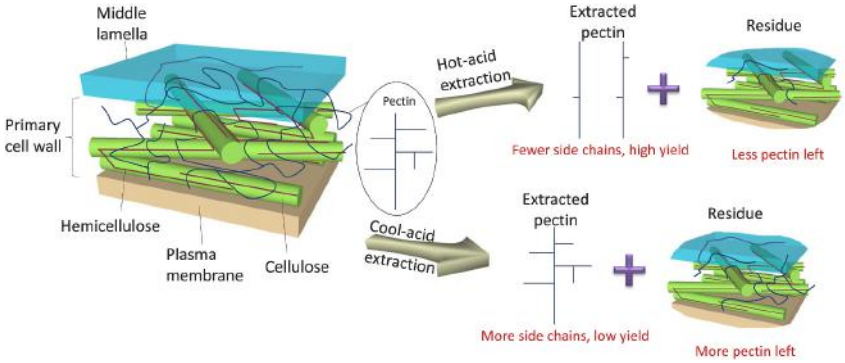

Fig. 2. Mechanism of the pectin extraction under different conditions.

Table 5
Average values of molecular weight and radius of pectin samples.

	Mw[a] (kDa)	Mn[b] (kDa)	Polydispersity (Mw/Mn)	Rz[c] (nm)
1	294.2 ± 0.3%	147.4 ± 0.6%	1.996 ± 0.7%	45.8 ± 0.6%
2	463.3 ± 0.7%	336 ± 0.8%	1.379 ± 1.05%	67.8 ± 0.7%
3	204.2 ± 0.2%	87.6 ± 1%	2.331 ± 0.99%	42.1 ± 0.5%
4	100.6 ± 0.3%	43.6 ± 1.3%	2.305 ± 1.35%	34.9 ± 0.9%
5	205.8 ± 0.2%	85.3 ± 1.4%	2.412 ± 1.36%	41.9 ± 0.4%
6	689.1 ± 0.8%	401 ± 0.6%	1.718 ± 0.96%	68.5 ± 0.8%

[a] Mw: weight-average of Molar mass.
[b] Mn: number-average of molar mass.
[c] Rz: z-average of root mean square radius of gyration.

canning acidic water was relatively fixed. However, utilizing this kind of polysaccharide which now mostly recovered from citrus canning effluent is advantageous for environment. Thus, the other properties of this polysaccharide were necessary to evaluate.

3.3.1. Molecular weight analysis

Table 5 shows the results of molecular weight by weight-average and number-average molecular weight. They both show the sample 2 and 6 have a much higher molecular weight, and their z-average of root mean square radiuses of gyration are also larger, further confirming the side chain hydrolysis occurred during high-temperature extraction, which reduced the molecular weight and size. The samples from high-temperature extraction show a higher polydispersity, indicating the hydrolysis was occurred in different degree.

The molecular weight distribution is shown in Fig. 3A. The samples extracted from the high-temperature experiment 1, 3, 4, and 5 all distribute in the relatively low-molecular weight region. And they all have a broad distribution consistent with the larger polydispersity (Table 5). The log-log plots of RMS radius *vs.* molar mass (Fig. 3B) indicate all the samples are branched since the slope values of linear fitting that aren't 0.333, 0.5–0.6 or 1.0 (Podzimek & Vlcek, 2001). Low temperature extracted samples show larger slope values than high temperature extracts indicating they have different structure conformations.

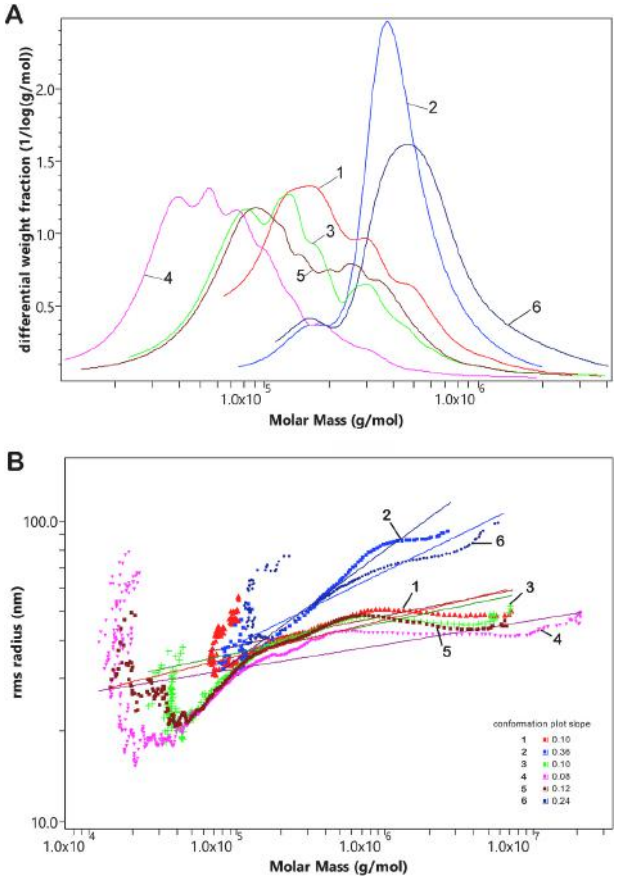

Fig. 3. (A) Molar mass distribution by differential weight fraction. (B) Conformation plot of RMS (root-mean-square) radius vs. molar mass.

3.3.2. Rheology properties

Pectin is widely used as a thickener or gelling agent in food industry. The viscosity curves were presented in Fig. 4A. The data was fitted well by the Herschel-Bulkley model with $R^2 > 0.98$. Table 6 shows the parameters of the fitting model. All of them are non-Newtonian pseudo-plastic fluid since $n < 1$. All curves showed shear thinned behavior, caused by the weakening of pectin intermolecular forces with increased shear rate (Peng et al., 2016). The consistency index *K* corresponds to the apparent viscosity. Sample 2 and 6 show obvious higher *K* values than sample 1, 3, 4, and 5, and the apparent viscosity of the sample 1, 3, 4, and 5 are consistent with the published result whose sample was also extracted under high temperature (Wang & Lü, 2014), confirming that low-temperature extracted pectin could be a better food thickener.

Fig. 4B is the image recorded by camera under the same operation. Sample 2 and 6 contain more bubbles in the solutions indicating the solutions have high viscosity which makes the bubbles hard to escape. And the shorter flow distance of sample 2 and 6 also clearly show the solutions were highly viscous. The high viscosity is probably caused by the samples being highly branched and with large molecular weight.

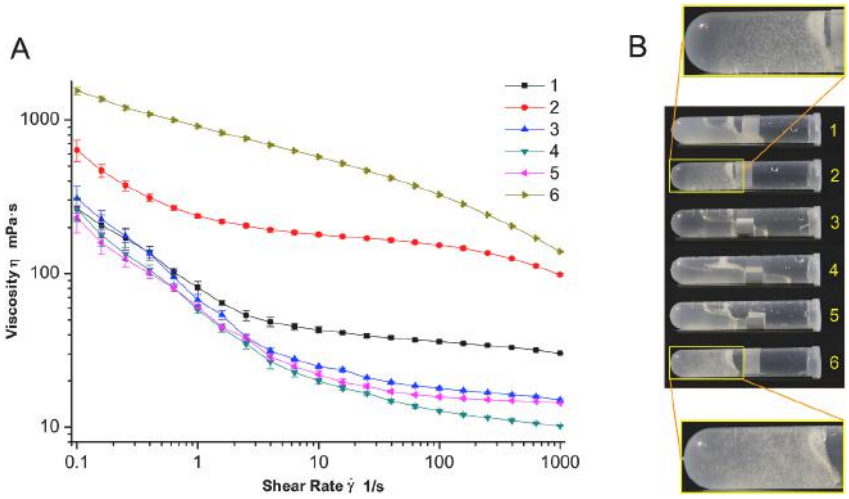

Fig. 4. (A) The flow behaviors of the pectin (aq.) extracted under different conditions. (B) Sample 2 and 6 show a higher viscosity by containing more bubbles and flowing less distance in a fixed time (5 s).

Table 6
Rheological parameters of samples 1 to 6 fitted with Herschel-Bulkley model.

Samples	σ_0 (mPa)	K (mPa·s^n)	n	R^2
1	24.68	49.85	0.93	1.00
2	33.61	215.01	0.91	0.98
3	32.91	28.59	0.90	1.00
4	25.04	26.99	0.84	0.99
5	22.44	28.38	0.88	0.99
6	3.43	909.25	0.79	0.98

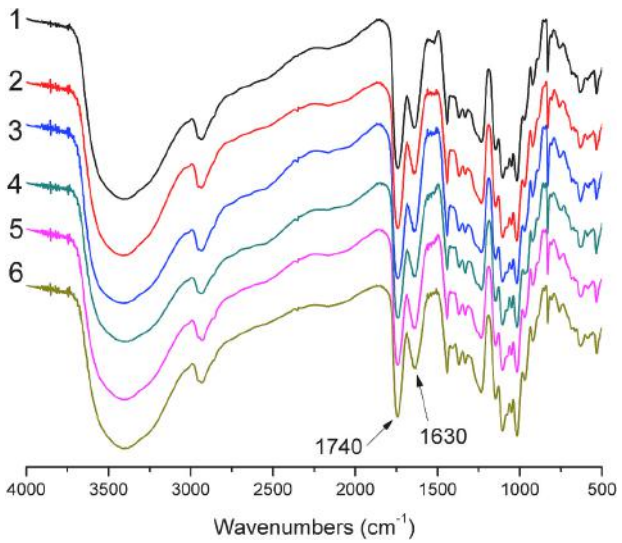

Fig. 5. FTIR spectra of the six pectin samples extracted under different conditions.

Although the esterification degree of pectin could also affect the viscosity (Thakur, Singh, Handa, & Dr. Rao, 1997), the FTIR spectra shown in Fig. 5 indicate their degree of methoxylation (DM) were similar according to the carboxylic ester and carboxylic acid signals at 1740 and 1630 cm^{-1}, respectively (Chatjigakis et al., 1998). The calculated DM values for samples from 1 to 6 were 54.53%, 57.04%, 55.71%, 53.77%, 52.01%, and 54.02%, respectively. Thus, the DM with similar value should have little impact on the samples' rheological properties.

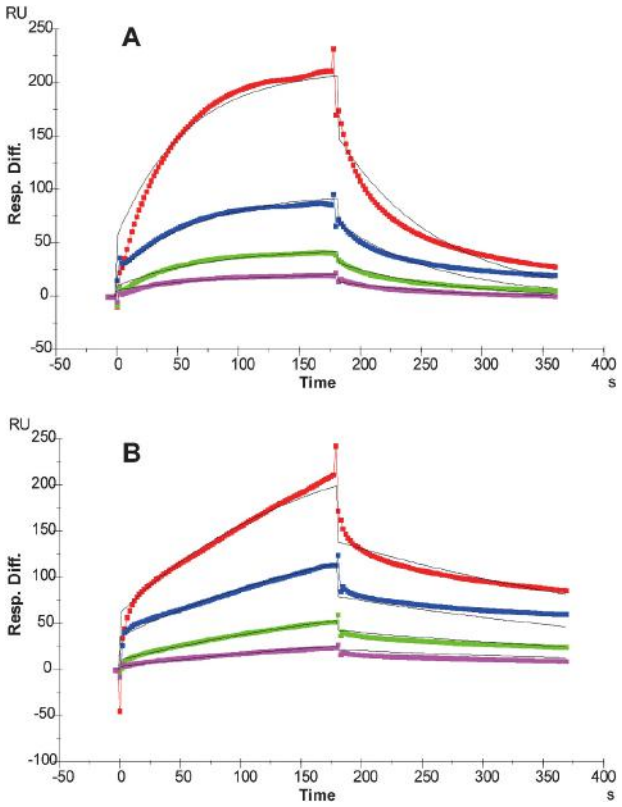

Fig. 6. SPR sensorgrams of (A) sample 1 and (B) sample 2 binding with Gal-3. Concentrations of (A) and (B) both were (from top to bottom): 5, 2.5, 1.25 and 0.63 μM, respectively. The black curves are the fitting curves using models from BIAevaluation 4.1.1.

3.3.3. *Binding character of pectin and galectin-3*

Galectin-3 (Gal-3) is a β-galactoside binding and multifunctional lectin. It has been regarded as a target because of its important roles in

many diseases, such as cancer (Smith et al., 2019; Song et al., 2014), fibrosis (Martínez-Martínez et al., 2015), and so on. Many researches presented Gal-3 blocking for disease inhibition. Pectin, containing β-galactoside, was widely reported as a Gal-3 inhibitor (Zhang et al., 2016a). In the current study, sample 1 and sample 2 representing high-temperature and low-temperature extracted pectin, respectively, were determined the binding affinity with Gal-3. The SPR measurement as a comparable method (Zhang et al., 2016b) was applied and the sensorgrams are shown in Fig. 6. The binding affinity expressed as K_D was calculated after curves fitting. The K_D of sample 1 is 10.9 μM, while sample 2 show a stronger binding by the K_D = 3.87 μM, close to the modified citrus pectin (Zhang et al., 2016b). The above results suggest the low-temperature extracted pectin may have better biological activities on the view of benefitting Gal-3 related diseases.

4. Conclusions

Pectin is widely used in food industry. The commercial pectin contains more GalA content and less side chains compared with our previous citrus canning recovered pectin. The mechanism of pectin extraction was studied under several extraction conditions considering the factors of extraction temperature, pH, time, and raw material shape. The results support that extraction conditions do impact the pectin structure/properties and the temperature is the decisive factor. Pectin being extracted below 40 °C, an unusual low temperature rarely studied for pectin extraction, makes the pectin structure fantastic. It should be emphasized that the low-temperature extraction keeps the pectin molecule more intact, remaining more neutral sugar branches and may be more close to the original molecule in the cell wall. The low-temperature extracted pectin exhibits much higher viscosity and a stronger Gal-3 binding affinity than the commercial pectin, suggesting its better application in food industry as healthy thickener and gelling agent.

CRediT authorship contribution statement

Jianle Chen: Conceptualization, Methodology, Investigation, Writing - original draft. **Huan Cheng:** Formal analysis, Investigation. **Zijian Zhi:** Investigation. **Hua Zhang:** Investigation. **Robert J. Linhardt:** Writing - review & editing. **Fuming Zhang:** Formal analysis. **Shiguo Chen:** Supervision, Project administration. **Xingqian Ye:** Supervision, Conceptualization.

Declaration of competing interest

None.

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References

Andersen, N. M., Cognet, T., Santacoloma, P. A., Larsen, J., Armagan, I., Larsen, F. H., et al. (2017). Dynamic modelling of pectin extraction describing yield and functional characteristics. *Journal of Food Engineering, 192*, 61–71.

Chatjigakis, A. K., Pappas, C., Proxenia, N., Kalantzi, O., Rodis, P., & Polissiou, M. (1998). FT-IR spectroscopic determination of the degree of esterification of cell wall pectins from stored peaches and correlation to textural changes. *Carbohydrate Polymers, 37*(4), 395–408.

Chen, J., Cheng, H., Wu, D., Linhardt, R. J., Zhi, Z., Yan, L., et al. (2017). Green recovery of pectic polysaccharides from citrus canning processing water. *Journal of Cleaner Production, 144*, 459–469.

Ciriminna, R., Chavarria-Hernandez, N., Rodriguez Hernandez, A. I., & Pagliaro, M. (2015). Pectin: A new perspective from the biorefinery standpoint. *Biofuels Bioproducts & Biorefining-Biofpr, 9*(4), 368–377.

Coll-Almela, L., Saura-Lopez, D., Laencina-Sanchez, J., Schols, H. A., Voragen, A. G., & Ros-Garcia, J. M. (2015). Characterisation of cell-wall polysaccharides from Mandarin segment membranes. *Food Chemistry, 175*, 36–42.

Colodel, C., Vriesmann, L. C., Teofilo, R. F., & de Oliveira Petkowicz, C. L. (2018). Extraction of pectin from ponkan (Citrus reticulata Blanco cv. Ponkan) peel: Optimization and structural characterization. *International Journal of Biological Macromolecules, 117*, 385–391.

Kalapathy, U., & Proctor, A. (2001). Effect of acid extraction and alcohol precipitation conditions on the yield and purity of soy hull pectin. *Food Chemistry, 73*(4), 393–396.

Kaya, M., Sousa, A. G., Crepeau, M. J., Sorensen, S. O., & Ralet, M. C. (2014). Characterization of citrus pectin samples extracted under different conditions: Influence of acid type and pH of extraction. *Annals of Botany, 114*(6), 1319–1326.

Kliemann, E., de Simas, K. N., Amante, E. R., Prudêncio, E. S., Teófilo, R. F., Ferreira, M. M. C., et al. (2009). Optimisation of pectin acid extraction from passion fruit peel (Passiflora edulisflavicarpa) using response surface methodology. *International Journal of Food Science and Technology, 44*(3), 476–483.

Koubala, B. B., Kansci, G., Mbome, L. I., Crépeau, M. J., Thibault, J. F., & Ralet, M. C. (2008). Effect of extraction conditions on some physicochemical characteristics of pectins from "Améliorée" and "Mango" mango peels. *Food Hydrocolloids, 22*(7), 1345–1351.

Kurita, O., Fujiwara, T., & Yamazaki, E. (2008). Characterization of the pectin extracted from citrus peel in the presence of citric acid. *Carbohydrate Polymers, 74*(3), 725–730.

Mao, G., Wu, D., Wei, C., Tao, W., Ye, X., Linhardt, R. J., et al. (2019). Reconsidering conventional and innovative methods for pectin extraction from fruit and vegetable waste: Targeting rhamnogalacturonan I. *Trends in Food Science & Technology, 94*, 65–78.

Martínez-Martínez, E., Calvier, L., Fernández-Celis, A., Rousseau, E., Jurado-López, R., Rossoni, L. V., et al. (2015). Galectin-3 blockade inhibits cardiac inflammation and fibrosis in experimental hyperaldosteronism and hypertension. *Hypertension, 66*(4), 767–775.

Masmoudi, M., Besbes, S., Chaabouni, M., Robert, C., Paquot, M., Blecker, C., et al. (2008). Optimization of pectin extraction from lemon by-product with acidified date juice using response surface methodology. *Carbohydrate Polymers, 74*(2), 185–192.

Maxwell, E. G., Belshaw, N. J., Waldron, K. W., & Morris, V. J. (2012). Pectin – an emerging new bioactive food polysaccharide. *Trends in Food Science & Technology, 24*(2), 64–73.

May, C. D. (1990). Industrial pectins: Sources, production and applications. *Carbohydrate Polymers, 12*(1), 79–99.

Mohnen, D. (2008). Pectin structure and biosynthesis. *Current Opinion in Plant Biology, 11*(3), 266–277.

Monsoor, M. A., Kalapathy, U., & Proctor, A. (2001). Improved method for determination of pectin degree of esterification by diffuse reflectance Fourier transform infrared spectroscopy. *Journal of Agricultural and Food Chemistry, 49*(6), 2756–2760.

Morris, V. J., Belshaw, N. J., Waldron, K. W., & Maxwell, E. G. (2013). The bioactivity of modified pectin fragments. *Bioactive Carbohydrates and Dietary Fibre, 1*(1), 21–37.

Mottern, H. H., & Hills, C. H. (1946). Low ester pectin from apple pomace. *Industrial and Engineering Chemistry, 38*(11), 1153–1156.

Peng, X., Mu, T., Zhang, M., Sun, H., Chen, J., & Yu, M. (2016). Effects of pH and high hydrostatic pressure on the structural and rheological properties of sugar beet pectin. *Food Hydrocolloids, 60*, 161–169.

Petkowicz, C. L. O., Vriesmann, L. C., & Williams, P. A. (2017). Pectins from food waste: Extraction, characterization and properties of watermelon rind pectin. *Food Hydrocolloids, 65*, 57–67.

Podzimek, S., & Vlcek, T. (2001). Characterization of branched polymers by SEC coupled with a multiangle light scattering detector. II. Data processing and interpretation. *Journal of Applied Polymer Science, 82*(2), 454–460.

Smith, E., Stasenko, M., Rao, T., Feit, N., de Stanchina, E., White, T., et al. (2019). Blocking metastatic behavior of MUC16/CA-125-expressing cancer by targeting galectin-3. *Gynecologic Oncology, 154*, 46.

Song, L., Tang, J.-w., Owusu, L., Sun, M.-Z., Wu, J., & Zhang, J. (2014). Galectin-3 in cancer. *Clinica Chimica Acta, 431*, 185–191.

Strydom, D. J. (1994). Chromatographic separation of 1-phenyl-3-methyl-5-pyrazolone-derivatized neutral, acidic and basic aldoses. *Journal of Chromatography A, 678*(1), 17–23.

Thakur, B. R., Singh, R. K., Handa, A. K., & Dr Rao, M. A. (1997). Chemistry and uses of pectin - a review. *Critical Reviews in Food Science and Nutrition, 37*(1), 47–73.

Wang, X., & Lü, X. (2014). Characterization of pectic polysaccharides extracted from apple pomace by hot-compressed water. *Carbohydrate Polymers, 102*, 174–184.

Wang, W., Wu, X., Chantapakul, T., Wang, D., Zhang, S., Ma, X., et al. (2017). Acoustic cavitation assisted extraction of pectin from waste grapefruit peels: A green two-stage approach and its general mechanism. *Food Research International, 102*, 101–110.

Wicker, L., Kim, Y., Kim, M.-J., Thirkield, B., Lin, Z., & Jung, J. (2014). Pectin as a bioactive polysaccharide – extracting tailored function from less. *Food Hydrocolloids, 42*, 251–259.

Willats, W. G. T., Knox, J. P., & Mikkelsen, J. D. (2006). Pectin: New insights into an old polymer are starting to gel. *Trends in Food Science & Technology, 17*(3), 97–104.

Wu, D., Zheng, J., Mao, G., Hu, W., Ye, X., Linhardt, R. J., et al. (2019). Rethinking the impact of RG-I mainly from fruits and vegetables on dietary health. *Critical Reviews in Food Science and Nutrition*, 1–23.

Yapo, B. M., Robert, C., Etienne, I., Wathelet, B., & Paquot, M. (2007). Effect of extraction conditions on the yield, purity and surface properties of sugar beet pulp pectin extracts. *Food Chemistry, 100*(4), 1356–1364.

Zhang, H., Chen, J., Li, J., Yan, L., Li, S., Ye, X., et al. (2018). Extraction and characterization of RG-I enriched pectic polysaccharides from Mandarin citrus peel. *Food Hydrocolloids, 79*, 579–586.

Zhang, T., Lan, Y., Zheng, Y., Liu, F., Zhao, D., Mayo, K. H., et al. (2016a). Identification of the bioactive components from pH-modified citrus pectin and their inhibitory effects on galectin-3 function. *Food Hydrocolloids, 58*, 113–119.

Zhang, T., Zheng, Y., Zhao, D., Yan, J., Sun, C., Zhou, Y., et al. (2016b). Multiple approaches to assess pectin binding to galectin-3. *International Journal of Biological Macromolecules, 91*, 994–1001.