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Zhang, H, Chen, J, Li, J et al. (8 more authors) (2018) Extraction and characterization of RG-I enriched pectic polysaccharides from mandarin citrus peel. Food Hydrocolloids, 79. pp. 579-586. ISSN 0268-005X

https://doi.org/10.1016/j.foodhyd.2017.12.002

Extraction and characterization of RG-I enriched pectic polysaccharides from mandarin citrus peel

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Abstract. Pectin is extensively used as thickener and gelling agent in the food industry. Commercial pectin is usually comprised of homogalacturonans (HG) with small amount of rhamnogalacturonan-I (RG-I). However, recent studies have shown the presence of neutral sugars in the RG-I region can improve the bioactivity of pectin in cancer prevention, cardiovascular disease treatment and in fibrosis prevention. Thus, the present study aims at obtaining an RG-I enriched pectin from citrus peel by developing a mild sequential extraction method. This sequential treatment consists of acid extraction followed by alkaline treatment at room temperature. The yields of polysaccharides extracted by acid (PA) and then by base (PB) were 4.2 wt% and 18.9 wt%, respectively. Moreover, the structural and rheological properties of PA and PB were also explored. Monosaccharide assay verified PA and PB consisted mainly of RG-I structure based on values for GalA/Rha of 10.0 and 2.0, respectively. The GPC-MALLS showed that both PA and PB were branched and had molecular weights of 282 kDa and 743 kDa, respectively. AFM imaging directly confirmed their branched-chain morphology. NMR and FT-IR analyses demonstrated that they were typical pectic polysaccharides, and the esterification degree for PA was 56 %, but PB was not esterified. Rheological analysis showed that the two polysaccharides had similar thickening properties when compared to commercial pectin and could be potentially used as functional food ingredients.

Key words: extraction of pectin, citrus peel, SEC-MALLS, RG-I structure

1 Introduction

Pectin is a complex polysaccharide that is extensively distributed in the primary cell wall and middle lamella of all plant tissues (Ridley, O'Neill, & Mohnen, 2001; Thakur, Singh, Handa, & Rao, 1997). It contains multiple "domains or building blocks" varying mainly in linear-forms of homogalacturonans (HG), highly branched-forms of rhamnogalacturonan-I (RG-I) and in rhamnogalacturonan-II (RG-II)(Zhi, et al., 2017a). The HG form, consisting of a linear backbone of 1,4-linked-α-D-galacturonic acid (GalA) units, which are partially methyl esterified at the carboxylic acid or acetylated (Xu, et al., 2014). They are classified into two types, high methoxyl pectin (HMP) with a degree of methylation (DM) > 50% and low methoxyl pectin (LMP) with a DM < 50% based on their degree of esterification. HMP and LMP have different physicochemical characteristics and, thus, are used in different applications (Chan & Choo, 2013).

Pectin is widely used in the food industry as gelling, stabilizing and thickening agents in food systems such as jams and jellies, confectionery, and fruit juice (Thakur, et al., 1997). Other applications include the use of pectins as fat replacers, edible films, in drug delivery, and in tissue engineering. Pectin is in short supply with a global market having a trade value of more than $850 million in 2013 with an annual growth rate of ≥ 5% (Chen, et al., 2016). Commercial pectins are extracted mainly from citrus peels and apple pomace, and to a lesser extent, from sugar beet pulp (May, 1990; Yapo, Robert, Etienne, Wathelet, & Paquot, 2007). For the best gelling properties and quality control, commercial pectin usually mainly consists of HG region with small amount of RG-I region and their GalA content is usually required to be higher than 65%. During the conventional extraction process, pectin is extracted using a strong acid (i.e., sulfuric, phosphoric, nitric,

hydrochloric, etc.,) under elevated (60-100 °C) temperatures (Koubala, et al., 2008). An acid extraction process is most widely used to obtain commercial pectin with a high degree of esterification and high levels of HG regions, but this process also results in the degradation of side-chains (Harris & Smith, 2010; Levigen, Ralet, & Thibault, 2002). In addition, the acid extraction process is often accompanied by the hydrolysis of the acid-labile linkages between the GalA and rhamnose (Rha) residues in the RG-I region (Khalikov & Mukhiddinov, 2004; Levigen, et al., 2002). Recently, researchers have begun to take notice of the importance of the pectin side chain, not only as it relates to the bioactivities of the pectin, but also with respect to gel its forming properties. Pectins enriched in RG-I structure show improved activities in to preventing cancer, cardiovascular diseases and fibrosis, by showing improved interact with Galectin-3 (Gal-3) (de Boer, Voors, Muntendam, van Gilst, & van Veldhuisen, 2009; Li, Li, & Gao, 2014). Gal-3 is characterized by a carbohydrate recognition domain that naturally binds to specific carbohydrate molecular patterns of molecular receptors, inducing cell adhesion, migration, transformation, and apoptosis (Vladoiu, Labrie, & Stpierre, 2014). The side chain of RG-I pectin can occupy this recognition domain and inhibit Gal-3 activity (Gao, et al., 2013). There also have been several studies showing that pectic polysaccharides containing higher arabinose (Ara) and galactose (Gal) significantly inhibit hemagglutination (Sathisha, Jayaram, Harish Nayaka, & Dharmesh, 2007). Pectic polysaccharides extracted from potato pulp, which has a galactan-rich RG-I, promotes Bifidobacterium and Lactobacillus growth to a greater extent than FOS, and the bifidogenic properties of RG I-rich fraction had 1.5-times more than HG-rich fraction (Michalak, et al., 2012).

Some innovative technologies have been applied to extract pectin, to promote the extraction

efficiency, and minimize the use of chemicals during pectin extraction. The properties of pectin from Yuza pomace using conventional-chemical and combined physical-enzymatic methods have been compared by researchers (Lim, Yoo, Ko, & Lee, 2012). The pectin obtained using combined physical-enzymatic contained more neutral sugar (18%) than conventionally extracted pectin (11%). Ultrasound extracted pectin from grapefruit peel has a higher percentage of side chains than conventionally extracted pectin (W. Wang, et al., 2015). However, while most of these new approaches have been applied at the laboratory scale, they show that side chains might be retained by modification of the widely used extraction methods. Our research group previously analyzed the structure of pectic polysaccharides from citrus canning processing water, and the results indicated that the four polysaccharides were dominated with RG-I regions (Chen, et al., 2016). These pectic polysaccharides were obtained by sequential, low temperature, acid and alkali treatments. Thus, it is important to extensively explore the structure of pectic polysaccharides isolated from citrus peel under similar extraction conditions.

In the current study, we seek to retain pectin's branches though mild extraction conditions and obtain a pectin is primarily composed of an RG-I structure. The cell wall was hydrolyzed with hydrochloric acid at low temperature to obtain a highly branched HMP. A large proportion of pectin loosely combined with fiber left in the residue, so treatment with sodium hydroxide at low temperature afforded a highly branched LMP. The extracted pectic polysaccharides were characterized by monosaccharide compositional analysis, size exclusion chromatography with multi-angle laser light scattering (SEC-MALLS), Fourier-transform infrared (FT-IR), nuclear magnetic resonance (NMR) spectroscopy and atomic force microscope (AFM). Finally, the

rheological behavior and gel properties were evaluated. The results of this study suggest that at low temperature, HMP and LMP rich in RG-I, can be extracted from citrus peels as a potential functional ingredient.

2 Materials and methods

2.1 Citrus peel materials and chemicals

Citrus peel was provided by a citrus fruit canning factory in China and oven-dried at 105 °C for 24 h. Citrus peel was sufficiently ground to pass through a 200-mesh sieve and stored in desiccators at room temperature for subsequent pectin extraction. Monosaccharide standards, 1-phenyl-3-methyl-5-pyrazolone (PMP), commercial pectin（CP）and D_2O were all purchased from Sigma (China). All other used chemicals were of analytical grade.

2.2 Pectin extraction

The method for extracting pectic polysaccharides from citrus peel powder is shown in Fig 1. Citrus peel powder was added into 0.4 % HCl solution with the solid to liquid ratio of 1:30. The mixture was stirred at 28 °C for 40 min to destroy the cell wall and extract a portion of the pectic polysaccharides. After this extraction stage, the mixture was filtered through a 400-mesh filter bag to obtain residue and liquid. The liquid was then used to extract pectic polysaccharides (PA) after adjusting pH and precipitation with 95 % ethanol in the volume ratio of 1:1. The residue was re-suspended in the same volume of 0.6 % NaOH continuing the extraction procedure by magnetic stirring at 32 ° C for 10 min. At the end of extraction, a filter bag was used to obtain the liquid and the residue was discarded. The liquid underwent pH-adjustment and precipitation as well. After

precipitation, the retentate was washed with 95 % ethanol for 2~3 times followed by oven-dried at 55 °C for 24 h.

The pectin yield was calculated according to equation (1) and (2).

$$\text{Yield of PA (\%)} = \frac{Pure\ PA\ pectin(g)}{initial\ citrus\ peel\ powder(g)} * 100\% \quad (1)$$

$$\text{Yield of PB (\%)} = \frac{Pure\ PB\ pectin(g)}{initial\ citrus\ peel\ powder(g)} * 100\% \quad (2)$$

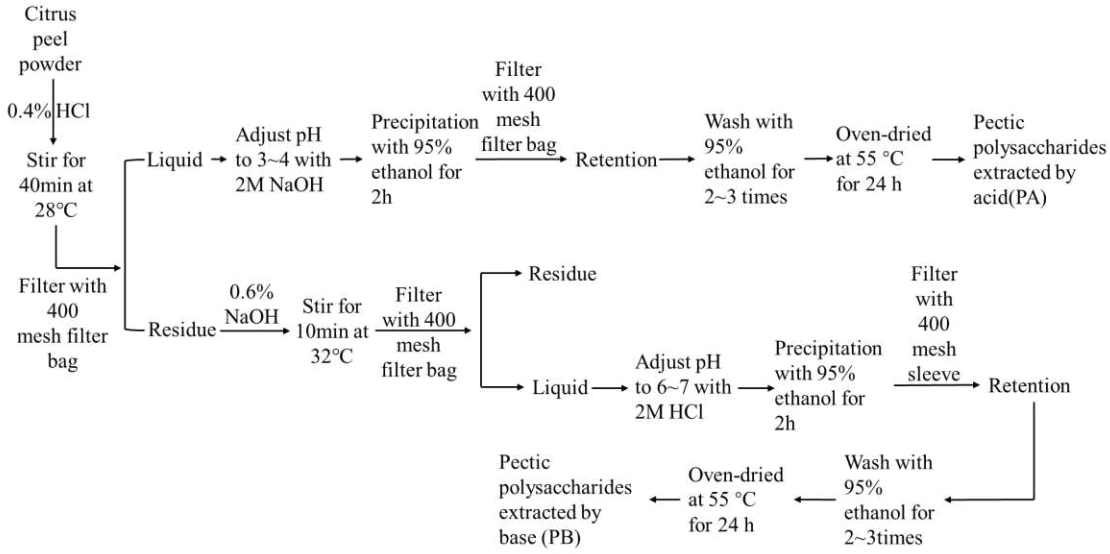


Fig 1. Flow chart for extraction of pectic polysaccharides

2.3 Monosaccharide analysis

Monosaccharide composition was analyzed by a 1-phenyl-3-methyl-5-pyrazolone (PMP)-high performance liquid chromatography (HPLC) method (Strydom, 1994) with modifications. Briefly, pectic polysaccharides samples (typically 2-3 mg) were first hydrolyzed with 2 M trifluoroacetic acid at 110 °C for 8 h in an ampoule, after which the acid was removed using a stream of nitrogen and neutralized with 0.1 M sodium hydroxide. The dry hydrolyzates were dissolved in 450 µL of 0.3 M sodium hydroxide and derivatized using 450 µL PMP solution (0.5 M, in methanol) at 70 °C

for 30 min. Finally, the mixtures were neutralized by 0.3 M hydrochloric acid, and 3 × 1 mL chloroform was used to extract excess reagent. The upper phase was filtered through a 0.22 μm membrane and 1 ml of the resulting solution was injected for analysis. Waters e2695 (Waters, US) with a Zorbax Eclipse XDB-C18 column (250 mm × 4.6 mm, 5 μm, Agilent, USA) was used to perform HPLC analysis at 25 °C, while detecting by 2489 UV/Vis Detector(Waters, US) at 250 nm. The mobile phases were: solvent A, 15 % acetonitrile with potassium phosphate buffer (0.05 M, pH 6.9), solvent B, 40% acetonitrile with the same buffer. The elution rate was 1 mL/min, relying on a gradient of B from 0% to 15% in the initial 10min, then from 15% to 25% in the next 20 min.

2.4 SEC-MALLS analysis

Pectic polysaccharides were dispersed in purified water to the concentration of 5 mg/ml. After filtering through a syringe-filter with pore size of 0.45 μm, 50 μL of solution was injected through a sample loop. The molar mass and root mean square (RMS) radius of gyration were determined through high-performance (HP) size exclusion chromatography (SEC) equipped with multi-angle laser light scattering (MALLS) (Wyatt Dawn Heleos-II, USA) and RI detector at 25 °C. Isocratic elution with 0.15 M NaCl solution at a flow rate of 0.5 mL/min was performed on the Shodex SB-806 HQ (Showa Denko KK, Japan). The molar mass was calculated using the dn/dc value of 0.1850 mL/g.

2.5 FT-IR spectroscopy and NMR analysis

The FT-IR spectrum of pectic polysaccharides was measured on a Nicolet iN10 instrument (Thermo Fisher Scientific, USA). Samples (~ 1 mg) were mixed with 200 mg KBr powder, ground and then pressed into pellets for FT-IR scanning in the frequency range of 4000–400 cm^{-1}. Data

obtained were processed by Origin 9.1 software.

For NMR analysis, pectic polysaccharides (10-15 mg) were suspended in 500 μL of D_2O (99.96 %) and freeze dried twice before dissolved in 500 μL of high-quality D_2O. The ^1H NMR spectra were collected by a DD2-600 (Agilent, USA) spectrometer at room temperature.

2.6 AFM test

The samples were dissolved in ultrapure water at a concentration of 1 mg/mL with continuous stirring for 2 h and subsequently incubating at 80 °C for 2 h. The stock solutions were next diluted by sodium dodecyl sulfate（SDS）solution, obtaining a mixed solution containing polysaccharides and SDS both 10 μg/mL. The diluted solutions were then stirred for 24 h and filtered through a 0.22-μm filter. After the sample was ready, a freshly cleaved mica substrate was immersed into the solution for ten minutes. Afterwards, three micas were rinsed and air-dried and then observed by AFM (XE70) using tapping mode.

2.7 Rheological measurements

The rheological properties of pectic polysaccharides were carried out by HAAKE RheoStress 6000 rheometer (Thermo Scientific, USA) with a 40 mm parallel plate. Different solutions at 0.75 % and 1.5 % for rheological tests were prepared by mixing pectin with distilled water under magnetic stirring for 1 h. After 12 h placing in room temperature, the samples were subjected to steady shearing at 25 °C with the shear rates ranged from 0.01-100 s^{-1}. Data were fitted to a power law model (equation 3).

$$\eta = k\gamma^{(n-1)} \quad (3)$$

In equation（3）, where η is apparent viscosity (mPa·s), k (mPa·s^n) is consistency index, γ is the

shear rate (s^{-1}) and n (dimensionless) is the flow behavior index.

3 Results and discussion

3.1 Yield and monosaccharide composition of PA and PB

The yield and monosaccharide composition of the pectic polysaccharides extracted from citrus peel by hydrochloric acid and sodium hydroxide are shown in Table 1. The yield of PA is 4 %, which is very low compared to the hot acid extraction method (20%-30%) (Palanisamy, Dhivya, & Kumaresan, 2014). The low extraction temperature leads to low pectin solubilization (Methacanon, Krongsin, & Gamonpilas, 2014). In this experiment, the temperature used to extract PA was 28 °C, very mild and only capable of destroying part of the linkage between fiber and pectin in the cell wall, but insufficient for extracting all of the pectic polysaccharides. The relatively higher yield obtained for PB compared to PA is partly ascribable to the initial treatment with acid and, thus, the remaining pectic polysaccharides in cell wall are better extracted. The total yield of PA and PB is 23 %, corresponding to the yield of pectin by subcritical water (19-22 %) and hot acid (20 % - 30 %) (Palanisamy, et al., 2014; X. Wang, Chen, & Xin, 2014).

In PA, the 57 % GalA, which constitutes the backbone of homogalacturonan (HG) and rhamnogalacturonan (RG) regions of pectin, occupies the largest proportion of monosaccharides. The value is lower than the content in commercial pectin（≥ 65 %. This may due to the mild extraction temperature that did not completely release pectin chains initially tightly bound to the cell wall matrix (Besson, Yapo, Maxwell Beugre, Koffi, & Gnakri, 2014). The neutral sugars, Rha, Gal, xylose (Xyl) and Ara represented the major components, consistent with the literature (X. Wang, et al., 2014). Rha and GalA are the main structural units of the RG region. Gal and Ara

correspond to the side chains of RG I, while Xyl mainly composed xylogalacturonan (XG). The GalA/Rha ratio for PA, consisting mainly of RG-I structure, as was 10.0 (Oosterveld, Beldman, Schols, & Voragen, 2000). This ratio was close to the RG dominated soybean pectin with a ratio for 3.4, and much lower than commercial citrus pectin having a ratio of 74 and murta pectin with a ratio of 66, and characterized by the predominance of long HG regions (Isabelle, et al., 2012; Taboada, et al., 2010).

The content of neutral sugars in PB were even higher than in PA, reaching to 77 %. Alkali extracted pectin is richer in neutral sugars than hot acid extracted pectin from a variety of different plant sources (Yapo, Lerouge, Thibault, & Ralet, 2007). Indeed, hot alkali conditions have been successfully applied for the recovery of intact RG-I, while HG is seriously degraded by elimination and oxidative peeling (Bonnina, et al., 2001). PB mainly consists of Rha, Gal, Ara and glucose (Glu). The absence of Xyl indicates that PB has little XG structure. The GalA/Rha ratio is 2.0, indicating that RG-I structure is dominant in PB. Since the PB (Gal + Ara)/Rha ratio of 4.91 is slightly higher than the ratio of 4.83 for PA, PB probably has a higher proportion of hairy regions and side chains than PA (X. Wang, et al., 2014).

Based on the yield and composition of PA and PB, we hypothesize that acid treatment destroys the cell wall and extracts some pectic polysaccharides loosely connected to the hemicellulose, while alkali completely extracts the remaining pectic polysaccharides. A schematic of the extraction process is shown in the Fig. 2 (Cosgrove, 2000).

Table 1. Yields and monosaccharide compositions of PA and PB

	Yield (wt. %)	Galacturonic acid (wt. %)	Neutral sugar composition (wt. %)				
			Rhamnose	Glucose	Galactose	Arabinose	Xylose
PA	4.2	57.4	5.8	-	4.3	23.6	8.9
PB	18.9	23.4	11.4	9.5	19.7	36.1	-

PA and PB refer to the citrus peel pectic polysaccharides extracted by hydrochloric acid and sodium hydroxide, respectively.

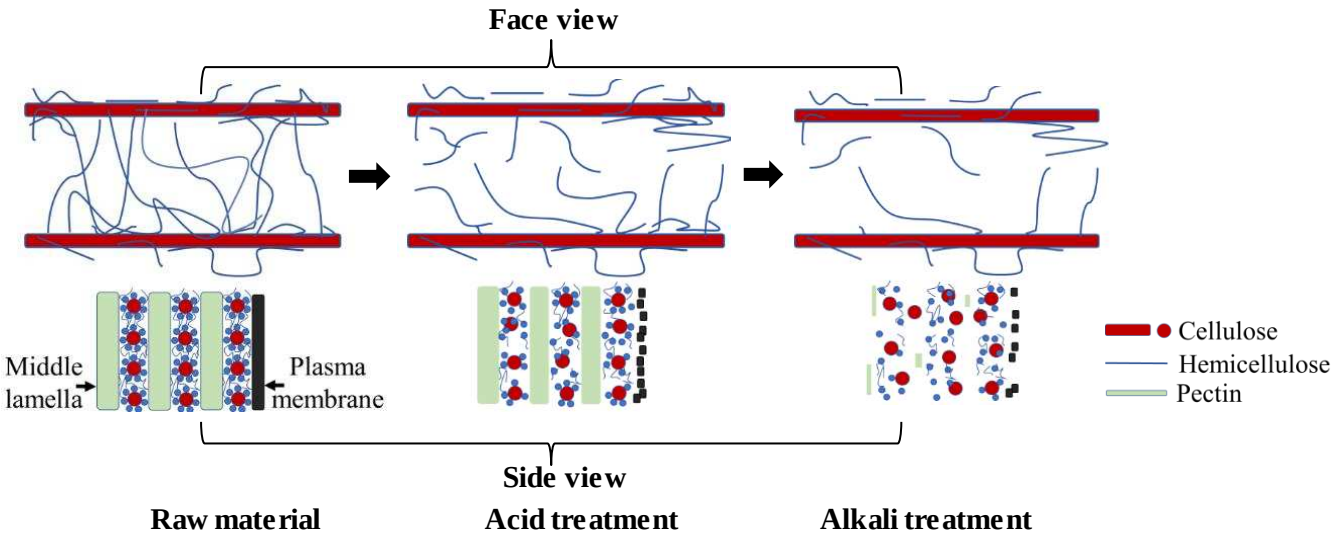


Figure 2. Schematic diagram of extraction process

3.2 SEC-MALLS-RI analysis

Chromatograms of molar mass distribution of PA and PB are shown in Fig. 3. The relative values were calculated by Astra 6.1 in Table 2 to obtain more accurate information about molecular size of the recovered polysaccharides. The weight average of molar mass (Mw) of PA was lower than that of PB. However, the z-average root mean square radius of gyration (Rz) of PA was higher than that of PB. This may be the result of different monosaccharide compositions and different molecular structures in the two types of polysaccharides (Chen, et al., 2016). Pectin polysaccharides containing higher Ara have a larger molecular weight but smaller Rz than those containing higher

GalA (Makoto, et al., 2008). The Rz of PA and PB of 28.6-49.5 nm are in good agreement with previously published data (Fishman, et al., 2015). Fishman et al., reports a molar mass of several low methoxyl citrus pectin in the range of 103-288 kDa, which were lower than PB. This may be due to the better preservation of side chains during the extraction. Polydispersity index is useful for appraising the molecular mass distribution of a polysaccharide. PB has a broader molecular mass distribution than PA (Table 2). The chain conformation of polysaccharides in aqueous solution can be calculated from the slope between RMS radius and molar mass (Fig. 4). Both PA and PB are predicted to be branched, since the slope value of linear fitting is not 0.333, 0.5-0.6 or 1.0 (Gnanasambandam & Proctor, 2000) and is consistent with the anomalous SEC phenomenon of plots bending upward at the low molar mass region (Podzimek, Vlcek, & Johann, 2001).

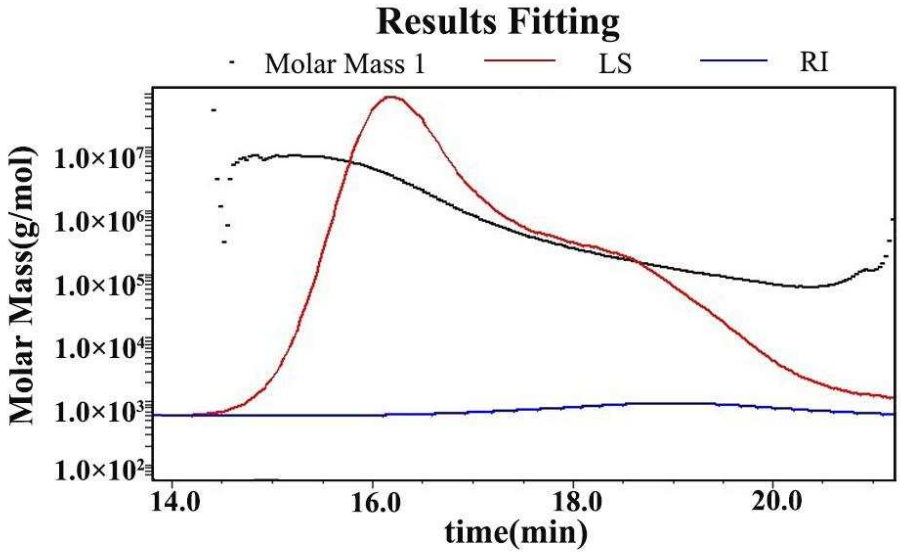
Table 2. Average values of molecular weights and radii of PA and PB

	Mw[a] (kDa)	Mn[b] (kDa)	Polydispersity (Mw/Mn)	Rz[c] (nm)
PA	282 (± 2 %)	109 (± 7 %)	2.6 (± 7.5 %)	49 (± 4 %)
PB	743 (± 2 %)	192 (± 13 %)	3.9 (± 12.9 %)	38 (± 5 %)

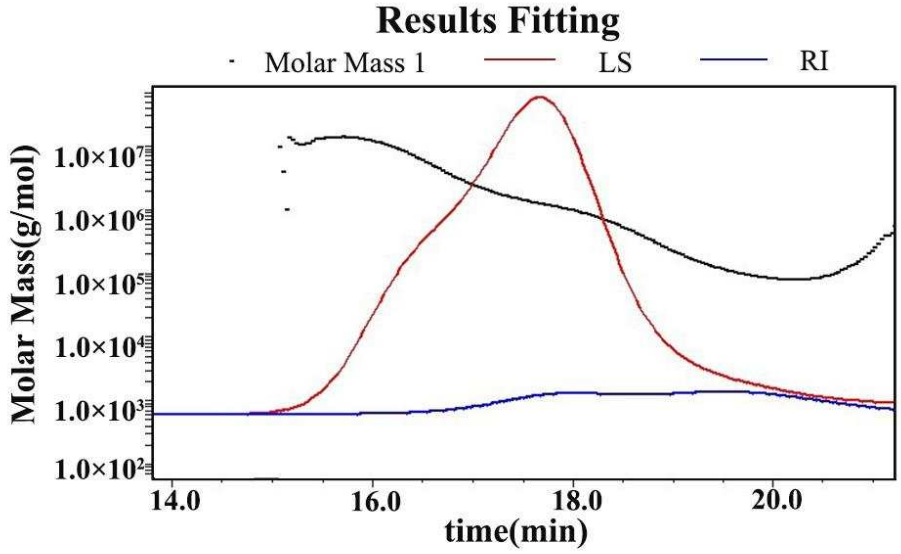
[a]Mw: weight-average of molar mass.

[b]Mn: number-average of molar mass.

[c] Rz: z-average of root mean square radius of gyration.



(a)



(b)

Figure 3. Chromatograms of molar mass distribution and molecular radius distributions of (a) PA and (b) PB.

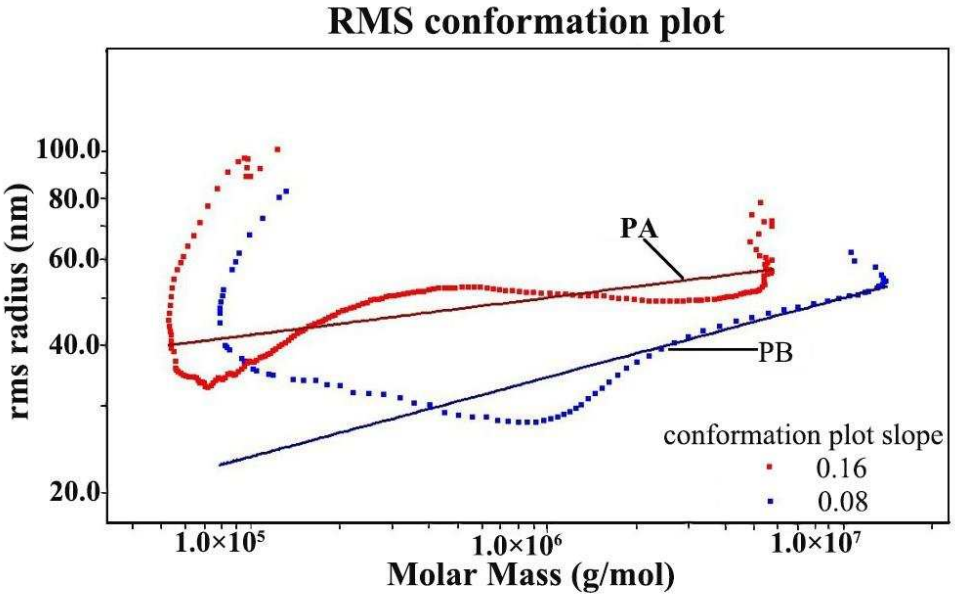


Figure 4. Chromatograms of chain conformation of aqueous PA and PB.

3.3 FT-IR analysis and NMR measurements

The FT-IR spectra show characteristic absorption peaks of pectic polysaccharides in both PA and PB (Fig. 5). The absorption peak at 3385 cm^{-1} (PA) or 3421 cm^{-1} (PB) is attributed to the stretching vibrations of hydroxyl groups (OH). The spectra of both PA and PB show a peak at 2933 cm^{-1} resulting from the C-H stretching of CH_2 groups. Moreover, the region between 1800 cm^{-1} and 1500 cm^{-1} is of special interest since it is important for evaluating the degree of esterification (DE) (Vriesmann, 2009). In spectra of PA, the average of the ratio of the peak area at 1745 cm^{-1} (COO-R) over the sum of the peak areas of 1745 cm^{-1} (COO-R) and 1633 cm^{-1} (COO-) is used to calculate a DE of 56 %. The value is relatively lower than pectin extracted using subcritical water (69 %-75 %) (X. Wang, et al., 2014) and pectin from orange peel or lemon peel (70 %) (Sousa, Nielsen, Armagan, Larsen, & Sørensen, 2015). The most significant parameters impacting the DE of a

pectin are pH and time (Levigen, et al., 2002). PB showed an extremely low level of esterification (DE = 10 %), as confirmed by the COO^- peak at 1610 cm^{-1}, consistent with the literature (Yapo, Lerouge, et al., 2007). At the industrial scale, low temperature alkali treatment is often applied to de-esterify HMP in the manufacture of LMP (Renard & Thibault, 1996). Therefore, the alkali extraction procedure may include the breakage of linkages between pectin and fiber and simultaneously result in the de-esterification of the pectin. Carboxylate groups also have symmetrical stretching band at 1442 cm^{-1} (PA) or 1415 cm^{-1} (PB) (Saberian, Hamidi-Esfahani, Gavlighi, & Barzegar, 2017). The peaks of PA and PB between 1000 and 1150 cm^{-1} corresponds the stretching vibrations C–OH side groups and the C–O–C glycosidic bond vibration (KačUráková, Capek, Sasinková, Wellner, & Ebringerová, 2000). The interpretation of the spectral region between 800-1200 cm^{-1} is generally difficult because it is the "finger print" region and is different for different polysaccharides (Hosseini, Khodaiyan, & Yarmand, 2016).

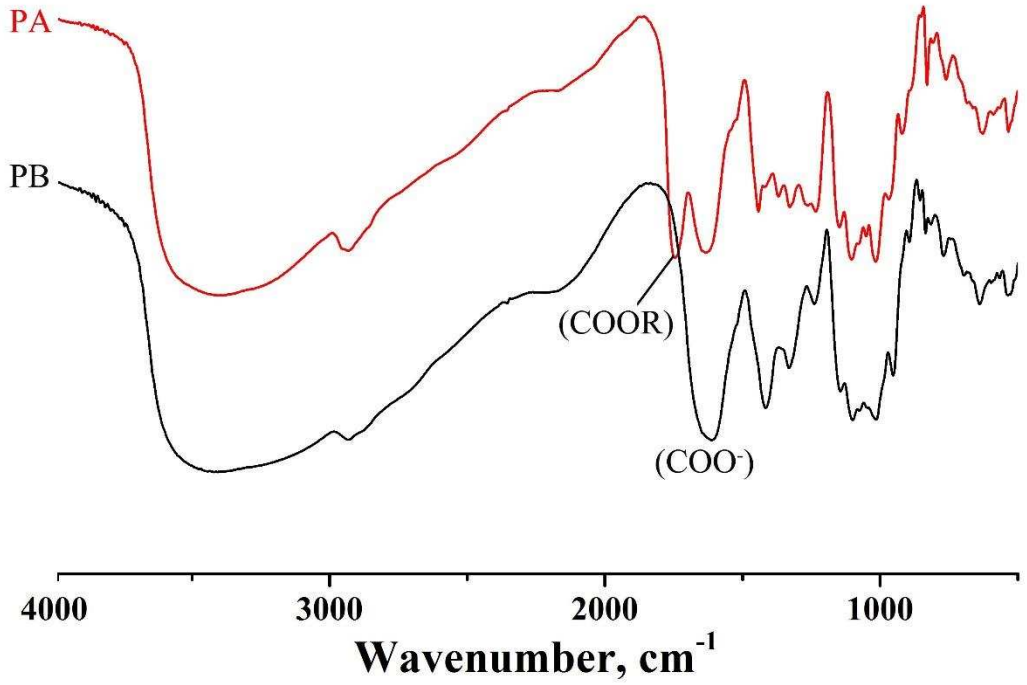


Fig. 5. FTIR spectra of PA (up) and PB (down)

Detailed structural information about the proton environment of the extracted pectic polysaccharides from citrus peel were obtained using ^{1}H NMR (Fig. 6). In the ^{1}H NMR spectrum of PA, a very intense signal at 3.68 ppm could be assigned to the methyl ester groups of the GalA carboxyl groups (Zhang, et al., 2013). Major signals observed in the spectrum of PA were assigned to the five protons of GalA (H-1, 4.97 ppm; H-2, 3.61 ppm; H-3, 3.88 ppm; H-4, 4.00 ppm and H-5, 4.34 ppm). In the anomeric region, the signals at 5.02 ppm and 4.84 ppm were attributed to the H-1 of Rha and H-1 of Gal, respectively (Zhi, et al., 2017b). There were additional peaks in the spectrum of PB that were difficult to assign. This may be due to the presence of larger amounts of Ara, Gal and Rha, almost equivalent to that of GalA. The H-1 signal of methylated GlcA residue is shifted to 5.03 ppm (Zhang, et al., 2013). Other signals from 3.5 to 4.3 ppm were too congested to

distinguish and will probably require 2D NMR to assign.

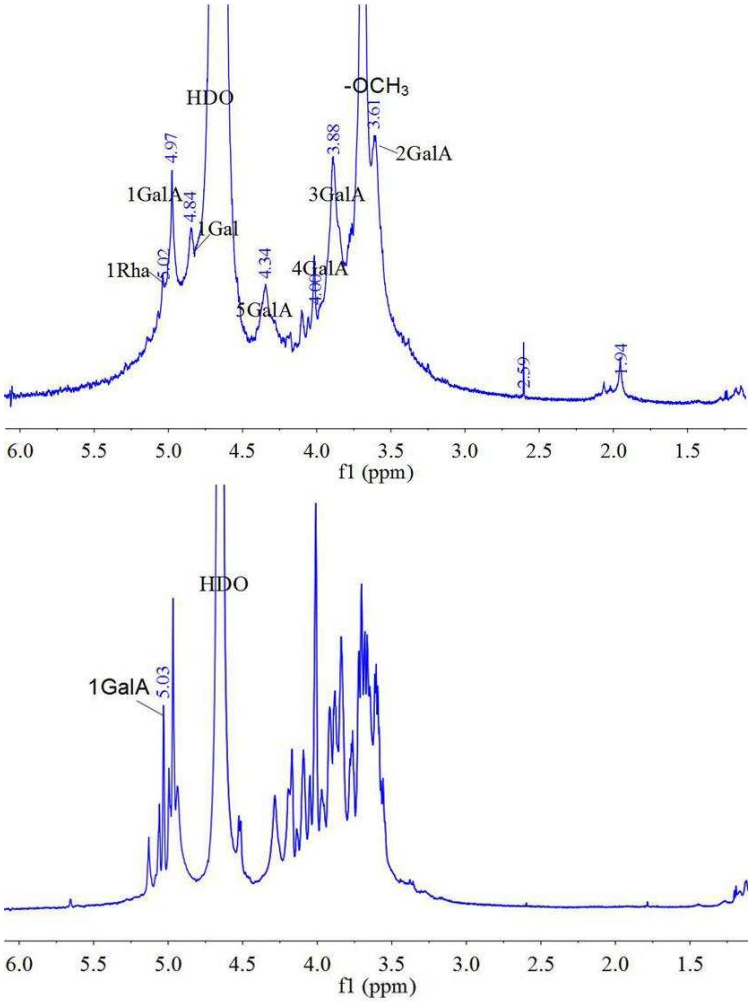
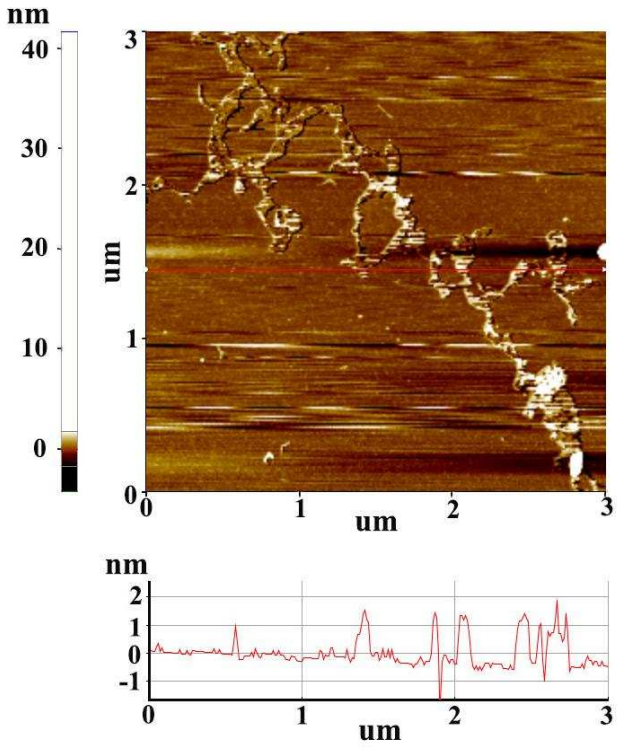


Fig. 6. The ^{1}H NMR spectrum of the PA (per panel) and the ^{1}H NMR spectrum of the PB (lower panel).

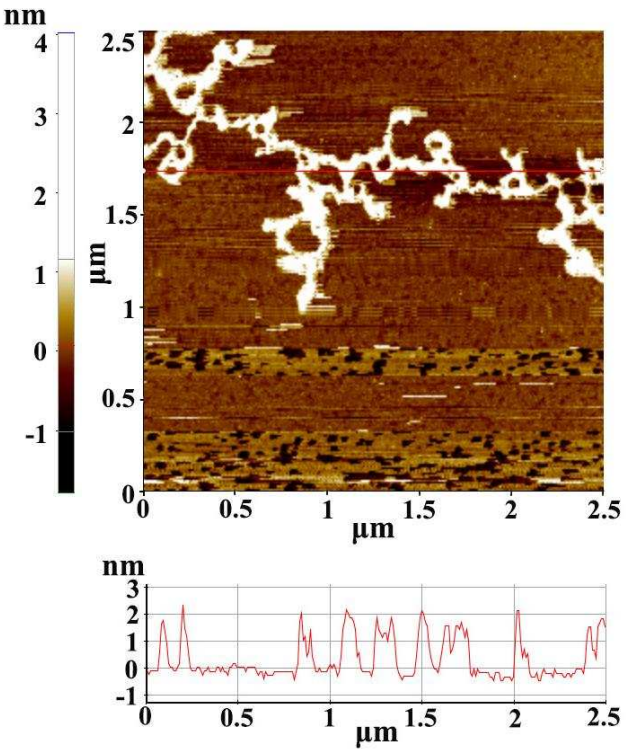
3.4 AFM image analysis

Atomic force microscopy (AFM) was next used to image the molecule shapes of PA, PB and CP. The red line in each image illustrates the location of the cross-sections that are profiled in the graph beneath the images. PA, PB and CP had heights of about 1.5 nm, 2 nm and 3 nm, respectively. However, the expected diameters of single polysaccharide strands, imaged by AFM and adopting

helical conformations, are from 0.5 to 0.8 nm (Round, Macdougall, Ring, & Morris, 1997). This suggests that the polysaccharide chains are slightly aggregated. In case of morphology, a chain-like structure is characteristic for these molecules. In addition, PB and PA are significantly branched, with many short side chains attached to the main bone, which are consistent with the chromatograms of the chain conformation.



(a)



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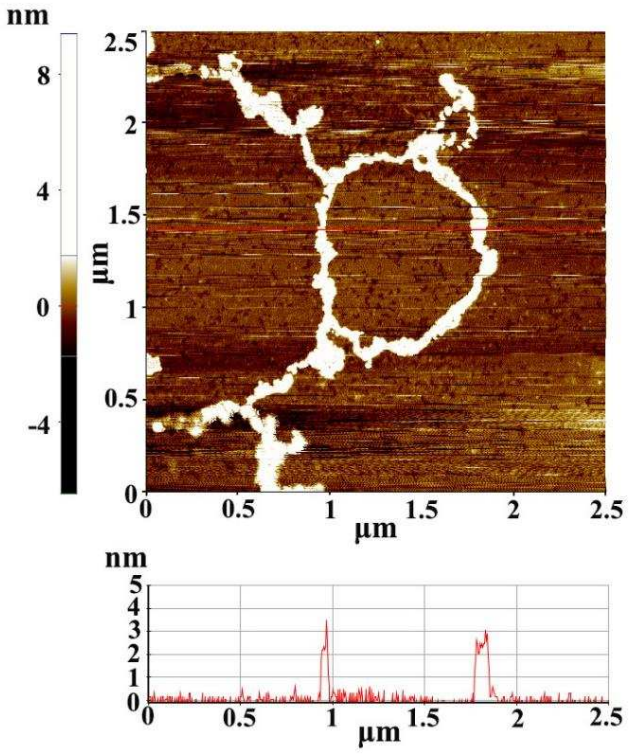

(c)

Fig. 7. Representative topographical AFM images of (a) PA, (b) PB and (c) CP

3.5 Rheological properties

The rheological curves of PA, PB and commercial citrus pectin (CP) at concentrations of 0.75 % and 1.5 % were used as a control and are shown in Fig. 8. All three of these samples show an obvious shear thinning phenomena and behave as pseudoplastic fluids. The flow curves were fitted by the power law model, which is often used to describe the flow behavior of polymers. In this model, the consistency coefficient can reflect the viscosity and flow resistance. The smaller the fluid characteristics index, the more obvious is the polymer pseudoplasticity. The parameters obtained were shown in Table 3. When the two sets of data are compared, we find that the

consistency coefficients of PA and PB were higher than that of CP at both concentrations. In terms of fluid characteristics index, there is a slight decrease in the three solutions when the concentration increases, suggesting the increased intertwining and breaking of polymer interactions. Rheological properties of pectin solutions depend on many structural parameters including molar mass and its distribution, the distribution of substituents (Sengkhamparn, et al., 2010), and the degree of branching (Hwang & Kokini, 1992). Thus, the relationship between these parameters and the resulting rheological behavior can be quite complex. The larger consistency of the coefficient values of PA and PB, when compared to CP may be due to the branched structures of PA and PB. PB has low GalA content and low degree of methylation, which may result in lower viscosity than PA. Higher concentrations of three solutions display increased viscosity (Fig. 6), which suggests that viscosity can be controlled by controlling the concentration of these polysaccharides.

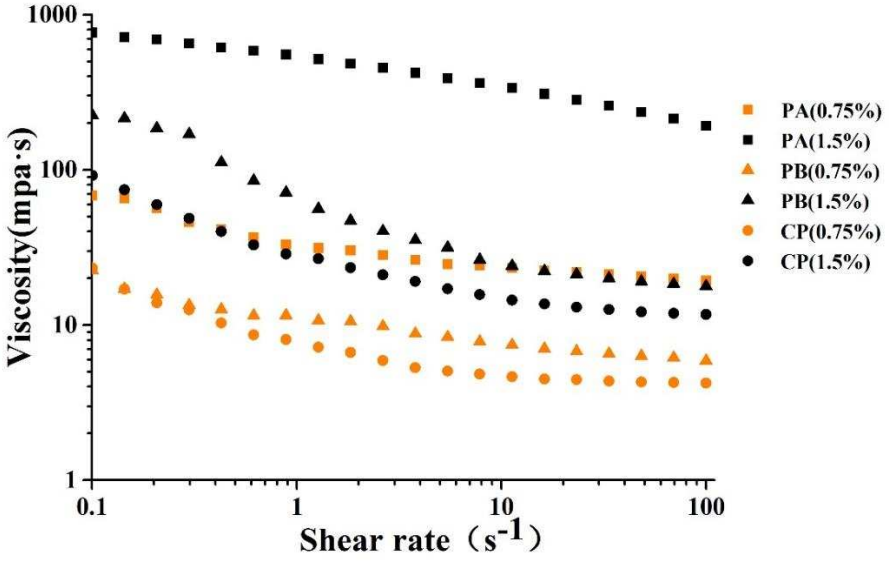

Fig. 8. The flow behavior of PA, PB, and CP at 0.75% and 1.5%.

Table 3 Parameters of flow curves after fitted to power law model

Concentration / Index	1.5 %			0.75 %		
	PA	PB	CP	PA	PB	CP
k	522	77	33	37	12	9
n	0.80	0.60	0.71	0.82	0.83	0.77
R^2	0.99	0.94	0.93	0.91	0.95	0.87

4 Conclusions

Because of the strong extraction conditions used in preparing commercial pectins, these mainly consists of HG structure. Recent studies have emphasized the importance of the HG-I domain for retaining desired biological activities. Therefore, the aim of this study was to prepare pectins enriched of HG-I domains. By controlling the acid extraction temperature at 28 °C and the alkali extraction temperature at 32 °C, we successfully obtained two types of pectic polysaccharides having predominantly HG-I structures. Monosaccharide compositional analysis showed both types, PA and PB, contained large proportion of neutral sugars. Additional structure information on these polysaccharides was obtained using SEC-MALLS, ^{1}H NMR and FT-IR. AFM images directly demonstrated their branched-chain morphology, consistent with the results by monosaccharide determination. Rheological analysis showed that both PA and PB polysaccharides had good thickening properties and could be potentially used as functional food ingredients.

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