Food Hydrocolloids 79 (2018) 579–586

Contents lists available at ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

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

Hua Zhang [a], Jianle Chen [a], Junhui Li [a], Lufeng Yan [a], Shan Li [a], Xingqian Ye [a], Donghong Liu [a], Tian Ding [a], Robert J. Linhardt [b], Caroline Orfila [c], Shiguo Chen [a, *]

[a] Zhejiang Key Laboratory for Agro-Food Processing, Department of Food Science and Nutrition, Fuli Institute of Food Science, Zhejiang University, Hangzhou 310058, China
[b] Center for Biotechnology & Interdisciplinary Studies, Department of Chemistry & Chemical Biology, Rensselaer Polytechnic Institute, Biotechnology Center 4005, Troy, NY 12180, USA
[c] School of Food Science and Nutrition, University of Leeds, Leeds LS2 9JT, UK

ARTICLE INFO

Article history:
Received 9 October 2017
Received in revised form
1 December 2017
Accepted 1 December 2017
Available online 5 December 2017

Keywords:
Extraction of pectic polysaccharides
Mandarin citrus peel
SEC-MALLS
RG-I structure

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 that RG-I region enriched pectic polysaccharides possess improved bioactivities, including cancer prevention, cardiovascular disease treatment and fibrosis. Thus, in this study, the isolation of mandarin peel pectic polysaccharides enriched in RG-I was evaluated through a sequential extraction method, consisting of acid followed by alkaline hydrolysis 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 composition analysis showed that PA and PB consisted mainly of branched RG-I with GalA/Rha values of 8.4 and 1.7, respectively, and high content of arabinose and galactose. The results of GPC-MALLS confirmed that both PA and PB were branched and had molecular weights of 282 kDa and 743 kDa, respectively. AFM imaging directly verified the branched-chain morphology of these polysaccharides. NMR and FT-IR analyses demonstrated that they were typical pectic polysaccharides, and the esterification degree of 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.

1. Introduction

Pectin is a complex polysaccharide widely distributed in the primary cell wall and middle lamella of most plant tissues (Ridley, O'Neill, & Mohnen, 2001; Thakur, Singh, Handa, & Rao, 1997). Pectin contains multiple domains including mainly linear homogalacturonans (HG) and highly branched rhamnogalacturonan-I (RG-I) and rhamnogalacturonan-II (RG-II) (Zhi et al., 2017). The HG form of pectin, consists of a linear backbone of 1,4-linked-α-D-galacturonic acid (GalA) units, which is partially methyl esterified at the carboxylic acid or acetylated (Xu et al., 2014). Pectins 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%. 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. The global market of pectin is growing, driven by demand in 'clean label' ingredients and pectin's demand exceeds its supply. The pectin market was valued more than $850 million in 2013 with a projected annual growth rate ≥5% (Chen et al., 2016). Commercial pectins are extracted mainly from citrus peel and apple pomace, and to a lesser extent, from sugar

* Corresponding author.
E-mail addresses: 21613072@zju.edu.cn (H. Zhang), 18511581374@163.com (J. Li), 11513026@zju.edu.cn (L. Yan), 11613001@zju.edu.cn (S. Li), psu@zju.edu.cn (X. Ye), dhliu@zju.edu.cn (D. Liu), tding@zju.edu.cn (T. Ding), linhar@rpi.edu (R.J. Linhardt), C.Orfila@leeds.ac.uk (C. Orfila), chenshiguo210@163.com (S. Chen).

https://doi.org/10.1016/j.foodhyd.2017.12.002
0268-005X/

beet pulp (May 1990; Yapo, Robert, Etienne, Wathelet, & Paquot, 2007). For the best gelling properties and quality control, commercial pectin consists mainly of HG with small amount of RG-I with GalA generally higher than 65%. During conventional extraction processes, 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 produce commercial HMP with high proportions of HG, 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 its gel forming properties. Pectins enriched in RG-I structure show improved activities in 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, & St-Pierre, 2014). The side chain of RG-I pectin can occupy this recognition domain and competitively inhibit the activity of Gal-3 (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 fructooligosaccharides, and the bifidogenic properties of RG I-rich fraction are 1.5-times higher 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 method 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 (Wang et al., 2015). However, most of these technologies are in laboratory stage and difficult to implement at large scale. A better strategy to retain the side chains may be to optimize 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 polysaccharides were dominated with RG-I regions (Chen et al., 2016). These pectic polysaccharides were obtained by sequential acid and alkali treatment of the canning water at low temperature. Therefore, we wanted to apply similar extraction conditions to extract pectic polysaccharides from mandarin peel, another abundant waste product of the citrus industry.

The aim of the study was to apply acid and alkaline extraction conditions at low temperature to extract branched RG-I rich pectins from mandarin peel. The first extraction treatment was with hydrochloric acid at low temperature (28 °C) to obtain branched HMP. The residue was then treated with sodium hydroxide at low temperature (32 °C) to obtain 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 branched HMP and LMP, with potential use as functional ingredients, can be extracted from citrus peel under lower than conventionally used temperature conditions.

2. Materials and methods

2.1. Citrus peel materials and chemicals

Mandarin citrus peel (*Citrus unshiu Marc.*) was provided by a citrus fruit canning factory in China and oven-dried at 105 °C for 24 h. Dried 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), pectin from citrus peel (GalA >74.0%, DM = 55%, Mw = 550 kDa) and D_2O were all purchased from Sigma -Aldrich (Shanghai, 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 (pH = 1.0) with the solid to liquid ratio of 1:30. The mixture was stirred at 28 °C for 40 min to loosen the cell wall structure and extract a portion of the pectic polysaccharides. After this extraction stage, the mixture was filtered through a 400-mesh filter bag. A first fraction of acid-extracted pectic polysaccharides (PA) was recovered from the acidic filtrate by adjusting pH to neutral and precipitation with 95% ethanol in the volume ratio of 1:1. The residue was resuspended in 0.6% NaOH (pH = 13.2, 1:30 solid to liquid ratio), 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 described previously to recover the pectins extracted with base (PB). After precipitation, both precipitates were washed with 95% ethanol 2–3 times to remove salt and oven-dried at 55 °C for 24 h.

The temperatures used for acid and alkaline treatments were chosen based on the processing conditions used in segment membrane removal in the citrus canning factory. Considering the best yield and the convenience of pH adjustment to a neutral value, the pH of acidic extract and basic extract was adjusted to ~3 or ~6 before precipitation with ethanol, respectively.

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

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

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

2.3. Monosaccharide analysis

Monosaccharide composition was analyzed by a 1-phenyl-3-methyl-5-pyrazolone (PMP)-high performance liquid

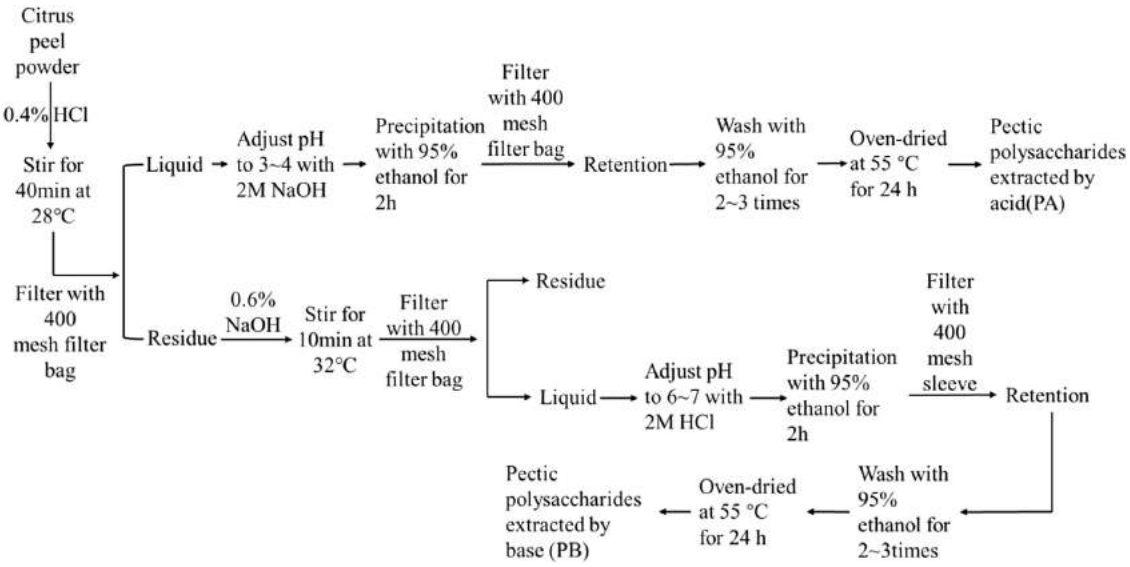

Fig. 1. Flow chart for extraction of PA and PB pectic polysaccharides.

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. 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 10 min, then from 15% to 25% in the next 20 min. Detection was with a 2489 UV/Vis Detector (Waters, US) at 250 nm.

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 spectra of pectic polysaccharides were 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 OriginPro 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

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 samples were ready, three freshly cleaved mica substrates were immersed into solutions for 10 min, respectively. Afterwards, three mica substrates were rinsed and air-dried and then observed by AFM (XE-70, Park Scientific Instruments, Suwon, Korea) using tapping mode in air at room temperature (humidity: 50%–60%). The probe is a classical silicon cantilever (Si_3N_4) with a spring constant of 0.2 N/m and a resonance frequency of approximately 13 kHz. XEI software was used for image manipulation.

2.7. Rheological measurements

The rheological properties of pectic polysaccharides were carried out using a 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 at room temperature, the samples were subjected to steady shearing at 25 °C with the shear rates ranged from 0.1 to 100 s^{-1}. Data were fitted to a power law model (equation (3)). The software used for fitting rheological data was OriginPro 9.1.

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

Table 1
Yields and monosaccharide compositions of PA and PB.

	Yield (wt. %)	Galacturonic acid (mol %)	Neutral sugar composition (mol %)				
			Rhamnose	Glucose	Galactose	Arabinose	Xylose
PA	4.2 ± 0.7	51.8 ± 1.0	6.2 ± 0.1	–	4.1 ± 0.1	27.5 ± 0.2	10.4 ± 0.1
PB	18.9 ± 2.3	20.3 ± 0.9	11.7 ± 0.1	8.9	18.5 ± 0.2	40.6 ± 1.1	–

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

In equation (3), where η is the apparent viscosity (mPa·s), k (mPa·s^n) is the 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 mandarin peel by hydrochloric acid and sodium hydroxide are shown in Table 1. The yield of PA was 4.2%, which is very low compared to the hot acid extraction method (20%–30%) (Palanisamy, Dhivya, & Kumaresan, 2014). This most likely due to the low extraction temperature leading to less loosening of the cell wall structure and smaller extent in pectin solubilization (Methacanon, Krongsin, & Gamonpilas, 2014). In this experiment, the temperature used to extract PA was 28 °C, which is very mild and only capable of disrupting part of the connecting between fiber and pectin in the cell wall, but insufficient for extracting most 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 23.1%, comparable to the yield of pectin obtained by subcritical water (19–22%) and hot acid (20%–30%) (Palanisamy et al., 2014; Wang, Chen & Xin, 2014).

In the PA fraction, GalA was the main monosaccharide, accounting for 51.8 mol%. The value is lower than the content in commercial pectin (≥65%). The neutral sugars, Rha, Gal, xylose (Xyl) and Ara represented the major components, consistent with the literature (Wang et al., 2014). Rha and GalA are the main structural units of the RG region. Gal and Ara are monosaccharides belonging to the side chains of RG-I, while Xyl mainly composed xylogalacturonan (XG). As the GalA/Rha ratio for PA was 8.4, suggesting that PA consists mainly of RG-I structure (Oosterveld, Beldman, Schols, & Voragen, 2000). This ratio was close to the ratio of RG-dominated soybean pectin (3.4) and much lower than that of the commercial citrus pectin (74) and murta pectin (66), which were 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 79.7%. Alkali extracted pectin is also 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 RG-I with low extent of degradation, while HG can be degraded through elimination and oxidative peeling (Bonnina et al., 2001). PB mainly consists of Rha, Gal, Ara and glucose (Glc). The absence of Xyl indicates that PB has little xyloglucan or XG structure. The GalA/Rha ratio is 1.7, indicating that RG-I structure is dominant in PB. Since the PB (Gal + Ara)/Rha ratio of 5.1 is the same as the ratio of 5.1 for PA and PB has more Rha, PB probably has a higher proportion of hairy regions and side chains than PA (Wang et al., 2014).

According to the yield and composition of PA and PB, we hypothesize that the acid treatment may extract pectic polysaccharides loosely connected to the hemicellulose, while alkali may extract pectic polysaccharides more tightly associated with the hemicelluloses. It is likely that some pectic polysaccharides remain in the residue, attached strongly to the hemicelullose and even cellulose network. A schematic diagram is shown in Fig. 2 (Cosgrove, 2000).

3.2. SEC-MALLS-RI analysis

Chromatograms of molar mass distribution of PA and PB are shown in Fig. 3. The relative values in Table 2 were calculated using Astra 6.1 to obtain more accurate information about the molecular size of the recovered polysaccharides. The weight average of molar

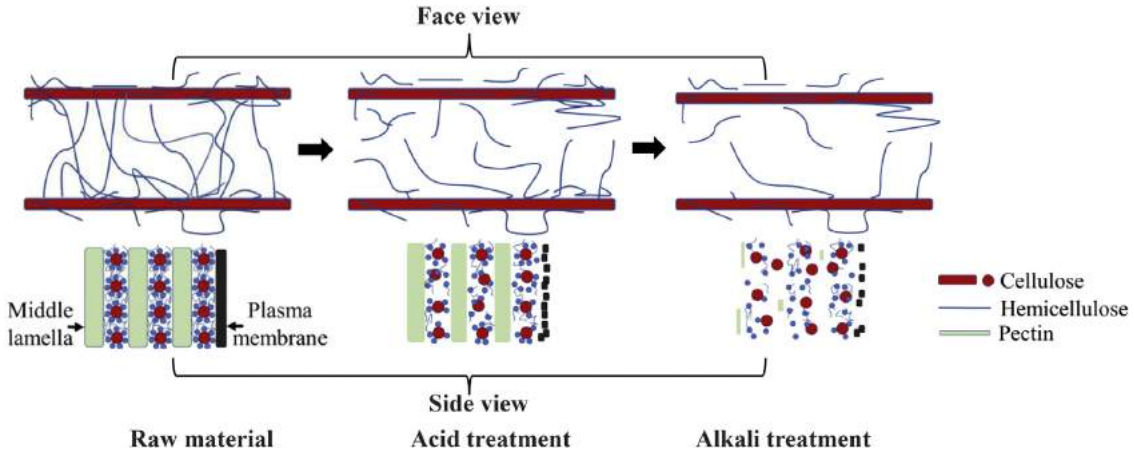

Fig. 2. Schematic diagram of extraction process.

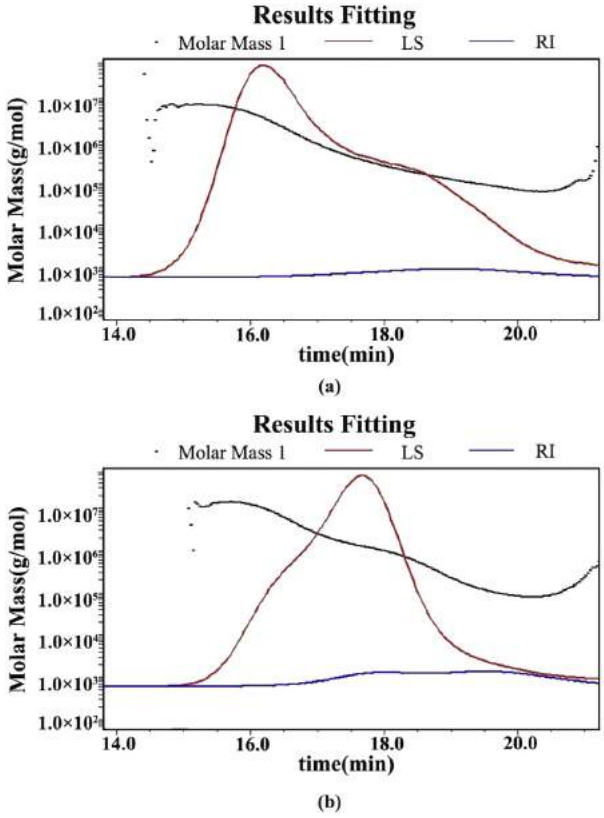

Fig. 3. Chromatograms of molar mass distribution and molecular radius distributions of (a) PA and (b) PB.

Table 2
Average values of molecular weights and radius 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.

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 are in good agreement with previously published data (Fishman et al., 2015). The molecular weight of PB is much higher than the previously published commercial citrus LMP (Fishman et al., 2015), which may due to the better preservation of side chains during the low temperature extraction in the present study. 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

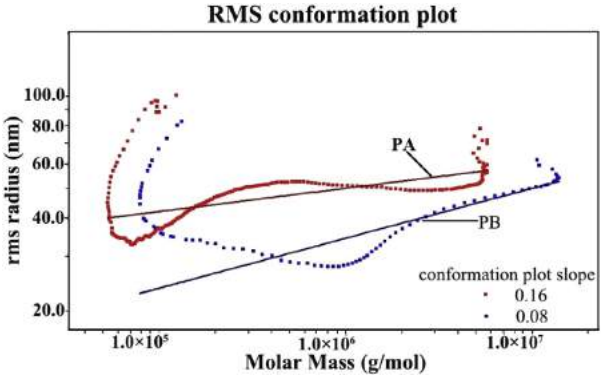

Fig. 4. Chromatograms of chain conformation of PA and PB in aqueous environment.

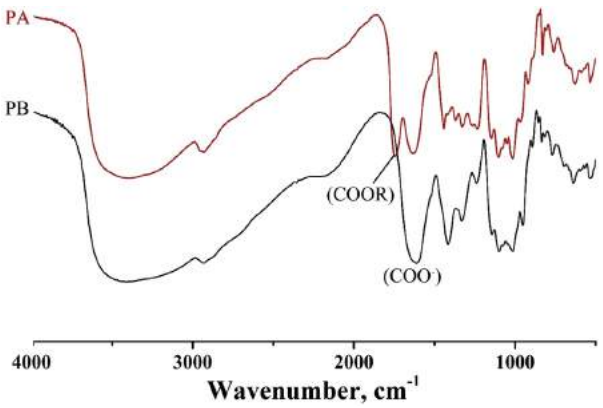

Fig. 5. FTIR spectra of PA and PB.

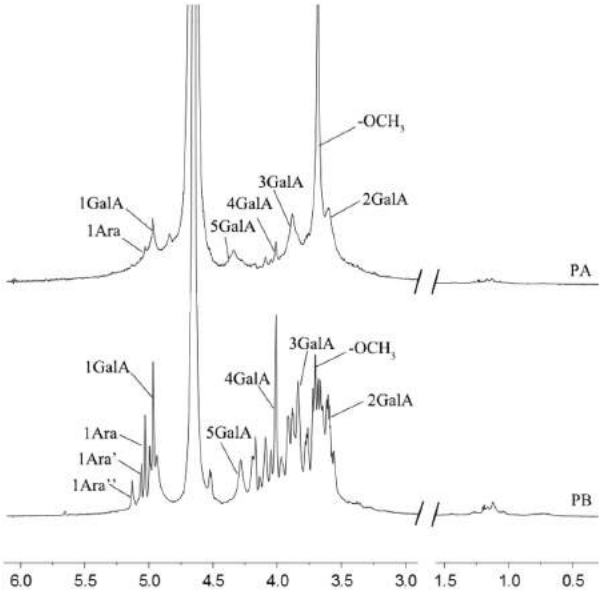

Fig. 6. The ^{1}H NMR spectrum of the PA and PB.

consistent with the anomalous SEC phenomenon of plots bending upward at the low molar mass region (Podzimek, Vlcek, & Johann, 2001).

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 methyl esterification (DE) (Vriesmann, 2009). In the spectrum 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-) was used to calculate a DE of 56%. The value obtained was relatively lower than pectin extracted using subcritical water (69 %–75%) (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, as confirmed by the COO^- peak at 1610 cm^{-1}, consistent with the literature (DE = 10%) (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 dissolving out of pectin 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} correspond to the stretching vibrations C–OH side groups and the C–O–C glycosidic bond vibration (KačUráková, Capek, Sasinková,

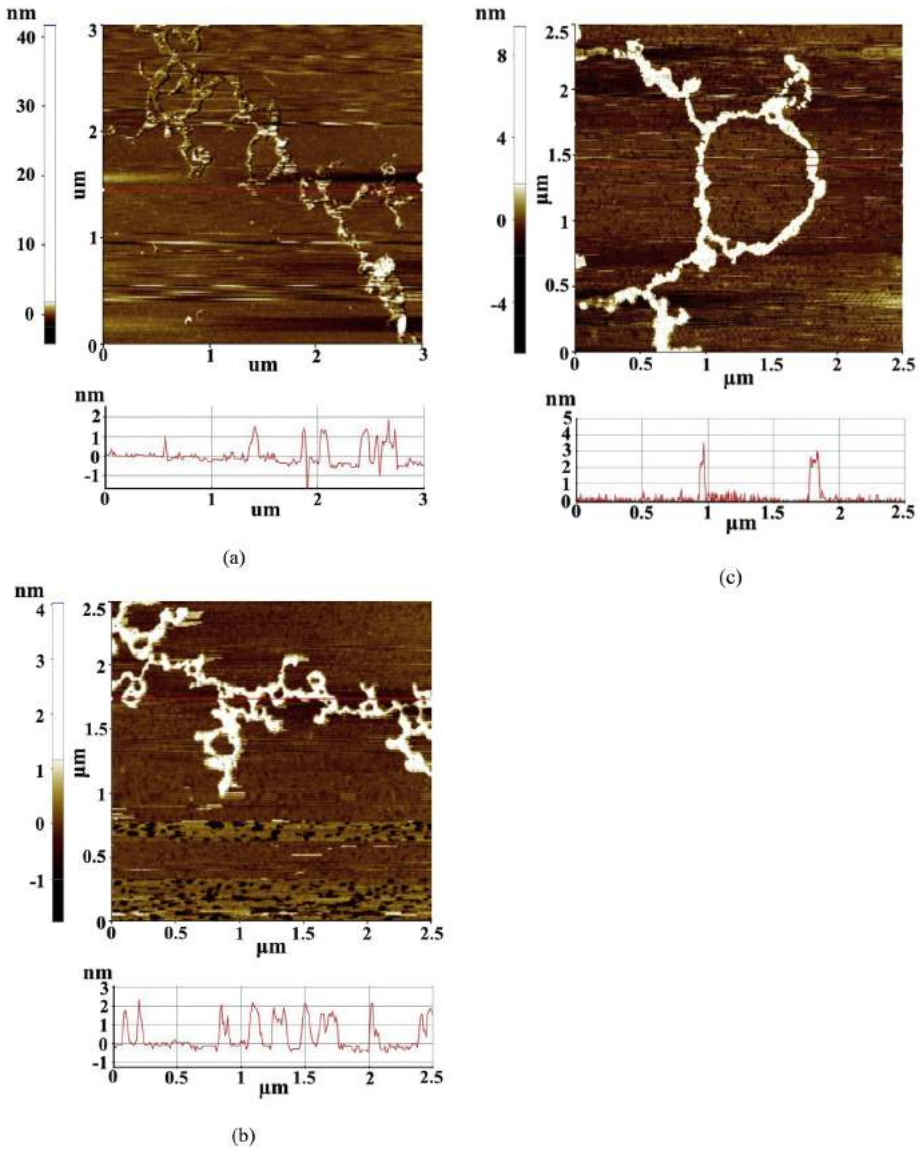

Fig. 7. Representative topographical AFM images of (a) PA, (b) PB and (c) CP.

Wellner, & Ebringerová, 2000). The interpretation of the spectral region between 800 and 1200 cm^{-1} is generally difficult because it is the "fingerprint" region and is different for different polysaccharides (Hosseini, Khodaiyan, & Yarmand, 2016).

Detailed structural information about the proton environment of the extracted pectic polysaccharides from mandarin peel was obtained using ^{1}H NMR (Fig. 6). The spectra showed that PA and PB had different structures. 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, while the equivalent signal in PB spectrum was weak, which was reasonable (Zhang et al., 2013). Signals at 1.14 and 1.22 were derived from methoxyl groups of O-2 and O-2,4 linked L-rhamnose, respectively (W. Wang et al., 2016). Major signals observed in the spectrum of PA and PB 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) (Zhi et al., 2017). In the anomeric region, the signals between 5.0 and 5.2 ppm were attributed to the H-1 of different types of Ara. The signals for Ara in the PB spectrum were larger than those in PA, which correspond to the results of monosaccharide compositional analysis. There were additional peaks in the spectrum of PB that were difficult to assign. This may due to the presence of larger amounts of Ara, Gal and Rha, almost equivalent to that of GalA.

3.4. AFM image analysis

Atomic force microscopy (AFM) was next used to image the molecule shapes of PA, PB and CP. The red lines in each image shown in Fig. 7 illustrate the location of the cross-sections that are profiled in the graph beneath the images. PA, PB and CP have 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 backbone, which are consistent with the chromatograms of the chain conformation.

3.5. Rheological properties

The rheological curves of PA, PB and commercial citrus pectin (CP) as a control at concentration 0.75% and 1.5% are shown in Fig. 8. All these samples show 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

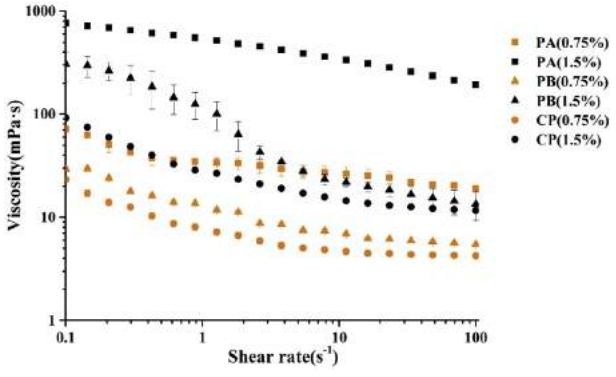

Fig. 8. The flow behavior of PA, PB, and CP at concentration of 0.75% and 1.5%.

Table 3
Parameters of flow curves obtained by fitting to power law model.

Index	Concentration					
	1.5%			0.75%		
	PA	PB	CP	PA	PB	CP
k (mPa·s^n)	522	99	33	38	14	9
n	0.80	0.48	0.71	0.84	0.75	0.77
R^2	0.99	0.96	0.93	0.93	0.94	0.87

of polymers. In this model, the consistency coefficient (k) can reflect the viscosity and flow resistance. The smaller the fluid characteristics index (n), the more obvious is the polymer pseudoplasticity. The parameters obtained are 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. Larger consistency coefficient values of PA and PB compared to CP, may due to macromolecular entanglements enhanced by 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 samples display increased viscosity (Fig. 8.), which suggests that viscosity can be controlled by controlling the concentration of these polysaccharides.

4. Conclusions

In this article, we described the extraction of highly branched RG-I fractions from mandarin peel. By lowering the acid extraction temperature to 28 °C and the alkali extraction temperature to 32 °C, the RG-I structure was preserved. The extracted pectic polysaccharides are rich in neutral side chains; and SEC-MALLS and AFM confirmed large-size polymers with branched morphologies. Rheological analysis showed that the two polysaccharides have good thickening properties superior to commercial citrus pectin and could be potentially used as functional ingredients in food. Further work is required to optimize the yield of extraction and to further understand their health benefits.

Acknowledgments

This work was financially supported by the Key Research and Development Project of Zhejiang Province, China (2015C02036) and Public Welfare Project of Zhejiang Province, China (2015C32088).

References

de Boer, R. A., Voors, A. A., Muntendam, P., van Gilst, W. H., & van Veldhuisen, D. J. (2009). Galectin-3: A novel mediator of heart failure development and progression. *European Journal of Heart Failure, 11*(9), 811–817.

Bonnina, E., Brunel, M., Gouy, Y., Lesagemeessen, L., Asther, M., & Thibault, J. (2001). Aspergillus Niger I-1472 and Pycnoporus cinnabarinus MUCL39533, selected for the biotransformation of ferulic acid to vanillin, are also able to produce cell wall polysaccharide-degrading enzymes and feruloyl esterases. *Enzyme & Microbial Technology, 28*(1), 70–80.

Chan, S. Y., & Choo, W. S. (2013). Effect of extraction conditions on the yield and chemical properties of pectin from cocoa husks. *Food Chemistry, 141*(4), 3752.

Chen, J., Cheng, H., Wu, D., Linhardt, R. J., Zhi, Z., Yan, L., et al. (2016). Green recovery

of pectic polysaccharides from citrus canning processing water. *Journal of Cleaner Production, 144*.
Cosgrove, D. J. (2000). Expansive growth of plant cell walls. *Plant Physiology & Biochemistry, 38*(1–2), 109–124.
Fishman, M. L., Chau, H. K., Qi, P. X., Hotchkiss, A. T., Garcia, R. A., & Cooke, P. H. (2015). Characterization of the global structure of low methoxyl pectin in solution. *Food Hydrocolloids, 46*(Supplement C), 153–159.
Gao, X., Zhi, Y., Sun, L., Peng, X., Zhang, T., Xue, H., et al. (2013). The inhibitory effects of a rhamnogalacturonan I (RG-I) domain from ginseng pectin on galectin-3 and its structure-activity relationship. *Journal of Biological Chemistry, 288*(47), 33953.
Gnanasambandam, R., & Proctor, A. (2000). Determination of pectin degree of esterification by diffuse reflectance Fourier transform infrared spectroscopy. *Food Chemistry, 68*(3), 327–332.
Harris, P. J., & Smith, B. G. (2010). Plant cell walls and cell-wall polysaccharides: Structures, properties and uses in food products. *International Journal of Food Science & Technology, 41*(s2), 129–143.
Hosseini, S. S., Khodaiyan, F., & Yarmand, M. S. (2016). Optimization of microwave assisted extraction of pectin from sour orange peel and its physicochemical properties. *Carbohydrate Polymers, 140*, 59.
Hwang, J., & Kokini, J. L. (1992). Contribution of the side branches to rheological properties of pectins. *Carbohydrate Polymers, 19*(1), 41–50.
Isabelle, B., Coutinho, P. M., Schols, H. A., Gerlach, J. P., Bernard, H., & De, V. R. P. (2012). Degradation of different pectins by fungi: Correlations and contrasts between the pectinolytic enzyme sets identified in genomes and the growth on pectins of different origin. *Bmc Genomics, 13*(1), 1–11.
KačUráková, M., Capek, P., Sasinková, V., Wellner, N., & Ebringerová, A. (2000). FT-ir study of plant cell wall model compounds: Pectic polysaccharides and hemicelluloses. *Carbohydrate Polymers, 43*(2), 195–203.
Khalikov, D. K., & Mukhiddinov, Z. K. (2004). Physicochemical principles of plant-cell protopectin decomposition by acid catalysts. *Chemistry of Natural Compounds, 40*(2), 101–114.
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.
Levigen, S., Ralet, M. C., & Thibault, J. F. (2002). Characterisation of pectins extracted from fresh sugar beet under different conditions using an experimental design. *Carbohydrate Polymers, 49*(2), 145–153.
Li, L. C., Li, J., & Gao, J. (2014). Functions of galectin-3 and its role in fibrotic diseases. *Journal of Pharmacology & Experimental Therapeutics, 351*(2), 336.
Lim, J., Yoo, J., Ko, S., & Lee, S. (2012). Extraction and characterization of pectin from Yuza (citrus junos) pomace: A comparison of conventional-chemical and combined physical–enzymatic extractions. *Food Hydrocolloids, 29*(1), 160–165.
Makoto, N., Takahiro, F., Sakie, N., Sayaka, I., Saphwan, A. A., Katsuyoshi, N., et al. (2008). Comparison of sugar beet pectin, soybean soluble polysaccharide, and gum Arabic as food emulsifiers. 1. Effect of concentration, pH, and salts on the emulsifying properties. *Food Hydrocolloids, 22*(7), 1254–1267.
May, C. D. (1990). Industrial pectins: Sources, production and applications. *Carbohydrate Polymers, 12*(1), 79–99.
Methacanon, P., Krongsin, J., & Gamonpilas, C. (2014). Pomelo (Citrus maxima) pectin: Effects of extraction parameters andits properties. *Food Hydrocolloids, 35*(3), 383–391.
Michalak, M., Thomassen, L. V., Roytio, H., Ouwehand, A. C., Meyer, A. S., & Mikkelsen, J. D. (2012). Expression and characterization of an endo-1,4-β-galactanase from Emericella nidulans in Pichia pastoris for enzymatic design of potentially prebiotic oligosaccharides from potato galactans. *Enzyme & Microbial Technology, 50*(2), 121–129.
Oosterveld, A., Beldman, G., Schols, H. A., & Voragen, A. G. J. (2000). Characterization of arabinose and ferulic acid rich pectic polysaccharides and hemicelluloses from sugar beet pulp. *Carbohydrate Research, 328*(2), 185–197.
Palanisamy, K., Dhivya, J. A. E., & Kumaresan, K. (2014). *Extraction and analysis of pectin from citrus Peels: Augmenting the yield from citrus limon using statistical experimental design* (Vol. 5, pp. 303–312).
Podzimek, S., Vlcek, T., & Johann, C. (2001). Characterization of branched polymers by size exclusion chromatography coupled with multiangle light scattering detector. I. Size exclusion chromatography elution behavior of branched polymers. *Journal of Applied Polymer Science, 81*(7), 1588–1594.
Renard, C. M. G. C., & Thibault, J. F. (1996). Degradation of pectins in alkaline conditions: Kinetics of demethylation. *Carbohydrate Research, 286*, 139–150.
Ridley, B. L., O'Neill, M. A., & Mohnen, D. (2001). Pectins: Structure, biosynthesis, and oligogalacturonide-related signaling. *Phytochemistry, 57*(6), 929.
Round, A. N., Macdougall, A. J., Ring, S. G., & Morris, V. J. (1997). Unexpected branching in pectin observed by atomic force microscopy. *Carbohydrate Research, 303*(3), 251–253.
Saberian, H., Hamidi-Esfahani, Z., Gavlighi, H. A., & Barzegar, M. (2017). Optimization of pectin extraction from orange juice waste assisted by ohmic heating. *Chemical Engineering & Processing Process Intensification, 117*(Supplement C), 154–161.
Sathisha, U. V., Jayaram, S., Harish Nayaka, M. A., & Dharmesh, S. M. (2007). Inhibition of galectin-3 mediated cellular interactions by pectic polysaccharides from dietary sources. *Glycoconjugate Journal, 24*(8), 497–507.
Sengkhamparn, N., Sagis, L. M. C., Vries, R. D., Schols, H. A., Sajjaanantakul, T., & Voragen, A. G. J. (2010). Physicochemical properties of pectins from okra (Abelmoschus esculentus (L.) Moench). *Food Hydrocolloids, 24*(1), 35–41.
Sousa, A. G., Nielsen, H. L., Armagan, I., Larsen, J., & Sørensen, S. O. (2015). The impact of rhamnogalacturonan-I side chain monosaccharides on the rheological properties of citrus pectin. *Food Hydrocolloids, 47*, 130–139.
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.
Taboada, E., Fisher, P., Jara, R., Zúñiga, E., Gidekel, M., Cabrera, J. C., et al. (2010). Isolation and characterisation of pectic substances from murta (Ugni molinae Turcz) fruits. *Food Chemistry, 123*(3), 669–678.
Thakur, B. R., Singh, R. K., Handa, A. K., & Rao, D. M. A. (1997). Chemistry and uses of pectin — a review. *Critical Reviews in Food Science and Nutrition, 37*(1), 47.
Vladoiu, M. C., Labrie, M., & St-Pierre, Y. (2014). Intracellular galectins in cancer cells: Potential new targets for therapy (review). *International Journal of Oncology, 44*(4), 1001.
Vriesmann, L. C. (2009). Polysaccharides from the pulp of cupuassu (Theobroma grandiflorum): Structural characterization of a pectic fraction. *Carbohydrate Polymers, 77*(1), 72–79.
Wang, Chen, Q., & Xin, L. (2014). Pectin extracted from apple pomace and citrus peel by subcritical water. *Food Hydrocolloids, 38*(3), 129–137.
Wang, W., Ma, X., Jiang, P., Hu, L., Zhi, Z., Chen, J., et al. (2016). Characterization of pectin from grapefruit peel: A comparison of ultrasound-assisted and conventional heating extractions. *Food Hydrocolloids, 61*(Supplement C), 730–739.
Wang, Ma, X., Xu, Y., Cao, Y., Jiang, Z., Ding, T., et al. (2015). Ultrasound-assisted heating extraction of pectin from grapefruit peel: Optimization and comparison with the conventional method. *Food Chemistry, 178*, 106.
Xu, Y., Zhang, L., Bailina, Y., Ge, Z., Ding, T., Ye, X., et al. (2014). Effects of ultrasound and/or heating on the extraction of pectin from grapefruit peel. *Journal of Food Engineering, 126*(4), 72–81.
Yapo, B. M., Lerouge, P., Thibault, J. F., & Ralet, M. C. (2007). Pectins from citrus peel cell walls contain homogalacturonans homogenous with respect to molar mass, rhamnogalacturonan I and rhamnogalacturonan II. *Carbohydrate Polymers, 69*(3), 426–435.
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, L., Ye, X., Ding, T., Sun, X., Xu, Y., & Liu, D. (2013). Ultrasound effects on the degradation kinetics, structure and rheological properties of apple pectin. *Ultrasonics Sonochemistry, 20*(1), 222–231.
Zhi, Z., Chen, J., Shan, L., Wang, W., Rui, H., Liu, D., et al. (2017). Fast preparation of RG-I enriched ultra-low molecular weight pectin by an ultrasound accelerated Fenton process. *Scientific Reports, 7*.