Soluble Polysaccharides Isolation and Characterization from Rabbiteye Blueberry (*Vaccinium ashei*) Fruits

Jia Deng,[a] Zheng-Jun Shi,[b,c] Xian-Zhong Li,[b] and Hui-Min Liu [a,b,*]

Five soluble polysaccharide fractions were sequentially extracted with water, EDTA, Na_2CO_3, 4% KOH, and 14% KOH solutions at room temperature for 4 h from cell wall material of rabbiteye blueberry (*Vaccinium ashei*) fruits, and their physicochemical properties were examined. The sequential treatments yielded a total 36.02% soluble polysaccharides of the dry cell wall material. HPAEC and spectroscopy (FT-IR, NMR) analyses indicated that water-, EDTA-, and Na_2CO_3-soluble polysaccharide fractions were mainly composed of pectins, followed by lower amounts of arabinogalactans and glucans, while the two KOH-soluble fractions were mainly composed of hemicelluloses. Homogalacturonan was proven to be the predominant component in the isolated blueberry fruit pectic substance. The isolated blueberry fruit hemicelluloses could be defined as a linear β-(1→4)-linked-xylopyranosyl, in which xylose was the predominant neutral sugar (69.98 to 77.16%), followed by lower amounts of galactose, glucose, arabinose, and mannose.

Keywords: Blueberry; Pectins; Hemicelluloses; Isolation; Characterization

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INTRODUCTION

Blueberry (*Vaccinium ashei*) fruits are known for their health-promoting substances and are thus gaining wide popularity with the public (Lila 2004; Wang *et al.* 2005). Like other berry fruits, blueberry fruits are rich sources of bioactive compounds with antimicrobial activities against human pathogens (Puupponen-Pimiä *et al.* 2001, 2005). These bioactive compounds include flavonoids, such as flavonols (Häkkinen and Törrönen 2000), anthocyanins, and others (Cao *et al.* 1998), all having antioxidant activity. It has been shown that dietary supplementation with blueberry fruit extracts may decrease the enhanced vulnerability to oxidative stress that accompanies aging (Joseph *et al.* 2005). Joseph reported that treatments with extracts from blueberries reduced oxidative stress and age-related declines in normal function *in vitro* and *in vivo* (Joseph *et al.* 1998). In recent decades, consumer consciousness of food nutritional value has increased and this awareness has increased the popularity of blueberries.

Pectins are an important family of heterogeneous polysaccharides in fruit cell walls (Bansal *et al.* 2011). The major pectic polysaccharide is homogalacturonan (HG), a linear homopolymer consisting of α-(1→4)-bound galacturonic acid (GalA) residues. The carboxyl moieties of the polymer are esterified to a certain degree with methanol at C-6 (Voragen *et al.* 2009). The degree and pattern of the methoxylation are important parameters for pectin functionality (Willats *et al.* 2006). Next to HG, two types of

rhamnogalacturonan (RG) polymers occur in the pectin structure. The backbone of rhamnogalacturonan I (RG-I) is composed of repeating units of [α-(1→4)-GalA-α-(1→2)-Rha]. The rhamnose units of RG-I can be substituted at O-4 with mainly galactosyl- and/or arabinosyl-containing neutral sugar side chains. Both single unit [β-D-(1→4)-Gal] and polymeric substitutions, such as arabinogalactan I and arabinan, have been identified in the side chains. Thirdly, rhamnogalacturonan II (RG-II) has a backbone made up of α-(1→4)-bound GalA residues, containing clusters of four different hetero-oligomeric side chains (Voragen *et al.* 2009). In addition, xylogalacturonan (XGA) is another pectic polysaccharide comprising a GalA backbone, substituted at O-3 with monomeric and short oligomeric β-D-Xyl side chains (Ralet *et al.* 2009). It is generally assumed that various constituent polymers of pectins are covalently linked, but the exact features are still under debate (Caffall and Mohnen 2009). As the main component of the middle lamella, pectins are of major importance for intercellular adhesion (Cosgrove *et al.* 2005), and thus for overall tissue structure.

Hemicelluloses are the second most abundant plant renewable material after celluloses (Sun *et al.* 2001). Unlike cellulose, hemicelluloses are not chemically homogeneous but rather a family of polysaccharides, which comprises xyloglucan, xylans (including glucuronoxylan, arabinoxylan, and glucuronoarabinoxylan), mannans (including glucomannan, galactomannan, and galactoglucomannan), and 1→3/1→4 β-glucans (Cosgrove *et al.* 2005; Fry *et al.* 2008). The principal sugars of hemicelluloses are D-xylose, L-arabinose, D-glucose, D-galactose, D-mannose, D-glucuronic acid, 4-*O*-methyl-D-glucuronic acid, D-galacturonic acid, and to a lesser extent, L-rhamnose, L-fucose, and various *O*-methylated neutral sugars (Lv *et al.* 2010). In the plant cell walls, hemicelluloses are associated with cellulose and lignin by hydrogen bonds and covalent bonds (mainly ether and ester linkages), respectively. Therefore, isolation of hemicelluloses in a pure form from plant cell walls involves hydrolysis of ester and ether linkages followed by extraction of them into aqueous media (Xu *et al.* 2007). It has been recently observed in tomato tissue that hemicellulose polymers are present at cell junctions, imparting a role in cell adhesion (Ordaz-Ortiz *et al.* 2009). Unlike pectins, hemicelluloses show minimal structural changes upon heating of plant-based foods (Sila *et al.* 2008). During fruit ripening, nevertheless, considerable changes of hemicellulose structures occur, indicated by substantial depolymerization of the hemicelluloses xyloglucan (Miedes and Lorences 2004; Yashoda *et al.* 2005).

Blueberry cell wall components have not been studied extensively. Rabbiteye blueberry contains more complex polysaccharides than highbush blueberry, and this difference may contribute to the toughness of rabbiteye blueberry and their longer fresh shelf-life (Silva *et al.* 2005). Quantitative and qualitative changes in the pectin composition of Bluetta blueberries were determined by Proctor and Peng (1989) during fruit development. It was found that the contribution of pectins to the total fruit mass decreased twofold with fruit maturation. Meanwhile, a reduction in dilute alkali soluble pectins from about 65% to 20% and a corresponding increase in water soluble pectins from about 20% to 60% of alcohol insoluble solid factions were observed. Vicente *et al.* (2007) analyzed the modifications in blueberry cell wall structure and composition at five different ripening stages to understand cell wall disassembly. The authors found that xylans, which are usually a minor hemicellulosic fruit wall component, are abundant in blueberry fruits. In addition, Vicente *et al.* also reported that little reduction in pectin polymer size occurred during blueberry ripening, while hemicelluloses size decreased as ripening progressed. Woodruff *et al.* (1960) reported a marked decline in the soluble

pectin content of Jersey blueberries and a simultaneous increase in pectin methylesterase activity during ripening.

Since research has tended to focus on the dietary value of blueberry fruits, only limited detailed information is available regarding the chemical components and their structure properties about cell wall polysaccharides of blueberry fruits. The objectives of the present study were therefore to isolate and characterize the soluble pectic and hemicellulosic polysaccharides in ripe blueberry fruits.

EXPERIMENTAL

Materials

Rabbiteye blueberries (*Vaccinium ashei*) 'Brightwell' were grown in the experimental orchard in Southwest Forestry University, Kunming, Yunnan, in China. The fruits were collected at their commercial harvest maturity according to ground color. After harvest, the fruits with size uniformity and an absence of defects were selected, taken to the laboratory, and immediately processed. All standard chemicals, such as monosaccharide and chromatographic reagents, were purchased from Sigma Chemical Company (Beijing, China).

Cell Wall Isolation

Cell wall material was extracted according to Redgwell *et al.* (2008) with some modifications. A sample of 50 g frozen fruit tissue was homogenized in 250 mL of 95% ethanol to extract simple sugars, and the slurry was boiled with continuous stirring for 30 min to inactivate enzymes. The homogenized sample was cooled and filtrated with Miracloth, and the residue was successively washed with 150 mL of 85% ethanol, 250 mL of chloroform: methanol (1:1, v/v), and 250 mL of acetone till the insoluble material became colorless. The residue, considered as cell wall material (CWM), was dried overnight at 37°C, weighed, and stored in a desiccator at room temperature until use.

Isolation of Polysaccharide Fractions

The sample was subjected to sequential extraction, as shown in Fig. 1, which makes it possible to obtain five soluble polysaccharide fractions: (1) water-soluble fraction (WSF), (2) ethylenediaminetetraacetic acid (EDTA)-soluble fraction (ESF), (3) Na_2CO_3-soluble fraction (NSF), (4) 4% KOH-soluble fraction (4KSF), and (5) 14% KOH-soluble fraction (14KSF). Briefly, samples of approximately 5.0 g were sequentially treated with 700 mL of water, 0.05 mol L^{-1} sodium acetate (pH 6.5) containing 0.05 mol L^{-1} EDTA, 0.05 mol L^{-1} Na_2CO_3 containing 0.01 mol L^{-1} $NaBH_4$, 4% KOH containing 0.1% $NaBH_4$, and 14% KOH containing 0.1% $NaBH_4$ for 4 h at room temperature with stirring. The extracted solutions were filtrated with Buchner funnel. After filtration, the pellet was washed three times with 100 mL of the same solution and dried overnight at 37°C. The supernatant was combined with washing liquor, neutralized to pH 6.5, and concentrated to less than 30 mL with a rotary evaporator under reduced pressure. After that, three volumes of ethanol were added to each concentrated solution with continuous stirring, and then the flocculent precipitate appeared. The flocculent precipitate was separated by centrifugation at 8500 × g and 4°C for 10 min, dialyzed against distilled water (M_w cut-off 3500 D, 48 h), freeze-dried, and labeled as WSF, ESF, NSF, 4KSF, and 14KSF, respectively. All the experiments were

performed at least in duplicate. Yields of the polysaccharide fractions were calculated on a dry weight basis related to the dried CWM samples. The relative standard deviation was observed to be lower than 4.50%.

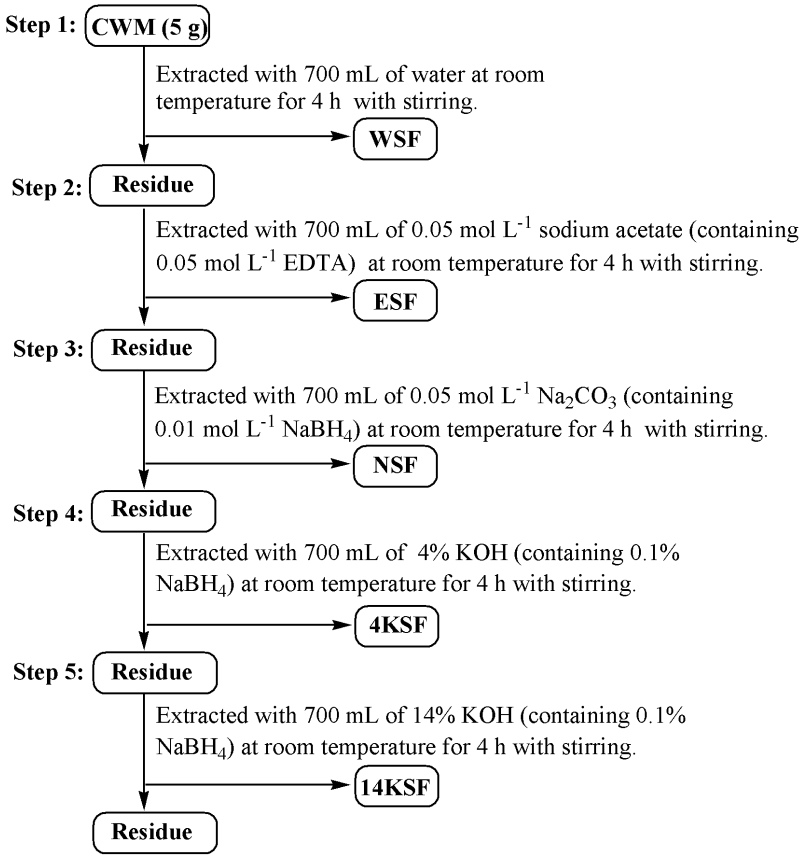

Fig. 1. Scheme for extraction of polysaccharide fractions from blueberry fruits

Characterization of the Polysaccharide Fractions

Molecular weights of the polysaccharide preparations were determined by gel permeation chromatography (GPC) on a PL aquagel-OH 50 column (300 × 7.7 mm, Polymer Laboratories Ltd.), calibrated with PL pullulan polysaccharide standards (peak average molecular weights of 783, 12200, 100000, and 1600000 g mol^{-1}, Polymer Laboratories Ltd.). A flow rate of 0.5 mL min^{-1} was maintained. The eluent was 0.02 and 0.15 M NaCl in 0.005 M sodium phosphate buffer at pH 7.5 for pectic (WSF, ESF, and NSF) and hemicellulosic fractions (4KSF and 14KSF), respectively. Detection was achieved with a Knauer differential refractive index detector (RID). The column oven temperature was kept at 30°C. Polysaccharides were dissolved with 0.02 M NaCl in 0.005 M sodium phosphate buffer, pH 7.5, at a concentration of 0.10%.

The neutral sugar compositions of the polysaccharide fractions were determined by hydrolysis with dilute sulfuric acid. A 4 to 6 mg sample of polysaccharides was hydrolyzed with 1.475 mL of 1 mol L^{-1} H_2SO_4 for 2.5 h at 105°C. After hydrolysis, the mixture was filtered, and the filtrate containing the liberated neutral sugars was analyzed by high-performance anion exchange chromatography (HPAEC) system (Dionex ICS 3000, U.S.) with a pulsed amperometric detector and an ion exchange Carbopac PA-1 column (4 × 250 mm). Neutral sugars were separated in 18 mM NaOH (carbonate free and purged with nitrogen) with postcolumn addition of 0.30 M NaOH at a rate of 0.5 mL

min^{-1}. Run time was 45 min, followed by 10 min elution with 0.2 M NaOH to wash the column and then a 15 min elution with 18 mM NaOH to re-equilibrate the column. Calibration was performed with standard solutions of L-rhamnose, L-arabinose, D-glucose, D-galactose, D-mannose, D-xylose, glucuronic acid, and galacturonic acid. The analyses were run twice, and the average values were calculated for all of the polysaccharide fractions.

Fourier transform infrared (FT-IR) spectra of the polysaccharide fractions were determined using a Thermo Scientific Nicolet iN10 FT-IR Microscope (Thermo Nicolet Corporation, Madison, WI, USA) equipped with a liquid nitrogen cooled MCT detector. The dried samples were ground and palletized using BaF_2, and their spectra were recorded in the range from 4000 to 700 cm^{-1} at 4 cm^{-1} resolution and 128 scans per sample. The fingerprint region was baseline corrected between 1900 and 750 cm^{-1}. Before data collection, a background scanning was performed for background correction.

Solution-state ^{13}C nuclear magnetic resonance (NMR) spectra were obtained on a Bruker AVIII 400 MHz spectrometer (Bruker, German). The sample of 80 mg was dissolved in 1.00 mL D_2O at 80°C before detection. The chemical shifts were calibrated relative to the signals from D_2O, used as an internal standard, at 4.7 ppm for the ^1H NMR spectra. ^{13}C NMR spectra were obtained at 25°C after 30000 scans. A 30° pulse flipping angle, a 9.2 μs pulse width, and a 2 s delay time between scans were used.

RESULTS AND DISCUSSION

Yield of Released Polysaccharide Fractions

Previous studies have shown that the blueberry fruit cell walls consist of several hemicellulosic and pectic polymers, which vary in structural characteristics (Silva *et al.* 2005), and that one step of treatment could only extract part of the polysaccharides from the raw materials (Bergmans *et al.* 1996). In view of these facts, fractionation techniques, such as sequential extraction with different solvents showing different dissolving capacities of cell wall components, were worth attempting to obtain more homogeneous soluble polysaccharide fractions and thus to further explore their structural properties. As shown by the data in Table 1, the sequential treatments of CWM of blueberry fruits with water, EDTA, Na_2CO_3, 4% KOH, and 14% KOH released 2.76, 6.43, 10.00, 9.69, and 7.14% soluble polysaccharides, respectively. The total yield of the five soluble polysaccharide fractions was 36.02% of the original CWM of blueberry fruits.

Table 1. Yield of Polysaccharide Fractions Solubilized During Successive Treatments of Rabbiteye Blueberry Fruits

Polysaccharide fractions	Yield (% dry matter, W/W)
Water-soluble fraction (WSF)	2.76
Ethylenediaminetetraacetic acid-soluble fraction (ESF)	6.43
Na_2CO_3-soluble fraction (NSF)	10.00
4% KOH-soluble fraction (4KSF)	9.69
14% KOH-soluble fraction (14KSF)	7.14
Total solubilized polysaccharides [a]	36.02
Residue	61.04
[a] Represents the total amounts of WSF, ESF, NSF, 4KSF, and 14KSF	

It should be noted that the polysaccharide fractions NSF, 4KSF, and 14KSF presented high yields of polysaccharides (10.00, 9.69, and 7.14% of the initial amount of CWM of blueberry fruits, respectively), which suggested that the treatment of alkaline solution at room temperature could significantly dissolve polysaccharides from CWM of blueberry fruits. The high solubility of fruit polysaccharides in alkaline aqueous solution resulted from the alkali function, because hydroxyl ions liberated from alkaline solution could cause swelling of the cell wall, disruption of intermolecular hydrogen bonds between cellulose and other polysaccharides, and hydrolysis of ester bonds which most likely play an important role in connecting the cell wall polysaccharides and lignin (Bergmans *et al.* 1996). It could be speculated that these differences in extractability of polysaccharides were the results of different structural properties of these polymers in the blueberry fruit cell walls.

Distribution of Molecular Weight

In order to investigate the molecular weights of the five soluble polysaccharide fractions extracted with different extraction solvents, the weight-average (M_w) and number-average (M_n) molecular weights, and polydispersity (M_w/M_n) of the polysaccharide fractions were analyzed by GPC, and the results are listed in Table 2. The molecular weight distributions of five polysaccharide fractions are also shown in Fig. 2. Obviously, the three-polysaccharide fractions WSF, ESF, and NSF, isolated with water, EDTA, and Na_2CO_3, respectively, had a high degree of polymerization with weight-average molecular weights between 33630 and 122510 g mol^{-1}. However, the soluble polysaccharide fractions 4KSF and 14KSF, isolated with 4% and 14% KOH containing 0.1% $NaBH_4$, respectively, had a much low degree of polymerization with weight-average molecular weights between 4290 and 8280 g mol^{-1}. These results suggested that the extraction of alkali-soluble polysaccharides with 4% and 14% KOH might result in noticeable degradation of polysaccharides.

Polydispersity is an important parameter of macromolecules in the chemical industry. In general, narrow polydispersity means better stability of physicochemical properties. Therefore, it is important to get polymers with a relatively narrow polydispersity from plants. As shown from the data in Table 2, the three soluble polysaccharide fractions (WSF, ESF, and NSF) had wider distributions of molecular weights (from 1.86 to 2.66), while the two soluble polysaccharide fractions (4KSF and 14KSF) showed narrow distributions of molecular weights (from 1.05 to 1.45).

Table 2. Weight-Average (M_w) Molecular Weights, Number-Average (M_n) Molecular Weights, and the Polydispersity (M_w/M_n) of the Isolated Polysaccharide Fractions from Rabbiteye Blueberry Fruits

	Polysaccharide Fractions				
	WSF	ESF	NSF	4KSF	14KSF
M_w	50900	122510	33630	4290	8280
M_n	19160	61820	18050	4080	5720
M_w/M_n	2.66	1.98	1.86	1.05	1.45

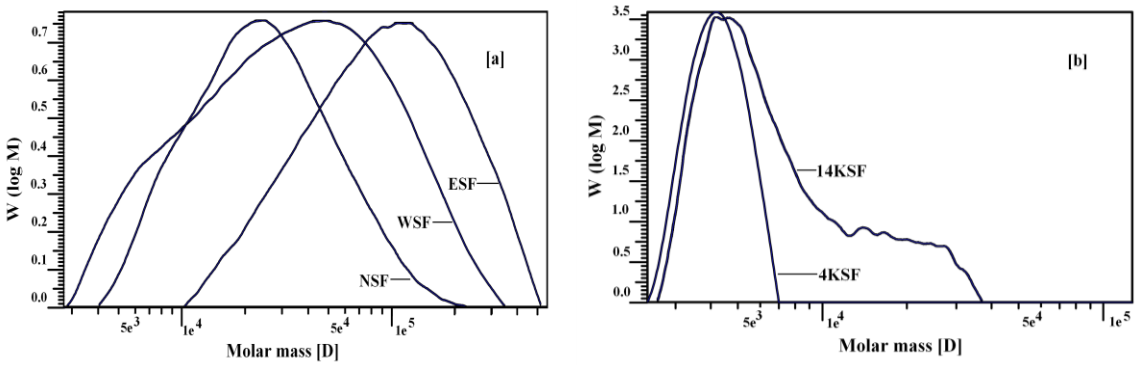

Fig. 2. Molecular weight distributions of pectic (a) and hemicellulosic fractions (b)

Content of Neutral Sugars and Uronic Acids

As mentioned above, composition of the polysaccharides can vary depending on the methods of isolation. To analyze the difference among these polysaccharide fractions sequentially isolated from blueberry fruits, the contents of neutral sugars and uronic acids of the five polysaccharide fractions were detected, and the HPAEC analysis data are illustrated in Table 3.

Table 3. Contents of Neutral Sugars and Uronic Acids (% Polysaccharides Sample, w/w) in the Isolated Polysaccharide Fractions

	Polysaccharide Fractions					
	WSF	ESF	NSF	4KSF	14KSF	Residue
Rhamnose	1.65	2.77	4.86	1.11	0.97	1.48
Arabinose	29.69	15.55	16.05	3.93	1.51	4.65
Galactose	10.96	8.57	9.34	10.53	5.82	4.77
Glucose	15.52	5.36	4.11	9.91	9.96	1.15
Xylose	3.63	3.41	7.45	69.98	77.16	85.5
Mannose	1.19	0.74	1.27	2.46	2.53	0.63
GalA	37.36	63.56	56.92	2.09	2.06	1.82

The water-soluble polysaccharide fraction was found to be enriched in neutral sugars, representing 62.64% of the total molar content of monosaccharides. Arabinose (29.69%), glucose (15.52%), and galactose (10.96%) were the predominant neutral sugars, and only a very small amount of rhamnose (1.65%) was detected in the water-soluble fraction. The molar concentration of GalA in WSF was 37.36%. According to the monosaccharide composition, it seems that homogalacturonan, glucans, arabino-galactans, and other water-soluble polysaccharides were extracted from blueberry fruits when treated with water (Taboada *et al.* 2010). In blueberry fruit CWM, the yield of WSF was lower than that in ESF and NSF fractions, which suggests the low proportion of this type of association in the blueberry fruit cell wall.

As can be observed in Table 3, the EDTA-soluble fraction showed the highest molar concentration of GalA (63.56%), suggesting the presence of a significant amount of pectic polymers in ESF. It is known that the EDTA-soluble pectin fraction is the homogalacturonan fraction associated with calcium ions in the cell wall (Prabasari *et al.* 2011). In the present study, arabinose was the most abundant neutral sugar in ESF, which showed very low (Ara + Gal)/GalA and significantly high GalA/Rha molar ratios of 0.38

and 22.95, respectively. According to these ratios, pectins extracted with 0.05 mol L^{-1} sodium acetate (pH 6.5) containing 0.05 mol L^{-1} EDTA should have a high proportion of homogalacturonan and a minor amount of arabinogalactans.

GalA was found to be the predominant component (56.92%) in the Na_2CO_3 soluble polysaccharide fraction, implying that NSF is also mainly composed of pectins. The GalA/Rha molar ratio of the Na_2CO_3 soluble polysaccharide fraction was lower (11.71) than that of fractions obtained from the other treatments. This fact indicated the presence of small amounts of rhamnogalacturonic regions in this pectic fraction. However, this fraction also showed a low (Ara + Gal)/GalA molar ratio of 0.45, suggesting that the polymeric backbones are not extensively branched with neutral sugar chains. This fact is related to the drastic extraction conditions employed, which cause polymer debranches (Taboada *et al.* 2010). In addition, it was found that the disruptive nature of this extraction approach yielded pectins with low average molecular weight, in comparison with those extracted with water and EDTA (Table 2).

As shown by the data in Table 3, the KOH soluble fractions were enriched in neutral sugars, representing 97.92 and 97.94% of the total monosaccharide content in 4KSF and 14KSF, respectively. Xylose was observed to be the predominant neutral sugar, followed by a lower amount of glucose and galactose, and only a small amount of uronic acid was presented in these two KOH-soluble fractions. The data for the specific neutral sugars composition implied that the two extracted polysaccharide fractions were mainly composed of xylan, which is in accordance with the report of hemicelluloses from blueberry (*Vaccinium* sp.) fruits (Vicente *et al.* 2007).

FT-IR Spectra

Fourier transform infrared spectroscopy could be applied to explore the physicochemical and conformational properties of carbohydrates (Kačuráková and Mathlouthi 1996; Mathlouthi *et al.* 1986). In this study, FT-IR spectroscopy was used for identification of the isolated polysaccharide types based on their typical spectral patterns in the 1200-800 cm^{-1} region (Kačuráková *et al.* 2000).

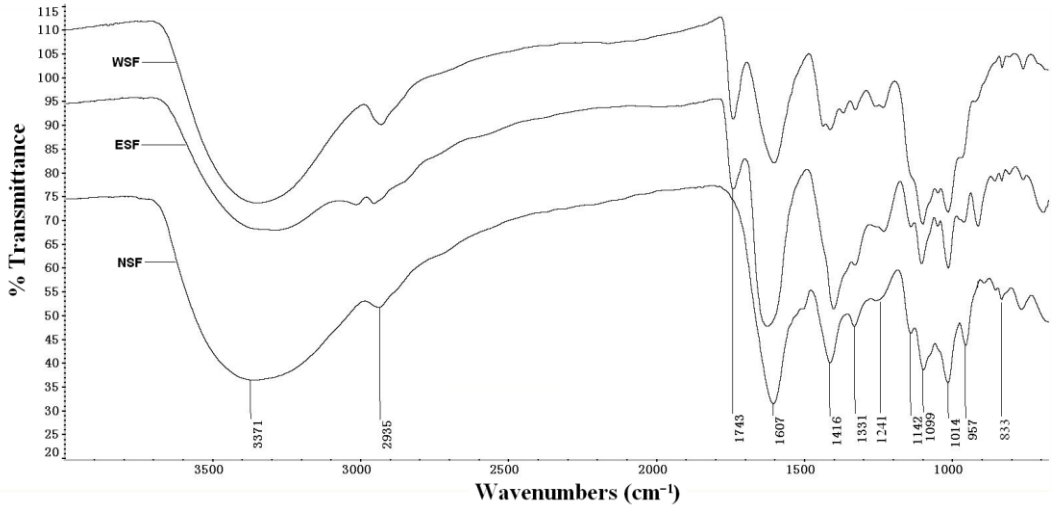

Fig. 3. FT-IR spectra of the isolated polysaccharide fractions WSF, ESF, and NSF

Figure 3 shows the FT-IR spectra of the polysaccharide preparations WSF, ESF, and NSF. The spectra showed minor changes in the peaks and absorption intensities when compared with the spectrum of standard pectins (Chatjigakis *et al.* 1998; Kamnev *et al.*

1998; Manrique and Lajolo 2002), suggesting the presence of predominant content of pectins in the three isolated polysaccharide fractions (WSF, ESF, and NSF). It is well known that the pectic substances belong to a class of carboxypolysaccharides, which differ from neutral polysaccharides, with an intense band in the region 1743 cm^{-1} (for salts around 1607 cm^{-1}) related to vibrations of the carboxyl group (Filippov 1992). From this point, the EDTA-soluble polysaccharide complexes contained much higher amounts of pectic substances than WSF and NSF, as shown by a significant absorption at 1607 cm^{-1} (salt), which was in correspondence with the chemical analysis results in Table 3. Disappearance of the ester band at 1743 cm^{-1} in NSF is undoubtedly caused by the full saponification of acetyl groups and methyl esters, indicating that the Na_2CO_3-soluble pectic polysaccharides obtained in this study are fully de-esterified. The bands at 1416, 1331, and 1241 cm^{-1} represent C-H stretching, OH, and C-O bending vibration in pectic polysaccharides (Kačuráková *et al.* 1998); such fingerprint is characteristic of homogalacturonic acid (not of rhamnogalacturonan-I or arabinogalactan). Absorptions at 1142 and 1099 cm^{-1} are both assigned to the coupling of C-O, C-C, and O-H bond stretching, bending, and asymmetric stretching of the C-O-C glycosidic bridge (Aguirre *et al.* 2009). The presence of arabinosyl side chains is shown by the low intensity peak at 1142 cm^{-1} (Manrique and Lajolo 2002), corresponding to the results obtained from sugar analysis. Absorbance at 1014 cm^{-1} is assigned to the vibration of C-O-H deformation, and absorbance at 957 cm^{-1} is assigned to C-H bending (Sebastiana *et al.* 2009). Another characteristic absorbance peak, the characteristic "anomeric region" absorption band for α-linkage in pectic polysaccharides, was identified by the weak peak at 833 cm^{-1}.

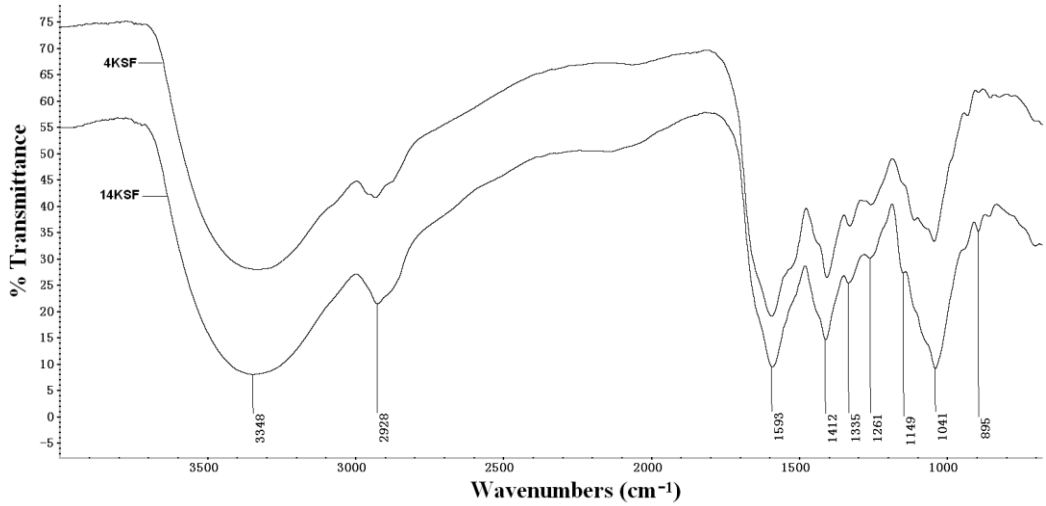

Fig. 4. FT-IR spectra of the isolated polysaccharide fractions 4KSF and 14KSF

Figure 4 shows the FT-IR spectra of 4KSF and 14KSF obtained by extraction with KOH from pectin-free residue of blueberry fruits. It was found that most absorption bands of the two isolated polysaccharide fractions were rather similar, indicating an analogous structural property between the two soluble polysaccharide fractions. The bands at 1412, 1335, 1261, 1149, 1041, and 895 cm^{-1} are associated with hemicelluloses (Sun *et al.* 2004). The three bands at 1412, 1335, and 1261 cm^{-1} represent the C–H and C–O bending or stretching frequencies. The prominent absorption at 1041 cm^{-1} is attributed to the glycosidic linkage (C–O–C) contributions in xylans (Cao *et al.* 2011). This strong absorption implies that the two KOH-soluble polysaccharide fractions are

mainly composed of xylans, which is in good agreement with the sugar analysis results of the two hemicellulosic fractions, in which the predominant content of xylose was 69.80 and 77.16%, respectively. In the anomeric region (950-700 cm^{-1}), a small band at 895 cm^{-1}, which is due to the C-1 group frequency or ring frequency, is indicative of β-glycosidic linkages between xylose units in the hemicelluloses. Obviously, the intense band at 1593 cm^{-1} is mainly due to the absorbed water, since the hemicelluloses usually have a strong affinity for water, and in the solid state these macromolecules may have disordered structures that can easily be hydrated (Chaikumpollert *et al.*, 2004; Kačuráková *et al.*; 1998; Sun *et al.*, 2004). Meanwhile, this strong signal is also partially caused by C=O stretching vibration of ionic carboxyl groups of the saponified pectin residues present in the hemicellulosic fractions (Sun and Hughes 1999; Tamaki *et al.* 2008).

^{13}C-NMR Spectra

NMR spectroscopy has proven to be a powerful tool to assay and identify the polymer backbone and the type of side chain branching along the backbone. To further elucidate the structural characteristics of the polysaccharide polymers extracted from blueberry fruits, the pectic fraction ESF and hemicellulosic fraction 4KSF were investigated by ^{13}C NMR, and their NMR spectra are shown in Fig. 5.

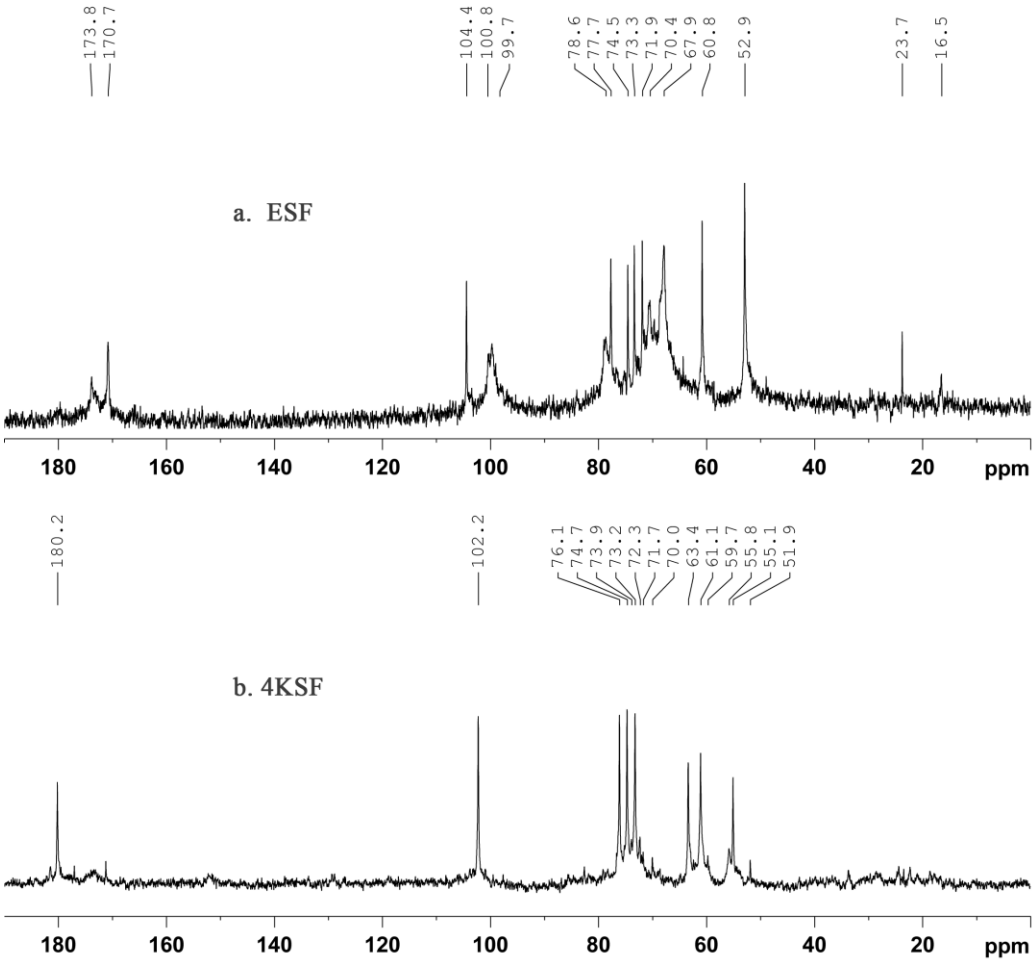

Fig. 5. ^{13}C-NMR spectra (in D_2O) of the polysaccharide fractions ESF (a) and 4KSF (b)

Figure 5 (a) shows the ^{13}C NMR spectrum of EDTA-soluble pectic polysaccharides (ESF). As shown in the spectrum, the dominant peaks at δ 99.7, 77.7, 74.5, 73.3, and 71.9 ppm are ascribed to C-1, C-4, C-5, C-3, and C-2 of α-(1→4)-linked-GalA residues, respectively (Habibi *et al.* 2004). In the low field region, typical signals were observed for the C-6 carboxyl group of GalA units at 173.8 and 170.7 ppm. The occurrence of two carboxyl signals suggested the presence of free and esterified carboxyl groups of α-D-GalA (Wang *et al.* 2005). A signal at 52.9 ppm was assigned to methyl groups binding to carboxyl groups of GalA (Keenan *et al.* 1985). The dominant signals of α-(1→4)-linked-GalA residues confirmed that NSF is composed of a homogalacturonan backbone (Dong *et al.* 2010), which was in good agreement with the sugar and FT-IR analysis results. The strong signals at δ 104.4, 67.9, and 60.8 ppm correspond to C-1, C-2, and C-6 of β-D-galactopyranosyl residues (Patra *et al.* 2012), respectively. The weak resonances at δ 78.6 and 70.4 ppm might arise from C-3 and C-5 of arabinofuranose residues (Dong *et al*. 2010), respectively. The signals of β-D-galactopyranosyl and arabinofuranose indicate a probable proportion of arabinogalactans in ESF. Characteristic signal at δ 23.7 ppm was easily identified to C-6 from the rhamnopyranose methyl group (Sun *et al.* 2010).

Figure 5 (b) presents the ^{13}C NMR spectrum of 4% KOH-soluble hemicellulosic polysaccharide fraction (4KSF). The spectrum exhibits five major signals corresponding to β-D-(1→4)-linked-xylan. The signal at 102.2 ppm originates from the anomeric region in a β-configuration (Wen *et al.* 2011), as shown in the FT-IR spectra, while the signals at δ 76.1, 74.7, 73.9, and 63.4 ppm correspond to C-4, C-3, C-2, and C-5 of β-D-(1→4)-linked-xylopyranosyl units (Shi *et al.* 2011), respectively. Signals at δ 180.2 and 55.1 ppm arise from C-6 and binding methyl groups of α-D-GalA (Bushneva *et al.* 2002; Catoire *et al.* 1998), respectively, which was in accordance with the aforementioned sugars and FT-IR analysis results.

Thus, on the basis of the results described above and those of previous literature (Mukhiddinov *et al.* 1990; Vicente *et al.* 2007), it can be concluded that ESF from blueberry fruits belongs to homogalacturonan with a β-1-4-galactan side chain, and 4KSF belongs to xylan. However, more research is required for a further characterization of the detailed structural properties of homogalacturonan and xylan.

CONCLUSIONS

1. The sequential treatments of blueberry fruit CWM with water, EDTA, Na_2CO_3, 4% KOH, and 14% KOH yielded 36.02% soluble pectic and hemicellulosic polysaccharides of the dry cell wall material.
2. The water-, EDTA-, and Na_2CO_3-soluble pectic polysaccharide fractions isolated from blueberry fruits were mainly composed of homogalacturonan, followed by a minor amount of arabinogalactans and glucans.
3. The two KOH-soluble fractions isolated from blueberry fruits were mainly composed of hemicelluloses, in which xylose was the predominant neutral sugars (69.98 to 77.16%), followed by lower amounts of galactose, glucose, arabinose, and mannose. The isolated hemicellulosic polysaccharide fractions could be defined as β-(1→4)-linked-xylopyranosyl.

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