Eur Food Res Technol (2011) 233:731–734
DOI 10.1007/s00217-011-1568-8

ORIGINAL PAPER

Fractionation and structural characterization of haw pectin oligosaccharides

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Received: 6 January 2011 / Revised: 20 August 2011 / Accepted: 22 August 2011 / Published online: 3 September 2011

Abstract Although haw pectin and its hydrolysates are believed to have various functions in food industries, correlation of the structure with the functionality of oligomers has not yet been clarified. In the present study, haw pectin oligosaccharides were fractionated, and their structural characterizations were analyzed. The results showed that ultrafiltered haw pectin hydrolysates yielded 11 fractions (F1 ~ F11) on a DEAE-Sephadex A-25 column chromatograph. One fraction (F1) was galacturonic acid, whereas other fractions (F2 ~ F11) were oligosaccharides. Structural analysis using nuclear magnetic resonance and gas chromatography with mass spectrometry showed that these oligosaccharides (F2 ~ F11) are oligogalacturonide family with degrees of polymerization of 2–11, linked by α–(1 → 4) linkages. The information obtained could be used in the exploitation and utilization of haw pectin and its derivational oligomers.

Keywords Haw · Pectin · Oligogalacturonide · *Crataegus pinnatifida* Bge

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Introduction

The haw (*Crataegus pinnatifida* Bge) fruit, which belongs to the rose family, is always processed into jam and/or jelly in China, because of its high pectin content [1]. Previously, pectin extracted from haw fruit was found to contain two regions: the homogalacturonan region and the neutral sugar riches hairy region in its molecule [2, 3]. Focusing on the various functions and applications of pectin as food additives, we carried out a series of researches about pectin, such as those involving manufacturing technology [1], properties and the biological activities, and so on, and confirmed that haw pectin oligosaccharides have strong antibacterial activity [4] and they modulates lipid metabolism in the body [5]. Thus, haw pectin oligosaccharides have great application prospects in the development of food additives and functional food. In the current paper, the fractionation and structural features of haw pectin oligosaccharides were studied, in order to provide the basic data for a wider and deeper knowledge of haw pectin utilization.

Materials and methods

Sample preparation

Haw pectin was obtained from haw fruit through hot water extraction [1] and subjected to the enzymolysis in 0.02 mol L^{-1} acetate buffer at pH 3.5 and 50 °C with 1% substrate concentration. A dose of 0.2 U/mL commercially available pectinase (*endo*-polygalacturonase containing pectinesterase from *Aspergillus niger*, DSM Food Specialties, China) immobilized on an agar gel support [6] was used to cleave haw pectin for 2 h [7]. After enzyme hydrolysis, the reaction solution was processed by

ultrafiltration using a hollow fiber membrane with a molecular cutoff weight of 6 kDa (Tianjin Motimo, China) [7, 8]. The filtrate (haw pectin hydrolysate) was then subjected to the column chromatograph (4.6 × 30 cm, HCO_3^- form) of DEAE-Sephadex A-25. The 10 g haw pectin hydrolysate was applied onto the column. The column was first washed with distilled water to remove the neutral sugar and then eluted with a linear concentration gradient of NH_4HCO_3 (0.1–0.75 and 2.0 mol L^{-1}), and 10 mL fractions were collected.

General analysis

The fractionated oligosaccharides were completely reduced [9] and methylated using the Hakomori method modified by Harris et al. [10]. The methylated oligosaccharide was hydrolyzed with 90% formic acid, then with 0.5 mol L^{-1} H_2SO_4. The resulting partially methylated sugars thus obtained were converted into their alditol acetates [11] and then analyzed by gas chromatography with mass spectrometry (GC–MS). A Shimadzu QP-5000 (Japan) gas chromatograph–mass spectrometer was used together with a Shimadzu CBP-10 M25-025 capillary column (0.22 mm × 25 m). The column temperature was programmed at 160–200 °C at 2 °C/min. Spectra were recorded at an ionizing potential of 70 eV.

^1H and ^{13}C-NMR were performed in D_2O using Varian FT-NMR (200 MHz) with an acquisition time (AQ) of 3.72 s, a pulse delay (PD) of 3.74 s for ^1H-NMR, and 0.18 s AQ and 1.498 s PD for ^{13}C-NMR. 3-(Trimethylsilyl)-1-propanesulfonate was used as the internal standard.

For the analysis of the chemical properties of oligosaccharides, the total sugar content was determined using the phenol–H_2SO4 method [12], galacturonic acid (GalA) content using the *m*-hydroxyldiphenol method [13] and reducing galacturonic acid content by the Milner–Avigad method [14]. The average degree of polymerization (DP) was calculated as the ratio of total galacturonic acid content to the reducing galacturonic acid.

Silica gel plate (Qingdao haiyang chemical, China) was used for thin-layer chromatography (TLC) analysis. Sample was developed by the solvents of n-butanol, acetic acid and H_2O (4/6/3, v/v/v), spotted by 50% sulfuric acid.

Results and discussion

DEAE-Sephadex A-25 chromatography (Fig. 1) yielded 11 major fractions F1 ~ F11. The elution position of F1 matches that of standard galacturonic acid. Considering the DP and the total sugar and uronic acid contents in Table 1, F1 was a monosaccharide of GalA, whereas F2 ~ F11 were oligosaccharides. A linear correlation was found between the elution volume and deduced DP (Fig. 2); the major sugar component of F2 ~ F11 was uronic acid (Table 1), and the main component of haw pectin was polygalacturonan [2], which indicated that F2 ~ F11 might be the oligogalacturonides with the DP of 2–11, respectively. Additionally, F2 ~ F7 (with higher yields) were the respective single spot with the linear array in the TL-chromatogram, which provided further evidence that they were pure oligogalacturonides with the DPs of 2–7.

Methylation analysis (Table 2) of F5 and F6 showed that F5 was a penta-oligogalacturonide and F6 was a hexa-oligogalacturonide. Tables 3 and 4 showed the ^1H and ^{13}C-NMR results of F5 and F6 (relatively higher in Yields). In ^1H-NMR of F5 (Fig. 3), the signals of the anomeric proton (δ5.36, H1-α; δ4.66, H1-β) formed reducing terminal of GalA and the signal at 5.09 ppm indicative of the glycosidic linkage of α-(1 → 4)-GalA [15–18] were observed. The ^{13}C-NMR signals at 95.13 ppm (C1-α), 99.11 ppm (C1-β) and 102.85 ppm (C1-α) in ^{13}C-NMR of F5 were contributed

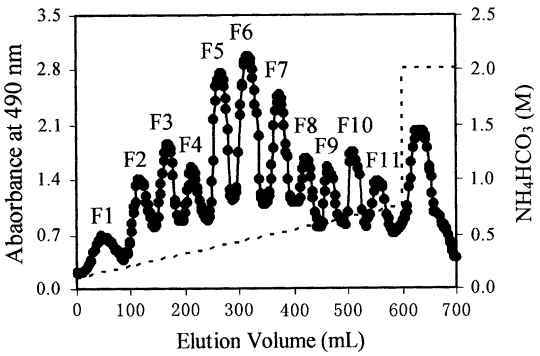

Fig. 1 Elution profile of haw pectin hydrolysates on a column of DEAE-Sephadex A-25. Column, 4.6 × 30 cm (HCO_3^-); solvents, 0.1–0.75 and 2.0 mol L^{-1} NH_4HCO_3; fraction, 10 mL. *Closed circles* total sugar; *broken line* NH_4HCO_3

Table 1 Chemical properties of the fractions F1 ~ F11

Fractions	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11
Total sugar (%)[a]	98.7	99.4	99.0	99.3	100.3	99.1	99.0	98.4	98.5	99.3	98.6
Uronic acid (%)[a]	98.4	97.6	98.8	98.2	99.7	97.4	98.6	98.1	98.4	99.0	97.1
DP	1.1	2.2	2.9	4.2	5.0	6.0	6.9	7.8	8.9	9.9	11.1
Yield (%)	4.5	5.6	8.1	6.1	11.4	13.3	10.0	6.0	5.5	7.4	4.9

[a] Each value is the mean of three replicates, and variability was less than 5%

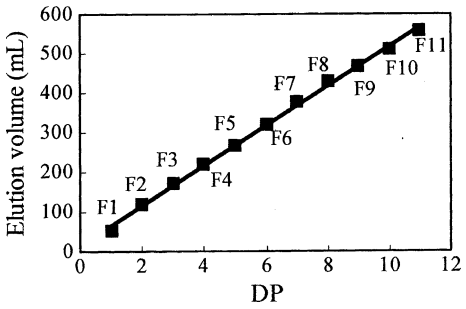

Fig. 2 Relationship between the elution position and DP of haw pectin oligosaccharides

Table 2 Methylation analysis of oligosaccharides F5 and F6

	Residue	Position of OMe group	Linkage	Molar ratio
F5	Galactose	2, 3, 4, 6	Terminal	1.0
		2, 3, 6	4	4.1
F6	Galactose	2, 3, 4, 6	Terminal	1.0
		2, 3, 6	4	4.9

Table 3 ^{1}H-NMR data (δ in ppm, J in Hz) of oligosaccharides F5 and F6

	Linkage	H-1α ($J_{1,2}$)	H-1β ($J_{1,2}$)
F5	→4)-α-GalA	5.36 (3.66)	4.66 (8.0)
	→4)-α-GalA-(1→	5.09 (4.2)	
	α-GalA-(1→	5.09 (4.2)	
F6	→4)-α-GalA	5.36 (3.66)	4.66 (8.6)
	→4)-α-GalA-(1→	5.09 (4.2)	
	α-GalA-(1→	5.09 (4.2)	

Table 4 ^{13}C-NMR data (δ in ppm) of oligosaccharides F5 and F6

	Linkage	C1	C4	C6
F5	→4)-α-GalA	95.13α	81.11	174.01α
		99.11β		175.62β
	→4)-α-GalA-(1→	102.85	81.11	174.92
	α-GalA-(1→	102.85	75.62	174.92
F6	→4)-α-GalA	95.14α	81.13	173.52α
		99.12β		174.05β
	→4)-α-GalA-(1→	102.76	81.13	174.96
	α-GalA-(1→	103.13	75.65	174.96

by the GalA anomeric carbon at the reducing terminal and the C1 of GalA with α-(1 → 4) linkages [18–21], respectively. In addition, because the chemical shift of C4 of GalA would shift its signal toward the lower field by the substitution at O-4 [22], a signal at 75.62 ppm was assigned to the C4 of GalA in the non-reducing terminal, and a signal at 81.11 ppm was assigned to the C4 of the reducing terminal

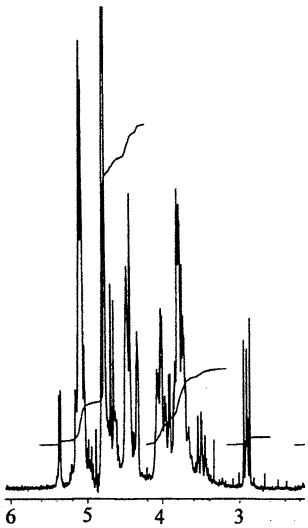

Fig. 3 ^{1}H-NMR spectrum of oligosaccharide F5

and middle GalA residues in F5. The results of NMR in F6 were similar to those in F5 (Tables 2, 3). These results indicated that F5 was a penta-α–(1 → 4)-oligogalacturonide and F6 was a hexa-α–(1 → 4)-oligogalacturonide. The integration rations δ5.09/δ5.36 (H-1 of glycosidic GalA/H-1 to the reducing terminal GalA) were 3.86 (F5) and 5.01 (F6) in the NMR spectra, which further supported these conclusions.

Integrating all the results mentioned above, it might suggest that haw pectin oligosaccharides of F2 ~ F11 obtained from DEAE-Sephadex A-25 column chromatograph might be the oligogalacturonide family with the DP of 2–11, respectively, linked by α–(1 → 4) linkages. Such results may provide the useful data for the research and development of utilizing approaches of haw pectin and its derivatives.

Acknowledgments This study was supported by National Natural Science Foundation of China (Grant No. 31071512) and Liaoning Provincial Natural Science Foundation of China (Grant No. 20102084).

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