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Isolation and structural characterization of water unextractable arabinoxylans from Chinese black-grained wheat bran

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ABSTRACT

Water unextractable polysaccharide from bran of a Chinese black-grained wheat variety was isolated with saturated barium hydroxide as extraction medium. The fraction composed mainly of arabinose (36.53%) and xylose (61.31%), indicating the presence of pure arabinoxylans. The arabinoxylan isolates exhibited the high average molecular weight (Mw) of 3.81×10^5 and an intermediate Ara/Xyl (A/X) ratio of 0.60. The fraction consisted of a β-D-(1→4)-linked xylan backbone, which was mono- or disubstituted with α-L-arabinofuranosyl (Ara*f*) groups at position *O*-3, *O*-2 or at both *O*-2 and *O*-3 positions. The xylan backbone contained a relatively high amount of unsubstituted xylopyranosyl (Xyl*p*) residues (57.71%) and low amount of disubstituted Xyl*p* residues (6.22%). About 22.0% of Xyl*p* residues were monosubstituted at *O*-3 position. α-L-Ara*f* side groups were prevalently (1→3)-linked to β-D-Xyl*p* residues as single units. Moreover, small quantities of 2-, and 5-linked α-L-Ara*f* indicated the presence of short arabinan side chains.

1. Introduction

Cereal bran has received considerable attention as a source of dietary fiber. This fibrous component influences the processing of cereal grains and their end-product quality (Migliori & Gabriele, 2010; Rouau, El-Hayek, & Moreau, 1994). Moreover, their consumption has been associated with lower risk of diseases such as diabetes, coronary heart disease, colon cancer, and other bowel disorders (Brownlee Iain, 2011; Lu, Walker, Muir, Mascara, & O'Deira, 2004; Reddy et al., 2000; Slavin, 2003).

Cereal dietary fiber, which is mostly insoluble, consists primarily of structural polymers, such as arabinoxylan (pentosan), lignin, cellulose, and other non-starch polysaccharides (Muralikrishna & Rao, 2007). Arabinoxylan (AX), a neutral non-starch polysaccharide from cereal grains, has been shown to contain a linear backbone of β-D-(1→4)-xylopyranosyl units, to which α-L-arabinofuranosyl substituents are attached through *O*-3, *O*-2 or both *O*-2 and *O*-3 linkages (Vinkx, Stevens, Gruppen, Grobet, & Delcour, 1995). Additionally, ferulic acid can be esterified to some of the arabinose side-chains at position *O*-5 and create cross-links with other cell wall components to yield insoluble complexes (Shyamprasad & Muralikrishna, 2007).

Wheat bran is one of the rich sources of dietary fiber and contains high concentrations of arabinoxylans (Izydorczyk & Biliaderis, 1995). Because of the role in the preparation of dough and baking performance and their properties for application as food additive, wheat arabinoxylans have been the subject of a significant number of investigations (Hoffmann, Kamerling, & Vliegenthart, 1992; Nandini & Salimath, 2002; Revanappa, Nandini, & Salimath, 2010; Saulnier, Sado, Branlard, Charmet, & Guillon, 2007). An important physiological effect of wheat bran arabinoxylans is its fecal bulking capacity and large bowel protection function, which is ascribed to its high water holding capacity and its low degradability in the digestive tract for stimulating a healthy colonic production of short chain fatty acid (SCFA) (Stevens & Selvendran, 1988; Vardakou et al., 2008).

Since AX are physically and chemically associated with each other and with other cell wall components, such as lignin and cellulose, most of them are water unextractable and can only be extracted with alkaline media. An extraction technique that facilitates the fractionation and characterization of cereal polysaccharides has been developed by using aqueous saturated barium hydroxide (Gruppen, Hamer, & Voragen, 1991). It preferentially extracts arabinoxylan from water unextractable material of cereal grain. The use of this solvent for the extraction of water unextractable arabinoxylans from wheat, rye, barley, and corn fiber has been described (Izydorczyk, Macri, & MacGregor, 1997).

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The fine structural features and molecular weight of polysaccharides are of fundamental importance since they will influence certain physicochemical properties of the polymers and therefore the technological performance and functional properties of the products (Cui, 2005). Important structural characteristics of arabinoxylans are the degree of xylan polymerization, the A/X ratio, and the substitution pattern (Vinkx et al., 1995). Several molecular structures have been proposed for different cereal arabinoxylans. However, little information is available about the structure of water unextractable AX from pigmented wheat bran and associated physicochemical properties are not known presently.

The black-grained wheat variety was derived from a distant hybridization between different species collected in China. Seed coat of this particular wheat genotype is black, and the main pigment component of this wheat seed was an anthocyanin phenolic compound (Li, Shan, Sun, Corke, & Beta, 2005). The anthocyanin constituents and the associated antioxidant capacity of Chinese black-grained wheat were investigated for comparison with common wheat controls, which showed that Chinese black-grained wheat exhibited considerably higher 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity than common wheat controls did (Hu, Cai, Li, Corke, & Kitts, 2007; Li et al., 2005).

Black-colored foods hold an important position in Chinese food culture and enjoy wide acceptance in the marketplace. Many studies on black-seeded cereals, for instance, black rice, black sesame, black sorghum, and black soybean, have shown that they are possessed of potential health benefits. In this study, water unextractable arabinoxylan from a Chinese black-grained wheat variety was isolated with saturated barium hydroxide, and specific structural features was identified by using partial acid hydrolysis followed by methylation analysis and nuclear magnetic resonance (NMR) spectroscopy.

2. Experimental

2.1. Plant materials

Chinese black-grained wheat bran was provided by Research and Development Company of Hedong Wumai (Yuncheng, China).

2.2. Preparation of water unextractable arabinoxylans isolates CBA

Bran samples (300 g) were grounded and were refluxed twice with 80% aqueous ethanol for 30 min to remove colored materials and inactivate endogenous enzymes. To remove residual starch, the dried ethanol treated residue was extracted twice with water at 50 °C for 30 min followed by centrifugation (3000 rpm, 20 min). Then, autoclave extraction (121 °C, 40 min) was performed twice for isolation of water extractable arabinoxylans.

The remaining water insoluble residue, after autoclave extraction, was further extracted with alkaline as described by Nilsson, Saulnier, Andersson, and Aman (1996). Water unextractable arabinoxylans was isolated under continuous stirring (16 h, room temperature, RT) with 400 mL saturated aqueous barium hydroxide containing 1% (w/v) sodium borohydride to prevent alkaline degradation. After centrifugation (3000 rpm, 20 min), the residue was re-extracted with another 200 mL of the same solvent (6 h, RT) and centrifuged. The supernatants were acidified with acetic acid to pH 5.0, concentrated and precipitated by ethanol (final concentration 60%, v/v). The resulting precipitate was dissolved in water and dialyzed extensively against distilled water (Mw cut-off 12,000 Da, 72 h) and then freeze-dried. The resultant extract was referred to as the CBA fraction.

2.3. General methods

The total protein content was determined according to the Lowry method (Lowry, Rosebrough, Farr, & Randall, 1951). Total neutral sugar content was determined by the reaction with phenol in the presence of sulfuric acid using xylose as standard. Neutral monosaccharide composition was analyzed according to the following procedure: polysaccharides were hydrolyzed with 2 M trifluoroacetic acid (TFA) at 121 °C for 1.0 h into monosaccharide. Their corresponding alditol acetates were analyzed by gas chromatography equipped with glass capillary column (Φ 0.32 mm × 30 m) packed with 3% OV1701 on Chromosorb W-AW DMCS (80–100 mesh) at a temperature program of 150–190 °C (10 °C/min) to 240 °C (3 °C/min). The percentage of monosaccharides in the sample was calculated from the peak areas using response factors.

2.4. Molecular weight determination

The molecular weight was determined by the high performance size exclusion chromatography (HPSEC), which was performed on a Waters HPLC system, including two serially linked Ultrahydrogel™ Linear (Φ7.8 mm × 300 mm) columns, a Waters 2410 RI detector and an on-line degaser. The mobile phase was 0.1 M $NaNO_3$, and the flow rate was 0.9 mL/min. The sample (2–5 mg) was dissolved in water (0.2 mL). A 20 μL sample was injected in each run. The molecular weight was estimated by reference to the calibration curve made from a Dextran T-series standard of known molecular weight (6100, 16,500, 26,290, 40,000, 84,000, 158,000).

2.5. Partial acid hydrolysis

Fragment CBA (150 mg) was hydrolyzed with 0.1 M trifluoroacetic acid (TFA) for 1 h at 100 °C. After cooling, TFA was evaporated under a stream of N_2. The hydrolysate was dissolved in distilled water and was dialyzed against distilled water (Mw cut-off 3500 Da). The retentate and dialysate were concentrated and then lyophilized, giving a high-molecular weight fraction (CBA-PH) and a low-molecular weight fraction (CBA-PL).

2.6. Determination of the glycosidic linkage composition

The glycosidic linkage analysis was determined by methylation and gas chromatography–mass spectroscopy (GC–MS). Methylation analysis was carried out according to the method of Ciucanu and Kerek (1984) with slight modifications (Cui, 2005). The methylated product was then converted into partially methylated alditol acetates (PMAA) by hydrolysis, reduction, and acetylation. PMAA solution was injected onto a GC–MS system equipped with a trace mass spectrometer (ThermoQuest Finnigan, San Diego, CA) and an OV1701 capillary column (0.25 mm × 30 m, 0.25 mm film thickness). The carrier gas was helium. The temperature program was 3 °C/min gradient from 150 to 250 °C. Energy of ionizing electrons was 70 eV. Peak identification was based on retention times using partially methylated alditol acetates as standards. The percentage of the methylated sugars was estimated as ratios of the peak areas (total ion current).

2.7. ^{1}H and ^{13}C NMR spectroscopy

The polysaccharide samples were exchanged 3 times in D_2O (at concentrations of approximately 40 mg/mL), with intermediate freeze-drying. Finally, samples were dissolved in D_2O. ^{1}H and ^{13}C NMR spectroscopy were performed on a Brucker AMX400 NMR spectrometer (Germany) using standard pulse sequences with

Table 1
Yields and sugar composition of fractions from CBA by partial acid hydrolysis.

Fragments	Yield (wt.%)	Content of the sugar residues (wt.%)				Ara/Xyl
		Ara	Xyl	Glc	Gal	
CBA	5.84[a]	36.53	61.31	2.16	Trace	0.60
CBA-PH	62.71[b]	19.56	80.42	Trace	n.d.[c]	0.24
CBA-PL	21.38[b]	73.16	23.35	3.49	Trace	–

[a] Of the plant raw material.
[b] Of the parent CBA.
[c] Not detected.

5 mm tubes at 65 °C. 1,4-Dioxane was used as an internal chemical-shift reference for spectra. Two-dimensional spectra (COSY, TOCSY, HSQC and NOESY) were recorded using standard pulse sequences from the Bruker library.

3. Results and discussion

3.1. Preparation of water unextractable arabinoxylans isolates CBA

Water unextractable arabinoxylans fraction (CBA) was obtained by extraction of Chinese black-grained wheat bran residue (left after removal of water extractable polysaccharides) with saturated barium hydroxide. Yield and sugar composition of CBA were given in Table 1. The yield of the fraction was 5.84% (w/w) of the initial amount of bran material. It consisted predominantly of polysaccharides (78.49%) and contained 11.26% proteins. The neutral sugar identified were predominantly arabinose (36.53%) and xylose (61.31%), only minor amounts of glucose were present in this fraction. The sum of arabinose and xylose residues accounted for more than 90% of the composite sugars of the isolated polysaccharide fraction, indicating the presence of pure arabinoxylans. The arabinose to xylose ratio (A/X) was 0.60, which was lower than the ratio of 0.71 previously reported for barium hydroxide extract of wheat bran arabinoxylans (Schooneveld-Bergmans, Beldman, & Voragen, 1999) but higher than the ratio of 0.54 reported for the extract of rye bran arabinoxylans (Nilsson et al., 1996).

3.2. Partial acid hydrolysis

To study the linkages between backbone and side chains of polysaccharides, the native fraction CBA was partially hydrolyzed with 0.1 M TFA. Partial acid hydrolysis resulted in two subfractions of CBA: high-molecular weight (CBA-PH) and low-molecular weight (CBA-PL) ones. The sugar composition of fractions was listed in Table 1. CBA-PH, yielded 62.71% of the parent CBA, composed of xylose and arabinose and possessed a declined A/X ratio of 0.24. Compared with CBA, the amount of arabinose (19.56%) decreased considerably whereas the amount of xylose (80.42%) increased, suggesting that xylose residues present in the backbone which were not susceptible to mild acid hydrolysis, whereas arabinose residues were attached to the backbone as the branches and easy to be hydrolyzed. This hydrolysis behavior was in accordance with previous observations that furanosyl rings are considered as weak glycosidic linkages, which can be easily hydrolyzed by acid. Furthermore, terminal linkages at non-reducing ends are hydrolyzed more rapidly than internal glycosidic linkages (Cui, 2005).

For the low-molecular weight fraction CBA-PL, the sugar composition presented in Table 1 showed that arabinose residues (73.16%) were the predominant monosaccharide sugar, followed by xylose residues (23.35%), confirming the presence of xylan backbone in the fraction CBA, which mainly substituted by the branches of arabinose residues.

3.3. Molecular size distribution

To investigate the hydrolysis extent of polymer chain, the fragment CBA and the treated fragment CBA-PL were analyzed using HPSEC to determine how the treatment affected the molecular weight. Fig. 1 shows the elution profiles and molecular weight distributions of the samples. A peak in the elution profile of CBA indicated large amounts of arabinoxylans were at high molecular weight region.

As the hydrolysis reaction preceded, partially hydrolyzed product in fraction CBA-PH eluted at longer retention time. Compared with the original fraction CBA, a broadening of the molecular weight distribution appeared and there existed a decrease in the hydrodynamic volume, corresponding to the decrease of average molecular weight. There was significant difference in Mw between the two fractions, with the original material CBA exhibiting the higher Mw of 3.8×10^5, whereas CBA-PH was characterized by the considerably lower Mw of 8.0×10^4 (Fig. 1), which was most probably caused by some degradation of the backbone. The A/X ratio also decreased simultaneously from 0.60 to 0.24 during reaction due to the decrease of arabinose residues content. Thus, it showed that partial acid hydrolysis of CBA resulted in the debranching of the backbone with removal of arabinose substituents and consequently coupled with loss of molecular weight.

3.4. Determination of the glycosidic linkage composition

Table 2 shows the glycosidic linkage composition and substitution pattern of the original extract CBA and partial acid hydrolyzed fraction CBA-PH, as determined by methylation analysis.

Methylation analysis confirmed that the original extract CBA comprised pure arabinoxylans with arabinose and xylose residues.

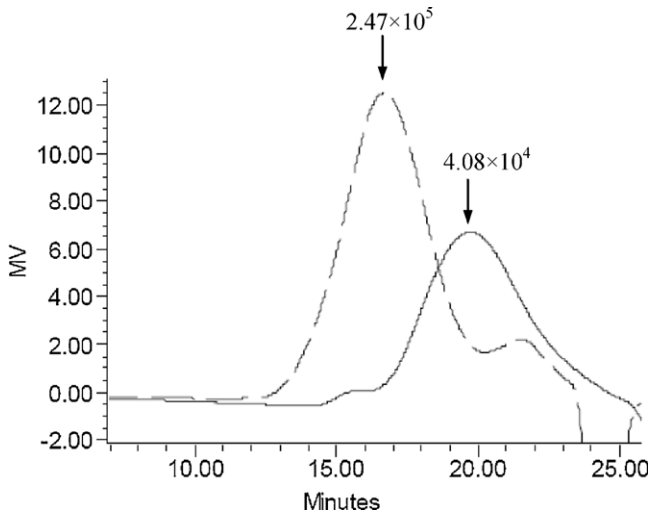

Fig. 1. High performance size exclusion chromatography of fraction CBA (- - -) and partial acid hydrolyzed fraction CBA-PH (———).

Table 2
Glycosidic linkage composition of methylated CBA and CBA-PH.

Residues	Linkage	PMAA	Mol.% [a]		Mol.% [b]	
			CBA	CBA-PH	CBA	CBA-PH
L-Ara*f*	T-	2,3,5-Me_3-Ara*f*	77.02	62.09	30.07	12.25
	1,2-	3,5-Me_2-Ara*f*	17.11	22.55	6.68	4.45
	1,5-	2,3-Me_2-Ara*f*	5.87	15.36	2.29	3.03
	Total		100	100	39.04	19.73
L-Xyl*p*	T-	2,3,4-Me_3-Xyl*p*	14.07	19.38	8.58	15.55
	1,4-	2,3-Me_2-Xyl*p*	57.71	75.23	35.18	60.39
	1,3,4-	2-Me-Xyl*p*	19.24	n.d.[c]	11.73	n.d.
	1,2,4-	3-Me-Xyl*p*	2.76	5.39	1.68	4.33
	1,2,3,4-	Xyl*p*	6.22	n.d.	3.79	n.d.
	Total		100	100	60.96	80.27
Unsub/Sub Xyl					2.05	13.95
Di/Mono Xyl					0.28	0.00

[a] Relative molar ratio, calculated from the ratio of peak areas.
[b] Mol.% of the parent CBA and CBA-PH, respectively.
[c] Not detected.

The degree of branching and mode of substitution in arabinoxylans were investigated by monosaccharide and glycosidic linkage analyses. Xyl*p* residues, formed the main chain with 1→4 linkage, were present in three forms: unsubstituted, monosubstituted and disubstituted (Table 2). Over 50% of the Xyl*p* residues (57.71%) were present as unsubstituted residues, as revealed by the high content of 2,3-Me_2-Xyl*p*. Approximately 22.0% of the Xyl*p* units were monosubstituted, predominantly at the *O*-3 position (19.24%) as revealed by 2-Me-Xyl*p*. Another 6.22% of the Xyl*p* residues were disubstituted at *O*-2 and *O*-3 position, and the remaining Xyl*p* residues (14.07%) were terminal. So the relative composition ratio of mono-, di-, and unsubstituted Xyl*p* in the arabinoxylan fraction was 22.0:6.22:57.71 (Table 2), with a high proportion of unsubstituted to substituted Xyl*p* (2.05), and a very low proportion of di- to monosubstituted Xyl*p* residues (0.28).

Isolated CBA from black-grained wheat bran was low in disubstituted and high in mono- and unsubstituted Xyl*p* residues. Izydorczyk and Biliaderis (1994) obtained a high molecular weight AX F55 from wheat, exhibiting a low degree of disubstituted Xyl*p* residues (9.8%) and a high degree of monosubstituted Xyl*p* residues (20.8%). The presence of relatively large amounts of terminal xylose was reported before for wheat bran (10.7%) (Schooneveld-Bergmans et al., 1999), rye bran (8.9%) (Ebringerova, Hromádková, Petráková, & Hricovini, 1990) and barley bran (6.9%) (Izydorczyk et al., 1997), which indicate more complicated structural features of side chains in the alkali-extractable arabinoxylan. It has been suggested that terminal Xyl*p* units might be linked through arabinose residues to the xylan backbone (Vinkx et al., 1995). A disaccharide side chain, 2-*O*-*β*-D-Xyl*p*-*α*-L-Ara*f*, attached at position *O*-3 of the main chain, was proved to exist in cereal arabinoxylans (Höije et al., 2006; Pastell, Virkki, Harju, Tuomainen, & Tenkanen, 2009).

Regarding the arabinose residues, the proportion of terminal, 1,2-, 1,5-linked arabinose was, respectively, in the ratio of 77.02:17.11:5.87 (Table 2). The high proportion of terminal arabinose residues suggested that most of the terminal arabinose residues attached to the xylan backbone directly as single units. Yet 22.98% of the arabinose residues were non-terminal, which was prevalently 1,2- and 1,5-linked. Substituted arabinose residues appeared to be present in small quantities, indicating the presence of short arabinosyl side chains. Short oligomeric side-chains, consisting of two or more arabinosyl residues linked via 1→2, 1→3, and 1→5 linkages, have been reported for some branched arabinoxylans from what, rye, and sorghum (Izydorczyk & Biliaderis, 1995).

Compared with the original extracts CBA, partial acid hydrolyzed fraction CBA-PH was enriched in xylose (80.27%) and the amount of arabinose (19.73%) decreased considerably. All the results agree well with the previous sugar composition analysis. Fraction CBA-PH consisted of 75.23% unsubstituted xylose, and 5.39% monosubstituted xylose residues. *O*-3 mono substituted and disubstituted xylose residues disappeared, whereas the content of unsubstituted xylose residues increased, indicating the branching points of the xylan backbone with arabinose substituents appeared to be at the *O*-3 position of 1,3,4-linked xylose residues. Since the relative amount of *O*-2 monosubstituted xylose residues (5.39%) increased, the residues linked to this position appeared to be more resistant to acid hydrolysis than the arabinose linked to the *O*-3 position of xylose. This corresponded with the previous report on AX of barley husk (Höije et al., 2006). The relative amount of terminal xylose (19.38%) increased slightly after acid treatment, which was also probably caused by some degradation of the backbone. This was indicated by a distinct decrease in the hydrodynamic volume, which was shown by HPSEC analysis (Fig. 1).

Methylation analysis (Table 2) also showed that after partial acid hydrolysis, most terminal arabinose residues were removed and there was a minor change in the amount of 1,2- and 1,5-linked arabinose residues in CBA-PH, which suggested that the side chains consisted by these two kinds of arabinose residues were stable in weak acid condition. The results indicated that most of arabinose residues were located at the non-reducing end of the branches. Compared with the content of mono- (11.73%) and disubstituted xylose (1.68%), the high molar ratio of terminal arabinose (30.07%) suggested that in addition to connecting directly with *O*-3, *O*-2 or *O*-2 and *O*-3 position of 1,4-linked xylose in the backbone, more than 10% T-L-Ara*f* should be attached to *O*-2 or *O*-5 position of arabinose residues. These arabinose residues formed into short arabinan chains.

3.5. NMR spectroscopy analysis

The 1D and 2D NMR spectra of the fragments were analyzed respectively to determine the primary structure. The anomeric protons were used as starting points for the assignment of proton signals by COSY and TOCSY experiments, and carbon signals were assigned from HSQC experiments. The inter-residue linkages were established from NOESY spectra. According to the characteristic signals, the ^{1}H and ^{13}C spectra of the fraction CBA were completely assigned and the corresponding chemical shifts of signals were summarized in Table 3.

Chemical shifts were assigned by comparison with previously reported literature data (Gruppen, Hamer, & Voragen, 1992; Hoffmann et al., 1992). The ^{1}H spectra of the fragments were given in Fig. 2. Signals at 5.6–5.0 ppm were corresponded to the anomeric protons of *α*-arabinofuranose (Hoffmann et al., 1992). The spec-

Table 3
Chemical shifts for the resonances of glycosyl residues of CBA in ^{1}H and ^{13}C NMR spectra.

Glycosyl residues	Chemical shifts, δ (ppm)				
	H1/C1	H2/C2	H3/C3	H4/C4	H5/C5
α-L-Ara*f*-(1→3 mono	5.40/108.16	4.20/81.40	3.92/77.81	4.20/85.29	3.75/3.77/62.01
α-L-Ara*f*-(1→3 di	5.29/108.53	4.20/81.67	3.92/78.00	4.20/85.29	3.75/3.77/61.88
α-L-Ara*f*-(1→2 di	5.22/109.16	4.16/82.01	3.95/77.10	4.12/84.79	3.75/3.77/61.88
β-*D*-Xyl*p*-(1→	4.57/103.12	3.37/73.51	3.45/76.24	3.62/69.80	3.99/3.32/65.82
→4)-β-*D*-Xyl*p*-(1→	4.50/102.20	3.34/73.27	3.58/74.30	3.78/76.99	3.34/4.11/63.57
→3,4)-β-*D*-Xyl*p*-(1→	4.52/101.79	3.45/73.54	3.75/78.02	3.82/74.31	3.34/4.11/63.56
→2,4)-β-*D*-Xyl*p*-(1→	4.60/–[a]	3.47/–	3.64/–	3.81/–	4.07/3.30/–
→2,3,4)-β-*D*-Xyl*p*-(1→	4.65/100.52	3.59/78.81	3.84/77.40	3.84/74.31	3.34/4.11/63.13

[a] Not determined.

trum of the sample CBA showed three major peaks in the anomeric region (Fig. 2A). The predominant signal at 5.40 ppm was assigned to terminal arabinose residues linked to *O*-3 of monosubstituted xylose residues. An NOE from H-1 of Ara*f* to the H-3 at 3.75 ppm showed that the α-L-Araf residue was (1→3)-linked to the β-D-Xyl*p* residue of the xylan backbone (figure not shown). The other two major signals at 5.22 and 5.29 ppm originated from the terminal α-L-Ara*f* substituents linked to both *O*-2 and *O*-3 of the same xylose residues, respectively, which suggested the presence of disubstituted β-D-Xyl*p* units in the main chain (Hoffmann et al., 1992).

In the anomeric region of 5.6–5.0 ppm, some minor signals were overlapped with the three major peaks and thus could not be assigned clearly. However, the spectrum of partial acid hydrolyzed fraction CBA-PH revealed the presence of other sugar signals of

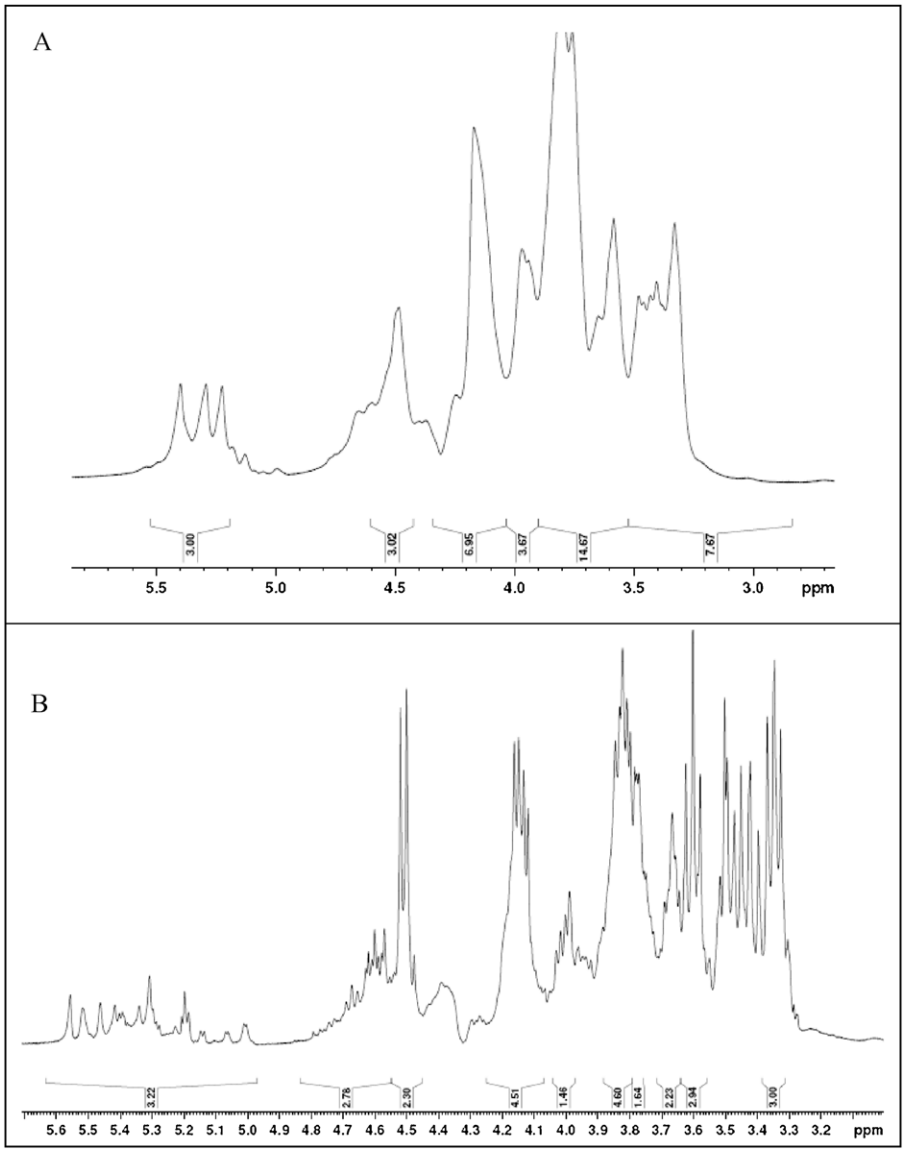

Fig. 2. ^{1}H NMR spectra of fraction CBA (A) and partial acid hydrolyzed fraction CBA-PH (B).

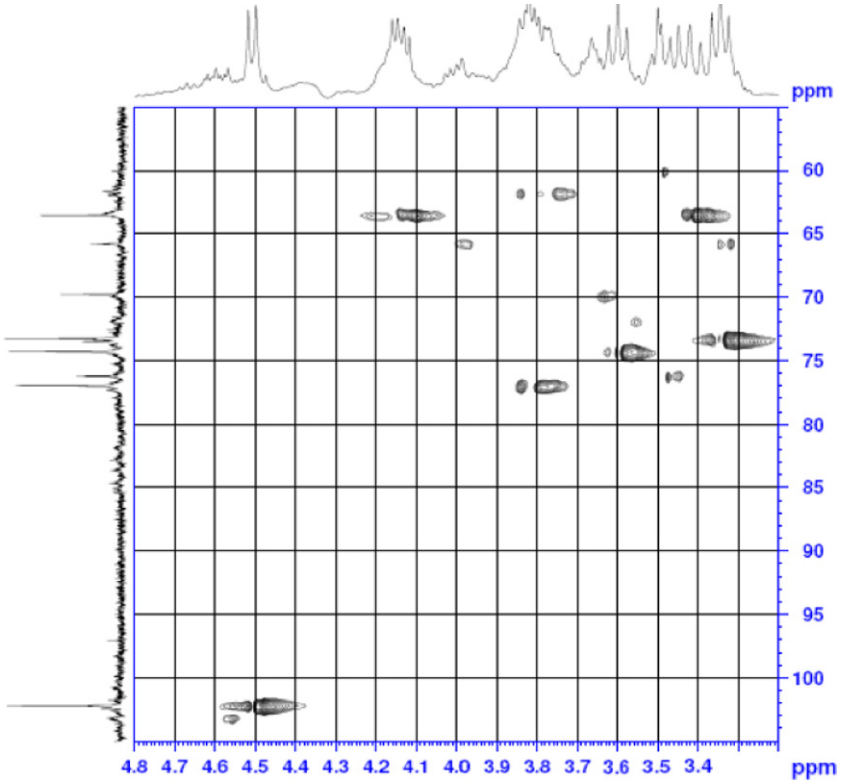

Fig. 3. $^{1}H/^{13}C$ HSQC spectrum of partial acid hydrolyzed fraction CBA-PH.

low intensity. In the proton spectrum of fraction CBA-PH (Fig. 2B), partial signals of terminal α-L-Ara*f* almost disappeared and some minor peaks became relatively distinct. Compared with these changes, a weak signal at about 5.31 ppm was originated from the α-L-Ara*f* residue (1→2)-linked with the monosubstituted β-D-Xyl*p* residue (Pastell et al., 2009). The peak at 5.52 ppm indicated the presence of α-L-Araf unit in a disaccharide side chain 2-*O*-β-D-Xyl*p*-α-L-Ara*f* attached to the *O*-3 of the β-D-Xyl*p* residue, as earlier published by Höije et al. (2006). 4-O-Me-α-D-Glc*p*A side chain has been reported to be present in wheat bran AX. However, no evidence of Glc*p*A in the present samples was found in the proton spectra.

For the Xyl*p* residues, the anomeric signals at 4.4–4.7 ppm were due to the un-, mono- and disubstituted β-D-Xyl*p* residues. The large signal at 4.50 ppm arose from the anomeric proton of the unsubstituted Xyl*p* residues, and the two signals at 4.52 and 4.60 ppm were characteristic of the anomeric protons of β-D-Xyl*p* residues monosubstituted by α-L-Ara*f* groups at C-3 and C-2, respectively. The anomeric signal of the disubstituted Xyl*p* residue resonated at 4.61 ppm was also observed in the spectra. The connectivity from H-1 to H-5 was clearly established from COSY and TOCSY spectra (Table 3), and these data were confirmed by heteronuclear HSQC spectrum.

In the anomeric region of ^{13}C NMR spectra, signals at 108–110 ppm were assigned to the C-1 of terminal or branched arabinofuranosyl units, and the signals at 100–103 ppm corresponded to the anomeric carbons of terminal or branched xylopyranose residues (figure not shown). Resonance signals in the region of 75–86 ppm were corresponded to C-2–C-4 of α-L-Ara*f* units, while the signals of C-2–C-4 of β-D-Xyl*p* appeared in the region of 65–85 ppm (Cui, 2005). Resonance signals in the region of 60–64 ppm arose from C-5 of terminal arabinofuranosyl units (61.88/62.01 ppm) and C-5 of terminal xylopyranose residues (63.56 ppm). According to the literature data published for arabinoxylan, ^{1}H and ^{13}C NMR spectroscopy allowed detection of the terminal, 4-, 3,4-, 2,4- and 2,3,4-substituted β-xylopyranose residues in the fragment CBA. In addition, the residues of the terminal α-arabinofuranose were identified completely (Table 3).

The $^{1}H/^{13}C$-HSQC (Fig. 3) and TOCSY spectra of fragments demonstrated the presence of regions consisting of β-1,4-linked xylan. In the ^{1}H and ^{13}C NMR spectra of fraction CBA-PH, the signals of α-arabinofuranose almost disappeared and became simple, whereas a group of intensive signals were observed for the residues of β-1,4-linked xylose. According to the literature (Gruppen et al., 1992), these resonances were characteristic of β-1,4-D Xylan which occupied the main part of the fraction CBA-PH.

4. Conclusion

Water unextractable polysaccharide from bran of a Chinese black-grained wheat variety was isolated at room temperature with saturated barium hydroxide as extraction medium. The fraction yielded 5.84% (w/w) of the initial bran material and composed mainly of arabinose (36.53%) and xylose (61.31%), indicating the presence of pure arabinoxylans. The arabinoxylan isolates exhibited the high average molecular weight (Mw) of 3.81×10^5 and an intermediate A/X ratio of 0.60.

The structural characterization of the isolated arabinoxylan (CBA) indicated that the isolates CBA consisted of a β-D-(1→4)-linked xylan backbone, which was mono- or disubstituted with α-L-arabinofuranosyl groups at position *O*-3, *O*-2 or at both *O*-2 and *O*-3 positions. Glycosidic linkage analyses showed that the fraction CBA was a less branched macromolecule and the xylan backbone contained a relatively high amount of unsubstituted xylopyranosyl residues (57.71%) and low amount of disubstituted xylose residues (6.22%). About 22.0% of xylose residues in the backbone were monosubstituted at *O*-3 position by α-arabinose side groups leading a low ratio of di- to monosubstituted xylose residues (0.28). Substitution of Xyl*p* occurred not only through *O*-3 or *O*-2 mono-, *O*-2 and *O*-3

disubstitution by terminal Ara*f* as single units, but also through 2-, 5-linked Ara*f* as short arabinan side chains.

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