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# Structural features of two heteroxylan polysaccharide fractions from wheat bran with anti-complementary and antioxidant activities

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ABSTRACT

Wheat bran is a rich source of bioactive substances ascribed to its arabinoxylan component. Two water-soluble arabinoxylans were sequentially extracted from wheat bran. WB1, released during enzymatic digestion of starch and protein, contained medium-branched arabinoxylan (A/X = 0.88) consisting of 3-*O*-substituted (22%), di-substituted (19.8%) and 58% unsubstituted Xyl*p* residues. It was slightly contaminated with (1 → 3,1 → 4)-β-glucan and arabinogalactan, and free of protein. WB2 extracted with 0.5% NaOH contained ~95% arabinoxylan (A/X = 1.09). WB2 and two 5% NaOH-extracted arabinoxylans were rich in protein and phenolic compounds. All radical-scavenging assays indicated a relation with the protein and total phenolics contents. The protein-free WB1 displayed the highest hydroxyl radical scavenging effect indicating the distinct role of phenolic acids. The immunomodulatory activity of WB1 was somewhat lower, whereas, that of WB2 higher in comparison to the immunogenic polysaccharide PMII. The arabinoxylans have the potential as immuno-enhancing and antioxidant additives in functional foods.

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## 1. Introduction

Dietary fiber intake provides many health benefits. Individuals with high intakes of dietary fiber appear to be at significantly lower risk for developing coronary heart disease, stroke, hypertension, diabetes, obesity, and certain gastrointestinal diseases (Anderson et al., 2009). The broad research activities in the field of immunologically active polysaccharides concerned not only those from medicinal plants, but also of from cereals and grasses (Hromádková & Ebringerová, 2003; Patel et al., 2007; Paulsen, 2001). Many of the civilization diseases are connected with oxidative stress which is combined with free radical formation. Therefore, antioxidants became of interest also in connection with the immune system as reflected by the increasing number of reports dealing with both the immunological and antioxidant activities of polysaccharides (Nergard et al., 2005; Rao & Muralikrishna, 2006).

Soluble dietary fiber such as mixed-linkage (1 → 3,1 → 4)-β-D-glucan and arabinoxylans (AX) may affect inflammatory processes and immune responses by several mechanisms (Hromádková, Košťálová, & Ebringerová, 2008; Samuelsen, Rieder, Grimmer, Michaelsen, & Knutsen, 2011).

Industrial wheat bran is one of the most representative available hemicellulose-rich by-products and, hence, a rich source of dietary fibers. Crude extracts from wheat bran contain a complex of acidic and neutral AX and co-extracted mixed-linkage β-glucan and arabinogalactans contaminated in various proportions with other cell wall components (protein, phenolics, lipids). Quantitatively, most of the acidic AX in grains is located in the outer grain layers, which contribute about 25% of the dry weight of the grain (Hollmann & Lindhauer, 2005), whereas, the neutral AX is accumulated in the aleurone and endosperm layers (Hoffmann, Kamerling, & Vliegenthart, 1992). Cereal AX xylans have the main chains composed of (1 → 4)-linked β-D-xylopyranosyl (β-Xyl*p*) residues which are mainly substituted with (1 → 2)- and/or (1 → 3)-linked α-L-arabinofuranosyl (α-Ara*f*) residues, and can also carry single α-D-glucopyranosyluronic acid or its 4-O-methyl ether (acidic AX), and acetyl substituents (Ebringerová, Hromádková, & Heinze, 2005). Ferulic and *p*-coumaric acids may be ester-linked to AX at position 5 of some of their α-Ara*f* side chains (Ishii, 1997).

Usually, the antioxidant activity of AX has been ascribed to contaminating and/or covalently bound phenolics, such as in case of feruloylated arabinoxylans or feruloylated xyloligosaccharides (Hromádková et al., 2008; Rao & Muralikrishna, 2006; Wang, Sun, Cao, & Wang, 2010). AXs from wheat bran showed activity in lymphocyte transformation and complement-fixing tests (Patel et al., 2007).

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The aim of the present study was to isolate AX from industrial wheat bran under mild conditions minimizing destructive effects of the used chemicals and other treatments which might cause loss of bioactivity. The composition, main structural features and molecular properties of the obtained AX will be characterized by chemical and spectral methods and the bioactivity evaluated by antioxidant and immunomodulating activity in *vitro tests*.

## 2. Experimental

### 2.1. Materials and chemicals

The wheat bran (*Triticum aestivum*) was a commercially available domestic product. The air-dried bran was grinded and passed through a 0.5 mm sieve. Crude AX fractions (D2-I and D5-I) were preparations from a previous study (Hromádková et al., 2008). They were isolated from wheat bran by extraction with 5% NaOH at 60 °C with and without assistance of ultrasound for 10 min. The extracts were exhaustively dialyzed using cellulose membrane (MWCO, 3.5 kg/mol, Serva) and lyophilized.

The stable free radical 1,1-diphenyl-2-picryl-hydrazyl (•DPPH) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich (Germany). Gallic acid and 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) were obtained from Fluka (Germany), the Folin–Ciocalteu's phenol reagent and $K_2S_2O_8$ from Merck (Darmstadt, Germany) and 2,2′-azino-bis(3-ethylbenthiazoline-6-sulfonic acid) salt (ABTS$^{\bullet+}$) from Polysciences, Inc. (Warrington, PA). $NaBD_4$ and 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) were from Sigma Aldrich (Milwaukee, WI). All other used chemicals were of analytical grade.

### 2.2. Extraction and purification of polysaccharides

The air-dried wheat bran was exhaustively pre-extracted by Soxhlet with chloroform–ethanol (65:35, v/v) to remove lipophilic and colored extractive compounds. The resulting extractive-free wheat bran (WB) was treated with acetate buffer (pH 5.4) at 70 °C for 30 min under stirring, cooled and, then the enzymatic digestion of starch was performed with the α-amylase from *Aspergillus oryzae* (60.5 U/mg) and amyloglycosidase (50 U/mg) at 37 °C for 43 h under stirring. After inactivation of the enzymes (100 °C for 10 min), the mixture was centrifuged at 5000 rpm for 15 min and filtered. The extract was adjusted to pH 7.5 with NaOH, and Pronase (from *Streptomyces griseus*) was added to the sample solution. The solution was mixed at 27 °C for 48 h. The enzymes were inactivated by heating the mixture at 100 °C for 10 min. The insoluble part was removed by centrifugation and the supernatant was extensively dialyzed with deionized water (MWCO, 3.5 kg/mol, Serva) and lyophilized (yield 2.2%). The digesting step with Pronase was repeated two times yielding the purified polysaccharide fraction WB1 (yield 1.35%).

The fiber residue of wheat bran was further extracted with 0.5% NaOH at 60 °C for 1 h. The insoluble material was separated by centrifugation at 7000 rpm for 15 min and the supernatant was neutralized to pH 6.8, dialyzed (MWCO, 3.5 kg/mol) and lyophilized (yield 4.3%). The separated fraction was dissolved in buffer (pH 7.5), digested three times with Pronase as in case of the former sample yielding fraction WB2 (yield 1.7%). All treatments after digestion with Pronase were performed under the same conditions as described in the extraction procedure with acetate buffer.

### 2.3. General methods

Sugar analysis of the polysaccharides was performed after hydrolysis with 2 M trifluoroacetic acid (reflux, 2 h). The neutral sugar composition of the hydrolysates was determined by gas chromatography on a Hewlett-Packard Model 5890 Series II chromatograph equipped with a PAS-1701 column (0.32 mm × 25 m), the temperature program of 110–125 (2 °C/min) to 165 °C (20 °C/min) and flow rate of hydrogen 20 $cm^3$/min in form of the alditol trifluoroacetates. The uronic acid content was determined by the 3-hydroxydiphenyl assay using glucuronic acid as standard (Blumenkrantz & Asboe-Hansen, 1973) and identified by paper chromatography (p.c.) (Ebringerová, Hromádková, Petráková, & Hricovíni, 1990). The nitrogen content (%N) was obtained by elemental analysis (Elemental Analyser, Perkin-Elmer Model 240). The content of total of phenolic compounds (TP) was performed using the modified Folin–Ciocalteau method (Yu et al., 2002) using gallic acid as standard. The TP content was expressed as gallic acid equivalents. The measurements of TP, protein, uronic acids and neutral sugars were performed in triplicate.

The molecular characterizations of samples were investigated by HPSEC method (a high-performance size-exclusion chromatographic method). The samples (3 mg/mL) were dissolved in the eluent at room temperature for 1 day and centrifuged before analysis at 6000 rpm for 5 min. The HPLC system (Shimadzu, Wien, Austria) comprised a high pressure pump LC-10AD, membrane degasser (GT-104), an injector (Rheodyne 77251), an UV–vis detector (SPD-10AV) and a differential refractometer (RID-6A). Columns HEMA-BIO 300 (8 mm × 250 mm; 10 μm sorbent particle size; Tessek, Prague, Czech Republic) were used. The UV detector was used at $\lambda_0$ = 280 nm. The mobile phase 0.1 M $NaNO_3$ was used at a flow rate 0.4 mL/min, and weight molecular weight ($M_w$) obtained using a calibration from pullulan standards P10-P800 (Shodex Standard P-28, Macherey-Nagel, Germany). The elution profiles were recorded by Class-VP chromatography software.

### 2.4. FTIR and NMR spectroscopies

Fourier transform infrared spectra (FTIR) of polysaccharides were measured using the Nicolet-Magna 750 spectrophotometer (Nicolet, Madison, USA) with DTGS detector and OMNIC 3.2 software (Nicolet, Madison, USA) in the form of KBr pellets (2 mg sample/200 mg KBr). The NMR spectra were measured at 40 °C in $D_2O$ (99.96% D) solutions using a Varian 400 MR spectrometer operating at 400 MHz for protons and 100 MHz for $^{13}C$ nuclei. Acetone was used as an internal standard ($\delta$ 2.225 for $^1H$ and $\delta$ 31.07 for $^{13}C$). Homonuclear $^1H/^1H$ correlation spectroscopy (COSY), and heteronuclear $^{13}C$–$^1H$ correlation experiments (HSQC, HMBC) were run using the standard Varian pulse sequence. Experimental data were processed with the MestReNova 7.0 software.

### 2.5. Methylation analysis

Methylation analysis of WB1 was performed according to Ciucanu and Kerek (1984), modified for polysaccharides (unpublished results). After methylation with methyl iodide in the solid NaOH/DMSO medium, the isolated product was hydrolyzed by 4 M TFA under reflux for 6 h, reduced with $NaBD_4$, converted into alditol acetates and analyzed by GLC–MS on FINNIGAN SSQ 710 spectrometer equipped with SP 2330 column (0.25 mm × 30 m) at 80–240 °C, 70 eV, 250 mA, and ion-source temperature 150 °C.

### 2.6. Antioxidant capacity tests

#### 2.6.1. Assay of DPPH radical-scavenging activity

Free radical scavenging ability (RSA) of the arabinoxylan fractions against the stable free DPPH radical (•DPPH) was determined spectrophotometrically (Rao & Muralikrishna, 2006); using the Spectronic 20 Genesys device. Briefly, 1 mL of polysaccharide sample (0.33–6.67 mg/mL in water) was mixed with 1 mL of a freshly

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