JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Structural Comparison of Arabinoxylans from Two Barley Side-Stream Fractions

LEENA PITKÄNEN,*[,†] PÄIVI TUOMAINEN,[†] LIISA VIRKKI,[†] VLADIMIR ASEYEV,[§] AND MAIJA TENKANEN[†]

Department of Applied Chemistry and Microbiology, P.O. Box 27, FIN-00014 University of Helsinki, Finland, and Department of Chemistry, P.O. Box 55, FIN-00014 University of Helsinki, Finland

The structures of barley (*Hordeum vulgare*) arabinoxylans isolated from two industrial side fractions, barley husks (BH) and barley fiber (BF), were characterized. Arabinoxylans were extracted with saturated barium hydroxide after enzymatic pretreatment. Barium hydroxide was selective toward arabinoxylans, and only a minor amount of glucose-containing material was coextracted. Acid methanolysis followed by gas chromatography, ^{1}H NMR spectroscopy, and specific enzymatic treatments followed by anion exchange chromatography with pulse amperometric detection (HPAEC-PAD) revealed that the chemical structure of barley husk arabinoxylan (BHAX) clearly differed from that of barley fiber arabinoxylan (BFAX). BFAX was more branched, containing more β-D-xylopyranosyl (β-D-Xyl*p*) residues carrying α-L-arabinofuranosyl (α-L-Ara*f*) units at both O-2 and O-3 positions. BHAX, on the other hand, contained more 2-*O*-β-D-Xyl*p*-α-L-Ara*f* substituents than BFAX. BHAX and BFAX also differed with respect to the hydrodynamic properties investigated with multidetector size exclusion chromatography. BFAX had a higher weight-average molar mass and larger hydrodynamic volume, the latter indicating less dense conformation than BHAX. M_n, M_w/M_n, R_h, and the Mark–Houwink *a* value were also determined for both arabinoxylans.

KEYWORDS: Barley; arabinoxylans; α-arabinofuranosidase; chemical structure; d*n*/d*c*; endo-(1→4)-β-D-xylanase; hydrodynamic volume; molar mass; pullulan; size exclusion chromatography

INTRODUCTION

Interest in the utilization of biomass has increased during recent decades. Side-stream waste material from agriculture such as cereal husks and straw could be exploited as a source of biopolymers. During the manufacture of barley starch and potable ethanol, large amounts of barley husks and barley fiber are coproduced. Both of these side-fractions currently have no productive use other than livestock feeding. However, both the barley fiber and barley husks are rich in arabinoxylans and thus constitute a potential source of biopolymers. The quantity of barley side-stream fractions may significantly increase in the future due to ethanol production for transportation fuel purposes. Also, when biowaste accumulation problems are taken into account, the development of economically efficient ways to utilize agricultural wastes will soon be an important task. The chemical composition and physical properties of biopolymers need to be thoroughly studied to develop new industrial applications.

After cellulose, hemicelluloses are the second most abundant renewable bioresource produced by plants. Classically, hemicelluloses are defined as cell-wall polysaccharides closely associated with cellulose that can be extracted with alkaline reagents (*1*). Arabinoxylans consist of a (1→4)-linked β-D-xylopyranosyl (β-D-Xyl*p*) backbone with α-L-arabinofuranosyl (α-L-Ara*f*) substituents attached at position O-2, O-3 or both and constitute the main hemicellulosic material in cereals. Both the amount and the degree of substitution of arabinoxylans vary according to the cereal species and the part of the kernel (*2*). In general, arabinoxylans in the outer parts of the kernel are less substituted than those in the inner parts. Whole grain barley contains 6.6% (dry weight) arabinoxylans with an arabinose-to-xylose ratio of 0.42. The arabinoxylan content of barley endosperm is lower, being 1.42% with an arabinose-to-xylose ratio of 0.67 (*3*). Arabinoxylans are especially abundant in aleurone cells. Barley aleurone cells contain 85% arabinoxylans with an arabinose-to-xylose ratio of 0.58 (*4*). According to methylation analysis, alkali-soluble arabinoxylan from hull-less barley grain contains 42% of substituted xylose units, of which 43% are O-2 and O-3 disubstituted xylose residues, 34% are O-3 monosubstituted xylose residues, and 22% are O-2 monosubstituted xylose residues (*5*). The results of Trogh et al. (*6*) obtained with ^{1}H NMR spectroscopy and gas chromatography for alkali-soluble arabinoxylan from hull-less barley flours were slightly different: 48.5% of xylose units were substituted, 47% of these with O-2 and O-3 disubstituted xylose residues, 25%

* Corresponding author (telephone +358 9 191 58 392; fax +358 9 191 58 475; e-mail leena.m.pitkanen@helsinki.fi).
[†] Department of Applied Chemistry and Microbiology.
[§] Department of Chemistry.

10.1021/jf800315q CCC: $40.75 © 2008 American Chemical Society
Published on Web 06/13/2008

with O-3 monosubstituted xylose residues, and 28% with O-2 monosubstituted xylose residues. Very few studies have been carried out on the chemical composition of barley husks, which may contain from one-third (*7*) to 46% arabinoxylan (*8*), with an arabinose-to-xylose ratio from 0.18 to 0.37 (*8, 9*).

High-performance size exclusion chromatography (HPSEC) combined with multiple detectors is a powerful tool for polymer analysis, providing information about the molar mass and molecular size as well as the parameters concerning conformation. Biopolymers such as cereal arabinoxylans have mainly been analyzed with systems containing only a refractive index detector using conventional calibration with narrow molar mass standards (*10, 11*). Saake et al. (*12*) used the universal calibration for oat spelt xylan. Multiple detector systems including a light scattering detector have also been used to analyze arabinoxylans (*13, 14*). The biggest limitation in the use of a light scattering detector to determine the average molecular mass of arabinoxylans is their poor solubility and impurities. Both factors complicate the precise determination of the refractive index increment value (d*n*/d*c*), which is constant for certain polymer−solvent combinations and required for light scattering studies. The square of the *dn*/d*c* value is included in the value of the optical constant *K* (eq 2) included in the basic equation of light scattering (eq 1) (*15*).

$$\frac{Kc}{R_\theta} = \frac{1}{M_w P(\theta)} + 2A_2 c \quad (1)$$

$$K = \left(\frac{4\pi^2 n_0}{\lambda_0 N_A}\right)\left(\frac{dn}{dc}\right)^2 \quad (2)$$

In eqs 1 and 2, c is the solution concentration, R_θ is the intensity of light scattered at the angle θ, A_2 is the second osmotic virial coefficient, n_0 is the refractive index of the solvent, and λ_0 is the wavelength of the incident light. At infinite dilution and zero angle, the angular dependence of the scattered light $P(\theta) = R_\theta/R_{\theta=0} = 1$, and eq 1 yields the weight-average molar mass (M_w).

In addition, the determination of intrinsic viscosities ([η]) is conditional on accurate knowledge of the polymer concentration eluting from the column and clearly affects the reliability of the results calculated with universal calibration. In comparison with the conventional calibration, the universal calibration, and the molar mass calculation based on light scattering and/or viscometry, the conventional calibration is the only method independent of the sample recovery on the detectors. However, due to the lack of appropriate molar mass standards, molar mass calculation techniques based on light scattering and viscometry need to be developed for various polysaccharide samples.

The aim of this study was to isolate alkali-extractable arabinoxylans from the husks and fiber of barley using specific barium hydroxide extraction. Both the husks and fiber are side-stream fractions from the industrial ethanol process. Alkaline extraction was used to effectively isolate the arabinoxylans from the complex cell-wall matrix. Isolated arabinoxylans were characterized with monosaccharide composition analysis, ^{1}H NMR spectroscopy, enzymatic hydrolysis, and HPSEC, and the structures of arabinoxylans from two side fractions were compared. A HPSEC method for arabinoxylans was developed, with attention paid to difficulties encountered with this form of analysis for arabinoxylans. In addition, the chemical composition of barley husk and barley fiber samples was thoroughly analyzed.

MATERIALS AND METHODS

Barley (*Hordeum vulgare*) materials, husks (BH) and fiber (BF), used for the isolation of arabinoxylans were obtained from Altia Corp. (Koskenkorva, Finland). Both husks and fiber were byproducts from the industrial process producing potable ethanol and barley starch. Husks are recovered after cleaning of the grains and fiber, which contains cell-wall material of whole barley grain, after starch has been separated by wet-milling. Barium hydroxide (barium hydroxid-8-hydrat) was purchased from Merck (Darmstadt, Germany) and sodium borohydride ($NaBH_4$) from Fluka (Buchs, Switzerland). Sodium hydroxide prepared for HPLC was from Fluka. The anhydrous methanol used for methanolysis reagent was from Fluka and acetyl chloride from Merck (Schuchardt, Germany). Pyridine was obtained from Sigma-Aldrich Chemie (Steinheim, Germany). HPLC grade dimethyl sulfoxide (DMSO) for HPSEC was from Laboratory-Scan (Dublin, Ireland) and lithium bromide (LiBr) from Sigma-Aldrich Chemie. D-Xylose, D-arabinose, D-glucose, D-galactose, D-mannose, and L-rhamnose were from Merck (Darmstadt, Germany) and (1→4)-β-D-xylobiose and (1→4)-β-D-xylotriose from Megazyme (Bray, Ireland). D_2O (99.9%) for NMR measurements was from Merck. All other chemicals used were of analytical or HPLC grade.

Pepsin (0.7 FIP-U/mg) was from Merck and pancreatin from the swine pancreas (P-7545) from Sigma-Aldrich Chemie. Termamyl 120L α-amylase, Shearzyme [endo-(1→4)-β-D-xylanase, 49075 nkat/mL] and the α-L-arabinofuranosidase (AXH-m, 14590 nkat/mL) were obtained from Novozymes (Bagsvaerd, Denmark). The activities were determined according to the method of Virkki et al. (*16*). The other α-L-arabinofuranosidase (AXH-d3, E-AFAM2, activity given by the manufacturer = 200 U/mL = 3340 nkat/mL) was purchased from Megazymes.

Composition of BH and BF. The monosaccharide composition of BH and BF was determined with anion exchange chromatography after sulfuric acid hydrolysis (*17*). The barley samples were ground with a Tecator Cyclotec 1093 sample mill to reduce the particle size to <0.5 mm. Then, 200 mg of sample was mixed with 2 mL of 72% H_2SO_4 and prehydrolyzed at 30 °C in a water bath for 60 min. Prehydrolysis was stopped by adding 6 mL of water. Samples were transferred to 100 mL volumetric flasks with 50 mL of water and autoclaved for 40 min. The hydrolysis medium was filtered with glass filters (porosity 4), and the monosaccharides of the filtrate were analyzed with borate complex anion exchange chromatography using gradient elution with 0.3 and 0.9 M potassium tetraborate buffers, pH 9.2. D-Xylose, D-arabinose, D-glucose, D-mannose, D-galactose, and L-rhamnose were used as standards. The hydrolysis residue was recovered on the filters and determined gravimetrically with a procedure similar to the Klason lignin procedure. The total starch was measured using a Megazyme kit (AOAC method 996.11), which is based on enzymatic hydrolysis of the sample to glucose with thermostable α-amylase and amyloglucosidase. The content of cellulose and (1→3)(1→4)-β-D-glucan was calculated by subtracting the starch content from the total amount of anhydroglucose. The sum of anhydroxylose and anhydroarabinose is presented as the arabinoxylan content.

The amount of lipids was measured gravimetrically using Soxhlet extraction. The Kjehldal method was used for protein determination (6.25 × N). The ash content was measured gravimetrically after ashing the samples at 550 °C in a temperature-programmed muffle oven (Nabertherm, Germany). All analyses were performed in triplicate, except protein determination, which was carried out in duplicate.

Extraction of Arabinoxylans. Water-insoluble arabinoxylans were extracted from barley husks and fiber using a method by Virkki et al. (*18*) in which starch and proteins were digested enzymatically and arabinoxylans further extracted with saturated barium hydroxide (**Figure 1**). The ground barley samples (15 g) were suspended in water and kept in a boiling water bath for 15 min to gelatinize the starch. The gelatinized starch was degraded with 1.8 mL of Termamyl solution in a boiling water bath for 60 min. The pH of the suspension was adjusted with 1 M HCl to 1.5 for pepsin treatment (1.5 g) to degrade the proteins at 40 °C for 60 min. Digestion of proteins and lipids was further continued with 1.5 g of pancreatin at pH 6.8 (pH adjusted with 1 M NaOH) and a temperature of 40 °C for 60 min. The remaining carbohydrate material (water-insoluble fiber, WIS) was filtered through

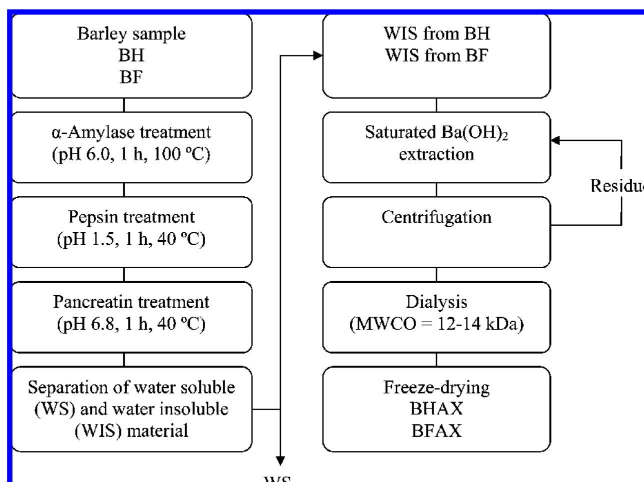

Figure 1. Schematic diagram of the isolation of arabinoxylans (AX) from barley husks (BH) and barley fiber (BF).

glass filters (porosity 2), washed with water, ethanol, and acetone, and dried in an oven. Saturated barium hydroxide with 0.26 M $NaBH_4$ was added to WIS (170 mL/g of WIS), and suspensions were stirred at room temperature overnight. The supernatant was collected after centrifugation, and the barium hydroxide extraction was repeated (80 mL/g of WIS). The suspensions were stirred for an hour and centrifuged, and the supernatant was collected again. The combined supernatants were neutralized with glacial acetic acid and dialyzed (MWCO 12000−14000, Medicell International Ltd., London, U.K.) against 0.2 M sodium acetate buffer, pH 5.0 (3 h), running tap water (3 days), and Milli-Q water (3 h). Barium hydroxide soluble material (alkali-extractable barley husk arabinoxylan, BHAX, and alkali-extractable barley fiber arabinoxylan, BFAX) was recovered after freeze-drying (Heto Dry winner DW 8-85, Gydevang, Denmark).

Composition of BHAX and BFAX. Monosaccharide composition analysis was performed using gas chromatography (GC) after acid methanolysis carried out according to the method of Sundberg et al. (*19*). The BHAX and BFAX samples were degraded to monosaccharides with hydrochloric acid in anhydrous methanol (prepared by adding 16 mL of acetyl chloride to anhydrous methanol in a 100 mL volumetric flask). The samples were kept at 100 °C in pear-shaped flasks for 3 h and neutralized with pyridin. The samples were trimethylsilylated prior to GC analysis. The monosaccharide standards (D-xylose, D-arabinose, D-glucose) were treated with acid methanolysis in the same way as the arabinoxylan samples and diluted to make a calibration curve. The areas of all peaks resulting from different anomers of each monosaccharide were summed for quantification. The samples and the standards were analyzed in triplicate. The GC instrument consisted of a Hewlett-Packard 5890 series II GC system with a flame ionization detector (FID), a HP 7673 series injector, and an autosampler (Hewlett-Packard, Palo Alto, CA). The column used was a HP-5 (30 m × 0.32 mm × 0.25 μm; Agilent Technologies, Foster City, CA). The temperature program was as follows: oven temperature, 70 °C, ramped at 2 °C/min to 175 °C and at 12 °C/min to 290 °C. The injection volume was 0.5 μL and the split ratio 1:30. The presence of uronic acids was checked with a Hewlett-Packard 6890 series gas chromatography coupled to an Agilent 5973 mass spectrometer (GC-MS) using chromatographic conditions similar to those for GC-FID.

The protein content of extracted xylans was calculated from the amount of nitrogen (6.25 × N) determined according to American Association of Cereal Chemists approved method 46-30 (*20*).

NMR Analysis of BHAX and BFAX Samples. For the NMR analysis the BHAX and BFAX samples were exchanged three times with D_2O and finally dissolved in 1 mL of pure D_2O. The ^1H spectra of arabinoxylan samples were obtained on a Varian Unity 500 spectrometer (Varian NMR Systems, Palo Alto, CA) operating at 500 MHz for ^1H. The measurements were performed at 50 °C, and ^1H chemical shifts (ppm) were referenced to an internal acetone signal at 2.225 ppm.

Enzymatic Treatments of BHAX and BFAX. Enzymatic treatments of BHAX and BFAX samples were carried out to obtain more information about the arabinose substitution of extracted arabinoxylans. The arabinoxylan samples (5 g/L) in 0.02 M sodium acetate buffer, pH 5.0, were first incubated with Shearzyme, using an endo-(1→4)-β-D-xylanase dosage of 10000 nkat/g of xylan, at 40 °C for 48 h. Hydrolysis was terminated by keeping the samples in a boiling water bath for 10 min. Both samples were further incubated separately with two different α-L-arabinofuranosidases (AXH-m and AXH-d3). AXH-m dosage was 5000 nkat/g of xylan and AXH-d3 dosage 1000 nkat/g of xylan. Reaction conditions for AXH treatments were similar (40 °C, 24 h), except that 0.02 M sodium acetate buffer, pH 5.0, was used for AXH-m and 0.05 M sodium phosphate buffer, pH 6.5, for AXH-d3.

High-performance anion exchange chromatography with pulse amperometric detection (HPAEC-PAD) was used after enzyme treatments to investigate the oligosaccharides present. The equipment consisted of an SSI pulse equalizer (Scientific Systems, Inc., model LP 21, State College, PA), two Waters 515 HPLC pumps (Waters Corp., Milford, MA), an autosampler Waters 717 Plus (Waters Corp.), a column and precolumn (CarboPac PA-100, 4 × 250 mm and CarboPac PA-100 guard, 4 × 50 mm, Dionex, Sunnyvale, CA), and a Decade pulse amperometric detector (Antec Leyden, Zoeterwoude, The Netherlands). Gradient elution with 1 M NaAc in 0.1 M NaOH was used for elution. Millenium[32] software (Waters Corp.) was used for instrument control and data handling. Further details of the HPAEC-PAD analysis of arabinooligosaccharides are provided by Rantanen et al. (*21*).

The formed arabinooligosaccharides were also qualitatively analyzed with offline electrospay ionization mass spectrometry (ESI-MS). The analysis was carried out using a high-performance liquid chromatograph (Agilent 1100, Agilent Technologies, Santa Clara, CA) combined with a mass spectrophotometer (Agilent XCT Plus ion trap). The oligosaccharide mixtures were directly injected into the mass spectrophotometer without column separation. Electrospray ionization (ESI) was performed in positive ion mode. The nebulizer gas (N_2) pressure was 30 psi, the drying gas flow and temperature were 8 L/min and 350 °C, respectively. The capillary voltage was set at 3270 V, capillary exit offset at 121 V, skimmer 1 potential at 40.0 V, and trap drive value at 61.2. Spectra were recorded as averages of four using ultra scan mode and a scan range from m/z 50 to 1500.

HPSEC Analysis of BHAX and BFAX. HPSEC analysis of the xylans was performed using 0.01 M solution of LiBr in DMSO as an eluent, which was recommended by the column manufacturer. The samples ($c = 4$ mg/mL) were dissolved in eluent at room temperature for 4 days and filtered before analysis with 0.45 μm syringe filters (GHP Acrodisc 13, Pall Corp., Ann Arbor, MI). The HPSEC equipment consisted of an integrated autosampler and pump module (GPCmax, Viscotek Corp., Houston, TX), two linear type columns (Shodex LF-804, 8 × 300 mm, Showa Denko, Tokyo, Japan) and a guard column (Shodex LF-G, 4.6 × 10 mm), a UV detector (Waters 486 Tunable Absorbance Detector, Milford, MA), a combined light scattering and viscometric detector (270 Dual Detector, Viscotek Corp.), and a refractive index (RI) detector (VE 3580, Viscotek Corp.). Light scattering detector ($\lambda_0 = 670$ nm) included two scattering angles: 7° (low-angle light scattering, LALS) and 90° (right-angle light scattering, RALS). The Viscotek Dual Detector was calibrated with the pullulan standard ($M_w = 47300$, Polymer Laboratories, Shropshire, U.K.). The flow rate was 1 mL/min, and the columns were thermoregulated in a column oven (Croco-cil 100-040-220P, Cluzeau Info Labo, Sainte-Foy-la-Grande, France) at 40 °C. The average molar masses were calculated from the light scattering signal. The literature value for the refractive index increment (dn/dc) of 0.064 for xylans determined by Goring and Timell (*22*) was used for molar mass calculations. The UV detector at $\lambda_0 = 280$ nm was used mainly for monitoring the protein and lignin impurities. Intrinsic viscosities ($[\eta]$) were calculated on the basis of the viscosity signals obtained from the viscometric detector. All of the calculations regarding molar mass averages (M_w, M_n), polydispersity index (M_w/M_n), hydrodynamic radius (R_h), and the Mark−Houwink a value were done using the OmniSEC 4.2 software (Viscotek Corp.).

d*n*/d*c* Determination for Pullulan. The pullulan sample (Hayashibara, Okayama, Japan) used for refractive index increment (dn/dc)

Table 1. Chemical Composition of Barley Husks (BH) and Barley Fiber (BF) (Percent of Dry Weight ± SD, $n = 3$)

component	BH	BF
carbohydrates[a]	72.6 ± 0.5	59.2 ± 0.5
starch	31.9	9.77
cellulose + β-glucan	34.0	35.8
arabinoxylan	32.5	50.0
proteins	5.4 ± 1.3	12.6 ± 0.1
lipids	0.9 ± 0.2	2.8 ± 0.1
ash	4.7 ± 0.1	3.5 ± 0.2
Klason lignin	17.1 ± 0.4	18.6 ± 0.2
total	100.7	96.7

[a] Carbohydrate composition is depicted as percentage values from the total amount of glucose, xylose, and arabinose. Cellulose + β-glucan is calculated by subtracting starch content from the total amount of anhydroglucose. Arabinoxylan is presented as the sum of anhydroxylose and anhydroarabinose.

Table 2. Monosaccharide Composition of Barley Husks (BH) and Barley Fiber (BF) (Percent of Dry Weight ± SD, $n = 3$)

monosaccharide	BH	BF
Glc	47.8 ± 0.5	27.0 ± 0.2
Xyl	18.9 ± 0.3	19.8 ± 0.2
Ara	4.7 ± 0.2	9.8 ± 0.2
Man	0.4 ± 0.1	1.1 ± 0.0
Gal	0.7 ± 0.1	1.4 ± 0.0
Rha	0.1 ± 0.0	0.1 ± 0.0
total	72.6 ± 0.5	59.2 ± 0.5

measurements was a kind gift from Technical Research Centre of Finland (VTT). The purity of the sample was checked with ^{1}H NMR spectroscopy. The d*n*/d*c* of pullulan was determined at 20 °C using an Abbe refractometer (ABBE 60/ED). A He−Ne laser from the Spectra-Physics Lasers Inc. operating at $\lambda_0 = 632.8$ nm was the light source. Stock solutions of four different pullulan concentrations (20, 30, 40, 50 mg/mL) in DMSO containing 0.01 M LiBr were further diluted, and the refractive indices of the solutions were measured three times. Then the refractive indices were plotted versus the pullulan concentration and the d*n*/d*c* value was obtained from the slope of the fitted linear function.

RESULTS

Extraction of Water-Insoluble Arabinoxylans. The chemical composition of the barley materials is shown in **Table 1**. Barley husks (BH) contained much more starch than barley fiber (BF), which is most likely due to endosperm remains in BH. BF is material from which the starch has been recovered in a wet-milling process. The amount of arabinoxylan was calculated as a sum of anhydroxylose and anhydroarabinose. The arabinoxylan content of BF was higher in comparison with BH. BF also contained more protein, which was predictable. The Klason lignin content was almost equal in both fractions. The majority of monosaccharides in BH and BF consisted of glucose, xylose, and arabinose, and only small amount of other sugars were present (**Table 2**). The xylose content of both barley materials was almost equal, but the arabinose content in BF was double that in BH. The arabinose-to-xylose ratio was 0.25 for BH and 0.49 for BF. Arabinose mainly originates from arabinoxylans rather than arabinogalactan due to the low amount of galactose present in either of the barley fractions.

BH contained 65.5% of WIS and BF 71.4%.The arabinoxylan content of WIS (considering all arabinose and xylose as arabinoxylan) from BH and BF, respectively, was 12.2 and 18.4%. The monosaccharide composition of extracted material was analyzed using gas chromatography after decomposition

Table 3. Monosaccharide Composition of Extracted Barley Arabinoxylans from Barley Husks (BHAX) and Barley Fiber (BFAX) (Percent of Weight ± SD, $n = 3$)

monosaccharide	BHAX	BFAX
Xyl	45.8 ± 1.0	45.3 ± 1.2
Ara	13.0 ± 0.5	23.1 ± 0.6
Glc	3.67 ± 0.1	4.53 ± 0.2
total	62.5	72.9
Ara:Xyl	0.28 ± 0.0	0.51 ± 0.0

of material with acid methanolysis. The material mainly consisted of arabinoxylan (**Table 3**). The xylose content was similar in both BHAX and BFAX, but as expected BFAX contained more arabinose, which indicates a higher degree of substitution in comparison with BHAX. Only a low amount of glucose-containing material (6% of carbohydrates), that is, starch or (1→3)(1→4)-β-D-glucan, was left. The carbohydrate yield in acid methanolysis was 62.5% for BHAX and 72.9% for BFAX. These values are at the same level as the yields obtained for commercial arabinoxylan isolates (*16, 21*) and thus acceptable, taking into consideration the protein, ash, and moisture contents of isolated barley arabinoxylans. The protein contents of BHAX and BFAX were 6.6 and 8.1%, respectively.

NMR Spectroscopy. The α-anomeric regions of ^{1}H NMR spectra of polymeric BHAX and BFAX samples are illustrated in **Figure 2**. All anomeric ^{1}H NMR signals of arabinoxylans are found in the spectral region of 4.4−5.6 ppm and α-L-Ara*f* anomeric protons at 5.0−5.6 ppm (*23, 24*). ^{1}H NMR spectra of samples revealed a (1→4)-linked β-D-Xyl*p* main chain partly substituted with α-L-Ara*f*. Both spectra show three to five major peaks and some overlapping signals in the region of 5.2−5.6 ppm. Signals of low intensity are also found in the region of 5.05−5.16 ppm, but their reliable assignation could not be made.

The assignation of the main proton signals (A−E, **Figure 2**) is summarized in **Table 4**. The main peak in both spectra at 5.39 ppm (B) indicates the presence of an α-L-Ara*f* (1→3)-linkage to monosubstituted β-D-Xyl*p* residue. The signals at 5.28 and 5.22 ppm (D, E) originate from the (1→3)-linked and (1→2)-linked α-L-Ara*f* substituents on the doubly substituted β-D-Xyl*p* unit in the main chain (*25, 26*). The signal at 5.54 ppm (A) indicates the presence of a disaccharide side chain 2-*O*-β-D-Xyl*p*-α-L-Ara*f* attached to O-3 of xylopyranosyl residue (*27*). In both BHAX and BFAX the signal of the α-L-Ara*f* residue (1→2)-linked to the monosubstituted xylose unit (5.30 ppm, C) partly overlapped with that of the (1→3) α-L-Ara*f* residue in the doubly substituted xylose unit. Other overlapping α-anomeric signals are typical for complex structures of polymeric arabinoxylans and originate from α-L-Ara*f* residues attached to two consecutive substituted xylose residues (*24*). Due to these overlapping signals 4-*O*-methylglucuronic acid (5.26 ppm) found by Höije et al. (*27*) from barley husk was not observed in the ^{1}H NMR spectrum. However, no uronic acids were found in BHAX or BFAX samples according to GC-MS analyses.

Comparison of signal intensities in the ^{1}H NMR spectra shows that the main substitution in both samples was that of α-L-Ara*f* residues linked to O-3 of monosubstituted β-D-Xyl*p* residues of the main chain (B in **Figure 2**). When the signal intensities of α-L-Ara*f* residues linked to disubstituted xylose residues were compared, a significant difference between the samples was observed. In BHAX the degree of disubstitution to β-D-Xyl*p* units (5.28 and 5.22 ppm, D and E, respectively)

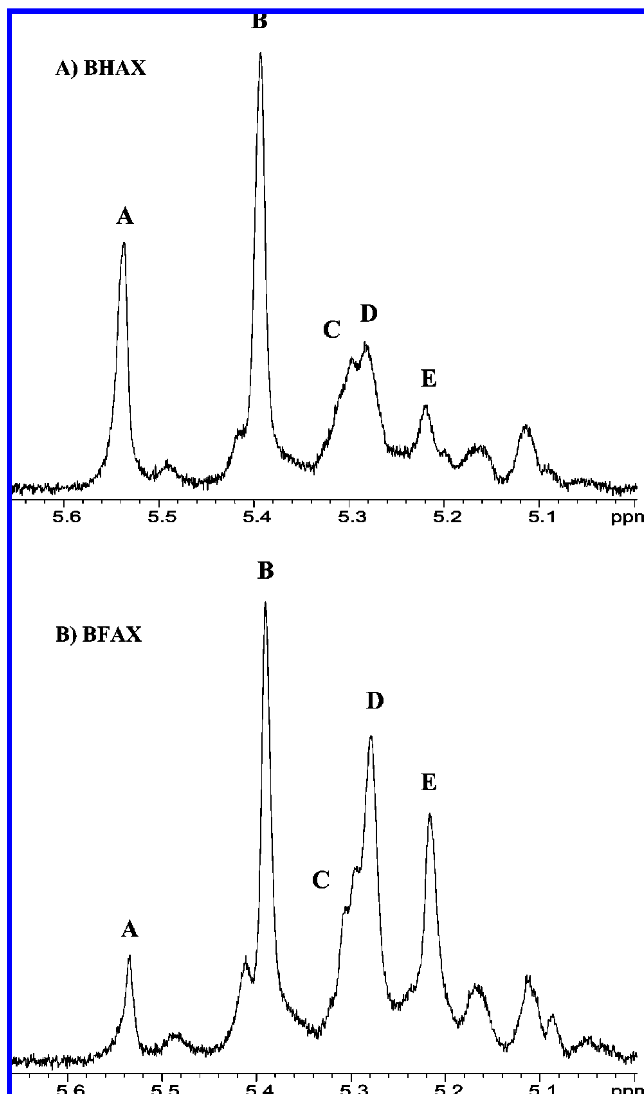

Figure 2. α-Anomeric region of ^{1}H NMR spectra of arabinoxylans from barley husks (BHAX) (**A**) and barley fiber (BFAX) (**B**) measured at 500 MHz in D_2O and referenced to internal acetone (2.225 ppm). The probe temperature was 50 °C. Peak assignation (A−E) is presented in **Table 4**.

Table 4. Chemical Shifts of Anomeric ^{1}H Resonances (ppm) of α-L-Ara*f* Units from Barley Husk Arabinoxylan (BHAX) and Barley Fiber Arabinoxylan (BFAX) Measured at 500 MHz in D_2O at 50 °C

sugar residue	peak label in **Figure 2**	BHAX	BFAX
β-D-Xyl*p*(1→2)-α-L-Ara*f*(1→3)[a]	A	5.54	5.53
α-L-Ara*f*(1→3) mono	B	5.39	5.39
α-L-Ara*f*(1→2) mono	C	5.30	5.30
α-L-Ara*f*(1→3) di	D	5.28	5.28
α-L-Ara*f*(1→2) di	E	5.22	5.23

[a] Disaccharide chain linked O-3 to monosubstituted Xyl*p*.

was lower than in BFAX. At the same time, the proportion of the disaccharide side chain found in both spectra at 5.54 ppm was lower in BFAX than in BHAX.

Enzyme-Assisted Structure Elucidation. Further information regarding arabinose substitution was obtained using specific enzymatic treatments with endo-(1→4)-β-D-xylanase followed by two α-L-arabinofuranosidases that differ in their substrate specificities. AXH-m liberates (1→2)- and (1→3)-linked α-L-Ara*f* residues from monosubstituted xylopyranosyl residues, whereas AXH-d3 acts solely on (1→3)-linked α-L-Ara*f* residues in disubstituted xylopyranosyl residues (*28, 29*). Shearzyme is a commercial endo-(1→4)-β-D-xylanase preparation that efficiently forms short-chain arabinoxylooligosaccharides from arabinoxylans (*21*). Treatment of BHAX and BFAX with Shearzyme resulted in the liberation of xylose, xylobiose, and longer oligosaccharides (**Figure 3A**,**D**). The main oligosaccharide peaks seen in the HPAEC-PAD chromatograms correspond to compounds with non- and monosubstituted xylose residues as oligosaccharides with disubstituted xylose residues have a much lower response factor in PAD and thus result in significantly smaller peaks.

Peak 6 with the retention time of 32 min in chromatograms A, C, D, and F may originate from trisaccharide α-L-Ara*f*-(1→3)-β-D-Xyl*p*-(1→4)-D-Xyl*p* or a tetrasaccharide consisting of three (1→4)-β-D-linked xylopyranosyl units with α-L-arabinofuranosyl substituent linked to the middle xylopyranosyl residue via (1→3) linkage (*21*). When samples after Shearzyme hydrolysis were further treated with AXH-m, a clear release of arabinose and disappearance of peak 6 with concominant formation of xylobiose and some xylotriose was detected (**Figure 3B**,**E**). Treatment of Shearzyme hydrolysate with AXH-d3 resulted in the liberation of arabinose but no clear disappearance of any oligosaccharide peak in the chromatogram (**Figure 3C**,**F**). Clearly more arabinose was formed from BFAX than from BHAX, indicating more double-substituted xylopyranosyl residues in the former. Peak 4 with the retention time of 29.5 min increased especially in chromatogram F. This peak most probably originates mainly from α-L-Ara*f*-(1→2)-β-D-Xyl*p*-(1→4)-D-Xyl*p*, thus indicating the action of AXH-d3 on diarabinosylxylobiose with a disubstituted xylopyranosyl residue.

Peak 5 at the retention time of 31 min found in all chromatograms, and thus not affected by either AXH, is presumably an oligosaccharide carrying a disaccharide side chain 2-*O*-β-D-Xyl*p*-α-L-Ara*f* attached to O-3 of xylopyranosyl residue. The intensity of peak 5, that is, the concentration of this oligosaccharide, is higher in BHAX hydrolysate than in BFAX hydrolysate, which is in accordance with the ^{1}H NMR data (**Figure 2**). ESI-MS analysis revealed that oligosaccharides remaining after Shearzyme and AXH-m treatments were mainly tetrasaccharides, as a strong signal at the position that corresponds to four pentose sugar units [$(M + Na)^+ = 569$, results not shown] was detected. Thus, peak 5 was tentatively identified as β-D-Xyl*p*-(1→2)-α-L-Ara*f*-(1→3)-β-D-Xyl*p*-(1→4)-D-Xyl*p*.

HPSEC Analysis. Pullulan was used to calibrate the combined laser light scattering and viscometric detector. The d*n*/d*c* value of pullulan in DMSO with 0.01 M LiBr, needed in the calibration procedure, was measured. The $dn/dc = 0.056 \pm 0.004$ mL/g was obtained from the slope of the linear fit as shown in **Figure 4**. The effect of LiBr salt at the concentration of 0.01 M in DMSO was studied by comparing the refractive indices of pure DMSO and DMSO containing salt. The difference between refractive indices was negligible, indicating that the refractive properties of both solvents were within the experimental error and that the d*n*/d*c* value defined in this study can also be used for pullulan in pure DMSO.

The HPSEC chromatograms of BHAX and BFAX differed clearly. The chromatograms of BHAX and BFAX had three different molecular populations that can be most clearly seen in RALS signal of BHAX sample, but the same segmentation pattern can also be seen in all other signals (**Figure 5**). The first molecular population eluted in the region of 12−14.5 mL, the second in 14.5−17 mL and the third in 17−21 mL. The second population was the prevailing one in both samples according to the RI signals. However, the intrinsic viscosity of this component is different between the two samples, which

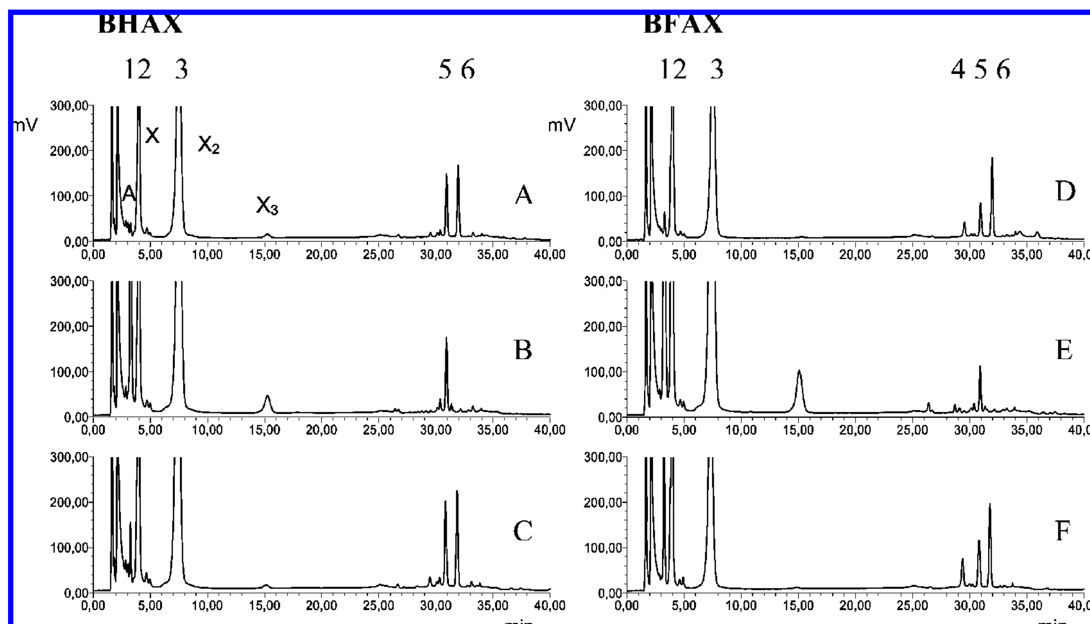

Figure 3. HPAEC-PAD chromatograms of oligosaccharides obtained from arabinoxylans from barley husks (BHAX) (**A**−**C**) and barley fiber (BFAX) (**D**−**F**) after sequential treatments with Shearzyme [endo-(1→4)-β-D-xylanase] and two α-L-arabinofuranosidases (AXH-m and AXH-d3): Shearzyme (**A**, **D**); Shearzyme followed by AXH-m (**B**, **E**); Shearzyme followed by AXH-d3 (**C**, **F**). Peak identification: 1, arabinose; 2, xylose; 3, xylobiose; 4, α-L-Ara*f*(1→2)-xylobiose; 5, β-D-Xyl*p*(1→2)-α-L-Ara*f*-xylobiose (tentative); 6, α-L-Ara*f*(1→3)-xylobiose.

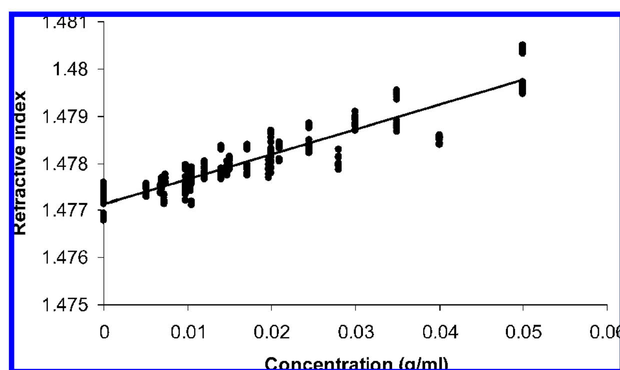

Figure 4. d*n*/d*c* plot for pullulan in DMSO with 0.01 M LiBr.

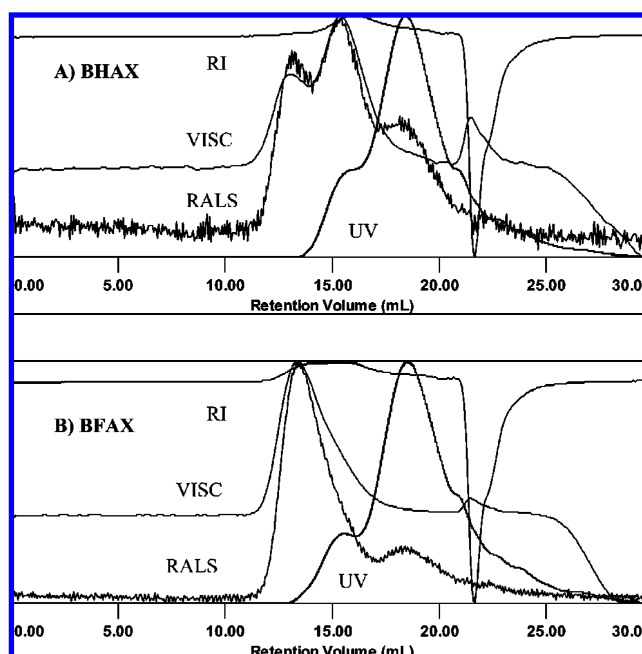

Figure 5. HPSEC chromatograms of arabinoxylans from barley husks (BHAX) (**A**) and barley fiber (BFAX) (**B**) analyzed in DMSO with 0.01 M LiBr. Signals: RI, refractive index; VISC, viscometer; RALS, right-angle light scattering; UV, ultraviolet.

can be seen in viscosity signals. The differences in intrinsic viscosities between the main polymeric components can also distinctly be perceived in the low molar mass region in the overlay figure of Mark−Houwink plot (**Figure 6**), where the conventional line represents the Mark−Houwink plot of BHAX and the dotted line that of BFAX.

The average molar masses were calculated using a light scattering detector (**Table 5**). The literature value of the d*n*/d*c* = 0.064 for xylans was used in the calculations. The sample recovery after the columns, based on an RI detector and given d*n*/d*c* value, was 62% for BHAX and 72% for BFAX. These values are in good agreement with the fact that both samples were not completely soluble in the HPSEC eluent. The average molar masses of BFAX were higher in comparison with BHAX. The M_w for BFAX was 81000 g/mol and that for BHAX 49300 g/mol. The molecular weight distributions of both arabinoxylans were broad, the polydispersity index being 3.3 for BHAX and 2.4 for BFAX. The intrinsic viscosity, hydrodynamic radius, and Mark−Houwink *a* value were slightly higher for BFAX than for BHAX.

DISCUSSION

The water-insoluble arabinoxylans from the husks and fiber of barley were isolated and their structures compared. The composition of barley husks and barley fiber, which are industrial side fractions and potential sources of arabinoxylans, were also analyzed. The special focus of this work was on the exploitation of HPSEC equipped with several detectors, including a light scattering detector and a viscometer, in the characterization of barley arabinoxylans.

Composition of Barley Fractions and Isolated Arabinoxylans. Barley husks primarily consist of cellulose, arabinoxylan, and lignin (*30*). Salomonsson et al. (*31*) analyzed the composition of five normal and high-lysine barley genotypes, including dehusked kernels and the husks. According to their studies,

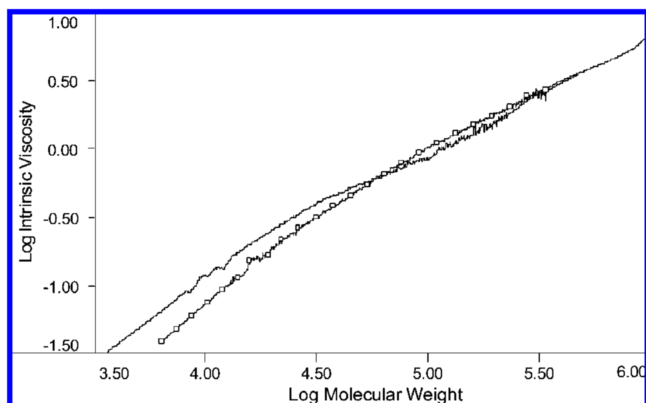

Figure 6. Mark−Houwink plots of arabinoxylans from barley husks (BHAX) and barley fiber (BFAX) (dotted line).

Table 5. HPSEC Results of Arabinoxylans from Barley Husks (BHAX) and Barley Fiber (BFAX)[a]

	BHAX	BFAX
M_w (g/mol)	49300	81000
M_n (g/mol)	15000	33700
M_w/M_n	3.3	2.4
[η] (dL/g)	0.45	0.73
R_h (nm)	6.0	8.9
a	0.75	0.89
sample recovery (%)	62	72

[a] Abbreviations: M_w, weight-average molar mass; M_n, number-average molar mass; M_w/M_n, polydispersity index; [η], intrinsic viscosity; R_h, hydrodynamic radius (determined with LS/viscometry method); *a*, Mark−Houwink exponent.

barley husks contain about 70% polysaccharides, 20% Klason lignin, 6% ash, and 5% crude protein, which corresponds well with our results. There was, however, one clear difference between the results of Salomonsson et al. and those of this study. Barley husks analyzed in this study contained a significant amount of starch (one-third of all the carbohydrates), which presumably originates from the starchy endosperm remains in the husk fraction after the industrial peeling process. The main components of barley kernel are starch (49.4−66.2%), dietary fiber (13.6−27.5%), and crude protein (9.3−12.9%) (*32*). BF material analyzed in the present study was obtained from a process in which water-soluble components, that is, starch and (1→3)(1→4)-β-D-glucans, were separated by wet-milling. BF thus mainly contained water-insoluble polysaccharides such as arabinoxylans and cellulose and proteins and lignin from the outer parts of the grain.

Barium hydroxide extraction was found to be specific toward arabinoxylans as reported earlier (*14*). Only a low amount of glucose-containing material, that is, starch, (1→3)(1→4)-β-D-glucan or cellulose, was coextracted. Arabinoxylan samples extracted from BF and BH represent well the arabinoxylans originally occurring on the cereal cell walls as indicated by the similar arabinose-to-xylose ratios of BH and BF (0.25 and 0.49) and the corresponding arabinoxylans BHAX and BFAX (0.28 and 0.51, respectively). The arabinose-to-xylose ratio of BHAX was somewhat higher than earlier reported for arabinoxylan from barley husks (0.20) (*9*), although Höije et al. (*8*) obtained a higher ratio of 0.37 for a BHAX sample that had been alkali-extracted after enzyme pretreatment. The arabinose-to-xylose ratio of BFAX was lower than reported for arabinoxylans from barley flours (0.72, 0.71) (*5, 6*) and for dehulled barley grains (*18*).

BFAX contained significantly more α-L-Ara*f* substituents than BHAX. According to ^{1}H NMR spectroscopy and enzyme-assisted HPAEC-PAD analysis, there is no significant difference in the content of monosubstituted α-L-Ara*f*-(1→3)-xylopyranosyl residues between BHAX and BFAX. Thus, the higher content of α-L-Ara*f* in BFAX mainly results from the greater amount of doubly substituted xylopyranosyl residues. Another clear difference identified by both ^{1}H NMR spectoscopy and enzyme-assisted HPAEC-PAD analysis was in the content of the longer substituent 2-*O*-β-D-Xyl*p*-α-L-Ara*f*, which was clearly higher in BHAX than in BFAX. This structural unit has previously been identified in arabinoxylan from corn cob (*33*), some milling fractions of rye (*34*), and barley husks (*24*), but not in arabinoxylan from barley grain. The content of xylopyranosyl residues carrying a monosubstituted α-L-Ara*f*-(1→2) substituent seemed low in both samples compared to contents obtained for barley and barley flours (*5, 6*).

HPSEC of Barley Arabinoxylans. HPSEC chromatograms of BHAX and BFAX differed distinctly from each other. In the BHAX sample, the proportion of the first eluting component is very low according to RI and viscosity signals. Actually that component probably has a higher intrinsic viscosity than the second component, but the viscometer signal is proportional to the concentration beside the intrinsic viscosity, which is the reason for such a low viscosity signal. BFAX sample contained more of that high-viscosity component (main viscosity peak) eluting before the component with the highest concentration. The Mark−Houwink plot shows that there is no density difference between the samples in high molar mass area (the area where the molar mass of the first component lies). By contrast, the BHAX sample has lower intrinsic viscosity in a wide range of lower molar mass, which indicates a slightly more compact structure of BHAX sample compared to the BFAX sample. In addition, the average intrinsic viscosity of BFAX was higher than that of BHAX, indicating the less dense conformation of BFAX over BHAX.

Especially the RALS signal in the BHAX chromatogram is trimodal. In some cases the prepeak in the light scattering signal before the peak from the main polymeric material may be an indication of aggregation. However, this was unlikely in the present study because of the strong signal from the viscometer. The peak eluted in the higher molar mass area could also result from starch impurities, but this is unlikely according to the low glucose level in both arabinoxylan samples. The UV signal at $\lambda_0 = 280$ nm appeared in both chromatograms in the low molar mass region. The signals were probably caused by lignin or protein impurities. Both lignins and proteins have a higher d*n*/d*c* value than that of arabinoxylan, which complicated the accurate determination of molar masses but was taken into consideration in the calculations.

The M_w for BHAX was 49300 g/mol and that for BFAX 81000 g/mol. The M_w of BHAX was thus slightly higher than recently published for barley husks using the DMSO system and universal calibration (34300−44700 g/mol) (*8*). An even lower weight-average molar mass has been calculated for alkaline-extracted oat spelt xylan (21900 g/mol) (*12*). The β-glucanase preparation, used in the process of Koskenkorva to enhance the separation of starch, may contain some xylanase side activity, which can have a reducing effect on the molar mass of BFAX. Molar mass data for alkali-extractable barley flour or whole grain could not be found in the literature. However, molar masses for water-soluble arabinoxylans of barley seemed to be higher, on the order of a few hundred thousand (*35*). The results obtained in this study demonstrate that light scattering and viscometry detections can be used to reliably calculate the molar mass of arabinoxylans.

The main difficulty in HPSEC analysis of arabinoxylans is their poor solubility. In HPSEC analyses of many polysaccharides reported in the literature, such as arabinoxylans, the time used for dissolving the polysaccharide sample is generally rather short. In many cases the dissolution time is only <2−3 h, which is, according to our preliminary dynamic light scattering (DLS) studies for pullulan in DMSO, too short a time for sufficient dissolution of a polysaccharide. Thus, turbidity of a polymer solution often is an indication of incomplete dissolution. In the present study, arabinoxylan samples were allowed to dissolve in DMSO containing 0.01 M LiBr at room temperature for 4 days prior HPSEC analyses. However, even after the 4 days of dissolution, both samples were still cloudy. Besides the incomplete dissolution, the filtration of samples for HPSEC may reduce the representativeness of the analyte under investigation. Losses most probably also occur during the filtration of rather viscous arabinoxylan samples.

Refractive Index Increments of Polysaccharides. The refractive index increments of many polysaccharides in commonly used SEC solvents have not been extensively studied. DMSO is a better solvent than water for polysaccharides such as dextrans and xylans due to the aggregation of polysaccharides in water (*36, 12*). In addition, DMSO is a strong hydrogen-bond acceptor. However, among the underivatized nonstarch polysaccharides, only the d*n*/d*c* values for dextrans and xylan in DMSO have been determined (*22, 37*). Basedow et al. investigated the dependence of the d*n*/d*c* of dextran on the wavelength of the light source, measuring the d*n*/d*c* value at four different wavelengths. The d*n*/d*c* of dextran in DMSO slightly decreased with increasing wavelength, being between 0.0760 and 0.0720 mL/g. Goring and Timell measured the d*n*/d*c* of wood 4-*O*-methylglucuronoxylan in DMSO at $\lambda_0 = 546$ nm, obtaining the value of 0.064 mL/g. Due to the relatively high refractive index of DMSO, d*n*/d*c* values in DMSO are generally rather low. Besides dextrans, pullulans are widely used as molecular mass standards in SEC, although their d*n*/d*c* values in DMSO have not previously been investigated. The d*n*/d*c* value obtained in this study (0.056 ± 0.004 mL/g) at $\lambda_0 = 633$ nm is in the same range as the d*n*/d*c* values of dextrans and wood xylan.

In conclusion, different polymeric arabinoxylans could be extracted from two industrial side-stream coproducts of barley. Extracted arabinoxylans were heterogeneous due to the unfractionated nature of barley husks and barley fiber. Both barley husk and fiber were rich in arabinoxylans and thus valuable sources for arabinoxylans.

ACKNOWLEDGMENT

Altia Corporation (Finland) is thanked for barley husks and fiber materials, Novozymes (Denmark) for α-L-arabinofuranosidase (AXH-m), and VTT (Finland) for the pullulan sample. Jaakko Arpiainen and Juha Simola and Jürgen Puls and Bodo Saake (BFH, the Federal Research Centre of Forestry and Forest Product in Hamburg, Germany) are thanked for help with the composition analyses of BH and BF. Michael Kurantz (U.S. Department of Agriculture, Philadelphia, PA) is acknowledged for protein analyses of BFAX and BHAX and Paul Clarke (Viscotek, U.K.) for valuable discussions regarding HPSEC analyses. Jouni Jokela and Matti Wahlsten are thanked for their help in the ESI-MS analyses.

LITERATURE CITED

(1) Aspinall, G. O. Structural chemistry of the hemicelluloses. *Adv. Carbohydr. Chem.* **1959**, *14*, 429–468.

(2) Aspinall, G. O. Chemistry of cell wall polysaccharides. *Biochem. Plants* **1980**, *3*, 473–500.

(3) Henry, R. J. Pentosan and (1→3), (1→4)-β-glucan concentrations in endosperm and wholegrain of wheat, barley, oats and rye. *J. Cereal Sci.* **1987**, *6*, 253–258.

(4) McNeil, M.; Albersheim, P.; Taiz, L.; Jones, R. L. Structure of plant cell walls. VII. Barley aleurone cells. *Plant Physiol.* **1975**, *55*, 64–68.

(5) Viëtor, R. J.; Angelino, S. A. G. F.; Voragen, A. G. J. Structural features of arabinoxylans from barley and malt cell wall material. *J. Cereal Sci.* **1992**, *15*, 213–222.

(6) Trogh, I.; Croes, E.; Courtin, C. M.; Delcour, J. A. Enzymic degradability of hull-less barley flour alkali-solubilized arabinoxylan fractions by endoxylanases. *J. Agric. Food Chem.* **2005**, *53*, 7243–7250.

(7) Garrote, G.; Dominguez, H.; Parajó, J. C. Production of substituted oligosaccharides by hydrolytic processing of barley husks. *Ind. Eng. Chem. Res.* **2004**, *43*, 1608–1614.

(8) Höije, A.; Gröndahl, M.; Tømmeraas, K.; Gatenholm, P. Isolation and characterization of physicochemical and material properties of arabinoxylans from barley husks. *Carbohydr. Polym.* **2005**, *61*, 266–275.

(9) Aspinall, G. O.; Ferrier, R. J. The constitution of barley-husk hemicellulose. *J. Chem. Soc.* **1957**, 4188–4194.

(10) Izydorczyk, M. S.; Macri, L. J.; MacGregor, A. W. Structure and physicochemical properties of barley non-starch polysaccharides. II. Alkali-extractable β-glucans and arabinoxylans. *Carbohydr. Polym.* **1998**, *35*, 259–269.

(11) Sarbu, A.; Goncalves, F.; Norberta de Pinho, M. Oat spelts xylan molecular mass estimation by size exclusion chromatography. *Carbohydr. Polym.* **2003**, *53*, 297–304.

(12) Saake, B.; Kruse, T.; Puls, J. Investigation on molar mass, solubility and enzymatic fragmentation of xylans by multi-detected SEC chromatography. *Bioresour. Technol.* **2001**, *80*, 195–204.

(13) Fishman, M. L.; Doner, L. W.; Chau, H. K.; Hoagland, P. D. Characterization of hemicellulose B from corn fiber by high-performance size-exclusion chromatography with on-line molar mass and viscometric detection. *Int. J. Polym. Anal. Charact.* **2000**, *5*, 359–379.

(14) Gruppen, H.; Hamer, R. J.; Voragen, A. G. J. Barium hydroxide as a tool to extract pure arabinoxylans from water-insoluble cell wall material of wheat flour. *J. Cereal Sci.* **1991**, *13*, 275–290.

(15) Kratochvíl, P. *Classical Light Scattering from Polymer Solutions*; Elsevier Science Publishers: Amsterdam, The Netherlands, 1987.

(16) Virkki, L.; Maina, H. N.; Johansson, L.; Tenkanen, M. New enzyme-based method for analysis of water-soluble wheat arabinoxylans. *Carbohydr. Res.* **2007**, *343*, 521–529.

(17) Puls, J. Substrate analysis of forest and agricultural wastes. In *Bioconversion of Forest and Agricultural Plant Residues*; Saddler, J. N., Ed.; CAB International: Wallingford, U.K., 1993; Vol. 9, pp 13−32.

(18) Virkki, L.; Johansson, L.; Ylinen, M.; Maunu, S.; Ekholm, P. Structural characterization of water-insoluble nonstarchy polysaccharides of oats and barley. *Carbohydr. Polym.* **2005**, *59*, 357–366.

(19) Sundberg, A.; Sundberg, K.; Lillandt, C.; Holmbom, B. Determination of hemicelluloses and pectins in wood and pulp fibers by acid methanolysis and gas chromatography. *Nord. Pulp Pap. Res. J.* **1996**, *11*, 216–219, 226.

(20) American Association of Cereal Chemists. *Approved Methods of the AACC*, 9th ed.; AACC: St. Paul, MN, 1995; method 46-30.

(21) Rantanen, H.; Virkki, L.; Tuomainen, P.; Kabel, M.; Schols, H.; Tenkanen, M. Preparation of arabinoxylobiose from rye xylan using family 10 *Aspergillus aculeatus* endo-1,4-β-D-xylanase. *Carbohydr. Polym.* **2007**, *68*, 350–359.

(22) Goring, D. A. I.; Timell, T. E. Molecular properties of six 4-*O*-methylglucuronoxylans. *J. Phys. Chem.* **1960**, *64*, 1426–1430.

(23) Gruppen, H.; Hoffmann, R. A.; Kormelink, F. J.; Voragen, A. G.; Kamerling, J. P.; Vliegenthart, J. F. Characterisation by 1H NMR spectroscopy of enzymically derived oligosaccharides from alkali-

extractable wheat-flour arabinoxylan. *Carbohydr. Res.* **1992**, *233*, 45–64.

(24) Hoffmann, R. A.; Kamerling, J. P.; Vliegenthart, J. F. G. Structural features of a water-soluble arabinoxylan from the endosperm of wheat. *Carbohydr. Res.* **1992**, *226*, 303–311.

(25) Hoffmann, R. A.; Leeflang, B. R.; de Barse, M. M. J.; Kamerling, J. P.; Vliegenthart, J. F. G. Characterisation by ^{1}H-n.m.r. spectroscopy of oligosaccharides, derived from arabinoxylans of white endosperm of wheat, that contain the elements →4)[α-L-Ara*f*-(1→3)]-*β*-D-Xyl*p*-(1→ or →4)[α-L-Ara*f*-(1→2)][α-L-Ara*f*-(1→3)]-*β*-D-Xyl*p*-(1→. *Carbohydr. Res.* **1991**, *221*, 63–81.

(26) Viëtor, R. J.; Hoffmann, R. A.; Angelino, S. A. G. F.; Voragen, A. G. J.; Kamerling, J. P.; Vliegenthart, J. F. G. Structures of small oligomers liberated from barley arabinoxylans by endoxylanase from *Aspergillus awamori*. *Carbohydr. Res.* **1994**, *254*, 245–255.

(27) Höije, A.; Sandström, C.; Roubroeks, J. P.; Andersson, R.; Gohil, S.; Gatenholm, P. Evidence of the presence of 2-*O*-*β*-D-xylopyranosyl-α-L-arabinofuranose side chains in barley husk arabinoxylan. *Carbohydr. Res.* **2006**, *341*, 2959–2966.

(28) Van den Broek, L. A. M.; Lloyd, R. M.; Beldman, G.; Verdoes, J. C.; McCleary, B. V.; Voragen, A. G. J. Cloning and characterization of arabinoxylan arabinofuranohydrolase-D3 (AXHd3) from *Bifidobacterium adolescentis* DSM20083. *Appl. Microbiol. Biotechnol.* **2005**, *67*, 641–647.

(29) Van Laere, K. M. J.; Voragen, C. H. L.; Kroef, T.; Van den Broek, L. A. M.; Beldman, G.; Voragen, A. G. J. Purification and mode of action of two different arabinoxylan arabinofuranohydrolases from *Bifidobacterium adolescentis* DSM 20083. *Appl. Microbiol. Biotechnol.* **1999**, *51*, 606–613.

(30) Viëtor, R. J. Structural characteristics of arabinoxylans from barley, malt and wort. Ph.D. dissertation, University of Wageningen, The Netherlands, 1992.

(31) Salomonsson, A.-C.; Theander, O.; Åman, P. Composition of normal and high-lysine barleys. *Swed. J. Agric. Res.* **1980**, *10*, 11–16.

(32) Oscarsson, M.; Andersson, R.; Salomonsson, A.-C.; Åman, P. Chemical composition of barley samples focusing on dietary fiber components. *J. Cereal Sci.* **1996**, *24*, 161–170.

(33) Kusakabe, I.; Ohgushi, S.; Yasui, T.; Kobayashi, T. Studies on the xylanase system of *Streptomyces*. Part X. Structures of the arabinoxylo-oligosaccharides from the hydrolytic products of corncob arabinoxylan by a xylanase from *Streptomyces*. *Agric. Biol. Chem.* **1983**, *47*, 2713–2723.

(34) Nilsson, M.; Saulnier, L.; Andersson, R.; Åman, P. Water unextractable polysaccharides from three milling fractions of rye grain. *Carbohydr. Polym.* **1996**, *30*, 229–237.

(35) Dervilly, G.; Leclercq, C.; Zimmermann, D.; Roue, C.; Thibault, J. F.; Saulnier, L. Isolation and characterization of high molar mass water-soluble arabinoxylans from barley and barley malt. *Carbohydr. Polym.* **2001**, *47*, 143–149.

(36) Merienne, S.; Busnel, J.-P.; Fricoteaux, F.; Prudhomme, J.-C. Size exclusion chromatography of dextrans in DMSO as eluent. *J. Liq. Chromatogr. Relat. Technol.* **2000**, *23*, 1745–1756.

(37) Basedow, A. M.; Ebert, K. H.; Ruland, U. Specific refractive index increments of dextran fractions of different molecular weights. *Makromol. Chem.* **1978**, *179*, 1351–1353.

Received for review January 31, 2008. Revised manuscript received April 14, 2008. Accepted April 24, 2008. The University of Helsinki Research Funds, Danisco Foundation, and CostD29 (Short-term Scientific Mission) are gratefully acknowledged for their financial support of this study.

JF800315Q