Studies on Rye (*Secale cereale* L.) Lines Exhibiting a Range of Extract Viscosities. 1. Composition, Molecular Weight Distribution of Water Extracts, and Biochemical Characteristics of Purified Water-Extractable Arabinoxylan

S. M. Ragaee,[†] G. L. Campbell,[‡] G. J. Scoles,[§] J. G. McLeod,[#] and R. T. Tyler*[,†]

Department of Applied Microbiology and Food Science, Department of Animal and Poultry Science, and Department of Plant Sciences, University of Saskatchewan, 51 Campus Drive, Saskatoon, Saskatchewan, Canada S7N 5A8; and Research Branch, Agriculture and Agri-Food Canada, P.O. Box 1030, Swift Current, Saskatchewan, Canada S9H 3X2

Five rye lines exhibiting a wide range of extract viscosities, along with commercial cultivars of rye and wheat, were compared with respect to their physical and chemical properties. Rye wholemeals contained significantly higher concentrations of total and soluble dietary fiber (TDF and SDF, respectively), total and water-extractable arabinoxylan (TAX and WEAX, respectively), and β-glucan than did wheat. Significant positive correlations were obtained between rye wholemeal extract viscosity and SDF content ($r = 0.90$, $p < 0.05$) and WEAX content ($r = 0.89$, $p < 0.05$). Gel permeation chromatography (GPC) of water extracts of rye wholemeals revealed the presence of a high molecular weight fraction (HMWF), which was found in higher concentration in the ryes than in wheat. A significant positive correlation ($r = 0.84$, $p < 0.05$) was observed between HMWF content (expressed as a proportion of the total carbohydrate in water extracts) and extract viscosity of rye wholemeals. Treatment of a rye wholemeal extract with xylanase, followed by GPC, indicated that the HMWF consisted primarily of WEAX. Successive treatment of a rye wholemeal extract with α-amylase, lichenase, protease, and xylanase confirmed that the viscosity of the extract was primarily related to its content of WEAX. WEAX was isolated from high, intermediate, and low extract viscosity ryes. Structural differences were observed among the three arabinoxylans using H NMR and high-pressure size exclusion chromatography with triple detection. The WEAX from high extract viscosity rye was a higher molecular weight macromolecule exhibiting a higher intrinsic viscosity, a larger radius of gyration, a larger hydrodynamic radius, and a lower degree of branching compared to WEAX from low and intermediate extract viscosity ryes.

Keywords: *Rye; extract viscosity; water-extractable arabinoxylan; gel permeation chromatography; H NMR; size exclusion high-pressure liquid chromatography; triple detector system*

INTRODUCTION

Cereal grains contain 5−15% of cell wall material (*1*). This fibrous component influences the processing of cereal grains and their end-use quality. The principal polysaccharide constituents of the cell wall in rye are arabinoxylan (at 7−12% of the kernel), β-glucan (1−2%), and cellulose (1−2%) (*2, 3*). Arabinoxylan consists of a linear backbone of (1→4)-β-D-xylopyranosyl (Xylp) units, to which α-L-arabinofuranosyl (Araf) substituents are attached through O-2, O-3, or O-2,3 linkages (*4*). The cell wall of wheat also contains arabinoxylan but at a lower concentration than rye (*5*). A primary distinction between rye and other cereals is that rye contains a higher percentage of water-extractable arabinoxylan (WEAX) that causes high viscosity when dispersed in water. Viscosity is affected by the molecular size, shape, and charge of the heterogeneous arabinoxylan (*6*).

Various studies have attributed the viscosity of rye to its content of WEAX (*7*−*9*). In other studies, genetic variation in rye extract viscosity was found to be closely related to the proportion of a high molecular weight fraction (HMWF) in the WEAX (*10*). Further research resulted in the development of rye lines having a wide range of extract viscosities, which has provided the base materials for studying the impact of viscosity in model systems (*11, 12*).

Several molecular structures have been proposed for different cereal arabinoxylans, including water-extractable wheat endosperm arabinoxylan (*13*), alkali-extractable wheat endosperm arabinoxylan (*14*), and alkali-extractable barley endosperm arabinoxylan (*15*). For rye endosperm arabinoxylan, Bengtsson et al. (*16*) proposed a distinct model involving either two types of arabinoxylan polymers or two distinct regions in the rye arabinoxylan molecule. The major polymer structure (arabinoxylan I) had a xylan chain substituted exclusively at O-3 of Xylp with Araf groups, whereas the minor polymer (arabinoxylan II) contained disubstituted O-2,3 Xylp residues. The successful separation of a minor fraction containing only un- and disubstituted Xylp residues by Vinkx et al. (*9*) favors the existence of

* Author to whom correspondence should be addressed [telephone (306) 966-5024; fax (306) 966-8898; e-mail tyler@duke.usask.ca].

[†] Department of Applied Microbiology and Food Science.

[‡] Department of Animal and Poultry Science.

[§] Department of Plant Sciences.

[#] Agriculture and Agri-Food Canada.

10.1021/jf001227g CCC: $20.00
Published on Web 04/06/2001

two separate polymers in rye arabinoxylan. Moreover, the flexibility of less substituted arabinoxylan might permit intermolecular alignment over short sequences of continuously unsubstituted Xylp residues, which would lead to the formation of H-bond-stabilized macrostructures. Chain conformation and intermolecular associations were found to have a direct bearing on certain physical and functional properties of these macromolecules (*6*).

In the current study, the compositions of rye wholemeals differing in extract viscosity were determined, as were the molecular weight distributions of their water extracts. Commercial cultivars of rye and wheat were included in the study for comparative purposes. Enzyme treatment was employed to confirm the contribution of WEAX to rye viscosity. Because the fine structure of arabinoxylan is of fundamental importance in determining its physicochemical properties, WEAX was isolated from high, intermediate, and low extract viscosity ryes. Structural information on the WEAX was obtained by proton nuclear magnetic resonance spectroscopy (H NMR) and by size exclusion high-pressure chromatography (HPSEC) with triple detection (refractive index, differential pressure, and light scattering detectors).

MATERIALS AND METHODS

Materials. Five rye (*Secale cereale* L.) lines exhibiting a range of extract viscosities (5−95 cP) were used in this study. Low (R5) and high (R95) extract viscosity spring ryes were grown at Saskatoon, SK, in 1997, and low (R10) and intermediate (R19 and R30) extract viscosity fall ryes were grown at Swift Current, SK, in 1996−1997. Each line was designated by the corresponding extract viscosity of the wholemeal (R = rye, number = extract viscosity in centipoise). Commercial samples of fall rye (cv. Prima) and hard red spring wheat (*Triticum aestivum* L. cv. CDC Teal) were obtained locally. Grains were ground using a Cyclone sample mill (UDY Corp., Fort Collins, CO) to pass a 0.5 mm screen prior to analysis. Moisture content of wholemeals and flours was determined according to AACC Method 44-15A (*17*).

Determination of Total and Soluble Dietary Fiber, β-Glucan, and Arabinoxylan. Total and soluble dietary fiber (TDF and SDF, respectively) contents of the wholemeals were determined according to the AOAC (*18*) procedure. β-Glucan content was determined using the enzymatic method of McCleary and Glennie-Holmes (*19*). Arabinose and xylose were determined in the wholemeals and water extracts after hydrolysis with sulfuric acid and derivatization of the sugars to their alditol acetates (*20*). The derivatized sugars were quantified on a Hewlett-Packard model 5880A gas−liquid chromatograph equipped with a fused silica DB-23 capillary column (30 m length and 0.25 mm i.d.) (J&W Scientific, Folsom, CA) and a flame ionization detector. The injector, column, and detector were maintained at 275, 230, and 300 °C, respectively. A standard mixture of arabinose, xylose, mannose, galactose, and glucose (Sigma Chemical Co., St. Louis, MO) was prepared for calibration; β-D-allose was employed as internal standard. Total and WEAX contents were estimated by summation of the arabinose and xylose contents (× 0.88) of the wholemeals and water extracts, respectively. All analyses were performed in duplicate, at least.

Viscosity Measurement. Heated (130 °C, 90 min) wholemeal was slurried in deionized water (1:5 flour/water, w/v) on a magnetic stirrer (6.5 rpm, 90 min, 25 °C) and then centrifuged (3000*g*, 10 min). The viscosity of the supernatant was determined using a Brookfield cone-plate viscometer (model LVTDCP-11, Brookfield Engineering Laboratories Inc., Stoughton, MA) equipped with spindle CP-40 and maintained at 25 °C.

Molecular Weight (MW) Distribution. Heated (130 °C, 90 min) rye and wheat wholemeals were extracted with water (as described above) but at a meal-to-water ratio of 1:10 (w/v). The mixtures were centrifuged (3000*g*, 10 min), and the supernatant was then filtered through 0.45 μm disposable filters (Millipore-HA, Millipore Co., Bedford, MA); 0.5 mL of the filtrate was loaded on a gel permeation column (Econo column 100 cm × 1.5 cm, Bio-Rad, Mississauga, ON) containing Sephacryl S-500 (Pharmacia, Uppsala, Sweden). The sugars were eluted with 0.3% NaCl at a flow rate of 12 mL/h. Fractions (4 mL) were collected, and an aliquot of each fraction was analyzed for total sugar content by using the phenol−sulfuric acid method (*21*). The column was calibrated with standard dextrans from *Leuconostoc mesenteroides* strain 13-512 (average MWs of approximately 10×10^3, 20×10^3, 126×10^3, 300×10^3, 575×10^3, and 4×10^6) (Sigma). Sugar content was calculated as glucose for dextran standards and as pentose for rye extracts. The content of a HMWF in each extract was expressed as a percentage of the total carbohydrate applied to the column.

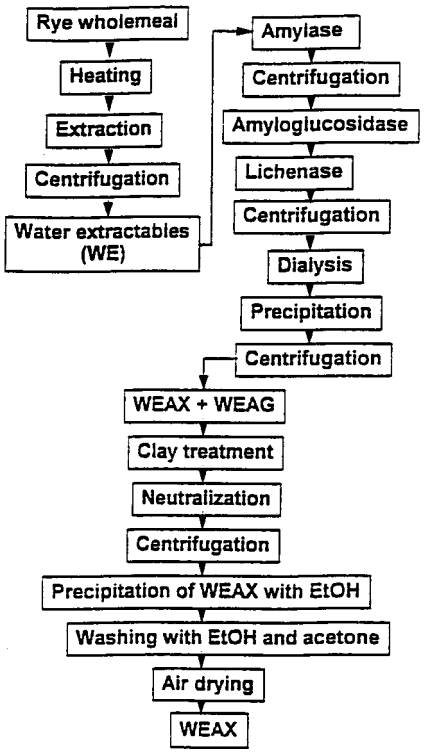

Figure 1. Procedure for isolation of WEAX.

Rye Extract Viscosity As Affected by α-Amylase, Protease, Lichenase, and Xylanase Treatment. High extract viscosity rye (R95) wholemeal was heated and extracted with water (1:10, w/v) as described previously. Several aliquots (1 mL) of the extract were incubated (in triplicate) with each enzyme (30 μL) at the optimum pH and temperature for each enzyme. Enzyme treatments were applied according to the method of Bhatty et al. (*22*) except that xylanase incubation times were 2, 4, 6, 8, 10, and 60 min; incubation times for the other enzymes were 10, 20, 30, 40, 50, and 60 min. The viscosities of all extracts were determined using a Brookfield cone-plate viscometer as described previously.

Xylanase Treatment of the HMWF. Low extract viscosity rye (R5) wholemeal was heated and extracted with water (1:10, w/v) as described previously. The extract was dialyzed against deionized water (12 h, 40 °C) using membrane tubing (MW cutoff of 6000−8000; Spectra Medical Industries Inc., Los Angeles, CA) to eliminate low molecular weight components. A 30 μL aliquot of the xylanase preparation used previously was added to 1.0 mL of the dialyzed extract and incubated for 15 min at 40 °C. The extract was filtered, and 0.5 mL of the supernatant was subjected to GPC as described previously. Samples of the untreated extract, before and after dialysis, were also filtered, and the supernatants were subjected to GPC.

Table 1. Extract Viscosity, TDF, TAX, WEAX, and β-Glucan Concentrations (Percent of Dry Matter) of Wholemeals from Experimental Ryes and from Commercial Cultivars of Rye and Wheat

	extract viscosity[a] (cP)	TDF (%)	SDF (%)	TAX (%)	WEAX (%)	WEAX (% of TAX)	β-glucan (%)	HMWF[b] (%)
				Experimental Ryes[c]				
R95	95.0 ± 1.9[d]	17.0 ± 0.1	5.2 ± 0.0	7.3 ± 0.0	2.5 ± 0.02	33.7	1.9 ± 0.01	38.7 ± 0.5
R30	30.0 ± 1.1	16.2 ± 0.3	4.0 ± 0.0	6.8 ± 0.1	2.0 ± 0.02	30.1	2.5 ± 0.01	34.2 ± 0.4
R19	19.0 ± 0.9	16.8 ± 0.5	4.6 ± 0.1	6.7 ± 0.2	2.0 ± 0.01	30.0	1.9 ± 0.06	32.0 ± 1.3
R10	9.9 ± 0.2	14.7 ± 0.2	4.0 ± 0.0	5.6 ± 0.0	1.3 ± 0.01	23.4	2.0 ± 0.01	20.9 ± 0.4
R5	4.9 ± 0.2	16.5 ± 0.2	3.8 ± 0.2	6.7 ± 0.0	1.5 ± 0.01	22.1	1.8 ± 0.08	20.1 ± 0.5
				Commercial Cultivars				
rye	12.5 ± 0.3	15.2 ± 0.1	4.1 ± 0.2	7.0 ± 0.2	1.8 ± 0.01	25.2	2.1 ± 0.08	27.5 ± 0.6
wheat	2.3 ± 0.1	11.6 ± 0.2	1.4 ± 0.1	4.9 ± 0.1	1.0 ± 0.02	20.3	0.6 ± 0.01	16.4 ± 0.0

[a] Wholemeal-to-water ratio of 1:5 (w/v). [b] The proportion of the total carbohydrate loaded on the column represented by the high molecular weight fraction. [c] Each line is designated R (rye) followed by its wholemeal extract viscosity (in cP). [d] Mean ± standard deviation ($n = 3$).

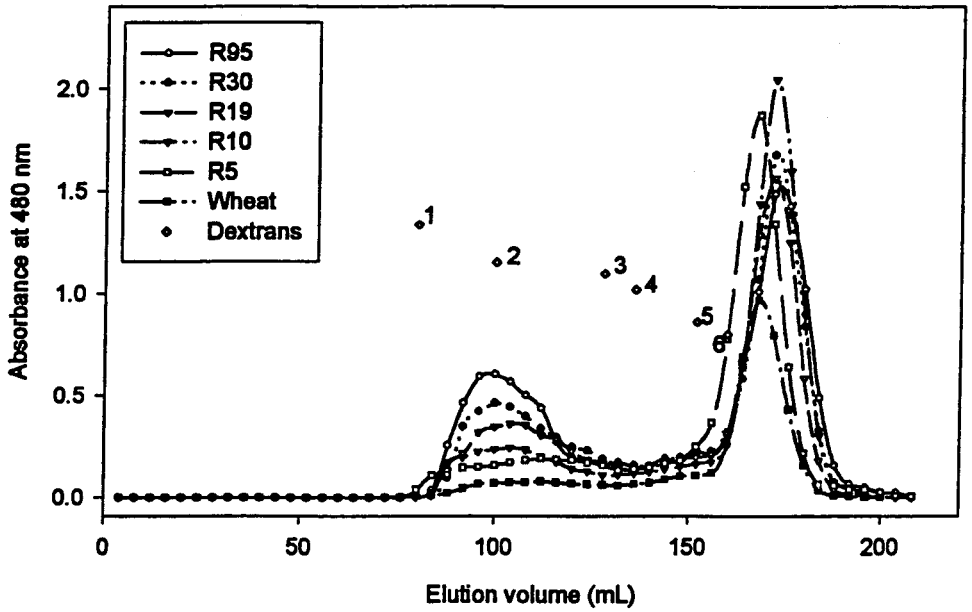

Figure 2. MW distributions of water extracts (1:10 w/v) of wholemeals from experimental ryes and wheat determined by GPC using Sephacryl S-500. Each rye line is designated R (rye) followed by its wholemeal extract viscosity (in cP). Dextran standard MW values (approximate): 1, 4×10^6; 2, 575×10^3; 3, 300×10^3; 4, 126×10^3; 5, 20×10^3; 6, 10×10^3.

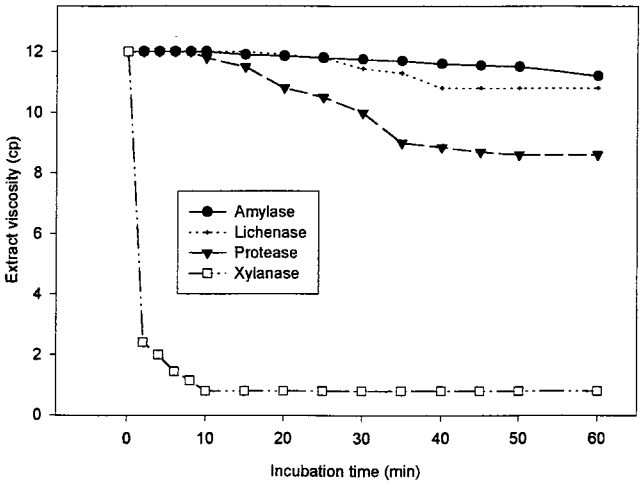

Figure 3. Effect of amylase, lichenase, protease, and xylanase treatment on the viscosity of a water extract from R95 rye wholemeal. The ratio of wholemeal to water was 1:10 (w/v).

Isolation of WEAX. The procedure used for the preparation of water extractables (WE) and purified WEAX is presented schematically in Figure 1. Wholemeals from high (R95), intermediate (R30), and low (R5) extract viscosity ryes were heated (130 °C, 90 min) and then extracted with water (1:10, w/v) as described previously. The extracts were incubated with α-amylase (1.2 mL, 2900 units/mL) (Sigma) at 90 °C for 30 min to hydrolyze contaminating starch and to denature protein. The mixture was cooled (25 °C) and centrifuged (3000*g*, 15 min), and the supernatant was collected and then incubated with amyloglucosidase (0.3 mL, 200 units/mL) (Sigma) at 60 °C for 12 h. The mixture was cooled (25 °C) and then recentrifuged (3000*g*, 20 min). The supernatant was incubated with lichenase (1.0 mL, 50 units/mL) (*β*-glucan kit, Biocon, Lexington, KY) for 2 h at 40 °C before dialysis (48 h, 4 °C) using membrane tubing (as described previously) to eliminate low molecular weight components. The dialyzed supernatant was treated with a clay suspension (montmorillonite, Sigma) to remove protein (15:1 clay-to-protein ratio, w/w). The pH of the supernatant was adjusted to 3.0 before stirring for 30 min on a magnetic stirrer (as described previously). The suspension was neutralized (pH 7.0) and centrifuged (10000*g*, 30 min) to remove the clay−protein complex. Ethanol (95%) was added (to a final ethanol concentration of 80%), and the solution was stirred for 30 min (as described previously), left overnight at 4 °C, and centrifuged (10000*g*, 30 min). The sediment (predominantly WEAX) was resolubilized in water (300 mL), and WEAX was separated from water-extractable arabinogalactan (WEAG) by ethanol precipitation (final ethanol concentration of 65%). The precipitated WEAX was recovered by centrifugation (10000*g*, 30 min), washed with ethanol and acetone, and then air-dried.

Composition and MW Distribution of the Isolated WEAX. Purified WEAX was analyzed for moisture (vacuum oven, 60 °C, 7 h), ash [AACC Method 08-30 (*17*)], *β*-glucan (described previously), starch (*23*), and protein (*24*) using bovine serum albumin as a reference protein. Monosaccharide compositions of the hydrolyzed WEAX and viscosities of 1% aqueous solutions of the WEAX were determined as described previously. Purified WEAX was solubilized in water at 4 °C

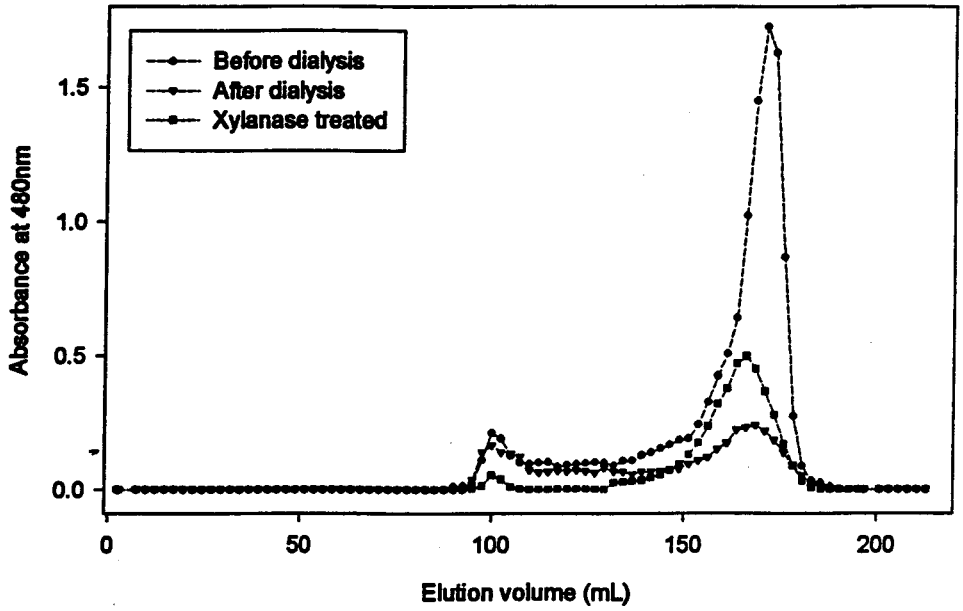

Figure 4. MW distribution of a water extract from R5 rye at a wholemeal-to-water ratio of 1:10 (w/v) before dialysis, after dialysis, and after dialysis and xylanase treatment.

at an arabinoxylan/water ratio of 0.1:100 (w/v), filtered, and subjected to GPC as described previously.

H NMR. Pure WEAX from high (R95), intermediate (R30), and low (R5) extract viscosity ryes was dissolved in D_2O (99.9%) with stirring (120 min, 4 °C), followed by lyophilization. This step was repeated to remove extraneous H signals from the spectrum. The deuterium-exchanged dry material was finally dissolved in D_2O (2.0 mg/mL). H NMR spectra were recorded on a 500 MHz instrument (Bruker Analytic GmbH, Rheinstetten/Karlsruhe, Germany) at 67 °C. The pulse repetition time was 0.01 s, with the number of scans varying from 100 to 500. Chemical shifts were referenced to an acetone internal standard (δ 2.2).

HPSEC with Triple Detection. Pure WEAX from high (R95), intermediate (R30), and low (R5) extract viscosity ryes was analyzed using a size exclusion high-pressure liquid chromatograph equipped with a Mix Bed column (VisoGel GMPWXL, Viscotek Co., Houston, TX) and a triple detector system (Viscotek model T60). The triple detector system consisted of refractive index, laser light scattering photometer, and viscometer (differential pressure) detectors connected in series. The column and the triple-detector system were maintained at ambient temperature (~25 °C). The light scattering angle was 90°, and the laser wavelength was 670 nm. Samples were dissolved in 0.05 M phosphate buffer (pH 7.0) at a concentration of 0.15%. The injection volume was 78 μL, and the samples were eluted with 0.1 M $NaNO_3$ at a flow rate of 0.5 mL/min. On-line detection of the radius of gyration (R_g) and the hydrodynamic radius (R_h) (*25*) were achieved using the light scattering photometer. A Mark−Houwink plot (*26*, *27*) was produced by plotting the logarithm of intrinsic viscosity versus the logarithm of MW.

RESULTS AND DISCUSSION

Extract Viscosity, TDF, SDF, TAX, WEAX, and β-Glucan. All rye wholemeals were higher in TDF, SDF, TAX, WEAX, and β-glucan than wheat wholemeals (Table 1). Åman et al. (*28*) reported similar results for TDF (15.9% in rye and 11.1% in wheat), TAX (9.1 and 6.0%), and β-glucan (1.8 and 0.8%). Extract viscosity of rye was weakly correlated with TDF and TAX contents and strongly correlated with SDF ($r = 0.90$, $p < 0.05$) and WEAX ($r = 0.89$, $p < 0.05$) contents. No significant association was evident between extract viscosity and β-glucan content of the rye lines. Wheat exhibited very low viscosity values. When WEAX was calculated as a percentage of TAX (Table 1), the differences among ryes were more obvious. The results support the view that WEAX determines rye extract viscosity (*7*−*9*).

Table 2. Yield, Viscosity, and Composition[a] of WEAX Isolated from High (R95), Intermediate (R30), and Low (R5) Extract Viscosity Ryes (Percent Dry Basis)

	R95[b]	R30	R5
yield (g/100 g of meal)	1.6 ± 0.0[c]	1.5 ± 0.0	0.7 ± 0.0
viscosity (cP) of 1% solution	566.0 ± 2.8	76.0 ± 0.2	32.4 ± 0.3
xylose	62.8 ± 0.6	66.9 ± 0.4	63.2 ± 0.6
arabinose	37.9 ± 0.2	36.7 ± 0.4	38.1 ± 0.2
arabinoxylan[d]	88.6 ± 0.3	91.2 ± 0.4	89.2 ± 0.4
protein	4.3 ± 0.1	3.8 ± 0.0	4.1 ± 0.1
ash	1.7 ± 0.1	0.8 ± 0.0	1.1 ± 0.0
A/X[e]	0.60	0.55	0.60

[a] Arabinose and xylose were determined in hydrolyzed isolates. [b] Each line is designated R (rye) followed by its wholemeal extract viscosity (in cP). [c] Mean ± standard deviation ($n = 3$). [d] Arabinoxylan = (arabinose + xylose) × 0.88. [e] Arabinose-to-xylose ratio.

GPC of Rye and Wheat Wholemeal Water Extracts. Several studies have indicated that variation in rye extract viscosity was more closely related to the MW distribution of WEAX (*9*, *10*, *29*) than to the quantity of WEAX present. In this study, water extracts from rye and wheat wholemeals were fractionated on Sephacryl S-500 (Figure 2). An HMWF eluted between 75 and 140 mL, corresponding to a weighted average MW of 500000. A low molecular weight fraction (weighted average MW of 12000) eluted between 140 and 200 mL. The HMWF was confirmed by GLC to contain only arabinose and xylose, whereas the low molecular weight fraction contained arabinose and xylose in addition to other sugars (results not shown). The proportion of the total sugars loaded on the column represented by the HMWF (%HMWF) varied among samples (20−39%), as shown in Table 1. The viscosity of the water extracts was strongly correlated ($r = 0.84$, $p < 0.05$) with %HMWF in the extract. The %HMWF in the wheat extract was low compared to that in the rye extracts.

Enzyme Treatment of Rye Extracts. Treatment of the water extract from R95 with either α-amylase or

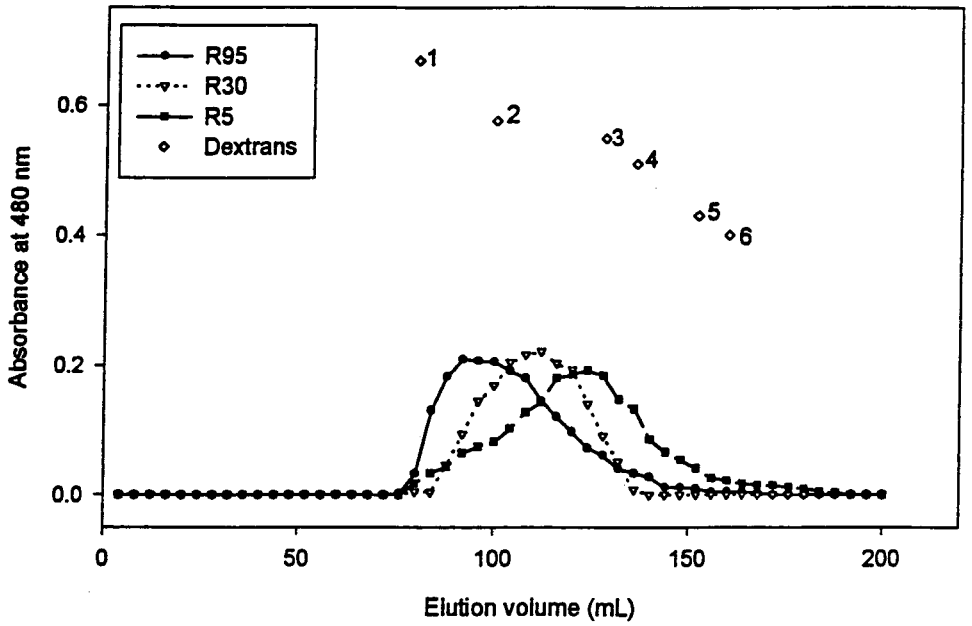

Figure 5. MW distribution of WEAX isolated from R5, R30, and R95 ryes determined by GPC using Sephacryl S-500. Each rye line is designated R (rye) followed by its wholemeal extract viscosity (in cP). Dextran standard MW values (approximate): 1, 4×10^6; 2, 575×10^3; 3, 300×10^3; 4, 126×10^3; 5, 20×10^3; 6, 10×10^3.

lichenase did not significantly reduce its viscosity (Figure 3), indicating the small contributions of starch and β-glucan to the viscosity of the extract. The slight reduction in extract viscosity resulting from the addition of protease may have been due to hydrolysis of peptide bridges between arabinoxylan and other constituents. Similar results were obtained by Ebringerovà et al. (*30*), who observed a decrease in the average MW of the WEAX−protein complex isolated from rye bran after incubation with Pronase. A very marked reduction in the viscosity of the extract resulted from treatment with xylanase. These results confirm that the viscosity of the rye extract was principally due to its content of WEAX. Similar results have been reported by others (*8*, *9*, *29*, *31*, *32*).

Xylanase Treatment of a High MW Arabinoxylan Fraction. Dialysis of the water extract from low extract viscosity rye (R5) removed most of the low MW components (Figure 4). Subsequent incubation of the dialyzed rye extract with xylanase markedly reduced the %HMWF, indicating that the HMWF was primarily arabinoxylan.

Yield, Viscosity, Composition, and MW of Purified WEAX. The procedure employed for the isolation of WEAX from rye was a combination of several existing procedures. The α-amylase/amyloglucosidase treatment was employed by Loosveld et al. (*33*). Delcour et al. (*34*) applied α-amylase treatment followed by clay treatment. Precipitation of WEAX with aqueous ethanol (65%, v/v, ethanol/extract), leaving arabinogalactan in the supernatant, was employed by Cleemput et al. (*35*).

The lowest yield (Table 2) of isolated WEAX was obtained from the low extract viscosity (R5) rye wholemeal (0.7 g/100 g of meal); the yields from R30 and R95 were similar (1.5 and 1.6 g/100 g, respectively). Despite the similarity in the degree of purification of the three WEAX, there were significant differences in the viscosities of their 1% aqueous solutions, ranging from 32 cP for R5 to 566 cP for R95.

Analysis of the isolated WEAX confirmed a high level of purity (Table 2). Arabinose and xylose were the only sugars present in significant amounts in hydrolysates of the isolated WEAX. Arabinoxylan accounted for ~90% of the WEAX. The A/X ratio of the WEAX from R30 (0.55) was slightly lower than that of WEAX from R5 or R95 (0.60 in each case). The residual protein in the WEAX may have resulted from protein covalently attached to WEAX (*30*).

Purified WEAX from the three ryes exhibited single peaks when subjected to GPC, eluting between 80 and 150 mL (Figure 5). There were significant differences in average MW among the three WEAX, with WEAX from R5 exhibiting the lowest MW (weighted average of 269000), WEAX from R30 an intermediate MW (weighted average of 476000), and WEAX from R95 the highest MW (weighted average of 730000).

H NMR. The H NMR spectra of the WEAX isolated from R95, R30, and R5 are presented in Figure 6. The absence of peaks between δ 4.7 and 4.8 in the spectra indicated no contamination by β-glucan (*16*, *35*). There was also no contamination with arabinogalactan as indicated by the absence of a peak at δ 5.26 (*33*). More detailed representations of the L-arabinofuranosyl anomeric proton regions 5.4−5.2 of the WEAX are presented in Figure 7. In this region, the WEAX exhibited three major peaks. The first peak (δ 5.4) resulted from the anomeric protons of α-L-Araf linked to O-3 of Xylp residues of the arabinoxylan (*9*, *16*, *35*−*37*). The remaining two peaks (δ 5.30 and δ 5.23) represent the anomeric protons of Araf linked to O-2 and O-3 of the same Xylp residue (*9*, *16*, *35*−*37*).

The relative distribution of mono- and disubstituted Xylp residues in the three WEAX was calculated by quantitative integration of the anomeric proton peaks of the individual Araf residues (*35*), assuming that all Araf residues were terminally linked (as shown by the spectra). Purified WEAX from R5 had a higher percentage of disubstituted Xylp residues (28%) compared to WEAX from R30 and R95 (18 and 15%, respectively) (Table 3). Purified WEAX from R30 had a high percentage of monosubstituted Xylp residues (59%) compared to WEAX from R5 and R95 (49 and 51%, respectively). The highest percentage of unsubstituted Xylp residues

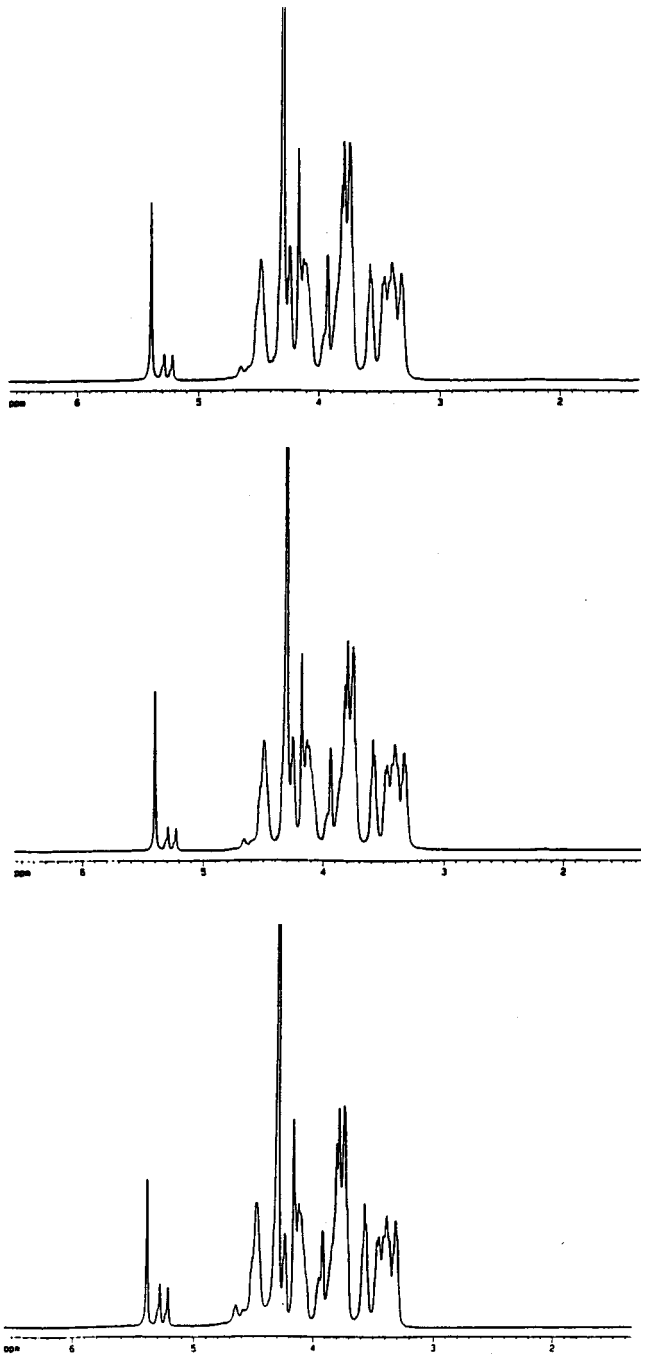

Figure 6. H NMR spectra of purified WEAX from R95 (top), R30 (middle), and R5 (bottom) rye wholemeals.

(34%) was obtained for purified WEAX from R95, compared to 23 and 24% for WEAXs from R5 and R30, respectively. According to Wyatt (*25*), a high degree of disubstitution of a polymer will cause its hydrodynamic volume to be small and its density to be high, which would be expected to generate low viscosity in aqueous solution; this was the case for WEAX from R5 and R30. Similarly, Courtin and Delcour (*38*) reported that high MW WEAX isolated from wheat flour, which exhibited high viscosity, was low in disubstituted and high in mono- and unsubstituted Xylp residues compared to a lower MW WEAX purified from a wheat arabinoxylan concentrate. Izydorczyk and Biliaderis (*39*) obtained (from wheat) a high MW, high viscosity WEAX fraction exhibiting a low degree of disubstituted Xylp residues and a high degree of monosubstituted Xylp residues.

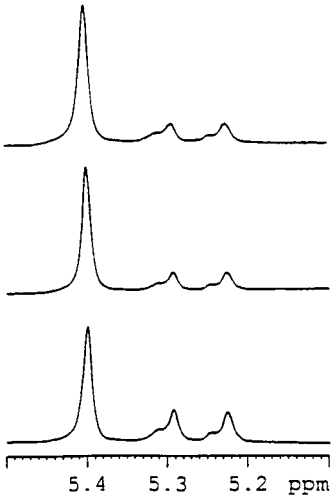

Figure 7. Arabinose anomeric proton regions of the H NMR spectra of purified WEAX from R95 (top), R30 (middle), and R5 (bottom) rye wholemeals.

Table 3. Fine Structure of Purified WEAX Isolated from High (R95), Intermediate (R30), and Low (R5) Extract Viscosity Rye Wholemeals, As Determined by H NMR and HPSEC with Triple Detection

parameter	R95[a]	R30	R5
Xylp			
unsubstituted (%)	34	24	23
monosubstituted (%)	51	59	49
disubstituted (%)	15	18	28
$Xylp_{disub}/Xylp_{monosub}$[b]	0.29	0.31	0.35
MW[c]	494950 ± 23971[d]	280400 ± 18809	199050 ± 5162
R_g[e] (nm)	54.5 ± 1.2	37.1 ± 1.2	29.1 ± 0.5
R_h[f] (nm)	41.9 ± 0.9	28.5 ± 1.0	22.4 ± 0.4
IV (dL/g)	10.2 ± 0.2	6.2 ± 0.3	4.3 ± 0.2
a value[g]	0.57 ± 0.0	0.72 ± 0.0	0.67 ± 0.0

[a] Each line is designated R (rye) followed by its wholemeal extract viscosity (in cP). [b] Ratio of disubstituted xylose residues to monosubstituted xylose residues. [c] Weighted average molecular weight. [d] Mean ± standard deviation ($n = 2$). [e] Weighted average radius of gyration. [f] Weighted average hydrodynamic radius. [g] Slope of Mark−Houwink plot.

Despite differences in viscosity and in the degree of mono- and disubstitution, the WEAX from R5 and R95 exhibited similar A/X ratios. This could be related to differences in the level of a proposed arabinan (*30*) or to the shorter chain length (i.e., lower MW) of the more highly branched WEAX from R5. Similar A/X ratios were observed for low and high viscosity WEAX isolated from rye bran (*30*), despite higher disubstitution in the low viscosity fraction. Izydorczyk and Biliaderis (*6*) reported that the proportion of disubstituted residues was not related to the A/X ratio and varied considerably among various WEAX.

HPSEC with Triple Detection. The structures of WEAXs isolated from R95, R30, and R5 were further characterized using HPSEC coupled with refractive index, light scattering, and intrinsic viscosity measurements. The outputs obtained from the three detectors (refractive index, light scattering, and differential pressure) for the WEAX are presented in Figure 8.

The weighted average MWs of the WEAX as determined by light scattering were 495000, 280000, and 199000 for R95, R30, and R5, respectively. The breadth of the peaks corresponding to each WEAX indicates that the WEAX were mixtures of molecules having a range of molecular weights, which agrees with results obtained by others (*13*, *39*) for wheat WEAX. The MW values

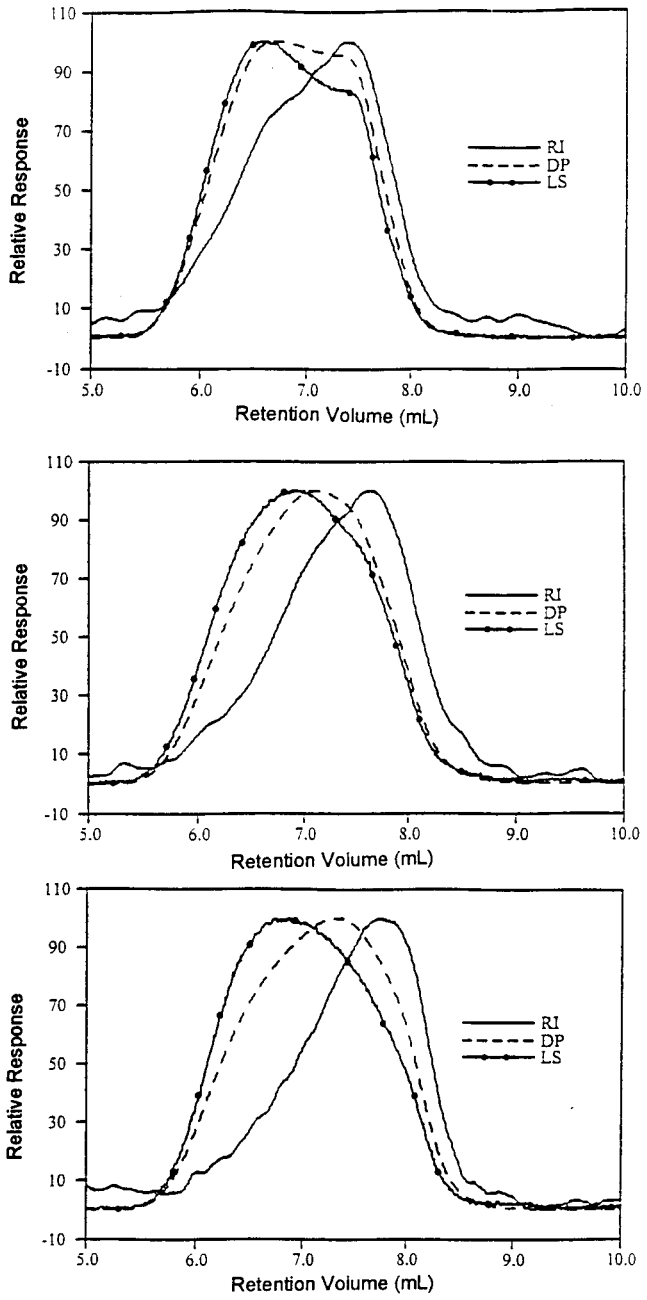

Figure 8. Triple detector chromatograms of purified WEAX from R95 (top), R30 (middle), or R5 (bottom) rye wholemeal, as determined by HPSEC with triple detection. RI, refractive index detector; DP, differential pressure detector; LS, light scattering detector.

obtained with the light scattering detector were substantially lower than corresponding values obtained by GPC (Figure 5). In GPC, molecules are separated according to their hydrodynamic volumes; thus, the nonavailability of MW markers similar in structure to the WEAX tested may have led to overestimation of MW by this technique (*27*). This may explain the discrepancies observed between the two MW determination methods. Significant differences in the radius of gyration (R_g) among the three WEAX were observed (Table 3), with R_g being proportional to extract viscosity. A similar trend was observed for the hydrodynamic radius (R_h). R_g is said to be influenced by the conformation of a macromolecule and dependent upon its internal mass distribution, whereas R_h reflects the end-to-end size of the molecule in solution (*25*). The higher R_h value

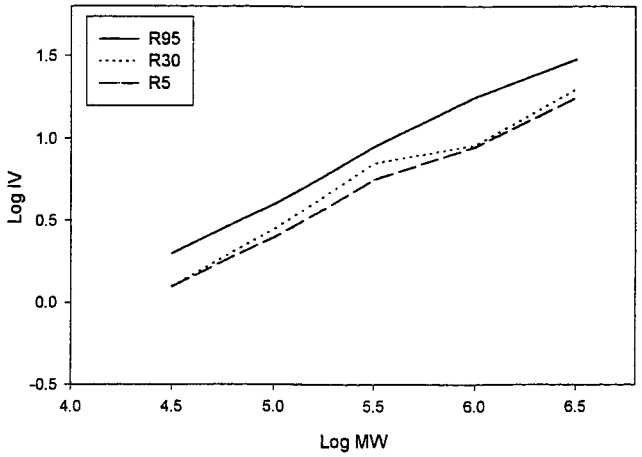

Figure 9. Mark−Houwink plots of purified WEAX from R95, R30, or R5 rye, as determined by HPSEC with triple/detection.

obtained for WEAX from R95 supports the interpretation that this polymer is able to occupy a larger space in aqueous solution as a consequence of being a less dense molecule, resulting in an ability to exert higher viscosity. Conversely, the smaller R_g and R_h of WEAX from R30 and R5, in addition to their lower MW, confirmed their compactness and, hence, the lower viscosities of solutions of these polymers.

The intrinsic viscosities of the three arabinoxylans were 10.2, 6.2, and 4.3 dL/g for WEAX from R95, R30, and R5, respectively (Table 3). This provides further support for the conclusion that the relatively high viscosity of WEAX from R95 was related to its higher MW, larger R_g and R_h, and lower degree of branching. Mark−Houwink plots were obtained by plotting the intrinsic viscosity values of the WEAX against MW (Figure 9). Structural differences among the three WEAX were predicted from the slopes (*a* values) of the plots (Table 3). The high viscosity WEAX from R95 exhibited an *a* value of 0.57 nm, which was lower than that obtained for WEAX from R5 (0.67 nm) or R30 (0.72 nm). A high *a* value is considered to be characteristic of a polymer chain with restricted flexibility (*6*, *30*). In contrast, a low *a* value, as obtained for WEAX from R95, is characteristic of an unperturbed configuration (*6*, *30*). These results confirmed again that WEAX from R95 was a long-chain, extended macromolecule with a relatively low degree of branching.

Conclusions. The development of rye lines exhibiting a wide range of extract viscosities afforded the opportunity to characterize the factors that contribute to rye extract viscosity. The viscosities of rye wholemeal water extracts varied substantially. Extract viscosity of rye wholemeals was positively correlated with SDF and WEAX contents. The MW distributions of water extracts indicated the presence of both high and low MW components in water extracts from rye and wheat. High viscosity was related to a high content of WEAX in the water extracts, the %HMWF in particular. Xylanase treatment of the rye water extract indicated that the HMWF was mainly arabinoxylan. Other enzyme treatments confirmed that viscosity was generated primarily by the HMWF.

WEAX isolated from ryes differing in extract viscosity exhibited structural differences, in that high viscosity was associated with a high MW, a large R_g and R_h, and a low degree of branching.

ABBREVIATIONS USED

R5 and R10, low extract viscosity ryes; R19 and R30, intermediate extract viscosity ryes; R95, high extract viscosity rye; TDF, total dietary fiber; SDF, soluble dietary fiber; TAX, total arabinoxylan; WEAX, water-extractable arabinoxylan; WEAG, water-extractable arabinogalactan; A/X, arabinose-to-xylose ratio; GPC, gel permeation chromatography; HPSEC, high-pressure size exclusion chromatography; IV, intrinsic viscosity; MW, molecular weight; HMWF, high molecular weight fraction; %HMWF, proportion of the high molecular weight fraction; R_g, radius of gyration; R_h, hydrodynamic radius; Xylp, xylopyranosyl residue; Araf, arabinofuranosyl residue.

ACKNOWLEDGMENT

The technical assistance provided by D. Hassard and D. Hay was greatly appreciated.

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Received for review October 10, 2000. Revised manuscript received February 1, 2001. Accepted February 1, 2001. Financial support provided by the Agriculture Development Fund and the Value-Added Crops Strategic Research Program, Saskatchewan Agriculture and Food, Regina, SK, is gratefully acknowledged.

JF001227G