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Depolymerization Degree of Water-Extractable Arabinoxylans in Rye Bread: Characteristics of Inbred Lines Used for Breeding of Bread Cultivars

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ABSTRACT: The water-extractable arabinoxylans (WE AXs) present in rye bread govern its viscous properties, which may be related to reduced risk of cardiovascular diseases and diabetes. Breads made from rye cultivars generally exhibit higher AX-dependent extract viscosities (Cyran, M. R.; Saulnier, L. *Food Chemistry* **2012**, *131*, 667−676) when compared with those produced from inbred lines used for their breeding. To give further details about this trend, the WE AXs were isolated from breads of lines and structurally characterized by HPSEC and ^{1}H NMR spectroscopy. The extract viscosities of endosperm and whole-meal breads were usually comparable, in contrast to those made from rye cultivars with higher viscosity of endosperm bread. The WE AXs present in breads obtained from inbred lines were characterized by the higher degradation degrees than those in breads from cultivars, as indicated by their HPSEC-RI profiles. This was associated with considerably lower proportions of 2-Xyl*p* in their backbones. Besides, a level of endoxylanase activity in flours from inbred lines was much higher than that in flours from cultivars. Breeding of hybrid rye cultivars for production of high-viscosity bread requires the proper components. They may be preliminarily selected from populations with high WE AX contents and relatively low levels of endoxylanase activity by using the overall viscosity test for starting flours. However, further measurement of AX-dependent extract viscosity in test breads made from such lines may verify their usefulness completely.

KEYWORDS: *rye (Secale cereale L.) inbred lines, whole-meal bread, endosperm bread, extract viscosity, arabinoxylans, structure, molecular weight, endoxylanase activity*

INTRODUCTION

The global recommendation to increase the daily amount of dietary fiber (DF) in the human diet is mostly related to an increased consumption of whole-grain products. In comparison to products made from refined flour, they contain a higher level of DF, including also the bioactive components located in the outer layers of the grain. However, there are intra- and interspecies differences in the content and composition of DF in cereals, which are modified by environment.[1,2] Currently, the DF level in cereal-based products is a primary indicator of their health-promoting properties. A higher DF content of items consumed usually means a higher proportion of DF components, predominantly arabinoxylans (AXs) and β-glucans, which undergo bacterial fermentation in the large intestine, and/or higher proportion of unfermented DF residue. The water-extractable (WE) DF polysaccharides are readily fermented in the cecum and proximal colon as opposed to water-unextractable (WU) counterparts, especially highly heterogeneous AXs. Their extent of fermentation depends on structural features and the type of links with other grain components.[3,4] The partial fermentation of DF by intestinal bacteria results in the production of short-chain fatty acids, which decrease the pH of the colon and modify the gut microbiota composition, thus providing health benefits to the host.[5] It has been demonstrated that certain alkali-extractable (AE) AXs with higher molecular weight and uniquely substituted arabinose side chains are capable of sustaining the linear fermentation profile of short-chain fatty acids.[3] Besides, the residual DF present in the colon may absorb some carcinogenic substances that are excreted from the human organism.[6] The above properties of DF along with those implicated by the presence of antioxidants and other bioactive components in cereal grains are thought to be protective against some types of cancer, such as gastric and colonic as well as breast and prostate cancers.[7]

On the other hand, it has been well documented that the consumption of products rich in WE DF polysaccharides may increase the digesta viscosity in the small intestine, which forms a diffusion barrier and reduces the activity of digestive enzymes. This in itself restricts the rate of digestion and nutrient absorption, also resulting in lowered serum cholesterol and glucose levels that are related to a reduced risk of developing cardiovascular disease and diabetes.[8,9] Among cereal DF polysaccharides, such health properties have been claimed mainly for viscous β-glucans from oat[10,11] and barley.[12,13]

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However, recent studies in humans provided evidence that also WE AXs from wheat, triticale, and rye exhibit health benefits in terms of glucose and lipid metabolism.[14−18] Furthermore, it has been shown that corn bran AX, extracted under alkaline conditions and cross-linked by laccase to enhance its viscosity, significantly reduced the postprandial blood glucose level in rats.[19]

The concentration of WE AXs in rye (2.6−4.0%) is about 5 times higher than in wheat (0.36−0.83%).[20,21] Consequently, the mean overall extract viscosity reported for commercial rye cultivar (13 cP) was distinctly higher than that of wheat (2.3 cP), although the highest variability (5−95 cP) was observed in rye lines.[22] Nevertheless, the viscous properties of cereal-based products are also dependent on the processing used, during which the content and molecular characteristics of WE AXs and β-glucans may be altered.[17,23] Generally, this is ascribed to the hydrolytic action of AX- and β-glucan-degrading enzymes associated with the grain, on both WE and WU fractions of these polysaccharides.[24]

Among bread cereals rye is second only to wheat. Whole-meal rye bread has been shown to be effective in reducing serum cholesterol levels in men with moderately elevated serum cholesterol.[18] A clinical trial in healthy subjects demonstrated that not only whole-meal rye bread but also that produced from white rye flour was characterized by a lower postprandial insulin response and beneficial blood glucose profile in comparison to those of white wheat breads without and with rye bran supplementation.[25] Moreover, Rosén et al.[26] recently revealed that whole-meal breads made from some rye cultivars may be more insulin-saving than others, probably due to differences in the content of DF and other bioactive components.

Our latest study demonstrated that production of high-viscosity bread by a straight dough method is dependent on the rye genotype used.[27] The extract viscosity ascribed to AXs of endosperm bread is usually higher; however, it may be comparable to or even 2 times higher than that of whole-meal bread made from the same rye cultivar. This is due to the much lower endoxylanase activity in the endosperm flour than in whole meal. There are also differences in the fine structure of WE AXs present in the endosperm and outer layers of the grain that result in a relatively lower extent of degradation of these polysaccharides in endosperm bread, when compared with those in whole-meal bread with higher WE AXs content.[28]

The present study was carried out to investigate depolymerization degree of WE AXs in rye bread and its impact on the viscous properties of endosperm and whole-meal breads produced by a straight dough method from rye inbred lines, which, in contrast to rye cultivars, exhibit a wide range of WE AX contents and activities of AX-degrading enzymes. Another aim was to indicate the possible strategies for selection of inbred lines as components in the breeding of hybrid rye cultivars suitable for production of high-viscosity bread.

MATERIALS AND METHODS

Rye Samples. The material consisted of six homozygous inbred lines of winter rye (L_1−L_6), which were multiplied in the isolation plots at the Plant Breeding and Acclimatization Institute plant station in Radzikow, Poland. All inbred lines were developed by subsequent self-pollination from Polish populations of rye and selected with respect to combining ability and agronomic traits. They represent elite parent materials and some of them are used in commercial hybrid rye production.

Rye grains, conditioned to 12% moisture content, were milled in a Quadrumat Senior mill (Brabender Instruments, Inc., Duisburg, Germany). The endosperm and whole-meal flours with 50−60 and 95−98% extraction rates, respectively, were obtained by standard milling, that is, break rolling, sifting, and reduction rolling. The whole-meal flours were obtained by reduction rolling until a particle size similar to that of endosperm flour was achieved, as indicated by sieve analysis.

Test Breads. Both types of bread were produced by the straight dough method with yeast and lactic acid, as described previously by Cyran and Ceglinska.[27] The freeze-dried bread samples were milled to a particle size of 0.5 mm and stored in plastic bags with airtight closure at −20 °C, until they were analyzed.

Isolation of Purified Water-Extractable Dietary Fiber Fractions. Freeze-dried bread samples (140 g) were suspended in deionized water (1:5 w/v) and mixed for 60 min at 37 °C with a mechanical stirrer (CAT, Staufen, Germany). The supernatants were centrifuged (20 min, 10000*g*, room temperature), and the pellets were resuspended in deionized water (500 mL) and mixed for 30 min at 37 °C. After they were centrifuged (20 min, 10000*g*, room temperature), both supernatants were combined. The crude extracts were purified from starch and protein contaminants using a two-step incubation with α-amylase from porcine pancreas (EC 3.2.1.1, Sigma-Aldrich), amyloglucosidase from *Aspergillus niger* (EC 3.2.1.3, Roche Diagnostics, Mannheim, Germany), and proteinase K from *Tritirachium album* (EC 3.4.21.64, Roche Diagnostics), as described by Cyran et al.[29] After digestion of starch and protein, the extracts were dialyzed against deionized water using membrane tubing (MW cutoff of 6000−8000, Bioron, Łódź, Poland). Aliquots of each purified extract were freeze-dried and are further referred to as WE.

Fractionation by Graded Ammonium Sulfate Technique. The remaining portions of the extracts were directly fractionated by graded ammonium sulfate precipitation.[29] Four subfractions were obtained from each extract and denoted WE.40, WE.60, WE.80, and WE.100, respectively, for materials collected at 40, 60, 80, and 100% saturation levels.

Chemical Analyses. All analyses were carried out in at least duplicate. Moisture and ash contents were analyzed by using AACC methods 44.15A and 46.11A, respectively.[30] Protein content (N × 6.25) was determined according to the Kjeldahl method using a Kjeltec Auto 1030 analyzer (Tecator, Höganäs, Sweden). Klason lignin was measured gravimetrically as the residue left after two-step acid hydrolysis (12 M sulfuric acid, 1 h, 35 °C; 1 M sulfuric acid, 3 h, 100 °C) and corrected for ash.[31]

Analysis of noncellulosic polysaccharides (NCP) present in bread samples was performed according to the method described by Englyst and Cummings[32] with some modifications.[27] The bread samples (150−200 mg) were suspended (1:25, w/v) in sodium acetate buffer (0.1 M, pH 5.0) and incubated with thermostable α-amylase (10 μL, 3000 U/mL, Megazyme, Bray, Ireland) in a boiling water bath for 1 h. The samples were cooled to 45 °C, mixed with amyloglucosidase and protease (40 μL, 3300 U/mL; and 20 μL, 350 U/mL, respectively; Megazyme) and incubated in a shaking water bath at 45 °C for 16 h. The WE NCP present in the supernatant were separated from WU polysaccharides by centrifugation (15500*g*, 20 min, at 4 °C) and precipitated with 4 volumes of 95% ethanol.

The monosaccharide compositions of the WE and WU NCP were analyzed by gas chromatography. Samples were hydrolyzed in 1 M sulfuric acid (100 °C, 2 h). The released monosaccharides were converted to alditol acetates and quantified on a wide-bore capillary column (Rtx-225, 30 m, 0.53 mm i.d.; Restek, Bellefonte, PA, USA) in an SRI 8610C gas chromatograph (SRI Instruments, Torrance, CA, USA) equipped with a flame ionization detector. The injector and detector were set at 230 °C, and hydrogen was used as carrier gas. The column was held at 180 °C for 2 min, ramped from 180 to 220 at 5 °C/min, and held at 220 °C for 10 min. *meso*-Erythritol was used as internal standard.

Arabinoxylan-Dependent Extract Viscosity. The extract viscosity was determined after synergistic action of starch- and protein-degrading enzymes.[27] Duplicates of 2.0 g of rye bread were

Table 1. Chemical Composition of Endosperm and Whole-Meal Rye Breads, Their Arabinoxylan-Dependent Extract Viscosities (AX-EV), and Endo-β-D-xylanse, α-L-Arabinofuranosidase, and β-D-Xylosidase Activity Levels in Starting Flours[a]

	L_1	L_2	L_3	L_4	L_5	L_6	L_1	L_2	L_3	L_4	L_5	L_6
			endosperm bread						whole-meal bread			
WE AX (% dm)	2.32	3.66	2.34	3.03	2.53	3.82	2.73	3.96	2.87	3.43	3.10	4.46
Ara/Xyl of WE AX	0.65	0.55	0.65	0.53	0.60	0.51	0.62	0.52	0.62	0.49	0.54	0.45
WU AX (% dm)	1.65	1.66	1.53	1.91	1.63	1.77	5.31	6.10	5.06	6.43	4.95	5.49
Ara/Xyl of WU AX	0.88	0.75	0.86	0.85	0.80	0.83	0.72	0.65	0.69	0.62	0.63	0.67
total NCP (% dm)	6.94	8.60	7.59	7.83	7.90	8.85	12.3	14.1	13.1	13.7	12.4	13.6
Klason lignin (% dm)	0.69	0.62	0.71	0.93	0.81	0.42	2.03	3.01	1.74	2.15	1.62	1.61
protein (% dm)	11.4	9.3	10.6	11.4	11.3	10.6	14.5	12.3	14.8	15.0	14.5	14.0
ash (% dm)	2.96	2.96	2.94	3.04	3.00	2.95	3.92	3.88	3.93	4.24	3.80	3.58
AX-EV (mPa s)	4.5	25.8	12.0	8.3	12.8	13.1	4.6	12.3	9.9	7.3	12.6	13.0
			endosperm flour						whole-meal flour			
endo-β-D-xylanase (EU)	0.91	0.34	0.33	0.86	0.37	0.35	2.02	1.03	0.66	1.99	0.48	1.22
α-L-arabinofuranosidase (nkat/g)	0.37	0.55	0.21	0.21	0.23	0.24	0.49	0.99	0.24	0.22	0.29	0.30
β-D-xylosidase (nkat/g)	0.46	0.63	0.39	0.27	0.28	0.25	0.54	0.91	0.40	0.29	0.33	0.30

[a]Results of chemical analyses were obtained from duplicates; the coefficient of variation was <4%. Results of arabinoxylan-degrading enzyme activities were obtained from quadruplicates; the coefficient of variation was <7%. AX = 0.88(%ara + %xyl). NCP = 0.90(%ara + %xyl + %man + % gal + %glc).

weighed in an erlenmeyer flask (100 mL) and suspended (1:5, w/v) in sodium maleate buffer (0.05 M, pH 6.0). The suspension was stirred in a shaking water bath for 1 h at 30 °C and then centrifuged for 20 min at 10000*g* at room temperature. An aliquot (4 mL) of the extract was mixed (in duplicate) with α-amylase (25 μL, 3000/U), amyloglucosidase (25 μL, 3300 U/mL), and protease (25 μL, 350/U) purchased from Megazyme. After 60 min of incubation at 30 °C, the viscosity of the extracts was measured in a Brookfield cone/plate viscometer model LVDV-II+ (Brookfield Engineering Laboratories Inc., Stoughton, MA, USA) maintained at 30 °C. The starch- and protein-degrading enzymes were checked for their side activities toward AX using the sodium maleate solution of a standard rye flour AX-I (Ara/Xyl ratio of 0.5), containing only singly substituted xylose residues in the chain.[29] No change in the viscosity was observed after α-amylase, amyloglucosidase, or protease treatment during 1 h.

Proton Nuclear Magnetic Resonance Spectroscopy (^{1}H NMR). Samples (10−15 mg) were dissolved in D_2O (0.7 mL, 99.8% D, Sigma-Aldrich, Poznań, Poland) using an ultrasonic water bath. ^{1}H NMR spectra were recorded at 85 °C on a Varian NMR System, DirectDrive 700 MHz. The chemical shifts are reported relative to internal sodium 3-(trimethylsilyl)propionate-d_4 standard at δ 0.00.

High-Performance Size-Exclusion Chromatography (HPSEC). The purified dietary fiber fractions and subfractions of rye breads were dissolved in ultrapure water (5 mg/mL) for 16 h at 40 °C using a rotary incubator, filtered through a 0.45 μm membrane, and injected at room temperature on a HPSEC system constituted of two Shodex OH-pack SB HQ 804 and 805 columns eluted at 0.7 mL/min with 0.05 M $NaNO_3$ containing 0.02% NaN_3. Online molar mass and intrinsic viscosity determinations were performed using a multiangle laser light scattering (MALLS) detector (mini-Dawn, Wyatt, USA), a differential refractometer (ERC 7517 A), and a differential viscometer (T-50A, Viscotek, USA).[33] M_w and polydispersity index $I = M_w/M_n$ were determined using ASTRA 1.4 software (Wyatt, USA) and $[\eta]$ using TRISEC software (Viscotek, USA). Concentrations were calculated using $dn/dc = 0.146$ mL/g; M_w and $[\eta]$ were calculated for polysaccharide population eluting between 11.5 and 17.5 mL.

Enzyme Activity Assays. Samples of rye flours (5.0 g) were homogenized in sodium acetate buffer (1:4 w/v, 0.1 M, pH 4.5, 2 × 30 s, cooled on an ice bath) using an Ultraturrax (IKA Labortechnik, Staufen, Germany).[34] Homogenates were centrifuged (15000*g*, 4 °C, 25 min) in a Kokusan H-2000A2 centrifuge (Kokusan Corp., Tokyo, Japan). The pellets were resuspended in sodium acetate buffer and homogenized and centrifuged as described above. The supernatants were combined and adjusted to the same volume with sodium acetate buffer. The apparent endo-β-D-xylanase activity was determined in the extracts (0.5 mL) using the Xylazyme AX tablets as a substrate (Megazyme) according to the procedure described by Cleemput et al.[35] and expressed in EU. One EU is the amount of enzyme needed to increase the extinction at 590 nm by 1.0, under the condition of the assay, in 2 h. The α-L-arabinofuranosidase and β-D-xylosidase activities were measured using *p*-nitrophenyl-β-D-xylopyranoside (p-X) and *p*-nitrophenyl-α-L-arabinofuranoside (p-A) (Sigma-Aldrich) as described by Rasmussen et al.[34] with some modifications. The reaction mixture, containing a crude extract (160 μL) and p-X or p-A (40 μL, 0.01 M in 0.1 M sodium acetate buffer, pH 4.5), was incubated at 30 °C for 20 min. The reaction was stopped by the addition of sodium carbonate (400 μL, 0.2 M), and the released *p*-nitrophenol was measured at 405 nm. In controls, assay buffer was substituted for extract or no substrate was added. All assays were made in quadruplicate. The activities of α-L-arabinofuranosidase and β-D-xylosidase activity were expressed in nanokatals (nkat) per gram of flour, 1 nkat producing 1 nmol of product per second at the pH and temperature of incubation.

Statistical Analysis. When relevant, samples were analyzed at least in duplicate and results expressed as mean values. Pearson correlation coefficients (r) were calculated between the examined parameters across rye inbred lines and types of bread. The influence of the WE AX content in breads and activities of AX-degrading enzymes in the starting flours on the bread AX-dependent extract viscosity was determined by multiple linear regression.

RESULTS AND DISCUSSION

Physicochemical Characteristics of Rye Breads and Starting Flours. The chemical composition of endosperm and whole-meal breads made from rye inbred lines by the straight dough method is shown in Table 1. The content of WE AXs in rye breads examined varied largely. It ranged from 2.73 to 4.46% in whole-meal bread and from 2.32 to 3.82% in endosperm bread, representing 82−92% of their amount found in corresponding whole-meal bread. Also, a large variation in the level of WU AXs was observed. They constituted only 31−42% of total AXs content in endosperm bread and 55−66% in whole-meal bread. In both types of rye bread, the WU AXs had higher degrees of branching with α-L-arabinofuranosyl residues than those from WE fractions, as indicated by their Ara/Xyl ratios. However, both WE and WU AXs in endosperm bread were characterized by the higher degrees of branching in comparison to counterparts present in whole-meal bread. The whole-meal bread had an almost 2 times higher level of total AXs than that of endosperm bread as well as higher contents of

Table 2. Yield and Composition (Percent) of Water-Extractable Fractions and Their Subfractions Isolated from Endosperm and Whole-Meal Breads Made from Rye Inbred Lines L_1, L_2, and L_6[a]

	L_1					L_2					L_6				
	WE	WE.40	WE.60	WE.80	WE.100	WE	WE.40	WE.60	WE.80	WE.100	WE	WE.40	WE.60	WE.80	WE.100
endosperm bread															
yield[b]	4.10	0.14	0.60	1.14	0.58	5.60	0.27	2.49	0.82	0.34	5.49	0.31	2.54	1.09	0.48
AX[b]	1.84	0.01 (1)	0.21 (15)	0.83 (57)	0.39 (27)	3.31	0.03 (1)	1.94 (67)	0.67 (23)	0.25 (9)	3.46	0.04 (1)	2.05 (66)	0.79 (25)	0.23 (7)
A/X	0.70	0.71	0.52	0.61	0.96	0.58	0.56	0.48	0.65	1.00	0.52	0.48	0.42	0.67	0.86
arabinose	20.9	4.0	13.7	31.3	37.7	24.8	3.8	28.9	36.7	41.6	24.5	5.1	27.3	32.9	25.4
xylose	30.0	5.6	26.1	51.4	39.5	42.4	6.8	59.7	56.8	41.4	47.1	10.8	64.4	49.1	29.4
mannose	9.9	1.2	1.0	1.5	9.9	3.0	0.5	0.0	0.0	1.0	2.6	0.8	0.1	0.1	1.1
galactose	2.7	0.7	0.0	0.0	0.3	2.3	0.3	0.0	0.0	0.6	2.3	0.7	0.1	0.1	0.6
glucose	12.6	4.2	21.7	9.3	4.1	9.0	4.1	7.6	3.9	3.3	11.0	4.0	8.6	3.0	5.0
total NCP	68.4	14.0	56.2	84.2	82.3	73.4	14.0	86.5	87.6	79.1	78.8	19.2	90.4	76.7	55.3
whole-meal bread															
yield[b]	4.75	0.16	0.98	1.12	0.32	6.06	0.20	2.12	1.10	0.31	5.50	0.31	2.14	1.24	0.33
AX[b]	2.02	0.03 (2)	0.53 (33)	0.84 (52)	0.21 (13)	3.45	0.08 (3)	1.67 (59)	0.87 (31)	0.23 (8)	3.52	0.14 (4)	1.77 (57)	0.97 (32)	0.21 (7)
A/X	0.64	0.64	0.49	0.64	0.89	0.54	0.48	0.48	0.57	0.72	0.49	0.36	0.41	0.57	0.76
arabinose	18.8	8.0	20.4	33.4	35.8	22.8	14.3	28.8	32.7	34.7	23.8	13.1	26.8	32.4	30.8
xylose	29.5	12.4	41.3	52.1	40.2	42.0	29.5	60.5	57.5	48.2	49.0	36.6	65.8	56.6	40.7
mannose	6.8	1.6	0.9	1.2	3.2	2.3	0.6	0.0	0.0	0.0	1.6	0.5	0.0	0.3	0.4
galactose	2.5	0.7	0.0	0.0	0.7	1.9	0.7	0.0	0.0	0.0	1.9	0.4	0.0	0.4	0.7
glucose	15.9	9.2	21.1	11.7	2.6	12.1	2.9	8.6	6.8	3.1	11.2	4.5	6.3	7.5	10.9
total NCP	66.2	28.7	75.3	88.6	74.1	72.9	43.1	88.2	87.2	77.4	78.7	49.6	89.0	87.4	75.0

[a]Results were obtained from duplicates; the coefficient of variation was <4%. Numbers in parentheses represent the percent of total AX recovered after ammonium sulfate fractionation. AX = 0.88(%ara + %xyl). NCP = 0.90(%ara + %xyl + %man + %gal + %glc). [b]Expressed as percent of rye bread dry matter.

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Klason lignin, protein, and ash, even though the highest AX-dependent extract viscosity was found for endosperm bread made from L_2, which was 2 times higher than that of corresponding whole-meal bread. In the remaining cases, there were no practical differences in the extract viscosity between endosperm and whole-meal breads made from the same line of rye. The AX-dependent extract viscosities of endosperm and whole-meal breads made from line L_6 with the highest content of WE AXs were 2 times lower than that of endosperm bread of line L_2; however, they were comparable with those of breads made from L_5 with much lower content of WE AXs. Again, the lowest extract viscosities were observed for breads made from L_1 with the lowest content of WE AXs, whereas breads made from L_3 with the same content of WE AXs had much higher extract viscosities. Clearly, this demonstrates that the amount of WE AXs in bread made from rye lines does not guarantee its high AX-dependent extract viscosity, although correlation coefficients between these parameters were relatively high, but not statistically significant ($r = 0.62$ and $r = 0.64$, respectively, for endosperm and whole-meal breads). The amounts of WE AXs in both types of rye bread were negatively correlated with their Ara/Xyl ratios ($r = -0.88$, $P < 0.05$), implying that an increase in their content in rye bread was accompanied by an increase in the proportion of AX population with a lower degree of branching. This relationship was also observed in starting endosperm flours and whole meals ($r = -0.92$, $P < 0.01$; and $r = -0.84$, $P < 0.05$, respectively) (results not shown).

The measurement of the overall water extract viscosity of flours, tested without the addition of starch- and protein-degrading enzymes, was useful only in the selection of lines with high WE AX content, as significant correlations were found between these traits for endosperm flour and whole meal ($r = 0.90$, $P < 0.05$; and $r = 0.87$, $P < 0.05$, respectively) (result not shown), whereas the extract viscosity of starting flours was not significantly correlated with AX-dependent extract viscosity of bread made from rye inbred lines. Among rye inbred lines with high WE AX content it was not able to discriminate the high-viscosity bread lines from those resulting in bread with much lower AX-dependent extract viscosity (results not shown). Nevertheless, the measurement of flour overall extract viscosity may be used for preliminary selection of rye lines with high WE AX content, being a first step in a selection toward high-viscosity bread lines. However, to completely verify their usefulness, the AX-dependent extract viscosity assay of test breads was necessary.

The endo-β-D-xylanase, α-L-arabinofuranosidase, and β-D-xylosidase activities in starting endosperm flours and whole meals are shown in Table 1. Excluding line L_5, the apparent endoxylanase activities of the remaining samples were 2−3 times higher in whole meals than in the corresponding endosperm flours, which is in agreement with previously reported data showing distinctly higher activity of this enzyme in the outer layers than in the inner parts of the cereal grains,[36,37] whereas line L_5 was differentiated by its low and comparable levels of endoxylanase activity in both types of flour, mostly implicated from its specific genetic background. Moreover, the whole-meal bread made from this line exhibited AX-dependent extract viscosity as high as those of L_2 and L_6 with the highest WE AX contents, suggesting its relatively low degradation degree of WE AXs. The endoxylanase activity in starting endosperm flours and whole meals was negatively correlated with bread AX-dependent extract viscosity ($r = -0.70$ and $r = -0.79$, respectively).

In the case of arabinofuranosidase and xylosidase activities, in general, there were no significant differences between whole meals and corresponding endosperm flours. Only line L_2 exhibited much higher activity levels of both enzymes in whole meal than in endosperm flour. Besides, in all samples examined practically the same activity levels of arabinofuranosidase and xylosidase were observed. The higher arabinofuranosidase and xylosidase activities in the endosperm flour and whole meal of line L_2, in comparison to those of the remaining samples, do not have any negative effect on the viscosity of resulting breads, as both breads made from L_2 were characterized by the high values of this parameter.

The results of multiple linear regression analysis showed that the WE AX content in bread and the levels of endoxylanase and arabinofuranosidase activities in a flour may explain 89% of the total variation in the AX-dependent extract viscosity of endosperm bread (38, 25, and 26%, respectively) (results not shown). In the case of whole-meal bread, only WE AX contents in the bread and the endoxylanase activity level in starting whole meal were the most influential factors, explaining 97% of variance in bread AX-dependent extract viscosity (41 and 56%, respectively).

Polysaccharide Content and Composition of WE Fractions and Their Subfractions. The purified dietary fiber fraction (WE), extracted with water at 37 °C from rye breads with diverse AX-dependent extract viscosities (L_1, L_2, and L_6), constituted 4.1−5.6% of dry matter in endosperm bread and 4.8−6.1% in whole-meal bread (Table 2). Their noncellulosic polysaccharide content ranged from 66 to 79% and that of AXs from 43 to 64%. The WE fractions isolated from breads made from L_1 were characterized by the lowest contents of these constituents. To isolate polymeric AX subfractions with different structural features, the parent WE fractions were fractionated by ammonium sulfate using its four saturation levels (40, 60, 80, and 100%), which resulted in four cell wall subfractions (WE.40, WE.60, WE.80, and WE.100, respectively). The AXs in WE.40 represented only 1% of their total amount recovered after consecutive ammonium sulfate fractionation. Thus, they were not further investigated. The remaining AX subfractions present in WE.60, WE.80, and WE.100 (AX-I, AX-II, and AX-III, respectively) are characterized by a different substitution pattern of their (1→4)-linked backbones, containing β-D-xylopyranosyl residues (Xyl*p*) partly substituted with terminal α-L-arabinofuranosyl residues (Ara*f*). The AX-I subfraction, dominating among WE AXs in rye endosperm flour and whole meal (70 and 62%, respectively),[38] is built almost exclusively of unsubstituted Xyl*p* (u-Xyl*p*) and monosubstituted Xyl*p* by Ara*f* attached through *O*-3 (3-Xyl*p*). In contrast, AX-II and AX-III subfractions with much higher degrees of branching contain both mono- and disubstituted Xyl*p* by Ara*f* at *O*-2 and *O*-3 positions (2,3-Xyl*p*). However, a branching degree of AX-III subfraction is higher than that of AX-II, mainly due to a greater proportion of the 2,3-Xyl*p* in the chain.[38]

Both types of bread made from line L_1 with extremely low AX-dependent extract viscosities showed marked differences in a relative distribution of the major AX-I subfractions, when compared to those made from rye lines L_2 and L_6 with high viscosity values. The proportions of AX-I were as low as 15% in endosperm bread of L_1 and 33% in the corresponding whole-meal bread, indicating its substantial degradation in both types

of bread. Consequently, there was a substantial increase in the proportions of AX-II and AX-III subfractions. In contrast, the proportions of AX-I found in breads made from L_2 and L_6 were as high as 67 and 66% in endosperm bread and 59 and 57% in whole-meal bread, respectively. Hence, the levels of remaining AX subfractions, AX-II and AX-III, were considerably lower. It has been shown recently that the amount of AX-I subfraction in rye bread as well as its relative proportion in the entire WE AX fraction were the principal factors controlling AX-dependent extract viscosity of rye breads made from rye cultivars.[28]

High-Performance Size Exclusion Chromatography. The HPSEC-RI profiles of the WE fractions isolated from endosperm and whole-meal breads made of three rye lines (L_1, L_2, and L_6) with low and high extract viscosities along with those from endosperm bread of population cultivar Diament and from whole-meal bread of hybrid cultivar Konto with the highest and the lowest viscosity (45 and 8 mPa s, respectively), recorded in our previous study,[27] are shown in Figure 1. They indicate the presence of two high molecular weight (HMW) populations, which were unresolved and eluted between 11.5 and 17.5 mL. The elution profiles of L_1 fractions illustrate the highest degradation degree of HMW populations, as their relative proportions were the lowest when compared to that of Diament, being especially rich in HMW polymers with high molecular weight peak that eluted at 13.7 mL. This may by related to the highest endoxylanase activity levels found in both endosperm flour and whole meal of L_1 (Table 1). The high Ara/Xyl ratios of WE AXs in breads made from L_1, somewhat higher of those in starting flours (results not shown), were rather unexpected, because a high branching degree usually means fewer regions with consecutive un-Xyl*p* in the chain, a favorable substrate for hydrolytic action of endoxylanase. It is clear that in this case other factors that may affect the depolymerization degree of HMW AXs must be taken into account.

Unlike the L_1 fractions, those of L_2 and L_6 exhibited much lower degradation degrees of HMW populations, but they were higher than that of Diament. Besides, they were enriched in HMW populations with lower molecular weight that eluted between 14.0 and 17.5 mL. The distinctly lower degradation degree of the HMW fraction from endosperm bread made of L_2 than that from corresponding whole-meal bread can be associated with substantial differences in the levels of endoxylanase activity between the endosperm flour and whole meal (Table 1). Despite the similar low levels of endoxylanase activity found in endosperm flours of L_2 and L_6, the degradation degree of HMW populations in resulting bread of L_2 was markedly lower than that of L_6. The lower Ara/Xyl ratio of the WE AXs isolated from endosperm bread of L_6, in comparison to that of L_2, could be one explanation.

The differences in degradation degrees of HMW populations present in breads made from rye lines were also evidenced by their weight-average molecular weight (M_w) and intrinsic viscosity (Table 3), which varied from 3.3 to 5.2 × 10^5 g mol^{-1} and from 223 to 467 mL g^{-1}, respectively, with the highest values found for WE fractions isolated from breads of L_2 and the lowest for those of L_1. Nevertheless, somewhat higher values of these parameters were observed for HMW populations from endosperm bread than from whole-meal bread, indicating their lower degrees of degradation, whereas significantly higher M_w and intrinsic viscosity were reported previously for six endosperm breads made from population rye cultivars (on average, 6.2 × 10^5 and 635 mL g^{-1}, respectively).[28]

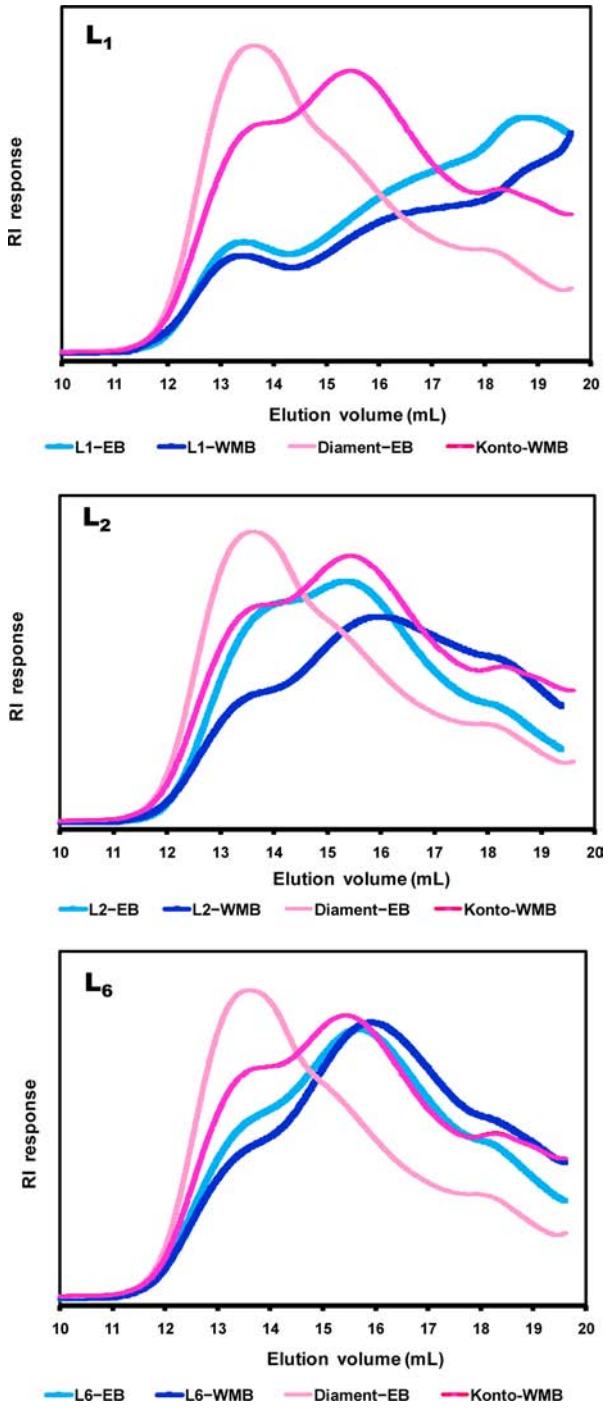

Figure 1. HPSEC-RI profiles of water-extractable fractions isolated from endosperm and whole-meal breads made from rye inbred lines L_1, L_2, and L_6 and from endosperm bread of population cultivar Diament and whole-meal bread of hybrid cultivar Konto (adapted from Cyran and Saulnier[28]).

Proton Nuclear Magnetic Resonance Spectroscopy. Figure 2 shows the ^{1}H NMR spectra of parent WE fractions and their ammonium sulfate precipitated subfractions isolated from endosperm bread made from lines L_1 and L_2 with low and high AX-dependent extract viscosity, respectively. Obviously, much stronger signal intensities of anomeric protons assigned to Ara*f* linked to *O*-2 and *O*-3 of the same Xyl*p* (δ 5.21 and

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Table 3. Weight-Average Molecular Weight (M_w), Polydispersity Index (M_w/M_n), and Intrinsic Viscosity ([η]) of Water-Extractable Populations with High Molecular Weight Isolated from Endosperm and Whole-Meal Breads Made from Rye Inbred Lines L_1, L_2, and L_6

	L_1	L_2	L_6
endosperm bread			
M_w (10^5g mol^{-1})	3.475 ± 0.270	5.172 ± 0.250	4.445 ± 0.150
M_w/M_n	1.752 ± 0.111	1.537 ± 0.094	1.712 ± 0.136
[η] (mL g^{-1})	243	467	404
wholemeal bread			
M_w (10^5 g mol^{-1})	3.300 ± 0.300	4.572 ± 0.220	4.112 ± 0.280
M_w/M_n	1.813 ± 0.098	1.651 ± 0.115	2.205 ± 0.075
[η] (mL g^{-1})	223	427	371

5.28, respectively)[39,40] were found in the spectra of parent fraction isolated from endosperm bread of rye line L_1 than in that of L_2. The same relationship could be observed in the spectra of AX-I subfractions, although these resonances were of very low intensities when compared with those of Ara*f* linked to *O*-3 of Xyl*p* (δ 5.39).[39,40] A close examination of the WE.60 spectra revealed the small splitting peaks at δ 5.23 and 5.29 in L_1 spectrum and their absence in L_2, suggesting some differences in the distribution of the double-substituted Xyl*p* in the AX chains; that is, two adjacent double-substituted Xyl*p* were more abundant in the former spectrum.[40] Besides, the spectra of the WE fraction and WE.100 subfraction from low-viscosity bread made from rye line L_1 were characterized by a strong resonance at δ 4.75, assigned to anomeric protons of nonacetylated 1→4-linked β-D-mannopyranosyl residues.[41,42] This was also in line with their exceptionally high mannose content in these isolates (10% for WE and WE.100) (Table 2) than in the corresponding fractions and subfractions from rye lines L_2 and L_6 (1−3%).

The mannose-containing polysaccharides, however, exclusively coprecipitated with the AX-III subfraction at 100% ammonium sulfate saturation, whereas arabinoxylan subfractions AX-I and AX-II, precipitated at 60 and 80% salt saturation, respectively, were associated with a mixed-linkage (1→3),(1→4)-β-D-glucan, as indicated by a small doublet at δ 4.74 and 4.75.[43] Nevertheless, an appreciable amount of glucose found in WE.60 from endosperm bread of L_1 (Table 2) does not correspond to very low signal intensities of (1→3),(1→4)-β-D-glucan anomeric proton signals observed in the ^{1}H NMR spectrum of this subfraction (Figure 2). This may imply the presence of other polysaccharides built of β-D-glucopyranosyl residues, presumably 1→4-linked, appearing at δ 4.54[41,42] and overlapped with high intensity signals of Xyl*p* anomeric protons (δ 4.48−4.66).[40] The distinctly high levels of mannose and glucose have been reported previously in WE nonstarch polysaccharide fractions isolated from the outer crust of white wheat bread.[42]

Apart from the mannose- and glucose-containing polysaccharides, the presence of other components, as indicated by the exceptionally strong resonance peaks of acetyl groups at δ 2.0−2.2,[44,45] methyl and methylene groups at δ 0.9 and 2.37 and aromatic rings at δ 7.27, 7.32, and 7.37 (not shown) differentiated well the WE.60 spectrum of L_1 from that of L_2. This may, to some extent, explain the relatively low polysaccharide contents found in the WE fraction and subfractions isolated from breads made from rye line L_1 (Table 2).

Evidently, the spectra of WE fractions and their WE.100 subfractions isolated from endosperm bread of rye inbred lines did not display any obvious resonances at δ 5.31 assigned to anomeric protons of Ara*f* linked to *O*-2 of the Xyl*p*, exceeding that of Ara*f* linked to *O*-3 of the doubly substituted Xyl*p* (δ 5.28),[46,47] in contrast to those made from hybrid and population rye cultivars reported in the preceding article.[28]

The relative proportions of un-, mono-, and disubstituted Xyl*p* (un-Xyl*p*, 3-Xyl*p*, 2-Xyl*p* and 2,3-Xyl*p*, respectively) in the AX backbones present in the fractions and subfractions from endosperm breads of L_1 and L_2 were calculated on the basis of the signal integration of the anomeric protons of Ara*f* linked to mono- and disubstituted Xyl*p* in the ^{1}H NMR spectra and the sugar analysis (Table 4). The relative ratio of 2-Xyl*p* was calculated on the basis of the differences in the signal intensities between the two peaks corresponding to the anomeric proton resonances of terminal Ara*f* linked to *O*-2 and *O*-3 of the same Xyl*p* (δ 5.21 and 5.28), as the anomeric protons of Ara*f* linked to *O*-2 of Xyl*p* (δ 5.31) overlap with that of Ara*f* linked to *O*-3 of the doubly substituted Xyl*p* (δ 5.28).[48] The chains of parent AX fraction isolated from endosperm bread of L_2 and its AX-I subfraction were enriched in the 3-Xyl*p*, whereas the level of 2,3-Xyl*p* in the parent AX fraction of L_1 was almost 2 times higher than that of L_2. Generally, it was due to a 3 times higher level of 2,3-Xyl*p* in its AX-I subfraction. Even though, the levels of doubly substituted Xyl*p* in the AX-III subfractions of endosperm breads made of rye lines L_1 and L_2 (38 and 35%, respectively) were much lower than those reported for hybrid and population rye cultivars (on average, 43 and 49%, respectively).[28] However, the most striking differences in the AX substitution pattern between lines and cultivars of rye were observed in the level of 2-Xyl*p*. The WE AXs present in endosperm bread made from rye cultivars had a 2−3 times higher proportion of 2-Xyl*p* (~8%) in the backbone, when compared with those of inbred lines (2−4%). In addition, they had markedly lower ratios of 2,3-Xyl*p*/2-Xyl*p* observed in WE fraction and AX-III subfraction (1.4−1.6 and 3.5−4.0, respectively) than those of the lines (3.8−4.0 and 6.5−6.8), indicating diversity in a substitution pattern of their chains.

It has been evidenced that in the case of bread made from rye cultivars the relative proportions of both 2-Xyl*p* and 2,3-Xyl*p* and the ratio of 2,3-Xylp/2-Xylp in the AX-III backbone, along with the level of dominating AX-I subfraction, are the key factors for the viscous potential of the bread.[28]

The breads made from rye inbred lines exhibited large variation in the AX-dependent extract viscosity, reflecting their ability to increase the digesta viscosity in the small intestine after their ingestion and, consequently, the specific health potential of components for breeding of rye cultivars with such characteristics. Despite lower WE AX contents, the endosperm breads generally showed AX-dependent extract viscosities

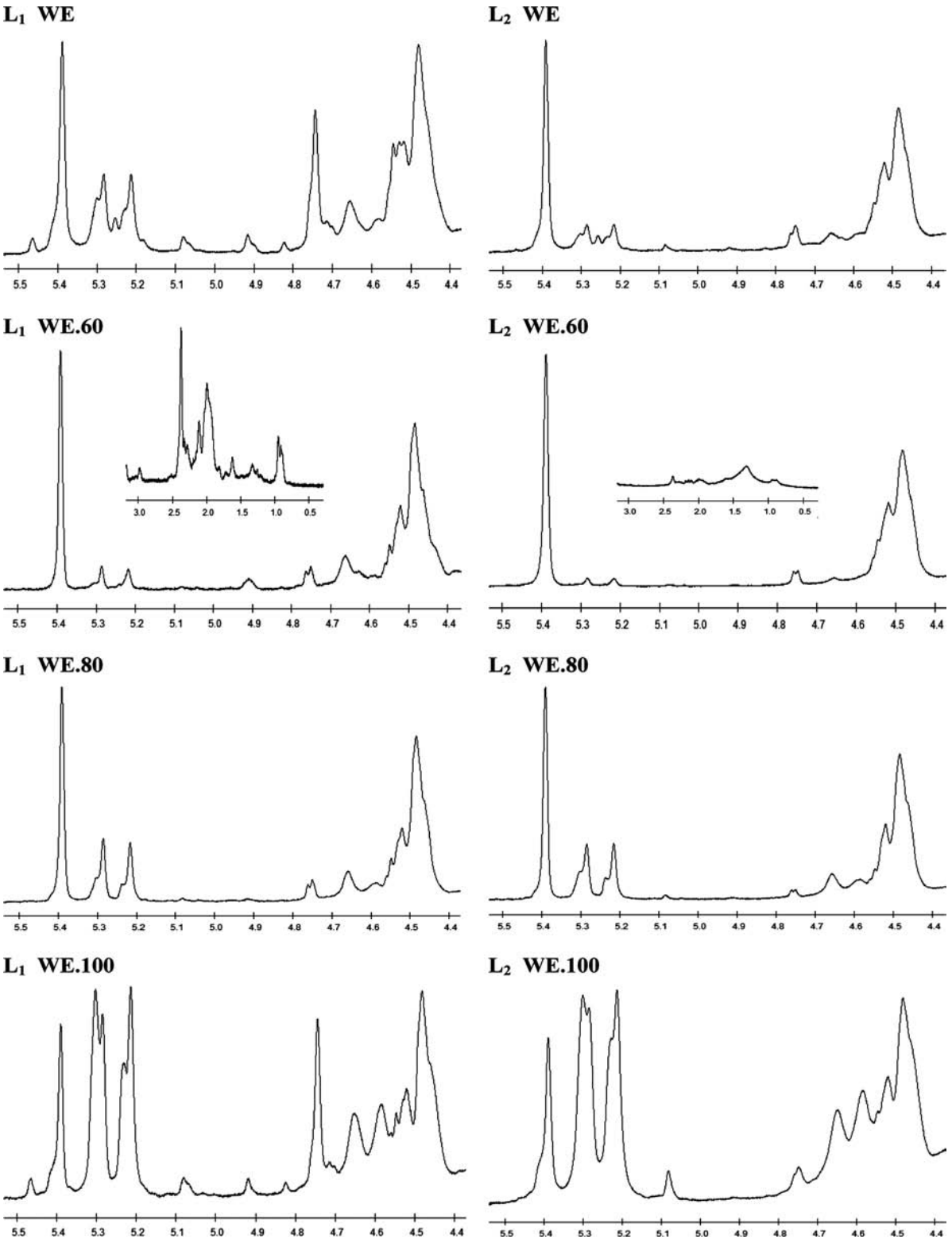

Figure 2. [1]H NMR spectra of arabinoxylans present in the parent water-extractable fractions and their subfractions isolated from endosperm breads made from rye inbred lines L_1 and L_2.

similar to those of corresponding whole-meal breads. This may be explained by, at least, a 2 times lower level of endoxylanase activity found in the endosperm flours than that in whole meals, which resulted in less intensive degradation of WE AXs in endosperm bread, evidenced by a slightly higher M_w and intrinsic viscosity of their HMW populations with lower polydispersity index. The high WE AX content in rye lines is prerequisite to obtaining the high-viscosity bread; however, it does not ensure the high value of this parameter. Among the factors controlling AX-dependent extract viscosity of rye bread, the degree of WE AXs breakdown during breadmaking also is particularly important, as an intensive degradation of these

Table 4. Relative Percentage of Un-, Mono-, and Disubstituted Xylose Residues (u-Xyl*p*, 3-Xyl*p*, 2-Xyl*p*, and 2,3-Xyl*p*) of WE AX Fractions and Subfractions Isolated from Endosperm Bread Made of Rye Inbred Lines L_1 and L_2 and Their Ratios of Substituted to Unsubstituted Xylose Residues (Sub/Unsub), 2,3-Xyl*p* to 3-Xyl*p* (2,3-Xyl*p*/3-Xyl*p*) and 2,3-Xyl*p* to 2-Xyl*p* (2,3-Xyl*p*/2-Xyl*p*)[a]

	L_1				L_2			
	WE AX	AX-I	AX-II	AX-III	WE AX	AX-I	AX-II	AX-III
u-Xyl*p*	46.9	55.5	52.5	40.5	50.8	53.9	49.9	37.6
3-Xyl*p*	32.0	37.0	32.6	17.4	38.1	44.0	34.2	19.3
2-Xyl*p*	4.2	0.1	1.4	5.6	2.3	0.1	1.0	5.5
2,3-Xyl*p*	16.9	7.4	13.5	36.5	8.8	2.0	14.9	37.6
sub/unsub	1.1	0.8	0.9	1.5	1.0	0.9	1.0	1.7
2,3-Xyl*p*/3-Xyl*p*	0.5	0.2	0.4	2.1	0.2	0.1	0.4	2.0
2,3-Xyl*p*/2-Xyl*p*	4.0		9.6	6.5	3.8		14.9	6.8

[a]AX-I, the arabinoxylan subfraction precipitated from WE AX solution at 40-60% ammonium sulfate saturation level; AX-II, the arabinoxylan subfraction precipitated from WE AX solution at 60-80% ammonium sulfate saturation level; AX-III, the arabinoxylan subfraction precipitated from WE AX solution at 80−100% ammonium sulfate saturation level.

polysaccharides may significantly limit the viscous potential of bread, even that with high WE AX level.

This study provided evidence that the proportion of 2-Xyl*p* in the WE AX backbones of breads made from rye inbred lines is much lower than in those from rye cultivars. Also, the most branched AX-III subfractions present in these breads are poorer in both 2-Xyl*p* and 2,3-Xyl*p* in comparison to those from rye cultivars. These differences in the fine structure of WE AXs may explain the relatively higher extent of their breakdown in bread produced from rye lines. However, it is not known whether the increased level of 2-Xyl*p* in the WE AX chain of rye cultivars results from a genetic background of the starting female and/or male components used for breeding of hybrid rye cultivars or from some other factors. Further study is needed to clarify this issue and gain new knowledge that could be used to support breeding strategies in the future.

Breeding of hybrid rye cultivars suitable for the production of bread with specific health benefits, related to increased digesta viscosity in the human small intestine after it ingestion, should be based on the appropriate components, both female and male, as well as their subsequent progenies, preliminarily selected in terms of their high overall extract viscosity. Nevertheless, further baking of test breads is necessary to verify their viscous potential, as the overall extract viscosity test of starting flours is not able to make a distinction between rye lines with high WE AX content with respect to resulting bread viscosity.

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Notes

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ABBREVIATIONS USED

Ara*f*, α-L-arabinofuranosyl residue; Ara/Xyl ratio, arabinose to xylose ratio; AX, arabinoxylan; DF, dietary fiber; HMW, high molecular weight; M_w, weight-average molecular weight; NCP, noncellulosic polysaccharides; WE, water-extractable fraction; WE AX, water-extractable arabinoxylan; WU, water-unextractable fraction; WU AX, water-unextractable arabinoxylan; Xyl*p*, β-D-xylopyranosyl residue.

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