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Association and Structural Diversity of Hemicelluloses in the Cell Walls of Rye Outer Layers: Comparison between Two Ryes with Opposite Breadmaking Quality

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Looking for potential quality indicators, which could be used in early selection of breeding materials, the structural features of cell wall arabinoxylans (AX) from outer layers of the grain (pooled shorts and bran fractions) were studied in two ryes with diverse breadmaking quality. The successive alkaline extraction of water-unextractable material with saturated $Ba(OH)_2$, followed by water and 1 and 4 M NaOH, resulted in four purified fractions, Ba, BaH, 1Na, and 4Na, respectively, that became water soluble after their isolation. The AX present in these fractions constituted ∼43, 12, 14, and 4% of their total amount recovered. Moreover, two xylan-enriched fractions, 1Na.P and 4Na.P (arabinose-to-xylose ratios, Ara/Xyl, of 0.07 and 0.19, respectively), were self-precipitated from both NaOH-extractable fractions. Polysaccharides of these fractions, containing mainly xylose, represented ∼16 and 1% of AX recovered. In the BaH and 1Na, AX coexisted with β-glucans, which predominated in the former protein-free fraction. On the contrary, hemicelluloses in the 1Na fraction were associated with protein as well. Further fractionation of the water-soluble materials by ammonium sulfate revealed that the parent AX populations in the Ba, BaH, and 1Na were composed of 3−4 subfractions with different degrees of substitution (Ara/Xyl of ∼0.4, 0.8, and 1.1), whereas 4Na was almost totally built of highly substituted AX (Ara/Xyl of 1.1). Despite a comparable proportion of un-, mono-, and disubstituted xylopyranosyl residues in the chain of $Ba(OH)_2$-extractable AX isolated from both ryes, the ^{1}H NMR and Fourier transform infrared demonstrated the marked differences in their spectral profiles, suggesting different substitution patterns of these dominating polysaccharides. The high molecular weight population present in the Ba fraction also differentiated well two ryes with opposite breadmaking quality.

KEYWORDS: Rye outer layers; hemicelluloses; fractionation; arabinoxylans; structural features

INTRODUCTION

In general, the cell walls of cereals consist of polysaccharides associated with minor components like proteins, phenolics, and, to a certain extent, minerals. Hemicelluloses, mainly AX and β-glucans surrounding the cellulose microfibrils as an amorphous matrix, represent a major group of cell wall polysaccharides in rye. They can be extracted by alkaline solutions that cleave predominantly ester and hydrogen linkages by which the different polymers are interconnected in the cell wall network, leaving cellulose microfibrils in the alkali-unextractable residue (*1*). Thus, the hemicelluloses released from water-unextractable material (WUM) by alkaline treatment are usually water soluble after their purification. However, in some cases when specific structural features allow their self-association, the aggregates formed during neutralization and dialysis are readily precipitated from aqueous solutions (*2*).

The structure of AX, the most important cell wall polysaccharides of rye, is highly variable. Its principal categorization is based on the ratio of side α-L-arabinofuranosyl (Ara*f*) branches to (1→4) linked β-D-xylopyranosyl (Xyl*p*) residues of a linear chain backbone (arabinose-to-xylose ratio, Ara/Xyl ratio). The Ara/Xyl ratios of the entire AX present in rye grain vary from 0.59 to 0.69. A similar range (from 0.56 to 0.68) was reported for the water-unextractable (WU) AX fraction, whereas the water-extractable (WE) polymers exhibited much higher variation in the Ara/Xyl ratio, which ranged from 0.58 to 0.85 (*3*). This parameter, however, usually expresses an average degree of arabinosylation of the overall AX population that is, in fact, composed of a range of AX subfractions with different proportions of substituted and unsubstituted xylopyranosyl units (u-Xyl*p*) (*4*, *5*). The WE-AX subfractions isolated from rye grain by graded ammonium sulfate precipitation had

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Ara/Xyl ratios in the range of 0.31−1.28 (*6*). A slightly wider range of this characteristic has been observed for WU-AX sequentially extracted by alkaline treatments (0.07−1.27) (*2*).

The structural diversity of AX is also related to both site and type of substitution by terminal Ara*f*. With respect to this, the Xyl*p* units occur as monosubstituted at *O*-3 (3-Xyl*p*) and, to some extent, at *O*-2 (2-Xyl*p*); besides, disubstituted ones at both *O*-2 and *O*-3 positions (2,3-Xyl*p*) are also abundant. Generally, the substituted Xyl*p* is present as one or two adjacent units isolated by u-Xyl*p* (*7*, *8*). However, somewhat longer blocks containing 3−4 residues of 2,3-Xyl*p* in succession have been found in WE-AX of rye (*9*). Whereas for wheat, they were identified in the structure of both WE- and WU-AX (*10*−*12*). Apart from single Ara*f* units, being the major side substituents of the xylan chain, some of them can form di- and trimeric side chains interlinked via (1→2), (1→3), or (1→5) linkages. These short chains of Ara*f* along with glucuronic acid (GlcA) residues and their 4-*O*-methylesters, acetyl, and phenolic acids groups are the minor substituents of AX. Furthermore, AX displays a wide distribution of molecular weights, which is also a significant element of their complex structure (*13*−*15*).

It is generally accepted that differences in AX structure, even relatively small, can result in changes of chain conformation and intermolecular association, which may have an impact on functionality of these polysaccharides. In rye breadmaking, starch and AX, the major water-binding components, control the water economy in dough and, consequently, have a strong influence on the quality of baked products. The relationships between the structure of rye WE-AX and their physicochemical and functional properties have been an interest of focus among cereal chemists (*9*, *16*−*18*), although it was much smaller in comparison to that in the most investigated wheat polymers. Nevertheless, for predominating WU-AX, such investigations are considerably limited, since before characterization they must be isolated by a relatively mild procedure, ensuring their minimum physical and chemical changes as well as an acceptable extraction efficiency. A lack of the consistent procedure, adapted for each type of material, is one of the important reasons that the structure of WU-AX is still not completely elucidated. On the other hand, their comparatively larger structural diversity than that of WE polymers, especially in the hemicellulose populations extracted by stronger alkaline treatments, is recognized as well (*2*).

Usually, sodium hydroxide solutions of different molar concentrations (1−4 M) have been used for extraction of WU polysaccharides from rye whole meal (*19*−*21*). Furthermore, an aqueous 1% ammonium hydroxide was introduced as an extractant for rye bran AX (*13*, *22*). Recently, saturated Ba(OH)$_2$ solution followed by water, 1 M KOH, and 4 M NaOH was used for sequential extraction of hemicelluloses from wheat and rye grain (*15*, *23*). More recently, after two first sequential extractions with saturated Ba(OH)$_2$ and water, Nilsson et al. (*2*) used 4 M KOH and water followed by 2 M KOH in a boiling water bath for extraction of WU polysaccharides from three milling fractions of rye grain. They distinguished AX populations differing in the degree of substitution with Ara*f*, indicated by their Ara/Xyl ratios. The authors concluded that only the relative distribution of certain structures differs in the above extracts, since, in general, the same signals were present in their proton nuclear magnetic resonance (^{1}H NMR) spectra. Up to now, however, such structures in the outer layers of rye have not been identified; their relative proportions in the alkali-extractable fractions are not known either. It is obvious that additional fractionation of each alkaline extract would be helpful in better characterization of these polysaccharides, making progress in our understanding of the cell wall organization. It has already been done for hemicelluloses originating from the central part of rye endosperm (*24*).

The present work, therefore, was undertaken to gain a new knowledge on the structure of hemicelluloses from the rye outer layers by applying the ammonium sulfate precipitation technique in the sequentially extracted alkali-extractable cell wall materials, and further structural characterization of more homogeneous subfractions were obtained. Moreover, in this investigation, we have compared the fine structure of hemicellulosic subfractions isolated from high and low breadmaking quality ryes to check if any notable differences exist between them.

MATERIALS AND METHODS

Consecutive Alkaline Extraction of WUM. The WUMs left from outer layers (combined shorts and bran fractions) of two Polish rye cultivars, Amilo and Nawid, with high and low breadmaking quality, respectively, after sequential water extraction with α-amylase and proteinase K (*6*) constituted a starting material for the extraction. The procedure of alkaline extraction was based partially on the method described by Gruppen et al. (*23*) for wheat hemicelluloses. The duplicate samples (20 g) of WUM were suspended in saturated Ba(OH)$_2$ (1:50 w/v) containing 1% (w/v) $NaBH_4$ using an Ultraturrax (IKA Labortechnik, Staufen, Germany), stirred continuously for 16 h at room temperature, and centrifuged (10000*g*, 20 min, room temperature). The residue was again mixed with the same extractant (500 mL), stirred for 4 h at room temperature, and centrifuged (10000*g*, 20 min). The combined supernatants were neutralized with glacial acetic acid, purified (as described below), and designated Ba. The remaining residue was suspended in deionized water (500 mL), treated with the Ultraturrax, stirred continuously for 16 h at room temperature, and centrifuged (10000*g*, 20 min). The pellet was re-extracted with water for 4 h at room temperature and centrifuged (10000*g*, 20 min). Both supernatants were combined, purified (as described below), and designated BaH. The residue, left after double extraction with saturated Ba(OH)$_2$ and double water extraction, was suspended in 1 M NaOH (500 mL) containing 0.1% (w/v) $NaBH_4$ using an Ultraturrax, stirred continuously for 16 h at room temperature, and centrifuged (10000*g*, 20 min). After a second extraction with 1 M NaOH for 4 h at room temperature and centrifugation, the supernatants were combined, neutralized with glacial acetic acid, purified (as described below), and designated 1Na. The last fraction, designated 4Na, was obtained by suspending the residue left after 1 M NaOH extraction in 4 M NaOH containing 0.1% (w/v) $NaBH_4$. All steps of the last alkaline treatment were conducted in the same way as described above.

Purification of AE Materials. The crude alkaline extracts (Ba, BaH, 1Na, and 4Na) were incubated with amyloglucosidase from *Aspergillus niger* (0.5 mL) (EC 3.2.1.3, Roche Diagnostics) and proteinase K from *T. album* (0.5 mL, 600 units/mL) (EC 3.4.21.64, Roche Diagnostics) at 40 °C overnight (pH 4.8, 0.03% NaN_3, to digest any residual starch and protein). After heat treatment (95 °C, 20 min) and centrifugation (10000*g*, 20 min), the supernatants were dialyzed against deionized water at 6 °C for 72 h using membrane tubing (M_W cutoff of 12000; Sigma-Aldrich). All precipitates formed during dialysis were separated by centrifugation (10000*g*, 20 min) and referred to as Ba.P, BaH.P, 1Na.P, and 4Na.P for materials sequentially isolated by saturated Ba(OH)$_2$, water, and 1 and 4 M NaOH, respectively.

Fractionation of Ba, BaH, 1Na, and 4Na. Aliquots of the purified alkaline extracts (200−600 mL) were freeze-dried. The remaining portions of the extracts were fractionated by graded ammonium sulfate precipitation, as previously described (*25*). Three subfractions were isolated from Ba at 60, 80, and 100% saturation level and denoted Ba.60, Ba.80, and Ba.100, respectively, for the saturation level of $(NH_4)_2SO_4$ at which subfractions were collected. Similarly, three or four subfractions were obtained from each of the remaining AE fractions (BaH, 1Na, and 4Na). They were designated in the same way.

Chemical Analyses. Moisture and ash contents were analyzed by AACC methods 44.15A and 46.11A, respectively (*26*). Protein content

Table 1. Yield and Composition (g/100 g of dm) of WUM Isolated from Rye Outer Layers[a]

	yield[b]	protein	ash	Klason lignin	polysaccharides[c]	Ara	Xyl	Man	Gal	Glc[d]	Cell	UA	Ara/Xyl
Amilo	31.3	13.4	2.54	9.5	57.5	14.8	26.6	1.1	1.9	12.6 (10.2)	6.9	0.9	0.56
Nawid	29.8	12.7	2.58	10.5	59.2	15.0	27.2	1.3	1.9	13.3 (10.4)	7.0	1.0	0.55

[a] Abbreviations: Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose, Glc, noncellulosic glucose; Cell, cellulosic glucose; and UA, uronic acids. [b] Expressed as weight percentage (dm) of rye outer layers. [c] Calculated as the sum of the experimentally obtained values for the constituent monosaccharides after their conversion to polysaccharides by using factors of 0.88 for pentoses and 0.9 for hexoses. [d] Values in parentheses represent β-glucan. Results were obtained from duplicates, and the coefficient of variation was less than 5%.

($N \times 6.25$) was determined by the Kjeldahl method using a Kjeltec Auto 1030 Analyzer (Tecator, Höganäs, Sweden). Starch was assayed enzymically according to the colorimetric method of Bach Knudsen et al. (*27*). The β-glucan content was evaluated according to the enzymatic method of McCleary and Glennie-Holmes (*28*). Klason lignin was measured gravimetrically as the residue left after two-step acid hydrolysis [72% (w/w) H_2SO_4, 1 h, 35 °C; 1 M H_2SO_4, 3 h, 100 °C] and corrected for ash (*29*). The content of uronic acids was determined by using the method of Scott (*30*). The monosaccharide composition was estimated by gas chromatography of alditol acetates obtained after hydrolysis with 1 M H_2SO_4 (2 h, 100 °C) (*31*). Alditol acetates were separated on a wide bore Rtx capillary column (30 m; 0.53 mm i.d.; Restek, Bellefonte, PA) in a Hewlett-Packard model 5890 Series II Plus gas chromatograph (Waldbronn, Germany) equipped with a flame ionization detector. The column was heated at 190 °C for 2 min, and then, the temperature program was 190−220 °C at 5 °C/min and 220 °C for 5 min. Output signals were collected and integrated by ChemStation software (Hewlett-Packard) using β-D-allose (Sigma-Aldrich) as the internal standard. The cellulose content was calculated as the difference between amounts of glucose determined with and without pretreatment with 72% (w/w) H_2SO_4 (*32*). The constituent sugars were expressed as polysaccharides, using polymerization factors of 0.88 for pentoses and 0.90 for hexoses. Phenolic acids, ferulic acid dehydrodimers, and dehydrotrimer were determined in the cell wall fractions after alkaline extraction under argon with 2 M NaOH at 35 °C for 30 min. The solution of 3,4,5-tri-methoxy-*(E)*-cinnamic acid (TMCA, Sigma-Aldrich) was added as an internal standard. Maize bran samples, included in each set of samples, were used as a reference material for ferulic acid dehydrodimers and dehydrotrimer analysis (*33*, *34*). The mixture was acidified to pH 2 with 2 M HCl and extracted with diethyl ether. Ether phases were evaporated to dryness, dissolved in MeOH/H_2O (50:50, v/v), and analyzed by high-performance liquid chromatography (HPLC) as described previously (*33*).

^{1}H NMR Spectroscopy. Cell wall fractions and subfractions were dissolved in D_2O (99.8%) with overnight stirring at room temperature and freeze-dried. Deuterium exchange was repeated once, and finally, material was redissolved in D_2O (10−20 mg/mL). ^{1}H NMR spectra (400 MHz) were recorded at 60 °C on a Bruker ARX spectrometer (Karlsruhe, Germany). Acetone was used as a standard (δ2.23 ppm).

High-Performance Size-Exclusion Chromatography (HPSEC) with Triple Detection. Samples were dissolved in 50 mM $NaNO_3$, containing 0.02% NaN_3 (5 mg/mL), with continuous stirring at room temperature, filtered over a 0.45 μm membrane, and injected at 25 °C on a HPSEC. The system was comprised of two Shodex OH-pak SB HQ 804 and 805 columns (Showa Denko K. K., Tokyo, Japan) eluted at 0.7 mL/min with 50 mM $NaNO_3$, containing 0.02% NaN_3. On-line molar mass analysis was performed using a multiangle laser light scattering (MALLS) detector (mini-Dawn, Wyatt Technology, Santa Barbara, CA, operating at three angles: 41, 90, and 138°), a differential refractometer (ERC 7517 A) ($dn/dc = 0.146$ mL/g), and a UV detector ($\lambda = 280$ nm). The 1.4 Astra software (Wyatt Technology) was used for data collection and calculations.

Fourier Transform Infrared Spectroscopy (FTIR) Spectroscopy. Samples were dissolved in deionized water at room temperature. Aliquots of the solutions (0.5 mL) were placed on a Teflon plate and dried in the oven at 40 °C. Dry films were removed from the plate and mounted in the atmosphere-controlled chamber of a Vector 22 Bruker spectrometer equipped with a DTGS detector. FTIR spectra were recorded in duplicate between 400 and 4000 cm^{-1} at 4 cm^{-1} resolution. Background spectra were taken in the empty chamber.

RESULTS AND DISCUSSION

Yield and Composition of WUM and AE Fractions. The WUMs obtained after sequential extraction with α-amylase and proteinase K constituted 31 and 30% of rye outer layers, for Amilo and Nawid, respectively (**Table 1**). These values are in accordance with the yield of WUMs previously reported for bran and intermediate milling fractions of rye grain (*2*). Polysaccharides, the major components of WUM, represented 58 and 59% of this fraction. They were mainly built of AX (64%, on average for both ryes), β-glucans (16%), and cellulose (11%). In addition to polysaccharides, the WUM contained substantial amounts of protein and Klason lignin. A very low level of residual starch was also detected (less than 0.5%, results not shown).

With successive alkaline treatment, four water-soluble AX fractions (Ba, BaH, 1Na, and 4Na) and two xylan precipitates (1Na.P and 4Na.P) were isolated from WUM of both rye samples (**Table 2**). The precipitates obtained during dialysis of the first two extracts, Ba and BaH, were not taken into consideration, since they represented a very low proportion of the material recovered with a trace level of polysaccharides (less than 4%). AX in the water-soluble hemicellulosic fractions represented 72% of their total amount recovered after extraction. Of the water-soluble AX, 57−60% was extracted with $Ba(OH)_2$. They had the lowest Ara/Xyl ratios in the Ba (0.61 and 0.62) and the highest in the 4Na fraction (0.93 and 1.00 for Amilo and Nawid, respectively), whereas the polymers present in the BaH and 1Na fractions were characterized by intermediate Ara/Xyl ratios ranging from 0.70 to 0.77. Two xylans with exceptionally low degree of arabinosylation, indicated by their Ara/Xyl ratios (0.07 and 0.18−0.20, respectively for 1Na.P and 4Na.P of both ryes), represented 18% of AX isolated. However, of the recovered xylans, 94% was precipitated from 1 M NaOH solution (1Na.P). As could be expected, AX in the Ba fraction constituted about 92% of polysaccharides. Although β-glucans dominated in the polysaccharide fraction from BaH (57−52%) (as revealed by content of noncellulosic glucose in **Table 2** and ^{1}H NMR, results not shown), the AX still represented a significant proportion of this fraction (39−43%). In opposite, the polysaccharide populations in 1Na were composed of 66−68% AX and 32−28% β-glucans. Unlike the first three hemicellulosic fractions, the fourth one (4Na) contained AX (74−78%) as well as some amount of cellulose (13−15%), while the highest proportion of cellulose (73%) was found in the material precipitated from 4 M NaOH solution (4Na.P). Its coexistence with xylan having a very low degree of branching (Ara/Xyl, 0.2) can be easily explained by their well-known ability to form an intermolecular association via hydrogen bonds between unsubstituted regions of the backbone in both polymers. The presence of cellulose only in the fractions released from

Table 2. Yield and Composition (%) of Cell Wall Fractions Isolated from WUM by Successive Alkaline Treatment and Unextractable Residue (Res)[a]

fraction	yield[b]	AX[c]	protein	Klason lignin	polysaccharides[d]	Ara	Xyl	Man	Gal	Glc[e]	Cell	Ara/Xyl
					Amilo							
Ba	20.7	43	7.2	0.8	72.1	28.6	46.8	0.5	1.3	4.6	ND	0.61
Ba.P	3.3				tr							
BaH	12.8	12	1.4	1.0	73.3	14.0	18.3	1.5	1.2	47.2	ND	0.77
BaH.P	2.0				tr							
1Na	10.6	13	11.8	6.4	57.6	17.6	25.0	1.0	0.6	20.7	ND	0.70
1Na.P	9.3	17	15.2	9.0	58.0	4.5	60.3	ND	ND	1.1	ND	0.07
4Na	3.5	4	2.0	NA	48.2	19.3	20.7	1.6	1.3	3.4	8.1	0.93
4Na.P	3.9	1	1.0	7.9	64.7	1.8	10.2	1.2	0.6	5.9	52.4	0.18
Res	12.2	10	1.9	13.3	55.5	16.8	14.0	ND	0.8	3.4	27.4	1.20
					Nawid							
Ba	21.6	42	9.2	0.9	70.3	27.9	45.2	0.5	0.9	5.2	ND	0.62
Ba.P	3.2				tr							
BaH	14.8	13	1.0	1.2	71.1	14.4	19.6	1.8	2.1	41.8	ND	0.73
BaH.P	2.4				tr							
1Na	15.0	15	12.9	7.5	48.0	16.0	21.0	1.0	1.0	15.2	ND	0.76
1Na.P	7.1	15	6.3	9.9	71.3	5.4	73.3	ND	ND	2.3	ND	0.07
4Na	3.4	4	3.1	NA	50.1	22.0	21.9	1.1	0.7	3.4	7.5	1.00
4Na.P	4.7	1	1.1	8.5	66.7	1.9	9.2	1.8	0.5	6.7	54.2	0.20
Res	11.6	10	1.9	14.0	52.7	16.8	13.8	ND	0.7	3.4	24.5	1.22

[a] Abbreviations: Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose, Glc, noncellulosic glucose; Cell, cellulosic glucose; NA, not analyzed; ND, not detected; and tr, traces. [b] Expressed as weight percentage of WUM. [c] On the basis of the amount of material recovered after extraction. All analysis was done at least in duplicate, and the coefficient of variation was less than 5%. [d] Calculated as the sum of the experimentally obtained values for the constituent monosaccharides after their conversion to polysaccharides by using factors of 0.88 for pentoses and 0.9 for hexoses.

WUM by 4 M NaOH treatment suggests that some AX are more closely associated with cellulose microfibrils, and for their extraction, much stronger alkaline conditions are required than those provided by saturated $Ba(OH)_2$ and 1 M NaOH. In addition to polysaccharides, the precipitated fractions, 1Na.P and 4Na.P, contained an appreciable amount of Klason lignin. Their level of protein, however, well-differentiated both fractions; the latter xylan represented almost a protein-free hemicellulosic fraction. It is worth noting that the fractions obtained by prolonged barium hydroxide extraction still contained some amounts of simple phenolics (396 and 443 μg g^{-1} for Amilo and Nawid, respectively) (results not shown). Interestingly, a ferulic acid dehydrotrimer (8-O-4′, 5-5′), its four dehydrodimers (8-O-4′, 8-5′, 5-5′, and 8-5′ benzofuran form) and ferulic acid constituted 44, 41, and 14% of total phenolics, respectively (on average for both rye samples). A minute amount of *p*-coumaric and sinapic acids was also detected in this fraction. Such a high proportion of tri- and diferulates that survived a relatively weak alkaline treatment may point to a higher resistance of tri- and dimeric ester cross-links in comparison to a single ester linkages between phenolic acids and Ara*f* in AX, since it is known that the level of this acid in cereals is appreciably higher than those of its dehyrodimers. As could be expected, no simple phenolics were detected in the other fractions obtained by much stronger alkaline treatments.

AX present in the residue left after alkaline extraction accounted for 10% of AX recovered. They displayed a much higher degree of substitution with Ara*f* (Ara/Xyl of 1.20 and 1.22) than the highly branched polymers extracted with 4 M NaOH.

Higher contents of protein and Klason lignin were detected in all corresponding fractions originating from rye with inferior breadmaking quality (Nawid), when compared with those from rye with superior breadmaking quality (Amilo). A similar trend was observed for the Ara/Xyl ratio between both sets of fractions. Except for the AX present in BaH, the remaining polymers isolated from Nawid were characterized by slightly higher Ara/Xyl ratios, indicating their higher degree of substitution, in comparison to those from Amilo.

Subfractions Obtained by Ammonium Sulfate Precipitation. For detailed structural characterization of the parent hemicellulosic fractions, 3−4 more homogeneous subfractions were isolated from each of them at 40, 60, 80, and 100% ammonium sulfate saturation. Their yields and chemical compositions are summarized in **Table 3**. It is evident that for the two first hemicellulosic fractions, Ba and BaH, as well as for 1Na from Amilo, the major subfractions were precipitated at 60% salt saturation and no material could be collected at 40% saturation. There was an increase in the proportion of hemicellulosic subfractions isolated at the highest saturation level, 100%, observed from Ba and BaH to 4Na fractions of both ryes. The analogous tendency, however much clear, was found in the proportions of AX with the highest degree of substitution (Ara/Xyl ∼1.0) that precipitated at the highest level of ammonium sulfate. Thus, the AX in the Ba.100 and BaH.100 represented 12−17 and 28−31% of total population recovered from Ba and BaH fractions, respectively. Their proportion increased up to 86 and 89% of the total AX recovered from 4Na fraction, for Amilo and Nawid. This holds also for 1Na fraction, in which the highest differences in the proportion of highly branched AX were revealed between both rye samples. For Nawid, however, the AX with the highest Ara/Xyl ratios of 0.90 and 0.98 was precipitated from the 1Na fraction at both 80 and 100% of salt saturation. They constituted 59% of the AX recovered from this fraction. Instead, for Amilo, such polymers were isolated only at the 100% saturation and represented 34% of the total population containing also AX with intermediate degree of substitution (Ara/Xyl, 0.78), which could not be precipitated from Nawid. Clearly, the intermediately branched AX (Ara/Xyl, 0.7−0.8) occurred in the parent Ba and BaH fractions, whereas the 4Na fraction was practically free of them. It contained a relatively low proportion of AX with somewhat lower Ara/Xyl ratio (0.47−0.57) that precipitated at three different saturation levels: 40, 60, and 80%.

Table 3. Yield of Subfractions Obtained from Alkaline Extracts by Ammonium Sulfate Precipitation at Different Saturation Levels (40, 60, 80, and 100%) and Their Distribution of AX, Ara/Xyl, and Contents of Polysaccharides and Protein

	Ba			BaH			1Na				4Na			
	60	80	100	60	80	100	40	60	80	100	40	60	80	100
							Amilo							
yield[a]	63.1	23.4	13.4	71.9	17.0	11.1		51.4	20.6	27.9	13.2	15.4	17.2	54.4
AX[a]	68.8	19.7	11.5	53.8	18.1	27.9		46.7	19.3	34.0	2.6	3.4	7.6	86.4
Ara/Xyl	0.38	0.74	1.03	0.44	0.76	1.07		0.31	0.78	1.00	0.47	0.49	0.54	1.04
polysaccharides[bc]	75.5	65.7	61.7	71.1	63.3	60.4		59.2	50.6	58.9	27.1	27.8	41.8	59.4
protein[b]	7.9	15.1	18.5	3.1	6.9	2.8		11.7	12.7	11.9	NA	NA	NA	7.7
							Nawid							
yield[a]	54.8	24.1	21.2	68.6	18.0	13.4	13.0	31.1	22.0	33.9	11.7	12.5	14.0	61.8
AX[a]	63.2	19.5	17.3	50.9	18.3	30.8	11.6	29.9	38.9	19.6	1.8	3.0	6.5	88.9
Ara/Xyl	0.38	0.73	1.04	0.37	0.73	1.08	0.34	0.44	0.90	0.98	0.48	0.52	0.57	1.08
polysaccharides[bc]	76.7	68.9	57.8	78.8	66.6	62.5	23.6	57.0	51.0	62.9	28.5	28.9	46.0	63.7
protein[b]	5.1	14.7	9.7	2.4	5.4	1.2	23.9	16.2	19.8	7.6	NA	NA	NA	4.3

[a] Expressed as weight percentage based on the amount of material recovered after fractionation. [b] Expressed as weight percentage (as is basis) of each subfraction; NA, not analyzed. [c] Calculated as the sum of the experimentally obtained values for the constituent monosaccharides after their conversion to polysaccharides by using factors of 0.88 for pentoses and 0.9 for hexoses.

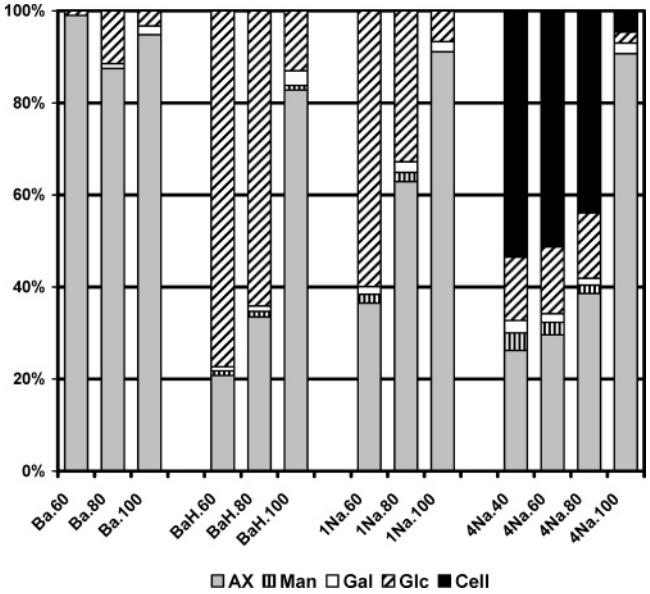

Figure 1. Composition of polysaccharides in the subfractions obtained from four AE fractions (Ba, BaH, 1Na, and 4Na) of rye outer layers from Amilo by ammonium sulfate precipitation at different saturation levels: 40, 60, 80, and 100%.

The AE subfractions were mainly composed of polysaccharides. Also, they contained some amounts of protein, especially those obtained from Ba and 1Na fractions (**Table 3**). The composition of polysaccharides, however, differed substantially among subfractions obtained from each extract (**Figure 1**). Almost pure AX populations were isolated from Ba extract at 60 and 100% ammonium sulfate saturation (99 and 95% of polysaccharides), while that precipitated at 80% saturation, in addition to AX, contained 12% polysaccharides built of non-cellulosic glucose. The two subsequent sets of polysaccharide subfractions, BaH and 1Na, were composed of β-glucans (as revealed by ^{1}H NMR, results not shown) and AX. β-Glucans dominated in subfractions obtained at 60% (BaH.60 and 1Na.60) and 80% salt saturation (BaH.80). The decrease in their proportion was evident from subfractions isolated at 60% to those precipitated at 100% saturation. The last set of subfractions (4Na) was the only one characterized by the presence of cellulose. The polysaccharide subfractions obtained from the 4Na fraction at 40, 60, and 80% saturation level contained 54, 51, and 44% cellulose, 26, 30, and 39% AX, respectively, and about 14% of other glucose-containing polymers. Similarly, as in the case of the previous fractions, AX represented a bulk of the last subfraction precipitated at 100% saturation, but on the contrary, a low level of cellulose (5%) was also found in this subfraction (4Na.100). The same relationships were noted for polysaccharide subfractions isolated from Nawid (results not shown).

^{1}H NMR Analysis of AX Fractions and Subfractions. The ^{1}H NMR spectra of parent Ba fractions and their subfractions isolated from both ryes are shown in **Figure 2**. The two regions of anomeric protons of Ara*f* (δ 5.2−5.4) and Xyl*p* (δ 4.4−4.7) are clearly discernible (*13*, *35*−*37*). The spectra of subfractions revealed that the entire AX fractions present in the Ba consisted of three populations differing in the relative proportion of the intensities of signals originating from anomeric protons of terminal Ara*f* linked to *O*-3 of Xyl*p* (δ 5.38) to those linked to *O*-2 and *O*-3 of the same Xyl*p* (δ 5.21 and 5.28). A strong resonance at δ 5.38 found in the subfractions isolated at 60% of salt saturation (Ba.60) indicates that a majority of the AX branching sites was represented by singly substituted Xyl*p*. A significant increase in the proportion of doubly substituted Xyl*p* is evidenced by a relative increase in the intensities of signals from Ara*f* linked to *O*-2 and *O*-3 of the same Xyl*p* observed from Ba.60 to Ba.100. However, the most striking difference between the spectra of Ba fraction and its subfractions obtained from both ryes was the presence of an additional resonance at δ 5.42 in those from Amilo, being especially prominent in the parent Ba fraction and Ba.60 subfraction. The ratio of its signal intensity to that of Ara*f* linked to *O*-3 of Xyl*p* (δ 5.38) was 0.67. Such resonance has been observed previously in the ^{1}H NMR spectra of hemicellulosic AX isolated from rye bran and whole meal (*13*−*15*). According to previous reports (*13*, *38*), the signals at δ 5.42 as well as those that appeared at δ 5.0−5.2 suggest the presence of short side chains of Ara*f* distributed along the backbone of the xylan core. The fact that Ara/Xyl ratio obtained by ^{1}H NMR (including resonance at δ 5.42) for Ba.60 (0.37) was in accord with that calculated from sugar analysis (0.38) supports the origin of the signal at δ 5.42 from the anomeric protons of Ara*f*. On the basis of the results of methylation analysis, Vinkx et al. (*15*) showed that up to 40% of total Ara*f* were substituted (2-, 3-, 5-, and 2,3-linked) in AX solubilized from rye whole meal by sequence of alkaline extractions. Comparing the ^{1}H NMR spectra with the results of

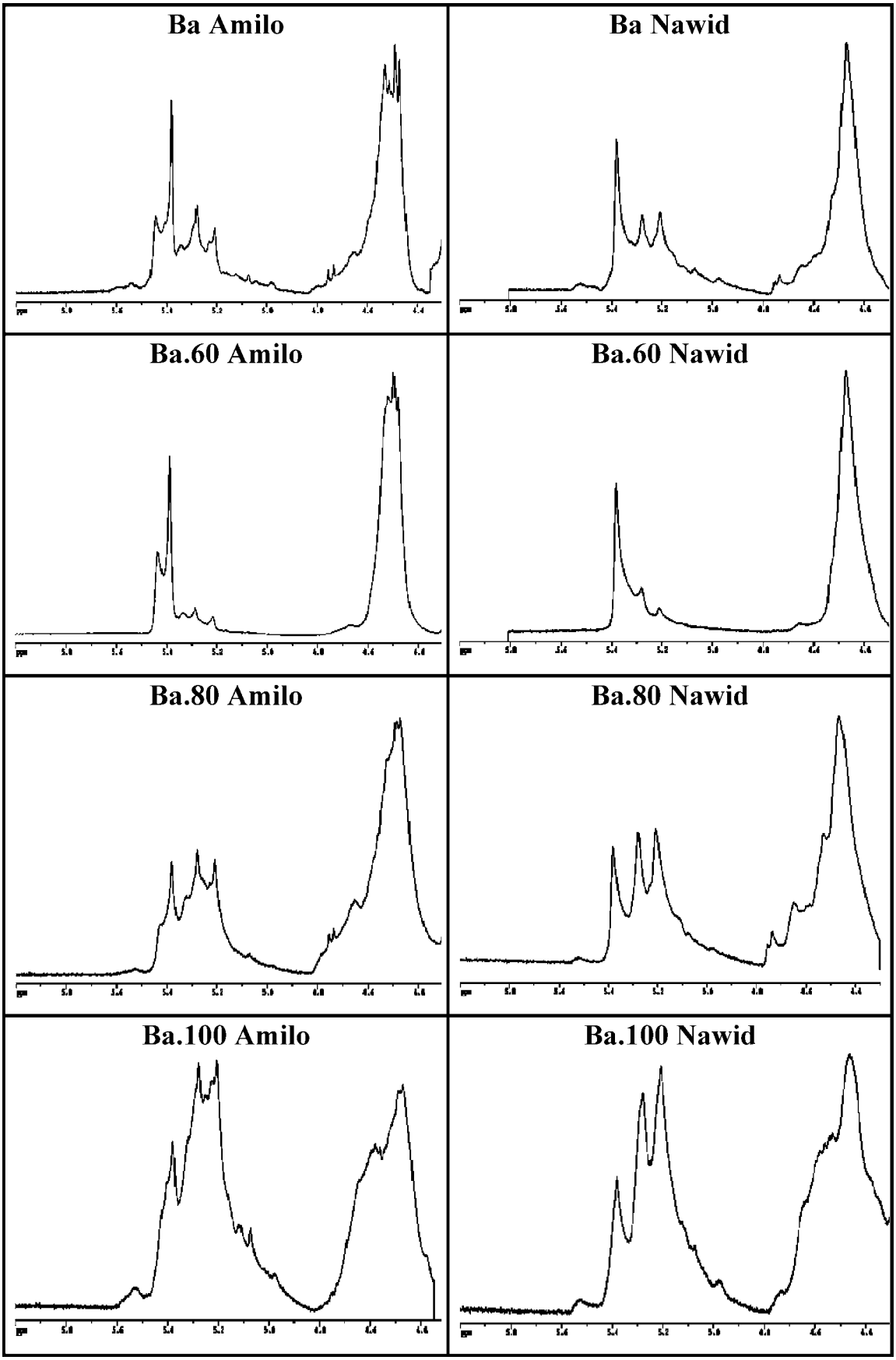

Figure 2. ^{1}H NMR spectra of the parent fractions extracted with barium hydroxide (Ba) and their subfractions obtained from outer layers of Amilo and Nawid.

methylation analysis published by these authors, it seems that the substantial levels of substituted Ara*f* in some AX fractions were coupled with comparatively high amounts of terminal Xyl*p* (~26% of total Xyl*p*), which are known as a minor element of

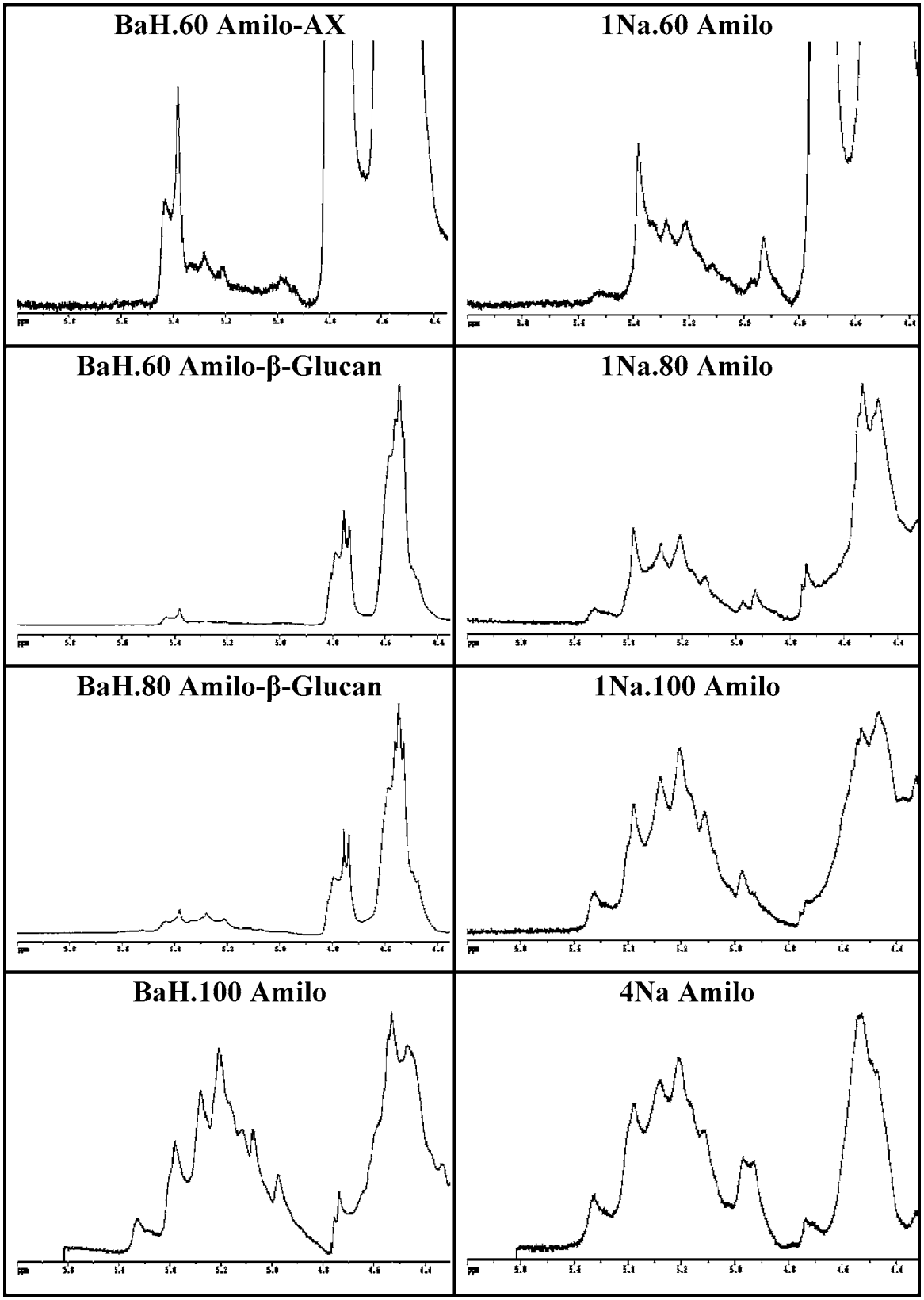

Figure 3. ^{1}H NMR spectra of the BaH and 1Na subfractions and the 4Na fraction isolated from outer layers of Amilo.

AX structure in cereals; however, they are more abundant in bran fraction. The short arabinan chains terminated by Xyl*p* or galactopyranosyl residues were reported previously for cereal heteroxylans of bran and husks (*39*, *40*).

The differences in substitution pattern of the $Ba(OH)_2$ extractable AX between both ryes are also evidenced by a different profile of signals in the Xyl*p* region of the Ba and Ba.60 spectra. For Amilo, the most intense multiple signals at δ 4.45−4.50 and 4.54, originating from unsubstituted and singly substituted Xyl*p*, respectively (*35*, *36*), are easily visible, whereas in this region of the corresponding spectra from Nawid, a broad single peak at δ 4.48 might indicate that, in general,

the longer sequences of unsubstituted Xyl*p* are more abundant in the major AX population (*37*). The obvious resonances at δ 4.45−4.47 of the unsubstituted Xyl*p* at the nonreducing end (*35*) in the Ba and Ba.60 spectra from Amilo and their virtual absence in those from Nawid suggest that terminal Xyl*p* make a difference to the structure of the major hemicellulosic AX populations of both ryes. A small resonance at δ 4.63−4.68 assigned to doubly substituted Xyl*p* (*35*, *36*) could be seen in the Ba.60 spectra of both ryes as well.

Figure 3 clearly showed that AX present in the BaH.60 subfraction displayed the same profile of signals from Ara*f* as those in the Ba.60, indicating that the majority of Xyl*p* was present as monosubstituted 3-Xyl*p* branches. However, in opposition to the material isolated with saturated $Ba(OH)_2$, the resonance at δ 5.42 was also observed in the BaH spectra from Nawid (results not shown). The AXs in the 1Na.60 subfraction as well as those present in BaH.80 and 1Na.80 were enriched in 2,3-Xyl*p*, as denoted by a slightly higher proportion of the signals originating from Ara*f* linked to *O*-2 and *O*-3 of the same Xyl*p* (δ 5.21 and 5.28) to those of Ara*f* linked to *O*-3 of Xyl*p* (δ 5.38), in comparison to the relative proportion of these signals found for Ba.60. Although the subfractions precipitated from the BaH and 1Na at 60 and 80% ammonium sulfate saturation contained an appreciable amount of β-glucans [as indicated by a level of glucose in **Figure 1** and the two groups of multiple resonances centered at δ 4.74 and 4.55 (*37*)], the AX recovered in these subfractions still represented a significant proportion of the total AX populations extracted by a consecutive alkaline treatment (19 and 21%, respectively for Amilo and Nawid). The ^{1}H NMR spectra demonstrated that the principal structural features of the AX populations with low and intermediate Ara/Xyl ratios (0.38−0.44 and 0.73−0.78), obtained from the parent Ba, BaH, and 1Na fractions by ammonium sulfate precipitation at lower saturation levels, might be successfully investigated by ^{1}H NMR spectroscopy and neutral sugar analysis, whereas among the subfractions with high Ara/Xyl ratio (0.98−1.08) that precipitated at 100% salt saturation (Ba.100, BaH.100, and 1Na.100), including 4Na fraction, a very specific fraction being almost exclusively built of highly branched AX (**Table 3**), only spectra of the Ba.100 subfraction in the region of Ara*f* undeniably reflected the basic structure of AX. The spectra of the remaining subfractions and that of 4Na fraction contained some additional peaks in the region of anomeric protons of Ara*f*. Furthermore, the region of Xyl*p* anomeric protons was not informative, mostly due to severe overlap of the broadening signals, suggesting a more complex structure. Considering the highly substituted xylan backbone in these preparations, it would be expected that, in part, the broadening of the peaks in both fingerprint regions of the spectrum or appearing of some additional resonances is related to the presence of neighboring branched Xyl*p* (*36*). The α-D-glucuronopyranosyl residues (Glc*p*A), which resonate at δ 5.32−5.37 (*41*), might be involved in the profile of Ara*f* signals as well, since the FTIR spectra of these materials clearly indicated their existence (results not shown). Moreover, the broad signals at δ 5.51, being especially prominent in the spectra of highly substituted arabinoxylan populations, suggest that short arabinan side chains may be more abundant in these materials (*41*). This is consistent with the results reported by Ebringerová et al. (*14*) for highly branched AX (Ara/Xyl ratio = 0.98) obtained from sodium chlorite-delignified rye bran by extraction with 4.5% NaOH. These authors showed that such AX was characterized by a high content of both branched Ara*f* (more that 30% of total Ara*f*) and nonreducing terminal Xyl*p* (about 22% of total Xyl*p*). The enrichment of the highly substituted AX in terminal Xyl*p* is also evident from the study of Vinkx et al. (*15*), who demonstrated that the AX population with a high Ara/Xyl ratio (1.06), which left in the solution after addition of ammonium sulfate to the $Ba(OH)_2$ extract of rye hemicelluloses, had a high content of terminal Xyl*p* (26%), whereas they represented only 5% of Xyl*p* in a population with a much lower Ara/Xyl ratio (0.55) that was precipitated from this solution.

Table 4. Relative Percentage of Unsubstituted and Differently Substituted Xylose Residues in AX from AE Fractions and Subfractions Obtained from WUM[a]

fraction/ subfraction	% of total Xyl*p*				sub/ un	di/ mono	-Ara*f*-(% of total Ara*f*)
	u-Xyl*p*	3-Xyl*p*	2,3-Xyl*p*	-Ara*f*-Xyl*p*[b]			
			Amilo				
Ba	57.8	23.2	13.7	5.3	0.73	0.60	17.2
Ba.60	72.1	17.8	4.3	5.8	0.39	0.24	30.9
Ba.80	55.6	14.8	25.9	3.7	0.80	1.75	9.6
Ba.100	38.9	19.1	36.3	5.7	1.57	1.90	11.0
BaH	48.5	25.8	19.7	6.0	1.06	0.76	15.4
BaH.60	67.9	20.0	6.8	5.3	0.47	0.34	23.9
BaH.80	52.1	19.7	21.5	6.7	0.72	1.11	17.5
1Na.60	77.3	14.4	8.3		0.30	0.57	
1Na.80	50.5	20.8	28.7		0.98	1.38	
			Nawid				
Ba	55.4	27.1	17.5		0.81	0.65	
Ba.60	69.3	23.7	7.0		0.44	0.30	
Ba.80	54.5	18.2	27.3		0.83	1.50	
Ba.100	37.6	21.0	41.4		1.66	1.97	
BaH	51.7	23.5	19.9	4.9	0.93	0.85	13.3
BaH.60	70.6	21.6	4.4	3.4	0.42	0.20	18.6
BaH.80	54.3	18.3	22.1	5.2	0.84	1.21	14.3
1Na.40	77.3	11.3	11.4		0.29	1.00	
1Na.60	68.5	20.5	11.0		0.46	0.54	
1Na.80	37.0	25.8	37.2		1.70	1.44	

[a] Abbreviations: u-Xyl*p*, unsubstituted β-(1→4)-linked D-Xyl*p*; 3-Xylp, β-(1→4)-linked D-Xyl*p* substituted with terminal α-L-Ara*f* at *O*-3; 2,3-Xyl*p*, β-(1→4)-linked D-Xyl*p* substituted with two terminal α-L-Ara*f* at *O*-2 and *O*-3; -Ara*f*-Xyl*p*, β-(1→4)-linked D-Xyl*p* substituted with short Ara*f* chain; sub/un, ratio of substituted to unsubstituted Xyl*p*; di/mono, ratio of 2,3-Xyl*p* to 3-Xyl*p*. [b] For calculation of the relative proportion, the presence of side chains built of two Ara*f* was assumed.

The marked differences in the structure of some AX populations are well-illustrated by their substitution patterns corresponding to the relative proportion of un-, mono-, and disubstituted Xyl*p* (u-Xyl*p*, 3-Xyl*p*, and 2,3-Xyl*p*) in their backbone chains (**Table 4**). The major AX subfractions isolated from the Ba and BaH fractions at 60% of salt saturation (Ba.60 and BaH.60) had only 28−30% of substituted Xyl*p* mostly represented by 3-Xyl*p* (75−83% of substituted Xyl*p*). A much lower level of substituted Xyl*p* (23%) was found for AX subfractions obtained from the 1Na fraction. However, these polymers were enriched in 2,3-Xyl*p*, and for Amilo, they required 60% level of saturation (1Na.60, 3-Xyl*p* represented 63% of substituted Xyl*p*) and only 40% saturation in the case of Nawid (1Na.40, 3-Xyl*p* represented 50% of substituted Xyl*p*). Except for 1Na subfractions from Nawid, there was a substantial rise in the proportion of di- to monosubstituted Xyl*p* observed from lowly to intermediately and highly substituted subfractions. As earlier pointed out by Ara/Xyl ratios (**Table 3**), the AX subfractions isolated from 1Na at 80% of ammonium sulfate saturation differed markedly in the proportion of sub- to unsubstituted Xyl*p* between both ryes (0.98 and 1.70, respectively, for Amilo and Nawid; representing intermediately and highly substituted polymers), although their proportions of 2,3-Xyl*p* to 3-Xyl*p* were similar. Assuming that short arabinan chains, suggested

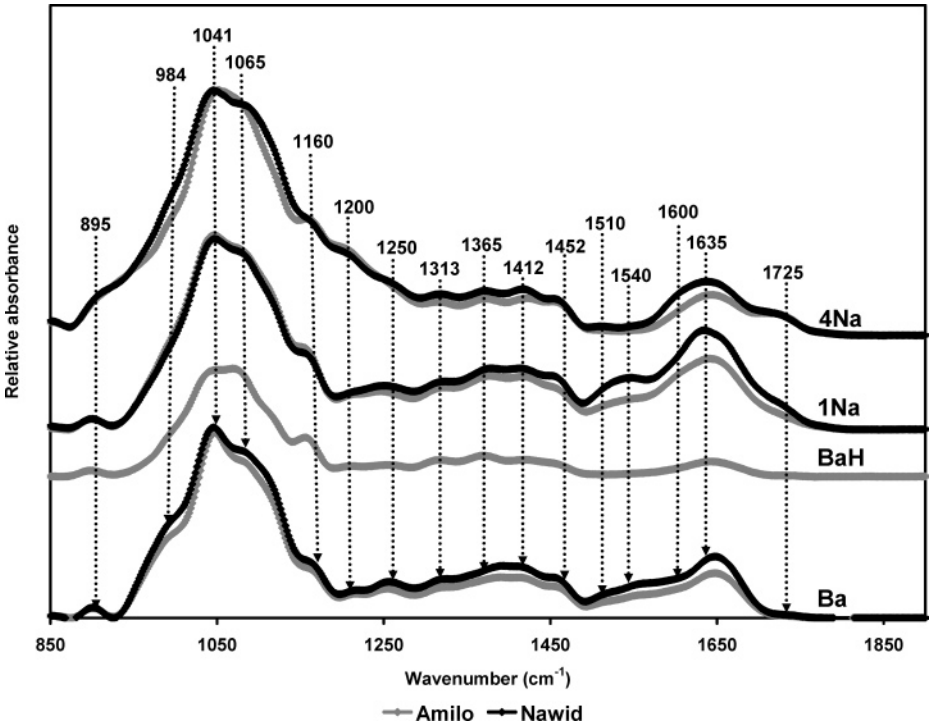

Figure 4. FTIR spectra of the hemicellulosic fractions obtained from outer layers of Amilo and Nawid by successive alkaline extraction.

from ^{1}H NMR data, are built of two Ara*f*, the calculated proportion of Xyl*p* carrying such substituents is low (3−7% of total Xyl*p*). On the other hand, the substituted Ara*f* units constitute an appreciable proportion of the total amount of these residues linked to Xyl*p* in the AX backbone, particularly in the lowly substituted subfractions.

FTIR Spectroscopic Analysis. The FTIR spectra of parent hemicellulosic fractions in the 1850 to 420 cm^{-1} region are shown in **Figure 4**. There were only small differences between Amilo and Nawid in the signal pattern of the Ba and 1Na fractions, which appeared in the carbohydrate region (1200 to 950 cm^{-1}) and more obvious in the amide I and II regions (1660 to 1600 and 1570 to 1510 cm^{-1}, respectively) of the 1Na spectra (*42*). On the basis of the results of sugar analysis (**Table 2**), the Ba spectra in the carbohydrate region represent a pure AX population with a relatively low degree of substitution (Ara/Xyl ratio, ∼0.6). An absorption band at 984 cm^{-1} is clearly visible in the spectra of these polymers. However, such signals could not be recognized in the 4Na spectra of highly substituted AX nor in the BaH and 1Na spectra of AX associated with β-glucans. It has previously been shown that the intensity of band at 990 to 984 cm^{-1} is strongly influenced by degree of substitution of the xylan backbone with Ara*f* (*43*, *44*). In the spectra of hemicellulosic fractions released from WUM by 1 and 4 M NaOH (1Na and 4Na), the bands at 1725 cm^{-1} arising from carbonyl stretching vibration of esters (*45*) indicate that AX present in these materials is enriched in GlcA residues in comparison with those in Ba and BaH fractions. This was earlier suggested by the results of ^{1}H NMR analysis (**Figure 3**). The partial association of highly substituted AX with some ligninlike components in the 4Na (virtually protein-free fractions) is evidenced by the overlapped bands characteristic of phenolic substances at 1635 cm^{-1} (assigned to carbonyl stretching conjugated with aromatic rings) and at 1600 cm^{-1} (due to aromatic ring of phenolic) (*46*). Much detailed examination of these spectra revealed the presence of signals at 1507 cm^{-1} corresponding to phenolic ring absorbance as well. This is supported by the perfectly matching HPSEC profiles of 4Na fractions obtained from both RI and UV detectors (results not shown).

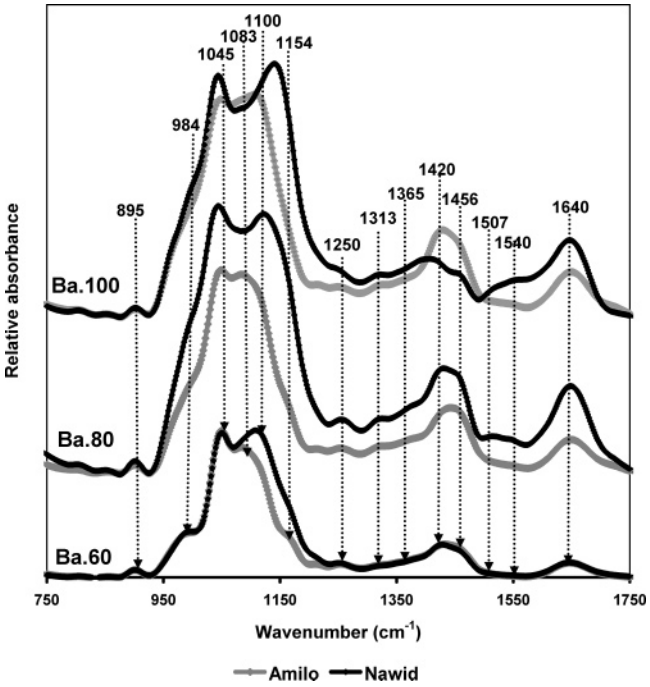

Figure 5. FTIR spectra of the Ba subfractions precipitated from outer layers of Amilo and Nawid at 60, 80, and 100% ammonium sulfate saturation.

Unlike the FTIR spectra of parent Ba fractions, those of Ba subfractions (Ba.60, Ba.80, and Ba.100) showed the significant changes in the spectral patterns between both ryes (**Figure 5**), suggesting differences in the pattern of AX substitution, as already indicated by ^{1}H NMR analysis of both parent Ba fractions and their subfractions. There were two major absorption bands in the polysaccharide fingerprint region (1200 to 950

cm^{-1}), in which dominating ring vibrations (C−C) are overlapped with stretching vibrations of side groups (C−OH) and the glycosidic bond vibrations (C−O−C) (*45*). The band at 1045 cm^{-1} appeared at the same frequency in all spectra of subfractions for both ryes. In opposite, the latter band was shifted from 1083 for Amilo to 1100 cm^{-1} for Nawid in the Ba.60 spectrum. It was manifested for all AX subfractions; however, the shift of this band was also observed from Ba.60 to Ba.100 (from 1083 to 1100 cm^{-1} and from 1100 to 1135 cm^{-1}, respectively, for Amilo and Nawid). A clear change in the profiles of absorption bands was found between ryes in the amide I and II regions for intermediately and highly substituted subfractions, which may imply some diversity of the proteinaceous material present in the corresponding cell wall preparations from both rye samples.

HPSEC. The size exclusion profiles of the hemicellulosic fractions reflected their multicomponent nature, and different polymer populations could be distinguished (**Figure 6**). The notable changes in the HPSEC profiles of polymers from high and low breadmaking quality ryes were found in the Ba and BaH fractions as shown by the different relative proportions of population with similar elution volume (**Table5**). On the contrary, the elution patterns of polymers extracted with 1 and 4 M NaOH were similar for both rye cultivars (**Table 5**, **Figure 6**). As previously mentioned, the profile of UV-absorbing substances went hand in hand with those recorded by the RI detector (mostly polysaccharides) throughout the entire elution volume of the 4Na from both rye samples (results not shown), suggesting an interaction between polysaccharides and ligninlike substances, as the level of protein was residual.

The average molecular weight values (M_w) are reported for the three populations of Ba (**Table 5**), whereas for other extracts BaH, 1Na, and 4Na, the presence of aggregates prevented a correct determination of M_w. The high molecular weight (HMW) polymers of Ba (fraction I, 11.5−13.5 mL) had a few times higher M_w than the other populations. The plot of M_w vs radius of gyration (results not shown) indicated a rigid rodlike conformation for these HMW populations. Furthermore, HMW polymers from Amilo had higher M_w than those from Nawid. However, such obvious differences in the M_w between both ryes were not observed in the other populations.

The HPSEC profiles obtained from both RI and UV detectors for Amilo subfractions are presented in **Figure 7**. The principal AX subfraction Ba.60 consisted of a large population in the HMW region (11−17 mL) and comparatively small peaks in the low molecular weight (LMW) region (18−21 mL) (as indicated by the RI detector). In contrast, all remaining subfractions had two polymer populations in both regions.

For the BaH and 1Na subfractions, which in addition to AX contained β-glucans, it was possible to point out the β-glucans peaks in the HPSEC profiles, since a substantial decrease in their level was observed from BaH.60 to BaH.100 and from 1Na.60 to 1Na.100 (as revealed by ^{1}H NMR and sugar analysis). They appeared in the LMW region at 17−19 mL as sharp and symmetric peaks, when compared to those of AX in the HMW region. It was evident that the relative proportion of the HMW AX was higher in the subfractions with a high degree of substitution (BaH.100 and 1Na.100) than that in the lowly and intermediately branched polymers.

Taking into account the profiles of both RI and UV detectors, the HMW populations of highly branched AX seem to be associated with UV-absorbing substances, as their profiles in the HMW region match each other. In the case of 1Na subfractions, this also holds for intermediately substituted AX.

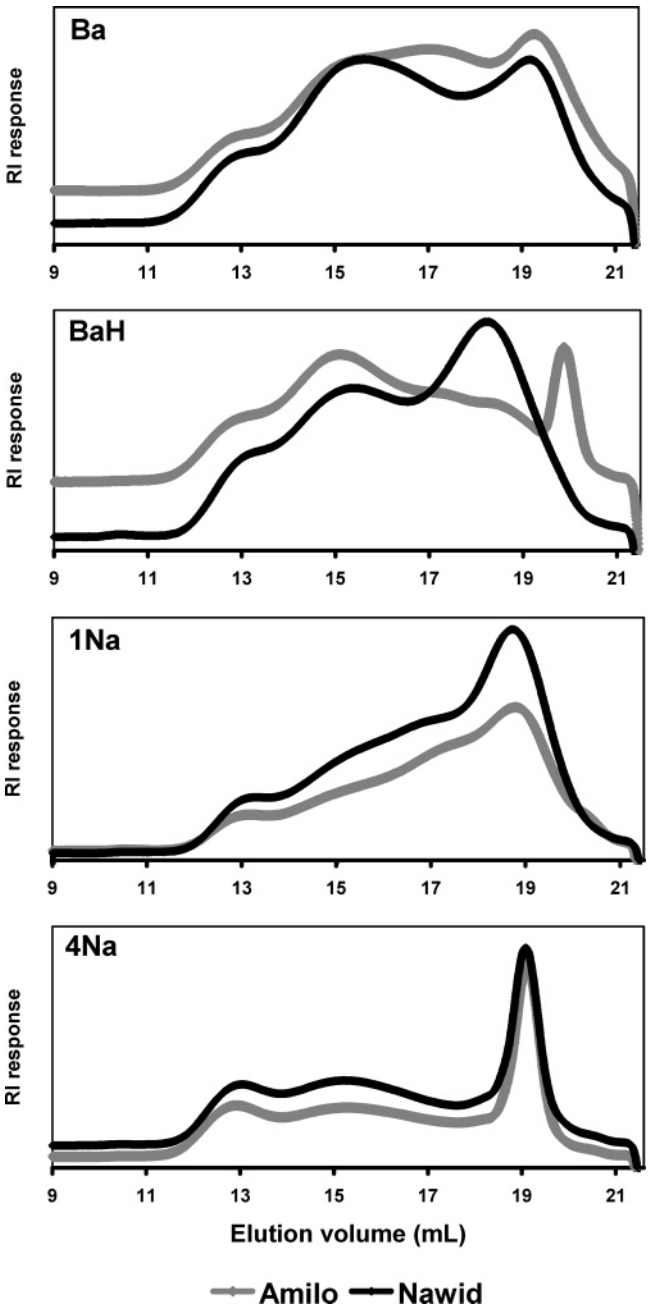

Figure 6. HPSEC profiles of the hemicellulosic fractions obtained from outer layers of Amilo and Nawid by successive alkaline extraction.

Because of very strong signals of UV-absorbing components in the LMW region of the 1Na subfractions, related to their substantial amounts of protein and ligninlike substances (**Table 2** and **3**), it is not possible to draw any conclusion about their interactions with LMW material.

Distribution of Hemicellulosic AX Substructures in the Rye Grain. In general, fractionation by graded ammonium sulfate of the parent hemicellulosic extracts resulted in more homogeneous AX populations as well as the populations with a similar degree of substitution with Ara*f*, exemplified by their Ara/Xyl ratio, were isolated from the parent fractions at the same level of salt saturation. Thus, on the basis of the results from fractionation of both central endosperm hemicelluloses (*24*) and those present in the outer layers of the rye grain, the relative distribution of the AX subfractions with different Ara/Xyl ratio in the whole grain was calculated and summarized in **Table 6**.

Table 5. Weight Average Molar Mass (M_w) of Major Polymeric Populations and Their Relative Proportions in AE Fractions Isolated from Rye Outer Layers

Ba			BaH		1Na		4Na	
population/ volume (mL)	M_w[a]	%[b]	volume (mL)	%[b]	volume (mL)	%[b]	volume (mL)	%[b]
				Amilo				
I (11.5–13.5)	1290000	10.4	11.5–13.5	17.5	11.5–13.5	8.7	11.5–13.5	18.6
II (13.5–18.0)	115000	61.0	13.5–16.5	51.5	13.5–17.5	48.0	13.5–18.0	45.0
III (18.0–20.0)	66000	28.6	16.5–19.0	31.0	17.5–20.0	43.3	18.0–20.0	36.4
				Nawid				
I (11.5–13.5)	950000	11.6	11.5–13.5	12.4	11.5–13.5	8.8	11.5–13.5	18.2
II (13.5–18.0)	106000	61.9	13.5–16.5	41.5	13.5–17.5	48.1	13.5–18.0	47.9
III (18.0–20.0)	74000	26.5	16.5–19.0	46.1	17.5–20.0	43.1	18.0–20.0	33.9

[a] Expressed as g/mol. [b] Relative proportions were calculated by integration of RI signals.

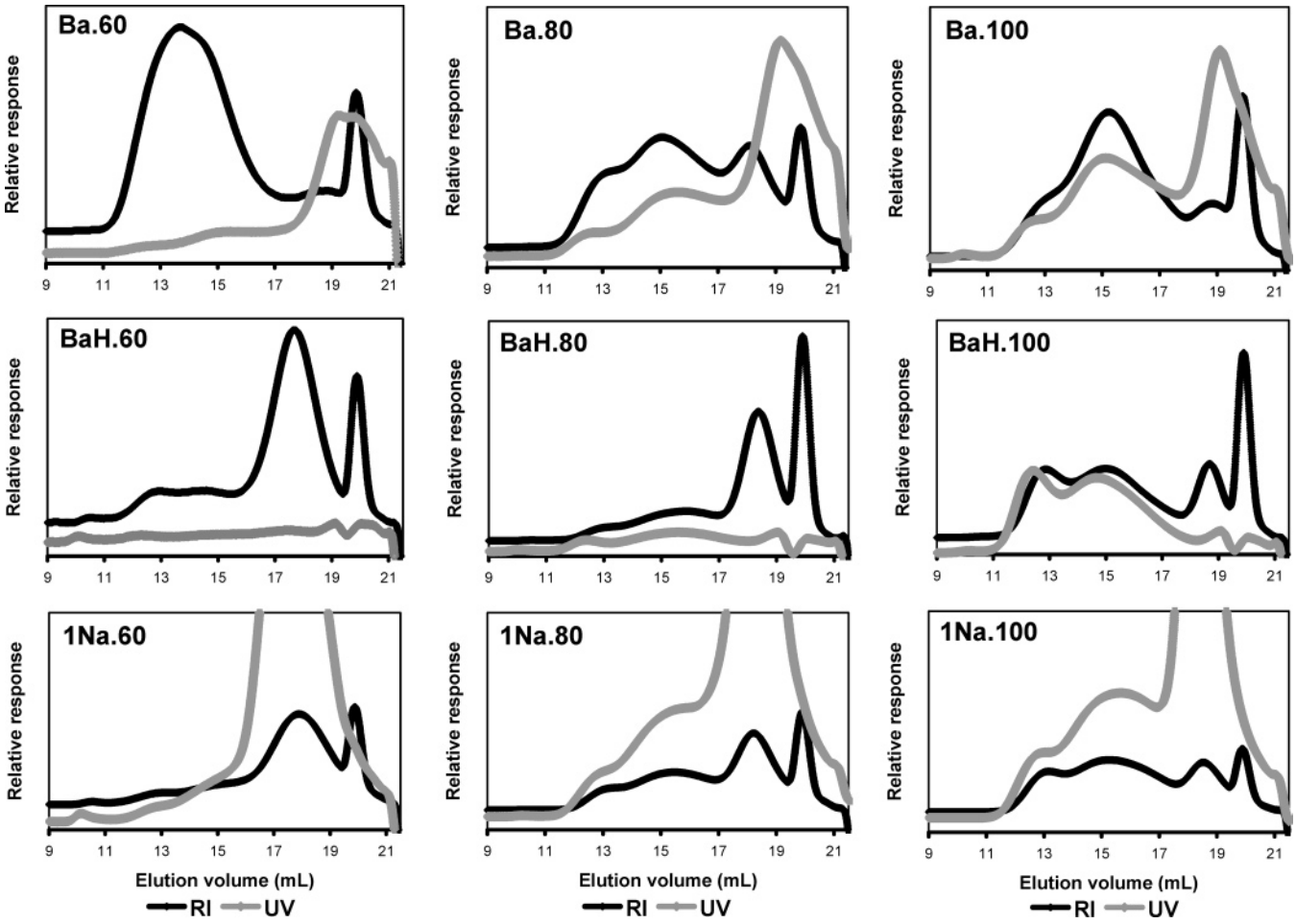

Figure 7. HPSEC-RI-UV profiles of the Ba, BaH, and 1Na subfractions precipitated from outer layers of Amilo at 60, 80, and 100% ammonium sulfate saturation.

The AX with intermediate and high degrees of substitution, isolated from central and outer layers of the grain at 80 and 100% ammonium sulfate saturation, had similar Ara/Xyl ratios. The yields of these subfractions, therefore, represent the sum of polymers from the inner and outer layers of the whole grain. Because the AX isolated from the outer layers at 60% saturation had significantly lower degrees of substitution (Ara/Xyl ratio, 0.31−0.44) than the corresponding polymers from the inner part of rye (0.48−0.54), both groups are presented separately. It was also observed for AX obtained from 1Na fraction at 40% ammonium sulfate saturation. Despite the differences in the degrees of substitution between polymers from both milling streams, all AX substructures that precipitated from the parent fractions at 60% salt saturation were practically composed of one branching unit, 3-Xyl*p*, whereas in the remaining populations of AX, a substantial level of 2,3-Xyl*p* was found, in addition to that of 3-Xyl*p*. It should be emphasized that highly substituted AX precipitated from AE extracts at 100% saturation contained both types of branched Xyl*p*, in contrast to the WE counterparts, having 2,3-Xyl*p* as only type of branching (*24*). Clearly, the central endosperm AXs were characterized by the higher proportion of subfractions with intermediate degrees of substitution obtained at 80% ammonium sulfate saturation, when compared with those of outer layers. However, the latter constituted 83−84% of total hemicellulosic AX recovered after fractionation, and consequently, they had a great impact on

Table 6. Relative Distribution and Structural Characteristics of Hemicellulosic AX Populations Isolated from Rye Grain by Sequential Alkaline Treatment Followed by Graded Ammonium Sulfate Precipitation and Xylans (1Na.P and 4Na.P) Self-Precipitated from Parent Extracts

			relative distribution (%)		
fraction/ subfraction	Ara/Xyl	major type of branching	flour[a]	outer layers	whole grain[b]
Ba.40	0.70	3-Xyl*p*, 2,3-Xyl*p*	0–1	ND	tr
Ba.60	0.48–0.50	3-Xyl*p*	24–26	ND	4
	0.38	3-Xyl*p*	ND	25–31	22–26
Ba.80	0.73–0.84	3-Xyl*p*, 2,3-Xyl*p*	20–24	8–9	10–11
Ba.100	1.03–1.08	3-Xyl*p*, 2,3-Xyl*p*	2–3	6–7	5–6
BaH.40	0.82–1.00	3-Xyl*p*, 2,3-Xyl*p*	1–3	ND	tr
BaH.60	0.52–0.53	3-Xyl*p*	2–6	ND	0–1
	0.37–0.44	3-Xyl*p*	ND	4–6	4–5
BaH.80	0.73–0.78	3-Xyl*p*, 2,3-Xyl*p*	4–18	2	2–5
BaH.100	0.95–1.13	3-Xyl*p*, 2,3-Xyl*p*	3–5	2–3	2–3
1Na.40	0.53–0.55	3-Xyl*p*, 2,3-Xyl*p*	2–4	ND	0–1
	0.34	3-Xyl*p*, 2,3-Xyl*p*	ND	0–1	0–1
1Na.60	0.49–0.54	3-Xyl*p*	8–12	ND	1–2
	0.31–0.44	3-Xyl*p*, 2,3-Xyl*p*	ND	2–3	2–3
1Na.80	0.74–0.90	3-Xyl*p*, 2,3-Xyl*p*	10–16	2–3	4
1Na.100	1.08–1.23	3-Xyl*p*, 2,3-Xyl*p*	2–4	4	4
1Na.P	0.07			20–21	16–18
4Na.100	1.04–1.08	3-Xyl*p*, 2,3-Xyl*p*		3–5	3–4
4Na.P	0.18–0.20			1–2	1–2
Res	1.20–1.22	3-Xyl*p*, 2,3-Xyl*p*		12	10

[a] Ref *24*. [b] Data recalculated from the values obtained for flour and for outer layers using the yields of both milling streams. Data represent minimum and maximum values for two rye cultivars, Amilo and Nawid; ND, not detected; tr, less than 0.5%.

overall distribution of AX in the whole grain. The lowly branched AX from outer layers of the rye grain that precipitated from barium hydroxide extract at 60% saturation level, Ba.60, constituted the most abundant substructure. Furthermore, its amount recovered from high breadmaking quality rye (1.20% of whole rye) was significantly higher than that of low breadmaking quality rye (0.78%). Also, the amount of xylans obtained from the 1Na extract (1Na.P) of Amilo, barely carrying any Ara*f* substituents, was much higher (0.80%) than that of Nawid (0.62%). Interestingly, these xylans represented the second most prominent substructure among the hemicellulosic AX isolated from AE extracts by ammonium sulfate fractionation. In the remaining subfractions, such differences between both ryes were less obvious. The intermediately substituted AX obtained from barium hydroxide extract at 80% salt saturation (Ba.80) and the highly branched polymers that left in the alkali-unextractable residue (Res) constituted 10−11% of the entire AX fraction. While, the levels of other substructures were relatively low. Certain losses of the materials should be borne in mind, as the recoveries of the WUMs were 78.3 and 83.8%, respectively, for Amilo and Nawid (**Table 2**). This could be explained by the possible depolymerization of the hemicellulosic materials, especially during prolonged extraction with strong alkali. Up to date, however, there is no better way to isolate polymers from the WU matrix.

The results of the present study provide insight into the diversity of hemicellulosic AX substructures and their association with other cell wall components of the rye grain. On the basis of the composition of each fraction, it can be concluded that the $Ba(OH)_2$-extractable AXs were, to some extent, connected to protein. It is of note, however, that simple phenolics, mostly ferulic acid and its dehydrodimers, known as native substituents of AX liberated by alkaline treatments, were also initially incorporated into hemicellulosic fractions. Therefore, for all fractions, their involvement in cross-linking between AX, protein, and/or ligninlike components should be considered as well. The water-extractable AX obtained from residue left after first alkaline extraction appeared to be attached to β-glucans, as both polymers extracted when, it is believed, β-glucans are released from insoluble complexes with Ba^{2+}. This is supported by the fact that there was no evident difference in the structure of predominating AX with low and intermediate degrees of substitution present in both hemicellulosic extracts, whereas the second population of both polysaccharides extracted with 1 M NaOH was associated with protein and ligninlike components. The latter substances along with cellulose coexisted with AX in 4 M NaOH-extractable fraction. This lignocellulosic complex and AX with the highest degree of substitution were also found in the alkali-unextractable residue left after consecutive extraction.

In addition to the overview of the AX substructures existing in the hemicellulosic fractions, the present work also pointed out some structural features of these polysaccharides, which could be tested as an index of breadmaking quality on a large number of samples to verify their usefulness in screening of breeding materials.

ABBREVIATIONS USED

WE, water-extractable fraction; WU, water-unextractable fraction; ^{1}H NMR, proton nuclear magnetic resonance; Ara/Xyl, arabinose-to-xylose ratio; HPSEC, high-performance size-exclusion chromatography; FTIR, Fourier transform infrared spectroscopy; Xyl*p*, xylopyranosyl residues; Ara*f*, arabinofuranosyl residues; HPLC, high-performance liquid chromatography; HMW, high molecular weight; LMW, low molecular weight.

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