

Structural Features of Arabinoxylans Extracted with Water at Different Temperatures from Two Rye Flours of Diverse Breadmaking Quality

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The water extractable (WE) arabinoxylans from two rye flours differing in baking quality were studied following sequential extraction with water at 4, 40, and 100 °C. Ammonium sulfate fractionation of the resulting WE fractions and subsequent analysis revealed substantial differences in the structure of the isolated materials. Furthermore, it allowed us to identify the factors contributing to arabinoxylan water extractability. Our results provide compelling evidence for the existence of separate polymers in rye WE arabinoxylans with different substitution degrees, ranging from quantitatively dominating, lowly substituted populations (arabinose to xylose ratio, Ara/Xyl \sim 0.5) to comparatively less abundant, highly substituted analogues (Ara/Xyl \sim 1.3). Generally, arabinoxylan water extractability was governed by the relative proportion of lowly and highly branched structures. A gradually increasing proportion of highly substituted populations was observed from cold to hot WE fractions. This was associated with the lower proportion of monosubstituted xylopyranosyl residues in the backbone, the higher proportion of disubstituted xylopyranosyl residues, and the higher level of substitution with feruloyl residues. Notable differences in the ratio of phenolic compounds to arabinose residues were observed between corresponding polymers isolated from rye flours of high and low baking quality, whereas the differences in their molecular weights were much less pronounced.

KEYWORDS: Rye flour; water extractable arabinoxylan; cell wall components; fractionation; arabinoxylan structure

INTRODUCTION

Arabinoxylans represent a considerable part of the building blocks of cell walls in cereals. These nonstarch polysaccharides are of growing importance in human diets with health-promoting characteristics (1–3) and are a major determinant in the performance of cereals in biotechnological processes (4, 5). They have been classified as WE or WU arabinoxylans. It is obvious that their extractability depends on the conditions used during extraction. In this respect, it is well-known that temperature greatly affects the proportion of WE arabinoxylans in the samples. Rye grain contains 7–12% of arabinoxylans, of which 20–25% can be extracted with water at room temperature (6) and 30–40% with hot water (7, 8). The arabinoxylan water extractability is also influenced by environment and genetic background (9, 10). As in the case of wheat (11), the level of WE nonstarch polysaccharides, and as a result, arabinoxylans in rye flour, is independent of the extraction rate during milling. In contrast, the content of their unextractable counterparts

increases gradually with the extraction rate (12). This explains the much higher hot water extractability (60–70%) of rye flour arabinoxylans (8, 13) in comparison to that of whole grain.

The arabinoxylan backbone consists of (1 \rightarrow 4)-linked β -D-Xylp, which is unsubstituted or, to a variable extent, mono- or disubstituted with α -L-Araf. It is generally accepted that the substitution degree of the arabinoxylan backbone with Araf as well as other substituents, e.g., phenolic acids, acetyl groups, and protein, influence its extractability (4, 5, 14). The predominant structure of rye WE arabinoxylans was described many years ago. Almost half of the Xylp in the chain was monosubstituted by L-arabinofuranosyl units at O-3 (3-Xylp), which occurred isolated, in pairs, and in sequences of three consecutive branched Xylp (15, 16). Later studies revealed that mono- and disubstituted Xylp occurred in different polymers or different regions of the same polymer. The two different structures found were designated arabinoxylan I and II (17, 18). In arabinoxylan I, 3-Xylp is virtually the only branching site (50% of total Xylp), whereas O-2 and O-3 double-substituted Xylp (2,3-Xylp) amounted for 60–70% of arabinoxylan II. Vinkx et al. (19) isolated nearly pure monosubstituted, disubstituted, and moderately substituted arabinoxylans. They concluded that rye WE arabinoxylan consists of a range of structures. The level of O-2

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monosubstituted Xylp (2-Xylp) varied significantly among these structures (20), despite the apparent absence of such linkages in previous observations (15, 17, 19). Diversity of arabinoxylan structure, therefore, is connected with the different proportion of four structural elements: un-, mono- (2-Xylp, 3-Xylp), and disubstituted (2,3-Xylp) xylose residues and their arrangement over the polysaccharide chain. In addition, molecular weight (MW) distribution, ferulic acid content, and interaction with other cell wall components are clearly important as well. Despite the above, the fine structure of arabinoxylan, which is essential for its functional behavior and physicochemical properties, is not well understood. The effect of differently substituted Xylp on arabinoxylan water extractability also remains unknown.

In the rye dough system, starch and arabinoxylans are thought to play a pivotal role, since rye proteins are quite soluble and do not contribute much to the baking performance of rye (9, 10). Rye arabinoxylans indeed affect the rheological properties of doughs and bread characteristics. In this respect, their viscosity-enhancing ability and water-holding and gelation potential are important (5, 21–23). In rye breeding, WE arabinoxylans are regarded as an important index of both feeding value (24, 25) and baking quality (13, 26). However, the high content of WE arabinoxylans in rye is favorable in breadmaking but adverse in animal feeding. The content of WE arabinoxylans in rye is controlled by many factors scattered throughout the genome, and the chromosomes 2R, 5R, and 6R are responsible for increased arabinoxylan content, while chromosome 3R is responsible for it reduced levels (27, 28). Results of a previous 3 year investigation in rye breeding materials showed a positive, significant correlation between the proportion of WE to WU arabinoxylans in rye flour and both flour and bread quality parameters. The ratio of water extract viscosity to WE arabinoxylan content was also highly correlated to these quality attributes (13). Water extracts containing the same level of arabinoxylans had distinctly different viscosity suggesting differences in their structure, molecular size, and/or interaction with the other cell wall components, which may be closely related to the baking quality of rye flour. In this respect, it is important to mention that rye breadmaking involves three phases, mixing, fermentation, and baking, which are performed at different temperatures. It seems therefore highly relevant to measure the levels of WE arabinoxylans in rye flour, not only at room temperature but also at higher temperatures, representative for the fermentation and baking phases, as well as to further fractionate the arabinoxylans solubilized at different temperatures. We have investigated the structural and molecular features of individual WE arabinoxylan subfractions isolated from two rye flours of diverse baking quality at different temperatures.

MATERIALS AND METHODS

Rye Flours. Two Polish rye cultivars, Amilo with high baking quality and Nawid with low baking quality, were selected from breeding materials grown in central Poland (DANKO, Plant Breeding Co., Laski) in 2000, based on the flour and bread quality parameters. Amilo is distinguished for its superior resistance to sprouting and very good baking quality, whereas Nawid is tolerant to low pH of soil and represents good level of lodging resistance. Samples were tempered to 14.0% moisture and milled on a Quadrumat Senior laboratory mill (Brabender, Duisburg, Germany) to obtain a straight grade flour. Milling yields (14.0% moisture base), protein contents, and ash contents (percent dm) were 58 and 63%, 7.2 and 7.6%, and, 0.9 and 1.0%, for Amilo and Nawid, respectively. Falling numbers were 309 and 126 s, respectively. Flours exhibited different peak viscosities (amylograph maxima, 820 and 270 BU) and temperatures of maximum viscosity (72 and 59 °C, respectively). Baking quality was evaluated based on

the following bread characteristics: overall outer appearance, crust cracking, blister under the crust, loaf volume, crust color, grain quality, and crumb texture.

Consecutive Water Extraction at Increasing Temperature. Prior to extraction, rye flour was suspended in 90% ethanol and boiled in a water bath under reflux for 1 h, to inactivate endogenous enzymes. The residue was filtered, washed with ethanol, and dried overnight at 40 °C. Inactivated rye flour (120 g), deionized water, and equipment required to perform extraction were stored at 4 °C overnight. Cold water extraction (1:5 w/v) was carried out for 1 h at 4 °C. After it was centrifuged (10 000g, 20 min, 4 °C), the residue was suspended in cold water and extraction was repeated once for 30 min. Both supernatants were combined, purified (as described below), and designated WE.I. The unextractable material was stirred with water (500 mL) at 40 °C, and extraction was performed twice, as described above. Combined supernatants were purified and designated WE.II. The material left after two consecutive water extractions was mixed with boiling water (500 mL) and thermostable α -amylase from *Bacillus licheniformis* (2 mL, 3000 units/mL) (EC 3.2.1.1, Megazyme International Ireland Ltd., Bray, Ireland) for 30 min at 100 °C followed by centrifugation (10 000g, 20 min, room temperature). The hot water extraction was repeated once with another portion of thermostable α -amylase (1 mL). After combined supernatants were purified, as described below, the starch and protein free materials were designated WE.III.

Purification and Fractionation of WE. The crude extracts were incubated with α -amylase from porcine pancreas (100 μ L) (EC 3.2.1.1, Sigma-Aldrich) and proteinase K from *Tritirachium album* (0.5 mL, 600 units/mL) (EC 3.4.21.64, Roche Diagnostics GmbH, Mannheim, Germany) at 40 °C overnight (pH 6.8, 10 mM CaCl₂, 0.03% NaN₃). After they were heat treated (20 min, 95 °C) and centrifuged (10 000g, 20 min, room temperature), the supernatants were dialyzed against deionized water (48 h, 6 °C) using membrane tubing (MW cutoff of 12 000; Sigma-Aldrich). The resulting extracts were incubated with amyloglucosidase from *Aspergillus niger* (0.5 mL, for WE.I and WE.II, 5 mL for WE.III) (EC 3.2.1.3, Roche Diagnostics GmbH) at 40 °C overnight (pH 4.8, 0.03% NaN₃), heat treated (20 min, 95 °C), and centrifuged (10 000g, 20 min, room temperature). The LMW components were eliminated by dialysis against deionized water at 6 °C. Aliquots of each extract (200–400 mL) were freeze-dried. The remaining extracts were directly fractionated by graded ammonium sulfate precipitation according to Izydorczyk et al. (29). The WE subfractions were subsequently precipitated from each extract by stepwise addition of (NH₄)₂SO₄, left overnight at 4 °C, and separated by centrifugation (10 000g, 20 min, 4 °C). The precipitated material was redissolved in water, dialyzed at 6 °C until free of (NH₄)₂SO₄ (judged by conductivity measurement), and freeze-dried. Collection was accomplished at 40, 60, 80, and 100% saturation level. Four subfractions were obtained from WE.I and denoted WE.I.40, WE.I.60, WE.I.80, and WE.I.100 (numbers refer to the saturation level of (NH₄)₂SO₄ at which subfractions were collected). WE.II yielded three subfractions denoted WE.II.60, WE.II.80, and WE.II.100 (no precipitate was observed at 40% saturation), and four subfractions (WE.III.40, WE.III.60, WE.III.80, and WE.III.100) were collected from WE.III.

Chemical Analyses. Moisture, ash, and protein contents in rye flours were determined by AACC methods 44.15A, 46.11A, and 08.12, respectively (30). Protein in purified polysaccharide materials was determined according to Lowry et al. (31) using bovine serum albumin as a standard. β -Glucan content was evaluated by the enzymatic method of McCleary and Glennie-Holmes (32) using a β -glucan assay kit (Megazyme International Ireland Ltd., Bray, Ireland).

Sugar analysis was performed after hydrolysis of samples with 1 M H₂SO₄ at 100 °C for 2 h followed by conversion of the monosaccharides to their alditol acetates (33). Samples were quantified on a Hewlett-Packard model 5890 Series II Plus gas chromatograph (Waldbronn, Germany) equipped with a 30 m \times 0.53 mm i.d. wide bore Rtx 225 capillary column (Restek, Bellefonte, PA) and flame ionization detector. The injector and detector were maintained at 230 and 250 °C, respectively. 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). β -D-Allose (Sigma-Aldrich) was used as

Table 1. Yield and Composition of Three Water Extracts (WE.I, WE.II, and WE.III) Obtained by Consecutive Extraction at Increasing Temperature from Two Experimental Rye Flours^a

fraction	yield ^b	arabinoxylans ^b	polysaccharides ^c	protein ^c	ash ^c	β -glucan ^c	molar composition ^d					
							Ara	Xyl	Man	Gal	Glc	Ara/Xyl
					Amilo							
WE.I	3.89	1.38	55.7	3.9	na	0.8	28.4	43.7	9.0	2.5	16.4	0.65
WE.II	0.93	0.33	60.7	7.2	na	4.8	26.5	40.7	12.5	1.6	18.8	0.65
WE.III	1.90	0.51	57.6	16.2	12.9	9.9	22.6	30.9	5.8	2.6	38.2	0.73
					Nawid							
WE.I	3.95	1.10	50.0	5.2	na	0.9	24.7	38.7	10.8	1.8	23.9	0.64
WE.II	0.60	0.17	52.1	10.4	na	5.6	24.5	37.6	16.1	1.5	20.2	0.65
WE.III	1.94	0.41	49.6	21.3	11.0	11.5	20.7	28.2	8.7	3.2	39.2	0.73

^a Abbreviations: arabinoxylans = $0.88 \times (\text{Ara} + \text{Xyl})$; Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glc, glucose; na, not analyzed. ^b Expressed as weight percentage of flour. Results are presented as means of two extractions; the coefficient of variation was less than 4%. ^c Expressed as weight percentage of WE.I, WE.II, and WE.III, respectively. Results obtained from triplicates; the coefficient of variation was less than 2%. ^d Expressed as percentage (mol/100 mol). Results obtained from triplicates; the coefficient of variation was less than 2%.

internal standard. The WE arabinoxylan content was estimated as 0.88 times the sum of monosaccharide arabinose and xylose. No correction was made for arabinose originating from arabinogalactan peptide because of the low relative abundance of the latter in rye water extracts. The polysaccharide content was calculated as the sum of constituent sugars.

Phenolic acids and their dehydromers were determined after alkaline extraction with 2 M NaOH at 35 °C for 30 min (under nitrogen, in the dark, with agitation). The solution of *o*-coumaric acid (1 mg/mL of 2 M NaOH) was then added as an internal standard. The mixture was acidified with HCl to pH 2 and extracted three times with diethyl ether. The combined extracts were evaporated to dryness under a stream of nitrogen at 35 °C, dissolved in methanol, and analyzed by HPLC as described previously (34). All analyses were performed at least in duplicate. All reagents were of at least analytical grade.

GPC. Samples (6.0–12.0 mg) were solubilized in 0.3% NaCl (3 mL) at room temperature overnight. The solution was filtered through a Profill 0.45 μ m disposable syringe filter (Alltech Associates Inc., Illinois), and the polysaccharide materials were fractionated with a Kontron Instruments 325 System HPLC (Milan, Italy) equipped with an autosampler 465 and on line monitoring with a refractive index detector (VDS Optilab, Berlin, Germany) and a Kontron Instruments 332 UV detector (280 nm). Samples were eluted isocratically with 0.3% NaCl (0.5 mL/min) at 30 °C on a 300 mm \times 8 mm i.d. Shodex SB-806 HQ GPC column (Showa Denko K. K., Tokyo, Japan) connected to a 50 mm \times 6 mm i.d. SB-800P guard column. The column was calibrated with Shodex standard P-82 pullulan standards (Showa Denko K. K.) with MWs of 78.8×10^4 , 40.4×10^4 , 21.2×10^4 , 11.2×10^4 , 4.73×10^4 , 2.28×10^4 , 1.18×10^4 , and 0.59×10^4 . Samples containing substantial amounts of polymeric glucose were solubilized in 0.3% NaCl and incubated with lichenase, (1 \rightarrow 3),(1 \rightarrow 4)- β -D-glucan-4-glucanohydrolase from *Bacillus subtilis* (EC 3.2.1.73, 50 μ L, 50 units/mL) (Megazyme International Ireland Ltd.) overnight at room temperature. After they were heat inactivated (20 min, 95 °C) and centrifuged (10 000g, 10 min, room temperature), the supernatants were applied to the GPC system as described above.

¹H NMR Spectroscopy. Samples were dissolved in D₂O (99.8% D) with overnight stirring at room temperature and freeze-dried. This step was repeated once, and finally, the deuterium-exchanged material was redissolved in D₂O (5 mg/mL) and centrifuged (10 000g, 10 min, room temperature) before analysis. ¹H NMR spectra were recorded on a Bruker 500 MHz spectrometer (Bruker, Karlsruhe, Germany) at 85 °C. Acetone was used as standard (δ 2.23 ppm).

RESULTS AND DISCUSSION

Yield and Chemical Composition of WE Materials. The extraction yields and analytical characteristics of the WE fractions obtained by sequential water extraction at 4 (WE.I), 40 (WE.II), and 100 °C (WE.III) are presented in **Table 1**. The material recovered in WE.I represented 3.9 and 4.0% of high

baking quality Amilo rye flour and low baking quality Nawid rye flour, respectively. Much less of these components was recovered in WE.II and WE.III; WE.II made up only 0.9 and 0.6%, while hot water extractable material constituted almost two times more, i.e., 1.9% of Amilo and Nawid, respectively. All fractions largely consisted of polysaccharides (50–61%) and proteins (4–21%).

The majority of arabinoxylans, 1.38 and 1.10% for Amilo and Nawid, respectively, was recovered in WE.I, while only 0.51 and 0.41% were recovered in WE.III and 0.33 and 0.17% were recovered in WE.II. The relative distribution of arabinoxylans over the three WE fractions was comparable in both flours: 62–66% of WE arabinoxylans were freely soluble, while only 10–15 and 23–24% required a higher extraction temperature. Total WE arabinoxylan contents were 2.22 and 1.68% in high and low baking quality flours, in agreement with earlier values for rye flours of differing breadmaking quality (13) as well as for whole meal ryes obtained by hot water extraction (18). Sequential water extraction at 40 and 65 °C earlier yielded various fractions of barley β -glucan and/or arabinoxylans (29, 35). It is clear from the above results that extraction temperature substantially influences the yield of WE material.

It is noteworthy that WE arabinoxylans extracted at 4 and 40 °C from both flour had equal arabinose to xylose (Ara/Xyl) ratios (\sim 0.64) indicating the same degree of branching. Hot WE arabinoxylans had higher substitution degrees (Ara/Xyl for both flours 0.73). These values are within the range previously reported for WE arabinoxylans from rye whole grain (0.57–0.68) (18) and flour (0.71) (8). It is important that in our study, the least soluble arabinoxylan fractions, extracted by hot water, had evidently a higher substitution degree than much easier soluble AX populations. Isolation of arabinoxylans by consecutive water extraction at increasing temperatures from rye flour indicates that these populations are noncovalently associated with one another and/or with the WU material and that the strength of the intermolecular linkages is different as they are brought into solution upon increasing temperature.

The WE.I fractions from both flours were characterized by the lowest protein content, 3.9 and 5.2%, and trace amounts of β -glucan (\sim 0.8%). The WE.II and WE.III contained progressively more protein and, concurrently, more β -glucans. It is of note that both fractions practically had the same protein to β -glucan ratio (ca. 1.6 and 1.9 for Amilo and Nawid) and that the largest quantity of protein was released at the highest temperature, despite its generally accepted protein denaturing effect. The polymers present in WE.I and WE.II were enriched

Table 2. Yield and Composition of Subfractions Isolated by Ammonium Sulfate Precipitation from Three Water Extracts of Rye Flour Amilo^a

subfraction	yield ^b	arabinoxylans ^b	polysaccharides ^c	protein ^c	molar composition ^d						
					Ara	Xyl	Man	Gal	Glc	total Ara + Xyl	Ara/Xyl
WE.I.40	0.09	0.03	48.7	7.6	25.7	43.5	4.0	1.7	25.1	69.1	0.59
WE.I.60	1.23	1.01	94.3	1.6	32.0	65.7	nd	nd	2.4	97.6	0.49
WE.I.80	0.32	0.22	80.6	2.5	44.9	49.6	4.1	nd	1.3	94.6	0.91
WE.I.100	0.14	0.07	57.5	2.3	53.1	41.4	3.8	nd	1.7	94.5	1.28
WE.II.60	0.38	0.22	84.4	3.6	25.7	52.3	4.6	nd	17.4	78.0	0.49
WE.II.80	0.17	0.09	74.7	2.4	31.6	42.6	21.7	nd	4.1	74.2	0.74
WE.II.100	0.11	0.04	42.1	2.7	46.8	42.3	7.7	nd	3.2	89.1	1.11
WE.III.40	0.13	0.01	28.5	16.4	8.1	13.6	6.3	1.9	70.1	21.7	0.59
WE.III.60	0.56	0.23	81.3	6.3	18.6	37.4	5.3	nd	38.7	56.0	0.50
WE.III.80	0.37	0.17	59.5	6.7	38.9	45.5	6.0	nd	9.6	84.4	0.85
WE.III.100	0.13	0.03	22.7	7.9	53.0	43.4	1.0	1.3	1.3	96.4	1.22

^a Abbreviations: arabinoxylans = $0.88 \times (\text{Ara} + \text{Xyl})$; Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glc, glucose; nd, not detected. ^b Expressed as weight percentage of flour. Results are presented as means of two extractions; the coefficient of variation was less than 4%. ^c Expressed as weight percentage of corresponding subfractions. Results obtained from triplicates; the coefficient of variation was less than 2%. ^d Expressed as percentage (mol/100 mol). Results obtained from triplicates; the coefficient of variation was less than 2%.

Table 3. Yield and Composition of Subfractions Isolated by Ammonium Sulfate Precipitation from Three Water Extracts of Rye Flour Nawid^a

subfraction	yield ^b	arabinoxylans ^b	polysaccharides ^c	protein ^c	molar composition ^d						
					Ara	Xyl	Man	Gal	Glc	total Ara + Xyl	Ara/Xyl
WE.I.40	0.09	0.02	34.3	14.9	19.8	33.5	4.0	nd	42.6	53.4	0.59
WE.I.60	1.08	0.82	84.5	2.4	31.2	65.8	nd	nd	3.0	97.0	0.47
WE.I.80	0.25	0.19	88.2	4.1	44.8	50.2	3.6	nd	1.4	95.0	0.89
WE.I.100	0.09	0.05	56.3	3.9	54.2	42.9	3.0	nd	nd	97.0	1.26
WE.II.60	0.25	0.10	62.6	5.9	23.5	46.4	9.1	nd	21.1	69.8	0.51
WE.II.80	0.10	0.05	77.8	5.0	28.1	37.1	25.3	nd	9.5	65.2	0.76
WE.II.100	0.10	0.02	49.8	4.4	36.8	37.5	18.6	nd	7.1	74.3	0.98
WE.III.40	0.18	0.01	14.7	20.6	12.1	16.8	10.2	nd	60.9	28.9	0.72
WE.III.60	0.52	0.19	82.7	9.9	16.0	31.6	8.7	nd	43.7	47.6	0.51
WE.III.80	0.34	0.13	50.0	7.5	35.5	41.9	9.6	nd	13.0	77.4	0.85
WE.III.100	0.17	0.06	42.3	8.9	48.0	42.0	3.1	1.6	5.3	89.9	1.14

^a Abbreviations: arabinoxylans = $0.88 \times (\text{Ara} + \text{Xyl})$; Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glc, glucose; nd, not detected. ^b Expressed as weight percentage of flour. Results are presented as means of two extractions; the coefficient of variation was less than 4%. ^c Expressed as weight percentage of corresponding subfractions. Results obtained from triplicates; the coefficient of variation was less than 2%. ^d Expressed as percentage (mol/100 mol). Results obtained from triplicates; the coefficient of variation was less than 2%.

in mannose. However, some care is due, since the mannose may have resulted from fructans, as reduction of the fructose formed in the hydrolysis step leads to both mannitol and glucitol. About 50% of glucose residues in WE.II and WE.III was from β -glucans, while only 7–9% in WE.I, indicating that other, easily soluble, glucose-containing polymers prevailed in the cold WE fractions.

Fractionation of WE Materials. Tables 2 and 3 list the yields and chemical characteristics of subfractions obtained by ammonium sulfate precipitation from the Amilo and Nawid WE fractions. The arabinoxylan populations that precipitated at 60% ammonium sulfate saturation were quantitatively dominant. The major subfraction, i.e., WE.I.60 isolated from both Amilo and Nawid, had the highest polysaccharide content (94 and 85%, respectively) and the lowest Ara/Xyl (~ 0.5). Apparently, structural analogous arabinoxylans were present in corresponding subfractions isolated from WE.II and WE.III at 60% saturation. The individual arabinoxylan recoveries (expressed as weight % of flour in Tables 2 and 3) show a decrease in the relative proportion of dominant, lowly branched populations, obtained at 60% ammonium sulfate saturation, from WE.I to WE.III. They constituted 76% of the total arabinoxylans recovered in WE.I from both flours, 63 and 59% in WE.II and 52 and 49% in WE.III, respectively, for Amilo and Nawid. The proportion of arabinoxylans with intermediate and high substitution degrees, isolated at 80 and 100% saturation, increased rapidly from WE.I to WE.III, with average values for both flours

from 17 to 36% and from 5 to 11%, for subfractions precipitated at 80 and 100% of salt saturation, respectively. Hence, these observations suggest that higher arabinoxylan water extractability might be related to higher proportions of dominant, lowly branched structures (Ara/Xyl ~ 0.5), whereas an increase in the proportion of more branched structures goes hand in hand with decreased arabinoxylan water extractability. It is of interest that wheat WE arabinoxylan with maximum solubility showed the same Ara/Xyl ratio (0.44–0.50) (14). The higher Ara/Xyl ratio of arabinoxylan extracted at higher temperature (Table 1) can be also explained by the fact that these highly substituted structures are possibly more embedded in the cell wall than the molecules with lower Ara/Xyl ratio. Once in solution, their solubility might be the same or even higher than that of lowly branched structures. From this point of view, our results do not go against the thesis of Andrewartha et al. (14).

Subfractions precipitated at 60, 80, and 100% of salt saturation from each extract contained almost equal protein content, although increasing from WE.I to WE.III. Clearly, the corresponding subfractions from Nawid, of inferior baking quality, had the higher protein content. At the lowest saturation level (40%), two small populations rich in protein (WE.I.40 and WE.III.40) were separated from cold and hot WE fractions (such population was absent in WE.II), which constituted only 0.1–0.2% of rye flours.

Structural Characterization of Polysaccharide Populations by ¹H NMR Spectroscopy. General Analysis of the ¹H NMR

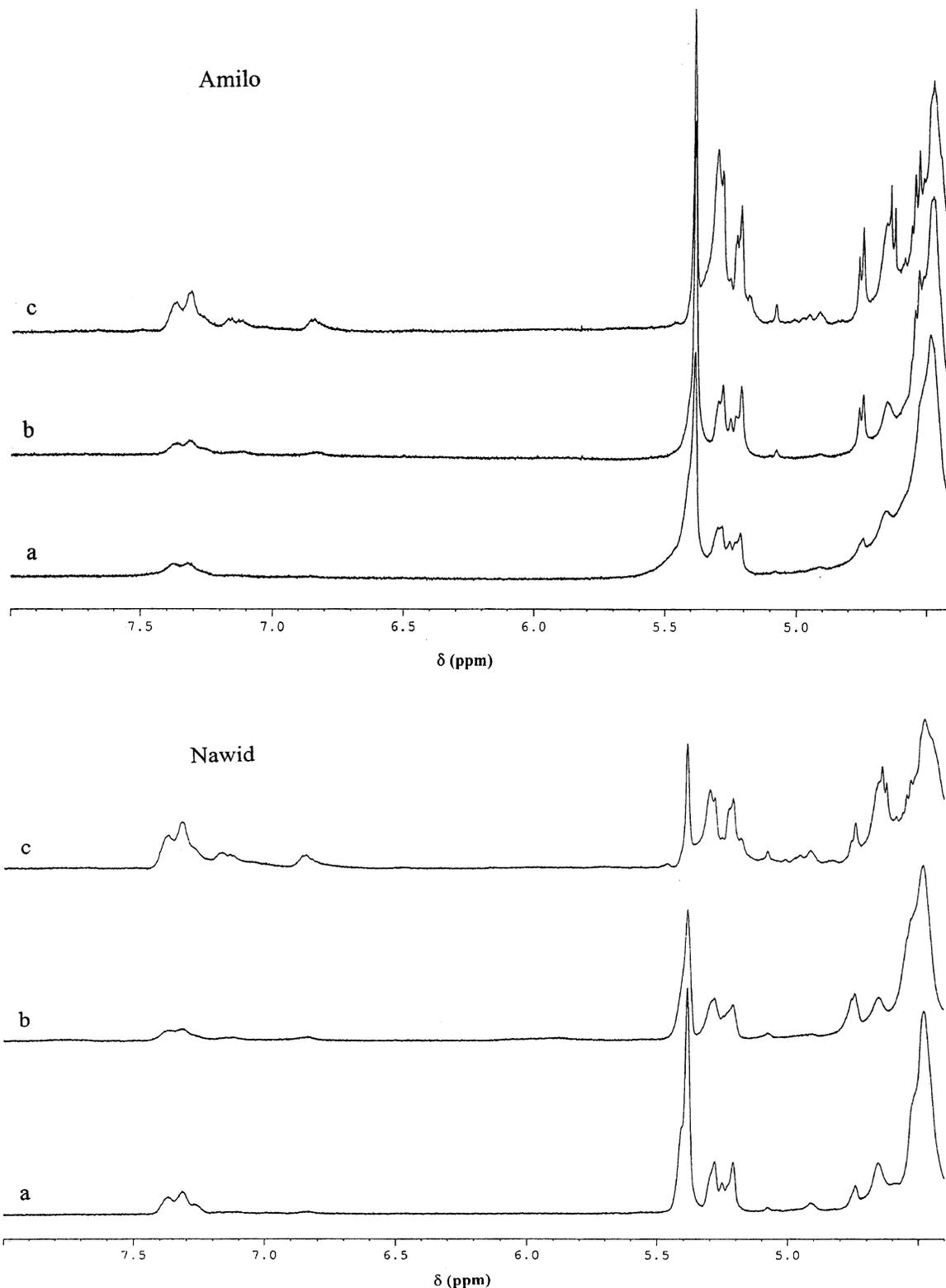


Figure 1. ^1H NMR spectra of the WE fractions obtained by successive water extraction at increasing temperature: (a) WE.I, 4 °C; (b) WE.II, 40 °C; and (c) WE.III, 100 °C.

Spectra. The ^1H NMR spectra of the unfractionated WE fractions isolated from both rye flours are shown in **Figure 1**. The signals of anomeric protons of terminal Araf linked to Xylp were prominent in all samples. Their chemical shifts were assigned by comparison with previously reported literature data (36–39). The resonance at $\delta 5.38$ ppm represents the anomeric protons of terminal Araf linked to *O*-3 of Xylp. The two signals of equal intensity at $\delta 5.21$ and $\delta 5.28$ ppm originate from the

anomeric protons of terminal Araf linked to *O*-2 and *O*-3 of the same Xylp. The doublet at $\delta 4.74$ and $\delta 4.75$ ppm, well-resolved in the spectra from Amilo, arises from the β -anomer in three-linked glucopyranosyl residues (Glc_p) in β -glucan (40). The increase from WE.I to WE.III in the relative intensities of signals from Araf linked to *O*-2 and *O*-3 of the same Xylp is clear in the spectra of both flours. However, they were represented by split peaks, indicating the presence of two

successive double-branched Xylp (37). The small contamination with arabinogalactan is indicated by the resonances at δ 5.25 ppm (41) and supported by sugar analysis. Unexpectedly, all spectra displayed clear resonances in the region of phenolic moieties, δ 6–8 ppm (34, 42), showing that phenolic compounds constitute a noticeable proportion of WE fractions. These signals were the most pronounced for hot WE fractions.

The ^1H NMR spectra of the individual subfractions precipitated from the three main WE fractions clearly showed that arabinoxylan present in each of them consists of series of structures (Figures 2 and 3). Generally, there was a decline in the relative intensities of resonances of Araf linked to *O*-3 of Xylp in spectra of subfractions precipitated with increasing ammonium sulfate saturation. This went hand in hand with increasing resonances of Araf linked to *O*-2 and *O*-3 of the same Xylp. Conspicuous splitting of peaks at δ 5.23 and δ 5.29 ppm in spectra of subfractions isolated at 40, 80, and 100% saturation indicates that two adjacent double-substituted Xylp were more abundant, whereas the lack of such peaks in WE.I.60 suggests that single arabinose branches occurred predominantly on isolated xylose units. A close examination of the WE.II.60 and WE.III.60 spectra revealed that the small splitting peaks could also be recognized in the former. The relatively intense signals of unsubstituted Xylp (δ 4.48 ppm), found in subfractions obtained at 60% saturation, might indicate the presence of longer sequences of unsubstituted Xylp (40). The WE.III.100 spectra from both rye flours were characterized by an extremely high peak at δ 5.30 ppm, which could be assigned to anomeric protons of Araf linked to *O*-2 of the Xylp (43, 44). To date, there is only one report on monosubstituted 2-Xylp in rye (20). The occurrence of such branching sites in the AX structure was further confirmed by methylation analysis (results not shown). Except for peaks of the arabinose anomeric protons, reflecting arabinoxylan structural features, the doublets at δ 4.74 and δ 4.75 ppm originating from β -glucans were readily noticeable. Surprisingly, in some spectra, such doublets were characterized by considerably lower resonance at δ 4.75 ppm or only single resonance at δ 4.74 ppm. In the spectrum of WE.II.80 from Nawid, which was enriched in glucose and mannose residues (Table 3), an extremely intense peak appeared at δ 4.74 ppm, in comparison with those from anomeric protons of Araf. However, in the corresponding subfraction from Amilo, with comparable level of mannose and a significantly lower level of glucose residues, this resonance was less pronounced with an accompanying small shoulder at δ 4.75 ppm. This might suggest different structures of mannose-containing polymers from Amilo and Nawid.

Much like the unfractionated materials, the spectra of the subfractions displayed clear resonances in the region of phenolic moiety, δ 6–8 ppm. This was particularly apparent for subfractions precipitated from cold and hot extracts at 40% ammonium sulfate saturation, WE.I.40 and WE.III.40, of both flours. In WE.III.40 spectra, however, much more intense peaks in this region were noted when compared to those from Araf anomeric protons. Phenolic acid analysis revealed the presence of ferulic acid and its dehydrodimers in cold WE subfractions precipitated at 40% saturation from both flours. The 8–8' coupled dehydrodimer was a major component. Nevertheless, the WE.I.40 from Nawid contained two times more ferulic acid (32.0 and 16.5 $\mu\text{g g}^{-1}$ for Nawid and Amilo, respectively) as well as five times more 8–8' coupled dehydrodimer (32.8 and 5.8 $\mu\text{g g}^{-1}$, respectively) than the corresponding subfraction from Amilo. Furthermore, both samples contained 5–5' (0.3 and 0.1 $\mu\text{g g}^{-1}$ for Nawid and Amilo, respectively) and 8-*O*-4' (0.2 $\mu\text{g g}^{-1}$ for

Table 4. Relative Percentage of Un-, Mono-, and Disubstituted Xylose Residues (u-Xyl, 2-Xyl, 3-Xyl, and 2,3-Xyl) of Arabinoxylans in WEs of Rye Flours Amilo and Nawid^a

Amilo						
fraction	u-Xyl	3-Xyl	2-Xyl	2,3-Xyl	di/mono	PhC/Ara
WE.I	45.5	44.1	nd	10.4	0.24	0.12
WE.II	48.0	38.9	nd	13.1	0.34	0.19
WE.III	45.7	19.2	16.1	19.0	0.54	0.33
Nawid						
fraction	u-Xyl	3-Xyl	2-Xyl	2,3-Xyl	di/mono	PhC/Ara
WE.I	48.4	39.3	nd	12.3	0.31	0.31
WE.II	50.2	34.6	nd	15.2	0.44	0.38
WE.III	48.9	18.0	7.9	25.2	0.97	0.72

^a Abbreviations: u-Xyl, unsubstituted β -(1 \rightarrow 4)-linked D-Xylp; 2-Xyl, β -(1 \rightarrow 4)-linked D-Xylp substituted with α -L-Araf at *O*-2; 3-Xyl, β -(1 \rightarrow 4)-linked D-Xylp substituted with α -L-Araf at *O*-3; 2,3-Xyl, β -(1 \rightarrow 4)-linked D-Xylp substituted with α -L-Araf at *O*-2 and *O*-3; di/mono, ratio of 2,3-Xyl to sum of 2-Xyl and 3-Xyl; PhC/Ara, the ratio of total resonance in phenolic acids region to total resonance from arabinose anomeric protons; nd, not detected.

both flours) dehydrodimers in small amounts. Because the presence of dehydrodimers in the cell wall provides evidence for its cross-linked structure, this might suggest a higher cross-linking of WE arabinoxylans from rye flour of inferior baking quality. Recently, the whole spectrum of ferulic acid dehydrodimers has been identified in soluble dietary fiber fraction from rye, in which 8–8' diferulic acid dominated as well (45). An attempt to identify the phenolic compounds in a subfraction obtained through hot extraction, WE.III.40, failed, despite much more intense signals in the region of phenolic moiety observed in the ^1H NMR spectra of these subfractions, as compared to corresponding resonances found for cold WE subfractions, WE.I.40 (Figures 2 and 3). This can be ascribed to different kinds of phenolic compounds present in WE.III.40, which could not be identified by the procedure used in our study.

Arabinoxylan Specific Analysis of the ^1H NMR Spectra. The proportion of unsubstituted (u-Xylp), mono- (3-Xylp, 2-Xylp), and disubstituted (2,3-Xylp) Xylp was calculated using both the ^1H NMR spectral data and the gas chromatography results, as described previously (38, 46, 47). The results for the main WE fractions from both flours are summarized in Table 4. The relative level of u-Xylp remained virtually constant, 46–48% and 49–50%, for Amilo and Nawid, respectively. In contrast, the proportion of 2,3-Xylp increased markedly from 10.4 and 12.3% in WE.I to 19.0 and 25.2% in WE.III, with a simultaneous decrease in the proportion of 3-Xylp from 44.1 and 39.3% in cold WE to 19.2 and 18.0% in hot WE. Hence, the ratio of di- to monosubstituted Xylp increased distinctly. Moreover, there is also clearly an increase in the ratio of phenolic compound resonances to total Araf signals (PhC/Ara) from cold to hot WE fractions. It is evident from these data that the arabinoxylan structure is one of the primarily factors determining its water extractability; however, in addition to that, the contribution of phenolic compounds is essential. The most striking is the fact that Amilo, besides distinctly lower values for ratios of di- to monosubstituted Xylp and PhC/Ara in the corresponding fractions, was characterized by two times higher proportion of 2-Xylp than Nawid. The importance of such structural elements for rye AX functionality is unknown.

The relative proportions of differently linked Xylp in arabinoxylans isolated after fractionation from each WE fraction are listed in Table 5. Generally, the increase in ammonium sulfate saturation yielded arabinoxylan subfractions with increasing

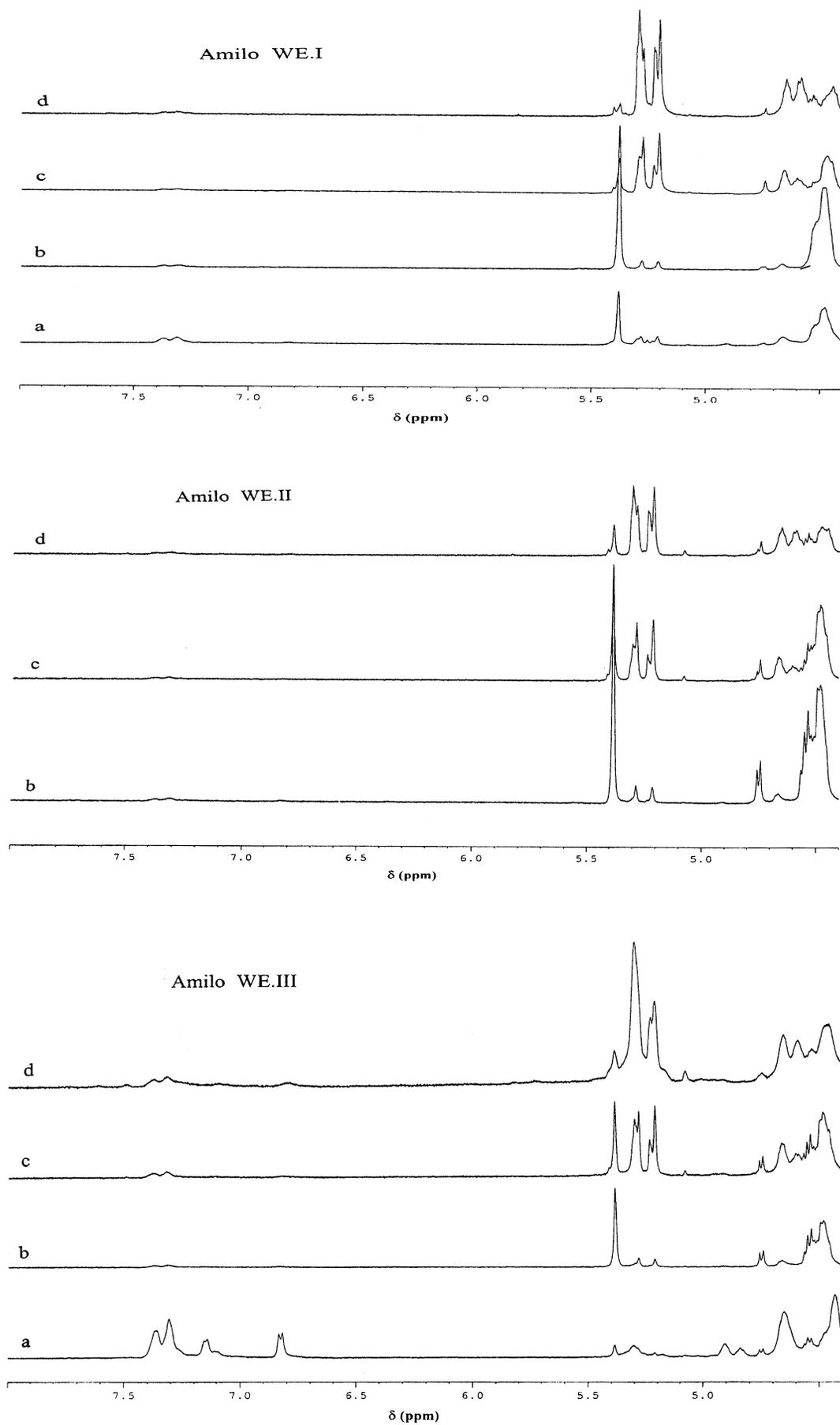


Figure 2. ^1H NMR spectra of the WE subfractions obtained from three main WE fractions of rye flour Amilo by ammonium sulfate precipitation at different saturation levels: (a) 40, (b) 60, (c) 80, and (d) 100%, respectively.

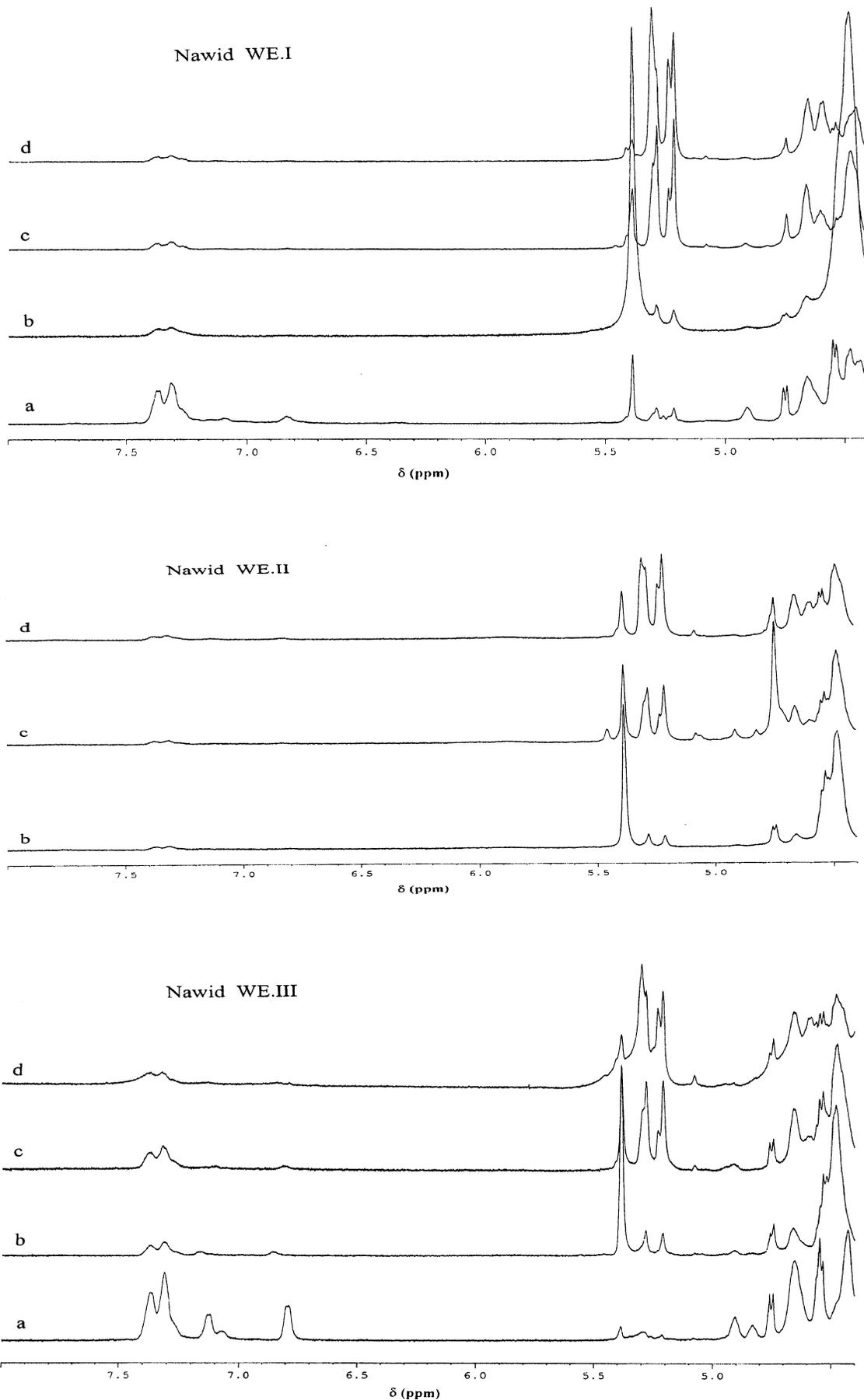


Figure 3. ^1H NMR spectra of the WE subfractions obtained from three main WE fractions of rye flour Nawid by ammonium sulfate precipitation at different saturation levels: (a) 40, (b) 60, (c) 80, and (d) 100%, respectively.

Table 5. Relative Percentage of Un-, Mono-, and Disubstituted Xylose Residues (u-Xyl, 2-Xyl, 3-Xyl, and 2,3-Xyl) of Arabinoxylans in WE Subfractions of Rye Flours Amilo and Nawid^a

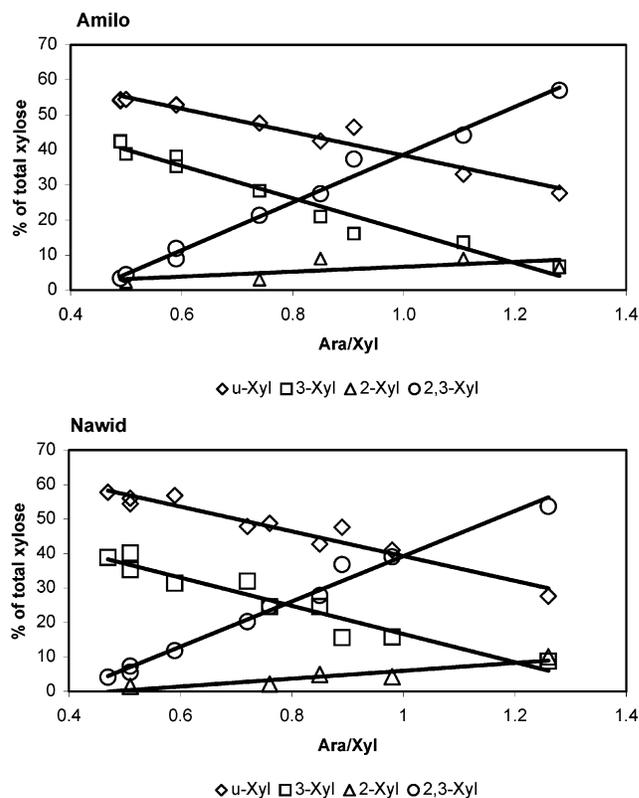
Amilo						
subfraction	u-Xyl	3-Xyl	2-Xyl	2,3-Xyl	di/mono	PhC/Ara
WE.I.40	52.9	38.2	nd	8.9	0.23	0.44
WE.I.60	54.0	42.6	nd	3.4	0.08	0.14
WE.I.80	46.5	16.1	nd	37.4	2.32	0.06
WE.I.100	29.5	6.8	6.5	57.2	4.30	0.03
WE.II.60	54.3	42.3	nd	3.4	0.08	0.19
WE.II.80	47.4	28.3	3.0	21.3	0.68	0.04
WE.II.100	33.1	13.7	9.0	44.2	1.94	0.10
WE.III.40	52.8	35.3	nd	11.9	0.34	4.37
WE.III.60	54.4	38.9	2.3	4.4	0.11	0.25
WE.III.80	42.5	21.0	9.0	27.5	0.91	0.16
WE.III.100	12.9	15.6	36.7	34.8	0.67	0.16

Nawid						
subfraction	u-Xyl	3-Xyl	2-Xyl	2,3-Xyl	di/mono	PhC/Ara
WE.I.40	56.8	31.4	nd	11.8	0.38	2.19
WE.I.60	57.1	38.8	nd	4.1	0.11	0.09
WE.I.80	47.6	15.6	nd	36.8	2.36	0.10
WE.I.100	27.6	8.8	10.0	53.6	2.85	0.07
WE.II.60	54.4	40.1	nd	5.5	0.14	0.10
WE.II.80	48.8	24.5	2.1	24.6	0.92	0.07
WE.II.100	41.0	15.8	4.2	39.0	1.95	0.09
WE.III.40	47.8	32.0	nd	20.2	0.63	7.06
WE.III.60	56.0	35.3	1.4	7.3	0.19	0.32
WE.III.80	42.7	24.6	4.9	27.8	0.94	0.36
WE.III.100	18.2	21.9	27.5	32.4	0.66	0.19

^a Abbreviations: u-Xyl, unsubstituted β -(1 \rightarrow 4)-linked D-Xylp; 2-Xyl, β -(1 \rightarrow 4)-linked D-Xylp substituted with α -L-Araf at O-2; 3-Xyl, β -(1 \rightarrow 4)-linked D-Xylp substituted with α -L-Araf at O-3; 2,3-Xyl, β -(1 \rightarrow 4)-linked D-Xylp substituted with α -L-Araf at O-2 and O-3; di/mono, ratio of 2,3-Xyl to sum of 2-Xyl and 3-Xyl; PhC/Ara, the ratio of total resonance in phenolic acids region to total resonance from arabinose anomeric protons; nd, not detected.

levels of disubstituted Xylp and 2-Xylp but decreasing amounts of u- and 3-Xylp. Similar observations were reported earlier for wheat, rye, and barley WE arabinoxylans fractionated with ammonium sulfate (19, 29, 48). The major populations, obtained at 60% saturation from three WE fractions, were almost pure monosubstituted arabinoxylans, containing only 3.4–4.4% of disubstituted Xylp for Amilo and 4.1–7.3% for Nawid. In contrast, the most soluble populations, which required 100% saturation to precipitate from WE.I, contained 57 and 54% disubstituted and low levels of monosubstituted Xylp, 3-Xylp (6.8 and 8.8%), and 2-Xylp (6.5 and 10%). Similar structural analogues were designated arabinoxylan I and II by Bengtsson et al. (18) for WE arabinoxylan populations of whole rye as well as reported by Vinkx et al. (19), however, as virtually pure monosubstituted and disubstituted arabinoxylans, which precipitated, respectively, at 50 and 100% of ammonium sulfate saturation. There was a clear rise in the level of 2-Xylp observed among subfractions obtained at increasing saturation level from 60 to 100% in WE.II and WE.III. However, subfractions from Amilo had an appreciable higher amount of this structural element than corresponding subfractions from Nawid. The arabinoxylans obtained at 80% salt saturation were characterized by intermediate level of u-Xylp and differently substituted Xylp in the chain.

Taking into account the three sets of subfractions (excluding WE.III.100), their substitution degrees, as reflected by Ara/Xyl ratios, were correlated with relative proportions of the four structural elements in the arabinoxylan backbone, i.e., u-, 2-, 3-, and 2,3-Xylp (Figure 4). Similar relationships were reported previously for wheat and rye WE arabinoxylans (44, 47).

**Figure 4.** Linear relationships between the relative proportion of differently linked xylose residues and the ratio of Ara/Xyl of WE arabinoxylans from two rye flours.

GPC. The GPC profiles obtained for WE fractions derived from Amilo and Nawid are shown in Figure 5. All profiles displayed a very broad peak in the HMW region and relatively narrow peaks in the LMW region. Peak MWs of the polymers that eluted in the HMW regions were much higher than those of the highest MW pullulan standard (78.8×10^4). Regrettably, there is no MW standard available that would be adequate for arabinoxylans. It follows that discrepancies between MW values obtained for rye WE arabinoxylans with pullulan standards and absolute MW determination exist (6, 49–51).

There was a slight shift in elution volume of HMW polymers from WE.I to WE.III toward higher hydrodynamic volumes (elution at 8.57, 8.45, and 8.20 mL for the Amilo samples and 8.63, 8.56, and 8.33 mL for the Nawid samples). This indicates that extraction at elevated temperatures yielded populations of somewhat higher MWs. The polysaccharide populations from Amilo had higher MWs than the corresponding populations from Nawid. The shift in MW was accompanied by changes in the GPC profiles in the HMW regions. The UV-absorbing materials eluted in the LMW region. These materials could be ascribed mostly to protein, as shown by considerable protein contents in WE fractions (Table 1), although phenolic acids cannot be excluded. It should be emphasized that UV-absorbing materials in the WE.II and WE.III profiles did not follow the carbohydrate elution profiles. In addition, there was a clear shift in their elution volumes toward the HMW region in comparison to the WE.I profiles, particularly pronounced for Nawid. This indicates that at least part of the UV-absorbing material, present in WE.II and WE.III, is not covalently linked to polysaccharide fraction, whereas in WE.I such material appeared to be strongly associated with the carbohydrate population. The association of LMW polysaccharides with protein was reported previously for whole grain of wheat and rye as well for wheat endosperm (49, 52).

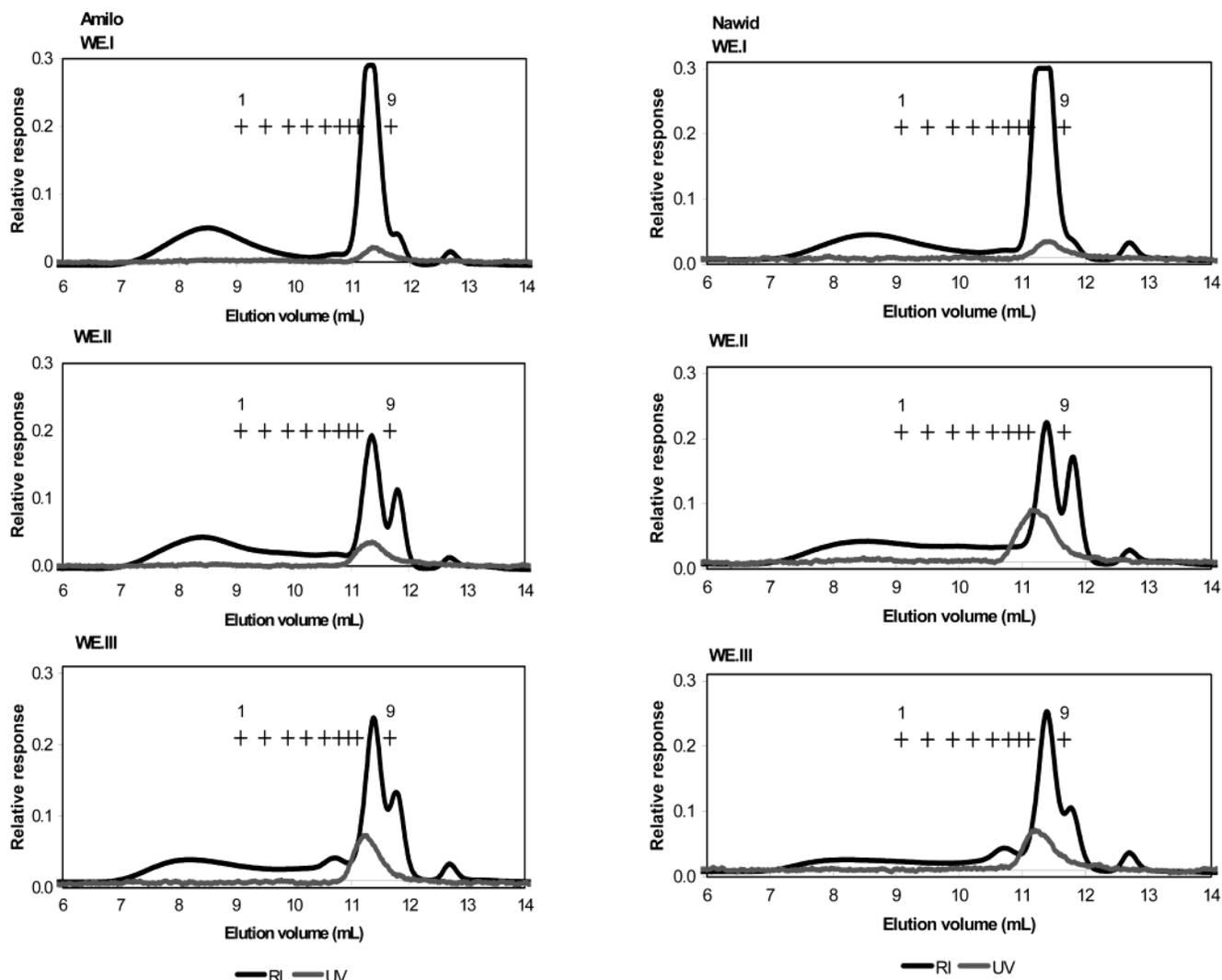


Figure 5. MW profiles of polysaccharides and UV-absorbing materials of three WE fractions (WE.I, WE.II, and WE.III) isolated from rye flours Amilo and Nawid. Pullulan calibration standards are (1) 78.8×10^4 ; (2) 40.4×10^4 ; (3) 21.2×10^4 ; (4) 11.2×10^4 ; (5) 4.73×10^4 ; (6) 2.28×10^4 ; (7) 1.18×10^4 ; (8) 0.59×10^4 ; and (9) glucose.

It is evident from the GPC profiles in HMW region that, excluding the subfractions precipitated at 40% ammonium sulfate saturation, these obtained at 60% saturation had generally the lowest MWs (Figures 6 and 7 for Amilo and Nawid, respectively). Increasing ammonium sulfate saturation resulted in more branched arabinoxylan subfractions with higher MWs. It is well-known that the overall strength of noncovalent interactions depends very much on the size of the interacting polymers. It seems, therefore, that subfractions precipitated at 80 and 100% saturation levels, with much higher MWs, might, despite their lower proportion, effectively influence the physicochemical properties of overall polysaccharide population. From this viewpoint, the WE.III fraction, with its highest MW and highest proportion of strongly branched subfractions, appears to be potentially more involved in the interactions with other components. It is equally known that the high MW of polymers is a primary factor of their high viscosity. Bengtsson et al. (18) in a study of WE arabinoxylans from rye grain from several countries concluded that arabinoxylan II, containing u- and 2,3-Xylp, had a stronger correlation to viscosity than arabinoxylan I, containing only u- and 3-Xylp. Recently, however, Ragaee et al. (51) observed that WE arabinoxylans from high extract viscosity rye had higher MWs displaying higher intrinsic viscosity and a lower proportion of 2,3-Xylp

as compared to WE arabinoxylans from low extract viscosity rye. Our observations support these results. It is believed that the terminal Araf substituents stiffen the arabinoxylan backbone into more extended conformations, which is directly related to its physicochemical properties. However, recent studies of wheat WE arabinoxylans suggest that the amount of ferulic acid dimers might be a major parameter responsible for variation in the macromolecular characteristics of arabinoxylans (47), which also agree with data from our study.

Clear differences were found in the elution volumes of HMW populations from Amilo and Nawid in the WE.I.40 subfractions, while the WE.III.40 subfraction profiles were almost similar for both flours (Figures 6 and 7). To point out the β -glucan peaks in the profiles of WE.III.40, subfractions enriched in glucose-containing polymers (Table 2), the samples were incubated with lichenase before GPC. The disappearance of the first peak eluted at 9.80 mL in WE.III.40 from Amilo after lichenase treatment indicates that β -glucan present in this subfraction had a higher apparent MW than arabinoxylan present as LMW polymer (results not shown). In contrast, the arabinoxylan in WE.III.60 subfraction eluted at 8.75 mL had a much higher MW than β -glucan eluted at 10.35 mL, which was totally degraded during incubation with lichenase. On the basis of calibration with pullulan standards, the MW of β -glucan from

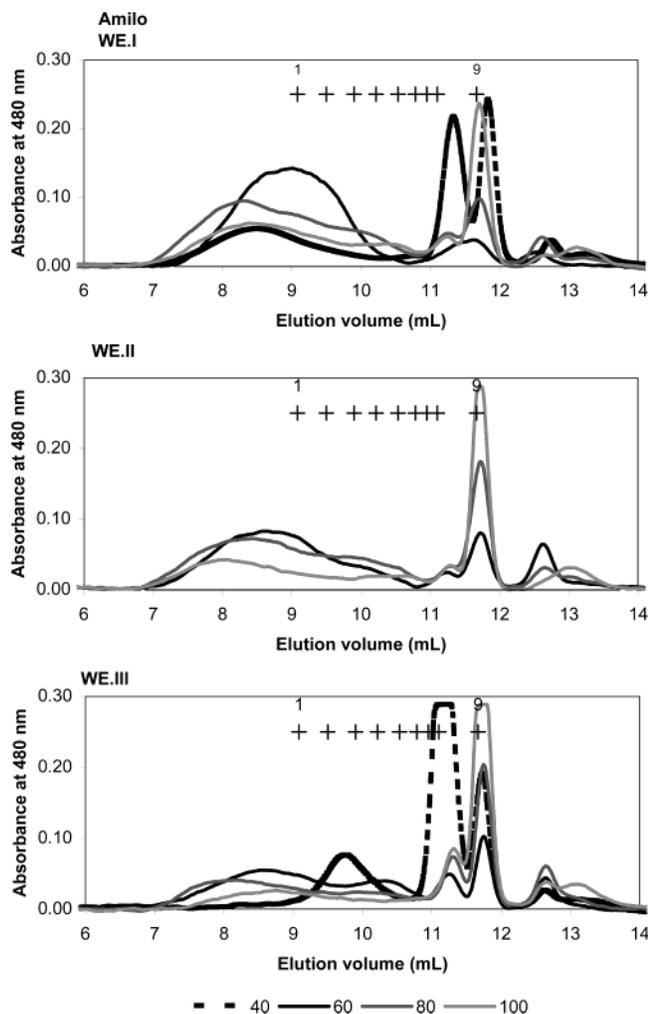


Figure 6. MW distribution of subfractions precipitated at 40, 60, 80, and 100% saturation of ammonium sulfate from three WE isolated from rye flour Amilo. Elution volumes of pullulan standards are as in Figure 5.

rye flour was calculated to be in the range $10\text{--}25 \times 10^4$. The MWs of arabinoxylans present in water extracts of rye flour, therefore, are generally much higher than those of β -glucans. Little is known about rye β -glucans and their impact on functionality of the WE fractions, in which arabinoxylans predominate. The noticeable amounts of β -glucans in arabinoxylan preparations as well as the marked differences in their concentrations in rye flours of diverse baking quality indicate that they should receive more attention in the future investigations on functionality of WE fractions.

ABBREVIATIONS USED

WE, water extractable fraction; WU, water unextractable fraction; Ara/Xyl, arabinose to xylose ratio; ^1H NMR, proton nuclear magnetic resonance; Xylp, xylopyranosyl residues; PhC/Ara, phenolic compounds to arabinose ratio; Ara_f, arabinofuranosyl residues; HPLC, high-performance liquid chromatography; GPC, gel permeation chromatography; HMW, high molecular weight; LMW, low molecular weight.

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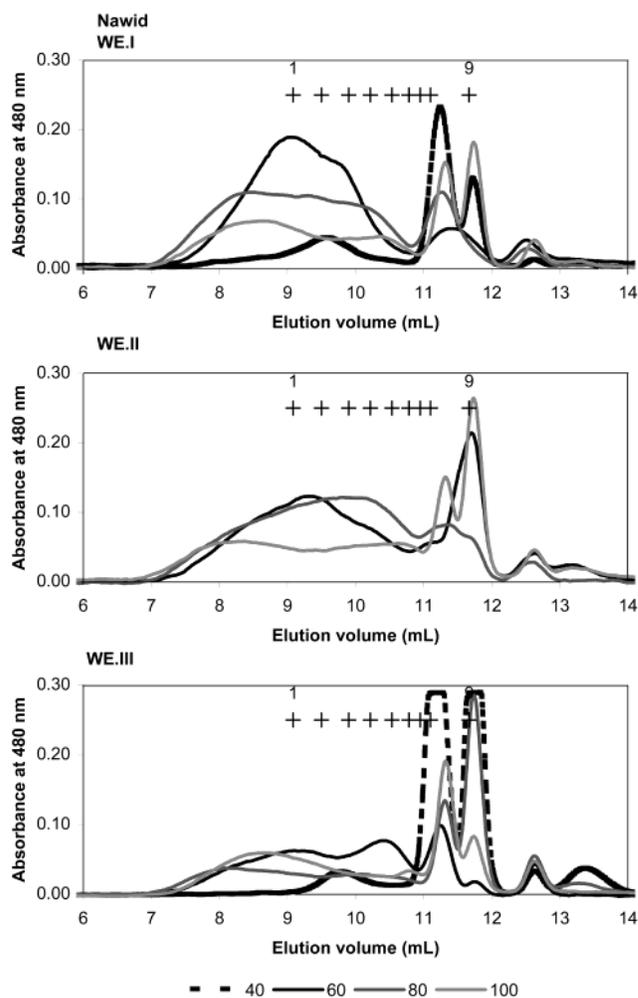


Figure 7. MW distribution of subfractions precipitated at 40, 60, 80, and 100% saturation of ammonium sulfate from three WE isolated from rye flour Nawid. Elution volumes of pullulan standards are as in Figure 5.

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LITERATURE CITED

- (1) Shinnick, F. L.; Mathews, R.; Ink, S. Serum cholesterol reduction by oats and other fiber sources. *Cereal Foods World* **1991**, *36*, 815–821.
- (2) Leinonen, K. S.; Poutanen, K. S.; Mykkänen, H. M. Rye bread decreases serum total and LDL cholesterol in men with moderately elevated serum cholesterol. *J. Nutr.* **2000**, *130*, 164–170.
- (3) Gråsten, S. M.; Juntunen, K. S.; Poutanen, K. S.; Gylling, H. K.; Miettinen, T. A.; Mykkänen, H. M. Rye bread improves bowel function and decreases the concentration of some compounds that are putative colon cancer risk markers in middle-aged women and men. *J. Nutr.* **2000**, *130*, 2215–2221.
- (4) Fincher, G. B.; Stone, B. A. Cell walls and their components in cereal grain technology. In *Advances in Cereal Science and Technology*; Pomeranz Y., Ed.; American Association of Cereal Chemists: St. Paul, MN, 1986; Vol. 8, pp 207–295.
- (5) Meuser, F.; Suckow, P. Nonstarch polysaccharides. In *Chemistry and Physics of Baking*; Blanshard, J. M. V., Frazier, P. J., Galliard, T., Eds.; Royal Society of Chemistry: London, U.K., 1986; pp 42–61.
- (6) Härkönen, H.; Pessa, E.; Suortti, T.; Poutanen, K. Distribution and some properties of cell wall polysaccharides in rye milling fractions. *J. Cereal Sci.* **1997**, *26*, 95–104.

- (7) Cyran, M.; Rakowska, M.; Wasilewko, J.; Buraczewska, L. Degradation of dietary fiber polysaccharides of rye in the intestinal tract of growing pigs used as a model animal for studying digestion in humans. *J. Anim. Feed Sci.* **1995**, *4*, 217–227.
- (8) Glitsø, L. V.; Bach Knudsen, K. E. Milling of whole grain rye to obtain fractions with different dietary fibre characteristics. *J. Cereal Sci.* **1999**, *29*, 89–97.
- (9) Drews, E.; Seibel, W. Bread-baking and other uses around the world. In *Rye: Production, Chemistry and Technology*; Bushuk, W., Ed.; American Association of Cereal Chemists: St. Paul, MN, 1976; pp 127–178.
- (10) Weipert, D. Rye and Triticale. In *Cereal Grain Quality*; Henry, R. J., Kettlewell, P. S., Eds.; Chapman and Hall: London, U.K., 1996; pp 205–224.
- (11) Delcour, J. A.; Van Win, H.; Grobet, P. J. Distribution and structural variation of arabinoxylans in common wheat mill streams. *J. Agric. Food Chem.* **1999**, *47*, 271–275.
- (12) Nyman, M.; Siljeström, M.; Pedersen, B.; Bach Knudsen, K. E.; Asp, N.-G.; Johansson, C.-G.; Eggum, B. O. Dietary fiber content and composition in six cereals at different extraction rates. *Cereal Chem.* **1984**, *61*, 14–19.
- (13) Cyran, M.; Cygankiewicz, A. Water-extractable and water-unextractable non-starch polysaccharides in rye flour: variability and relationship to baking quality parameters. *Cereal Res. Commun.* Submitted for publication.
- (14) Andrewartha, K. A.; Phillips, D. R.; Stone, B. A. Solution properties of wheat-flour arabinoxylans and enzymically modified arabinoxylans. *Carbohydr. Res.* **1979**, *77*, 191–204.
- (15) Aspinall, G. O.; Sturgeon, R. J. Cereal Gums. Part II. The constitution of an arabinoxylan from rye flour. *J. Chem. Soc. Part V* **1957**, 4469–4471.
- (16) Aspinall, G. O.; Ross, K. M. The degradation of two periodate-oxidised arabinoxylans. *J. Chem. Soc. Part II* **1963**, 1681–1686.
- (17) Bengtsson, S.; Åman, P. Isolation and chemical characterization of water-soluble arabinoxylans in rye grain. *Carbohydr. Polym.* **1990**, *12*, 267–277.
- (18) Bengtsson, S.; Andersson, R.; Westerlund, E.; Åman, P. Content, structure and viscosity of soluble arabinoxylans in rye grain from several countries. *J. Sci. Food Agric.* **1992**, *58*, 331–337.
- (19) Vinkx, C. J. A.; Reynaert, H. R.; Grobet, P. J.; Delcour, J. A. Physicochemical and functional properties of rye nonstarch polysaccharides. V. Variability in the structure of water-soluble arabinoxylans. *Cereal Chem.* **1993**, *70*, 311–317.
- (20) Vinkx, C. J. A.; Delcour, J. A.; Verbruggen, M. A.; Gruppen, H. Rye water-soluble arabinoxylans also vary in their contents of 2-monosubstituted xylose. *Cereal Chem.* **1995**, *72*, 227–228.
- (21) Jankiewicz, M.; Michniewicz, J. The effect of soluble pentosans isolated from rye grain on staling of bread. *Food Chem.* **1987**, *25*, 241–249.
- (22) Kühn, M. C.; Grosch, W. Baking functionality of reconstituted rye flours having different nonstarchy polysaccharide and starch contents. *Cereal Chem.* **1989**, *66*, 149–153.
- (23) Delcour, J. A.; Vanhamel, S.; Hosene, R. C. Physicochemical and functional properties of rye nonstarch polysaccharides. II. Impact of a fraction containing water-soluble pentosans and proteins on gluten-starch loaf volumes. *Cereal Chem.* **1991**, *68*, 72–76.
- (24) Raczynska-Bojanowska, K.; Rakowska, M.; Sitariski, J.; Rybka, K.; Zebalska, M.; Cyran, M. The soluble nondigestible compounds as an index in rye breeding for better protein digestibility. *J. Cereal Sci.* **1989**, *9*, 71–76.
- (25) Rakowska, M.; Raczynska-Bojanowska, K.; Kupiec, R. Studies on the antinutritive compounds in rye grain. V. Effect of polysaccharides complex on protein digestibility and feed utilization. *Pol. J. Food Nutr.* **1992**, *1/42*, 95–102.
- (26) Weipert, D. Pentosans as a selection traits in rye breeding. *Vortr. Pflanzzüchtg.* **1996**, *35*, 109–119.
- (27) Cyran, M.; Rakowska, M.; Miazga, D. Chromosomal location of factors affecting content and composition of nonstarch polysaccharides in wheat-rye addition lines. *Euphytica* **1996**, *89*, 153–157.
- (28) Boros, D.; Lukaszewski, A.; Aniol, A.; Ochodzki, P. Chromosome location of genes controlling the content of dietary fibre and arabinoxylans in rye. *Euphytica* **2002**, *128*, 1–8.
- (29) Izydorczyk, M. S.; Macri, L. J.; MacGregor, A. W. Structure and physicochemical properties of barley nonstarch polysaccharides — I. Water-extractable β -glucans and arabinoxylans. *Carbohydr. Polym.* **1998**, *35*, 249–258.
- (30) American Association of Cereal Chemists. *Approved Methods of the AACC*, 10th ed.; The Association: St. Paul, MN, 2000.
- (31) Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **1951**, *193*, 265–275.
- (32) McCleary, B. V.; Glennie-Holmes, M. Enzymatic quantification of (1 \rightarrow 3)(1 \rightarrow 4)- β -D-glucan in barley and malt. *J. Inst. Brew.* **1985**, *91*, 285–295.
- (33) Englyst, H. N.; Cummings, J. H. Simplified method for the measurement of total nonstarch polysaccharides by gas-liquid chromatography of constituent sugars as alditol acetates. *Analyst* **1984**, *109*, 937–942.
- (34) Saulnier, L.; Crépeau, M.-J.; Lahaye, M.; Thibault, J.-F.; Garcia-Conesa, M. T.; Kroon, P. A.; Williamson, G. Isolation and structural determination of two 5,5'-diferuloyl oligosaccharides indicate that maize heteroxylans are covalently cross-linked by oxidatively coupled ferulates. *Carbohydr. Res.* **1999**, *320*, 82–92.
- (35) Woodward, J. R.; Phillips, D. R.; Fincher, G. B. Water soluble (1 \rightarrow 3),(1 \rightarrow 4)- β -D-glucans from barley (*Hordeum vulgare*) endosperm. IV. Comparison of 40 °C and 65 °C soluble fractions. *Carbohydr. Polym.* **1988**, *8*, 85–97.
- (36) Hoffmann, R. A.; Geijtenbeek, T.; Kamerling, J. P.; Vliegthart, J. F. G. ¹H-N.m.r. study of enzymically generated wheat-endosperm arabinoxylan oligosaccharides: structures of hepta- to tetradeca-saccharides containing two or three branched xylose residues. *Carbohydr. Res.* **1992**, *223*, 19–44.
- (37) Hoffmann, R. A.; Kamerling, J. P.; Vliegthart, J. F. G. Structural features of a water-soluble arabinoxylan from the endosperm of wheat. *Carbohydr. Res.* **1992**, *226*, 303–311.
- (38) Cleemput, G.; Roels, S. P.; van Oort, M.; Grobet, P. J.; Delcour, J. A. Heterogeneity in the structure of water-soluble arabinoxylans in European wheat flours of variable bread-making quality. *Cereal Chem.* **1993**, *70*, 324–329.
- (39) Viëtor, R. J.; Hoffmann, R. A.; Angelino, S. A. G. F.; Voragen, A. G. J.; Kamerling, J. P.; Vliegthart, J. F. G. Structures of small oligomers liberated from barley arabinoxylans by endoxylanase from *Aspergillus awamori*. *Carbohydr. Res.* **1994**, *254*, 245–255.
- (40) Roubroeks, J. P.; Andersson, R.; Åman, P. Structural features of (1 \rightarrow 3),(1 \rightarrow 4)- β -D-glucan and arabinoxylan fractions isolated from rye bran. *Carbohydr. Polym.* **2000**, *42*, 3–11.
- (41) Loosveld, A.-M. A.; Grobet, P. J.; Delcour, J. A. Contents and structural features of water-extractable arabinogalactan in wheat flour fractions. *J. Agric. Food Chem.* **1997**, *45*, 1998–2002.
- (42) Ralph, J.; Quideau, S.; Grabber, J. H.; Hatfield, R. D. Identification and synthesis of new ferulic acid dehydrodimers present in grass cell walls. *J. Chem. Soc., Perkin Trans.* **1994**, *1*, 3485–3498.
- (43) Viëtor, R. J.; Angelino, S. A. G. F.; Voragen, A. G. J. Structural features of arabinoxylans from barley and malt cell wall material. *J. Cereal Sci.* **1992**, *15*, 213–222.
- (44) Vinkx, C. J. A. Structure and properties of arabinoxylans from rye. Dissertation, Katholieke Universiteit Leuven, Belgium, 1995.
- (45) Bunzel, M.; Ralph, J.; Marita, J. M.; Hatfield, R. D.; Steinhart, H. Diferulates as structural components in soluble and insoluble cereal dietary fibre. *J. Sci. Food Agric.* **2001**, *81*, 653–660.
- (46) Westerlund, E.; Andersson, R.; Åman, P.; Theander, O. Effects of baking on water-soluble nonstarch polysaccharides in white bread fractions. *J. Cereal Sci.* **1990**, *12*, 33–42.

- (47) Dervilly, G.; Saulnier, L.; Roger, P.; Thibault, J.-F. Isolation of homogeneous fractions from wheat water-soluble arabinoxylans. Influence of the structure on their macromolecular characteristics. *J. Agric. Food Chem.* **2000**, *48*, 270–278.
- (48) Izydorczyk, M. S.; Biliaderis, C. G. Influence of structure on the physicochemical properties of wheat arabinoxylan. *Carbohydr. Polym.* **1992**, *17*, 237–247.
- (49) Girhammar, U.; Nair, B. M. Certain physical properties of water soluble nonstarch polysaccharides from wheat, rye, triticale, barley and oats. *Food Hydrocolloids* **1992**, *6*, 329–343.
- (50) Nilsson, M.; Andersson, R.; Andersson, R. E.; Autio, K.; Åman, P. Heterogeneity in a water-extractable rye arabinoxylan with a low degree of disubstitution. *Carbohydr. Polym.* **2000**, *41*, 397–405.
- (51) Ragaei, S. M.; Campbell, G. L.; Scoles, G. J.; McLeod, J. G.; Tyler, R. T. Studies on rye (*Secale cereale* L.) lines exhibiting a range of extract viscosities. 1. Composition, molecular weight distribution of water extracts, and biochemical characteristics of purified water-extractable arabinoxylan. *J. Agric. Food Chem.* **2001**, *49*, 2437–2445.
- (52) Fincher, G. B.; Stone, B. A. A water-soluble arabinogalactan-peptide from wheat endosperm. *Aust. J. Biol. Sci.* **1974**, *27*, 117–132.

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