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Contents and Structural Features of Water-Extractable Arabinogalactan in Wheat Flour Fractions

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In Camp Remy Bühler MLU-202 roller mill flour fractions, the levels of water-extractable arabinoxylan (WE-AX) (0.30−0.41%, dry basis) were comparable to those of water-extractable arabinogalactan (WE-AG) (0.29−0.38%, dry basis). Minaret had more WE-AX (0.49−0.68%, dry basis) than WE-AG (0.27−0.36%, dry basis). The ratio of WE-AG to WE-AX for the different flour fractions varied between 0.83 and 1.03 for Camp Remy and between 0.46 and 0.57 for Minaret. For both wheat varieties, the percentage of WE-AX and WE-AG was higher for the second and third reduction roll fractions than for the three break and the first reduction roll fractions. There was little structural variation in WE-AG of different flour fractions. The arabinose to galactose (A/G) ratio (w/w) varied between 0.63 and 0.69 for Camp Remy WE-AG and between 0.66 and 0.72 for Minaret WE-AG. The molecular weight range of the water-extractable arabinogalactan-peptide (WE-AGP) isolated from the different flour fractions of Camp Remy and Minaret was 5×10^4−10×10^4. The ^{1}H NMR spectra of the WE-AGP isolated from the different flour fractions were comparable and display the following diagnostic peaks (300 MHz, D_2O, 85 °C): δ 5.26, anomeric protons of α-linked arabinofuranosyl residues; and δ 4.47−4.54, anomeric protons of β-linked galactose residues.

Keywords: *Arabinogalactan; arabinoxylan; wheat flour; milling*

INTRODUCTION

Water-extractable nonstarch polysaccharides of wheat consist of water-extractable arabinoxylan (WE-AX) and water-extractable arabinogalactan-peptide (WE-AGP). Considerable literature deals with the characterization of the WE-AX (Hoffmann et al., 1991; Izydorczyk and Biliaderis, 1993; Cleemput et al., 1993, 1995) and its function in the bread-making process (D'Appolonia, 1971; Hoseney, 1984; Meuser and Suckow, 1986; Roels et al., 1993). Less research has been performed on the isolation and characterization of WE-AGP of wheat.

Fincher and Stone (1974) found that the major part of WE-AGP of wheat flour consists of polysaccharide (92%) with an arabinose to galactose (A/G) ratio of 0.67, while Neukom and Markwalder (1975) reported an A/G ratio of 0.70. Westerlund et al. (1990) isolated a WE-AGP of wheat flour with an A/G ratio of 0.67. Izydorczyk et al. (1991) found, for WE-AGP isolated from eight Canadian wheat varieties, a relatively constant A/G ratio (0.66−0.75). Methylation studies and optical rotation data suggested that the galactan chain is linked β-D-(1−3) and/or β-D-(1−6) (Neukom and Markwalder, 1975). WE-AG is covalently associated with a hydroxyproline-rich peptide (8%) (Fincher et al., 1974). The peptide content of WE-AGP isolated from eight Canadian wheat varieties varied between 6.5 and 14.3% (Izydorczyk et al., 1991). According to Strahm et al. (1981) and McNamara and Stone (1981), a 4-hydroxyproline−galactoside linkage connects the arabinogalactan side chains to the peptide of a WE-AGP. Although to date no functional properties of WE-AGP of wheat have been described, it is not to be excluded that WE-AGP is important for the bread-making value of wheat.

The present work was thus carried out to investigate the distribution of WE-AX and WE-AG in different flour fractions of two wheat varieties (cv. Camp Remy and Minaret) and to document the structural variability of WE-AGP in different flour fractions.

MATERIALS AND METHODS

Materials. Two wheat varieties (Camp Remy and Minaret, harvest 1995) were obtained from AVEVE (Landen, Belgium). All reagents were of at least analytical grade. Specialty chemicals were α-amylase solution (Type XII-A, from *Bacillus licheniformis*, A3403, Sigma Chemical Co., St. Louis, MO), amyloglucosidase (Boehringer Mannheim, Mannheim, Germany), and β-D-allose (Sigma). Standard P-82 pullulans were purchased from Showa Denko K.K., Tokyo, Japan. Deuterium oxide (D_2O) was obtained from Acros Chimica, Geel, Belgium.

Flour Milling. Two European wheats (Camp Remy and Minaret) were conditioned to a final moisture content of 14.5%. The samples were milled on a Bühler MLU-202 (Uzwil, Switzerland) laboratory mill (Delcour et al., 1989) according to AACC Method 26-31 (American Association of Cereal Chemists, 1983). Three break roll fractions, B1, B2, and B3, and three reduction roll fractions, C1, C2, and C3, were obtained. The milling yields were 74.6 and 74.8% for Camp Remy and Minaret, respectively.

The ash contents of the different milling fractions were estimated according to AACC Method 08-01 (American Association of Cereal Chemists, 1983).

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Isolation and Purification of Water-Extractable Arabinoxylan and Water-Extractable Arabinogalactan-Peptide. The isolation was carried out at room temperature unless indicated otherwise. Samples of air-dry flour (200 g) were transferred into stainless steel bowls and heated in a drying oven (130 °C, 90 min) to inactivate endogenous enzymes. Before extraction with deionized water (1:5 w/v, 60 min), the flour was extracted with refluxing 80% ethanol as described by Fincher and Stone (1974). After centrifugation (3000*g*, 15 min), the aqueous supernatants were heated to 90 °C to precipitate soluble proteins. Residual starch was hydrolyzed by addition of 0.4 mL (7000 units) of α-amylase solution. The mixtures were kept at 90 °C for 30 min, cooled to room temperature, and centrifuged as above. After treatment with amyloglucosidase (120 units, 60 °C, 12 h), mixtures were cooled to room temperature and centrifuged again. After dialysis (48 h, 4 °C), WE-AX and WE-AGP were precipitated by stepwise addition of aliquots of ethanol (95%) to a final concentration of 80%. The mixtures were stirred for 30 min, kept at 4 °C overnight, and centrifuged (10000*g*, 30 min, 4 °C). Precipitates obtained were dissolved by shaking in 300 mL of deionized water for 60 min at room temperature. To separate WE-AX from WE-AGP, ethanol was added to a final concentration of 65% (v/v) as above. WE-AX was then precipitated and recovered according to the method of Cleemput et al. (1993), while WE-AGP was recovered from the supernatants (vacuum rotary evaporation, 50 °C).

Estimation of Water-Extractable Arabinoxylan and Water-Extractable Arabinogalactan in Wheat Flour Fractions. The monosaccharide composition of the water extracts of flour was estimated by gas−liquid chromatography. Extraction and hydrolysis procedures were essentially as described by Cleemput et al. (1993). Water extracts of heat-treated (cf. supra) flour samples (2.00 g) were obtained by suspension in water (1:10 w/v). After shaking (2 h, 30 °C) and centrifugation (3000*g*, 15 min), supernatants were hydrolyzed for 60 min in 4.0 M trifluoroacetic acid (TFA) at 110 °C. For the estimation of the monosaccharide composition of isolated WE-AX and WE-AGP, samples (15 mg) were directly hydrolyzed in 2.0 M TFA for 60 min at 110 °C. Alditol acetates prepared according to the method of Englyst and Cummings (1984) were separated on a Supelco SP-2380 column (30 m, 0.32 mm i.d., 0.2 μm film thickness) (Bellefonte, PA) in a Chrompack 9011 chromatograph (Middelburg, The Netherlands) equipped with a flame ionization detector. The carrier gas was He. Separation was at 225 °C, with injection and detection temperatures of 275 °C and β-D-allose as internal standard.

To calculate the percentage of WE-AX and WE-AG of the fractionated wheat products, the quantity of arabinose associated with WE-AX and that associated with WE-AG was calculated by combining (i) the data of the monosaccharide composition of the water extracts, (ii) the arabinose to xylose (A/X) ratios of isolated WE-AX, and (iii) the A/G ratios of isolated WE-AGP. WE-AX and WE-AG contents were respectively quantified as 0.88 and 0.89 times the sum of the contents of their constituent monosaccharides.

Protein Estimation. Protein contents were determined according to the method of Lowry et al. (1951) with bovine serum albumin as standard.

Gel Permeation Chromatography. Aliquots of the isolated WE-AGP of the different flour fractions (1.0 mg) were solubilized in 0.3% NaCl and centrifuged (10000*g*, 10 min). Solutions obtained (100 μL) were separated on a Jordi Aqueous GPC (Alltech, Bellingham, MA) glucose-bound column (100 nm, 250 × 10 mm) by elution with 0.3% NaCl (1.5 mL/min at room temperature). The eluate was monitored by using an R-400 refractive index detector (Waters Associates, Milford, MA). Molecular weight markers were Shodex standard P-82 pullulans (1.0 mg/mL) with molecular weights 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.

^{1}H Nuclear Magnetic Resonance Spectroscopy. ^{1}H NMR spectra were recorded on a Bruker 300 MHz Fourier transform spectrometer (Karlsruhe, Germany) at 85 °C. Samples were dissolved in D_2O, stirred for 120 min, and lyophilized. This step was repeated, and the resulting deuterium-exchanged dry material was finally dissolved in D_2O (1.0 mg/mL). Pulse repetition time was 2 s, and in a typical experiment, the number of scans was 7000.

Table 1. Yield and Ash Content (Percent of Wheat, Dry Basis) of the Different Milling Fractions of Camp Remy and Minaret

	Camp Remy		Minaret	
fraction[a]	yield	ash	yield	ash
bran	14.02	5.85	16.04	5.15
shorts	11.35	2.96	9.14	3.47
B1	13.55	0.47	11.43	0.47
B2	7.15	0.55	9.59	0.54
B3	0.96	0.90	1.92	na[b]
C1	40.25	0.38	43.99	0.47
C2	11.17	0.42	6.42	0.75
C3	1.55	0.67	1.47	na
flour	74.63	0.43	74.82	na

[a] B1, B2, and B3, break roll fractions; C1, C2, and C3, reduction roll fractions. [b] na, not analyzed.

Table 2. Monosaccharide Composition (Percent of Flour Fraction, Dry Basis) of the Water Extracts from the Different Milling Fractions of Camp Remy and Minaret

	Camp Remy					Minaret				
fraction[a]	Ara[b]	Xyl	Man	Gal	Glc	Ara	Xyl	Man	Gal	Glc
bran	0.35	0.42	0.68	0.71	3.83	0.32	0.42	0.49	0.57	3.31
shorts	0.54	0.61	0.66	0.89	6.41	0.62	0.83	0.78	1.20	6.24
B1	0.27	0.27	0.21	0.23	2.48	0.34	0.45	0.27	0.23	2.07
B2	0.25	0.23	0.15	0.23	2.20	0.31	0.37	0.24	0.23	1.82
B3	0.27	0.26	0.21	0.33	2.58	0.33	0.39	0.28	0.33	2.05
C1	0.26	0.26	0.18	0.22	3.31	0.32	0.40	0.25	0.24	2.10
C2	0.29	0.29	0.19	0.25	3.47	0.40	0.52	0.33	0.41	2.75
C3	0.31	0.32	0.23	0.33	4.58	0.41	0.53	0.42	0.49	3.73
flour	0.27	0.26	0.19	0.23	3.09	0.33	0.42	0.26	0.26	2.15

[a] B1, B2, and B3, break roll fractions; C1, C2, and C3, reduction roll fractions. [b] Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glc, glucose.

RESULTS AND DISCUSSION

Wheat Milling Fractions. Yields of laboratory wheat milling and ash contents of the fractions are listed in Table 1.

Monosaccharide Composition of the Different Milling Fractions. The monosaccharide composition of the water extracts from the different fractions of Camp Remy and Minaret shows the presence of glucose, xylose, arabinose, galactose, and mannose (Table 2). Because of the presence of mannose, the percentage galactose detected in the water extracts probably originates not only from the WE-AGP. Galactomannans (Fincher and Stone, 1986; Heredia et al., 1995) are present in wheat water extracts. Little is known about their structure.

Isolation and Purification of WE-AX and WE-AGP. Several methods were reported earlier for the separation of the water extractables into WE-AX and WE-AGP. Fincher and Stone (1974) used ammonium sulfate precipitation. Other researchers used diethylaminoethylcellulose−cellulose adsorption chromatography (Kündig et al., 1961; Medcalf et al., 1968; Lineback et al., 1977; MacArthur and D'Appolonia, 1980; Westerlund et al., 1990) or ethanol precipitation (Suckow et al., 1983; Cleemput et al., 1993). The isolation method developed in this study worked well for the different flour fractions. However, for the bran and the shorts, the separation into WE-AX and WE-AGP with an ethanol concentration of 65% was not sufficient (results not shown). Analysis of the monosaccharide

Table 3. Monosaccharide Composition (Percent), Protein Content (Percent), and Arabinose to Galactose Ratios (w/w) of the WE-AGP Isolated of the Different Milling Flour Fractions and Arabinose to Xylose Ratios (w/w) of WE-AX Isolated of the Different Milling Flour Fractions of (a) Camp Remy and (b) Minaret

	WE-AGP								WE-AX
fraction[a]	Ara[b]	Xyl	Man	Gal	Glc	Prot	AG	A/G	A/X
				(a) Camp Remy					
B1	28.13	1.87	0.84	41.71	1.32	16.22	62.16	0.65	0.50
B2	29.73	1.55	0.21	42.20	0.72	19.25	64.02	0.69	0.50
B3	24.65	3.60	0.10	36.05	1.91	20.05	54.02	0.64	0.46
C1	31.56	2.36	0.43	47.39	0.49	12.88	70.27	0.64	0.49
C2	31.95	2.75	0.95	45.31	1.08	14.39	68.76	0.68	0.48
C3	25.17	3.44	0.50	37.80	1.60	20.29	56.04	0.63	0.45
				(b) Minaret					
B1	30.57	2.30	0.36	44.58	0.49	12.87	68.88	0.66	0.49
B2	30.60	1.94	0.56	43.44	0.70	15.76	65.90	0.68	0.50
B3	25.99	2.58	0.31	35.69	0.89	25.96	54.90	0.69	0.50
C1	27.57	2.89	1.01	39.57	0.98	11.29	59.75	0.66	0.49
C2	26.83	3.54	1.00	36.82	0.99	23.62	56.65	0.68	0.47
C3	23.81	5.63	0.49	29.59	0.56	21.24	47.53	0.72	0.46

[a] B1, B2, and B3, break roll fractions; C1, C2, and C3, reduction roll fractions. [b] Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glc, glucose; Prot, protein; AG, 0.89 × (% Ara + % Gal); A/G, arabinose to galactose ratio; A/X, arabinose to xylose ratio.

Table 4. Arabinoxylan and Arabinogalactan Contents (Percent, Dry Basis) and Their Respective Ratios in the Water Extracts of the Different Milling Flour Fractions of Camp Remy and Minaret

	Camp Remy			Minaret		
fraction[a]	AX[b]	AG	AG/AX	AX	AG	AG/AX
B1	0.36	0.30	0.83	0.59	0.27	0.46
B2	0.30	0.29	0.97	0.49	0.27	0.55
B3	0.33	0.34	1.03	0.51	0.29	0.57
C1	0.34	0.30	0.88	0.52	0.28	0.54
C2	0.38	0.33	0.87	0.67	0.34	0.51
C3	0.41	0.38	0.93	0.68	0.36	0.53
flour	0.35	0.31	0.88	0.54	0.28	0.53

[a] B1, B2, and B3, break roll fractions; C1, C2, and C3, reduction roll fractions. [b] AX, 0.88 × (% Ara + % Xyl); AG, 0.89 × (% Ara + % Gal); AG/AX, AG to AX ratio.

composition of WE-AGP from different flour fractions for both varieties (Table 3) showed that, apart from arabinose and galactose, only small levels of xylose, mannose, and glucose were present.

There is little variation in A/G ratio (w/w) of WE-AGP over different flour fractions as it ranged from 0.63 to 0.69 and from 0.66 to 0.72 for Camp Remy and Minaret, respectively. The A/G ratios (w/w) for the WE-AGP in different flour fractions are comparable with those in the literature (Fincher and Stone, 1974; Neukom and Markwalder, 1975; Westerlund et al., 1990; Izydorczyk et al., 1991). In Table 3, the A/X ratios (w/w) of the isolated WE-AX of the milling flour fractions for both wheat varieties are given. These range from 0.45 to 0.50 and from 0.46 to 0.50 for Camp Remy and Minaret, respectively. Different flour fractions show little variation in A/X ratio of isolated WE-AX. For the two wheat varieties, the A/X ratios are comparable and lower than those reported by Suckow et al. (1983; six European wheats, 0.54−0.63), Izydorczyk et al. (1991; eight Canadian wheats, 0.53−0.71), and Cleemput et al. (1993; six European wheats, 0.51−0.61). Table 4 lists the WE-AX and WE-AG contents of the different flour fractions. For the two wheat varieties, the percentage of WE-AX and WE-AG is higher for the second and third reduction roll fractions. The amount of WE-AX present in different flour fractions of Camp Remy is comparable with the amount of WE-AG. Minaret has more WE-AX than

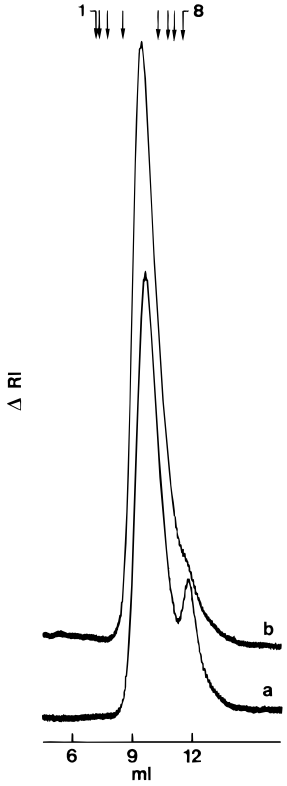

Figure 1. Gel permeation profile of WE-AGP of the first reduction roll fraction of (a) Camp Remy and (b) Minaret. Elution volumes of pullulan standards of molecular weight 78.8×10^4, 40.4×10^4, 21.2×10^4, 11.2×10^4, 4.73×10^4, 2.28×10^4, 1.18×10^4, and 0.59×10^4 (1 through 8, respectively) are indicated.

WE-AG. The ratio of WE-AG to WE-AX for the different flour fractions varies between 0.83 and 1.03 for Camp Remy and between 0.46 and 0.57 for Minaret.

Gel Permeation Analysis. Gel permeation profiles of the WE-AGP of the largest flour fraction, namely the first reduction roll fraction of Camp Remy and Minaret, are shown in Figure 1. The profiles of the other flour fractions are comparable. All fractions yielded a narrow peak in the molecular weight (MW) range 5.0×10^4−10.0×10^4 and a shoulder in a lower MW range ($\leq 5.0 \times 10^3$). The MW of the WE-AGP, obtained by calibration with pullulan standards, is in agreement with the value found by Izydorczyk et al. (1991). Lower MW values were found by Fincher and Stone (1974), Neukom (1976), and Strahm et al. (1981), who found MW for WE-AGP of, respectively, 2.2×10^4, 2.0×10^4−4.0×10^4, and 3.0×10^4−3.2×10^4. The MWs of the WE-AGP of the milling flour fractions B3 and C3 are somewhat higher than those for the milling fractions B1, B2, C1, and C3 for both varieties. There is little difference in WE-AGP MW between the two wheat varieties Camp Remy and Minaret. For Minaret, the shoulder peak of the low MW material is less pronounced.

^{1}H Nuclear Magnetic Resonance Spectroscopy. Figure 2 shows the ^{1}H NMR spectral data for the WE-AGP of the different flour fractions of Camp Remy. The WE-AGP ^{1}H NMR spectra are comparable for the six fractions.

The resonance at δ 5.26 arises from the anomeric protons in α-linked arabinofuranosyl residues (Westerlund et al., 1990). The peak with chemical shifts at δ 4.47−4.54 can be ascribed to the β-linked galactose residues. The small peak at δ 5.11 probably originates from α-arabinofuranose (Saulnier et al., 1992).

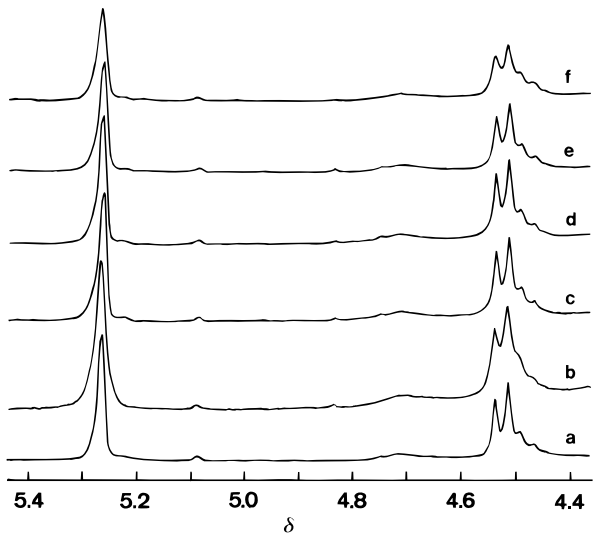

Figure 2. ^{1}H NMR spectra of WE-AGP of the first (a), second (b), and third (c) reduction roll fractions and of the first (d), second (e), and third (f) break roll fractions of Camp Remy. δ 5.26, anomeric protons of α-linked arabinofuranosyl residues; δ 4.47−4.54, anomeric protons of β-linked galactose residues.

The spectra further show that the WE-AGP isolates do not contain WE-AX (Bengtsson and Aman, 1990) and polymeric glucose (Gidley, 1985).

Conclusions. Unlike what can be observed for WE-AX, the percentage of WE-AG in different flour fractions is comparable for the two different wheat varieties, although the percentage of WE-AX and WE-AG is somewhat higher for the C2 and C3 fractions. There are differences in AG/AX ratios of the different flour fractions for the two wheat varieties. Since the percentage WE-AG is of the same order of magnitude of the percentage WE-AX for some varieties, the former components may deserve more scientific and practical interest.

ABBREVIATIONS USED

WE-AX, water-extractable arabinoxylan; WE-AG, water-extractable arabinogalactan; WE-AGP, water-extractable arabinogalactan-peptide; NMR, nuclear magnetic resonance; A/G, arabinose to galactose; A/X, arabinose to xylose; TFA, trifluoroacetic acid; MW, molecular weight; D_2O, deuterium oxide; Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glc, glucose; Prot, protein.

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