# Heterogeneity in the Structure of Water-Soluble Arabinoxylans in European Wheat Flours of Variable Bread-making Quality

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#### ABSTRACT

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The contents of water-soluble, enzyme-extractable, and total nonstarch polysaccharides (NSPs) were determined in six European wheat flours (Apollo, Slejpner, Sperber, Camp Remy, Minaret, and Soissons) with different mixing time and baking absorption characteristics and, hence, widely varying bread-making capacities. The NSP contents varied between 0.42 and 0.69, 1.20 and 1.73, and 1.35 and 2.25%, respectively. The water-soluble NSPs were extracted and fractionated by ethanol precipitation into arabinoxylans and arabinogalactans. In the arabinoxylan fractions,  $\beta$ -glucan occurred as a minor contaminant. The L-arabinose to D-xylose ratios of such arabinoxylans varied between 0.51 and 0.61, whereas the ratios of di- to monosubstituted D-xylose residues (estimated by  $^1$ H nuclear magnetic resonance) varied between 0.80 and 1.81. A major proportion of the xylan residues (63–66%) were not substituted by L-arabinose, whereas 13–21% and 16–24% of the D-xyloses were mono- or disubstituted,

respectively. From the <sup>1</sup>H nuclear magnetic resonance spectral data, it could be concluded that the disubstituted D-xyloses were either isolated or occurred as pairs in the arabinoxylan chain. Shodex B-806 chromatography yielded, for all arabinoxylan fractions, three peaks with estimated molecular weights between  $8.5 \times 10^5$  and  $5 \times 10^3$ , with only the relative proportions of the peaks, but not their molecular weights, varying with the variety. No apparent relationship was found between the molecular weight profiles and the L-arabinose substitution pattern in the arabinoxylans. Correlation coefficients between the analytical data obtained for the NSP content in the six European wheat flours and the baking absorption and mixing time of the corresponding flours increased when the structural variation in the arabinoxylans was taken into account, showing that increased knowledge of the arabinoxylan structure may lead to an enhanced understanding of the role of NSP in breadmaking.

It has been known for a long time that nonstarch polysaccharides (NSPs) from wheat flour have an impact on the breadmaking process (Pence et al 1950; Udy 1956, 1957; Jelaca and Hlynka 1972). Although conflicting ideas with regard to their exact role can be found in the literature (D'Appolonia et al 1970; D'Appolonia 1971, 1973; Casier et al 1973, 1979; Meuser and Suckow 1985), it is generally accepted that NSPs bind a significant amount of water (Kulp 1968, Jelaca and Hlynka 1971).

Lack of insight into the precise role of these secondary plant constituents can be ascribed, in part, to the great difficulty associated with the characterization of this heterogeneous class of compounds. Indeed, until quite recently, the compounds were separated mainly by making use of their different solubilities in water. The most logical separation was between water-soluble and water-insoluble NSP (Perlin 1951 a,b; Montgomery and Smith 1955; Kundig et al 1961; Medcalf et al 1968; Medcalf and Gilles 1968; D'Appolonia and Mac Arthur 1976).

Recently, several workers have used <sup>1</sup>H-nuclear magnetic resonance (NMR) spectroscopy for the structural characterization of water-soluble wheat (Hoffmann et al 1991, Izydorczyk and Biliaderis 1992) or rye (Bengtsson and Aman 1990, Vinkx et al 1993) arabinoxylan fractions. In particular, it was shown that mono- and disubstituted D-xylose residues occur in the arabinoxylan chain (Bengtsson and Aman 1990, Hoffmann et al 1991, Izydorczyk and Biliaderis 1992, Vinkx et al 1993), and their distribution on the xylan backbone was elucidated (Hoffmann et al 1992 a,b; Vinkx et al 1993). New developments make it possible to study structural variations in arabinoxylans of different wheat varieties (Andersson et al 1992), but we lack knowledge of the correlation between arabinoxylan structure and functionality of wheat varieties in breadmaking.

The purpose of this study was to investigate the structural variation in water-soluble NSP from six different European wheat flours and to investigate any correlation that might exist between

such variation and the well-characterized bread-making capacity of these flours (Roels et al 1993).

#### MATERIALS AND METHODS

#### **Flours**

Six European wheats (Apollo, Slejpner, Sperber, Camp Remy, Minaret, and Soissons) with varying bread-making potential were conditioned to 17% moisture and experimentally milled on a Buhler MLU-202 laboratory mill (Uzwil, Switzerland). The baking performance of these flours was evaluated over a wide range of mixing time and baking absorption levels after adjustment to constant protein level (Roels et al 1993).

# Isolation, Fractionation, and Purification of Water-Soluble Arabinoxylan

Samples of flour (150 g) were transferred into stainless steel bowls and heated in a drying oven (130°C, 90 min) to inactivate enzymes before extraction with water (5:1 v/w, 15 min, 30°C). Subsequent isolation procedures were accomplished at room temperature unless indicated otherwise. After centrifugation (3,000  $\times$  g, 15 min), the supernatant solution was heated to 90°C to precipitate the soluble proteins. Residual starch was hydrolyzed by adding 0.2 ml of  $\alpha$ -amylase (Type XII-A, from Bacillus licheniformis, A 3403, Sigma Chemical Co., St. Louis, MO). The solution was kept at 90°C for 30 min, cooled, and centrifuged as above. The resulting supernatant solution was fractionated with ethanol according to the method of Suckow et al (1983). The arabinoxylan component was isolated from the extract by stepwise addition of aliquots of ethanol (96%) to a final concentration of 65% (v/v). The mixture was stirred for 30 min, kept at  $4^{\circ}$ C overnight, and centrifuged (10,000  $\times$  g, 30 min,  $4^{\circ}$ C).

The precipitate obtained was dissolved in 150 ml of water and stirred for 30 min. Ethanol was added to a final concentration of 60% (v/v) as above. After stirring for 30 min, the mixture was stored at  $4^{\circ}$ C for 2 hr and centrifuged  $10,000 \times g$  as above. The precipitate was suspended in aliquots of ethanol (96%, 2×) and acetone (1×) with intermediate stirring (2 hr) and centrifugation (20,000 × g, 30 min, 4°C). The final precipitate was dried for 24 hr at  $45^{\circ}$ C (drying oven).

# **Chemical Analysis**

The water-soluble, enzyme-extractable, and total pentosan content of the six flours were estimated by gas-liquid chromatography following hydrolysis. Extracts were obtained as described by Hashimoto et al (1987) with some modifications. Different flour-

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to-solvent ratios were used (1:10 v/w for water-soluble and enzyme-extractable NSP and 1:5 v/w for total NSP). A 10% commercial pentosanase solution (Veron HE, Rohm Tech Darmstadt, Germany) was added to obtain the enzyme-extractable pentosan content. The hydrolysis step for estimating total pentosan content was performed with 2.0N trifluoroacetic acid for 120 min at 110°C. A compressed-yeast suspension of 0.25 g per milliliter of buffer was used to remove fermentable sugars.

The extracts for estimating the water-soluble and enzyme-extractable pentosan content were hydrolyzed with 2.0 N trifluoro-acetic acid for 60 min at 110°C. The monosaccharides were detected as alditol acetates. These were prepared using the method of Englyst and Cummings (1984). Gas-liquid chromatography was performed on a Hewlet Packard 5890 Series II chromatograph using a Supelco SP-2330 column (30 m × 0.75 mm). Injection and detection (flame-ionization detector) temperatures were 250°C. Separation was accomplished with two isothermal steps (190°C, 5 min and 210°C, 11 min), an intermediate step (20°C, 1 min), and a final temperature increase (15°C, 36 sec) to a temperature of 225°C. After two minutes at 225°C, the run was terminated. Inositol was used as internal standard. The monosaccharide compositions of the isolated arabinoxylan fractions were determined by the same hydrolysis and derivatization procedures.

Protein content was determined by the Lowry et al (1951) method, using bovine serum albumin as standard.

 $\beta$ -Glucan was extracted from the isolated arabinoxylan fractions with perchloric acid according to the method of Ahluwalia and Ellis (1984). The  $\beta$ -glucan content in the extracts was determined by fluorimetric detection with Calcofluor fluorescent dye and by a flow-injection analysis technique described by Jorgensen and Aastrup (1988).

# Gel-Permeation Chromatography

Samples (5.0 mg) of the isolated arabinoxylans were solubilized in 1.0 ml of water, filtered (0.45  $\mu m$ ), and separated on a Shodex B-806 column (50 m  $\times$  0.8 cm) by elution with water (1.0 ml/hr at 30°C). The refractive index of the eluate was monitored. Molecular weight markers were Shodex standard P-82 pullulan with molecular weights of 0.58  $\times$  10<sup>4</sup>, 1.22  $\times$  10<sup>4</sup>, 2.37  $\times$  10<sup>4</sup>, 4.80  $\times$  10<sup>4</sup>, 1.0  $\times$  10<sup>5</sup>, 1.86  $\times$  10<sup>5</sup>, 3.80  $\times$  10<sup>5</sup>, and 8.53  $\times$  10<sup>5</sup>

# <sup>1</sup>H-NMR Spectroscopy

 $^{1}\text{H-NMR}$  spectra were recorded on a Bruker 300-MHz FT-spectrometer (Bruker, Rheinstetten 4, Karlsruhe, Germany) at 85°C. The samples were prepared by dissolving the arabinoxylan fractions in D<sub>2</sub>O (99%), stirring for 120 min, and lyophilizing. This step was repeated once, and the dry material was dissolved in D<sub>2</sub>O (1.0 mg/ml).

# RESULTS AND DISCUSSION

# **NSP Content in the Flour Samples**

The water-soluble, enzyme-extractable, and total NSP content of six European wheat flours are shown in Table I. Total pentosan content of the six wheat varieties ranged from 1.35 to 2.25%, of which 30-40% is water soluble and 77-97% is enzyme extractable. Our results are in agreement with the values found by Hashimoto et al (1987), Shogren et al (1987) and Izydorczyk et al (1991). Shogren et al (1987) found a good correlation between

TABLE I
Water-Soluble (WS), Enzyme-Extractable (EEX), and Total (TOT)
Nonstarch Polysaccharide Contents (%, Dry Basis)
of Six European Wheat Flours

Flour	WS	EEX	ТОТ
Apollo	0.66	1.62	1.67
Slejpner	0.69	1.73	2.25
Sperber	0.61	1.52	1.75
Camp Remy	0.48	1.40	1.58
Minaret	0.56	1.52	1.59
Soissons	0.42	1.20	1.35

the water-soluble, enzyme-extractable, and total pentosan content. Correlations between the pentosan contents were also found in this work. There was a great variation in NSP content between the different wheat varieties.

We not only observed a variation in NSP content but also in the monosaccharide composition, as reflected in the ratios of L-arabinose to D-xylose listed in Table II. Such differences were also found in earlier comparative studies on the pentosan composition of different American and Canadian wheat varieties (Medcalf et al 1968, D'Appolonia and Mac Arthur 1975, Lineback et al 1977, Ciacco and D'Appolonia 1982, Izydorczyk et al 1991), indicating differences in pentosan structure between different wheat varieties. In addition to L-arabinose and D-xylose, D-galactose and D-glucose were detected in all three extracts, while D-mannose was found with the water-soluble and enzyme-extractable pentosans. It should be stressed that the L-arabinose values listed are not those of pure arabinoxylan fractions because the water-soluble arabinogalactan occurred in the samples as a minor contaminant (see below).

# Isolation and Chemical Analysis of the Arabinoxylans

Because a determination of the structure of the water-soluble arabinoxylan is not possible without a previous separation of the water solubles into arabinoxylans and arabinogalactans, this was an important step in our work.

Fractionation into arabinoxylans and arabinogalactans was reported earlier. Fincher and Stone (1974) used ammonium sulfate precipitation for this separation, and other researchers used diethylaminoethylcellulose-cellulose adsorption chromatography (Kundig et al 1961, Medcalf et al 1968, Lineback et al 1977, Mac Arthur and D'Appolonia 1980) or ethanol precipitation (Suckow et al 1983). According to the latter authors, an ethanol concentration of 65% gave the best separation into arabinoxylans and arabinogalactans.

In our study, ethanol precipitation yielded arabinoxylan fractions with less D-galactose contamination than did ammonium sulfate precipitation. Purified arabinoxylans from the six flours were, therefore, prepared according to the method of Suckow et al (1983).

The monosaccharide composition of the arabinoxylans is shown in Table III. Pentosan content was calculated as (% D-xylose + % L-arabinose)  $\times$  0.88. The arabinoxylans were almost free of D-galactose (maximum 2%), and 66-87% of the samples were pure arabinoxylan. A protein content of 7.0-13.0% was found in the fractions (Table IV). We did not observe a great difference in the ratio of L-arabinose to D-xylose in the arabinoxylans

TABLE II

Ratios of L-Arabinose to D-Xylose in Water-Soluble (WS),
Enzyme-Extractable (EEX), and Total (TOT) Nonstarch Polysaccharides
(%, Dry Basis) of Six European Wheat Flours

Flour	WS	EEX	тот
Apollo	0.73	0.62	0.59
Slejpner	0.78	0.63	0.67
Sperber	0.78	0.63	0.64
Camp Remy	0.86	0.66	0.65
Minaret	0.79	0.67	0.65
Soissons	1.02	0.78	0.76

TABLE III

Monosaccharide Composition of the Arabinoxylans
of Six European Wheat Flours<sup>a</sup>

Flour	ara (%)	xyl (%)	gal (%)	glu (%)	pent (%)	A/X
Apollo	31.5	61.8	2.3	2.5	82.1	0.51
Sleipner	28.1	52.6	0.8	2.7	71.0	0.53
Sperber	29.8	58.9	0.7	3.4	77.9	0.50
Camp Remy	31.4	59.0	1.4	6.9	79.6	0.53
Minaret	33.8	64.2	0.8	2.6	86.3	0.53
Soissons	28.2	46.6	1.2	9.0	65.8	0.61

<sup>&</sup>lt;sup>a</sup> ara = L-arabinose, xyl = D-xylose, gal = D-galactose, glu = D-gluose, pent =  $(\% \text{ ara} + \% \text{ xyl}) \times 0.88$ , A/X = L-arabinose-to-D-xylose ratio.

obtained from the different varieties (0.50-0.53), except for Soissons, which had a higher ratio (0.61). The magnitude of the ratios are in good agreement with the 0.54-0.63 ratios reported by Suckow et al (1983) for six European wheats (Diplomat, Carimulti, Vuka, Maris Huntsman, Benno, Disponent). In Canadian wheat varieties, Izydorczyk et al (1991) found more pronounced variation in ratios of L-arabinose to D-xylose (0.53-0.71) for the isolated arabinoxylans.

In our fractions, D-glucose was also detected. Part of this glucose originated from endosperm (1 $\rightarrow$ 3) and (1 $\rightarrow$ 4)-linked  $\beta$ -D-glucan (Bacic and Stone 1980) that is present in the fractions as a minor constituent (Table IV). This was also shown by <sup>1</sup>H-NMR (see below) and  $\beta$ -glucan analysis. Arabinoxylans with the highest glucose contamination had the highest  $\beta$ -glucan content. Thus, the arabinoxylan fraction from Soissons had a D-glucose content of 9.0% and 5.3% of which could be ascribed to  $\beta$ -glucan, whereas the arabinoxylan of Apollo contained 1.2%  $\beta$ -glucan and only 2.5% D-glucose.

# Structural Analysis of the Arabinoxylans by <sup>1</sup>H-NMR Spectroscopy

The H-NMR spectrum of the arabinoxylan from Soissons is shown in Figure 1. More details of the L-arabinofuranosyl (Araf) anomeric proton region (5.2-5.5 ppm) of the arabinoxylans from the six wheat varieties are shown in Figure 2. In this region, all samples display three peaks. The first peak, at 5.40 ppm, represents H-1 of Araf linked to O-3 of xylopyranosyl (Xylp) residues. The other two peaks of (theoretically) equal intensity at 5.30 and 5.23 ppm represent the anomeric protons of Araf linked to O-2 and O-3 of the same Xylp residue. The relative intensities of the Araf anomeric protons in di- and monosubstituted xyloses varies (Fig. 2). Table V lists the ratios of dito monosubstituted xyloses obtained by quantitative integration of the corresponding signals (Westerlund et al 1990). Values ranged from 0.87 to 1.81, indicating a large variation in pentosan characteristics between the varieties. The unresolved signals at the left side of the peaks at 5.30 and 5.23 ppm are probably the result of two neighboring disubstituted Xylp residues in the chain (Hoffmann et al 1992b, Vinkx et al 1993). Because these signals are present in all six spectra, it can be speculated that, in the six wheat varieties under study, disubstituted Xylp residues occur either isolated or paired in the arabinoxylan chain. The presence of a disubstituted Xylp residue next to a monosubstituted

TABLE IV
Protein and  $\beta$ -Glucan Content in the
Arabinoxylans of Six European Wheat Flours

Flour	Protein (%)	β-Glucan (%)
Apollo	11.22	1.2
Slejpner	10.73	2.2
Sperber	11.34	2.6
Camp Remy	8.21	4.6
Minaret	6.94	2.0
Soissons	13.08	5.3

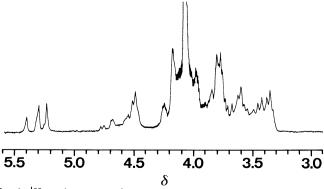


Fig. 1. <sup>1</sup>H nuclear magnetic resonance spectrum of the water-soluble arabinoxylan from Soissons wheat.

Xylp residue, exemplified by a signal downfield from 5.40 ppm, is not distinguishable in these spectra, although some spectra show a shoulder at about this value. This sequence has recently been reported for wheat arabinoxylans by Hoffmann et al (1992b). The broad doublet between 4.70 and 4.80 ppm originates from β-D-glucan (Bengtsson et al 1992), the intensity of which, in the different spectra, correlates with the β-D-glucan content in the samples.

The amount of unsubstituted, mono-, and disubstituted Xylp residues was calculated by combining the <sup>1</sup>H-NMR spectral data with the data from Table III. Results are given in Table V. A constant proportion of the Xylp residues in the arabinoxylan fraction of the different wheat varieties is unsubstituted. More

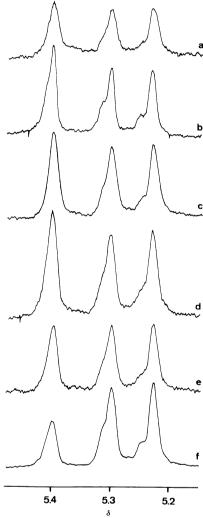


Fig. 2. The anomeric regions of the arabinose protons in the <sup>1</sup>H nuclear magnetic resonance spectra of arabinoxylans from six European wheat flours. Apollo (a), Slejpner (b), Sperber (c), Camp Remy (d), Minaret (e), Soissons (f).

TABLE V
Ratios of Di- and Monosubstituted Xyloses and Percentages
of Unsubstituted, Mono- and Disubstituted Xyloses
in Water-soluble Arabinoxylans of Six European Wheat Flours\*

Flour	Di/Mono	$X_0$	$X_1$	$X_2$
Apollo	0.87	65.2	18.6	16.2
Slejpner	0.80	63.1	20.5	16.4
Sperber	0.94	66.1	17.5	16.5
Camp Remy	0.84	63.4	19.9	16.7
Minaret	1.09	65.3	16.6	18.1
Soissons	1.81	63.2	13.1	23.7

<sup>&</sup>lt;sup>a</sup> Di/Mono = disubstituted/monosubstituted D-xylose,  $X_0$  = unsubstituted D-xylose,  $X_1$  = monosubstituted D-xylose,  $X_2$  = disubstituted D-xylose.

variation was found in the amount of mono- (13-20%) and disubstituted (16-24%) Xylp residues. The values are in accordance with those found for the water-soluble arabinoxylans from spring and winter wheat flours by Andersson et al (1992). It can be concluded that arabinoxylans from wheat flours of varying bread-making quality have different distributions of Araf residues along the backbone.

# Molecular Weight Distribution of the Arabinoxylans

The gel-permeation profiles of the six arabinoxylan fractions are shown in Figure 3. All fractions yielded two peaks in the high molecular weight range ( $> 8.5 \times 10^5$  to  $1.0 \times 10^5$ ), and one peak (Apollo, Soissons), or shoulder thereof (Slejpner, Sperber, Camp Remy, Minaret), in a lower molecular weight range ( $< 1.0 \times 10^5$ ).

The molecular weight values of the arabinoxylans, obtained by calibration with pullulan standards, are in agreement with values found by Mares and Stone (1973) and Izydorczyk et al (1991), who performed gel filtration on Sepharose 4B. For all varieties, the first two peaks elute at the same volume but vary in relative proportions, indicating that all varieties contain components of the same molecular weight. However, the content of these components in the isolated arabinoxylan fractions differs among the varieties.

Mares and Stone (1973) and Izydorczyk and Biliaderis (1992) showed that fractions of a water-soluble arbinoxylan with a higher ratio of L-arabinose to D-xylose, obtained by graded ammonium sulfate precipitation, have a higher proportion of lower molecular weight components. In our work, we found no such relationship between the known structure of the arabinoxylans of the different wheat varieties and their molecular weight distribution.

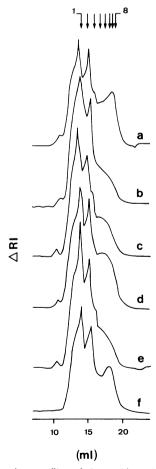


Fig. 3. Gel-permeation profiles of the arabinoxylan fractions from six European wheat flours. Apollo (a), Slejpner (b), Sperber (c), Camp Remy (d), Minaret (e), Soissons (f). Elution volumes of pullulan standards of molecular weight  $8.53 \times 10^5$ ,  $3.80 \times 10^5$ ,  $1.86 \times 10^5$ ,  $1.0 \times 10^5$ ,  $4.80 \times 10^4$ ,  $2.37 \times 10^4$ ,  $1.22 \times 10^4$ ,  $0.55 \times 10^4$  (1 through 8, respectively).

## Structural Features of NSP and Bread-making Capacity

The bread-making potential of the six European wheat varieties under study was recently investigated by Roels et al (1993) after adjustment of the flours to constant protein content by adding limited quantities of starch. The flours had varying mixing time (MT) and baking absorption (BA) characteristics and, hence, a widely varying bread-making capacity.

The authors estimated water-soluble pentosan content in the six flours and came to the following conclusions: 1) the BA increases when the water-soluble pentosan content decreases, and 2) MT required for optimum development of the dough is reduced with higher water-soluble pentosan content.

Our work generated data on the structural variation in the arabinoxylans, so we set out to correlate quantitative and qualitative NSP analytical data with differences in baking performance. After computation of such data for the flours as used by Roels et al (1993), we correlated average BA and MT data of the centerpoint combinations of each individual flour with the quantitative data for water-soluble, enzyme-extractable, and total NSP content in the six European wheat flours obtained in our work. As expected, BA (Table VI) and MT (Table VII) correlated negatively with the pentosan analytical data. This observation is, in part, in contradiction with the results of Shogren et al (1987), who found a positive correlation between BA and water-soluble pentosan content and MT at constant protein level.

The BA values are predicted with high probability (P < 0.05) from the water-soluble and enzyme-extractable NSP data, whereas the total NSP data correlate with much less probability, indicating that limits on dough manageability are imposed by the former

#### **TABLE VI**

Linear Regression Equations, Correlation Coefficients, and Probability Values for the Relationship of Baking Absorption (BA, %) to Percentage of Water-Soluble (WS) NSP, Enzyme-Extractable (EEX) NSP, Total (TOT) NSP, Water-Soluble Arabinoxylan (AX) and Arabinogalactan (AG), and Percentage Un (UN)-, Mono (MONO)-, and Disubstituted (DI) Xylose residues in Six European Wheat Flours<sup>a</sup>

r <sup>2</sup>	Prob Value	
0.70	0.0381	
0.72	0.0319	
0.42	0.1629	
0.76	0.0227	
0.43	0.1582	
0.71	0.0351	
0.54	0.0949	
0.96	0.0005	
	0.70 0.72 0.42 0.76 0.43 0.71 0.54	

<sup>&</sup>lt;sup>a</sup> Percentages of different classes of xylose residues in the flours were calculated from their contents in the arabinoxylans (Table V) and the xylose content in water-soluble arabinoxylans in the flours (data not listed).

### TABLE VII

Linear Regression Equations, Correlation Coefficients, and Probability Values for the Relationship of Mixing Time (MT, sec) to Percentage of Water-Soluble (WS) NSP, Enzyme-Extractable (EEX) NSP, Total (TOT) NSP, Water-Soluble Arabinoxylan (AX) and Arabinogalactan (AG), and Percentage Un (UN)-, Mono (MONO)-, and Disubstituted (DI) Xylose residues in Six European Wheat Flours\*

Linear Regression	r <sup>2</sup>	Prob Value
$MT = -595.12 \times WS + 440.6$	0.58	0.0795
$MT = -312.52 \times EEX + 540.3$	0.56	0.0863
$MT = -203.78 \times TOT + 442.3$	0.49	0.1220
$MT = -587.23 \times AX + 393.9$	0.54	0.0979
$MT = -1954.54 \times AG + 599.1$	0.17	0.4191
$MT = -1174.02 \times UN + 383.6$	0.57	0.0837
$MT = -3703.81 \times MONO + 358.0$	0.76	0.0232
$MT = -3850.57 \times DI + 359.3$	0.21	0.3657

<sup>&</sup>lt;sup>a</sup> Percentages of different classes of xylose residues in the flours were calculated from their contents in the arabinoxylans (Table V) and the xylose content in water-soluble arabinoxylans in the flours (data not listed).

classes of NSP. Subtracting arabinogalactan analytical figures from the amount of water-soluble NSP improves correlation of water-soluble arabinoxylan contents with BA values. Furthermore, it was clear from our data (P < 0.001) that the amount of disubstituted xyloses determines BA values to a great extent, although both mono- and unsubstituted xylose residues can also predict BA figures reasonably well. We need to stress, however, that such relationships will not necessarily hold for flour samples of different protein content as it is obvious that protein also contributes to BA values.

MT values can also be predicted from water-soluble and enzyme-extractable NSP data, although the probability that such relationships are correct is less than what was observed in correlations with BA (0.05 < P < 0.1). The analytical data for monoand unsubstituted xylose residues correlate quite well with the MT values. Here again, it is important to point out that the relationships were obtained for flour samples prepared under identical experimental conditions and adjusted to constant protein content. It is clear that, in daily practice, protein content will exert a great influence on MT values.

Furthermore, the complexity of factors governing BA and MT is great. Hence, we were surprised that, with the limited number of samples available, we obtained high  $r^2$  and low P values. Also, it seems important that further studies be undertaken to study the specific impact in baking of water-soluble NSP fractions enriched in mono- or disubstituted xyloses, and that the implications of the present findings on BA and MT values be compared with similar data on the impact of varying protein contents.

#### **CONCLUSIONS**

A large variation in water-soluble, enzyme-extractable, and total NSP content was found among European wheat flours of different bread-making capacity. NSP content was negatively correlated with the BA and MT characteristics of the corresponding flours. Not only did the NSP content vary, but differences in ratios of L-arabinose to D-xylose found between varieties indicated that the structure of the NSP varied.

Structures of the water-soluble NSP were further investigated. The arabinoxylan fractions were analyzed by gel-permeation chromatography. From the molecular weight profiles, it was clear that arabinoxylans in the different varieties contain components of the same molecular weight, but that the relative proportions of these components differs among the flours studied.

Estimation of the levels of un-, mono-, and disubstituted Xylp residues in the isolated arabinoxylans was possible by  $^1$ H-NMR spectroscopy analysis of these fractions and showed that differences in distribution of the Araf residues along the xylopyranosyl backbone exist among the varieties. Arabinoxylan fractions isolated from flours with good bread-making capacity have higher proportions of disubstituted Xylp residues. A significant and negative correlation ( $r^2 = 0.96$ ) was found between percent of disubstituted Xylp residues in the flour and BA, indicating the importance of the structure (other than content) of the NSP in determining the bread-making potential of the flour.

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