Variation in the Degree of D-Xylose Substitution in Water-Extractable European Durum Wheat (*Triticum durum* Desf.) Semolina Arabinoxylans

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Durum wheat (*Triticum durum* Desf.) semolina water-extractable arabinoxylan (TWEAX) (yield 0.28%, arabinose-to-xylose ratio (*A*/*X*) 0.62) was fractionated by a stepwise increase in ethanol concentration (up to 65%). The *A*/*X* ratios of the resulting fractions varied between 0.42 and 0.80. With increasing ethanol concentrations, increasing *A*/*X* ratios went hand in hand with a relative increase of low molecular weight compounds, indicating that high molecular weight compounds with a low *A*/*X* ratio are preferentially precipitated from alcohol/water mixtures. ^{1}H NMR showed that, whereas in TWEAX the levels of unsubstituted xyloses (X_0), monosubstituted (X_1), and disubstituted (X_2) xyloses were 63.1%, 11.8%, and 25.1%, respectively, fractions that precipitated with increasing ethanol concentrations had decreasing levels of X_0. Simultaneously, the level of X_1 decreased equally until it leveled of at ca. 10%. Concomitantly, the level of X_2 increased. The levels of X_0, X_1, and X_2 varied between 69.7% and 53.4%, 18.2% and 10.7%, and 12.2% and 35.9%, respectively.

Keywords: *Arabinoxylans, durum wheat, semolina, Triticum durum Desf.*

INTRODUCTION

Although the water-extractable arabinoxylans (AX) in bread-making wheat (*Triticum aestivum* L.) have been well studied (Hoffmann et al., 1991, 1992a,b; Izydorczyk et al., 1991; Izydorczyk and Biliaderis, 1992, 1993; Cleemput et al., 1993, 1995, 1997), they are poorly characterized in durum wheat (*Triticum durum* Desf.) and semolina (its milling product). Only a few reports deal with the content, structural characterization, and functional properties of water-extractable AX in this wheat species (Medcalf et al., 1968; D'Appolonia et al., 1970; Ciacco and D'Appolonia, 1982; Lempereur et al., 1997), and it is striking that recent breakthroughs in AX structural analysis such as the use of ^{1}H NMR spectroscopy have not been applied in the case of *T. durum* AX. This work, therefore, was aimed at characterization of the structure (and variabilities therein) of water-extractable AX in *T. durum* semolina. Because AX have a high water binding capacity (Kulp, 1968; Jelaca and Hlynka, 1971), it is not to be excluded that these components play a predominant role in the rheological properties of pasta doughs, their extrusion behavior as well as their drying characteristics. Variances in their contents as well as in their structures might, therefore, be of great importance in determining pasta dough processability. Furthermore, these components may also be important for pasta cooking quality (Liu et al., 1996).

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MATERIALS AND METHODS

Materials. Industrially prepared semolina from a blend of commercial durum wheat varieties was kindly supplied by Soubry NV (Roeselare, Belgium). It contained 14.5% moisture (AACC Method 44-15A) and, on a dry matter basis, 11.8% protein and 0.75% ash (AACC Methods 46-11A and 08-12, respectively).

Isolation and Purification of Total Water-Extractable AX. Water-extractable AX was isolated from durum semolina as described earlier (Cleemput et al., 1993) for wheat flour with the following modifications: (1) 2 kg of semolina that had been inactivated at 130 °C for 24 h was suspended in 6.0 L of deionized water and (2) the incubation with α-amylase (10.0 mL, Type XII-A, from *Bacillus licheniformis*, A 3403, Sigma Chemical Co., St. Louis, MO) was at 90 °C for 4 h. The AX in the resulting supernatant (4.40 L) were precipitated from the solution by dropwise addition of ethanol to a final concentration of 65% (Cleemput et al., 1993). This material is referred to as total water-extractable AX (TWEAX).

Fractionation of TWEAX by Ethanol Precipitation. TWEAX was separated into 5 fractions by graded ethanol precipitation. The fractionation was performed as described elsewhere (Cleemput et al., 1995) with some modifications. A sample (5.44 g) was dissolved in 2.0 L of water at 4 °C with continuous stirring. Aliquots of ethanol were added over a 30 min period with continuous stirring to a final concentration of 20% (v/v) ethanol. The mixture was stirred for an additional 30 min and kept overnight at 4 °C. The precipitated material is referred to as F_{0-20} and was recovered by centrifugation (10 000 g, 30 min, 4 °C). The ethanol concentration of the supernatant was increased to 30% in a similar manner, leading to F_{20-30}. Further increase in the ethanol concentration to 40%, 50%, and 65% led to fractions F_{30-40}, F_{40-50}, and F_{50-65}, respectively.

Sugar Analysis. Samples (15.0−16.0 mg) were hydrolyzed with 2.0 M trifluoroacetic acid (5.0 mL) for 60 min at 110 °C. *β*-D-Allose solution (1.0 mL, 1.0 mg/mL; Sigma Chemical Co.) was added as an internal standard. Derivatization was performed as outlined before (Englyst and Cummings, 1984). The

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Table 1. Yields, Ash, Protein, and Monosaccharide Contents, and Compositions of Arabinoxylan Fractions Obtained by Ethanol Precipitation of TWEAX (Means of Duplicate Analysis and Expressed on an As Is Basis)[a]

fraction	yield[b]	ash[c]	protein[c]	Ara[c]	Xyl[c]	Gal[c]	Glc[c]	AX (%)	sum	*A*/*X*
TWEAX	100	19.8	4.8	27.4	44.2	0.5	2.0	63.0	90.1	0.62
F_{0-20}	28.7	48.5	4.8	4.8	11.3	0.3	2.4	14.1	70.1	0.42
F_{20-30}	14.9	6.0	4.6	31.4	65.9		3.7	85.6	99.9	0.48
F_{30-40}	8.7	6.2	3.3	36.7	60.3		2.0	85.3	96.8	0.61
F_{40-50}	18.5	5.5	2.7	42.8	60.9		1.2	91.2	100.6	0.70
F_{50-65}	9.2	2.9	3.5	41.4	51.6	0.7	3.4	81.8	92.3	0.80

[a] Abbreviations: Ara, arabinose; Xyl, xylose; Gal, galactose; Glc, glucose; AX = 0.88(% Ara + % Xyl); *A*/*X*, arabinose-to-xylose ratio. [b] Yield is expressed as weight percentage of TWEAX. [c] Ash, protein, Ara, Xyl, Gal, and Glc are expressed as weight percentages.

resulting alditol acetates were injected (2 μL) into a Chrompack 9011 (Chrompack, Middelburg, The Netherlands) gas−liquid chromatograph equipped with a Supelco SP-2380 (Supelco inc., Bellefonte, PA) column (30 m, 0.32 mm i.d., 0.2 μm film thickness). Separation was at 225 °C, and the injection and detection (flame ionization detector) temperature was 275 °C.

Protein and Ash Contents. Protein contents were determined colorimetrically (Lowry et al., 1951) with bovine serum albumin as the standard. Ash contents were determined in duplicate according to AACC Method 08-01.

^{1}H NMR Spectroscopy. ^{1}H NMR spectra were recorded as outlined before (Cleemput et al., 1995). The proportions of unsubstituted (X_0) and mono- (X_1) and disubstituted (X_2) xylose residues were calculated by combining the ^{1}H NMR spectral data with the gas chromatography results as indicated in the Appendix (Westerlund et al., 1990). Chemical shifts were referenced to the internal standard acetone (δ 2.2).

Gel Permeation Chromatography. Aliquots of the isolated AX fractions (2.0 mg) were solubilized in 1.0 mL of Milli Q water containing 0.3% NaCl, filtered through 0.45 μm disposable fliters (Filterservice, Eupen, Belgium). Samples (20 μL) were separated with a Kontron Instruments 325 system (Milan, Italy) equipped with an HPLC autosampler 465 on a thermostated (30 °C) Shodex SB-804 HQ (Showa Denko K.K., Tokyo, Japan) gel permeation column (300 × 8 mm i.d.) connected with a SB-800P guard column (50 × 6 mm i.d.). Elution was performed with 0.3% NaCl in Milli Q water (0.3 mL/min). The refractive index of the eluate was monitored using a model 8110 refractive index detector (VDS Optilab, Berlin, Germany). Molecular weight markers were Shodex P-82 pullulan standards (Showa Denko K.K.) of molecular weight 7.88×10^5, 4.04×10^5, 2.12×10^5, 1.12×10^5, 4.73×10^4, 2.28×10^4, 1.18×10^4, 0.59×10^4, and glucose. Results are expressed as a mean of duplicate analysis.

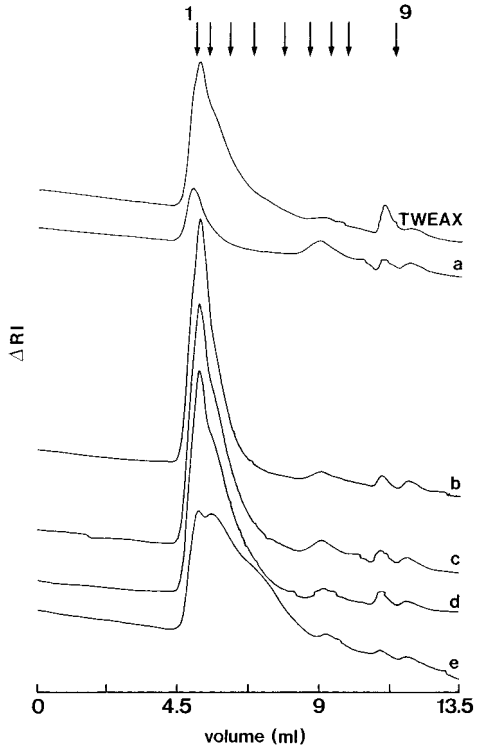

Figure 1. Gel permeation profiles of arabinoxylan fractions obtained by ethanol precipitation: TWEAX, (a) F_{0-20}, (b) F_{20-30}, (c) F_{30-40}, (d) F_{40-50}, (e) F_{50-65}. The refractive index (RI) of the eluate was monitored. Elution volumes of pullulan standards of molecular weight 7.88×10^5, 4.04×10^5, 2.12×10^5, 1.12×10^5, 4.73×10^4, 2.28×10^4, 1.18×10^4 , 0.59×10^4 Da, and glucose are indicated by numbers (1−9, respectively).

RESULTS AND DISCUSSION

Fractionation of Total Water-Extractable AX. The isolation procedure yielded 5.6 g of TWEAX corresponding to an extraction yield of 0.28%. The protein contents and monosaccharide compositions are listed in Table 1. Only small levels of galactose and glucose were present in the water-extractable material precipitated with an excess of ethanol. This points to low levels of arabinogalactan-peptide and α- and β-glucans in the AX fractions. Of further importance is that in agreement with earlier observations (Medcalf et al., 1968), the average *A*/*X* ratio of *T. durum* AX is higher than that of *T. aestivum* AX.

Graded ethanol precipitation yielded five AX fractions (F_{0-20}, F_{20-30}, F_{30-40}, F_{40-50}, and F_{50-65}). The yields, representing 79.9% of TWEAX, ash, protein contents, and monosaccharide data of the fractions are listed in Table 1. All the fractions contain comparable protein contents (2.7−4.8% range). Ash and AX contents are similar for the last four fractions but differ greatly from those of the F_{0-20} fraction. The very low AX concentration (14.1%, Table 1) can partially be explained by the very high ash content in this fraction (48.5%). Furthermore, the data show that increasing ethanol concentrations lead to the precipitation of fractions with an increasing *A*/*X* ratio (0.42−0.80). Similar results were obtained earlier for *T. aestivum* AX (Cleemput et al., 1995). The range of *A*/*X* ratios point to a large variability in the structure of *T. durum* AX.

Gel Permeation Chromatography. The gel permeation profiles of TWEAX, F_{0-20}, F_{20-30}, F_{30-40}, F_{40-50}, and F_{50-65} are shown in Figure 1. The molecular weight distribution of the TWEAX fraction shows a high molecular weight peak with a shoulder and tailing toward higher elution volumes, together with some material of low molecular weight. The elution volume of the material present in the first eluting peak was 5.12 mL, whereas the first peak in the profile of the F_{0-20} fraction eluted at 4.94 mL. The elution volumes of the material in the first peak of the fractions F_{20-30}, F_{30-40}, F_{40-50}, and F_{50-65} did not differ significantly (5.18 mL). With increasing ethanol concentration, the intensity of the shoulder at higher elution volumes than the first peak increased, resulting in a discrete peak (elution volume 5.62 mL) in the F_{50-65} fraction. This indicates

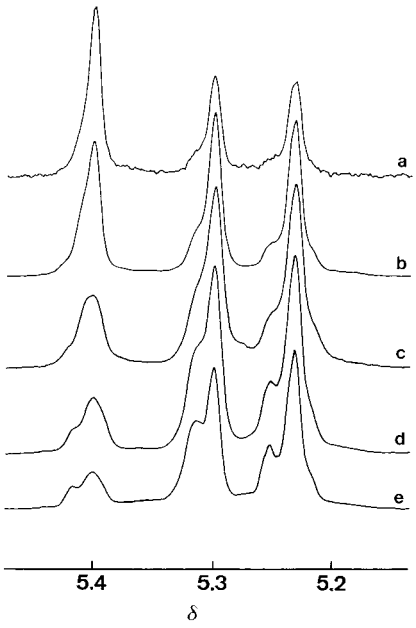

Figure 2. Arabinose anomeric proton regions of the ^{1}H nuclear magnetic resonance spectra of arabinoxylan fractions obtained by ethanol precipitation: (a) F_{0-20}, (b) F_{20-30}, (c) F_{30-40}, (d) F_{40-50}, (e) F_{50-65}.

that with increasing ethanol concentration, the average molecular weight of the precipitated fraction decreased. From this observation and the noticed increase in the *A*/*X* ratio with increasing ethanol concentration, it can be also concluded that compounds with a high molecular weight and low *A*/*X* ratio will preferentially precipitate from alcohol/water mixtures and that with increasing ethanol concentration, the molecular weight decreases while the *A*/*X* ratio increases. However, since the *A*/*X* ratios determined for the AX fractions obtained by ethanol precipitation are the averages of the *A*/*X* ratios of the high and low molecular weight material and their relative abundance, it is impossible at this stage to rationalize the relative impact of the molecular weight and *A*/*X* ratio on the precipitation behavior of AX.

^{1}H NMR Analysis. The arabinose anomeric proton region of the ^{1}H NMR spectra of the different AX fractions are shown in Figure 2. In this region (δ 5.2−5.5 ppm), all the fractions have three major peaks. The first peak (δ 5.40 ppm) represents the anomeric protons of α-L-arabinofuranosyl (Araf) linked to O-3 of xylopyranosyl (xylp) residues of the AX (Hoffman et al., 1992a,b). The two other peaks (δ 5.30 and δ 5.23 ppm) represent the anomeric protons of Araf linked to O-2 and O-3 of the same xylp residue (Hoffman et al., 1992a,b) and become relatively more important with increasing *A*/*X* ratio.

Of further note is that the absence of a signal at δ 5.26 ppm indicates that the AX fractions were not contaminated by arabinogalactan-peptide (Loosveld et al., 1997), a feature already clear from the low galactose levels in the different fractions (Table 1). As the fractions were contaminated with low levels of glucose and a small doublet occurred between δ 4.70 and 4.80 ppm (not shown), this is taken as evidence for the presence of low levels of β-glucan in the samples.

The proportion of X_0, X_1, and X_2 residues in the AX fractions was calculated by combining the ^{1}H NMR spectral data with the gas chromatographic results and are presented in Figure 3 as a function of the *A*/*X* ratios.

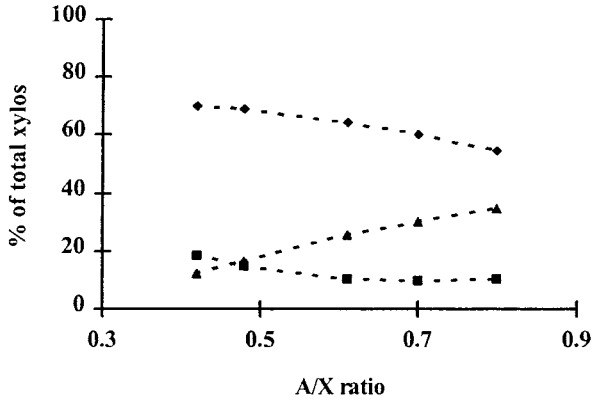

Figure 3. Relative amounts of xylose residues in water-extractable *T. durum* arabinoxylans as a function of *A*/*X* ratio: (◆) X_0, (■) X_1, (▲) X_2.

The levels of X_0 varied from 54.7% to 69.7%. A higher degree of substitution (decrease in X_0) was accompanied by a decrease in the proportion of X_1 (down to a plateau level of ca. 10%) and an increase in the proportion of X_2. The relationships between the *A*/*X* ratios and the different degrees of substitution, as shown in Figure 3, are in good agreement with those reported earlier (Gruppen et al., 1992) for *T. aestivum* AX.

The unresolved signals downfield of the δ 5.30 and 5.23 ppm peaks very likely result from two neighboring disubstituted xylp residues in the chain (Hoffmann et al., 1992b; Cleemput et al., 1993; Vinkx et al., 1993). It is logical that their relative intensity increases with higher levels of X_2 because the likelihood of having two adjacent X_2 residues obviously should increase with their overall level.

CONCLUSIONS

The present results confirm the earlier statement (Ciacco and D'Appolonia, 1982) that *T. durum* arabinoxylans contain more arabinose than their common wheat counterparts. They extend our further insight into structural features of these minor constituents of the most suited raw material for pasta products. However, many questions remain concerning their impact on semolina functionality. More work will be necessary to determine whether the difference in functionality between the proteins from *T. durum* and those from common wheats can, at least in part, be ascribed to the difference in the structure of the arabinoxylans in the two wheat classes. This may not be so unlogical when one takes into account that arabinoxylans clearly influence wheat functionality (Roels et al., 1993).

ABBREVIATIONS USED

A/*X*, arabinose/xylose; AX, arabinoxylan(s); Araf, L-arabinofuranosyl; NMR, nuclear magnetic resonance; TWEAX, total water-extractable arabinoxylan; xylp, xylopyranosyl; X_0, unsubstituted xylp residues; X_1, monosubstituted xylp residues; X_2, disubstituted xylp residues.

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APPENDIX: CALCULATION OF THE DISTRIBUTION OF X_0, X_1, AND X_2 FROM A COMBINATION OF GC AND ^{1}H NMR RESULTS

X = weight percentage of xylose

A = weight percentage of arabinose

X_0 = weight percentage of unsubstituted xylose residues

X_1 = weight percentage of monosubstituted xylose residues

X_2 = weight percentage of disubstituted xylose residues

$$X = X_0 + X_1 + X_2 \quad (1)$$

$$A = X_1 + 2X_2 \quad (2)$$

(1) From NMR spectra, integrate the peaks at δ 5.40 (X_1) and δ 5.30 and 5.23 ppm (X_2) and determine the ratio of $X_1/X_2 = a$ or $X_1 = aX_2$.

(2) From the GC analysis, determine the A/X ratio: $A/X = b$ or $b\mathrm{X} = A$

It follows that

$$A = aX_2 + 2X_2 = (a + 2)X_2 \quad (3)$$

$$b\mathrm{X} = (a + 2)\mathrm{X}_2 \quad (4)$$

Thus, from eq 4 it follows that $X_2/X = b/(a + 2)$ or

$$(X_1/a)/X = b/(a + 2) \quad (5)$$

Thus, from eq 5 it follows that $X_1/X = ab/(a + 2)$.

Since $1 = X_0/X + X_1/X + X_2/X$ (eq 6), it follows that $X_0/X = 1 - X_1/X - X_2/X$ or $X_0/X = 1 - [ab/(a + 2)] - b/(a + 2)$ or $X_0/X = 1 - (\mathrm{a} + 1)[b/(a + 2)]$.

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