Distribution of Carbohydrates in Gluten Fractions Isolated from European Wheats (*Triticum aestivum* L.) in a Batter System

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Glucose represented 89% of the total carbohydrate of gluten isolated in a batter system from wheat cultivars exhibiting varying breadmaking qualities. Galactose, arabinose (Ara), and xylose (Xyl) were the remaining carbohydrates (ca. 10% of the total carbohydrate content), while mannose was present in minor quantities (<1%). After Pronase treatment ca. 43% of the Ara and ca. 52% of the Xyl originating from arabinoxylan (AX) could be solubilized, indicating the presence of water extractable as well as water unextractable AX. Arabinogalactan-peptide (AGP) and small amounts of β-glucan were also present. Water extractable AX with a low Ara-to-Xyl ratio (A/X) and thus a low degree of substitution preferentially are incorporated in the gluten network probably as a result of alignment between glutenin polymers and AX in the stirring direction. In gluten from cultivars exhibiting high gluten protein recoveries (see accompanying paper; Roels et al. *J. Agric. Food Chem.* **1998**, *46*, 1344−1349), the levels of total gluten associated nonstarch polysaccharides (TOTGANSP), expressed as a percentage of total flour NSP, were also high. It was postulated that the gluten proteins of such cultivars agglomerate despite the presence of NSP since the gluten fractions with the best agglomeration properties contained the lowest amount of NSP. In the aforementioned gluten fractions the AGP/AX ratio was also unusually high, indicating that the undocumented role of AGP in wheat gluten agglomeration may well be underestimated.

Keywords: *Gluten agglomeration; gluten composition; arabinoxylan; arabinogalactan; Triticum aestivum*

INTRODUCTION

It is well-known that laboratory as well as industrially prepared vital wheat gluten, besides protein, contains a substantial amount of other flour components such as lipids (ca. 3.5−6.8%), minerals (ca. 0.5−0.9%), and carbohydrate material (ca. 7.0−16.0%, all on an as-is basis), the latter being mainly starch and to a lesser extent non-starch polysaccharides (NSP) (Roels, 1997). It is generally believed that the lipids strongly interact with gluten proteins during dough formation (MacRitchie, 1983) and are thus co-extracted from the doughs or batters prepared in gluten isolation. However, insight into the interactions between polysaccharides and gluten proteins is lacking. It has been shown that the starch level in gluten is inversely related to the protein content (Wadhawan, 1988; Saulnier et al., 1997; Roels et al., 1998), and its levels strongly depend on the washing efficiency during gluten isolation (Saulnier et al., 1997). The starch granules are therefore believed to be present in these gluten as a result of physical entrapment rather than as a result of specific interactions occurring at the gluten−starch granule interface. Although the presence of NSP in gluten has been known for more than 25 years (D'Appolonia and Gilles, 1971), and quantified in gluten (D'Appolonia and Gilles, 1971; Saulnier et al., 1997), it is still unknown if, and if so to what extent, these compounds can influence the agglomeration behavior of the gluten polymers. Much as with starch, the question arises whether these polysaccharides are incorporated in gluten matrix through physical entrapment or whether they specifically interact with the proteins during gluten agglomeration. A first type of specificity may originate from the fact that certain NSP (e.g. AGP) react specifically with certain gluten proteins (e.g. gliadins) while others do not. A second type of specificity may originate from the fact that subpopulations of NSP, exhibiting different structural features (e.g. AX with different degrees of substitution), are preferentially incorporated into the gluten matrix during isolation. McMaster and Bushuk (1983) concluded from the different monosaccharide compositions in alcohol soluble [containing mainly galactose (Gal), glucose (Glc), and arabinose (Ara)] and alcohol insoluble gluten fractions (containing mainly Glc) that there is some (first type) specificity in the association between flour NSP and gluten. In contrast, Saulnier et al. (1997) observed that the level of NSP is well correlated with the starch content which in turn depends on the washing efficiency during gluten isolation. The authors therefore concluded that the majority of NSP, like starch, are physically entrapped during gluten isolation.

In an effort to gain more insight into the factors determining gluten agglomeration behavior, vital wheat gluten was isolated from wheat flour samples of different varieties (Apollo, Slejpner, Camp Remy, Sperber, Minaret, and Soissons) of known breadmaking quality (Roels et al., 1993). In this work, it was hypothesized

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Table 1. Sugar Residue Composition (% Gluten, w/w, dm) of Carbohydrates in Undefatted Gluten Fractions Recovered from Different Sieves and Reconstituted Samples (Averages of Two Isolations) and A/X Ratios

cult[a]	sieve	$[Ara]_{AX}$[b]	$[Ara]_{AGP}$[c]	$[Xyl]_{AX}$[d]	[Man]	$[Gal]_{AGP}$[e]	$[Gal]_{GL}$[f]	[Glc]	total	A/X[g]
Ap	>400 μ	0.14	0.21	0.24	0.13	0.31	0.75	14.05	15.83	0.56
		***[i,j]	ns	***	ns	ns	ns	***	***	*
	>250 μ	0.28	0.20	0.46	0.14	0.29	0.72	19.65	21.73	0.60
		****[i,k]	ns	****	**	ns	ns	***	****	**
	>125 μ	0.82	0.21	1.35	0.22	0.31	0.66	23.71	27.28	0.61
		****[i,l]	ns	****	*	ns	*	****	****	**
	recon[h]	0.52	0.21	0.86	0.17	0.31	0.70	20.15	22.92	0.60
Sl	>400 μ	0.20	0.22	0.34	0.15	0.33	0.57	15.33	17.15	0.58
		***	ns	***	ns	ns	ns	*	*	***
	>250 μ	0.36	0.21	0.59	0.18	0.32	0.59	16.92	19.16	0.60
		****	ns	****	***	ns	*	***	***	**
	>125 μ	1.96	0.21	3.20	0.52	0.32	0.48	31.83	38.52	0.61
		****	ns	****	***	ns	**	***	***	***
	recon	0.67	0.22	1.10	0.25	0.33	0.55	19.69	22.81	0.61
Sp	>400 μ	0.16	0.21	0.29	0.11	0.31	0.51	18.05	19.64	0.57
		***	ns	***	ns	ns	*	ns	*	*
	>250 μ	0.34	0.21	0.57	0.13	0.31	0.48	21.01	23.05	0.60
		****	ns	****	***	ns	ns	****	****	**
	>125 μ	1.66	0.21	2.71	0.46	0.31	0.46	30.61	36.41	0.61
		****	ns	****	***	ns	ns	***	****	**
	recon	0.53	0.21	0.87	0.19	0.31	0.50	21.36	23.99	0.61
CR	>400 μ	0.14	0.21	0.25	0.13	0.31	0.46	19.34	20.83	0.56
		***	ns	***	ns	ns	ns	*	ns	***
	>250 μ	0.35	0.19	0.57	0.17	0.29	0.48	18.41	20.45	0.60
		***	*	***	*	*	**	****	****	**
	>125 μ	2.63	0.31	4.31	0.89	0.46	0.14	49.45	58.18	0.61
		***	ns	***	*	ns	**	****	****	***
	recon	0.37	0.21	0.63	0.19	0.32	0.43	22.04	24.20	0.59
Mi	>400 μ	0.15	0.20	0.26	0.13	0.30	0.66	17.30	19.00	0.57
		**	ns	**	ns	ns	**	**	**	ns
	>250 μ	0.23	0.22	0.40?	0.16	0.33	0.58	23.65	25.56	0.59
		**	ns	**	***	ns	ns	ns	ns	**
	>125 μ	0.77	0.20	1.26	0.26	0.31	0.55	26.87	30.22	0.61
		**	ns	**	**	ns	**	**	**	*
	recon	0.45	0.21	0.74	0.20	0.31	0.59	23.5	25.99	0.61
Ss	>400 μ	0.12	0.20	0.22	0.12	0.29	0.73	13.78	15.46	0.55
		***	**	***	ns	**	**	**	**	*
	>250 μ	0.29	0.23	0.48	0.16	0.34	0.63	20.17	22.29	0.60
		**	ns	**	ns	ns	*	**	**	**
	>125 μ	0.75	0.28	1.24	0.26	0.42	0.48	27.20	30.63	0.61
		***	*	***	**	*	**	**	**	*
	recon	0.49	0.25	0.81	0.21	0.37	0.57	22.53	25.23	0.60
6 cult	mean	0.51	0.22	0.84	0.20	0.33	0.56	21.55	24.19	0.60

[a] Cult, cultivar. Ap, Apollo; Sl, Slejpner; Sp, Sperber; CR, Camp Remy; Mi, Minaret; Ss, Soissons. "[ ]" Used for undefatted samples. Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glc, glucose. [b] $[Ara]_{AX}$, Ara associated with arabinoxylan. [c] $[Ara]_{AGP}$, Ara associated with arabinogalactan-peptide. [d] $[Xyl]_{AX}$, Xyl associated with arabinoxylan. [e] $[Gal]_{AGP}$, Gal associated with arabinogalactan-peptide. [f] $[Gal]_{GL}$, Gal associated with glycolipids. [g] A/X, $[Ara]_{AX}$ to $[Xyl]_{AX}$ ratio. [h] "recon", reconstituted gluten. [i] Level of statistical significance (P value) between observed means for different sieves; ns, not significant; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$; **** = $P < 0.0001$. [j] Statistical difference between sieve >400 μ and >250 μ. [k] Statistical difference between sieve >250 μ and >125 μ. [l] Statistical difference between sieve >400 μ and >125 μ.

that the observed differences in gluten aggregation and in dough development for breadmaking could be due to interferences of water extractable flour NSP (WEFNSP) disturbing the formation of a uniform gluten matrix, hence our special interest in the role of these polysaccharides during gluten isolation. The aforementioned flours were processed into vital wheat gluten and starch using a batter system (Roels et al., 1997). The resulting gluten fractions were recovered by freeze-drying, and their carbohydrate composition was determined to study the importance of these components in gluten agglomeration. This effort, so we hoped, would contribute to the understanding of wheat gluten agglomeration.

MATERIALS AND METHODS

Gluten Isolation. Gluten was isolated by means of a batter procedure described by Roels et al. (1998) and collected from sieves with decreasing pore size (400, 250, and 125 μ, respectively).

Gluten Defatting. Gluten samples (1.5 g) were extracted with two subsequent portions of 30.0 mL of water saturated *n*-butanol for 1 h at 100 °C with frequent intermediary shaking. After cooling to room temperature, the samples were centrifuged (10 min at 1250*g*). The residue was then washed on a sintered glass filter with subsequent 30.0 mL portions of ethanol, acetone, and diethyl ether, respectively. The samples were allowed to dry and were then gently ground with mortar and pestle.

Gluten Composition. Moisture and protein contents were determined according to AACC Methods 44-19 and 46-11A (N × 5.7), respectively (AACC 1995). Composition of carbohydrates was determined by acid hydrolysis followed by gas chromatography of alditol acetates by a modified procedure of Englyst and Cummings (1984) as described elsewhere (Veraverbeke, 1995).

Xylanase Activity of Enzyme Preparations. Xylanase activity of the enzyme preparations was measured using azurine-cross-linked xylan (Megazyme, Bray, Ireland). Activity was measured under the experimental conditions described hereafter.

Identification and Quantification of Water Extractable Gluten-Associated Carbohydrates. Gluten (100 mg) samples were weighed into screw-capped tubes and heated under conditions (130 °C for 64 h) that inactivated associated NSP-hydrolyzing enzymes but did not induce Mallard reactions (Roels, unpublished results). After cooling, gluten was suspended in 2.0 mL of deionized water containing 1.0 mg/mL of xylanase-free Pronase E (EC 3.4.24.4 from *Streptomyces griseus*; E. Merck, Darmstadt, Germany) and incubated at 37 °C for 24 h with continuous stirring. The mixtures were centrifuged (10 min at 10000*g*), and to 1.0 mL of the supernatant was added 2.0 mL of 3.0 M sulfuric acid. Hydrolysis and carbohydrate determination of the samples were performed as described above. A control containing all reagents but no gluten was run together with the samples.

Isolation and Fractionation of Water Extractable Gluten-Associated Non-Starch Polysaccharides (WEGANSP). Gluten (500 mg) samples were weighed into screw-capped tubes, heated as described above, cooled, and suspended in 10.0 mL of a Britton and Robinson universal buffer, pH 8.0 (McKenzie and Dawson, 1966), containing chloramphenicol (30 ppm), tetracycline (20 ppm), and 1.0 mg/mL of xylanase-free Pronase E (EC 3.4.24.4 from *S. griseus*; E. Merck, Darmstadt, Germany). Four drops of toluene were added, and the suspension was stirred continuously at 37 °C for 24 h. Enzyme was added a second time (2.5 mg in 5.0 mL of buffer), and the incubation was extended for another 24 h. The suspensions were centrifuged (10 min at 5000*g*). The supernatants were heated to 100 °C and cooled, and the pH was adjusted to 5.5 with 0.2 N HCl. Xylanase-free pullulanase [EC 3.2.1.41 from *Klebsiella pneumoniae*, 1.87 units in 40 μL of 3.2 M $(NH_4)_2SO_4$ solution; Sigma, St. Louis, MO] was added, and the mixtures were incubated at 30 °C for 16 h. The samples were then adjusted to pH 6.5 with 0.2 N NaOH and heated to 92 °C. Xylanase-free Termamyl solution (30 μL, Novo Nordisk, Bagsvaerd, Denmark) was added, and the mixtures were incubated for 30 min and heated to 100 °C. After cooling, the samples were filtered over DMCS-treated glass wool (Alltech, Deerfield, IL) and dialyzed (cutoff value 12−14 kDa) at 6 °C against deionized water for 93 h. Ethanol was added dropwise at room temperature and with continuous stirring to a final concentration of 80%. Precipitation was allowed to occur overnight at 6 °C. The samples were then centrifuged (30 min at 8000*g*, 6 °C). The supernatants were decanted, and the precipitates dissolved in deionized water and freeze-dried, resulting in a fraction F_{80}. Ethanol in the supernatants was removed by evaporation under reduced pressure (40 °C). The samples were made up to 50.0 mL and saturated as above with ethanol to a final concentration of 90%. After overnight storage at 6 °C, samples were centrifuged as above, yielding a precipitate and supernatant. The precipitate was dissolved in deionized water and freeze-dried, resulting in a fraction F_{90}. Ethanol was removed from the supernatant as above. Supernatants were then diluted with deionized water and finally freeze-dried, resulting in a fraction F_{SUP}.

Proton Nuclear Magnetic Resonance (^{1}H NMR) Spectroscopy. WEGANSP samples were dissolved in D_2O (99%), stirred overnight, and freeze-dried, and this step was repeated twice. The samples were finally dissolved in D_2O (ca. 1 mg/mL), and ^{1}H NMR spectra were recorded on a Bruker 300-MHz Fourier transform spectrometer at 85 °C. Pulse repetition was 1 s, and the number of scans was ca. 5000. The proportions of un-, mono-, and disubstituted xylose (Xyl) residues were obtained as outlined by Westerlund et al. (1990), i.e., by combining the ^{1}H NMR spectral data and gas chromatography results.

Zeleny Sedimentation. Zeleny sedimentation values of the wheat flours were determined according to Roels et al. (1993).

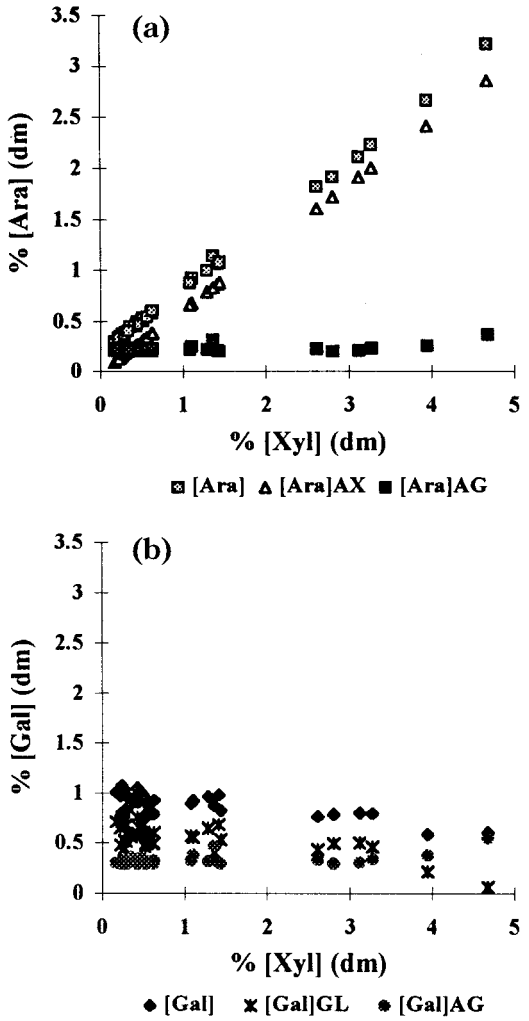

Figure 1. (a) Relationship between levels of Ara, Ara originating from AX, and Ara originating from AG with Xyl levels. (b) Variation in levels of Gal, Gal originating from AG, and Gal originating from GL with Xyl levels. Gluten samples were recovered from 400, 250, and 125 μ sieves.

Statistical Analysis. Statistical significances between means of observations were determined with *t* tests assuming unequal variances.

RESULTS AND DISCUSSION

Total Carbohydrate Composition. The results of the gluten carbohydrate composition and statistical analysis are summarized in Table 1. The total carbohydrate content of the reconstituted gluten isolated from the six European wheat cultivars averaged 24.2% (Table 1), which is higher than the average total carbohydrate content normally present in commercial wheat gluten (Wadhawan, 1988; Roels, 1997). The difference is mainly due to higher levels of Glc (mainly released by hydrolysis of residual starch) present in former samples. These higher levels, as expected, indicate that washing efficiency is better in industrial practice than in pilot facilities. The (average) levels of Glc found in gluten isolated from six varieties and in a set of 21 commercial gluten samples (Roels, 1997) represent 89% and 86% of the total carbohydrate content, respectively, which are comparable. The levels of Gal (Table 1) found in the reconstituted gluten in the present study and the aforementioned set of commercial gluten are comparable. Gal is a constituent of both AGP (Fincher and

Stone, 1974) and of glycolipids (GL) (Morrison, 1988) present in wheat flour, the latter being known to strongly associate with gluten proteins during dough-making (Olcott and Mecham, 1947). It is reasonable to assume that, once GL and gluten are associated, washing efficiency does not greatly influence the amount of GL bound, and hence the level of galactolipid Gal present in the gluten. The remaining carbohydrates were mainly Ara and Xyl (Table 1). Mannose (Man) was present only in small amounts.

Arabinoxylans and Arabinogalactan-Peptide. To calculate the levels of Ara associated with AX (Ara_{AX}) and with AGP (Ara_{AGP}) and the levels of Gal associated with AGP (Gal_{AGP}) and GL (Gal_{GL}), eight samples from different cultivars and from different sieves were selected to cover a wide range of Ara and Gal contents. The samples were defatted with water-saturated *n*-butanol at 100 °C as outlined above, and the residues were analyzed for carbohydrate compositions. After defatting, Gal contents [expressed on dry matter (dm)] were substantially lower (results not shown). For calculations (see eqs 1−6) the following assumptions were made:

(i) The defatting procedure removed all GL.

(ii) The Gal present in the defatted samples exclusively originates from AGP with an average A/G ratio of 0.67 (Fincher and Stone, 1974; Neukom and Markwalder, 1975; Westerlund et al., 1990; Izydorczyk et al., 1991; Loosveld et al., 1997).

(iii) All Xyl in the defatted samples originates from AX.

(iv) The ratio between AX and AGP does not change during defatting.

$$\{Xyl\} = 1.0603[Xyl] - 0.0802 \ (r^2 = 1.000;\ n = 8) \quad (1)$$

$$\{Ara\}_{AX} = 0.5480\{Xyl\} + 0.0211 \ (r^2 = 0.998;\ n = 8) \quad (2)$$

$$\{Ara\} = 0.9543[Ara] - 0.0309 \ (r^2 = 0.997;\ n = 8) \quad (3)$$

$$\{Ara\}_{AGP} = \{Ara\} - \{Ara\}_{AX} \quad (4)$$

$$[Ara]_{AGP} = 0.67[Gal] \quad (5)$$

$$[Gal]_{GL} = [Gal] - [Gal]_{AGP} \quad (6)$$

where Ara is the % (dm) arabinose, Ara_{AGP}, % (dm) arabinose associated with AGP, Gal, % (dm) galactose, Gal_{AGP}, % (dm) galactose associated with AGP, Gal_{GL}, % (dm) galactose associated with GL, Xyl, % (dm) xylose, "[ ]" are used for undefatted samples, and "{ }" are used for defatted samples.

Equations 1−3, derived from the carbohydrate compositions of the eight selected samples (undefatted and defatted), and eqs 4−6 were used to calculate the amounts of $[Ara]_{AX}$ and $[Ara]_{AGP}$ and the amounts $[Gal]_{AGP}$ and $[Gal]_{GL}$ in 36 gluten samples under study as follows: {Xyl} is calculated from [Xyl] with eq 1. $\{Ara\}_{AX}$ is computed using eq 2. $\{Ara\}_{AGP}$ is the difference between {Ara} (derived from eq 3) and $\{Ara\}_{AX}$ (eq 4). Because we assumed that the $[Ara]_{AX}/[Ara]_{AGP}$ ratio is the same in defatted and undefatted samples, $\{Ara\}_{AX}$ and $\{Ara\}_{AGP}$ can be converted to $[Ara]_{AX}$ and $[Ara]_{AGP}$. Finally, $[Gal]_{AGP}$ and $[Gal]_{GL}$ can be calculated using eqs 5 and 6, respectively. Results are presented in Table 1. For four varieties, the level of $[Ara]_{AGP}$ recovered from the different sieves is constant. For Camp Remy and Soissons, a slightly higher $[Ara]_{AGP}$ level on the >125 μ sieve is observed. $[Ara]_{AX}$ and $[Ara]_{AGP}$ are very slightly but significantly correlated ($r^2 = 0.284$; $P < 0.001$). Similar levels of $[Ara]_{AGP}$ are found in the reconstituted gluten for all varieties studied. The $[Gal]_{GL}$ levels decreased in gluten fractions recovered from the subsequent sieves with decreasing pore size. In our experiments, with decreasing pore size of the sieves, $[Ara]_{AX}$, $[Xyl]_{AX}$, [Man], and [Glc] levels in gluten increased while the $[Gal]_{GL}$ levels decreased (Table 1). The increase in $[Ara]_{AX}$ and $[Xyl]_{AX}$ contents in gluten fractions recovered from subsequent sieves with decreasing pore size is accompanied by a slight increase in A/X ratio.

We observed linear relationships between the monosaccharide equivalents present in gluten fractions recovered from the different sieves as shown in Figure 1a and b. The linear relationship ($r^2 = 1.000$) between (the measured) [Ara] or (calculated) $[Ara]_{AX}$ on one hand, and the measured [Xyl] on the other hand provides additional evidence for the presence of AX in wheat gluten confirming earlier reports (D'Appolonia and Gilles, 1971; Saulnier et al., 1997). It is striking that the linear relationship holds true for the whole set of gluten fractions originating from different varieties and different sieves. The (dimensionless) slope of this line (0.609) is indicative for the average A/X ratio of all the AX present and is in agreement with the A/X ratios shown in Table 1 for reconstituted gluten samples. This implies that (i) a homogeneous population of NSP with similar structures (and corresponding A/X ratios) agglomerate with gluten and that these polysaccharides are the same for all the varieties studied or (ii) that a heterogeneous population of AX with different structures (and hence different A/X ratios) are involved in the agglomeration of wheat gluten. The average A/X ratio is then determined by the A/X ratios and the level of the different polysaccharides present. The latter hypothesis is probably correct since it was shown that gluten contains both WE- and water unextractable (WU-) AX, and that WEGANSP consist as a population of AX with different A/X ratios (cf. infra).

Water Extractable Gluten-Associated Non-Starch Polysaccharides. The carbohydrate compositions of the material solubilized after Pronase treatment of different gluten fractions together with the results of the statistical analysis are shown in Table 2. Equations similar to those derived for total gluten-associated NSP (TOTGANSP) can be derived for the WEGANSP. They read as follows:

$$\{Xyl\} = 1.0032[Xyl] + 0.0211 \ (r^2 = 0.995;\ n = 8) \quad (7)$$

$$\{Ara\}_{AX} = 0.6457\{Xyl\} - 0.0261 \ (r^2 = 0.994;\ n = 8) \quad (8)$$

$$\{Ara\} = 1.0471[Ara] + 0.0787 \ (r^2 = 0.998;\ n = 8) \quad (9)$$

with symbols and dimensions as described above for eqs 1−6. Much as in the approach for the total carbohydrate composition, the distribution of Ara and Gal in water-extractable components released after Pronase treatment was calculated using eqs 4−9. These results are shown in Table 2. One can deduce from Tables 1 and 2 that the Pronase treatment released (on an average for the six cultivars) 43% of the $[Ara]_{AX}$, 45% of the $[Ara]_{AGP}$ and $[Gal]_{AGP}$, 52% of the [Xyl], and 18%

Table 2. Sugar Residue Composition (% Gluten, w/w, dm) of Water-Extractable Carbohydrates from Pronase Digests of Gluten Fractions Recovered from Different Sieves and Reconstituted Samples (Averages of Two Isolations) and A/X Ratios[a]

cult	sieve	$[Ara]_{AX}$	$[Ara]_{AGP}$	$[Xyl]_{AX}$	[Man]	$[Gal]_{AGP}$	$[Gal]_{GL}$	[Glc]	total	A/X
Ap	>400 μ	0.04	0.09	0.12	0.02	0.14	0.18	0.84	1.44	0.33
		**	ns	**	ns	ns	ns	ns	ns	*
	>250 μ	0.08	0.10	0.21	0.03	0.15	0.15	0.99	1.72	0.40
		****	ns	****	ns	ns	*	*	**	***
	>125 μ	0.28	0.11	0.57	0.04	0.17	0.07	2.16	3.41	0.50
		****	ns	****	ns	ns	*	*	**	**
	recon	0.18	0.11	0.38	0.04	0.16	0.12	1.53	2.52	0.47
Sl	>400 μ	0.08	0.09	0.20	0.02	0.13	0.10	1.15	1.77	0.38
		*	ns	*	ns	ns	ns	ns	ns	**
	>250 μ	0.14	0.09	0.32	0.03	0.13	0.15	0.93	1.79	0.44
		**	ns	**	*	ns	*	ns	**	***
	>125 μ	0.94	0.11	1.65	0.07	0.17	0.05	2.44	5.44	0.57
		**	ns	**	ns	ns	ns	ns	**	****
	recon	0.31	0.09	0.59	0.04	0.14	0.10	1.43	2.70	0.53
Sp	>400 μ	0.07	0.08	0.18	0.04	0.12	0.09	2.05	2.63	0.36
		**	ns	**	ns	ns	ns	*	ns	*
	>250 μ	0.13	0.11	0.29	0.04	0.16	0.13	1.60	2.44	0.43
		***	ns	***	ns	ns	ns	**	**	***
	>125 μ	0.83	0.09	1.47	0.06	0.13	0.13	2.67	5.39	0.56
		***	ns	***	*	ns	ns	***	**	**
	recon	0.25	0.09	0.49	0.05	0.13	0.11	2.11	3.23	0.51
CR	>400 μ	0.06	0.11	0.16	0.02	0.16	0.03	1.84	2.39	0.38
		**	ns	**	ns	ns	*	ns	ns	**
	>250 μ	0.11	0.11	0.26	0.03	0.16	0.06	1.57	2.31	0.42
		***	**	***	**	**	**	**	***	****
	>125 μ	1.21	0.14	2.09	0.10	0.21	0.00	4.15	7.90	0.58
		***	*	***	***	*	ns	***	***	****
	recon	0.17	0.11	0.34	0.02	0.17	0.03	2.03	2.87	0.50
Mi	>400 μ	0.06	0.09	0.17	0.04	0.14	0.12	1.13	1.76	0.37
		**	ns	**	ns	ns	ns	ns	ns	**
	>250 μ	0.11	0.10	0.26	0.02	0.14	0.11	1.34	2.09	0.42
		*	ns	*	ns	ns	ns	ns	ns	***
	>125 μ	0.37	0.11	0.71	0.05	0.17	0.07	2.22	3.70	0.52
		**	ns	**	ns	ns	ns	ns	*	****
	recon	0.22	0.10	0.44	0.04	0.15	0.09	1.79	2.83	0.50
Ss	>400 μ	0.03	0.07	0.11	0	0.10	0.20	0.98	1.49	0.28
		***	ns	***	ns	ns	ns	*	***	*
	>250 μ	0.10	0.10	0.25	0.02	0.15	0.17	1.48	2.27	0.42
		*	**	*	*	**	*	**	****	***
	>125 μ	0.32	0.14	0.62	0.04	0.21	0.08	2.40	3.80	0.51
		**	**	**	ns	**	*	***	****	**
	recon	0.20	0.11	0.41	0.03	0.17	0.13	1.85	2.90	0.49
6 cult	mean	0.22	0.10	0.44	0.04	0.15	0.10	1.79	2.84	0.50

[a] For legend, see Table 1.

of $[Gal]_{GL}$. The higher amount of [Xyl] released than that of $[Ara]_{AX}$ and the lower A/X ratio (Table 2) of the solubilized material compared to that of the total (Table 1) indicates that the enzyme treatment solubilized AX with an A/X ratio lower than that of the average population. It is furthermore striking that only half of the AGP was extracted after Pronase treatment. On the other hand, only 18% of the Gal associated with GL and 8% of the Glc was recovered in the extracted material. Twelve percent of the total level of carbohydrates present in wheat gluten under study could be solubilized after Pronase treatment. From the data, it is clear that gluten contains WE- as well as WUAX. Indeed, microscopic examination of gluten fractions recovered from the different sieves stained with 0.2% Fast Green FCF and 0.2% Calcofluor white M2R revealed the presence of endosperm cell walls and small bran particles (results not shown).

The A/X ratios obtained for WEGANSP (Table 2) are in agreement with those reported by Cleemput et al. (1995). For the variety Camp Remy, fractionated WE flour AX (WEFAX) had A/X ratios between 0.36 and 0.82 or between 0.47 and 0.89, depending on the isolation and fractionation method used (Cleemput et al., 1995). Moreover, the A/X ratios of WEGANSP in the reconstituted gluten samples (Apollo, 0.47; Slejpner, 0.53; Sperber, 0.51; Camp Remy, 0.50; Minaret, 0.50; Soissons, 0.49) are comparable (except for Soissons) to those reported for purified WEFAX in the same varieties (Apollo, 0.51; Slejpner, 0.53; Sperber, 0.50; Camp Remy, 0.53; Minaret, 0.53; Soissons, 0.61) (Cleemput et al., 1993). On the basis of these comparable A/X ratios, one can state that the populations of WEAX in wheat flour extracts and in reconstituted gluten are alike. In the case of Soissons, only part of the WEAX population (with an average A/X ratio lower than the average A/X ratio of the whole WEAX population) is readily incorporated into gluten network.

The A/X ratios of WEGANSP increased with the decreasing pore size of the sieves (Table 2), indicating that gluten (isolated in a batter process with uniaxial stirring) most easily interacts with WEAX with a low degree of substitution. If we consider gluten formation in our isolation procedure to be an alignment of uniaxi-

Table 3. Sugar Residue Composition (%, w/w) and Apparent[a] A/X Ratios of WEGANSP Fractions from Different Sieves Obtained by Ethanol Precipitation (Averages of Duplicate Experiments)[b]

frac[c]	cult	sieve	[Ara]	[Xyl]	[Man]	[Gal]	[Glc]	total	A/X
F_{80}[d]	CR	>400 μ	7.54	9.57	0.66	5.71	1.77	25.26	0.79
		>250 μ	9.78	13.06	0.73	5.68	3.18	32.43	0.75
		>125 μ	24.16	40.15	0.67	2.47	9.63	77.09	0.60
	Ss	>400 μ	5.56	6.56	0.73	5.75	1.61	20.21	0.85
		>250 μ	9.08	11.19	0.79	6.20	2.51	29.77	0.81
		>125 μ	12.76	18.28	0.57	4.33	3.26	39.21	0.70
F_{90}[e]	CR	>400 μ	6.49	7.12	0.98	6.21	2.20	23.00	0.91
		>250 μ	7.49	9.42	0.93	4.85	2.36	25.05	0.80
		>125 μ	12.32	19.50	0.84	2.23	4.92	39.80	0.63
	Ss	>400 μ	6.57	7.02	0.59	6.79	2.46	23.43	0.94
		>250 μ	7.93	9.30	0.73	6.05	3.94	27.95	0.85
		>125 μ	12.09	15.65	1.17	5.30	3.83	38.03	0.77
F_{SUP}[f]	CR	>400 μ	0.94	0.83	0.33	2.71	4.49	9.31	1.14
		>250 μ	1.17	0.99	0.27	3.17	0.48	6.08	1.18
		>125 μ	2.74	3.03	0.31	1.60	2.21	9.90	0.91
	Ss	>400 μ	0.99	0.76	0.28	3.42	2.60	8.06	1.30
		>250 μ	1.17	0.96	0.29	3.64	1.03	7.08	1.22
		>125 μ	1.59	1.38	0.31	3.54	1.48	8.30	1.15

[a] Ara originating from AX and AGP. [b] For legend, see Table 1. [c] "frac", fraction. [d] F_{80}, fraction precipitated at a final ethanol concentration of 80%. [e] F_{90}, fraction precipitated at a final ethanol concentration of 90%. [f] F_{SUP}, fraction soluble in 90% ethanol.

ally stirred polymers, it can be easily understood that the more linear structures (and in the case of WEAX thus with a low A/X ratio) preferentially take part in this agglomeration process and as a result are incorporated. This may also explain the improvement in gluten agglomeration and yields when cellulases and hemicellulases (Weegels et al., 1991, 1992) are used in gluten isolation process, where AX at a low degree of substitution are more susceptible to these enzymes (Cleemput, 1996).

Fine Structure of WEGANSP. To gain further insight into structural variations among AX incorporated into gluten network, six samples were selected (three sieves from the cultivars Camp Remy and Soissons) and WEGANSP were isolated as described above. The carbohydrate compositions of the resulting fractions F_{80}, $F_{90,}$ and F_{SUP} are shown in Table 3. The carbohydrate content of the fractions decreased with increased ethanol concentration (Table 3). The apparent A/X ratios (with Ara originating from AX and AG) decrease with decreasing pore size. Due to the low amount of AX present, ^{1}H NMR spectra could not be recorded for the F_{SUP} fractions. Spectral data for the F_{80} and F_{90} fractions of Camp Remy and Soissons are shown in Figure 2a and b. It can be seen that in all F_{80} and F_{90} fractions, both AX and AGP are present and they occur in different ratios. Indeed, the signal at δ 5.26 can be ascribed to terminal arabinofuranose residues (Ara*f*) in AGP (Westerlund et al., 1990). The peak at δ 5.40 represents the H-1 of Ara*f* linked to O-3 of xylopyranose residues (Xyl*p*), and the peaks at δ 5.30 and 5.23 stem from the anomeric protons of Ara*f* linked to O-2 and O-3 of the same Xyl*p* residue (Hoffmann et al., 1992a,b), indicating the presence of AX. Also evident from these spectra is the presence of β-D-glucan, as indicated by a broad doublet between δ 4.70 and 4.80 (Cleemput et al., 1995).

The relative intensity of the peaks indicating the presence of β-glucan increases for both varieties as the pore size of the sieves from which the gluten recovered, decreases. The proportion of Ara*f* from AGP in the total amount of Ara*f* (from AGP and AX) decreased with decreasing pore size. When similar fractions for the two cultivars (see also Table 4) are compared, it can be seen that fractions from Soissons contain higher levels of AGP than comparable fractions from Camp Remy (except for F_{90}, >250 μ). Information on the structures of AX and true A/X ratios was obtained by combining gas chromatography data with ^{1}H NMR spectral data. These results are presented in Table 4. From this Table it can be seen that, in fractions F_{80} and F_{90}, the proportion of AGP [as indicated by the Ara*f*(AGP)/Ara*f*(AGP + AX) ratio] was high on the first (>400 μ) sieve and then decreased. Further, it can be calculated that the AGP/AX ratio on the first sieve (where gluten exhibiting the best agglomeration properties is retained) is higher ($P < 0.002$) than what is normally observed in wheat water extracts. Indeed, AGP/AX values in gluten fractions recovered from the first sieves are as follows for Apollo, 1.44; Slejpner, 0.79; Sperber, 0.80; Camp Remy, 1.23; Minaret, 1.00; and Soissons, 1.21, whereas AGP/AX ratios (% of dm) between 0.41 and 1.00 with a mean of 0.53 were found for water extracts of 18 European wheat flour samples (Loosveld et al., 1998). For reconstituted gluten samples used in this study, lower AGP/AX values ($P < 0.01$) than those found in wheat water extracts (Loosveld et al., 1997) were obtained (0.48, 0.26, 0.30, 0.55, 0.38, and 0.46 for Apollo, Slejpner, Sperber, Camp Remy, Minaret, and Soissons, respectively). From the above data, it is clear that the undocumented role of AGP in wheat gluten agglomeration may well be underestimated.

As already mentioned, true A/X ratios of water extractable gluten-associated arabinoxylans (WEGAAX) increase with decreasing pore size. This implies a higher degree of substitution of AX recovered in gluten fractions as the pore size of the sieves decrease. Indeed, the higher A/X ratios arise from a decrease in the proportion of Xyl*u* residues and an increase in the proportion of Xyl*m* and Xyl*d* residues (Table 4). The increase is more pronounced for the Xyl*d* residues than for the Xyl*m* residues. It is also worthwhile to note that a concentration of 80% ethanol, used to quantitatively precipitate AX and AGP from water extracts of wheat flour (Loosveld et al., 1997), was insufficient to precipitate all WEGANSP. Part of the water extractable gluten-associated arabinogalactan-peptide (WEGAAGP) as well as a portion of WEGAAX remains soluble at this ethanol concentration. It is therefore tempting to speculate that at least part of the WEFNSP were altered during gluten isolation, resulting in structures exhibiting different physicochemical properties.

Technological Implications. From a technological point of view, the level of NSP associated with wheat gluten may determine the quantity of energy required to dry isolated gluten. Figure 3a shows how [Ara], $[Ara]_{AX}$, $[Ara]_{AGP}$, and [Xyl] are related to the amount of water retained (g/g, dm) by wet gluten fractions recovered from different sieves. Although these components are only minor constituents, the amount of water bound by wet gluten can be predicted in a better way from their levels [Ara ($r^2 = 0.939$), Xyl ($r^2 = 0.941$)] than from the protein ($r^2 = 0.742$) or Glc contents ($r^2 = 0.731$) (Figure 3b). Moreover, protein content (% of dm) and the amount of water bound are inversely related, indicating other gluten components play a more important role in water retention. When [Ara] is separated from $[Ara]_{AX}$ and $[Ara]_{AGP}$, it can be seen that the amount of water retained is determined by the AX since the correlation coefficient of the regression line between

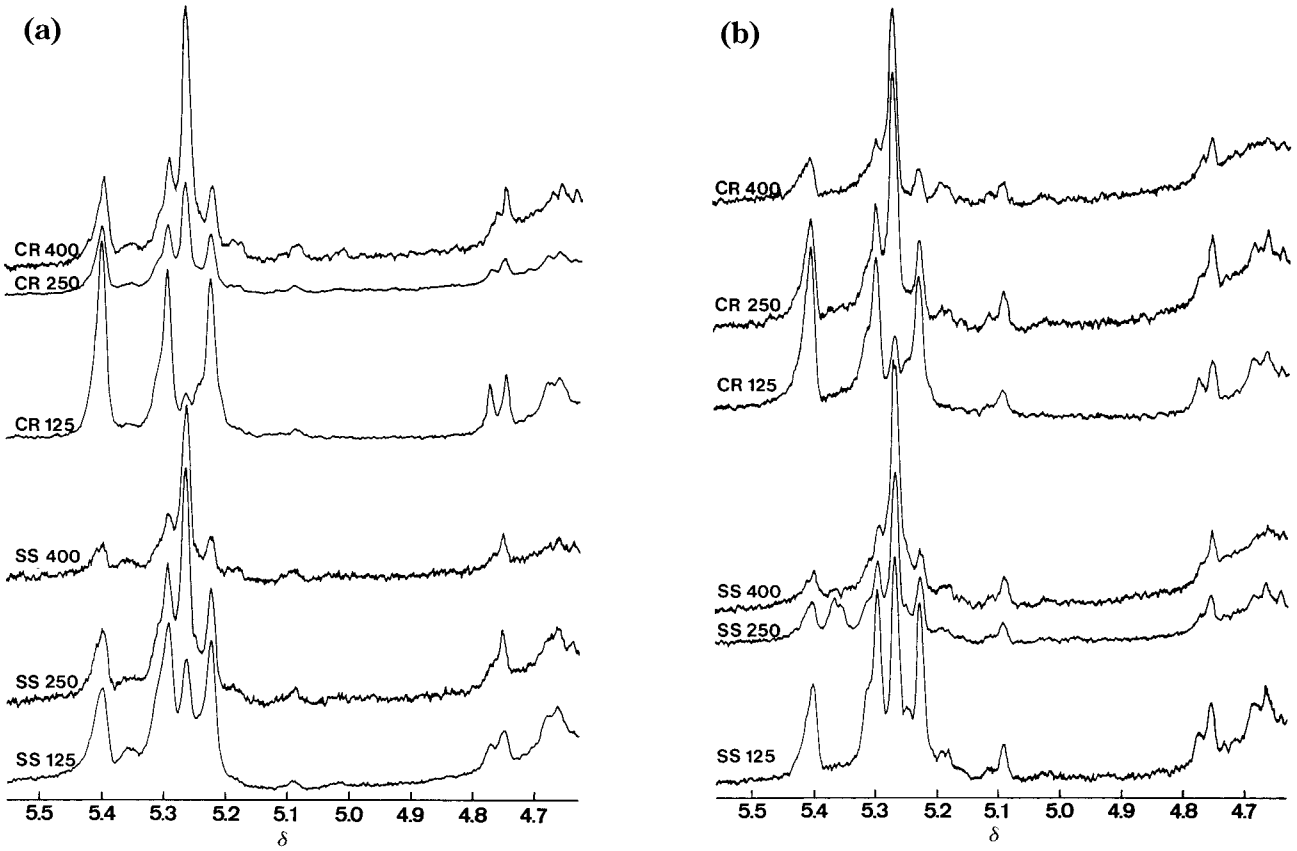

Figure 2. (a) Anomeric proton region of the ^{1}H NMR spectra (D_2O, 85 °C, 300 MHz) of water extractable gluten-associated non-starch polysaccharides precipitated at 80% ethanol from gluten samples isolated from Camp Remy (CR) and Soissons (Ss). Numbers represent 400, 250, and 125 μ sieves, respectively. (b) Anomeric proton region of the ^{1}H-NMR spectra (D_2O, 85 °C, 300 MHz) of water extractable gluten-associated non-starch polysaccharides precipitated at 90% ethanol from gluten samples isolated from Camp Remy (CR) and Soissons (Ss). Numbers represent 400, 250, and 125 μ sieves, respectively.

Table 4. Percentages of Mono- (Xyl*m*), Di- (Xyl*d*), and Unsubstituted Xyl*p* (Xyl*u*) Residues in AX, AGP/AX Ratio, and True A/X Ratios in Fractions Obtained by Ethanol Precipitation of WEGANSP Isolated from Gluten Fractions Recovered from Different Sieves

frac[a]	cult[b]	sieve	Ara*f*(AGP)/ Ara*f*(AGP + AX)[c]	AGP/ AX[d]	A/X	Xyl*m*	Xyl*d*	Xyl*u*
F_{80}	CR	>400 μ	46.7	0.64	0.42	18.2	11.9	69.9
		>250 μ	32.1	0.39	0.51	20.0	15.4	64.6
		>125 μ	5.0	0.05	0.57	20.3	18.4	61.2
	Ss	>400 μ	53.3	0.82	0.40	15.5	12.0	72.4
		>250 μ	39.7	0.54	0.49	15.2	16.8	67.9
		>125 μ	20.5	0.23	0.56	19.2	18.1	62.6
F_{90}	CR	>400 μ	56.8	0.92	0.39	20.8	9.3	69.9
		>250 μ	43.3	0.59	0.45	20.8	12.2	67.0
		>125 μ	10.3	0.10	0.57	22.3	17.2	60.5
	Ss	>400 μ	59.0	1.00	0.38	12.7	12.8	74.5
		>250 μ	40.7	0.59	0.51	16.6	17.0	66.4
		>125 μ	28.8	0.35	0.55	15.2	19.9	64.9

[a] F_{80}, fraction precipitated at a final ethanol concentration of 80%. F_{90}, fraction precipitated at a final ethanol concentration of 90%. [b] cult, cultivar; CR, Camp Remy; Ss, Soissons. [c] Ara*f*(AGP)/ Ara*f*(AGP + AX), proportion of arabinogalactan arabinofuranoside residues in the total amount of arabinogalactan-peptide and arabinoxylan arabinofuranoside residues (in %). [d] AGP/AX, ratio between the amount of arabinogalactan-peptide and arabinoxylans.

the amount of water retained and [Ara] or $[Ara]_{AX}$ is the same (0.940), while that for $[Ara]_{AGP}$ drops to 0.277. For $[Ara]_{AX}$ and [Xyl], intercepts of the regression lines are identical, reflecting the identical origin (AX) of these monosaccharides. In this view, the intercept (1.77 g of water/g of dm) would be the amount of water retained by gluten components other than AX (especially protein material and starch), i.e., when no AX is present.

Protein−Carbohydrate Interactions. *Gluten−NSP Interactions.* The relationship between the levels of TOTGANSP associated with gluten (as a percentage of the TOTFNSP) isolated from six wheat cultivars and gluten protein recoveries (GPR; Roels et al., 1997) is shown in Figure 4. The level of TOTGANSP associated with gluten varied from 3.9% (Apollo) to 11.6% (Soissons). These levels are comparable with those that can be estimated from data presented by D'Appolonia and Gilles (1971) after taking into account the protein contents of their NSP preparations and assuming a TOTNSP content of 2% for an average baker's patent flour. Values between 8% and 12% are thus obtained from their data. From Figure 4, it can be seen that a fairly good linear relation exists between the amount of NSP that associates with gluten (expressed as a percentage of TOTFNSP) and the GPR, Soissons exhibiting a somewhat different behavior in this respect. We may infer that high GPR's are obtained when high percentages of the TOTFNSP interact with gluten proteins, and thus NSP favoring gluten agglomeration. However, it can also be stated that with higher GPR's, more NSP originally present in flour associate with gluten proteins, and good processable gluten agglomerate despite the presence of these NSP. Three pieces of evidence for the latter hypothesis can be pointed out: (i) NSP contents are low in gluten fractions where agglomeration readily occurs (>400 μ); (ii) GPR's increase when the Zeleny sedimentation value of flour

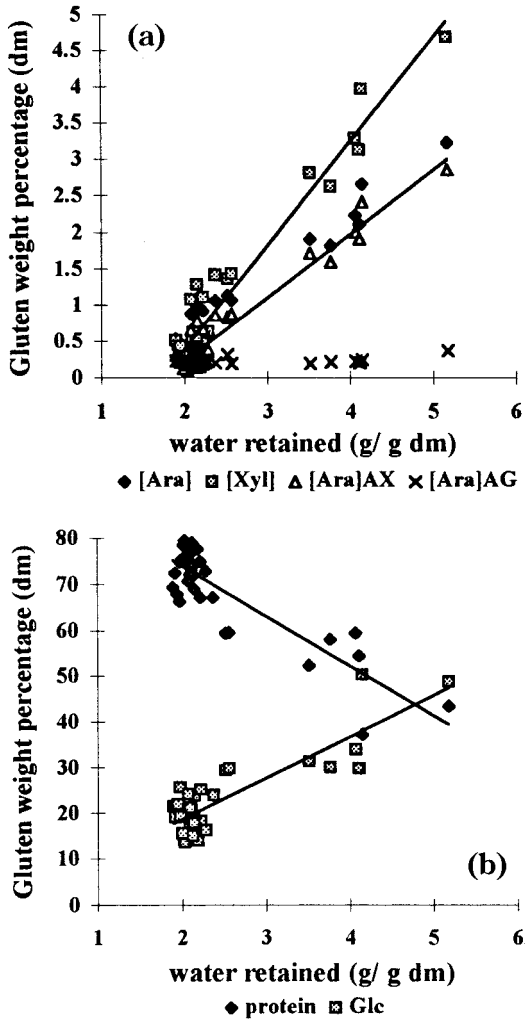

Figure 3. (a) Relationship between the levels of Ara, Xyl, Ara originating from AX, and Ara originating from AG and the amount of water retained in isolated gluten fractions from six European wheat cultivars. (b) Relationship between the protein levels (see the accompanying paper: Roels et al., 1998) and Glc levels and the amount of water retained in isolated gluten fractions from six European wheat cultivars.

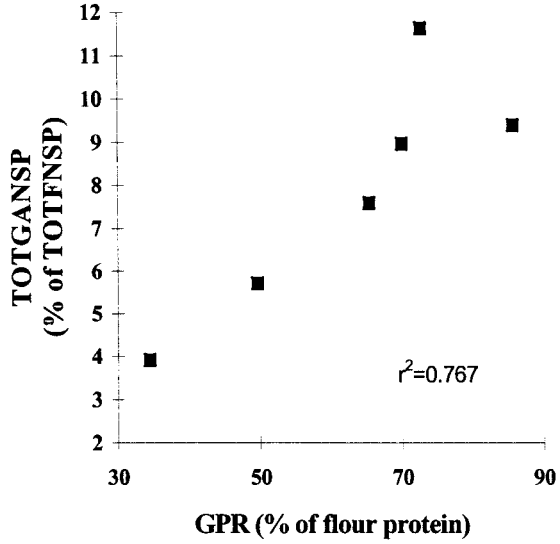

Figure 4. Level of total nonstarch polysaccharides (NSP) associated with gluten (expressed as a percentage of the total flour NSP) in relation to gluten protein recovery (GPR) (expressed in g of protein/100 g of flour protein) for reconstituted gluten isolated from six European wheat cultivars.

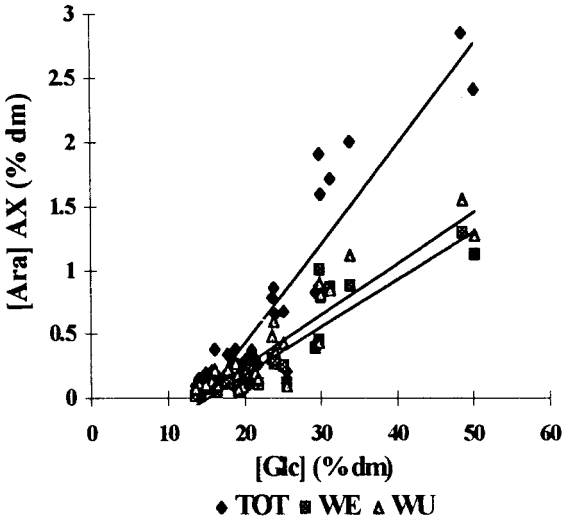

Figure 5. Relationship between Ara levels originating from total (TOT), water extractable (WE) AX, and water unextractable (WU) AX, and Glc levels in gluten fractions isolated from six European wheat cultivars.

increases [the Zeleny sedimentation value is inversely related to the WEFNSP content (Roels et al., 1993)]; and (iii) the use of NSP degrading enzymes during gluten isolation increases gluten recovery from flours with poor gluten agglomeration properties. For flours containing gluten with good agglomeration properties, the effect of added hemicellulases or cellulases was indeed much less pronounced (Weegels et al., 1991, 1992).

The question arises whether the interactions between gluten and starch on one hand and between gluten and NSP on the other are specific or due to physical inclusion of these components in gluten matrix. It was shown that, when gluten was isolated using a batter system with uniaxial stirring, AX with a low A/X ratio is incorporated preferentially in the gluten network. This interaction is probably induced by the uniaxal stirring which favors an alignment between the glutenin molecules and NSP with a low degree of branching. The structural features of AX determine their ease of incorporation, and thus one can conclude that these interactions are specific. In contrast, linear relationships exist between Ara_{AX} originating from TOTGANSP, WEGANSP, and WUGANSP (% dm) and the level of Glc (% dm) with correlation coefficients (r^2) of 0.853, 0.846, and 0.837, respectively (Figure 5). Since a higher washing efficiency diminishes both starch and NSP contents in gluten, it may be safe to assume that some of the interactions between gluten and starch or NSP are the result of physical entrapment rather than being of a specific nature, as suggested by Saulnier et al. (1997).

Gluten−Galactolipid Interactions. Linear relationships between the levels of [Gal], $[Gal]_{AGP}$, and $[Gal]_{GL}$ and the contents of different protein classes (Roels et al., 1997) were examined. Correlations were rather low but were always best between different Gal levels and the glutenin level, more so than between the residue and Gal levels and even less so in the case of gliadin and Gal levels. No correlation was found between the Gal levels and the level of physiological proteins (results not shown). When total Gal level was separated into $[Gal]_{AGP}$ and $[Gal]_{GL}$, correlations between $[Gal]_{GL}$ and the levels of different protein classes (except for the albumin + globulin fraction) increased, whereas those

between $[Gal]_{AGP}$ level and the aforementioned protein class levels decreased. From these observations, it can be concluded that galactolipids preferentially interact with glutenin molecules, or that gliadins containing associated galactolipids are preferentially incorporated in glutenin structure. The latter hypothesis is in agreement with results of Zawistowska et al. (1985), who found Glc as the only major carbohydrate component in glutenin preparations and therefore excluded the presence of galactolipids in glutenin. It has been identified that the carbohydrate present in gliadins prepared from untreated flour was mainly Gal from digalactosyldiglyceride (Bushuk, 1986).

CONCLUSIONS

Substantial amounts of carbohydrates were detected in gluten isolated by a batter process at a pilot scale. Glc (mainly arising from starch) was the most abundantly present monosaccharide, followed to a lesser extent by Gal, Ara, and Xyl. Although a minor constituent, the amount of AX associated with gluten strongly determines the amount of water bound during isolation and can be an important factor in industrial practice with regard to the energy required to dry gluten. The TOTFNSP retained in gluten varied from 3.9% to 11.6%. In gluten from cultivars exhibiting high GPR's, the proportion of NSP (in % of flour NSP) was also high. Both WE- and WUAX were present in gluten. Although A/X ratios of WEGAAX were in line with those determined for wheat flour, higher ethanol concentrations were necessary for WEGAAX precipitation and recovery was not quantitative. Higher proportions of AGP in NSP were present in gluten fractions exhibiting the best agglomeration properties. In the batter isolation procedure with uniaxial stirring, AX with a low A/X ratio (indicating high levels of Xyl*u*) was preferentially incorporated. The A/X ratio of the incorporated WEGAAX increased (indicating an increasing degree of substitution of the xylan backbone) with decreasing pore size of the sieves. Since the A/X ratio of the WEGAAX gradually increases as the pore size of sieves decreases, a specific interaction between these AX and glutenin molecules exists. In contrast, the observation that the levels of Ara originating from TOTGANSP, WEGANSP, and WUGANSP are related to the Glc levels indicates that AX incorporation may result from physical entrapment rather than being of a specific nature. The observed correlation between Gal_{GL} and glutenin levels in different gluten fractions probably indicates that gliadins containing associated galactolipids are preferentially incorporated in the glutenin polymers.

ABBREVIATIONS USED

AGP, arabinogalactan-peptide; A/G, arabinose-to-galactose ratio; Ara, arabinose; Ara_{AGP}, arabinose associated with arabinogalactan-peptide; Ara_{AX}, arabinose associated with arabinoxylan; Ara*f*, arabinofuranose residue; AX, arabinoxylan; A/X, arabinose-to-xylose ratio; dm, dry matter; Gal, galactose; Gal_{AGP}, galactose associated with arabinogalactan-peptide; Gal_{GL}, galactose associated with glycolipids; GL, glycolipids; Glc, glucose; GPR, gluten protein recovery; Man, mannose; NSP, non-starch polysaccharides; RECON, reconstituted gluten; TOTFNSP, total flour non-starch polysaccharides; TOTGANSP, total gluten-associated non-starch polysaccharides; WE, water extractable; WEAX, water extractable arabinoxylans; WEFAX, water extractable flour arabinoxylans; WEFNSP, water extractable flour non-starch polysaccharides; WEGAAGP, water extractable gluten-associated arabinogalactan-peptide; WEGAAX, water extractable gluten-associated arabinoxylans; WEGANSP, water extractable gluten-associated non-starch polysaccharides; WU, water unextractable; WUAX, water unextractable arabinoxylans; WUGANSP, water unextractable gluten-associated non-starch polysaccharides; WUNSP, water unextractable non-starch polysaccharides; Xyl, xylose; Xyl*d*, disubstituted xylopyranose residue; Xyl*m*, monosubstituted xylopyranose residue; Xyl*p*, xylopyranose residue; Xyl*u*, unsubstituted xylopyranose residue.

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LITERATURE CITED

American Association of Cereal Chemists. *Approved Methods of the AACC*, 9th ed.; AACC: St. Paul, MN, 1995; Method 44-19, approved April 1961; Method 46-11A, approved October 1976.

Bushuk, W. Protein−lipid and protein-carbohydrate interactions in flour−water mixtures. In *Chemistry and Physics of Baking*; Blanshard, J. M. V., Frazier, J. P., Galliard, T., Eds; The Royal Society of Chemistry: London, 1986; Chapter 11.

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 breadmaking quality. *Cereal Chem.* **1993**, *70*, 324−329.

Cleemput, G.; van Oort, M.; Hessing, M.; Bergmans, M. E. F.; Gruppen, H.; Grobet, P. J.; Delcour, J. A. Variation in the degree of D-xylose substitution in arabinoxylans extracted from a European wheat flour. *J. Cereal Sci.* **1995**, *22*, 73−84.

Cleemput, G. Characterisation of water-extractable nonstarch polysaccharides and non-starch polysaccharide hydrolysing enzymes in European wheat flours. Ph.D. Dissertation, Leuven, Belgium, 1996.

D'Appolonia, B. L. and Gilles, K. A. Pentosans associated with gluten. *Cereal Chem.* **1971**, *48*, 427−436.

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.

Fincher, G. B.; Stone, B. A. A water-soluble arabinogalactan-peptide from wheat endosperm. *Aust. J. Biol. Sci.* **1974**, *27*, 117−132.

Hoffmann, R. A.; Geijtenbeek, T.; Kamerling, J. P.; Vliegenthart, J. F. G. ^{1}H-NMR study of enzymatically generated wheat-endosperm arabinoxylan oligosaccharides: Structures of hepta- to tetradecasaccharides containing two or three branched xylose residues. *Carbohydr. Res.* **1992a**, *223*, 19−44.

Hoffmann, R. A.; Kamerling, J. P.; Vliegenthart, J. F. G. Structural features of a water-soluble arabinoxylan from the endosperm of wheat. *Carbohydr. Res.* **1992b**, *226*, 303−311.

Izydorczyk, M.; Biliaderis, C. G.; Bushuk, W. Comparison of the structure and composition of water-soluble pentosans from different wheat varieties. *Cereal Chem.* **1991**, *68*, 139−144.

Loosveld, A.-M. A.; Grobet, P.; Delcour, J. A. Contents and structural features of water-extractable arabinogalactan in wheat flour fractions. *J. Agric. Food Chem.* **1997**, *45*, 1998−2002.

Loosveld, A.-M. A.; Maes, C.; Grobet, P. J.; Delcour, J. A. Structural variation and levels of water-extractable arabinogalactan-peptide in European wheat flours. *Cereal Chem.* **1998**, submitted for publication.

MacRitchie, F. Role of lipids in baking. In *Lipids in Cereal Technology*; Barnes, P. J., Ed.; Academic Press Inc: London, 1983; Chapter 8.

McKenzie, H. A.; Dawson R. M. C. pH, buffers and physiological media. In *Data for Biochemical Research*; Dawson, R. M. C., Elliott, D. C., Elliott, W. H., Jones, K. M., Eds.; Clarendon Press: Oxford, 1966; Chapter 20.

McMaster, G. J. and Bushuk, W. Protein−carbohydrate complexes in gluten: Fractionation and proximate composition. *J. Cereal Sci.* **1983**, *1*, 171−184.

Morrison, W. R. Lipids. In *Wheat Chemistry and Technology*; Pomeranz, Y., Ed.; AACC: St. Paul, MN, 1988; Chapter 7.

Neukom, H.; Markwalder, H. Isolation and characterization of an arabinogalactan from wheat flour. *Carbohydr. Res.* **1975**, *39*, 387−389.

Olcott, H. S.; Mecham, D. K. Characterization of wheat gluten. I. Protein−lipid complex formation during doughing of flours. Lipoprotein nature of the glutenin fraction. *Cereal Chem.* **1947**, *24*, 407−414.

Roels, S. P.; Cleemput, G.; Vandewalle, X.; Nys, M.; Delcour, J. A. D. The bread volume potential of variable quality flours of constant protein level is determined by factors governing the mixing time and baking absorption levels. *Cereal Chem.* **1993**, *70*, 318−323.

Roels, S. P. Factors governing wheat and wheat gluten functionality in breadmaking and gluten/starch separation. Ph.D. Dissertation, Katholieke Universiteit Leuven, Belgium, 1997.

Roels, S. P.; Sindic, M.; Deroanne, C.; Delcour, J. A. Protein composition and agglomeration tendency of gluten isolated from European wheats (*Triticum aestivum* L.) in a batter system. *J. Agric. Food Chem.* **1998**, *46*, 000−000.

Saulnier, L.; Andersson, R.; Åman, P. A study of the polysaccharide components in gluten. *J. Cereal Sci.* **1997**, *25*, 121−127.

Veraverbeke, W. Samenstelling en functionaliteit van vitale gluten. Thesis, Katholieke Universiteit Leuven, Belgium, 1995.

Wadhawan, C. K. Fundamental studies on vitality of gluten for breadmaking. Ph.D. Dissertation, Manitoba, Winnipeg, Canada, 1988.

Weegels, P. L.; Marseille, J. P.; Voorpostel, A. M. B. Enzymes as a processing aid in the separation of wheat into starch and gluten. In *Gluten Proteins*; Bushuk, W., Tkachuk, R., Eds.; AACC: St. Paul, MN, 1991; pp 199−203.

Weegels, P. L.; Marseille, J. P.; Hamer, R. J. Enzymes as a processing aid in the separation of wheat flour into starch and gluten. *Starch* **1992**, *44*, 44−48.

Westerlund, E.; Andersson, R.; Åman, P.; Theander, O. Effects of baking on water-soluble polysaccharides in white bread fractions. *J. Cereal Sci.* **1990**, *12*, 33−42.

Zawistowska, U.; Békés, F.; Bushuk, W. Involvement of carbohydrates and lipids in aggregation of glutenin proteins. *Cereal Chem.* **1985**, *62*, 340−345.

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