Physicochemical and Bread-Making Properties of Low Molecular Weight Wheat-Derived Arabinoxylans

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To gain understanding on the functional role of arabinoxylans in bread-making, low molecular weight arabinoxylan (LMW-AX), isolated from a commercial wheat-derived product, and high molecular weight arabinoxylan (HMW-AX), isolated from wheat flour as such, were thoroughly characterized and tested for their impact on dough and bread-making in two European wheat flours. Dough development characteristics, as assessed by mixography, were affected considerably by the addition of HMW-AX and, to a lesser extent, by LMW-AX. Small-scale bread-making tests showed that neither water-holding capacity nor oxidative gelation or viscosity was the main factor governing LMW-AX functionality in bread-making. These findings imply that other mechanisms dictate the impact of LMW-AX and probably also of HMW-AX in bread-making.

Keywords: *Wheat; arabinoxylan; low molecular weight; bread-making*

INTRODUCTION

Over the past two decades, a lot of effort has been devoted to the wheat and rye nonstarch polysaccharides arabinoxylan (AX) and arabinogalactan peptide (AGP). Interest in these flour components was generated by early investigations reporting the possible functional role of these biopolymers in bread-making (Sandstedt et al., 1939; Pence et al., 1950; Cawley, 1964). In this context, AX has received most of the attention.

The structure of both water extractable and water unextractable AX (WE-AX and WU-AX, respectively) is an extended xylan backbone chain, with variable arabinose substitutions on the xylose O-2 and/or O-3 position(s). The arabinose-to-xylose ratio (A/X ratio) is a direct measure for the degree of substitution and is therefore an important structural characteristic. Vinkx et al. (1993, 1995) reported A/X ratios as low as 0.21 and as high as 1.43 for rye WE-AX and WU-AX, respectively.

AX has a large water-holding capacity, imparts viscosity to water, and has the ability to gel when oxidant is added (Baker et al., 1943; Cawley, 1964; Jelaca and Hlynca, 1971; Izydorczyk and Biliaderis, 1992).

Published results concerning the impact of AX on bread quality and dough characteristics are far from coherent and conclude to negative (Roels et al., 1993), neutral (D'Appolonia et al., 1970), or impressive positive (Casier et al., 1973; Michniewics et al., 1992) effects. A lot of these differences may well be caused by different starting materials and isolation techniques for the AX, different bread-making recipes and procedures, and also impurities. The past few years, though, a consensus, acknowledging the importance of AX, has grown. A recent tool that contributes to that consensus is the use of purified microbial endoxylanases, which cut the xylan backbone to a variable extent. The fact that some of these endoxylanases are widely used in bread improver mixtures strongly points to the influence of AX in bread-making.

Despite the large number of publications on the relationship between WE-AX and bread-making, elaborate views on how they would influence bread-making are scarcely found. On the basis of results obtained through reconstitution experiments, Cawley (1964) concluded that the *viscosity* conferred by water extractable pentosans on dough was responsible for the positive functional effect of water solubles on bread-making, as several other types of plant gums gave results similar to those noted with pentosans. A second line of thinking is based on the strong *water-holding capacity* of AX in bread recipes, as exemplified by increased baking absorption and changed dough characteristics (Pence et al., 1950, 1951; Meuser and Suckow, 1986; Michniewics et al., 1991, 1992). This, together with the finding of Roels et al. (1993) that flour baking absorption is positively correlated with bread volume, tempts one to conclude that adding AX gives rise to volume increase by increasing baking absorption. Third, *gelling* of AX under the influence of oxidants affects both viscosity and water-holding capacity (Neukom and Markwalder, 1978) and could lead to or fortify a gas-holding network in dough. A fourth hypothesis suggests that *interaction phenomena* between AX, be it endogenous or added, and protein are responsible for functional effects observed. Gluten AX interactions are indeed documented (Udy, 1957; Hoseney et al., 1969; Michniewics et al., 1991; Roels et al., 1998), but whether they are evaluated as positive or negative for bread-making quality depends on the experimental setup (Jelaca and Hlynca, 1972; Roels et al., 1993).

The purpose of this study was to increase understanding on how WE-AX might influence bread-making by comparing structural, physicochemical, and bread-making properties of low molecular weight AX (LMW-AX), isolated from a commercial wheat-derived product, and

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high molecular weight AX (HMW-AX), isolated from wheat flour.

MATERIALS AND METHODS

Chemicals. Wheat pentosan concentrate (WPC), a byproduct of the industrial wheat starch−gluten separation process, was obtained from Pfeifer & Langen (Dormagen, Germany). All reagents were of at least analytical grade. Specialty chemicals were α-amylase solution (type XII-A, from *Bacillus licheniformis*, A3403, Sigma Chemical Co., St. Louis, MO), horseradish peroxidase (P, Sigma), β-D-allose (Sigma), amyloglucosidase (Boehringer Mannheim, Mannheim, Germany), and ferulic acid (Fluka AG, Basel, Switzerland). Standard P-82 pullulans were purchased from Showa Denko K.K., Tokyo, Japan. Deuterium oxide (D_2O) was obtained from Acros Chemica (Geel, Belgium). Clay (montmorillonite K10) was purchased from Aldrich (Bornem, Belgium).

Flours. Wheats (Soissons and Estica, harvest 1996) were obtained from AVEVE (Landen, Belgium). Conditioning was to 14% moisture for 24 h at room temperature. Additional water was added 30 min prior to milling to reach a final moisture content of 14.5%. The samples were experimentally milled on a Buhler MLU-202 laboratory mill (Uzwill, Switzerland) according to AACC Method 26-31. Milling yield, protein content [percent dry matter (dm), AACC Method 46-13] and ash content (percent dm, AACC Method 08-01), were 69.4 and 73.0%, 10.3 and 11.5%, and 0.50 and 0.64% for Soissons and Estica, respectively.

Chemical Composition of Native and Isolated Samples. Monosaccharide composition of WPC, flours, flour water extractable material, and isolated AX was estimated by gas−liquid chromatography as described by Loosveld et al. (1997) with, for calculation of AX present, correction for the presence of AGP based on an arabinose-to-galactose ratio of 0.7 (Loosveld et al., 1997). AX content was then defined as 0.88 times the sum of monosaccharide xylose and corrected arabinose. Ferulic acid was estimated as described by Delcour et al. (1989). A micro-Kjeldahl procedure according to AACC Method 46-13 (N × 5.7) was used for protein assessment. Moisture contents were determined according to AACC Method 44-15A.

Isolation and Purification of WE-AX from Wheat Flour. The isolation was based on the method of Loosveld et al. (1997) with Estica flour. Instead of subsequent drying of the AX precipitate with ethanol and acetone washings, it was redissolved in deionized water and lyophilized. The obtained material, further referred to as EST-AX, was ground and sieved through a 250 μm sieve.

Purification of WE-AX from WPC. WPC (50.0 g) was solubilized in deionized water (1:10 w/v). Clay was added to this solution as a watery suspension (20% w/v) to obtain a clay/protein ratio of 15:1. The pH of the mixture was immediately adjusted to 3.5 with NaOH (0.5 M) to obtain a maximal adsorption of protein to the clay and to prevent acid hydrolysis from taking place. The mixture was stirred for 30 min and centrifuged (10000*g*, 30 min, 15 °C). The residue, which consisted of the clay/protein complex, was discarded. The WE-AX in the supernatant was precipitated under stirring by slow and continuous addition of ethanol (95%) to a final concentration of 60%. After ethanol addition, the mixture was stirred for 30 min and kept overnight at 4 °C. Following centrifugation (10000*g*, 30 min, 4 °C), the residue, consisting of WE-AX, was dissolved in water (250 mL) and lyophilized. The obtained material, labeled WPC-AX, was ground and sieved through a 250 μm sieve.

Fractionation of WPC. For structural characterization, WPC (5.0 g) was solubilized in deionized water (1:10 w/v). Clay treatment was as described above. Fractionation was then carried out by stepwise increase of the ethanol concentration. To minimize concentration effects from influencing the precipitation behavior, each fraction was precipitated from the original clay-treated supernatant, instead of being consecutively precipitated from the previous supernatant. The first fraction was defined as the fraction that precipitated at an ethanol concentration of 60% and is referred to as F_{60}. The second fraction was obtained by using an ethanol concentration of 65%, redissolving the precipitate in 50 mL of water, and bringing the medium to 60% ethanol. The precipitate was removed as above, and the remaining material was precipitated by adding excess ethanol. The fraction is designated F_{60-65}. The same procedure was followed for the third fraction (F_{65-80}). The last fraction (F_{80}) was recovered from the supernatant of fraction 3 by rotary evaporation. Figure 1 outlines the fractionation procedure. The chemical compositions of all four fractions were determined.

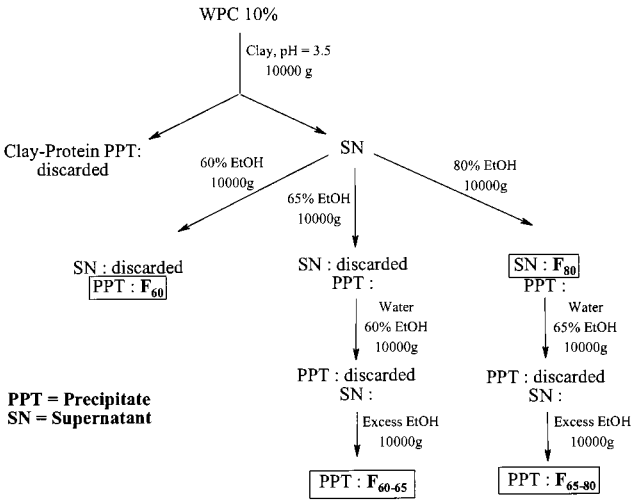

Figure 1. Outline of the fractionation procedure on WPC: F_{60}, AX fraction that precipitates at 60% EtOH; F_{60-65}, AX fraction that precipitates between 60 and 65% EtOH; F_{65-80}, AX fraction that precipitates between 65 and 80% EtOH; F_{80}, AX fraction that does not precipitate at 80% EtOH.

Gel Permeation Chromatography (GPC). Aliquots of WPC and isolated samples (1.0 mg) were solubilized in 0.3% NaCl and centrifuged (10000*g*, 10 min). Solutions obtained (20 μL) were separated on a Shodex SB-804 HQ GPC column by elution with 0.3% NaCl (0.5 mL/min at 30 °C) and monitored with a refractive index detector. Molecular weight markers were Shodex standard P-82 pullulans (1.0 mg/mL) with molecular weights of 78.8×10^4, 40.4×10^4, 21.2×10^4, 11.2×10^4, 4.73×10^4, 2.28×10^4, 1.18×10^4, and 0.59×10^4 and glucose.

^{1}H Nuclear Magnetic Resonance Spectroscopy. ^{1}H NMR spectra were recorded with a Bruker 300 MHz Fourier transform spectrometer (Karlsruhe, Germany) at 85 °C. Samples were dissolved in D_2O, stirred for 120 min, and lyophilized. This step was repeated and the resulting deuterium-exchanged dry material was finally dissolved in D_2O (1.0 mg/mL). Pulse repetition time was 2 s, and, in a typical experiment, the number of scans was 5000.

Viscosity and Gelling Capacity. Viscosity of 1.0% solutions was measured with an Ostwald type viscometer at 30 °C. To 5.0 mL of sample solution was added 0.3 mL of deionized water in the viscometer. After mixing, measurements were carried out. Gelling capacity was then assessed as follows: to 5.0 mL of sample solution were added 0.1 mL of H_2O_2 solution (0.39 g/mL), 0.1 mL of horseradish peroxidase solution (0.2 purpurogallin unit), and 0.1 mL of deionized water in the viscometer. After mixing, the solution was allowed to stand for 30 min before measurements were started.

Mixograph Measurements. Soissons and Estica flours were used for the mixograph and bread-making tests. On the basis of the 500 BU farinograph absorption value found, mixograms (National Manufacturing, Lincoln, NE) were recorded to determine optimal mixing requirement. Mixograms of flours with WPC, WPC-AX, and EST-AX (3.0% substitution level) were taken at baking absorption levels as defined in the bread-making test.

Bread-Making. Wheat loaves (10 g) were produced in triplicate using the procedure described by Finney (1984).

Table 1. Monosaccharide Composition (Percent), Protein Content (Percent), Ferulic Acid Content (Percent), Corrected Arabinose to Xylose Ratios (w/w), and Percentage of Un-, Mono-, and Disubstituted Xylose of Native WPC, of Estica and Soissons Flour, and of the AX Isolated from These Substrates

sample	Ara	Xyl	Gal	Glc	protein	AG[a]	AX	A/X	X_0	X_1	X_2	FA
					(A) WPC							
WPC	27.47	36.01	9.68	7.14	29.47	14.53	49.89	0.57	65.0	13.4	21.6	
WPC-AX	41.20	59.41	2.49	1.85	1.51	3.79	87.00	0.66	60.7	12.1	27.1	0.08
					(B) Estica							
Estica flour	0.95	1.47	0.40	75.21	11.54	nr	nr	nr	nr	nr	nr	
EST-AX	34.52	67.12	1.45	1.68	3.55	2.20	88.55	0.50	67.5	15.1	17.4	0.18
					(C) Soissons							
Soissons flour	0.70	0.88	0.38	68.96	10.30	nr	nr	nr	nr	nr	nr	

[a] AG, 0.89 × [(%Gal × 0.7) + %Gal]; AX, 0.88 × [(%Ara − 0.7 × %Gal) + %Xyl]; A/X, (%Ara − 0.7 × %Gal)/%Xyl.

Doughs were mixed with a 10-g pin mixer (National Manufacturing). Ingredients, other than flour, yeast, and water, were sugar (sucrose, 6.0%) and salt (1.5%). When the bread formula was supplemented with isolated material (0−3.0% range), this was done by removing a weight fraction of flour equal to the weight fraction of material added (both 14.0% moisture base). When substitution was made, baking absorption was adjusted to a combination of optimum handling properties and loaf volume, which was determined in several preliminary baking trails by an experienced baker. Mixing time was kept constant. Fermentation with fresh yeast (0.53 g) was 90 min, final proof was 35 min, and baking was 13 min at 232 °C. Volume readings were as described by Vanhamel et al. (1991), whereby (as cited by the authors) three independent volume readings gave a reasonable power to discriminate between items that differ in volume by at least 1 cm^3.

RESULTS AND DISCUSSION

Chemical Composition of WPC, Estica Flour, and Soissons Flour. As can be seen in Table 1, WPC is indeed rich in AX and AGP, with a combined content of >60%. The remaining part of the sample consists mainly of water extractable protein material and, to a lesser extent, polymeric glucose. More detailed analysis of the monosaccharide composition shows that the A/X ratio, corrected for the presence of AGP, is only slightly above the range of A/X found in 10 wheat flour AX samples (0.49−0.55; Cleemput et al., 1997). Xylose substitution patterns, as measured with ^1H NMR spectroscopy, are in line with the values for wheat WE-AX published by Cleemput et al. (1997), with a somewhat high value for disubstituted xylose. The analytical results for total Soissons and Estica flours are also given in Table 1. WE-AX content is 0.31 and 0.44%, respectively.

Chemical Composition of the Different Isolated Fractions. Because of the composition of WPC, an isolation method was developed, using clay (Pence et al., 1950) and ethanol precipitation as major steps. The clay used is very specific in binding protein material and leaves the AX population virtually unchanged. The protein content of WPC is reduced by almost 90% by the one-step clay treatment (results not shown). The chemical compositions of WPC-AX and EST-AX are displayed in Table 1. Very similar purification results are obtained. The major difference in composition between these two isolates is the A/X ratio, which is 0.66 for WPC-AX and 0.50 for EST-AX. Compared to EST-AX, the isolation yield for WPC-AX was low though. Only 27.1% of all AX present in WPC could be recovered in the isolate (Table 2). To understand why the yield was this low, and to further characterize WPC, fractionation was carried out.

Fractionation of WPC. The fraction obtained at an ethanol concentration of 60% (F_{60}) contains mainly AX

Table 2. AX Content (Percent), A/X Ratio, AG Content (Percent), Protein Content (Percent), and Percentage of Total Yield of the Different Fractions Obtained from WPC by Graded EtOH Precipitation, Expressed on Sum of Analytes

fraction[a]	AX[b]	A/X	AG	protein	percentage of yield[c]
F_{60}	91.75	0.70	4.39	3.86	27.1
F_{60-65}	55.27	0.55	37.22	7.52	26.1
F_{65-80}	68.02	0.53	18.81	13.17	28.4
F_{80}	44.69	0.49	2.43	52.91	18.4

[a] F_{60}, fraction <60% EtOH; F_{60-65}, fraction >60−<65% EtOH; F_{65-80}, fraction >65−<80% EtOH; F_{80}, fraction <80%. [b] AX, 0.88 × [(%Ara − 0.7 × %Gal) + %Xyl]; A/X, (%Ara − 0.7 × %Gal)/%Xyl; AG, 0.89 × [(%Gal × 0.7) + %Gal]. [c] Yield calculated as AX present in fraction to AX present in starting material on same weight base.

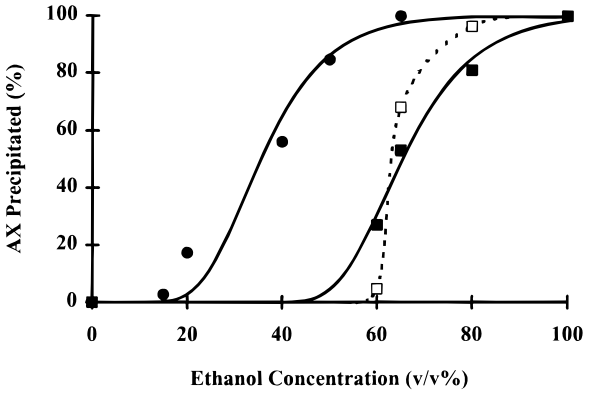

Figure 2. Cumulative precipitation plot of AX with increasing EtOH concentration for (a) AX from standard wheat flour (Cleemput et al., 1995; ●), (b) AX from WPC (■), and (c) AGP from WPC (□).

(Table 2). The yield for this fraction is very low and far from nearly complete precipitation, which takes place in regular flour extracts (Fincher and Stone, 1974). Fraction 2 (F_{60-65}) contains the largest part of AGP and a yield of AX comparable to that of fraction 1. Fraction 3 (F_{65-80}) contains surprisingly more AX and less AGP than fraction 2. The same holds true for the last fraction (F_{80}), in which both are present to a lesser extent. When these data are compared to the results of Fincher and Stone (1974) and Cleemput et al. (1995) for WE-AX isolated from wheat flour, it is clear that the ethanol precipitation curve (Figure 2a) has shifted significantly (Figure 2b) and overlaps with the ethanol precipitation curve for AGP (Figure 2c), the precipitation range of which corresponds well to the one published by Fincher and Stone. A second difference concerns the A/X ratios. Cleemput et al. (1995) found A/X ratios rising from 0.36 to 0.82 in a range increasing from 15

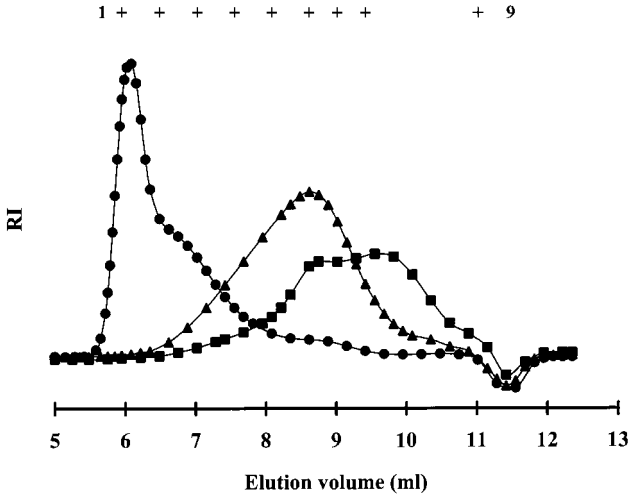

Figure 3. Gel permeation profile of (a) WPC (■), (b) WPC-AX (▲), and (c) EST-AX (●). Elution volumes of pullulan standards of molecular weight (1) 78.8×10^4, (2) 40.4×10^4, (3) 21.2×10^4, (4) 11.2×10^4, (5) 4.73×10^4, (6) 2.28×10^4, (7) 1.18×10^4, (8) 0.59×10^4, and (9) glucose are indicated.

Table 3. Physicochemical Properties of WPC and of AX Isolated from WPC and Estica Wheat Flour

	WPC	WPC-AX	EST-AX
$MW_{min,app}$	150	150	3000
$MW_{peak,app}$	5000−17000	20000	770000
$MW_{max,app}$	580000	>788000	>788000
$MW_{na,app}$	2100	10700	316300
$MW_{wa,app}$	18500	50300	555300
η_{sp}	0.03	0.13	1.41
η_{gel}	0.03	0.13	2.43

to 65% ethanol. Similar results for ammonium sulfate precipitation are reported by Izydorczyk and Biliaderis (1992). For WPC, the order is reversed as the values drop from 0.70 for F_{60} to 0.49 for F_{80}. These results will be further discussed below.

GPC. The profile of WPC (Figure 3a) shows that the AX present is of low apparent molecular weight (MW_{app}) with peak values ranging from 17000 to 5000. In comparison with EST-AX (Figure 3c; Table 3) this means a minimum 45-fold decrease in peak MW_{app}. The profile for WPC-AX (Figure 3b) indicates that the isolation procedure removes a large part of the smallest molecules out of the WPC sample. MW_{app} is raised to a peak value of 20000. On the basis of the gel permeation profiles, apparent weight-average MW ($MW_{wa,app}$) and apparent number-average MW ($MW_{na,app}$) were calculated according to the formulas (Flory, 1953)

$$MW_{wa,app} = \sum_{i=a}^{b} C_i(MW_i) / \sum_{i=a}^{b} C_i$$

$$MW_{na,app} = \sum_{i=a}^{b} C_i / \sum_{i=a}^{b} C_i / MW_i$$

where i is the time index (in seconds), C_i is the ratio of the amount of monomers eluting at time i to the total amount of monomers, and MW_i is the molecular weight corresponding to time i. Results are presented in Table 3. More than a peak value, $MW_{wa,app}$ allows one to assess the real impact of a MW distribution of polymers on their physical properties. It is clear from Table 3 that removal of proteins and the smallest polysaccharide molecules from WPC increases its $MW_{wa,app}$ almost 3-fold. The difference in $MW_{wa,app}$ between WPC-AX and EST-AX is >1 order of magnitude. For WPC, WPC-AX, and EST-AX the ratio of $MW_{na,app}$ over $MW_{wa,app}$ ranges from 0.11 over 0.21 to 0.56, respectively, and indicates an increasing level of monodispersity (1.0 represents a monodisperse product and 0 indicates infinite polydispersity). These characteristics clearly justify WPC-AX to be considered LMW-AX and EST-AX HMW-AX.

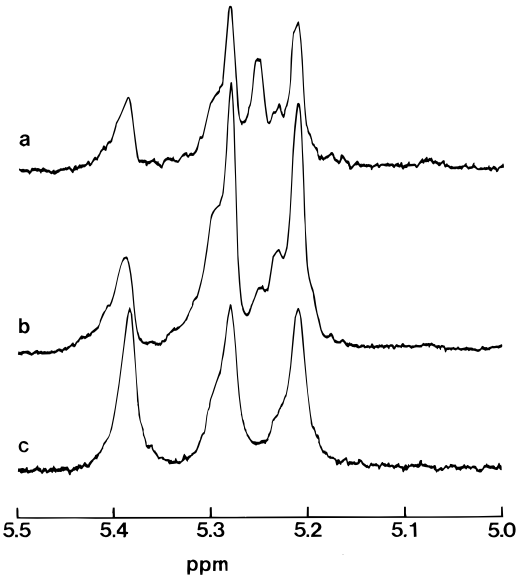

Figure 4. ^{1}H NMR spectra of (a) WPC, (b) WPC-AX, and (c) EST-AX: δ 5.39, anomeric protons of arabinose monosubstituted on xylose; δ 5.22 and 5.24, anomeric protons of arabinose disubstituted on xylose.

The rationale for these strong differences most probably can be found in the origin of WPC, being a byproduct of gluten−starch separation. Streams of this kind can easily be contaminated with bacteria, as demonstrated by Yin and Walker (1992). Enzymic degradation, whether by microbial growth, potentially used processing enzymes, or endogenous enzymes, is therefore the most plausible explanation for the phenomena observed. Further evidence for enzymic degradation stems from the fact that the observed decrease of the A/X ratio with increase of the ethanol concentration of the precipitating medium is opposite to results published by Fincher and Stone (1974) and Cleemput et al. (1995) for unmodified wheat water extractable AX. Indeed, many endoxylanases hydrolyze less substituted AX more readily than more substituted AX (Coughlan and Hazelwood, 1993). The broader precipitation range observed above is then due to smaller AX molecules, the higher A/X ratios due to the fact that more substituted AX, which are less susceptible to attack, are of higher average molecular weight and for that reason precipitate in the 60% ethanol fraction. The fact that there is still AX that precipitates at ethanol concentrations >80% indicates that the hydrolysis has progressed significantly. As WPC is a spray-dried product, assessment of endoxylanase activity present in WPC could not support or reject this hypothesis. Not unexpectedly, virtually no endoxylanase activity could be found in WPC (data not presented).

^{1}H Nuclear Magnetic Resonance Spectroscopy. Figure 4 shows the spectral data for WPC, WPC-AX, and EST-AX. The resonance at δ 5.39 arises from the chemical shift of anomeric protons of arabinose mono-

Table 4. Effect of Addition of WPC, WPC-AX, and EST-AX on Baking Absorption (Percent) and Loaf Volume (Percent) for the Wheat Flours Soissons and Estica[a]

addition level (%)	WPC		WPC-AX		EST-AX	
	BA[b]	volume	BA	volume	BA	volume
			(A) Soissons			
0	55.8	100.0 ± 1.5 a	55.8	100.0 ± 1.5 a	55.8	100.0 ± 1.5 a
1	55.3	107.1 ± 1.1 b	55.3	104.5 ± 2.0 b	57.3	110.4 ± 1.5 b
2	54.8	112.2 ± 2.9 c	54.8	107.7 ± 1.9 bc	59.3	117.8 ± 1.0 c
3	53.8	116.4 ± 2.6 c	54.8	111.5 ± 1.4 c	60.8	117.4 ± 1.8 c
			(B) Estica			
0	58.0	100.0 ± 2.1 a	58.0	100.0 ± 2.1 a	58.0	100.0 ± 2.1 a
1	57.0	100.9 ± 0.9 a	57.5	104.1 ± 1.0 b	59.0	103.6 ± 2.3 a
2	56.0	100.3 ± 2.0 a	57.0	107.8 ± 0.6 c	60.5	108.3 ± 1.1 b
3	55.0	99.5 ± 1.3 a	56.5	104.5 ± 0.7 b	62.0	113.9 ± 1.0 c

[a] Volume is presented as the mean ± SD ($n = 3$). Means within the same column and for the same flour not followed by a common letter differ significantly ($P < 0.05$). Volume is expressed as percentage of the reference volume (addition level 0%), which is set to 100%. Reference volumes for Soissons and Estica are 52 cm^3 and 49 cm^3, respectively.

substituted on xylose (Hoffmann et al., 1992). Peaks at δ 5.22 and 5.24 show the presence of disubstituted xylose, with the shoulder on both peaks indicating the occurrence of two disubstituted xylose moieties next to each other. Table 1 shows that xylose disubstitution (X_2) rises from 17.4% for EST-AX over 21.6% for WPC to 27.1% for WPC-AX. Unsubstituted xylose levels (X_0) drop in the same order from 67.5 to 60.7%. Comparison of these results with those published by Cleemput et al. (1995) shows that the values observed for WPC and for WPC-AX, especially, deviate significantly from the averages found by Cleemput et al. (1995) for water extractable arabinoxylan from wheat. They can be interpreted as further indication for the hypothesis elaborated above.

Viscosity and Gelling Capacity. As could be expected, the viscosity values vary for the different isolated samples (Table 3). EST-AX has the highest viscosity, which correlates well with the high $MW_{wa,app}$ found for this AX isolate. The viscosity of WPC nearly equals that of water.

WPC and WPC-AX do not gel. Although the ferulic acid content of WPC-AX was less than half that of EST-AX, the nongelling must probably be ascribed to their low molecular weights. The AX isolation procedure according to Loosveld et al. (1997) preserved the capacity of EST-AX to gel.

Mixograph Measurements. Soissons and Estica flours were selected on the basis of their suitability for bread-making and their different characteristics as determined by mixograph (Figure 5, S1 and E1) and farinograph (results not shown) measurements. These indicate that Soissons flour is stronger than Estica flour when dough-forming is evaluated. Considering that the Payne Glu-1 scores (Payne et al., 1987) are 10 and 3 for Soissons and Estica, respectively, the gluten network must be an important factor in this respect.

Baking absorption levels used for the mixograph measurements on the native flours and the flours with WPC, WPC-AX, and EST-AX substitution can be found in Table 4. Manual determination of baking absorption for the latter were chosen over assessment of baking absorption with the farinograph for several reasons. First of all, it can be questioned whether the 500 BU baking absorption is well-defined for flours complemented with AX, which can have a strong influence on water distribution in a dough. Second, the farinograph cannot replace a baker's evaluation of dough characteristics such as stickiness, which are important for real-life baking conditions. Finally, the technique is not unprecedented (Roels et al., 1993).

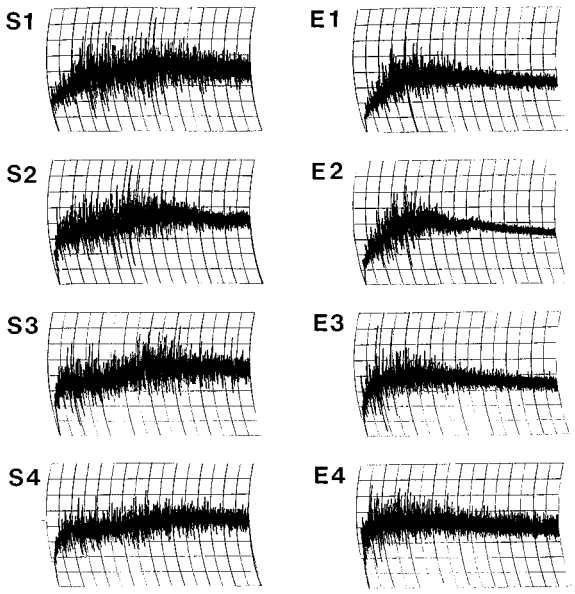

Figure 5. Mixograms at 14% mb and optimal baking absorption of (S1) Soissons, (S2) Soissons + 3% WPC, (S3) Soissons + 3% WPC-AX, (S4) Soissons + 3% EST-AX, (E1) Estica, (E2) Estica + 3% WPC, (E3) Estica + 3% WPC-AX, and (E4) Estica + 3% EST-AX.

As can be seen, the baking absorption for WPC and WPC-AX substitution is somewhat lower than for the reference (indicated as "0% addition level"). Substitution with 3.0% EST-AX increases baking absorption considerably. Mixograms are displayed in Figure 5 (S1 to S4 and E1 to E4). The times to peak were 270 and 150 s for Soissons and Estica, respectively (S1 and E1); the corresponding values in the case of substitution with 3% WPC were 210 and 135 s (S2 and E2). Both flours show considerably less resistance to overmixing when WPC is brought in. When 3.0% WPC-AX is used (S3 and E3), initial development of the dough is quickened and for Estica peak time decreases (120 s). Soissons does not show a clear shift of time to peak (255 s) but demonstrates a narrowing and flattening of the profile, which is even more pronounced in the EST-AX−Soissons combination (S4). Substitution of 3.0% EST-AX in Estica (E4) increases initial development even more strongly than for WPC-AX and diminishes peak time to 100 s. The profile itself gets a little broader and the dough more resistant to overmixing.

When the profiles of WPC substitution are compared to those of WPC-AX and EST-AX, it can be assumed that WPC-induced characteristics are a combined effect of the three major fractions present in WPC (AX, protein, and AGP). Decreased resistance to overmixing is caused by the protein fraction as removal of this fraction from WPC by clay treatment re-establishes the resistance for both Estica and Soissons (result not shown). The observed decrease of time to peak for Estica upon substitution with AX is in agreement with the results found by Pence et al. (1951) and Yin and Walker (1992) and in contrast to those of Jelaca and Hlynca (1971), Vanhamel et al. (1993), and Biliaderis et al. (1995) for HMW-AX. A tentative explanation for the faster development of the dough upon substitution with WPC-AX and, even more, EST-AX might be the increase in viscosity of the liquid phase caused by the arabinoxylans. When the viscosity is increased, the lubricating effect of the water diminishes and dough particles more strongly interact, which forces the dough to develop more rapidly. As EST-AX imparts higher viscosity than WPC-AX, the effect could be more pronounced for the former. Yin and Walker (1992) suggested that faster dough development on addition of AX might be attributed to weak secondary bonds forming between AX and gluten molecules.

Test Bread-Making. The results for Soissons (Table 4, control loaf, 52 cm^3) show that substitution with WPC can improve bread volume significantly (+16% for 3.0% substitution), although baking absorption is decreased at the same time. Because of its high protein content, bread-making with WPC does not allow firm conclusions on the functional role of AX present in the sample. Using WPC-AX, baking absorption was depressed little and volume increase was up to 12%. Loaf volume was highest when 2% EST-AX was introduced to the bread formula (18%). Baking absorption increase here was also substantial (9%). Apart from loaf volume, other bread characteristics were altered only in a minor way. Statistical calculations indicate that, for all three samples, addition level is strongly correlated with loaf volume response ($R^2 = 0.91$, 0.91, and 0.84 for WPC, WPC-AX, and EST-AX addition, respectively) and regression coefficients correspondingly differ significantly from zero. Comparison of WPC-AX and EST-AX shows significantly ($P < 0.05$) different slopes for regression. Except for WPC substitution, for which no volume effects were found, the observations and results found for Estica flour (control loaf, 49 cm^3) were similar to those obtained for Soissons flour. Loaf volume increases for WPC-AX substitution were identical for both flours, except for the 3.0% level. Correlations between substitution level and loaf volume found were $R^2 = 0.01$, 0.54, and 0.95 for WPC, WPC-AX, and EST-AX, respectively. Regression coefficients were significantly different from zero ($P < 0.05$) for WPC-AX and EST-AX.

To explain the different effects of WPC in Soissons and Estica bread-making, one might hypothesize that the strong gluten network of Soissons [as indicated by Figure 5 (S1) and a Glu-1 score of 10] is weakened by the addition of protein present in WPC and thus became more extensible. In Estica, which has an average strength gluten network [Figure 5 (E1) and a Glu-1 score of 3], the effect of protein and AX might have canceled each other. The effects of WPC-AX on bread-making were rather unexpected. On the basis of all the data given in the literature, it was expected to find little or no volume increasing effects with a sample that is hydrolyzed to such extent. Experiments by Cawley (1964), McCleary (1986), and Rouau et al. (1994), although on endogenous AX, would even suggest that strongly hydrolyzed AX might impart negative effects on dough and bread characteristics. The results for the high molecular weight EST-AX substitution are in line with those found by Jelaca and Hlynca (1972), Casier et al. (1973), Michniewics et al. (1992), and Biliaderis et al. (1995).

The results found in these experiments indicate that the hypotheses proposed in the literature until now are not sufficient to explain the functional properties shown by LMW-AX during bread-making. High water-holding capacity resulting in higher *baking absorption* for AX-enriched flours (Meuser and Suckow, 1986; Michniewics et al., 1991; Biliaderis et al., 1995) cannot be the determining factor for LMW-AX functionality, because, even at baking absorption levels lower than that for the control, loaf volume increase is observed. In contrast to the ideas of some authors (Hoseney, 1984; Meuser and Suckow, 1986), *gelling* does not explain the results found either, as gelling capacity is nonexistent for WPC-AX (Table 3). This finding is coherent with that of Delcour et al. (1991), who stated that removal of ferulic acid from rye AX did not impair the effect that the AX had on bread-making. Furthermore, the hypothesis by Roels et al. (1993) about hydrophilic hindrance of AX during gluten formation is hard to combine with the findings above. On the other hand, the results of Jelaca and Hlynca (1972) on positive *interaction phenomena* between AX and gluten could be an explanation for the observed effects. The results of Cawley (1964) and Rouau et al. (1994), pointing to *viscosity* increase as a reason for better baking results on gluten starch loaves and flour, respectively, are at first glance not consistent with results obtained here either. Indeed, even for LMW-AX with a specific viscosity 11 times lower than for HMW-AX, increase in loaf volume could be found. However, when the solubilizing behavior of the different AX samples was assessed, it seems that the rate at which the AX solubilizes is related to its degree of degradation (unpublished results). WPC solubilizes almost immediately, WPC-AX takes a few hours to go in solution, and EST-AX has to be hydrated overnight. It is possible that only part of the lesser or nondegraded AX becomes hydrated during mixing and fermentation. This would imply that the viscosity increase in the dough by AX substitution is smaller than would be expected and that the ratio of specific viscosity from EST-AX over WPC-AX obtained in water is altered in dough.

With the above in mind, one must equally consider that isolated and subsequently added AX might behave differently from AX naturally present in wheat flour. The above-mentioned views on AX functionality might explain the behavior of endogenous AX and might account for the effects of exogenous HMW-AX, but they fail to explain the above-illustrated functional role of LMW-AX in bread-making. If LMW-AX exerts functional effects by mechanisms other than those by which HMW-AX does, this also means that the endoxylanase bread-improving mechanism might involve more than just influencing water-holding capacity or viscosity. Further research will have to point out what other mechanisms govern the functionality of AX.

Conclusion. Results show that purified AX, when introduced in a lean bread formula, does increase loaf volume. This is true not only for HMW-AX isolated as such from flour, as also shown previously by other authors, but also for LMW-AX. The hypotheses that AX exerts its functional role by means of its macromolecular structure, gelling capacity, or influence on water distribution and water availability in dough are not able to explain the above findings. Viscosity increase could not be ruled out completely as a factor governing LMW-AX functionality.

ABBREVIATIONS USED

AG(P), arabinogalactan (peptides); Ara, arabinose; AX, arabinoxylans; A/X ratio, arabinose-to-xylose ratio; dm, dry matter; EST-AX, Estica flour arabinoxylan isolate; FA, ferulic acid; Gal, galactose; Glc, glucose; GPC, gel permeation chromatography; HMW-AX, high molecular weight arabinoxylans; ^{1}H NMR, proton nuclear magnetic resonance; LMW-AX, low molecular weight arabinoxylans; MW_{app}, apparent molecular weight; $MW_{max,app}$, maximum apparent molecular weight; $MW_{min,app}$, minimum apparent molecular weight; $MW_{peak,app}$, apparent molecular weight at peak value; $MW_{na,app}$, apparent number-average molecular weight; $MW_{wa,app}$, apparent weight-average molecular weight; nr, not relevant; WE-AX, water extractable arabinoxylans; WPC, wheat pentosan concentrate; WPC-AX, wheat pentosan concentrate arabinoxylan isolate; WU-AX, water unextractable arabinoxylans; X_0, X_1, and X_2, amount of, respectively, un-, mono-, and disubstituted xylose as pecentage of total amount of xylose; Xyl, xylose; η_{sp}, specific viscosity; η_{gel}, specific viscosity after treatment with peroxidase and O_2.

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LITERATURE CITED

American Association of Cereal Chemists. *Approved Methods of the AACC*, 8th ed.; The Association: St. Paul, MN, 1983.

Baker, J. C.; Parker, H. K.; Mize, M. D. The pentosans of wheat flour. *Cereal Chem.* **1943**, *20*, 267−280.

Biliaderis, C. G.; Izydorczyk, M. S.; Rattan, O. Effect of arabinoxylans on bread-making quality of wheat flours. *Food Chem.* **1995**, *5*, 165−171.

Casier, J. P. J.; De Paepe, G.; Brummer J.-M. Einfluss der wasserunlöslichen weizen- und roggen-pentosane auf die backeigenschaffen von weizenmehlen und andere rohstoffen (Influence of water unextractable wheat and rye pentosans on the baking characteristics of wheat flour and other raw materials). *Getreide, Mehl, Brot.* **1973**, *27*, 36−44.

Cawley, R. W. The role of wheat flour pentosans in baking. II Effect of added flour pentosans and other gums on gluten-starch loaves. *J. Sci. Food Agric.* **1964**, *15*, 834−838.

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.; Booij, C.; Hessing, M.; Gruppen, H.; Delcour, J. A. Solubilisation and changes in molecular weight distribution of arabinoxylan and protein in wheat flours during bread-making, and the effects of endogenous arabinoxylan hydrolyzing enzymes. *J. Cereal Sci.* **1997**, *26*, 55−66.

Coughlan, M. P.; Hazelwood, G. P. β-1,4-D-Xylan degrading enzyme system: biochemistry, molecular biology and applications. *Biotechnol. Appl. Biochem.* **1993**, *17*, 259−289.

D'Appolonia, B. L.; Gilles, K. A.; Medcalf, D. G. Effect of water soluble pentosans on gluten-starch loaves. *Cereal Chem.* **1970**, *47*, 194−204.

Delcour, J. A.; Vinkx, C. J. A.; Vanhamel, S.; Block, G. G. A. G. Combined monitoring of UV absorbance and fluorescence intensity as a diagnostic criterion in reversed-phase high-performance liquid chromatographic separations of natural phenolic acids. *J. Chromatogr.* **1989**, *467*, 149−157.

Delcour, J. A.; Vanhamel, S.; Hoseney, R. C. Physicochemical and functional properties of rye non-starch polysaccharides. II. Impact of a fraction containing water-soluble pentosans and proteins on gluten-starch loaf volumes. *Cereal Chem.* **1991**, *68* (1), 72−76.

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

Finney, K. F. An optimized, straight-dough, bread-making method after 44 years. *Cereal Chem.* **1984**, *61* (1), 20−27.

Flory, P. L. *Principles of Polymer Chemistry*; Cornell University Press: London, 1953; pp 273−292.

Geissmann, T.; Neukom, H. On the composition of the water-soluble wheat flour pentosans and their oxidative gelation. *Lebensm. Wiss. Technol.* **1973**, *6*, 54−62.

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

Hoseney, R. C. Functional properties of pentosans in baked foods. *Food Technol.* **1984**, *38*, 114−117.

Hoseney, R. C.; Finney, K. F.; Pomeranz, Y.; Shogren M. D. Functional (breadmaking) and biochemical properties of wheat flour components. II Role of water-solubles. *Cereal Chem.* **1969**, *46*, 117−125.

Izydorczyk, M. S.; Biliaderis, C. G. Influence of structure on the physicochemical properties of wheat arabinoxylan. *Carbohydr. Polym.* **1992**, *17*, 237−247.

Jelaca, S. L.; Hlynca, I. Water binding capacity of wheat flour crude pentosans and their relation to mixing characteristics of dough. *Cereal Chem.* **1971**, *48*, 211−222.

Jelaca, S. L.; Hlynca, I. Effect of wheat-flour pentosans in dough, gluten and bread. *Cereal Chem.* **1972**, *49*, 489−495.

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

Meuser, F.; Suckow, P. Non-starch polysaccharides. In *Chemistry and Physics of Baking*; Blanshard, J. M. V., Frasier P. J., Gaillard, T., Eds.; Royal Society of Chemistry: London, 1986; pp 42−61.

Michniewics, J.; Biliaderis, C. G.; Bushuk, W. Effect of added pentosans on some physical and technological characteristics of dough and gluten. *Cereal Chem.* **1991**, *68* (3), 252−258.

Michniewics, J.; Biliaderis, C. G.; Bushuk, W. Effect of added pentosans on some bread properties of wheat. *Food Chem.* **1992**, *43*, 251−257.

Neukom, H.; Markwalder, H. U. Oxidative gelation of wheat flour pentosans: a new way of cross-linking polymers. *Cereal Foods World* **1978**, *23*, 374−376.

Payne, P. I.; Nightingale, M. A.; Krattiger, A. F.; Holt, L. M. The relationship between HMW glutenin subunit composition and the bread-making quality of British-grown wheat varieties. *J. Sci. Food Agric.* **1987**, *40*, 51−65.

Pence, J. W.; Elder, A. H.; Mecham, D. K. Preparation of wheat flour pentosans for use in reconstituted doughs. *Cereal Chem.* **1950**, *27*, 60−66.

Pence, J. W.; Elder, A. H.; Mecham, D. K. Some effects of soluble flour components on baking behaviour. *Cereal Chem.* **1951**, *28*, 94−104.

Roels, S. P.; Cleemput, G.; Vandewalle, M.; Nys, M.; Delcour, J. A. Bread volume potential of variable-quality flours with constant protein level as determined by factors governing mixing time and baking absorption levels. *Cereal Chem.* **1993**, *70* (3), 318−323.

Roels, S. P.; Grobet, P. J.; Delcour, J. A. The distribution of carbohydrates in gluten fractions isolated from European wheats (*Triticum aestivum* L.) in a batter system. *J. Agric. Food Chem*. **1998**, *46*, 1334−1343.

Rouau, X.; El-Hayek, M.-L.; Moreau, D. Effect of an enzyme preparation containing pentosanases on the bread making quality of flours in relation to changes in pentosan properties. *J. Cereal Sci*. **1994**, *19*, 259−272.

Sandstedt, R. M.; Jolitz, C. E.; Blish, M. J. Starch in relation to some baking properties of flour. *Cereal Chem*. **1939**, *16*, 780−792.

Udy, D. C. Interactions between proteins and polysaccharides of wheat flour. *Cereal Chem*. **1957**, *34*, 37−46.

Vanhamel, S.; Van den Ende, L.; Darius, P.; Delcour, J. A. A volumeter for breads prepared from 10 g of flour. *Cereal Chem*. **1991**, *68*, 170−173.

Vanhamel, S.; Cleemput, G.; Delcour, J. A.; Nys, M.; Darius, P. L. Physicochemical and functional properties of rye nonstarch polysaccharides. IV. The effect of high molecular weight water soluble pentosans on wheat-bread quality in a straight-dough procedure. *Cereal Chem*. **1993**, *70*, 306−311.

Vinkx, C. J. A.; Reynaert, H. R.; Grobet, P. J.; Delcour, J. A. Physicochemical and functional properties of rye non starch polysaccharides. V. Variability in the structure of water-soluble Arabinoxylans. *Cereal Chem*. **1993**, *70* (3), 311−317.

Vinkx, C. J. A.; Stevens, I.; Gruppen, H.; Grobet, P. J.; Delcour, J. A. Physicochemical and functional properties of rye non starch polysaccharides. VI. Variability in the structure of water-unextractable arabinoxylans. *Cereal Chem*. **1995**, *72* (4), 411−418.

Yin, Y.; Walker, C. E. Pentosans from gluten-washing wastewater: isolation, characterisations, and role in baking. *Cereal Chem*. **1992**, *69* (6), 592−596.

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