Quantitative and Qualitative Study of Arabinogalactan-Peptide during Bread Making

Anne-Marie A. Loosveld,*,† Caroline Maes,† Piet J. Grobet,‡ and Jan A. Delcour†

Laboratory of Food Chemistry, Katholieke Universiteit Leuven, and Center for Surface Chemistry and Catalysis, Katholieke Universiteit Leuven, Kardinaal Mercierlaan 92, B-3001 Heverlee, Belgium

The quantitative and qualitative changes of water-extractable arabinogalactan-peptide (WE-AGP) during a straight dough wheat bread-making process in the flour from two wheat varieties Skirlou and Soissons were investigated. The extractability of WE-AGP remains constant or increases slightly as a result of the bread-making process (increases of 12% for Skirlou and 10% for Soissons). This contrasts with the great increase in extractability of the water-extractable arabinoxylan (WE-AX) (29% for Skirlou and 77% for Soissons of the initially present WE-AX). No changes in arabinose substitution of WE-AX (0.47−0.50 for Skirlou, 0.52−0.54 for Soissons) and WE-AGP (0.66−0.70 for Skirlou, 0.69−0.72 for Soissons) occurred during the bread-making process. In contrast to differences found in molecular weight distribution of WE-AX, no differences in molecular weight distribution of WE-AGP as a result of processing were observed. Apparent molecular weights of 2.4×10^4 and 2.2×10^4 were found for WE-AGP samples of flour and doughs at different stages of the bread-making process for Skirlou and Soissons, respectively. The ^{1}H NMR spectra (300 MHz, D_2O, 85 °C) of the WE-AGP isolated from flour, dough, and bread fraction were comparable. Taken together, the above data suggest that, unlike what can be observed in gluten agglomeration in dilute systems (Roels, S. P., et al. *J. Agric. Food Chem.* **1998**, *46*, 1334−1343), there is no specific interaction between WE-AGP and gluten proteins in a straight dough bread-making process.

Keywords: *Arabinogalactan-peptide; bread making; wheat flour; nonstarch polysaccharides*

INTRODUCTION

It has been known for a long time that nonstarch polysaccharides (NSPs) from wheat flour have an important impact on the bread-making process (Pence et al., 1950; Udy, 1956, 1957; Jelaca and Hlynka, 1972). Such NSPs consist of arabinoxylans (AXs) and water-extractable arabinogalactan-peptides (WE-AGPs).

A lot of research was done on the impact of AXs on dough manageability and bread quality (D'Appolonia et al., 1970; Casier et al., 1973; Michniewics et al., 1992; Roels et al., 1993). Studies investigating the effects of bread making on flour NSPs (D'Appolonia, 1973; Westerlund et al., 1989, 1990; Rouau, 1993; Rouau et al., 1994; Cleemput et al., 1997) were also carried out and focused on the AXs. Of importance in this context is that the presence of endogenous NSPs hydrolyzing enzymes in wheat flour was illustrated (Cleemput et al., 1995), that differences in molecular weight distribution as a result of processing were observed in gel permeation profiles of water-extractable arabinoxylans (WE-AXs) and that such differences in part could be ascribed to enzymic hydrolysis (Cleemput et al., 1997).

Loosveld et al. (1997) showed that, for some wheat varieties, the content of water-extractable arabinogalactan (WE-AG) is of the same magnitude as that of WE-AX.

Furthermore, Roels et al. (1998) for six wheat varieties recently documented the intervention of WE-AGP in wheat gluten agglomeration. In gluten fractions with the best agglomeration properties, the WE-AGP/WE-AX ratio was higher than what is normally observed in wheat water extracts (Roels et al., 1998).

Despite the above, which may imply that WE-AGPs have a functional role in bread making, data on the fate of WE-AGP during bread making are rather scarce. In this context, Westerlund et al. (1989, 1990) studied the WE-AGP and found that this NSP fraction became less extractable during the baking step (Westerlund et al., 1989) in a straight dough bread making process. Isolated WE-AGPs of dough, crumb, and inner and outer crust showed variation in the arabinose/galactose (*A*/*G*) ratio (0.55−0.67) (Westerlund et al., 1990).

To increase insight in the functional role of this flour minor constituent and its possible interaction with gluten proteins, we undertook a profound study of the water extractability of WE-AGP during the bread-making process. Structural changes in WE-AGP during the bread-making process were also investigated to study the potential presence and/or impact of endogenous WE-AGP hydrolyzing enzymes.

MATERIALS AND METHODS

Flours. Two wheat flours (Skirlou, Soissons; Belgian harvest 1996) (AVEVE, Landen, Belgium) were conditioned to a final moisture content of 14.5%. The samples were experimentally milled on a Bühler MLU-202 laboratory mill (Uzwil, Switzerland) according to AACC method 26-31 (American Association of Cereal Chemists, 1983). The milling yields were 70 and 72%, respectively. Ash (AACC method 08-01; American Association of Cereal Chemists, 1983) and protein content (N × 5.7) were 0.65 and 0.54% (dry basis) and 11.5 and 12.1% (dry basis) for Skirlou and Soissons, respectively.

* Author to whom correspondence should be addressed [telephone (+32) 16 32 14 84; fax (+32) 16 32 19 97; e-mail annemie.loosveld@agr.kuleuven.ac.be].

† Laboratory of Food Chemistry.

‡ Center for Surface Chemistry and Catalysis.

10.1021/jf9805350 CCC: $15.00
Published on Web 11/21/1998

Table 1. Arabinoxylan and Arabinogalactan Extracted from Flour and Doughs Isolated at Different Stages of the Bread-Making Process (Percent of Flour, Dry Basis)

	Skirlou			Soissons	
stage[c]	WE-AX[a] (stdev)	WE-AG[b] (stdev)	stage[c]	WE-AX[a] (stdev)	WE-AG[b] (stdev)
(0)	0.69 (0.02)	0.33 (0.02)	(0)	0.31 (0.01)	0.31 (0.01)
(1)	0.70 (0.01)	0.33 (0.01)	(1)	0.38 (0.01)	0.33 (0.01)
(2)	0.71 (0.02)	0.34 (0.02)	(2)	0.36 (0.01)	0.36 (0.02)
(3)	0.74 (0.02)	0.35 (0.02)	(3)	0.40 (0.01)	0.36 (0.02)
(4)	0.75 (0.02)	0.36 (0.02)	(4)	0.41 (0.03)	0.34 (0.03)
(5)	0.74 (0.01)	0.36 (0.01)	(5)	0.41 (0.01)	0.36 (0.01)
(6)	0.74 (0.01)	0.37 (0.01)	(6)	0.41 (0.01)	0.34 (0.01)
(7)	0.76 (0.02)	0.36 (0.01)	(7)	0.44 (0.02)	0.36 (0.02)
(8)	0.89 (0.01)	0.37 (0.01)	(8)	0.55 (0.01)	0.34 (0.02)

[a] WE-AX: $0.88 \times [(\%\ \text{Xyl} \times A/X) + \%\ \text{Xy}]$. [b] WE-AG: $0.89 \times \{[(\%\ \text{Ara} - (\%\ \text{Xyl} \times \text{A/X})] + [[(\%\ \text{Ara} - (\%\ \text{Xyl} \times \text{A/X})]/A/G]\}$. [c] (0) Flour, (1) end of kneading, (2) 30 min of fermentation, (3) 60 min of fermentation, (4) 105 min (first punch), (5) 155 min (second punch), (6) 180 min (molding), (7) 235 min (before baking), and (8) 259 min (end of the baking).

Optimal baking absorptions were deduced from farinograms recorded with a 50 g mixing bowl (Brabender, Duisberg, Germany) according to AACC-method 54-21 (American Association of Cereal Chemists, 1983) and were 56.9 and 55.1% for Skirlou and Soissons, respectively. Mixing times were recorded at the farinograph absorptions with a 10 g mixograph (National Mfg., Lincoln, NE) (AACC-method 54-40A; American Association of Cereal Chemists, 1983) and were 80 and 225 s for Skirlou and Soissons, respectively.

Test Baking and Sampling. Wheat loaves (100 g of flour) were produced using the Finney (1984) method with the farinograph and mixograph-deduced parameters. Doughs were mixed with a 100 g pin mixer (National Mfg., Lincoln, NE). Ingredients, other than flour and water, were 6% sugar (sucrose), 1.5% salt, and 3% shortening (Crisco, Procter and Gamble, Cincinnatti, OH). Fermentation with Fermipan yeast (0.76 g, Gist Brocades, Delft, The Netherlands) was 180 min and the final proof was 55 min at 30 °C. Baking was 24 min at 215 °C. Dough samples (from 100 g of flour) were taken (1) at the end of kneading, after (2) 30 min, (3) 60 min, (4) 105 min (after first punch), (5) 155 min (after second punch), (6) 180 min (after molding), (7) 235 min (before the baking process) and (8) 259 min (end of the baking process). The samples were immediately frozen in liquid nitrogen and subsequently freeze-dried. The freeze-dried samples were gently milled with a pestle and mortar. The bread-making experiment was done in triplicate.

Estimation of Percentages of Water-Extractable Arabinoxylan and Water-Extractable Arabinogalactan at Different Stages of the Bread-Making Process. The WE-AX and WE-AG contents of flour and dough at different stages of the bread-making process were determined with all extractions in triplicate. Gas chromatography determinations on the resulting extracts were carried out in duplicate and calculated as described earlier by Loosveld et al. (1997). The calculation method includes a combination of the data of the monosaccharide composition of water extracts of the samples, the arabinose/xylose (A/X) ratios of isolated WE-AX and the A/G ratios of isolated WE-AGP. The WE-AX and WE-AG contents were calculated, respectively, as 0.88 and 0.89 multiplied by the sum of the contents of their constituent monosaccharides (see also Table 1), thus taking into account the water uptake upon hydrolysis of these polysaccharides.

Isolation and Purification of Water-Extractable Arabinoxylan and Water-Extractable Arabinogalactan-Peptide. For extraction of WE-AX and WE-AGP from intermediates at different stages of the bread-making process, samples were first refluxed with 80% ethanol (Fincher and Stone, 1974) to denature protein. The residue was air-dried and gently milled with a pestle and mortar. WE-AX and WE-AGP were then isolated from the samples as outlined by Loosveld et al. (1997). Thus, deionized water extracts were heated at 90 °C to precipitate soluble protein, starch was removed from the filtrates by α-amylase and amyloglucosidase followed by dialysis, and to the retentates ethanol (95%) was stepwise added to a concentration of 80%. The precipitates obtained (WE-AX and WE-AGP) were removed by centrifugation and dissolved in deionized water.

Ethanol was added to a final concentration of 65% (v/v) as above to precipitate WE-AX. The WE-AGPs were recovered from the supernatants by vacuum rotary evaporation (40 °C) and freeze-drying. The composition of the WE-AGP and WE-AX fractions were determined by gas−liquid chromatography following hydrolysis and conversion to alditol acetates (Loosveld et al., 1997).

Protein contents of isolated WE-AGP were determined (Lowry et al., 1951) with bovine serum albumin as standard.

Gel Permeation Chromatography. Changes in apparent molecular weight distributions of the WE-AX and WE-AGP during the bread-making process were determined by gel permeation chromatography. Samples (2.0 mg/mL) were solubilized in 0.3% NaCl, filtered (0.45 μm), and separated on a Shodex B-804 (Showa Denko K. K., Tokyo, Japan) gel permeation column (8 mm × 300 mm) by elution with 0.3% NaCl (0.5 mL/min at 30 °C). The refractive index of the eluate was monitored using a Refractive Index Detector Model 8110 (VDS Optilab, Berlin, Deutschland) detector. Molecular weight markers were Shodex standard P-82 pullulans (2.0 mg/mL) (Showa Denko K. K., Tokyo, Japan) with molecular weights of 78.8×10^4, 40.4×10^4, 21.2×10^4, 11.2×10^4, 4.73×10^4, 2.28×10^4, 1.18×10^4, and 0.59×10^4.

^{1}H NMR 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 (Acros Chimica, Geel, Belgium), 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 approximately 7000.

RESULTS AND DISCUSSION

Quantitative Changes of Water-Extractable Arabinoxylan and Water-Extractable Arabinogalactan-Peptide during Bread Making. In Table 1, percentages WE-AX and WE-AG extracted from doughs at different stages of bread making are given. The values for the WE-AX of Skirlou indicate no solubilization during mixing, a slight solubilization during fermentation, and significant solubilization during baking. For Soissons, an increase of WE-AX after mixing, some solubilization of WE-AX during fermentation, and a significant solubilization as a result of baking were observed. Although the absolute increase in levels of WE-AX were comparable for both Skirlou (0.20%) and Soissons (0.24%) bread making, they were, respectively, approximately 29 and 77% of the initially present WE-AX for the same flours (0.69 and 0.31%, respectively). Rouau (1993) and Rouau et al. (1994) reported an increase in water-extractable NSP after mixing as a result of physical phenomena. Little solubilization during fermentation was also found by Cleemput et al. (1997). These results are in contrast with Rouau et al. (1994) who found significant solubilization during fermentation. In agreement with our observations, Westerlund et al. (1989) and Cleemput et al. (1997) found that water-unextractable arabinoxylan was rendered partly extractable during baking.

In the case of the WE-AG, the extractability seems to slightly increase (approximately 12 and 10% for Skirlou and Soissons, respectively) as a result of the bread-making process, although the increase is not significant. These results are in contrast with those of

Table 2. Monosaccharide Composition (Weight Proportion, %), Protein Content (%), Arabinose to Xylose Ratio, and Arabinoxylan Content (%) of WE-AX Isolated of Doughs at Different Stages of the Bread-Making Process

stage[b]	*Ara*[a]	*Xyl*	Man	Gal	Glc	*Prot*	*A/X*	*AX*
				(a) Skirlou				
(0)	*34.3*	*67.5*	0.2	1.5	2.5	*3.7*	*0.49*	*88.7*
(1)	*30.1*	*62.2*	0.4	0.6	1.6	*5.4*	*0.48*	*80.8*
(2)	*28.7*	*55.8*	0.6	1.4	0.8	*9.9*	*0.50*	*73.5*
(3)	*29.0*	*59.7*	0.6	1.1	3.4	*10.0*	*0.47*	*77.4*
(4)	*30.2*	*62.3*	0.5	1.1	4.4	*5.1*	*0.47*	*80.7*
(5)	*30.5*	*63.5*	0.4	1.0	2.4	*5.0*	*0.47*	*82.1*
(6)	*29.2*	*58.9*	0.6	1.5	2.7	*8.9*	*0.48*	*76.6*
(7)	*29.9*	*59.7*	0.7	1.6	1.1	*7.5*	*0.48*	*77.9*
(8)	*30.2*	*63.4*	0.4	0.6	4.7	*2.9*	*0.47*	*82.0*
				(b) Soissons				
(0)	*37.2*	*69.2*	0.2	1.1	3.4	*5.7*	*0.53*	*93.0*
(1)	*27.4*	*45.4*	1.2	5.3	5.4	*16.2*	*0.52*	*60.8*
(2)	*27.2*	*43.9*	1.9	6.0	4.0	*19.0*	*0.52*	*58.9*
(3)	*29.5*	*47.1*	1.9	6.0	4.2	*14.9*	*0.54*	*63.7*
(4)	*29.2*	*47.0*	1.8	6.3	4.3	*19.6*	*0.53*	*63.2*
(5)	*30.9*	*51.9*	1.0	5.3	5.1	*15.2*	*0.52*	*69.6*
(6)	*29.7*	*49.6*	1.4	5.1	4.6	*15.9*	*0.53*	*66.7*
(7)	*30.2*	*50.6*	1.5	5.1	4.2	*16.1*	*0.53*	*68.0*
(8)	*30.8*	*52.0*	1.0	4.9	8.6	*6.9*	*0.53*	*69.8*

[a] Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glc, glucose; Prot, protein; A/X, (%Ara − 0.7 × %Gal)/Xyl; AX, 0.88 × [(%Ara − 0.7 × %Gal) + %Xyl]. [b] (0) Flour, (1) end of kneading, (2) 30 min of fermentation, (3) 60 min of fermentation, (4) 105 min (first punch), (5) 155 min (second punch), (6) 180 min (molding), (7) 235 min (before baking), and (8) 259 min (end of baking).

Table 3. Monosaccharide Composition (Weight Proportion, %), Protein Content (%), Arabinose to Galactose Ratio, and Arabinogalactan Content of WE-AGP Isolated of Doughs at Different Stages of the Bread-Making Process

stage[b]	*Ara*[a]	Xyl	Man	*Gal*	Glc	*Prot*	*A/G*	*AG*
				(a) Skirlou				
(0)	*34.5*	2.8	1.4	*48.0*	2.1	*10.3*	*0.69*	*72.2*
(1)	*27.5*	6.4	3.0	*35.1*	2.8	*18.4*	*0.69*	*52.8*
(2)	*26.6*	7.7	3.1	*33.5*	3.6	*16.8*	*0.66*	*49.6*
(3)	*28.5*	4.9	3.0	*36.9*	2.3	*16.4*	*0.70*	*56.0*
(4)	*28.2*	11.8	1.1	*33.3*	3.3	*16.6*	*0.67*	*49.4*
(5)	*26.2*	5.5	0.7	*33.8*	3.2	*20.2*	*0.69*	*51.0*
(6)	*28.5*	4.9	0.7	*37.1*	3.8	*16.5*	*0.70*	*56.2*
(7)	*27.4*	4.6	0.8	*36.5*	3.9	*17.0*	*0.68*	*54.7*
(8)	*28.8*	6.1	1.4	*37.4*	5.6	*11.7*	*0.69*	*56.3*
				(b) Soissons				
(0)	*36.1*	2.1	0.3	*48.8*	1.4	*11.0*	*0.72*	*74.5*
(1)	*27.1*	1.9	1.0	*36.9*	1.5	*21.0*	*0.70*	*56.9*
(2)	*27.8*	2.0	2.5	*38.0*	2.2	*18.8*	*0.70*	*58.6*
(3)	*30.2*	1.5	1.7	*41.6*	2.4	*15.5*	*0.70*	*63.9*
(4)	*28.3*	1.8	1.3	*38.9*	2.1	*19.8*	*0.70*	*59.8*
(5)	*29.9*	2.3	0.7	*40.3*	2.1	*19.1*	*0.71*	*62.5*
(6)	*28.8*	2.4	0.5	*39.1*	2.3	*20.3*	*0.70*	*60.4*
(7)	*28.5*	2.1	0.4	*39.3*	2.0	*21.3*	*0.69*	*60.3*
(8)	*29.8*	2.6	0.8	*41.1*	4.8	*12.4*	*0.69*	*63.1*

[a] Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glc, glucose; Prot, protein; *A/G*, (%Ara − *A/X* × %Xyl)/% Gal; AG, 0.89 × [(%Ara − %Xyl × *A/X*) + %Gal]. [b] (0) Flour, (1) end of kneading, (2) 30 min of fermentation, (3) 60 min of fermentation, (4) 105 min (first punch), (5) 155 min (second punch), (6) 180 min (molding), (7) 235 min (before baking), and (8) 259 min.

Westerlund et al. (1989) who found lower percentages of arabinogalactan in bread fractions than in their doughs. For flours of six wheat varieties, Roels et al. (1998) documented the intervention of WE-AGP in wheat flour agglomeration. The WE-AGP/WE-AX ratio was higher in gluten fractions with the best coagulation properties than in wheat water extracts pointing to a preferential inclusion or interaction with WE-AGP. In contrast, our results point to an enhanced extractability rather than to an entrapment or a specific interaction.

Qualitative Changes of Water-Extractable Arabinoxylan and Water-Extractable Arabinogalactan-Peptide during Bread Making. *Monosaccharide and Protein Composition of WE-AX and WE-AGP.* The monosaccharide compositions of the isolated WE-AX and WE-AGP are listed in Tables 2 and 3. The WE-AXs isolated out of dough samples at different stages of the bread-making process are less pure than those isolated from flour especially for the flour of the wheat variety Soissons as follows from the monosaccharide composition and protein content data which were criteria for purity of the isolated samples. For Skirlou, 73.5−82.1% and, Soissons, 58.9−69.8% of the isolated material consisted of WE-AX. Among the different dough samples, only very small changes in arabinose/xylose (*A/X*) ratio (0.47−0.50 for Skirlou, 0.52−0.54 for Soissons) were observed. Similar observations for WE-AX were made by Cleemput et al. (1997) who observed a low purity of WE-AX isolated of doughs and who found also little variation in *A/X* among the different dough samples.

WE-AGPs isolated from dough samples are also less pure than those isolated from flour. For Skirlou, 49.4−56.3% and, for Soissons, 56.9−63.9% of the isolated material consisted of WE-AG. Among the different dough samples, only very small changes in arabinose/galactose (*A/G*) ratio (0.66−0.70 for Skirlou, 0.69−0.72 for Soissons) were observed. Westerlund et al. (1990) found larger variation in *A/G* ratio 0.55−0.67) for WE-AGP isolated of white flour, dough, crumb, and crust.

Some components interfere with the isolation af WE-AX and WE-AGP out of dough samples. Westerlund et al. (1990) also found lower carbohydrate contents in pentosan fractions isolated from doughs, crumb, and inner crust than those isolated from white flour. In our work, the protein contents of flour WE-AX were 3.7 and 5.7% for Skirlou and Soissons, respectively. In the dough and bread WE-AX samples, protein contents varried from 2.9 to 10% and from 6.9 to 19.6% for the same flours. The protein content of WE-AGP from flour, of which, as follows from literature data that the major part is associated into the WE-AG as a peptide (Fincher and Stone, 1974), was 10.3 and 11.0% for Skirlou and Soissons, respectively. When WE-AGPs were isolated from intermediates and final products in the bread-making process, their contents ranged from 11.7 to 20.2% and from 12.4 to 21.3 for Skirlou and Soissons bread making, respectively.

Changes in Molecular Weight of the Arabinogalactan-Peptide during Different Stages of the Bread-Making Process. No differences in molecular weight distribution as a result of processing were observed in the gel permeation profiles of the WE-AGP isolated from flour, doughs at different stages of bread-making process, and bread from flour of the wheat varieties Skirlou and Soissons (Figure 1). In contrast to differences found in molecular weight distribution of the isolated WE-AX as a result of processing (Cleemput et al., 1997) and confirmed in this work (results not shown), no differences were found in profiles of WE-AGP isolated of doughs at different stages of the bread-making process. Apparent molecular weights of 2.4×10^4 and 2.2×10^4 were found for WE-AGP samples of flour and doughs at different stages of the bread-making process for Skirlou and Soissons, respectively. An apparent molecular weight range of WE-AGP isolated of different

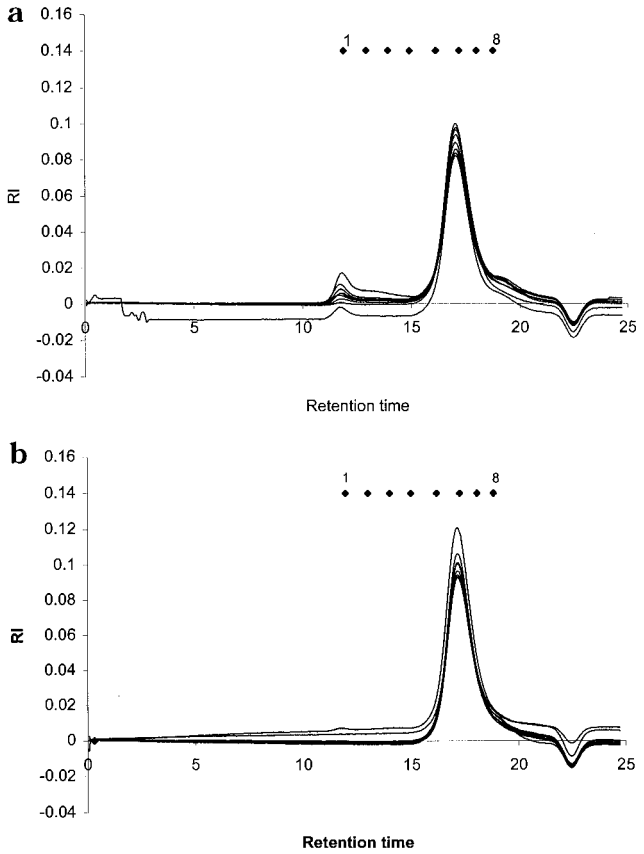

Figure 1. Gel permeation profiles of WE-AGP isolated of flour, doughs at different stages of the bread-making process and bread for the flours of the wheat varieties (a) Skirlou and (b) Soissons. Elution volumes of pullulan standards of molecular weight 78.8×10^4, 40.4×10^4, 21.2×10^4, 11.2×10^4, 4.73×10^4, 2.28×10^4, 1.18×10^4, and 0.59×10^4 (1−8, respectively) are indicated.

flour samples of 5.0×10^4 to 10.0×10^4 was found in earlier experiments (Loosveld et al., 1997). Analysis of the same pure WE-AGP samples with the Shodex B-804 column used in the present work gave apparent molecular weights of approximately 2.5×10^4. The differences found in apparent molecular weights are therefore due to differences in gel permeation column.

^{1}H NMR Spectroscopy. In the case of Skirlou flour bread making, the ^{1}H NMR profiles of WE-AGP isolated from dough at the end of kneading and bread are comparable with the ^{1}H NMR profile of WE-AGP isolated from flour (Figure 2). The resonance at δ 5.26 arises from the anomeric protons in α-linked arabinofuranosyl residues (Westerlund et al., 1990). The peak with chemical shifts at δ 4.47−4.54 can be ascribed to the β-linked galactose residues. In contrast to the ^{1}H NMR profile of the WE-AGP isolated from a dough at the end of kneading, the ^{1}H NMR profile of the WE-AGP isolated from bread shows two doublets of anomeric protons at δ 4.75 and 4.54. According to Westerlund et al. (1990), the latter peaks can be ascribed to signals of the anomeric protons of mannose and glucose, respectively.

CONCLUSIONS

The extractability of WE-AG seems to slightly increase as a result of the bread making. A greater solubilization of WE-AX was observed. In the latter case, part of the water-unextractable AX become extractable during the bread-making process. As it is generally assumed that wheat flour does not contain water-unextractable AGP the increased solubility should be ascribed to physical phenomena.

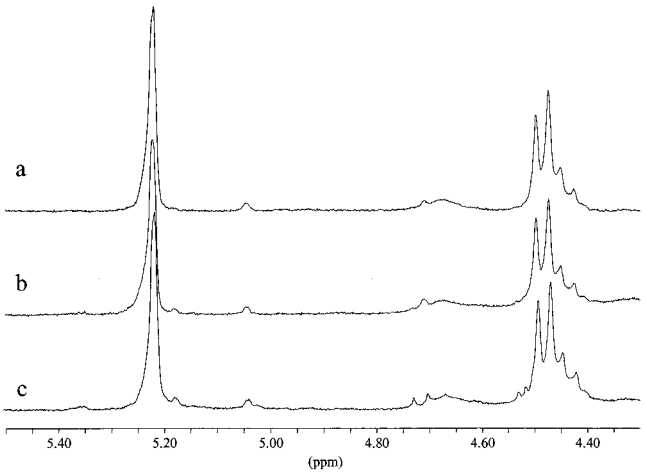

Figure 2. ^{1}H NMR spectra of WE-AGP isolated of (a) flour, (b) a dough at the end of kneading, and (c) bread of the wheat variety Skirlou. δ 5.26, anomeric protons of α-linked arabinofuranosyl residues; δ 4.47−4.54, anomeric protons of β-linked galactose residues.

Only very small differences in *A*/*G* ratios of isolated WE-AGP of flour and doughs isolated at different stages of the bread-making process were observed. In contrast to changes in molecular weight distribution of WE-AX during bread making, no differences in molecular weight distributions as a result of processing were observed. If present, WE-AGP hydrolyzing enzymes do not hydrolyze WE-AGP during the bread-making process.

The present work furthermore suggests that, unlike what was observed in a study on factors governing gluten agglomeration during wheat fractionation in a more dilute system (Roels et al., 1998), there is no specific entrapment of WE-AGP neither in dough formation nor in the other steps of the bread-making process.

ABBREVIATIONS USED

AX, arabinoxylan; WE-AX, water-extractable arabinoxylan; WE-AGP, water-extractable arabinogalactan-peptide; WE-AG, water-extractable arabinogalactan; NMR, nuclear magnetic resonance; Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glc, glucose; A/X, arabinose-to-xylose ratio; A/G, arabinose-to-galactose ratio; NSP, nonstarch polysaccharide.

LITERATURE CITED

American Association of Cereal Chemists. *Approved Methods of the AACC*, 8th ed.; The Association: St. Paul, MN, 1983; method 26-31, first approval April 1961, final approval Oct 1988; method 08-01, final approvel April 1961, revised Oct 1976 and Oct 1981; method 54-21, first approval April 1961, revised Oct 1982; method 54-40A, first approval April 1961, revised Oct 1988.

Casier, J. P. J.; De Paepe, G. M. J.; Brummer, J. Einfluss der wasserunlöslichen Weizen- und Roggen-Pentosane auf die Backeigenschaften von Weizenmehlen und anderen Rohstoffen. *Getreide Mehl Brot.* **1973**, *27*, 36−44.

Cleemput, G.; Bleukx, W.; Van Oort, M.; Hessing, M.; Delcour, J. A. Evidence for the presence of arabinoxylan hydrolyzing enzymes in European wheat flours. *J. Cereal Sci.* **1995**, *22*, 139−145.

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

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.

D'Appolonia, B. L. Comparison of pentosans extracted from conventional and continuous bread. *Cereal Chem*. **1973**, *50*, 27−36.

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

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

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

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

Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. Y. Protein measurement with the Folin phenol reagent. *J. Biol. Chem*. **1951**, *193*, 265−275.

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

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.

Roels, S. P.; Cleemput, G.; Vandewalle, X.; 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*, 318−323.

Roels, S. P.; Grobet, P. J.; Delcour, J. A. 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. Investigations into the effects of an enzyme preparation for baking on wheat flour dough pentosans. *J. Cereal Sci*. **1993**, *18*, 145−157.

Rouau, X.; El-Hayek, M.-L.; Moreau, D. An 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.

Udy, D. C. The intrinsic viscosities of the water-soluble components of wheat flour. *Cereal Chem*. **1956**, *33*, 67−74.

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

Westerlund, E.; Theander, O.; Andersson, R.; Aman, P. Effects of baking on polysaccharides in white bread fractions. *J. Cereal Sci*. **1989**, *10*, 149−156.

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

Received for review May 20, 1998. Revised manuscript received October 6, 1998. Accepted October 8, 1998. The Vlaams Instiutuut voor de Bevordering van het Wetenschappelijk-Technologisch Onderzoek in de Industrie (Brussels) is acknowledged for financial support.

JF9805350