NOTE

Characterization of the Carbohydrate Part of Arabinogalactan Peptides in *Triticum durum* desf. Semolina

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Large differences exist in the functionality of common wheat (*Triticum aestivum* L.) and durum wheat (*Triticum durum* Desf.) milling products. Common wheat flours are mainly used for bread and biscuit (cookie) production, while *T. durum* wheat semolina is mostly used as raw material for pasta and couscous. Differences in the suitability of both cereals for yielding high quality products in these processes have been ascribed to the processability of the two cereals in milling and to gluten contents or characteristics that differ significantly between the two wheat families.

The nonstarchy polysaccharides (NSP), minor constituents of both *durum* and *aestivum* wheat, consist mainly of arabinoxylan (AX) and arabinogalactan peptide (AGP). Information already exists about the structural differences of AX in *aestivum* and *durum* wheat. AX from *T. durum.* contains a higher proportion of arabinose than those from *aestivum* AX, indicating a more branched structure (Medcalf and Gilles 1968; Medcalf et al 1968; Ciaccio and D'Appolonia 1982; Roels et al 1999).

Aestivum AGP consist of a polysaccharide (92%) backbone of β-1,6 and β-1,3-linked D-galactopyranosyl residues. They are substituted with α–L-arabinofuranosyl residues (Fincher et al 1974; Neukom and Markwalder 1975; Loosveld et al 1998). This carbohydrate structure is covalently linked to a protein moiety rich in hydroxyproline, serine, threonine, and glycine (Fincher et al 1983). Because of low molecular weights and their structure, all AGP are water-extractable (Clarke et al 1979). Contents in common wheat flour are reported as 0.24–0.33% (Loosveld et al 1998).

In contrast to what is known concerning structural differences of AX in *T. aestivum* and *T. durum* wheats, little is known about the *T. durum* AGP. Therefore, we have determined the contents of AGP in *T. durum* semolina. After purification, we made a thorough structural analysis (GC, H^1-NMR, and methylation). In doing so, we focused on the carbohydrate part of AGP. Contents of AGP are given as arabinogalactans (AG) as it is assumed that the protein moiety only represents a minor part of the AGP, as in *aestivum* AGP (Fincher et al 1974). The results of this study could lead to a more basic understanding of AGP in general and the composition and structure in *durum* wheat. In further work, a detailed study of the protein moiety can be executed to competely characterize the AGP in *durum* wheat.

MATERIALS AND METHODS

Chemicals

All reagents were at least analytical grade. Specialty chemicals were heat-stable α-amylase (Termamyl 120 LS, Novo Nordisk, Bagsvaerd, Denmark) and amyloglucosidase (Boehringer Mannheim, Mannheim, Germany). Units were defined by the respective suppliers for both enzymes. β-D-Allose and α-L-arabinofuranosidase were obtained from Sigma (St. Louis, MO) and Megazyme (Bray, Ireland), respectively.

Semolinas

Durum wheat semolina A (semA) and semolina D (semD) were from the Canadian cultivar AC Avonlea and breeding line DT666, respectively (harvest year 1999, Canadian Grain Commission, Winnipeg, Canada). Semolina S (semS) was from a blend of Greek and French durum wheats (harvest year 1999, Soubry, Roeselare, Belgium). Samples were stored at 4°C until used. Protein content (N × 5.7) was determined by a Kjeldahl procedure (Approved Method 46-11A, AACC 2000) as 13.1, 14.3, and 13.6% (db), respectively. Ash content was determined (Approved Method 08-01) as 0.81, 1.00, and 0.93% (db) for semA, semD, and semS, respectively. Moisture content was determined (Approved Method 44-15A) as 14.1, 13.9, and 14.0% for semA, semD, and semS, respectively. For comparison purposes in some experiments, we also used *T. aestivum* commercial wheat flour Uno (Ceres, Vilvoorde, Belgium).

Inactivation of Endogenous Enzymes in Semolina

Semolina was refluxed in ethanol (95%) for 2 hr. After cooling, the ethanol was removed by vacuum rotary evaporation (45°C), and the material was air-dried. The material was crushed with a mortar and pestle until it passed a 250-μm sieve. This is referred to as inactivated material.

Purification of AGP

Extracts were made from the inactivated material (80.0 g) with deionized water (1:5, w/v, 60 min, 4°C). The suspension was centrifuged (8,000 × *g*, 15 min, 4°C) and the supernatant was boiled for 10 min. Following a Termamyl (3,000 Units, 30 min, 90°C) treatment and a centrifugation step (3,000 × *g*, 15 min, 15°C), the supernatant was treated with amyloglucosidase at pH 4.5 (50 Units, 12 hr, 60°C), centrifuged (8,000 × *g*, 40 min, 15°C) and then boiled (10 min). After a last centrifugation step (8000 × *g*, 40 min, 15°C) to remove the denatured proteins, the supernatant was dialyzed (48 hr, 4°C) and freeze-dried to obtain the NSP material. From this material, WE-AX were precipitated with ethanol precipitation (65%) following the method at Suckow et al (1973). After centrifugation (10,000 × *g*, 30 min, 15°C), AGP were subsequently precipitated by stepwise addition of ethanol to a final concentration of 80% to the supernatant as performed by Loosveld et al (1998). The precipitated AGP material was freeze-dried and is referred to as purified AGP. Purified AGP material (50 mg) was incubated with α-L-arabinofuranosidase as in Loosveld et al (1998), except that incubation time was extended to 24 hr.

Determination of Sugar Composition of Polysaccharides

For the determination of water-extractable carbohydrates in inactivated samples, 2.0 g of inactivated material was extracted with 20 mL of deionized water (60 min, 4°C). Extractions were performed fourfold. After centrifugation (3000 × *g*, 15 min, 4°C), the supernatant (2.5 mL) was hydrolyzed (60 min, 110°C) with 2.5 mL of 4.0*M* trifluoroacetic acid (TFA), resulting in a TFA concentration of 2.0*M*.

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TABLE I
Nonstarch Polysaccharide (NSP) Composition of Inactivated Semolinas and Purified Arabinogalactan Peptide (AGP) and AGP After Treatment with α-L-Arabinofuranosidase[a]

	Composition of Sample (%db)		Composition of Purified AGP		Composition of Purified AGP and Treated with Arabinofuranosidase	
	%AX	%AG	%AG	A/G	%AG	A/G
SemA			74.1	0.66	46.0	0.01
Total	1.83	0.24				
Water extract	0.38	0.24				
SemD			62.6	0.70	56.9	0.02
Total	1.87	0.25				
Water extract	0.38	0.25				
SemS			64.4	0.69	54.7	0.02
Total	2.17	0.30				
Water extract	0.41	0.30				
Max. CV (%)	8	8	6	6	7	5

[a] AX = arabinoxylans, AG = arabinogalactans, A/G = arabinose/galactose substitution, max CV = maximal coefficient of variation.

TABLE II
Glycosidic Linkage Composition of the Water-Extractable Arabinogalactan Peptide (AGP), Calculated from Methylation Analysis Results

	T. durum		*T. aestivum*	
	SemA	SemD	SemS	Uno[a]
%t-gal	3.1	3.6	2.9	2.5
%1,3-gal	8.6	8.9	8.6	9.1
%1,6-gal	15.4	15.0	13.2	11.3
%1,3,6-gal	73.0	72.5	75.4	77.2
%t-ara	91.7	91.8	90.4	89.7
%1,5-ara	5.2	6.3	7.2	5.6
%1,3,5-ara	1.3	1.9	2.4	2.5
%1,2,3,5-ara	1.8	nd	nd	2.2

[a] Standard.

For the determination of total carbohydrate content of inactivated materials, 50 mg of inactivated material was hydrolyzed (120 min, 110°C) with 5.0 mL of TFA (2.0*M*). After cooling, the hydrolysate was centrifuged (3000 × *g*, 15 min).

Purified AGP material (15 mg) was hydrolyzed (60 min, 110°C) with 5.0 mL of TFA (2.0*M*) for carbohydrate analysis. All analyses were done at least in duplicate.

Alditol acetates were prepared according to the method of Englyst and Cummings (1984) and were separated on a Supelco SP-2380 (Bellefonte, PA) column (30 m, 0.32 mm, i.d., 0.2 μm film thickness) in a chromatograph (Chrompack 9011, Middelburg, The Netherlands) equipped with a flame-ionization detector. The carrier gas was He. Separation was at 225°C with injection and detection temperatures of 275°C. β-D-allose was used as internal standard (1.0 mL added with a concentration of 1.0 mg/mL).

The xylose (xyl), arabinose (ara), and galactose (gal) data was used in the determination of the AX and arabinogalactan (AG) levels and the A/G ratio (the arabinose to galactose ratio or substitution degree of AG) as calculated by Loosveld et al (1998). AG levels are a good estimation of the level of AGP because it can reasonably (based on the gel permeation behavior) be assumed that, much as in *T. aestivum* (Fincher et al 1974), the peptide component makes up only a minor proportion of the polymer.

Gel Permeation Chromatography

Purified AGP (6.0 mg) were solubilized in 0.3% NaCl (3.0 mL) and centrifuged (10,000 × *g*, 10 min). The solution was filtered (0.45 μm) and the AGP was fractionated on a Shodex SB-804 HQ (Showa Denko K.K., Tokyo, Japan) GPC column (300 × 8 mm) by elution with 0.3% NaCl (0.5 mL/min). The refractive index of the eluate was monitored using a VDS Optilab detector (Berlin, Germany). Molecular weight (MW) markers were Shodex standard P-82 pullulan standards (Showa Denko K.K., Tokyo, Japan) with MW 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 made approximate calculations of MW possible.

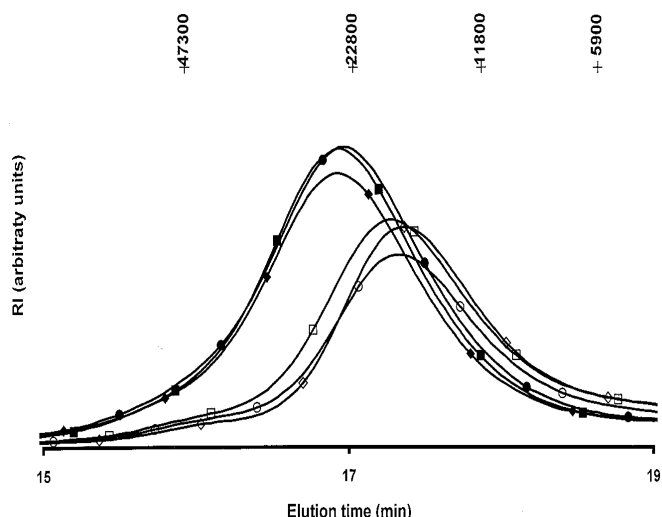

Fig. 1. Gel permeation chromatography profiles of AGP material, purified from samples semA ●, semD ■, and semS ◆, respectively and of arabinofuranosidase-treated AGP material of semA ○, semD □, and semS ◊, respectively. RI = signal measured with a refractive index dectector. Molecular weight markers are indicated above the graph.

^{1}H Nuclear Magnetic Resonance Spectroscopy

^{1}H-NMR spectra were recorded on a 300-MHz Fourier transform spectrometer (Bruker, Karlsruhe, Germany) at 85°C as in Loosveld et al (1998). Chemical shifts were referenced to the internal standard acetone (2.2 ppm).

Methylation Analysis

The method of Hakomori (1964), modified by Sandford and Conrad (1966), was used for methylation analysis as in Loosveld et al (1998). After methylation, the samples were dialyzed against water, dried in a rotary evaporator, hydrolyzed (2.0*M* TFA, 1 hr, 121°C), and converted to alditol acetates (Gruppen et 1992). Identification of the compounds was confirmed by gas chromatography-mass spectroscopy using a CP Sil 19 CB capillary column (25 m, 0.25 mm, i.d., 0.2 μm film thickness) (Chrompack, Middelburg, The Netherlands) in a Hewlett Packard 6890 gas chromatograph coupled to an HP 5973 mass-selective detector and using an HP Chem station (Hewlett-Packard, Amstelveen, The Netherlands). The temperature program was 160–185°C at 0.5°C/min, 185–230°C at 10°C/min, and 230°C for 5.5 min.

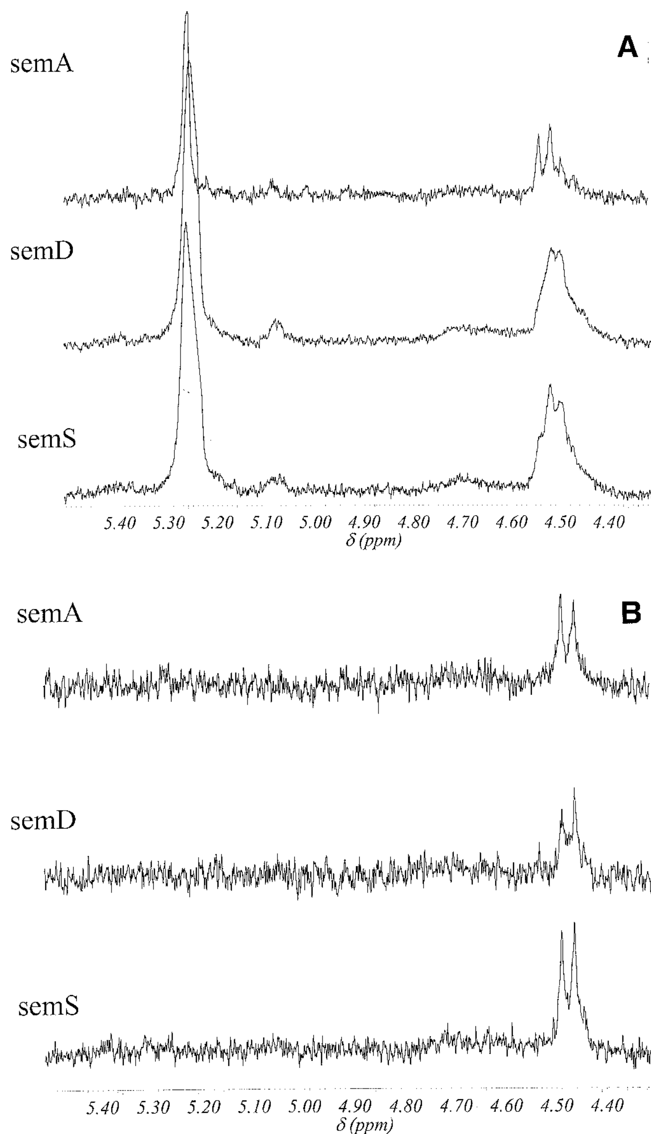

Fig. 2. A, Anomeric proton regions in the ^{1}H-NMR spectra of AGP material, purified from semA, semD, and semS, respectively. **B,** Arabinofuranosidase-treated AGP material of semA, semD, and semS, respectively.

RESULTS AND DISCUSSION

Carbohydrate Levels in Semolinas and Purified AGP

The AG contents of the three semolinas (Table I) varied between 0.24 and 0.30% for semA and semS, respectively. These values are in accordance with earlier findings for *T. aestivum* flour. Loosveld et al (1998) obtained AG content values of 0.24–0.33%, while Andersson et al (1994) obtained slightly lower values of 0.15–0.21%.

A/G ratios of the purified *T. durum* semolina AGP were 0.66–0.70 which agree with the values for *T. aestivum* flour (Fincher et al 1974; Neukom and Markwalder 1975; Izydorczyk et al 1991; Loosveld et al 1998).

The arabinofuranosidase treatment resulted in a substantial loss of arabinose from the *T. durum* AGP, A/G values as low as 0.02 and 0.01 were seen for the resulting products.

Gel Permeation Chromatography

The molecular weight (MW) profiles of the *T. durum* AGP are given in Fig. 1. Approximate calculations yielded apparent peak MW of 22,000, 23,000, and 23,000 for semA, semD, and semS AGP, respectively. Analogies were found between the MW from *T. durum* semolina AGP and those from *T. aestivum* flour (Fincher et al 1974; Neukom 1976; Strahm et al 1981; Loosveld et al 1998). The arabinofuranosidase treatment reduced the apparent peak MW. MW of 17,000, 17,500 and 16,500 were obtained for semA, semD, and semS AGP, respectively.

^{1}H Nuclear Magnetic Resonance Spectroscopy

The large signal at 5.26 ppm (Fig. 2A) is assigned to the anomeric proton in terminal arabinose residues in AGP (Westerlund et al 1990). This is validated by the observations made for the α-L-arabinofuranosidase-treated AGP (Fig. 2B), where this signal was absent. The signal between 4.60 and 4.40 ppm has been ascribed to the β-1,3- or β-1,6-galactopyranosyl residues (Loosveld et al 1998). Similar observations were made for the AGP from the three semolinas, and results were highly comparable with those for *T. aestivum* AGP (Loosveld et al 1998).

Methylation Analysis

Methylation analysis results made determination of percentage distributions of the different galactose linkages in AGP possible. The major part, with a range of 72.5–75.4% for *T. durum* AGP, was made up of galactopyranosyl units trisubstituted at the 1-, 3- and 6- positions (Table II). Comparison with the data for *T. aestivum* AGP (obtained from Uno commercial flour mix) showed higher proportions of 1,3,6-galactopyranosyl and 1,3-galactopyranosyl linkages and lower proportions of terminal galactopyranosyl and 1,6-galactopyranosyl linkages. Therefore, it can be concluded that small differences in linkage composition exist between *T. durum* and *T. aestivum* AGP. Percentages of terminal arabinofuranosyl units of *T. durum* and *T. aestivum* AGP were comparable (Table II) and, in both cases, they were the major arabinofuranosyl linkages.

CONCLUSIONS

With the techniques used here little difference in structure was detected among the *T. durum* AGP from different blends and when comparing them with *T. aestivum* AGP. The only structural differences between *T. durum* and *T. aestivum* AGP were detected with methylation analysis, indicating small differences in structure. It follows that the difference in structure between *T. durum* and *T. aestivum* AX, is larger than that between their AGP.

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