Comparison of the Structure and Composition of Water-Soluble Pentosans from Different Wheat Varieties[1]

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ABSTRACT

Water-soluble pentosans were isolated from flours of eight wheat varieties belonging to several Canadian classes. The pentosans were fractionated by $(NH_4)_2SO_4$ precipitation into arabinoxylans and arabinogalactans. The arabinoxylan from flours of Canada western red spring and Canada prairie spring had the lowest degree of branching, and that from the Canada utility class the highest. Among varieties, the degree of branching of the arabinogalactans was relatively constant, except for the Canada western red spring variety Katepwa, which was substantially lower. The peptide moieties of the arabinogalactans contained high levels of alanine and hydroxyproline. *Cis*- and *trans*-isomers of ferulic acid were found in the arabinoxylans. The ferulic acid content of arabinoxylans from different varieties varied significantly; the highest amount was found in the arabinoxylan of the Canada western soft spring variety Fielder. Purified arabinoxylans showed intervarietal differences in the molecular weight distribution by Sepharose CL-4B chromatography; the arabinoxylan from Katepwa had the highest apparent molecular weight. In contrast, the arabinogalactan-peptide fractions had similar molecular weights as determined by Sephacryl S-300 gel filtration.

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The major nonstarch polysaccharides of wheat flour are the pentosans, comprised of arabinoxylans and arabinogalactans (Neukom 1973). The water-soluble fraction of pentosans makes up between 0.5 and 0.8% of wheat flour. The structure of the pentosan components has been a subject of many research investigations and is generally well-known. Arabinoxylans consist of linear xylans with single L-arabinose side residues, whereas arabinogalactans have a galactan chain backbone that is highly branched with single L-arabinose residues as well as with low molecular weight peptides (Amado and Neukom 1985). Continuing interest in wheat flour pentosans has been stimulated by the many functional properties that have been ascribed to them. Their high water-absorbing capacity (Jelaca and Hlynka 1971, Shelton and D'Appolonia 1985) and ability to affect the rheological properties of doughs and bread (Jelaca and Hlynka 1972, Kim and D'Appolonia 1977, McCleary 1986) are of major technological importance in breadmaking. However, the functional performance of pentosans, particularly as it relates to their molecular structure, is not yet fully understood.

The properties and composition of pentosans from different wheat flours have been discussed in several reports. The studies of Medcalf et al (1968) on nonstarch carbohydrate constituents of durum and hard red spring wheat flours fractionated by diethylaminoethyl-cellulose chromatography suggested a more branched structure for the durum wheat pentosans compared with their hard red spring counterparts. D'Appolonia and MacArthur (1975) isolated pentosans from three hard red spring wheat varieties of conventional height and five semidwarf varieties and found some differences in their carbohydrate composition. According to Lineback et al (1977), the monosaccharide composition of fractionated pentosans (by diethylaminoethyl-cellulose chromatography) from hard red spring, hard red winter, and soft red winter wheat flours were also different. Ciacco and D'Appolonia (1982) confirmed previous results on the degree of branching of pentosans from different wheat flours and also reported on differences in the gelling capacity of pentosans. The present article is part of an investigation undertaken to determine the polymeric structure and chemical composition of pentosans and their purified constituents (arabinoxylans and arabinogalactans) of various wheat flours to elucidate their functional role in breadmaking and to assess their implication in the intervarietal differences in the bread-making potential of wheat flours.

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MATERIALS AND METHODS

Flours

The grain of eight wheat varieties of approximately the same protein content was selected from the 1987 crop grown in various locations in western Canada (Table I). Each sample was milled on a Buhler laboratory mill after tempering to a moisture content appropriate for each particular wheat class. The yields of straight grade flour ranged from 67.5 to 75.2%.

Extraction, Purification, and Fractionation of Pentosans

The water-soluble pentosans were extracted and purified according to the procedures used by Fincher and Stone (1974) and by Crowe and Rasper (1988), with some modifications. Flours were blended with three volumes of distilled water at 20°C for 5 min. After centrifugation (10,000 × *g*, 20 min), the aqueous extracts were immediately heated (95°C, 5 min) to inactivate endogenous hemicellulases and to denature water-soluble proteins. After centrifugation, residual proteins in the supernatant were removed by adsorption on Vega Clay (Pembina Mountain Clay, Winnipeg, MB). The extracts were stirred with the clay (20 g per liter of extract) for 30 min and then centrifuged (10,000 × *g*, 20 min). Salivary α-amylase (type IX A, EC 3.2.1.1, 1,290 units per milligram of protein, Sigma Chemical Co, St. Louis, MO) was used to eliminate starch contaminants from the extracts.

TABLE I
Composition of Flours Used for Isolation of Water-Soluble Pentosans

Wheat Class and Variety	Protein (%) (N × 5.7; 14%, mb)	Ash (%)	Total Pentosans (%, db)	Water-Soluble Pentosans (%, db)
Canada prairie spring				
HY 355	10.5	0.38	1.83 ± 0.10[a]	0.55 ± 0.02[a]
HY 320	10.0	0.39	1.96 ± 0.05	0.63 ± 0.02
Oslo	11.1	0.38	1.60 ± 0.05	0.50 ± 0.01
Canada utility				
Glenlea	12.1	0.48	1.67 ± 0.07	0.55 ± 0.01
Canada western soft white spring				
Fielder	10.0	0.39	1.86 ± 0.05	0.60 ± 0.02
Canada western red winter				
Norstar	11.3	0.33	13.7 ± 0.07	0.54 ± 0.01
Unregistered (hard red spring)				
Marshall	12.7	0.41	1.76 ± 0.07	0.62 ± 0.02
Canada western red spring				
Katepwa	12.1	0.42	2.06 ± 0.04	0.68 ± 0.02

[a]n = 3 ± SD.

After incubation with α-amylase (pH 6.5, 37°C, 48 hr), the solutions were dialyzed against distilled water (12,000–14,000 mol wt cutoff, 4°C, 48 hr); the enzyme was inactivated by heat (95°C, 30 min) and removed by centrifugation (10,000 × *g*, 20 min). The supernatants were then lyophilized. Incubation with α-amylase and all subsequent purification steps were repeated to minimize the presence of low molecular weight starch contaminants. The purified pentosans were fractionated into arabinoxylans and arabinogalactans by precipitation of the arabinoxylan with saturated ammonium sulfate (grade I, Sigma Chemical Co.) according to the method of Fincher and Stone (1974).

Chemical Analyses

The content of total and water-soluble pentosans in flours was determined by the phloroglucinol method of Douglas (1981). Protein content of the water-soluble pentosans, arabinoxylans, and arabinogalactans was determined by the method of Lowry et al (1951), using bovine serum albumin as a standard (Sigma Chemical Co.).

Residual α-D-glucan in pentosan preparations was assayed according to the dual-enzyme micromethod of Banks et al (1970), using commercial α-amylase (Tenase, Miles Lab, Elkhart, IN) and glucoamylase (Diazyme L-200, Miles Lab) preparations. Glucose was subsequently quantitated by a modified enzymatic method using glucose oxidase-peroxidase-chromogen (Biliaderis 1980).

Phenolic acids were liberated by treatment of pentosans or their fractions (150–200 mg) with 10 ml of 2*N* NaOH for 4 hr under nitrogen at 20°C. The hydrolysate was acidified to pH 2.0 with HCl and extracted twice with hexane (high-performance liquid chromatography [HPLC] grade) at a hexane-to-water phase ratio of 1:1. Free phenolic acids in the water layer were then extracted three times with diethyl ether-ethyl acetate (1:1, HPLC grades) at a solvent-to-aqueous phase ratio of 1:1 (Krygier et al 1982). The three organic extracts were combined and dehydrated with anhydrous sodium sulfate, filtered, and evaporated to dryness under vacuum at 30°C. The residue was dissolved in a known amount of methanol (HPLC grade) and analyzed according to a modified method of Hagerman and Nicholson (1982) by HPLC using a Supercosil LC-18 column (33 × 4.6 mm, 3-μm-diameter particles, 37°C; Supelco Canada, Ltd., Oakville, ON) and an absorbance detector (model 441, Waters Associates, Milford, MA) operated at 280 nm. Retention times and peak area were obtained with a Chromatopac C-R3A data processor (Shimadzu Corporation, Kyoto, Japan). The separation was obtained by a multistep gradient of acetate buffer (0.05 *M*, pH 4.7) and methanol. The run was programmed isocratically for 5 min with 5% methanol at 1 ml/min, followed by a 5-min linear gradient to 15% methanol. Subsequently, 15% methanol was run isocratically for 5 min, followed by a 10-min gradient to 30% methanol, and finally maintained at this level for the next 10 min. Hydroxybenzoic acid (used as an internal standard) and other phenolic acids were purchased from Sigma Chemical Co. *Cis*-isomers of ferulic and cinnamic acids were obtained by exposure of the *trans*-forms (1 m*M*) to UV light for 2 hr (Hartley and Jones 1975).

The content of feruloyl groups was also estimated spectrophotometrically by direct absorbance measurements at 375 nm of freshly prepared solutions of pentosans and their fractions in 0.07*M* glycine-sodium hydroxide buffer (pH 10) using the molar extinction coefficient of 31,600 (Fry 1982).

The relative amounts of component monosaccharides in the pentosans, arabinoxylans, and arabinogalactans were determined by HPLC after hydrolysis with 1*M* H_2SO_4 for 2 hr at 100°C and neutralization with barium carbonate. A Waters Associates chromatograph, equipped with an M 6000A solvent delivery system, and a model 441 refractive index detector were used for the analysis. Retention times and peak areas were obtained with a Hewlett Packard 3390A integrator. All samples (20 μl) were run isocratically at a flow rate of 0.6 ml/min, using degassed distilled water as the eluent, through an Aminex HPX-87P column (300 × 7.8 mm; Bio-Rad Labs, Richmond, CA) at 85°C in conjunction with a guard column.

The amino acids were determined as their ninhydrin derivatives after acid hydrolysis (6*M* HCl) by an amino acid analyzer (LKB Biochrom 4151 Alpha Plus amino acid analyzer, LKB/Biochrom, Science Park, Cambridge, England) equipped with a stainless steel column (200 × 4.6 mm i.d.) packed with LKB Ultropac 8 cation exchange resin (Na^+ form), particle size 8.0 ± 0. 5 μm (Rotter et al 1989). All amino acid standards except 4-hydroxyproline (Sigma Chemical Co.) were purchased from Pierce Chemical Co., Rockford, IL.

Chromatography

Gel permeation chromatography of pentosans and arabinoxylans was performed on a Sepharose CL-4B column (2.5 × 85 cm). Chromatography of arabinogalactans was performed on a Sephacryl S-300 column (2.5 × 85.0 cm). Elution was done with degassed 0.3% sodium chloride solution containing 0.05% sodium azide at a flow rate of 25 ml/hr at 25°C. Samples (10 mg) dissolved in the same buffer were applied onto the column, and fractions of 5 ml were collected. Total and void volumes were determined with xylose and Blue Dextran, respectively. Other molecular weight markers used in the Sepharose CL-4B chromatography were linear dextrans T-500, T-150, and T-70 (mol wt 466,000, 143,000, and 69,000, respectively) (Pharmacia Ltd, Montreal, PQ). Eluent fractions were analyzed for total carbohydrates by the phenolsulfuric method (Dubois et al 1956) and for proteins by the procedure of Lowry et al (1951). Feruloyl groups were monitored by spectrophotometry at 375 nm after the pH of the fractions was adjusted to 10 with 1*M* NaOH (Thibault and Rombouts 1986).

RESULTS AND DISCUSSION

The total pentosan content (Table I) of the eight wheat varieties ranged from 1.37 to 2.06%; of that total, the water-soluble component was within 30.1–39.4%. No relationship was apparent between wheat class and total pentosan content. However, the four varieties of the harder classes (Katepwa, Glenlea, Norstar, and Marshall) contained somewhat more soluble pentosans.

The extraction and purification procedures for water-soluble pentosans used in this study aimed to ensure maximum purity and avoid possible chemical or physical degradation of these materials. After heat-induced denaturation of water-soluble proteins, soluble proteins decreased substantially by treatment of the flour extracts with the Vega Clay. Approximately 75–80% of the residual proteins were removed by adsorption on this clay. The proteinaceous material in the purified pentosans (Table II) ranged from 4 to 5% for most samples, with the exception of Fielder (12.5%) and Marshall (16.0%), although the same purification scheme was followed in all cases. Nevertheless, the purified pentosans in this study were significantly freer from contaminating proteins than preparations of earlier reports by Lin and Pomeranz (1968), D'Appolonia and MacArthur (1975), Patil et al (1975), Lineback et al (1977), Yeh et al (1980), and Ciacco and D'Appolonia (1982).

Table II also compares the amounts of nitrogenous compounds in the two fractions of pentosans, arabinoxylan and arabinogalactan, prepared by ammonium sulfate fractionation. The values

TABLE II
Protein Content of Water-Soluble Pentosans and Their Fractions[a]

Variety	Pentosans	Arabinoxylans	Arabinogalactans
HY 355	4.7 ± 0.1	1.3 ± 0.1	9.1 ± 0.1
HY 320	4.0 ± 0.1	1.6 ± 0.1	6.6 ± 0.1
Oslo	5.2 ± 0.1	1.9 ± 0.1	8.5 ± 0.1
Glenlea	5.0 ± 0.1	2.2 ± 0.1	6.5 ± 0.2
Fielder	12.5 ± 0.3	8.7 ± 0.2	10.2 ± 0.2
Norstar	5.7 ± 0.1	2.9 ± 0.1	7.0 ± 0.2
Marshall	16.0 ± 0.3	8.2 ± 0.2	14.3 ± 0.2
Katepwa	6.0 ± 0.1	1.7 ± 0.1	7.0 ± 0.1

[a]%, dry basis; n = 3 ± SD.

of protein content in the arabinoxylans were low (1.3–2.9%) except those of Fielder (8.7%) and Marshall (8.2%). The protein content in arabinogalactans was much higher than those in arabinoxylans, which is in agreement with previous reports (Fincher and Stone 1974, Neukom and Markwalder 1975) and consistent with the structure of the arabinogalactan peptide (Fincher et al 1974, Strahm et al 1981). However, an unusually high protein content was found for arabinogalactans isolated from Fielder and Marshall (10.2 and 14.3%, respectively).

Starch is a common contaminant of pentosan preparations derived from wheat flours. The double treatment with salivary α-amylase lowered the amount of contaminating starch substantially but did not result in elimination of all α-D-glucans present (Table III). The pentosan yield following the first enzyme digestion (0.49–0.70%) is in close agreement with the values reported by D'Appolonia and MacArthur (1975) and Ciacco and D'Appolonia (1982). Despite the fact that identical hydrolysis conditions for α-D-glucans were adopted in all cases, the amount of starch removed varied with the wheat variety. Pentosans from Glenlea, Norstar, and Katepwa contained slightly higher amounts of residual starch than other preparations. The genetic and/or technological significance of these findings remains to be discovered.

The ratios of component monosaccharides present in the unfractionated pentosans and in their fractions are shown in Tables IV and V. The whole pentosans were composed of arabinose, xylose, and galactose. Small amounts of glucose and traces of fructose were also detected (Table IV). Substantial intervarietal differences were observed. The ratio of xylose to arabinose, indicative of the degree of branching of arabinoxylans, may play an important role in the physical properties of these constituents since branching is known to affect the conformation of these biopolymers in solution (Andrewartha et al 1979). The arabinoxylan of the Canada western red spring (Katepwa) and Canada prairie spring (HY 355, HY 320) varieties had the lowest degree of branching; the Canada utility variety Glenlea and the hard red spring variety Marshall had the highest. It is difficult to compare our results with those reported in previous investigations (Cole 1967, Medcalf et al 1968, D'Appolonia and MacArthur 1975, Ciacco and D'Appolonia 1982) because of the different procedures used for fractionation of the water-soluble wheat pentosans. In general, the arabinogalactans (Table V) appeared to be more branched than the arabinoxylans, as indicated by their relative proportion of arabinose. The degree of branching was relatively constant among varieties, except for the Katepwa arabinogalactan, which was less branched than the other samples.

The content of feruloyl groups in the soluble pentosan preparations and their arabinoxylan fractions was determined

TABLE III
Yields and Starch Content of the Water-Soluble Pentosans

Variety	First Digestion with α-Amylase		Second Digestion with α-Amylase	
	Yield[a]	Starch[b]	Yield[a]	Starch[b]
HY 355	0.53	3.64 ± 0.20	0.35	0.98 ± 0.07
HY 320	0.66	2.53 ± 0.06	0.45	0.42 ± 0.03
Oslo	0.67	5.20 ± 0.06	0.48	1.14 ± 0.04
Glenlea	0.52	15.97 ± 0.52	0.37	5.56 ± 0.05
Fielder	0.63	4.08 ± 0.12	0.45	2.50 ± 0.04
Norstar	0.51	12.04 ± 0.81	0.35	4.30 ± 0.11
Marshall	0.49	11.79 ± 0.13	0.37	2.50 ± 0.28
Katepwa	0.70	12.00 ± 0.20	0.43	3.50 ± 0.28

[a]%, on a flour basis.
[b]%, on a pentosan basis; n = 3 ± SD.

TABLE IV
Ratios of Component Sugars in Whole Pentosans

Variety	Arabinose	Xylose	Galactose	Glucose	Fructose
HY 355	1.00	0.97	0.62	0.28	Trace
HY 320	1.00	1.04	0.52	0.07	Trace
Oslo	1.00	0.82	0.58	0.08	Trace
Glenlea	1.00	0.92	0.57	0.25	Trace
Fielder	1.00	0.81	0.66	0.29	Trace
Norstar	1.00	0.78	0.59	0.19	Trace
Marshall	1.00	0.81	0.58	0.17	Trace
Katepwa	1.00	0.95	0.55	0.22	Trace

TABLE V
Ratios of Component Sugars in Arabinoxylans and Arabinogalactans

Variety	Arabinoxylans			Arabinogalactans			
	Arabinose	Xylose	Glucose	Arabinose	Galactose	Glucose	Xylose
HY 355	1.00	1.89	0.32	1.00	1.39	0.02	0.05
HY 320	1.00	1.75	0.09	1.00	1.42	0.01	0.00
Oslo	1.00	1.52	0.04	1.00	1.39	0.01	0.04
Glenlea	1.00	1.41	0.04	1.00	1.39	0.64	0.04
Fielder	1.00	1.58	0.14	1.00	1.34	0.07	0.00
Norstar	1.00	1.56	0.04	1.00	1.44	0.53	0.02
Marshall	1.00	1.43	0.05	1.00	1.44	0.01	0.01
Katepwa	1.00	1.67	0.12	1.00	1.52	0.58	0.00

TABLE VI
Phenolic Acid Content[a] in Pentosans and in Arabinoxylans

Variety	Total Phenolics				Component Phenolic Acids in Arabinoxylans			
	Pentosans		Arabinoxylans					
	I[b]	II[c]	I[b]	II[c]	*cis*-Ferulic	*trans*-Ferulic	*trans*-Cinnamic	Ratio *cis*:*trans*
HY 355	0.80[d] ± 0.05	0.86 ± 0.01	1.27 ± 0.03	1.24 ± 0.02	0.41 ± 0.01	0.83 ± 0.01	Trace	1:2.05
HY 320	0.85 ± 0.03	0.83 ± 0.02	1.43 ± 0.05	1.39 ± 0.01	0.42 ± 0.01	0.97 ± 0.01	0.005	1:2.35
Oslo	0.58 ± 0.05	0.48 ± 0.05	0.89 ± 0.05	0.82 ± 0.01	0.25 ± 0.01	0.57 ± 0.01	Trace	1:2.22
Glenlea	0.63 ± 0.06	0.56 ± 0.02	1.00 ± 0.05	0.87 ± 0.03	0.28 ± 0.01	0.59 ± 0.02	0.005	1:2.15
Fielder	0.92 ± 0.05	0.85 ± 0.03	1.37 ± 0.05	1.37 ± 0.01	0.44 ± 0.01	0.93 ± 0.01	Trace	1:2.14
Norstar	0.80 ± 0.05	0.70 ± 0.04	1.30 ± 0.05	1.14 ± 0.01	0.38 ± 0.01	0.76 ± 0.01	0.005	1:2.02
Marshall	0.46 ± 0.04	0.35 ± 0.04	0.86 ± 0.05	0.63 ± 0.01	0.20 ± 0.01	0.42 ± 0.01	Trace	1:2.09
Katepwa	0.75 ± 0.05	0.81 ± 0.03	1.10 ± 0.03	1.16 ± 0.01	0.32 ± 0.01	0.84 ± 0.01	Trace	1:2.63

[a]Milligrams per gram of material.
[b]Spectrophotometric measurements of solutions after adjustment of pH to 10.0 with glycine-NaOH at 375 nm; extinction coefficient 31,000 (Fry 1982).
[c]By high-performance liquid chromatography.
[d]n = 3 ± SD.

because of the potential implication of these groups in the cross-linking of wheat flour pentosans by reaction with oxidizing agents (Neukom and Markwalder 1975). It has been suggested that this reaction may contribute to the modification of the rheological properties of dough on addition of oxidizing agents (Hoseney 1984). The ferulic acid content values, determined by direct spectrophotometry (375 nm) were in close agreement with the total phenolic content obtained by HPLC (Table VI); apparently, ferulic acid was the main bound phenolic constituent found in arabinoxylans. Ferulic acid was not detected in the arabinogalactan fractions.

The HPLC chromatograms (Fig. 1) of deesterified phenolics of the pentosan and arabinoxylan preparations indicated the presence of both *cis*- and *trans*-isomers of ferulic acid. A control experiment with standard *trans*-ferulic acid showed that no *trans*- to *cis*-isomerization occurred during saponification and extraction of phenolics. These results, therefore, suggest that both *cis*- and *trans*-isomers of ferulic acid are present in the native wheat pentosans. This concurs with the findings of Guillon and Thibault (1988) on feruloylated pectins from sugar beet pulp and of Hartley and Jones (1976) on isolated cell walls of *Lolium multiflorum*. Small amounts of *trans*-cinnamic acid were also found in some preparations (Fig. 1, Table VI). The ferulic acid content of arabinoxylans from various flours varied significantly. The lowest amount was found in arabinoxylans from Marshall, Glenlea, and Oslo; the highest in Fielder, followed by HY 320, HY 355, Katepwa, and Norstar. Despite differences in the total content of ferulic acid, the ratios of *cis*- to *trans*-isomers were relatively constant for all samples (1:2.0–1:2.4), with the exception of Katepwa (1:2.6). It is difficult at this point to speculate on the relative importance of these isomers in the gelation process of arabinoxylans. More detailed studies on the fine structure, location, and isomer type of feruloyl groups along the chain backbone are needed to establish relationships between molecular structure and gelation properties of this polymer.

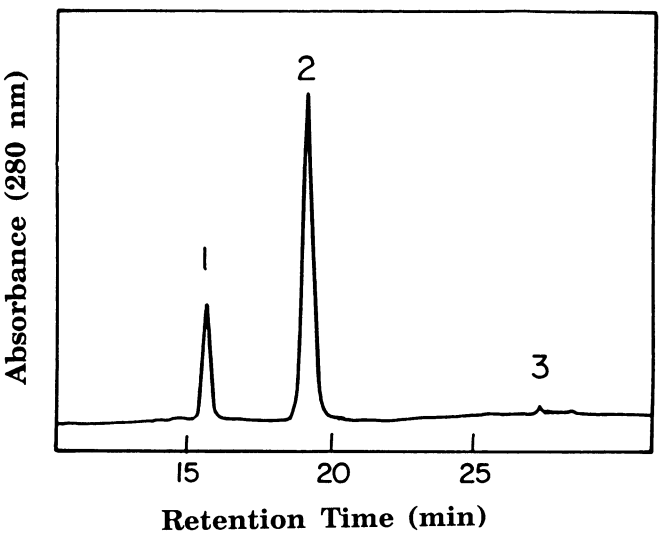

Fig. 1. High-performance liquid chromatogram of deesterified phenolic acids from arabinoxylan (Katepwa). 1, *cis*-ferulic acid; 2, *trans*-ferulic acid; 3, *trans*-cinnamic acid.

Gel permeation chromatography of the water-soluble pentosans on a Sepharose CL-4B column yielded two major peaks: a broad peak in the apparent molecular weight range of 5×10^5–5×10^6 and a narrower peak with an apparent molecular weight of about 7×10^4 (Fig. 2). The carbohydrate material eluted in the shoulder of the arabinogalactan peak contained some residual α-D-glucans present in the pentosan preparations. While proteins coeluted mainly with the carbohydrate material of the arabinogalactan component (as expected, considering its structure), a small portion of proteinaceous material was detected in the elution profile of the arabinoxylan. The distribution of ferulic acid corresponded closely with the broad peak of arabinoxylan.

The Sepharose CL-4B chromatography of the ammonium sulfate-precipitated arabinoxylans revealed distributions of molecular weight as broad as those of their counterparts in the whole pentosans and showed no evidence for arabinogalactan

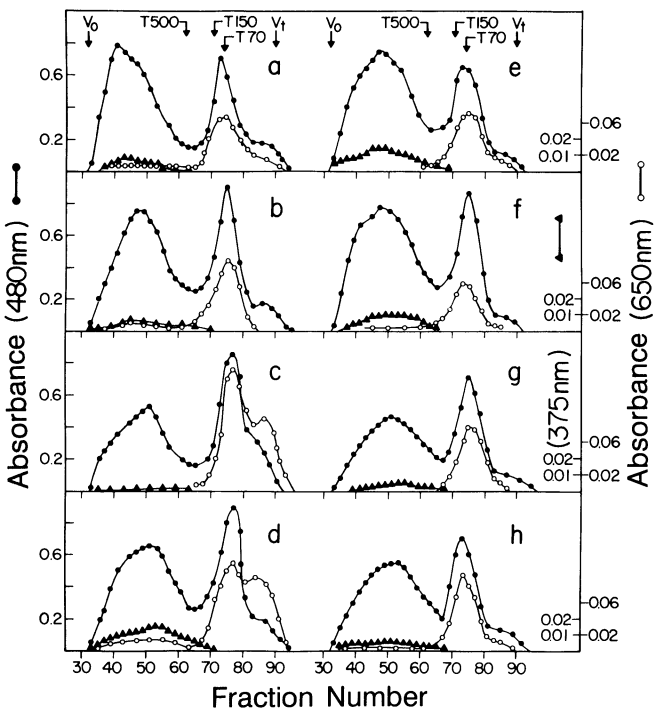

Fig. 2. Chromatography on a Sepharose CL-4B column (2.5 × 85 cm, eluted with 0.3% NaCl and 0.05% NaN_3, flow rate 25 ml/hr, 25°C) of pentosans isolated from various flours. a, Katepwa; b, Glenlea; c, Marshall; d, Fielder; e, HY 355; f, HY 320; g, Oslo; h, Norstar. Absorbance at 480, 375, and 650 nm corresponds to eluting carbohydrates (●), ferulic acid (▲), and proteins (○), respectively.

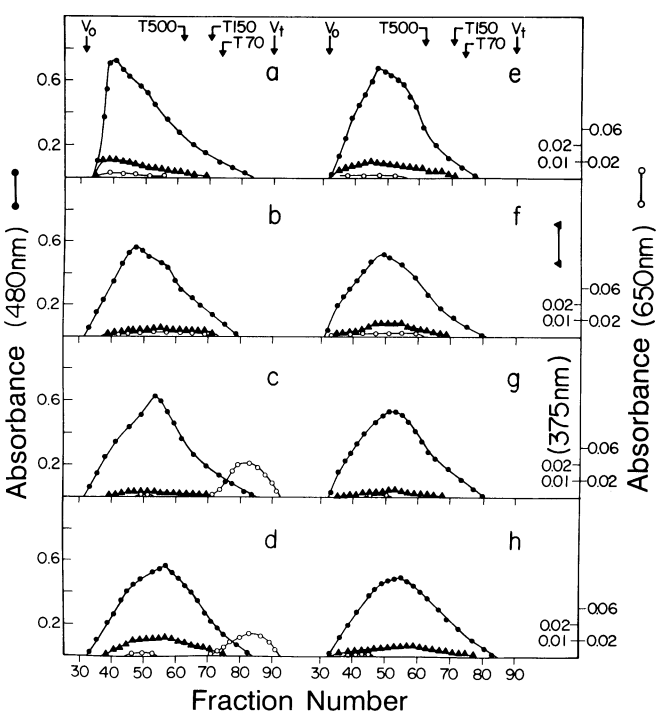

Fig. 3. Chromatography on a Sepharose CL-4B column (2.5 × 85 cm, eluted with 0.3% NaCl and 0.05% NaN_3, flow rate 25 ml/hr, 25°C) of arabinoxylans isolated from various flours. a, Katepwa; b, Glenlea; c, Marshall; d, Fielder; e, HY 355; f, HY 320; g, Oslo; h, Norstar. Absorbance at 480, 375, and 650 nm corresponds to eluting carbohydrates (●), ferulic acid (▲), and proteins (○), respectively.

TABLE VII
Amino Acid Composition of Arabinogalactan Peptides[a]
(mol/100 mol of Amino Acids)

Amino Acids	HY 355	HY 320	Oslo	Glenlea	Fielder	Norstar	Marshall	Katepwa
Alanine	21.8	20.8	20.3	18.8	15.3	18.8	13.8	18.0
Hydroxyproline	14.3	14.1	13.1	11.8	6.6	12.3	4.8	10.3
Isoleucine	2.0	1.8	2.0	2.1	2.4	2.2	2.8	2.3
Leucine	2.0	1.6	2.5	3.3	5.7	3.1	5.9	5.0
Methionine	1.2	1.2	1.1	1.3	1.6	1.4	2.0	1.1
Phenylalanine	1.1	1.0	1.4	1.3	1.8	1.4	1.9	1.5
Proline	3.5	5.4	4.7	6.3	6.1	5.4	6.8	4.4
Valine	5.1	5.9	5.4	5.3	5.5	5.4	6.1	5.7
Cystine	1.4	1.8	1.7	1.4	2.3	1.5	2.4	1.7
Glycine	4.1	4.2	4.6	5.1	7.0	4.5	6.7	5.2
Serine	9.4	9.0	9.8	9.2	8.5	9.7	8.7	9.6
Threonine	6.8	6.8	6.7	6.5	5.1	6.4	5.4	6.1
Tyrosine	3.9	3.2	2.9	3.1	2.8	3.0	2.3	3.7
Aspartic acid	7.3	7.5	7.8	8.2	7.9	8.1	7.8	7.8
Glutamic acid	12.8	12.4	12.9	12.3	14.4	12.6	15.7	13.0
Arginine	0.8	1.0	0.9	1.5	3.3	1.7	3.3	1.6
Lysine	1.7	2.1	1.8	2.2	3.0	2.2	3.6	2.5
Histidine	0.3	0.4	0.4	0.3	0.7	0.5	0.7	0.5

[a]Data represent means of triplicate analyses; the standard error of the mean was less than 6.0% of the mean values in all cases.

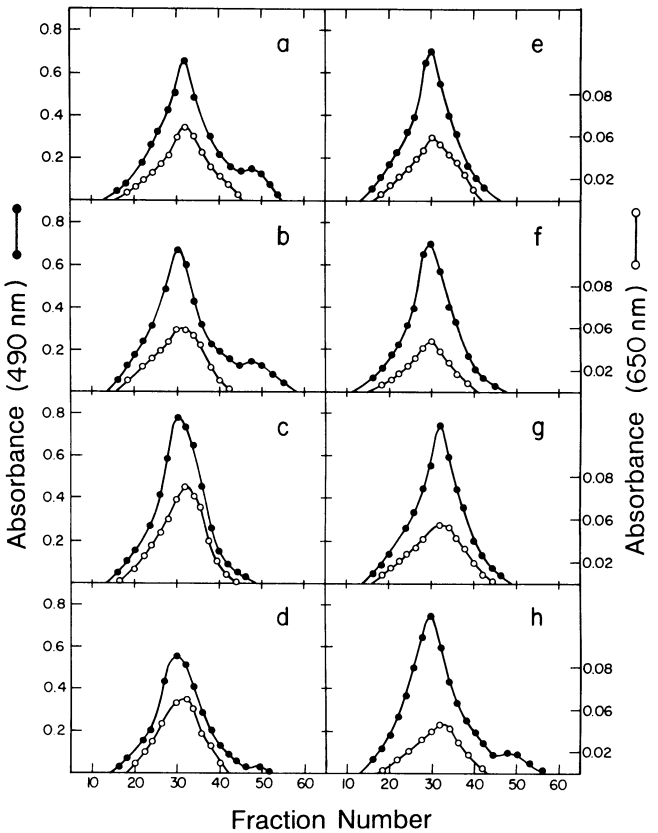

Fig. 4. Chromatography on a Sephacryl S-300 column (2.5 × 85 cm, eluted with 0.3% NaCl and 0.05% NaN_3, flow rate 25 ml/hr, 25°C) of arabinogalactans isolated from various flours. a, Katepwa; b, Glenlea; c, Marshall; d, Fielder; e, HY 355; f, HY 320; g, Oslo; h, Norstar. Absorbance at 490 and 650 nm corresponds to eluting carbohydrates (●) and proteins (○), respectively.

(Fig. 3). Therefore, ionic strength manipulation with this salt ("salting out") provides an effective means of fractionating the two polymeric constituents of water-soluble pentosans. Among varieties, differences were noted in the elution peak volume and size distribution of the arabinoxylans (Figs. 2 and 3). The most notable was the arabinoxylan from Katepwa, which eluted, in large portion, in the vicinity of the void volume, indicating a high molecular weight. The chromatograms of HY 355 and HY 320 also showed a greater proportion of high molecular weight species compared with those of Norstar, Fielder, Marshall, Oslo, and Glenlea. The proteinaceous material eluting in fractions 75–92 of the chromatograms of Marshall and Fielder (Fig. 3c and d) represents contaminating proteins; this is consistent with the data in Table II. The higher amounts of protein in pentosans of Marshall and Fielder is suprising, considering the similar protein contents among the various wheat flours (Table I) and the common purification scheme used for all pentosan preparations.

A finer gel medium (Sephacryl S-300) was also used to assess differences in the size and/or distribution of the low molecular weight arabinogalactan-peptide. However, the chromatograms of isolated arabinogalactan-peptides from the various flours were similar (Fig. 4). The small molecular weight material eluting in the shoulder of the peak of Katepwa, Glenlea, and Norstar contained α-D-glucans.

The protein in the arabinogalactan fraction of the eight varieties was subjected to amino acid composition analysis (Table VII). The results clearly indicate that this protein is distinctly different from wheat storage proteins because of its relatively high content of alanine and hydroxyproline and relatively low content of glutamic acid and proline. The high content of hydroxyproline is consistent with the report of Strahm et al (1981), according to which this amino acid is covalently linked to galactose, constituting the linkage between the arabinogalactan chain and the peptide moiety. Some variation in the peptide composition among wheat varieties is evident, but it is not clear whether this reflects genotypic differences or a small variation in arabinogalactan-peptide purity among the samples. Further research is needed to elucidate the structure of this peptide and to determine its functional role, if any, in the bread-making process.

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LITERATURE CITED

AMADO, R., and NEUKOM, H. 1985. Minor constituents of wheat flour: The pentosans. Pages 241-251 in: New Approaches to Research on Cereal Carbohydrates. R. D. Hill and L. Munck, eds. Elsevier Science Publishers: Amsterdam.

ANDREWARTHA, K. A., PHILLIPS, D. R., and STONE, B. A. 1979. Solution properties of wheat-flour arabinoxylans and enzymatically modified arabinoxylans. Carbohydr. Res. 77:191.

BANKS, W., GREENWOOD, C. T., and MUIR, D. D. 1970. The characterization of starch and its components. Part 2. The semi-micro

estimation of the starch content of cereal grains and related materials. Starch/Staerke 22:105.
BILIADERIS, C. G. 1980. Physicochemical studies on legume starches. Ph.D. dissertation. University of Saskatchewan, Saskatoon.
CIACCO, C. F., and D'APPOLONIA, B. L. 1982. Characterization of pentosans from different wheat flour classes and of their gelling capacity. Cereal Chem. 59:96.
COLE, E. W. 1967. Isolation and chromatographic fractionation of hemicelluloses from wheat flour. Cereal Chem. 44:411.
CROWE, N. L., and RASPER, V. F. 1988. The ability of chlorine and chlorine-related oxidants to induce oxidative gelation in wheat flour pentosans. J. Cereal Sci. 7:283.
D'APPOLONIA, B. L., and MacARTHUR, L. A. 1975. Comparison of starch, pentosans, and sugars of some conventional-height and semidwarf hard red spring wheat flours. Cereal Chem. 52:230.
DOUGLAS, S. G. 1981. A rapid method for the determination of pentosans in wheat flour. Food Chem. 7:139.
DUBOIS, M., GILLES, K. A., HAMILTON, J. K., REBERS, P. A., and SMITH, F. 1956. Colorimetric method for determination of sugars and related substances. Anal Chem. 28:350.
FINCHER, G. B., and STONE, B. A. 1974. A water-soluble arabinogalactan-peptide from wheat endosperm. Aust. J. Biol. Sci. 27:117.
FINCHER, G. B., SAWYER, W. H., and STONE, B. A. 1974. Chemical and physical properties of arabinogalactan-peptide from wheat endosperm. Biochem. J. 139:535.
FRY, S. C. 1982. Phenolic components of the primary cell wall. Biochem J. 203:493.
GUILLON, F., and THIBAULT, J. F. 1988. Further characterization of acid- and alkali-soluble pectins from sugar beet pulp. Lebensm. Wiss. Technol. 21:198.
HAGERMAN, A. E., and NICHOLSON, R. L. 1982. High-performance liquid chromatographic determination of hydroxycinnamic acids in the maize mesocotyl. J. Agric. Food Chem. 30:1098.
HARTLEY, R. D., and JONES, E. C. 1975. Effect of ultraviolet light on substituted cinnamic acids and the estimation of their *cis* and *trans* isomers by gas chromatography. J. Chromatography 107:213.
HARTLEY, R. D., and JONES, E. C. 1976. Diferulic acid as a component of cell walls of *Lolium multiflorum*. Phytochemistry 15:1157.
HOSENEY, R. C. 1984. Functional properties of pentosans in baked foods. Food Technol. 38(10):114.
JELACA, S. L., and HLYNKA I. 1971. Water-binding capacity of wheat flour crude pentosans and their relation to mixing characteristics of dough. Cereal Chem. 48:211.
JELACA, S. L., and HLYNKA, I. 1972. Effect of wheat-flour pentosans in dough, gluten, and bread. Cereal Chem. 49:489.
KIM, S. K., and D'APPOLONIA, B. L. 1977. Effect of pentosans on the retrogradation of wheat starch gels. Cereal Chem. 54:150.
KRYGIER, K., SOSULSKI, F., and HOGGE, L. 1982. Free, esterified, and insoluble-bound phenolic acids. 1. Extraction and purification procedure. J. Agric. Food Chem. 30:330.
LIN, F. M., and POMERANZ, Y. 1968. Characterization of water-soluble wheat flour pentosans. J. Food Sci. 33:599.
LINEBACK, D. R., KAKUDA, N. S., and TSEN, C. C. 1977. Carbohydrate composition of water-soluble pentosans from different types of wheat flours. J. Food. Sci. 42:461.
LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L., and RANDALL, R. J. 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193:265.
McCLEARY, B. V. 1986. Enzymic modification of plant polysaccharides. Int. J. Biol. Macromol. 8:349.
MEDCALF, D. C., D'APPOLONIA, B. L., and GILLES, K. A. 1968. Comparison of chemical composition and properties between hard red spring and durum wheat endosperm pentosans. Cereal Chem. 45:539.
NEUKOM, H. 1973. Chemistry and properties of the non-starchy polysaccharides (NSP) of wheat flour. Lebensm. Wiss. Technol. 6:59.
NEUKOM, H., and MARKWALDER, H. 1975. Isolation and characterization of an arabinogalactan from wheat flour. Carbohydr. Res. 39:387.
PATIL, S. K., TSEN, C. C., and LINEBACK, D. R. 1975. Water-soluble pentosans of wheat flour. I. Viscosity properties and molecular weights estimated by gel filtration. Cereal Chem. 52:44.
ROTTER, R. G., FROHLICH, A. A., MARQUARDT, R. R., and MILLS, P. A. 1989. Estimation of fungal contamination of cereal grains determined by measuring glucosamine concentration. Can. J. Anim. Sci. 69:235.
SHELTON, D. R., and D'APPOLONIA, B. L. 1985. Carbohydrate functionality in the baking process. Cereal Foods World 30:437.
STRAHM, A., AMADO, R., and NEUKOM, H. 1981. Hydroxyproline-galactoside as a protein-polysaccharide linkage in a water soluble arabinogalactan-peptide from wheat endosperm. Phytochemistry 20:1061.
THIBAULT, J., and ROMBOUTS, F. 1986. Effects of some oxidizing agents, especially ammonium peroxysulfate, on sugar-beet pectins. Carbohydr. Res. 154:205.
YEH, Y. F., HOSENEY, R. C., and LINEBACK, D. R. 1980. Changes in wheat flour pentosans as a result of dough mixing and oxidation. Cereal Chem. 57:144.

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