

# Effect of Molecular Size on Physical Properties of Wheat Arabinoxylan

Marta S. Izydorczyk\* and Costas G. Biliaderis

Food Science Department, University of Manitoba, Winnipeg, Manitoba R3T 2N2, Canada

A water-soluble wheat arabinoxylan was fractionated by molecular sieve chromatography into five polysaccharide fractions with limiting viscosities of 8.5 (F1), 6.2 (F2), 4.3 (F3), 3.8 (F4), and 3.4 (F5) dL/g. Compositional,  $^{13}\text{C}$  NMR, and  $^1\text{H}$  NMR analyses revealed very little structural variation among these fractions. The flow behavior of arabinoxylan fractions in dilute and concentrated solutions showed two critical concentrations ( $c^*$  and  $c^{**}$ ). Oxidative gelation of the arabinoxylan fractions, involving intermolecular cross-linking via feruloyl residues, was followed by dynamic rheometry. Almost linear relationships between the storage modulus ( $G'$ ) of the gel network and polymer concentration were found for all fractions. However, while  $G'$  values of F1 and F2 increased very rapidly with increasing polymer concentration, F3, F4, and F5 exhibited much less concentration dependence of  $G'$ . Positive linear relationships between rigidity ( $G'$ ) of cross-linked arabinoxylan gels and intrinsic viscosity  $[\eta]$  of arabinoxylan fractions were found. The effectiveness of cross-linking reactions,  $n_{e(\text{exp})}/n_{e(\text{theor})}$  (ratio of cross-link density experimentally determined to the theoretical value), increased with increasing molecular size of the arabinoxylan fractions. Fractions that formed networks with a high density of cross-linking (F1 and F2) were the most effective in their ability to entrap and hold water. The arabinoxylan fractions were also effective in stabilizing protein foams against thermal disruption in the order  $\text{F1} > \text{F2} > \text{F3} > \text{F4} > \text{F5}$ .

## INTRODUCTION

Arabinoxylan is the major nonstarch polysaccharide of wheat endosperm. Although it constitutes only a small portion (1-1.5%) of the wheat flour, this hydrocolloid has stimulated considerable interest due to its water absorption, viscosity enhancing, and gelling properties (Meuser and Suckow, 1986). The ability of arabinoxylan to form highly viscous solutions has an impact on the rheological behavior of doughs as well as the loaf volume and texture of bakery products (Meuser and Suckow, 1986; Delcour et al., 1991). The hydration properties of native arabinoxylans have a direct influence on the ability of flours to absorb water, while the high water-holding capacity of the cross-linked polymers (obtained via oxidative coupling of feruloyl residues) may affect the distribution of moisture among dough constituents and thereby alter the rheological properties of dough and bread systems (Meuser and Suckow, 1986; Izydorczyk et al., 1991a). Another important functional property ascribed to arabinoxylans is their possible involvement in gas retention by dough (Hoseney, 1984). Arabinoxylans, due to their viscosity and interfacial activity, may add to the elasticity and strength of the films surrounding the gas cells, thereby resulting in an even bread crumb texture (Izydorczyk et al., 1991a).

The composition and properties of arabinoxylans have been studied (Amado and Neukom, 1985; Girhammar et al., 1986; Izydorczyk et al., 199a,b), but the effect of molecular weight on the physical properties of these polysaccharides has not been systematically investigated. In the present work, arabinoxylans of different molecular sizes were obtained by gel filtration chromatography, and their solution and rheological properties were studied. An attempt was also made to identify relationships between molecular size and some physical properties of these water-soluble polymers.

## MATERIALS AND METHODS

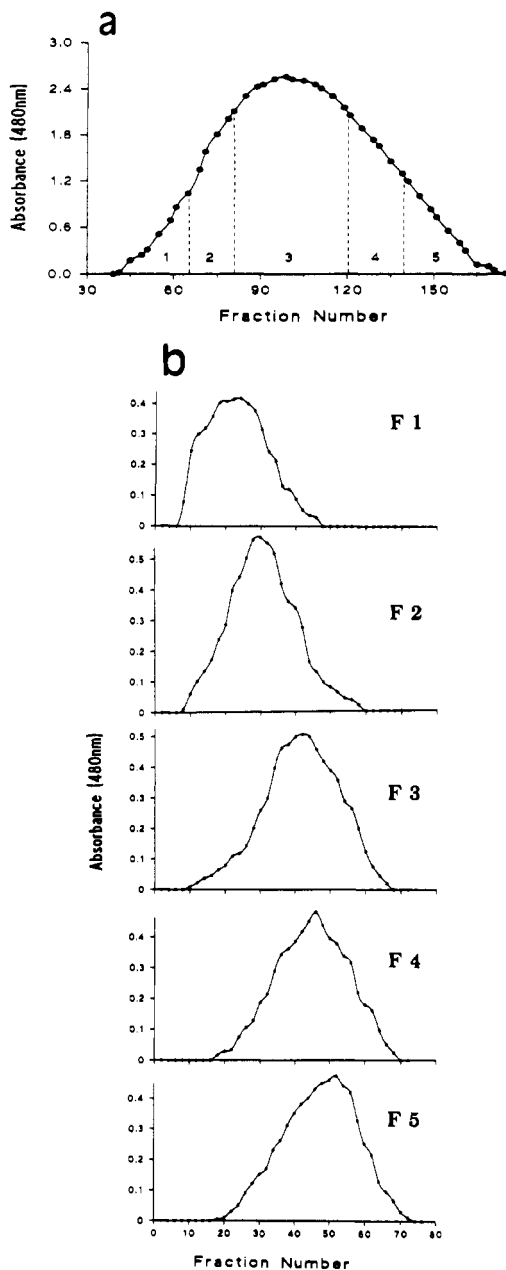
**Fractionation of Arabinoxylan.** Arabinoxylan was extracted and purified from a flour of Canada Western Red Spring wheat (variety Katepwa) according to the procedure previously

described (Izydorczyk et al., 1990). Preparative gel permeation chromatography was used to fractionate the arabinoxylan into various molecular mass fractions. Aliquots (50 mL) of the polysaccharide solution (1% w/w in 0.3% NaCl) were injected into a column (5.0  $\times$  90.0 cm) of Sepharose CL-2B (Pharmacia Fine Chemicals, Uppsala, Sweden) and eluted with degassed 0.3% NaCl containing 0.05%  $\text{NaN}_3$  at a flow rate of 40 mL/h at 25  $^\circ\text{C}$ . Effluent fractions (15 mL) were monitored for total carbohydrates according to phenol-sulfuric method (Dubois et al., 1956). A typical elution profile obtained is shown in Figure 1a. The procedure was repeated a number of times, and individual fractions from the various runs were pooled as indicated in Figure 1a to give five fractions, corresponding to five different molecular mass components present in the polysaccharide. Each fraction was concentrated in a rotary evaporator, dialyzed to remove all traces of NaCl and  $\text{NaN}_3$ , and finally freeze-dried. The fractions (10 mg) were rechromatographed on the analytical column (2.5  $\times$  85 cm) containing Sepharose CL-2B and eluted with degassed 0.3% NaCl at a flow rate of 30 mL/h; samples of 5.0 mL were collected.

**Chemical Analyses.** Protein content in arabinoxylans was determined according to the method of Lowry et al. (1951) using bovine serum albumin as a standard. The relative amounts of monosaccharides were determined by HPLC (Aminex HPX-87 column, 85  $^\circ\text{C}$ , flow rate 0.6 mL/min using deionized and degassed  $\text{H}_2\text{O}$ ) after hydrolysis with 1 M  $\text{H}_2\text{SO}_4$  for 2 h at 100  $^\circ\text{C}$  and neutralization with barium carbonate (Izydorczyk et al., 1991b). Phenolic acids were analyzed by HPLC as described previously (Izydorczyk et al., 1991b).

**NMR Spectroscopy.**  $^1\text{H}$  NMR spectra of arabinoxylan fractions (0.5% w/v in  $\text{D}_2\text{O}$ ) were recorded on a Bruker AM 300 spectrometer (300 Hz) at 85  $^\circ\text{C}$ . The chemical shifts were measured relative to sodium 3-(trimethylsilyl)tetradecatripropionate. Approximately 256 pulses were collected; pulse repetition time was 7.05 s and radio frequency pulse angle 68.6 $^\circ$ .  $^{13}\text{C}$  NMR spectra were obtained at 85  $^\circ\text{C}$  using the same instrument (75.9 MHz). The fractions (2% w/w) were dissolved in  $\text{H}_2\text{O}$  containing 10%  $\text{D}_2\text{O}$ . Approximately 30 000 pulses were collected; pulse repetition time was 1.24 s and radio frequency pulse angle 80.0 $^\circ$ . Chemical shifts are expressed in parts per million downfield from external  $\text{Me}_4\text{Si}$  but were actually measured by reference to internal 1,4-dioxane ( $\delta = 67.4$  ppm).

**Rheological Measurements.** For determination of the critical concentrations and coil overlap parameters the apparent viscosity of aqueous solutions of the arabinoxylan fractions (0.03-



**Figure 1.** (a) Elution profile of unfractionated arabinoxylan on the preparatory Sepharose CL-2B column (5.0 × 90.0 cm, 0.3% NaCl and 0.05%  $\text{NaN}_3$ , flow rate 40 mL/h, 25 °C, 15-mL fractions). (b) Elution profiles of arabinoxylan fractions on the analytical Sepharose CL-2B column (2.5 × 85.0 cm, 0.3% NaCl and 0.05%  $\text{NaN}_3$ , flow rate 30 mL/h, 25 °C, 5-mL fractions).

1.00% w/v) were measured with Ubbelohde capillary viscometers at 25 °C. All measurements were taken at the Newtonian region as verified by tests with viscometers of varying capillary diameters.

Steady shear viscosity measurements were also performed on a rotational Bohlin VOR rheometer (Bohlin Reology, Edison, NJ). Samples (2.0% w/v) were subjected to shear sweeps between 0.3 and 924  $\text{s}^{-1}$  at 20.0 ± 0.1 °C.

Arabinoxylan gels were obtained by adding horseradish peroxidase [0.22 purpurogallin unit (PU)/mL] and  $\text{H}_2\text{O}_2$  (1.5 ppm) to solutions of arabinoxylan fractions (0.20–2.50% w/v). The development of gel structure was monitored by small deformation oscillatory measurements using the Bohlin rheometer. After addition of the oxidant, the samples were placed between the parallel plates (30-mm diameter, 1-mm gap); a thin layer of light paraffin oil was added to cover the sample and prevent evaporation during measurements. The dynamic rheological parameters used to evaluate the gel networks were the storage modulus ( $G'$ ), loss modulus ( $G''$ ), and  $\tan \delta$  ( $\tan \delta = G''/G'$ ). All

measurements were conducted at 15.0 ± 0.1 °C at a frequency of 1 Hz and a maximum input strain of 4% for up to 4 h; due to relatively fast reaction kinetics, the  $G'$  of the cross-linked networks was reaching plateau values after 20–30 min. The theoretical and experimental values of the number of elastically effective covalent entanglement points ( $n_e$ ) were calculated from the equations developed by Flory (1953)

$$G' = [(f - 2)/2]n_eRT = [(f - 2)/2](c/M_e)RT$$

$$n_{e(\text{exp})} = 2G'/(f - 2)RT \quad n_{e(\text{theor})} = c/M_e$$

where  $G'$  is the plateau storage modulus,  $f$  is the functionality of the cross-linking agent (ferulic acid),  $c$  is the total polymer concentration ( $\text{kg}/\text{m}^3$ ),  $R$  is the gas constant,  $T$  is the absolute temperature, and  $M_e$  is the molecular weight between two cross-link points ( $\text{kg}/\text{mol}^{-1}$ ). The value of  $f$  was assumed to be 4, whereas the theoretical value of  $M_e$  was calculated for each fraction, thus taking into account the slight differences in the content of feruloyl groups among the fractions.

**Hydration Capacity of Cross-Linked Arabinoxylan.** Cross-linked arabinoxylans were prepared by addition of horseradish peroxidase (0.22 PU/mL) and  $\text{H}_2\text{O}_2$  (1.5 ppm) to polysaccharide solutions (1.0% w/v). The resulting gels were disrupted, washed with  $\text{H}_2\text{O}$ , and centrifuged for 10 min at 20000g. The gels were then washed with 80% ethanol, and the precipitated polysaccharides were filtered, dried, and finely ground. The dry product ( $\approx 20$  mg) was mixed with an excess of distilled  $\text{H}_2\text{O}$ , and after swelling for 48 h, the hydrated gel was filtered and weighed. The degree of hydration was expressed as grams of  $\text{H}_2\text{O}$  absorbed per gram of dry cross-linked polymer.

**Surface Tension Measurements.** The decrease of the surface tension of water with addition of arabinoxylan fractions was monitored with a Fisher surface tensiometer (Fisher Scientific, Winnipeg, MB). Solutions of 0.5% (w/v) concentration were placed in a dish (diameter 5.5 cm, depth 4 cm); the changes in surface tension were recorded every 5 min until the values reached equilibrium. The surface tension of distilled water was 72.0 ± 0.5 dyn/cm. Deesterified arabinoxylan (fraction F3 only) was obtained after treatment of the polymer with NaOH: 0.15 g of the material was dissolved in 10.0 mL of 2 N NaOH, and after 3 h at room temperature (under  $\text{N}_2$ ), the solution was acidified (pH 2.0) with HCl, dialyzed, and freeze-dried. The surface tension of the deesterified arabinoxylan was determined as described above.

**Foam Stabilization.** The effect of the arabinoxylan fractions on formation and stabilization of foam formed by a surface active protein [bovine serum albumin (BSA)] was studied by adding 0.25 mL of arabinoxylan solutions (1.0% w/v) to 1.0 mL of 2% BSA. The solutions were mixed well (30 s), and the foam volume was recorded immediately. The test tubes were subsequently placed in a water bath at 95 °C and the changes in foam volume noted after 3 min of heating.

## RESULTS AND DISCUSSION

The arabinoxylan from wheat endosperm, because of its polydispersity, exhibits a broad molecular mass distribution upon gel permeation chromatography on Sepharose CL-2B. Five fractions of different molecular size were obtained by fractionation of the native arabinoxylan using molecular sieve chromatography. The profiles of the individual arabinoxylan fractions on the analytical column, presented in Figure 1b, show a progressive decrease in the molecular size in the order F1 > F2 > F3 > F4 > F5. The obtained limiting viscosity values of these fractions (Table I) also indicate the same order of decreasing molecular size. The yields of the five fractions, their protein contents, and their monosaccharide compositions are given in Table I. A total recovery of 76.4% was obtained, and in view of the numerous steps involved in the experimental procedure this value was considered acceptable, assuming that an equal proportion of polysaccharide was lost to all five fractions. Most of the material

Table I. Physical and Chemical Characteristics of Arabinoxylan Fractions

fraction	yield, <sup>a</sup> %	[ $\eta$ ], dL/g	protein, %	ferulic acid, mg/g	Glu	Xyl	Ara	Xyl/Ara
unfrac		5.4	1.41 $\pm$ 0.05	nd <sup>b</sup>	3.4	59.3	37.3	1.50
F1	6.4	8.5	2.12 $\pm$ 0.02	1.15 $\pm$ 0.05	0	59.2	40.8	1.45
F2	12.1	6.2	1.20 $\pm$ 0.01	1.37 $\pm$ 0.06	0	59.6	40.4	1.47
F3	38.6	4.3	1.23 $\pm$ 0.02	1.23 $\pm$ 0.07	3.1	58.4	38.5	1.52
F4	13.6	3.8	1.83 $\pm$ 0.02	1.43 $\pm$ 0.10	4.3	57.9	37.8	1.53
F5	7.1	3.4	2.01 $\pm$ 0.02	1.30 $\pm$ 0.02	4.8	58.3	36.8	1.58

<sup>a</sup> Yield of fractions recovered from total material applied onto the column. <sup>b</sup> Not determined.

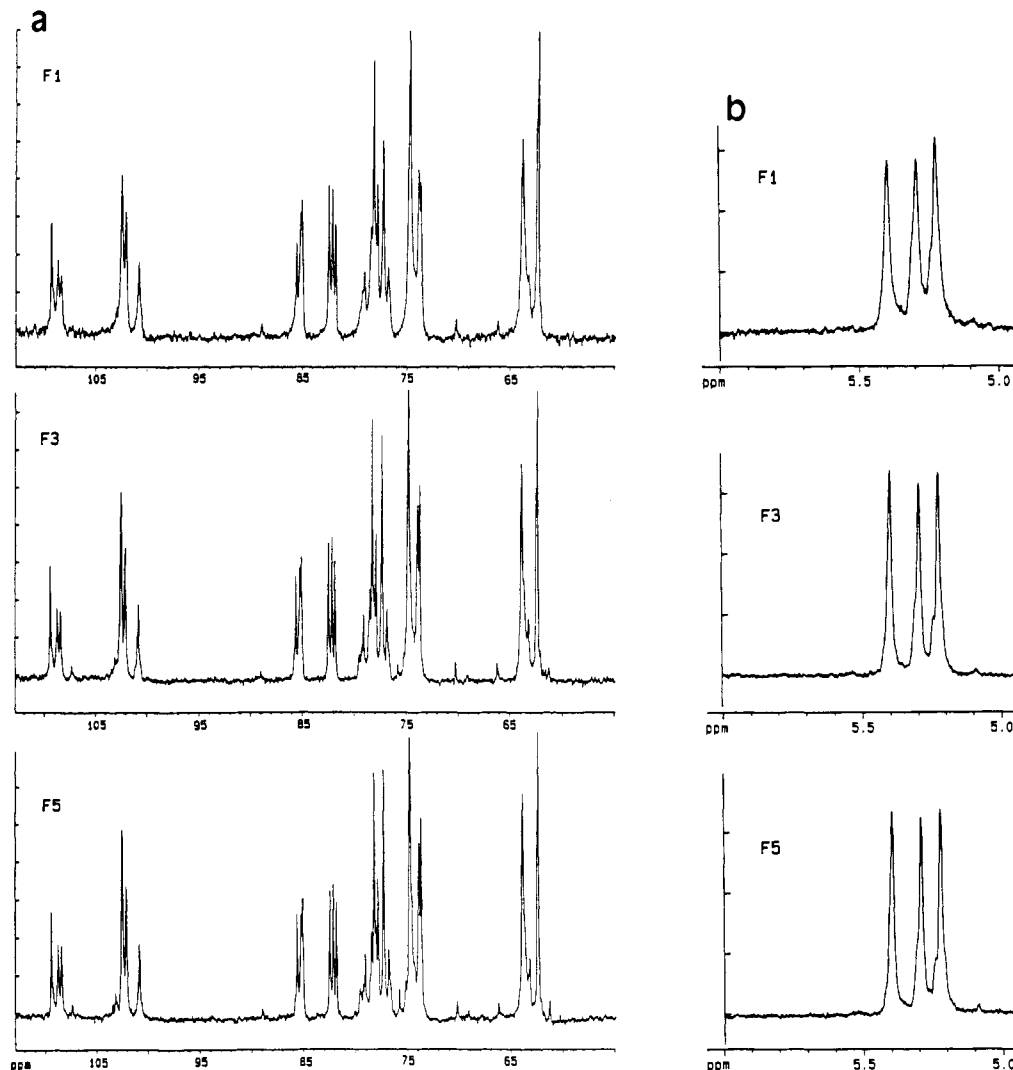
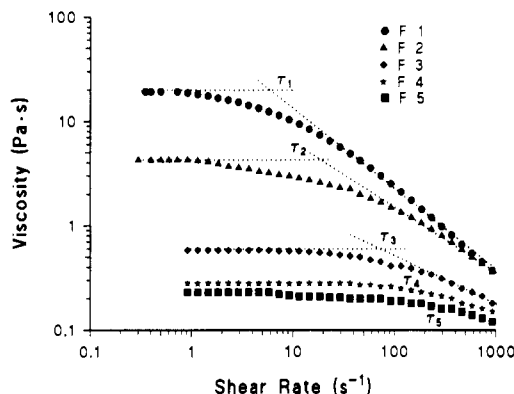


Figure 2. (a) <sup>13</sup>C NMR spectra of arabinoxylan fractions (10% D<sub>2</sub>O). The chemical shifts were assigned relative to 1,4-dioxane. (b) <sup>1</sup>H NMR spectra of arabinoxylan fractions (D<sub>2</sub>O). The chemical shifts were assigned relative to Me<sub>3</sub>SiC<sub>2</sub>D<sub>2</sub>CO<sub>2</sub>Na.

was recovered in fraction F3 (38.6%). Unfractionated arabinoxylan consisted mainly of xylose and arabinose, with a relative molar ratio of the two sugars 1.50:1.00. Small amounts of glucose and proteins were also found. After fractionation, the proteinaceous material was almost equally distributed among the fractions, with the exception of F1 and F5 which contained slightly higher amounts of protein. There was a small increase in the molar ratio of xylose to arabinose from F1 to F5, suggesting a slight decrease in the degree of substitution with decreasing molecular size. Glucose was detected only in the lower molecular mass fractions (F3, F4, and F5). It is likely that this monosaccharide originates from a small molecular weight glucan contaminant, rather than that being an integral part of the arabinoxylan structure. In contrast with a previous report (Yeh et al., 1980) that ferulic acid is confined to the high molecular weight part of polydis-

persed wheat arabinoxylan preparations, our results showed equal amounts of this phenolic for all fractions (Table I).

Only minor structural differences among the arabinoxylan fractions were shown by NMR spectroscopy. According to the data published by Bock et al. (1984), Ebringerova et al. (1990), and Izydorczyk and Biliaderis (1992), the resonances in the region of 108–110 ppm of the <sup>13</sup>C NMR spectra are attributed to anomeric carbons of  $\alpha$ -L-arabinofuranosyl residues (Figure 2a). The peaks at 109.4 and 108.7 ppm correspond to C-1 carbons of  $\alpha$ -L-Araf residues linked to a single xylose at O-2 and O-3, respectively, whereas the peak at 108.4 ppm corresponds to C-1 of  $\alpha$ -L-Araf linked to a xylose residue at O-3 only. As shown by the spectra in Figure 2a, the relative intensity of the peak at 108.4 ppm slightly increased from F1 to F5, indicating somewhat lower amounts of single  $\alpha$ -L-Araf



**Figure 3.** Effect of shear rate on the apparent viscosity of aqueous solutions of the arabinoxylan fractions (2.0% w/v) at 20 °C.

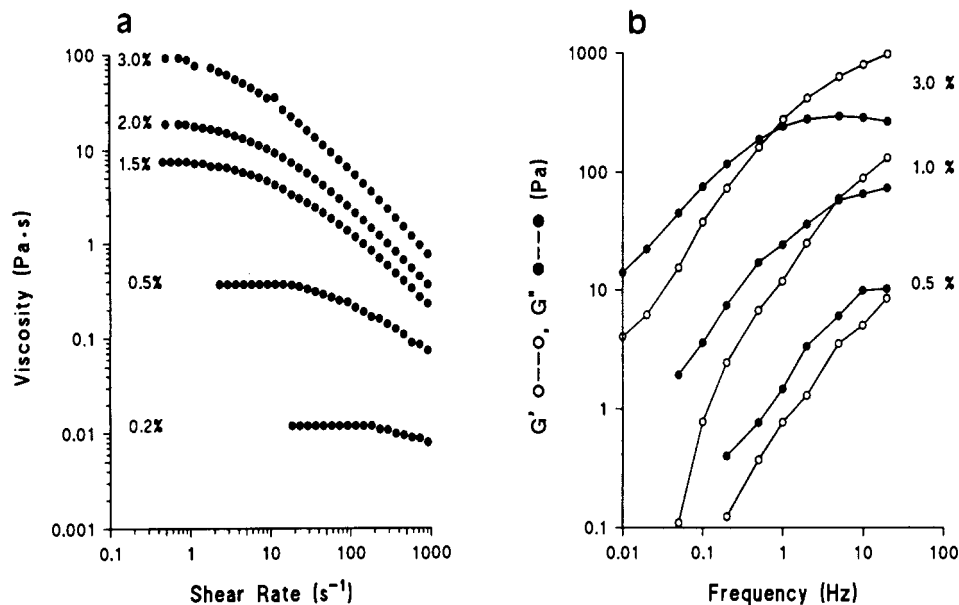
linked to xylose residues at O-3 for fraction F1. This observation was confirmed by the  $^1\text{H}$  NMR spectra (Figure 2b). The intensity of the resonance at 5.4 ppm, which has been assigned to the anomeric proton of Ara $\beta$  linked to O-3 of the xylopyranosyl residues, was slightly smaller for F1 than for F3 and F5. In agreement with the  $^{13}\text{C}$  NMR spectra, no distinguishable differences were observed in the amounts of  $\alpha$ -L-Ara $\beta$  residues linked to the same xylose at O-2 and O-3 (signals 5.22 and 5.28 ppm, respectively, in the proton spectra) among the fractions. The anomeric carbons of xylose residues are responsible for the peak multiplicity at 100–103 ppm in the  $^{13}\text{C}$  NMR spectra. According to the recently published work of Hoffmann et al. (1991), the resonance at 100.7 ppm corresponds to C-1 of  $\beta$ -D-Xylp residues doubly substituted (1 $\rightarrow$ 3 and 1 $\rightarrow$ 2) with arabinose. The 101.9 ppm resonance has been assigned to C-1 of unsubstituted xylose residues adjacent to mono- and/or disubstituted xyloses at their nonreducing end. The remaining unsubstituted xyloses as well as the monosubstituted (1 $\rightarrow$ 3) xylose residues contribute to the signal at 102.4 ppm. The multiplicity of peaks at 60–85 ppm is due to resonances of nonanomeric carbons of both, xylose and arabinose; because of the complex structure of wheat arabinoxylan, some of these resonances have not yet been fully resolved.

**Viscosity.** The viscosity vs shear rate curves for arabinoxylan fractions dissolved in  $\text{H}_2\text{O}$  (2.0% w/v) are compared in Figure 3. Although a shear thinning behavior was observed for all samples, the overall pattern of the flow curves differed substantially. Fractions F1 and F2 were most affected by changing the shear rate, whereas fractions F3, F4, and F5 exhibited Newtonian behavior at low shear rates. In fact, the beginning of shear rate zone marked by  $\tau$  ( $\tau$  being the inverse of shear rate,  $\dot{\gamma}_c$ , at which the apparent viscosity becomes shear rate dependent) (Castelain et al., 1987) was progressively shifted toward higher shear rates in the order of decreasing molecular weight of the samples. Also, the power law indices  $n$  ( $n$  being defined as  $\eta = k\dot{\gamma}^{n-1}$ , where  $\eta$  is viscosity and  $\dot{\gamma}$  shear rate) were 0.16, 0.35, 0.63, 0.74, and 0.79 for F1, F2, F3, F4, and F5, respectively, thus showing that shear thinning behavior was most pronounced for F1 and F2. The flow curves of F1 in dilute and concentrated solutions are presented in Figure 4a. It is noteworthy that a shear thinning behavior was observed for a polymer concentration as low as 0.2% (w/v). Dynamic rheological measurements also indicated that only fractions F1 and F2 exhibit elastic properties. The variation of the loss ( $G''$ ) and storage ( $G'$ ) moduli with frequency showed that with increasing polymer concentration the viscoelastic behavior of F1 changes from that of a viscous solution ( $G''$

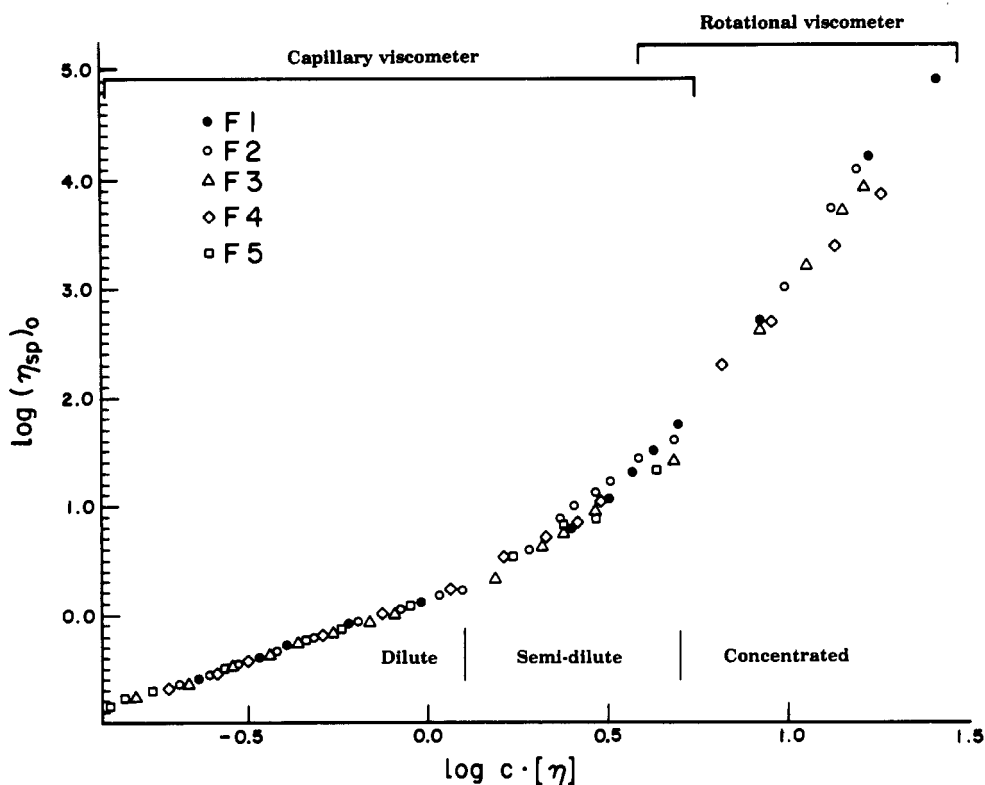
$> G'$  at all frequencies) to weakly elastic ( $G' > G''$  at higher frequencies) (Figure 4b). Also, the beginning of the elastic zone, when  $G'$  becomes greater than  $G''$ , was progressively shifted toward lower frequencies with increasing polymer concentration. Similar behavior was observed for fraction F2. Fractions F3, F4, and F5, on the other hand, exhibited behavior of dilute solutions, with  $G''$  exceeding  $G'$  at all frequencies, even at concentrations as high as 5.0% (w/v) (results not shown).

The concentration dependence of the specific viscosity at zero shear rate ( $\eta_{sp}$ ) $_0$  for the arabinoxylan fractions is shown in Figure 5, the concentration being expressed by the dimensionless parameter  $c[\eta]$ . Such plots are classically used for polymer solutions to take into account differences between hydrodynamic dimensions among different samples and to determine the transition from the dilute to the concentrated regimes. Close examination of the data reveals two critical concentrations,  $c^*$  and  $c^{**}$ , which delimit three distinct regimes of ( $\eta_{sp}$ ) $_0$  dependence on concentration: dilute, semidilute, and concentrated with the slopes being 1.1, 2.0–2.6, and 3.7–3.9, respectively (Table II). The intermediate zone between the dilute ( $c < c^*$ ) and concentrated ( $c > c^{**}$ ) domains is not observed with randomly coiled polysaccharides; for such polymers the  $\log(\eta_{sp})_0$  vs  $\log c[\eta]$  curve can be divided in two straight lines, with slopes of approximately 1.0 and 3.5 at low and high polymer concentrations, respectively (Castelain et al., 1987). In contrast, the existence of three domains appears to be characteristic of polymers with rigid, rodlike conformation (Cuvelier and Launay, 1986; Chauveteau, 1986). It seems that for rod polymers the transition between the dilute and concentrated regimes is smoother than for flexible polymers. The first critical concentration ( $c^*$ ) is related to the onset of coil overlap; above  $c^*$ , the contraction of the coils progressively brings the chains to their unperturbed dimensions, and beyond the second critical concentration ( $c^{**}$ ) the chain dimensions become independent of concentration (Castelain et al., 1987). The transition domain at intermediate concentrations has already been reported for some synthetic polymers as well as for xanthan and hydroxymethylcellulose (Cuvelier and Launay, 1986). The observed rheological behavior of arabinoxylan is not surprising, since this polymer has been shown to assume a fully extended rodlike shape in solutions (Andrewartha et al., 1979). In the dilute zone, where the hydrodynamic units do not overlap, a good superposition of data for all fractions on the same straight line with a slope of 1.1 was observed (Figure 5; Table II). In the intermediate concentration zone,  $c^* < c < c^{**}$ , a progressive decrease of the slopes from 2.6 to 2.0 for F1–F5 was shown (Table II). In the concentrated domain, the differences in terms of slopes are not surprising. Above  $c^*$  and  $c^{**}$  the flow behavior of macromolecules is dictated greatly by the intermolecular association of the coils. The differences in flow characteristics in these domains may arise from structural variations among the fractions. Small differences in the branching patterns (smooth vs hairy regions) and/or length of branches (which are only partly taken into account by the term  $c[\eta]$ ) may affect the chain stiffness of these polymers and, therefore, their flow characteristics. According to the theoretical predictions, the two critical concentrations  $c^*$  and  $c^{**}$ , progressively increased from F1 to F5, being lowest for the highest molecular weight fraction F1 and highest for the lowest molecular weight fraction F5 (Table II).

**Gelation.** The ability of arabinoxylan fractions to form three-dimensional gel networks after treatment of the samples with an oxidizing agent (peroxidase/ $\text{H}_2\text{O}_2$ ) was



**Figure 4.** (a) Effect of shear rate on the apparent viscosity of fraction F1 at various polymer concentrations. (b) Frequency dependence of storage ( $G'$ ) and loss ( $G''$ ) moduli for fraction F1 at various polymer concentrations.



**Figure 5.** Concentration dependence of zero shear specific viscosity ( $\eta_{sp}$ )<sub>0</sub> for aqueous solutions of arabinoxylan fractions defining the three concentration domains.

investigated in this study by small amplitude shear stress oscillatory testing. Figure 6 shows the effect of polymer concentration on the elastic properties of gels, whereas Table III summarizes the additional rheological parameters of these networks. As illustrated in Figure 6, almost linear relationships between the storage modulus ( $G'$ ) and polymer concentration were found for all fractions. However, while  $G'$  values of fractions F1 and F2 increased very rapidly with increasing polymer concentration, fractions F3, F4, and F5 exhibited much lower concentration dependence of the storage modulus. It is also evident that the ability of arabinoxylans to form gel networks is related to their molecular weight. At any polymer concentration, the storage modulus was much higher for fractions with

higher molecular weight. In fact, significant correlations ( $p < 0.01$ ) between the rigidity of the cross-linked arabinoxylan gels, as manifested by  $G'$ , and the intrinsic viscosity ( $[\eta]$ ) of the native polysaccharides at three different concentration levels were found (Figure 7).

Although the storage modulus is a good indicator of gel strength, another parameter,  $\tan \delta$ , which describes the relative changes of  $G'$  and  $G''$  with respect to each other, is also commonly used to characterize cross-linked networks. A high value of  $\tan \delta$  ( $>1$ ) implies a liquidlike character of a viscoelastic system, while a low value ( $<0.1$ ) points to a more elastic character (Ross-Murphy, 1984). As shown in Table III, values of  $\tan \delta$  below 0.1 were obtained generally for F1, F2, and F3, indicating strong

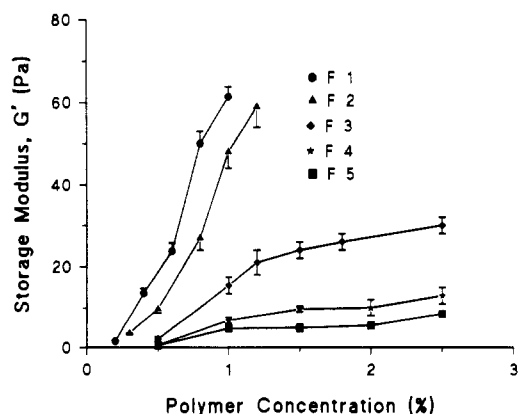
**Table II. Critical Concentrations and Slopes of the Regression Lines  $\log(\eta_{sp})_0$  vs  $\log c[\eta]$  in the Three Flow Regimes for Arabinoxylan Fractions**

fraction	$c^*$ , g/dL	$c^{**}$ , g/dL	slopes		
			$c < c^*$	$c^* < c < c^{**}$	$c > c^{**}$
F1	0.17	0.27	1.1	2.6	3.9
F2	0.20	0.30	1.1	2.4	3.7
F3	0.28	0.73	1.1	2.2	3.8
F4	0.29	1.05	1.1	2.0	3.9
F5	0.30	nd <sup>a</sup>	1.1	2.0	nd

<sup>a</sup> nd, not determined.**Table III. Rheological Parameters and Cross-Link Density of Arabinoxylan Networks<sup>a</sup>**

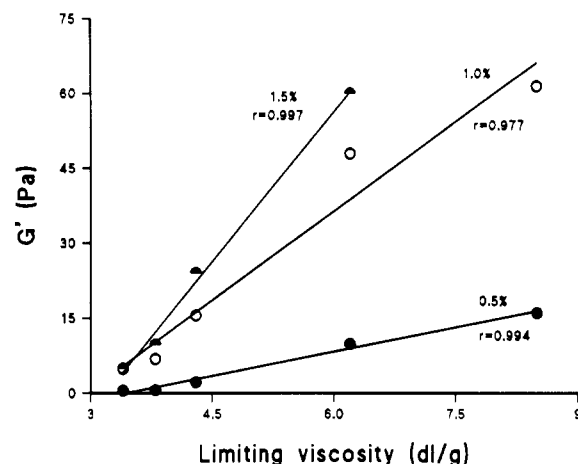
fraction	polymer concn, %	$G'$ , Pa	$\tan \delta$	$n_{e(\text{exp})}$	$n_{e(\text{theor})}$	$n_{e(\text{exp})}/n_{e(\text{theor})}$
				$\times 10^{-3}$ , mol m <sup>-3</sup>	$\times 10^{-2}$ , mol m <sup>-3</sup>	
F1	0.20	1.59 ± 0.10 <sup>b</sup>	0.22	0.66	1.2	0.055
	0.40	13.50 ± 1.50	0.07	5.6	2.4	0.232
	0.60	23.80 ± 2.20	0.07	9.9	3.6	0.271
	0.80	50.10 ± 3.00	0.06	21.0	4.8	0.440
	1.00	61.40 ± 5.00	0.10	27.0	5.9	0.460
F2	0.3	3.59 ± 0.03	0.21	1.5	2.1	0.071
	0.5	9.84 ± 1.50	0.06	4.1	3.5	0.117
	0.8	27.00 ± 3.50	0.06	11.3	5.6	0.200
	1.0	48.00 ± 4.25	0.07	20.0	7.0	0.280
F3	1.2	59.20 ± 5.00	0.13	25.0	8.4	0.300
	0.5	2.19 ± 0.52	0.17	0.9	3.1	0.029
	1.0	15.50 ± 2.05	0.08	6.5	6.3	0.103
	1.2	21.44 ± 3.20	0.08	8.9	7.6	0.117
	1.5	24.07 ± 4.05	0.16	10.0	9.5	0.105
F4	1.8	26.70 ± 4.00	0.41	11.0	11.5	0.095
	2.5	30.90 ± 5.50	0.70	13.0	15.9	0.082
	0.5	0.63 ± 0.02	0.51	0.3	3.7	0.007
	1.0	6.80 ± 0.80	0.12	2.8	7.5	0.037
	1.5	9.73 ± 1.20	0.26	4.1	11.2	0.037
F5	2.0	10.20 ± 3.00	0.62	4.2	14.9	0.028
	2.5	13.30 ± 2.5	0.76	5.5	18.6	0.029
	0.5	0.54 ± 0.05	0.60	0.2	3.4	0.006
	1.0	4.93 ± 1.00	0.19	2.0	6.7	0.030
	1.5	4.97 ± 1.05	0.49	2.1	10.1	0.021
	2.0	5.53 ± 1.08	0.94	2.3	13.5	0.017
	2.5	8.55 ± 0.90	0.97	3.6	16.9	0.020

<sup>a</sup> Solutions of arabinoxylan fractions were treated with horseradish peroxidase (0.22 PU/mL) and H<sub>2</sub>O<sub>2</sub> (1.5 ppm); the reported values are those obtained after 3 h of reaction. <sup>b</sup>  $n = 3 \pm \text{SD}$ .



**Figure 6.** Concentration dependence of storage modulus  $G'$  for arabinoxylan fractions after 1 h of treatment (plateau values) with peroxidase (0.22 PU/mL) and H<sub>2</sub>O<sub>2</sub> (1.5 ppm) at 15 °C (frequency 1.0 Hz, strain less than 4%).

gel networks only for these fractions. Interestingly, the relationship between  $\tan \delta$  and polymer concentration did not parallel that of  $G'$  vs polymer concentration. With initial rising of polymer concentration, the values of  $\tan \delta$  decreased, reached a minimum at an intermediate



**Figure 7.** Relationships between storage modulus ( $G'$ ) of cross-linked gels (plateau values) and limiting viscosity ( $[\eta]$ ) of arabinoxylan fractions at three levels of polymer concentration.

concentration, and rose thereafter. The effect was especially pronounced with fractions F3–F5. The intermediate concentration of the polymer corresponding to the lowest value of  $\tan \delta$  appears, therefore, to be the optimal one leading to the formation of the best cross-linked network. It seems that at a very low polymer concentration there are not enough chains available for extensive intermolecular interactions and cross-linking, whereas at higher concentrations the effectiveness of cross-linking might be reduced due to restrictions in chain diffusion. Both cases would lead to networks of high  $\tan \delta$ . According to the theory of rubber elasticity (Flory, 1953), the value of the plateau storage modulus obtained by mechanical measurements is directly related to the number of elastically effective chains per unit volume. Table III contains the values for the number of effective cross-links calculated theoretically ( $n_{e(\text{theor})}$ ) as well as from the  $G'$  values ( $n_{e(\text{exp})}$ ). The high values of  $n_{e(\text{theor})}$  correspond to an ideal network where all of the available feruloyl moieties participate in the cross-links among network chains. In reality, gels prepared by cross-linking of macromolecules in solution contain a significant amount of network imperfections and, therefore, the ratio  $n_{e(\text{exp})}/n_{e(\text{theor})}$  reflects the effectiveness of the cross-linking reaction (Kulicke and Nottelman, 1989). As can be gathered from Table III, the effectiveness of cross-linking for the high molecular weight fractions F1 and F2 lies between 5 and 46% and between 7 and 30%, respectively, and were much greater than for the remaining lower molecular weight fractions. With initial increases in concentration of these polymers, the ratio  $n_{e(\text{exp})}/n_{e(\text{theor})}$  rose rapidly, but it started to level off at higher concentrations. In contrast, fractions F3–F5 exhibited maximum number of effective cross-links at intermediate concentrations, followed by a decline with further concentration increases. Interestingly, the intermediate concentrations of arabinoxylans with the highest numbers of effective cross-links corresponded well with the lowest  $\tan \delta$  values which are indicative of strong networks for such cross-linked hydrogels.

**Water-Holding Capacity.** Cross-linked arabinoxylans do not dissolve in an aqueous environment but instead swell and imbibe large amounts of water. As can be seen from Figure 8, the amount of water absorbed and held varied significantly among the fractions. Cross-linked networks of fractions F1 and F2 exhibited much higher water-holding capacity than those of F4 and F5. The ranking of the samples in terms of their ability to imbibe water coincides with the cross-link density of arabinoxylan networks. It seems that fractions which formed

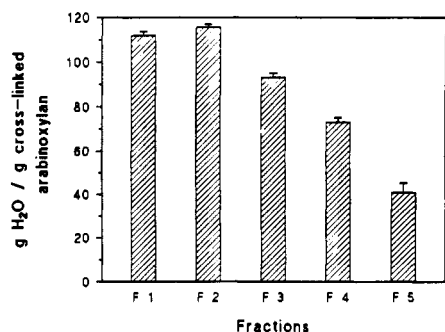


Figure 8. Water-holding capacity of cross-linked arabinoxylan fractions.

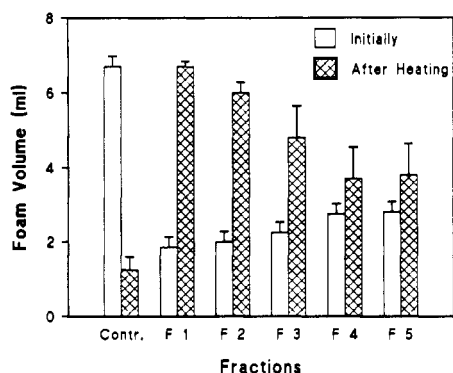


Figure 9. Effect of arabinoxylan fractions on the foam volume of bovine serum albumin (BSA) before and after heating (3 min, 95 °C). Arabinoxylan solutions (0.25 mL of 1.0% w/v) were added to 2% BSA (1 mL) and mixed well.

networks with a high degree of cross-linking (as indicated by a relatively high ratio  $n_{e(\text{exp})}/n_{e(\text{theor})}$ ; Table III) are more effective in their ability to entrap and hold water. The fact that sample F1 (with the highest cross-link density) held less water than F2 suggests, however, that there is an optimal network density above which the degree of swelling (i.e., the amount of water allowed to penetrate the network) is impeded and consequently the water-holding capacity decreases.

**Interfacial Properties.** Although polysaccharides are not generally considered as surface active agents, it has been previously shown that arabinoxylans from various wheat flours can lower the surface tension of water (Izydorczyk et al., 1991a). In the present study all fractions displayed almost equal capacities to reduce the surface tension ( $\sigma$ ) of water regardless of their molecular weight. The  $\sigma$  values of solutions containing 0.5% (w/v) arabinoxylans were 54.7, 54.8, 52.6, 52.9, and 50.0 dyn/cm for F1, F2, F3, F4, and F5, respectively. Deesterification of F3, which resulted in a complete removal of feruloyl residues, only slightly altered the surface properties of this fraction ( $\sigma = 54.0$  dyn/cm). This finding suggests that ferulic acid does not affect the interfacial properties of the arabinoxylan fractions.

The protective action of arabinoxylan fractions against thermal disruption of foams formed by the surface active protein (BSA) is demonstrated by the results of Figure 9. The control solution of 2% (w/v) BSA exhibited high foaming power (as indicated by the large foam volume) but low foam stability upon heating. Addition of arabinoxylans to BSA generally reduced the initial foam volume. Since the formation of foam is usually impeded by increasing viscosity of the liquid medium, the highly viscous fractions F1 and F2 did indeed exert the greatest negative effect on the foamability of BSA solutions. The presence of arabinoxylan in BSA solutions has proven,

however, beneficial during the thermal treatment. The ranking of arabinoxylan fractions in terms of their increasing ability to protect gas cells against thermal disruption coincided with the increasing viscosity of these polymers. Fraction F1 was the most effective in stabilizing the foam during heating and fraction F5 the least.

**Conclusions.** The findings of the present study clearly indicate that the molecular weight of wheat arabinoxylans is an important determinant of their physical properties in an aqueous environment. For instance, the ability of these water-soluble polymers to stabilize protein foams during heating increased with their size. High molecular weight arabinoxylans also exhibited a greater potential to form rigid cross-linked hydrogels with large water-holding capacity. In view of the observed molecular size-physical property responses, arabinoxylans from various wheats, differing in their size and polydispersity, could add to the variability in functional properties of their flours and the respective dough systems.

#### ACKNOWLEDGMENT

Support from the Natural Sciences and Engineering Research Council of Canada in the form of operating and equipment grants is gratefully acknowledged.

#### LITERATURE CITED

- Amado, R.; Neukom, H. Minor constituents of wheat flour. The pentosans. In *New Approaches in Research on Cereal Carbohydrates*; Hill, R. D., Munck, L., Eds.; Elsevier Science Publishers: Amsterdam, 1985.
- Andrewartha, K.; Philips, D. R.; Stone, B. A. Solution properties of wheat-flour arabinoxylans and enzymatically modified arabinoxylans. *Carbohydr. Res.* 1979, 77, 191-204.
- Bock, K.; Pederson, C.; Pederson, H. Carbon-13 nuclear magnetic resonance data for oligosaccharides. *Adv. Carbohydr. Chem. Biochem.* 1984, 42, 193-225.
- Castelain, C.; Doublie, J. L.; Lefebvre, J. A study of the viscosity of cellulose derivatives in aqueous solutions. *Carbohydr. Polym.* 1987, 7, 1-16.
- Chauveteau, G. Fundamental criteria in polymer flow through porous media. *Adv. Chem. Ser.* 1986, No. 213.
- Cuvelier, G.; Launay, B. Concentration regimes in xanthan gum solutions deduced from flow and viscosity properties. *Carbohydr. Polym.* 1986, 6, 321-333.
- Delcour, J. A.; Vanhamel, S.; Hosney, R. C. Physicochemical and functional properties of rye nonstarch polysaccharides. II. Impact of a fraction containing water-soluble pentosans and proteins on gluten-starch loaf volumes. *Cereal Chem.* 1991, 68, 72-76.
- Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A.; Smith, F. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* 1956, 28, 350-356.
- Ebringerova, A.; Hromadkova, Z.; Petrakova, E.; Hricovini, M. Structural features of a water-soluble L-arabino-D-xylan from rye bran. *Carbohydr. Res.* 1990, 160, 259-265.
- Flory, P. J. In *Principles of Polymer Chemistry*; Cornell University Press: Ithaca, NY, 1953.
- Girhammar, U.; Nakamura, M.; Nair, B. M. Water-soluble pentosans from wheat and rye—chemical composition and some physical properties in solution. In *Gums and Stabilisers for the Food Industry 3*; Phillips, G. O., Wedlock, D. J., Williams, P. A., Eds.; Elsevier Applied Science: London, 1986.
- Hoffmann, R. A.; Roza, M.; Maat, J.; Kamerling, J. P.; Vliegert, J. F. G. Structural characteristics of the cold-water-soluble arabinoxylans from the white flour of the soft wheat variety Kadet. *Carbohydr. Polym.* 1991, 15, 415-430.
- Hosney, R. C. Functional properties of pentosans in baked foods. *Food Technol.* 1984, 38, 114-117.
- Izydorczyk, M. S.; Biliaderis, C. G. Influence of structure on the physicochemical properties of wheat arabinoxylan. *Carbohydr. Polym.* 1992, 17, 237-247.

- Izydorczyk, M. S.; Biliaderis, C. G.; Bushuk, W. Oxidative gelation studies of water-soluble pentosans from wheat. *J. Cereal Sci.* **1990**, *11*, 153-169.
- Izydorczyk, M. S.; Biliaderis, C. G.; Bushuk, W. Physical properties of water-soluble pentosans from different wheat varieties. *Cereal Chem.* **1991a**, *68*, 145-150.
- Izydorczyk, M. S.; Biliaderis, C. G.; Bushuk, W. Comparison of the structure and composition of water-soluble pentosans from different wheat varieties. *Cereal Chem.* **1991b**, *68*, 139-144.
- Kulicke, W.-M.; Nottelmann, H. Structure and swelling of some synthetic, semisynthetic, and biopolymer hydrogels. *Adv. Chem. Ser.* **1989**, No. 223.
- Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **1951**, *193*, 265-275.
- Meuser, F.; Suckow, P. Non-starch polysaccharides. In *Chemistry and Physics of Baking*; Blanshard, J. M. V., Frazier, P. J., Galliard, T., Eds.; The Royal Society of Chemistry; Burlington House: London, 1986.
- Ross-Murphy, S. B. Rheological methods. In *Biophysical Methods in Food Research*; Chan, H. W.-S., Ed.; Blackwell Scientific Publications: Oxford, U.K., 1984.
- Yeh, Y. F.; Hosney, R. C.; Lineback, D. R. Changes in wheat flour pentosans as a result of dough mixing and oxidation. *Cereal Chem.* **1980**, *57*, 144-148.

Received for review June 25, 1991. Accepted January 2, 1992.

Registry No. Arabinoxylan, 9040-27-1.