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Influence of structure on the physicochemical properties of wheat arabinoxylan

Marta S. Izydorczyk & Costas G. Biliaderis

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

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A water-soluble arabinoxylan from wheat flour was purified and fractionated by a graded ammonium sulphate fractionation technique. The yields (based on the total amount of the material recovered) of the four polysaccharide fractions obtained at 60% (F60), 70% (F70), 80% (F80), and 95% (F95) saturation with $(NH_4)_2SO_4$ were 58·8%, 29·2%, 7·6%, and 4·4%, respectively. Monosaccharide, ^{1}H-NMR, and ^{13}C-NMR analyses revealed differences in structural characteristics among the fractions. F60 had the lowest arabinose:xylose ratio (0·58), followed by F70 (0·71), F80 (0·85), and F95 (0·88). The relative amount of Xyl*p* residues doubly substituted at O-2 and O-3 with Ara*f* increased substantially from F60 to F95, whereas the amount of unsubstituted and monosubstituted Xyl*p* decreased in the same order. The content of ferulic acid was much higher in F60 than in the remaining samples; F60 also showed the highest intrinsic viscosity (4·70 dl/g) followed by F70 (4·20 dl/g), F80 (3·16 dl/g), and F95 (1·90 dl/g). Calculated coil overlap parameter values ($c^*[\eta]$: 1·20–1·30) were much lower than those of other random coil polysaccharides, implying a rather stiff/extended chain conformation. Oxidative gelation of arabinoxylan fractions was probed by dynamic rheometry. Fraction F60 formed rigid gel networks, as assessed by the storage modulus (G') and tan δ (G''/G') values, while F70 and F80 exhibited poor gelling capacity. The arabinoxylan fractions were also effective in stabilizing protein foams against thermal disruption in the order of F60 > F70 > F80.

INTRODUCTION

Water-soluble wheat flour arabinoxylan is a high molecular weight component of pentosans, a heterogeneous mixture of non-starch polysaccharides of wheat endosperm. Since the early methylation studies of Perlin (1951) and Montgomery & Smith (1955) it has been generally believed that wheat arabinoxylan consists of a linear chain backbone of $(1\rightarrow4)$-β-xylopyranosyl units to which single α-L-arabinofuranosyl residues are attached through O-2 and/or O-3 atoms of the xylose residues. Also feruloyl groups esterified to arabinose residues are present in this polysaccharide. The relatively high ratio of arabinose to xylose confers a rigid rod-like conformation to the molecule and it is partly responsible for the high viscosity of arabinoxylan solutions (Andrewartha *et al.*, 1979). Arabinoxylan also exhibits a high degree of polydispersity as revealed by broad molecular weight distributions upon chromatography on Sepharose 4B (Fincher & Stone, 1974). Structural differences in terms of ferulic acid content, ratio of constituent monosaccharides and molecular size have been shown among arabinoxylans of different wheat varieties (D'Appolonia & MacArthur, 1975; Lineback *et al.*, 1977; Ciacco & D'Appolonia, 1982; Izydorczyk *et al.*, 1991*a*).

The continuing interest in arabinoxylans arises from the undeniable role they play in dough development and their beneficial effect on loaf properties. McCleary (1986) has shown that degradation of these polymers *in situ* leads to formation of doughs with low strength and poor consistency which result in loaves with reduced height and unacceptable crumb structure. The functional properties of arabinoxylans in baked products are thought to be exerted through their high water absorbing capacity and their ability to form hydrated networks either via chain entanglements

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(concentration-dependent effect) or via covalent cross-linking between ferulic acid residues present in adjacent arabinoxylan chains (free radical mediated process) (Meuser & Suckow, 1986). While previous studies have focused on the structure and/or functional properties of arabinoxylans, there has been very little attempt to establish relationships between molecular structure and physicochemical properties of this polysaccharide. In the present report, water-soluble arabinoxylan from wheat endosperm was fractionated by stepwise ammonium sulphate precipitation and several fractions of diverse molecular structure and molecular size were obtained. The solution properties and rheological behavior of these materials were investigated and related to their molecular characteristics.

EXPERIMENTAL

Isolation, purification, and fractionation of arabinoxylans

The water-soluble pentosans were extracted from a Canada Western Red Spring wheat flour and purified according to the procedure of Izydorczyk *et al.* (1990). The arabinoxylan component was isolated from the purified pentosans by precipitation with saturated $(NH_4)_2SO_4$ (grade I, Sigma Chemical Co, St Louis, MO) (Fincher & Stone, 1974). The precipitate was then redissolved in hot water, dialyzed against distilled H_2O for three days and freeze-dried (yield: 0·30% of flour).

The purified arabinoxylan was fractionated by a graded ammonium sulphate fractionation technique. The material was dissolved (0·2% w/v) in phosphate buffer (0·1 M, pH 7) and ammonium sulphate was added slowly (2 h) up to 60% saturation; the solution was allowed to stand overnight at 20°C. The precipitated polysaccharide was collected by filtration on a glass fiber paper, redissolved in H_2O and dialyzed until free of $(NH_4)_2SO_4$; fraction designated as F60. The saturation level of $(NH_4)_2SO_4$ in the remaining filtrate was subsequently adjusted to 70%, 80%, and 95%, stepwise. The corresponding precipitated polysaccharide fractions are designated as F70, F80, and F95, respectively.

Chemical analyses

Protein content of the arabinoxylan fractions was determined by 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°C, flow rate 0·6 ml/min using deionized and degassed H_2O) after hydrolysis with 1M H_2SO_4 for 2 h at 100°C and neutralization with barium carbonate. Phenolic acids were analyzed as described previously (Izydorczyk *et al.*, 1991a).

Gel permeation chromatography was performed on a Sepharose CL-4B column (2·5 × 85 cm). Solutions of arabinoxylan (3 ml, 0·5% w/v in 0·3 NaCl) were applied into the column and eluted with degassed 0·3% NaCl containing 0·05% NaN_3 at a flow rate of 25 ml/h at 25°C. The void and total volumes of the column were determined with Blue Dextran and xylose, respectively. Effluent fractions (5 ml) were monitored for total carbohydrates by the phenol-sulphuric method (Dubois *et al.*, 1956).

NMR spectroscopy

^{1}H-NMR spectra (300 Hz) were recorded at 85°C on a Bruker AM 300 FT spectrometer. Approximately 256 pulses were collected; pulse repetition time was 7·05 s and r.f. pulse angle 68·6°. Arabinoxylan fractions (0·5% w/v) were dissolved in D_2O. Sodium 3-trimethylsilyl-tetradeuteriopropionate was used as a reference.

Natural-abundance proton decoupled ^{13}C-NMR spectroscopy (75·9 MHz) was performed at 85°C using the above instrument. The fractions (2·0% w/v) were dissolved in H_2O containing 10% D_2O. Approximately 30 000 pulses were collected; pulse repetition time was 1·245 s and r.f. pulse angle 80·0°. Chemical shifts (δ) are expressed in ppm downfield from external Me_4Si but were actually measured by reference to internal 1,4-dioxane (δ = 67·4 ppm).

Rheological measurements

Viscosity measurements of aqueous solutions of arabinoxylan fractions (0·03–1·0% w/v) were performed using Ubbelohde viscometers (International Research Glassware, Kenilworth, NJ) at 25 ± 0·05°C. All measurements were taken at the Newtonian (zero shear rate) range as verified by tests with viscometers of different capillary diameter. A Bohlin VOR rheometer (Bohlin Reologi, Edison, NJ) was used to determine viscosity as a function of shear rate. All measurements were carried out at 25 ± 0·1°C using a concentric cylindrical geometry; the radii of the inner bob and the outer rotor were 7 and 7·7 mm, respectively. Samples (2·0% w/v) were subjected to shear sweeps between 3 and 924 s^{-1}; the reported values are averages of three replicates.

Solutions of increased viscosity or gels were obtained by treating the arabinoxylan fractions (various polymer concentrations) with horseradish peroxidase (0·22 purpurogallin units/ml) and H_2O_2 (1·5 ppm). Gel structure development was monitored with the Bohlin VOR rheometer operated in the oscillatory mode. Immediately after addition of the oxidant, the samples were placed between the parallel plates (30 mm diameter, 1 mm gap); excess material was removed and a thin layer of mineral oil was added to cover the upper plate and prevent evaporative losses throughout the measurements. All measurements were performed at 15 ± 0·1°C at a frequency of 1·0 Hz using a torsion bar of 19·3 g cm and maximum input strain of 4% for periods up to 4 h. The monitored parameters included storage modulus (G'), loss modulus (G''), dynamic viscosity (η'), and tan δ (G''/G'). The dynamic properties of the networks formed after gelation were also examined as a function of oscillatory frequency (0·02-20·0 Hz).

Effect of arabinoxylan on stability of protein foams

The effect of the arabinoxylan fractions on formation and stabilization of foam formed by a surface active protein (bovine serum albumin) were studied by adding 0·25 ml arabinoxylan solutions (1·0% w/v) to 1 ml of a 2% BSA solution. The solutions were mixed well (30 s) and the volume of foam 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 composition of the arabinoxylan obtained from the water-soluble pentosans of Canadian Hard Red Spring flour is given in Table 1. The polysaccharide was composed mainly of xylose and arabinose. Small amounts of glucose and protein were also detected. The high degree of substitution of the xylan backbone with arabinose residues is characteristic of wheat endosperm arabinoxylans, and the ratio of arabinose to xylose varies usually between 0·83–0·50, depending on the origin (wheat variety) of arabinoxylan (D'Appolonia & MacArthur, 1975; Lineback *et al.*, 1977; Ciacco & D'Appolonia, 1982; Izydorczyk *et al.*, 1991a). It is still unclear, however, whether the small amounts of glucose and proteins are an integral part of the arabinoxylan structure or whether they are mere contaminants.

Stepwise addition of ammonium sulphate to the solution of purified arabinoxylan resulted in four polysaccharide fractions. Yields and composition of each fraction are presented in Table 1. Based on the total amount of material recovered after fractionation, the majority (58·8%) of the polysaccharide precipitated at 60% saturation with $(NH_4)_2SO_4$. Another fraction (29·2% yield) was recovered after adjustment of the saturation level to 70%. Two small fractions (7·6 and 4·4% yields) were obtained at 80% and 95% saturation with $(NH_4)_2SO_4$, respectively. Fraction F60 contained substantially more protein material than the other fractions, and it had the least substituted xylan backbone, as revealed by the lowest arabinose to xylose ratio. The increasing arabinose to xylose ratio is indicative of a progressive increase in the degree of substitution from F60 to F95.

The content of ferulic acid also varied significantly among the fractions (Table 2). Fraction F60 contained more than twice the amount of ferulic acid than that in the remaining samples. Moreover, there was a shift in

Table 1. Yield and composition of native arabinoxylan and its fractions

Arabinoxylan	Yield[a] (%)	Protein (%)	Molar composition (%)			
			Glu	Xyl	Ara	Ara/Xyl
Unfractionated	—	2·67 ± 0·08[b]	3·8	57·0	39·2	0·68
F60	58·8	3·90 ± 0·02	4·8	60·3	35·9	0·58
F70	29·2	0·93 ± 0·02	—	58·6	41·4	0·71
F80	7·6	1·20 ± 0·02	1·5	53·3	45·2	0·85
F95	4·4	1·19 ± 0·02	2·8	51·7	45·5	0·88

[a] Based on total amount of material recovered.
[b] Lowry method, $n = 3 \pm$ SD.

Table 2. Ferulic acid content of arabinoxylan fractions

Fraction	Ferulic acid (mg/g)[a]			
	Total	*cis-*	*trans-*	*cis:trans*
F60	1·88 ± 0·03	0·72 ± 0·04	1·16 ± 0·02	1:1·61
F70	0·88 ± 0·01	0·44 ± 0·01	0·44 ± 0·01	1:1·00
F80	0·66 ± 0·05	0·35 ± 0·04	0·31 ± 0·03	1:0·88
F95	0·70 ± 0·04	0·36 ± 0·05	0·34 ± 0·01	1:0·94

[a] HPLC method, mg/g arabinoxylan.

the relative amounts of *cis* and *trans* isomers in the fractions; ferulic acid in the *trans* form was predominant in the F60, while almost equimolar amounts of *cis* and *trans* ferulic acid were found for the other fractions.

Differences among fractions were also revealed by gel filtration chromatography on Sepharose CL-4B (Fig. 1). Unfractionated arabinoxylan yielded a broad non-symmetrical peak. Although most of the arabinoxylan eluted in the vicinity of the void volume, part of it extended into the region of small molecular weight. The elution profile of fraction F60 was similar to that of unfractionated arabinoxylan, except that it lacked the low molecular weight species. In contrast, the majority of fraction F95 eluted in the low molecular weight region. However, even for the F95 fraction there is evidence for high molecular weight eluting species. Fractions F70 and F80 eluted in between the two extreme peaks of F60 and F95. The differences in the molecular weight distributions among fractions were also confirmed by limiting viscosity measurements (Table 3). Fraction F60 showed the highest $[\eta]$ (4·70 dl/g) followed by F70 (4·20 dl/g) and F80 (3·19 dl/g). An unusual low $[\eta]$ (1·90 dl/g) was found for F95.

NMR spectroscopy

^{1}H-NMR analysis of the arabinoxylan fractions (Fig. 2) revealed basically two groups of signals: one in the region of 5·22–5·40 ppm and the other at 4·40–4·80 ppm. According to the recent studies by Hromadkova *et al.* (1987), Bengtsson & Aman (1990), Ebringerova *et al.* (1990) and Herath *et al.* (1990) these resonances are attributed to anomeric protons of α-L-arabinofuranosyl and β-D-xylopyranosyl residues, respectively. The resonance at 5·40 ppm can be unequivocally assigned to H-1 of Ara*f* linked to O-3 of xylopyranosyl residues; the relative intensity of this signal decreased from F60 to F95. Anomeric protons of Ara*f* linked to O-2 and O-3 of the same Xyl*p* residue are responsible for peaks at 5·22 and 5·28 ppm, respectively. Since the relative intensity of the resonances at 5·22 and 5·28 ppm increase from F60 to F95, the relative amount of doubly substituted xyloses in these fractions must increase in the same order. It has also been suggested from methylation studies that the $\beta(1 \rightarrow 4)$ xylopyranosyl backbone of some arabinoxylans can be substituted, in addition to single Ara*f* residues, with short chains of two or more Ara*f* residues which are interlinked via $(1 \rightarrow 2)$, $(1 \rightarrow 3)$, or $(1 \rightarrow 5)$ linkages (Ebringerova *et al.*, 1990; Herath *et al.*, 1990). The unresolved signals at 5·25 and 5·41 ppm, visible especially in the spectra of F70, F80, and F95, could arise from the presence of small amounts of arabinosyl chains in these fractions. The complexity of substitution of the xylopyranosyl residues is also reflected by the broad peak around δ 4·50; the

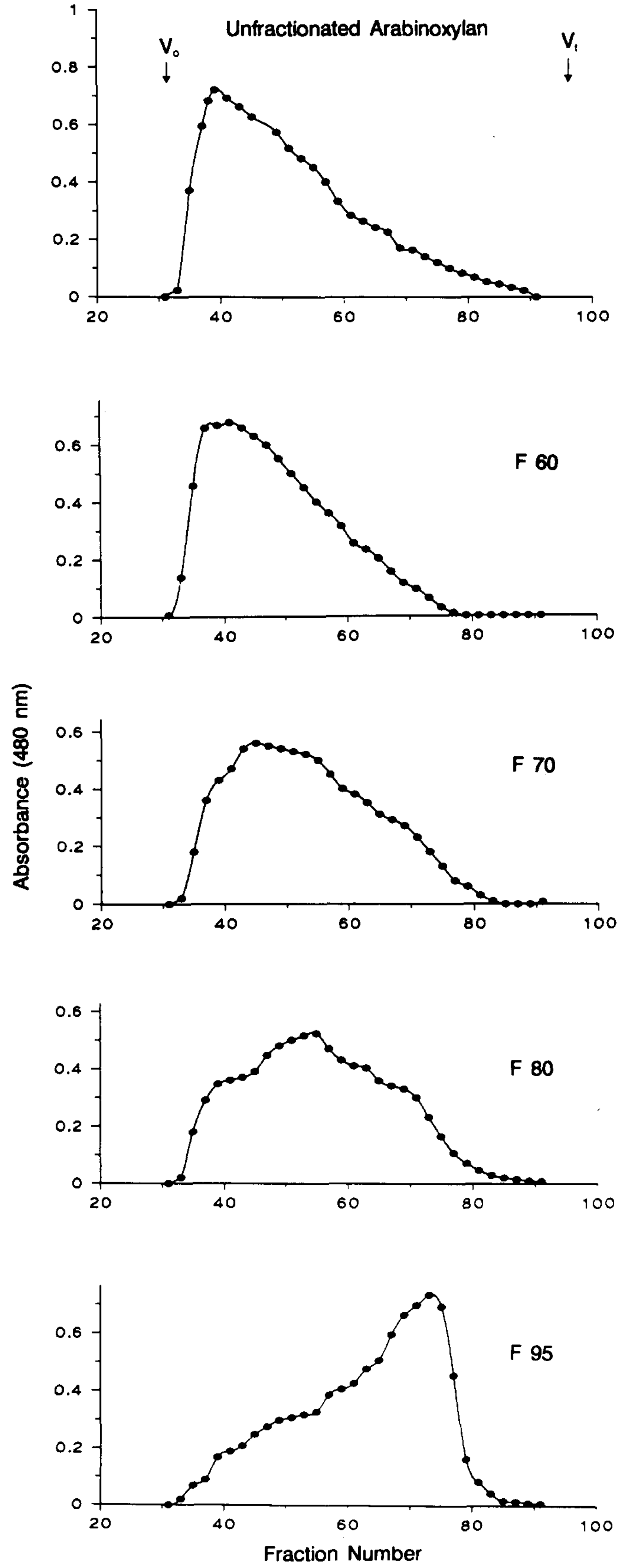

Fig. 1. Gel filtration chromatography on Sepharose CL-4B (2·5 × 85 cm, 0·3% NaCl and 0·05% NaN$_3$, flow rate 25 ml/h, 25°C) of the unfractionated arabinoxylan and its fractions obtained by fractional precipitation with $(NH_4)_2SO_4$.

Table 3. Limiting viscosity and coil overlap parameters corresponding to the dilute and entangled domains

Arabinoxylan fraction	$[\eta]$ (dl/g)	c^{*a} (g/100 ml)	Coil overlap ($c^*[\eta]$)	Slope[b]	
				Dilute domain	Entangled domain
F60	4·70	0·26	1·24	1·13	2·19
F70	4·20	0·31	1·30	1·12	2·04
F80	3·16	0·38	1·20	1·07	2·00
F95	1·90	ND[c]	ND	ND	ND

[a] Critical concentration, c^*.
[b] Slopes of log $(\eta_{sp})_0$ versus log $c[\eta]$ on both sides of the critical concentration.
[c] Not determined.

peak at 4·67 ppm was assigned to the double branched xylopyranosyl residues (Ebringerova *et al.*, 1990). In agreement with the monosaccharide analysis, integrations of the signals in the anomeric regions revealed a lower ratio of arabinose to xylose for the F60 compared to the other samples.

The differences in the structural features of the arabinoxylan fractions were also confirmed by ^{13}C-NMR spectra (Fig. 3). Assignments of the signals were based on published data of Bock *et al.* (1984) on xylo-oligosaccharides and Ebringerova *et al.* (1990) on rye arabinoxylan. The resonances at 109·4 and 108·7 ppm correspond to C-1 of α-L-Ara*f* residues linked to a single xylose at O-2 and O-3, respectively, whereas that at 108·4 ppm to C-1 of α-L-Ara*f* linked to a xylose residue at O-3 only. In agreement with the proton spectra (δ 5·40) the relative intensity of the latter peak progressively decreased from F60 to F95. The peak multiplicity for C-1 of xylose at 100–103 ppm is also indicative of the complex branching pattern of the wheat arabinoxylan. Resonances at 102·4, 101·9, and 100·7 ppm correspond to unsubstituted, monosubstituted (1 → 3), and doubly substituted (1 → 3 and 1 → 2) β-D-Xyl*p*, respectively. Changes in the relative intensities of these resonances further support the notion that the amount of unsubstituted xylose decreases and that of doubly substituted xylose increases from F60 to F95. Other regions of resonances assigned in the carbon spectra (Bengtsson & Aman, 1990) are at 85·0–85·1, 82·0–82·4, 78·1, and 62·2 ppm for C-4, C-2, C-3, and C-5 of α-arabinofuranosyl residues, respectively. Resonances around 63·5 and 63·7 ppm correspond to C-5 of substituted and unsubstituted xylose residues, respectively; note the relative changes in the intensities of these peaks from F60 to F95. Because of the complex structure of wheat arabinoxylan, the multiple resonances in the regions of 73·6–74·7 (signal contribution from C-2, C-3, and C-4 of β-D-Xyl*p*) and 76·7–79·0 ppm (signal contribution from C-3 and C-4 of β-D-Xyl*p* and C-3 of α-L-Ara*f*) cannot be fully resolved. Finally, signals at 66·1 and 70·1 ppm correspond to C-5 and C-4, respectively, of the terminal non-reducing β-D-Xyl*p* residues (Bock *et al.*, 1984). The progressive increase in the size of these peaks from F60 to F95 is consistent with a decreasing molecular weight of these fractions as evidenced by their molecular weight distributions (Fig. 1) and their $[\eta]$ values (Table 3).

Concentration dependence of zero shear rate viscosity

The solution behavior of arabinoxylan fractions (F60, F70, and F80) was examined by measuring the 'zero shear' specific viscosity $(\eta_{sp})_0$ at various concentrations of the polysaccharide. At low polymer concentrations (dilute domain), $(\eta_{sp})_0$ increased approximately linearly with increasing concentration, but at higher concentrations (entangled domain) the slopes changed abruptly to much higher values (Table 3; Fig. 4). This transition is characteristic for polysaccharides whose behavior in solution is influenced by the extent to which individual polymer molecules interact with each other (Launay *et al.*, 1986). The abrupt increase in the concentration dependence of $(\eta_{sp})_0$ corresponds to the onset of coil overlap between the polymer chains, and the critical concentration (c^*) at which it occurs depends on the volume occupied by each molecule. The differences in the values of c^* among the arabinoxylan fractions most likely reflect differences in the chain length and/or backbone stiffness among these polymers. The dimensionless coil overlap parameter ($c^*[\eta]$), a measure of the total volume occupied by all coils within the polymer solution regardless of their type and molecular weight (Morris *et al.*, 1981), was also obtained from the plot of log $(\eta_{sp})_0$ versus log $c[\eta]$ (Fig. 4, inset). The values of coil overlap parameter for all arabinoxylan fractions were much lower than those reported for other random coil polysaccharides (dextran, carboxymethylamylose, high mannuronate alginate, lambda carrageenan, ($c^*[\eta] \simeq 4$; Morris *et al.*, 1981) but close to those found for relatively stiff and extended polymers like hyaluronate ($c^*[\eta] \simeq 2{\cdot}5$; Morris *et al.*, 1981), xanthan gum and succinoglycan ($c^*[\eta] \simeq 0{\cdot}8$; Gravanis *et al.*, 1987) or guar gum ($c^*[\eta] \simeq 1{\cdot}3$;

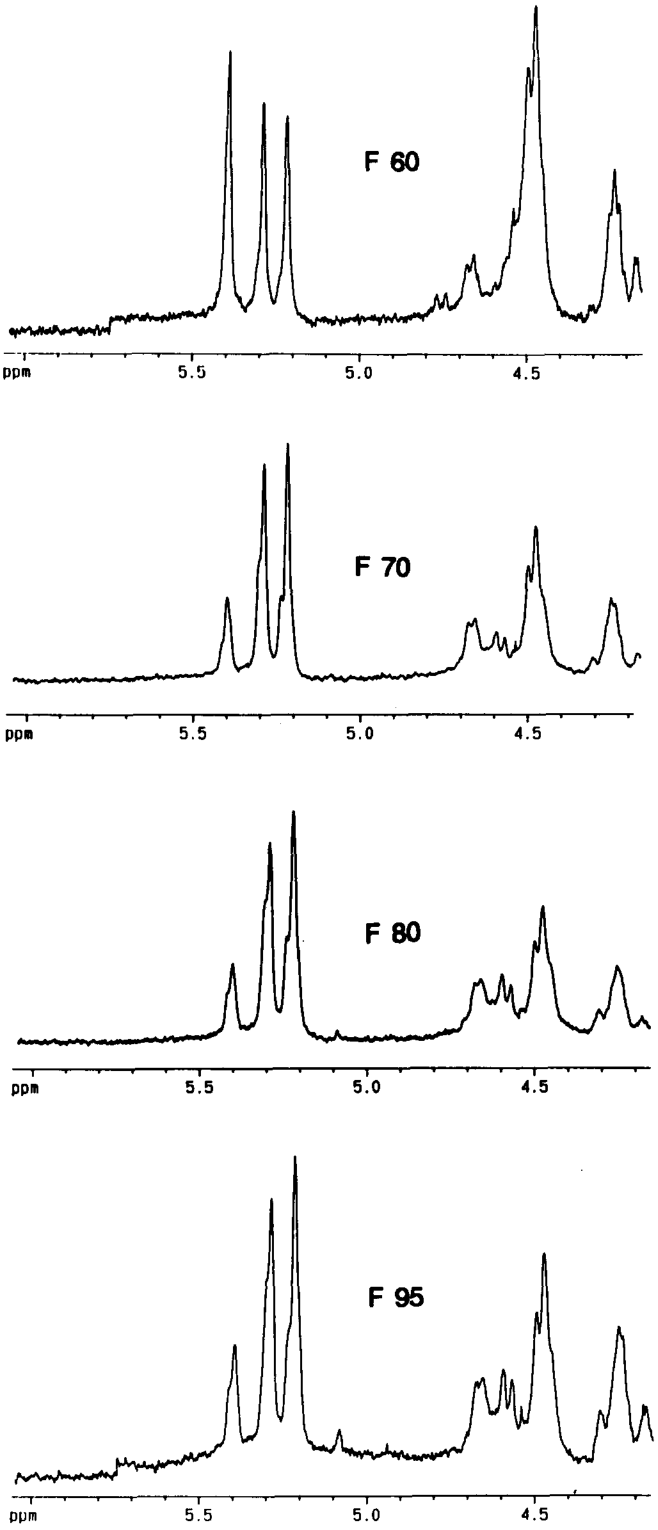

Fig. 2. ^{1}H-NMR spectra of arabinoxylan fractions (D_2O). The chemical shifts were assigned relative to $Me_3SiC_2D_2CO_2Na$.

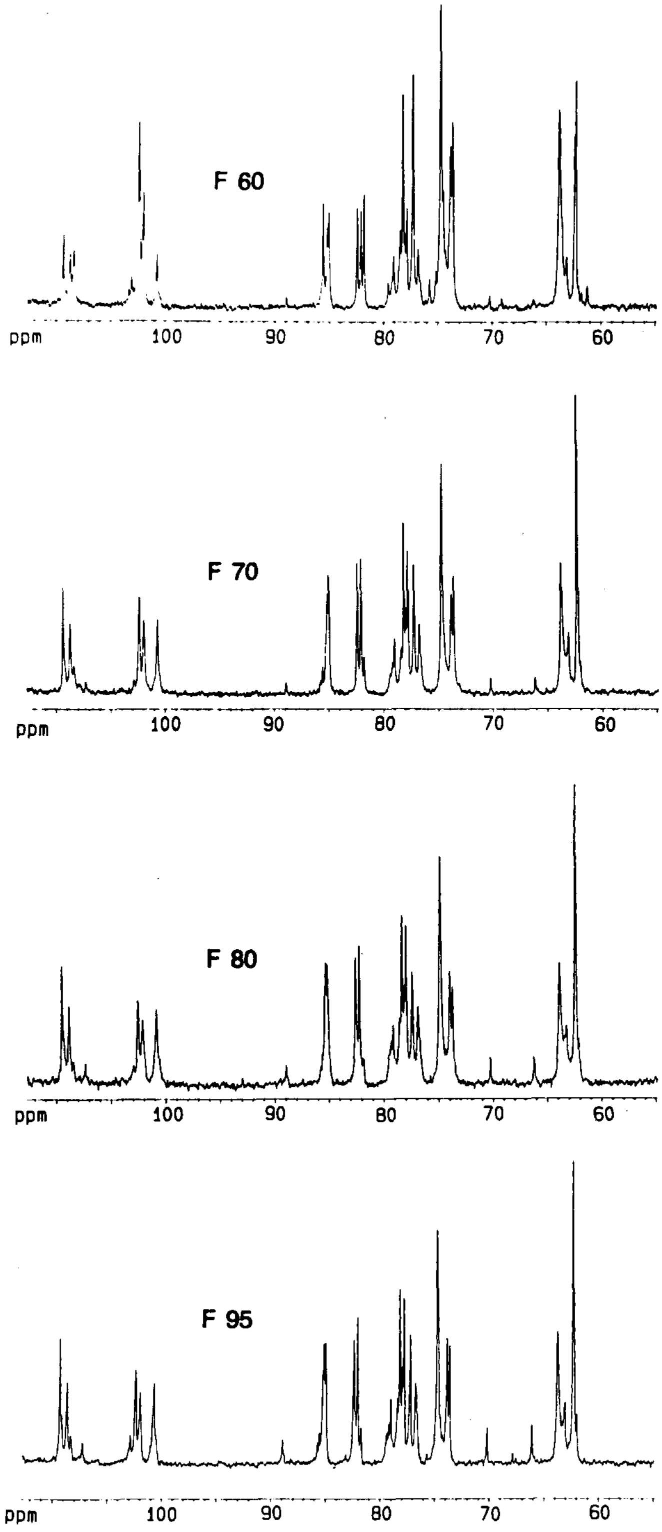

Fig. 3. ^{13}C-NMR spectra of arabinoxylan fractions (10% D_2O). The chemical shifts were assigned relative to 1,4-dioxane.

Doublier & Launay, 1981). This observation is in accord with the notion that wheat arabinoxylan assumes a fully-extended rod-like shape in solution (Andrewartha *et al.*, 1979).

Shear rate dependence of viscosity

Figure 5 shows the dependence of apparent viscosity on shear rate for aqueous solutions (2·0% w/v) of

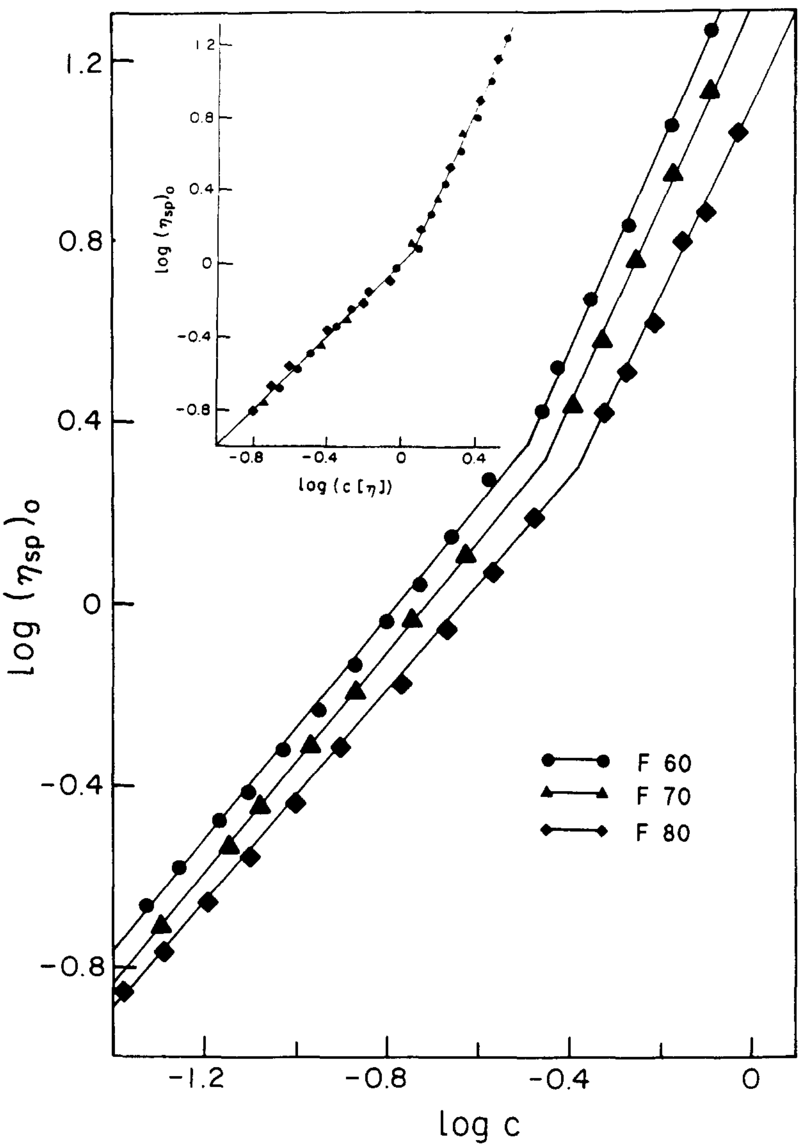

Fig. 4. Concentration dependence of 'zero shear' specific viscosity ($(\eta_{sp})_0$) for aqueous solutions of arabinoxylan fractions. Inset: 'zero shear' specific viscosity as a function of the overlap parameter ($c[\eta]$) for F60, F70, and F80.

arabinoxylan fractions at 25°C. Since the measurements were taken at a concentration above c^*, all samples (except F95) exhibited reduction in the viscosity with increasing shear rates. As expected, F60 with the highest apparent viscosity showed more pronounced shear thinning than the F70 and F80 fractions. The F95 exhibited a Newtonian-like behavior at this concentration.

Gelation

It is well known that arabinoxylans, when treated with free radical-generating agents, have the ability to form networks (gels or viscous solutions, depending on the polymer concentration and cross-link density) via covalent cross-linking involving feruloyl groups of adjacent arabinoxylan chains (Neukom & Markwalder, 1978). In this study, the gelling potential of the arabinoxylan fractions was investigated by small amplitude shear strain oscillatory testing. Figure 6 shows typical profiles of G' and G'' versus time for the F60 and F80 fractions following addition of peroxidase (0·22 PU/ml) and H_2O_2 (1·5 ppm). Most kinetic plots indicated that structure development proceeded via a rapid rise in the moduli values, followed by very little change in the rheological parameters over longer reaction times. While G' values exceeded those of G'' for all concentrations of F60, the G' was significantly lower than G'' in the case of F70, F80, and F95 over the entire course of the reaction. Typical frequency sweeps of the dynamic moduli (G', G'') and viscosity (η') for the F60 (0·75%) and F80 (5·0%) after 2 h of reaction with the oxidant are presented in Fig. 7. These plots did reveal the different nature of the networks formed by these

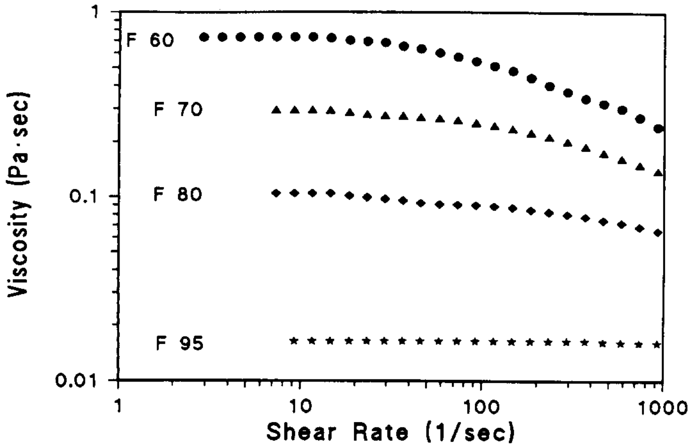

Fig. 5. The effect of shear rate on the apparent viscosity of aqueous solutions of arabinoxylan fractions (2·0% w/v) at 25°C.

materials, concurring with the data of Fig. 6. The behavior of F60 is typical of a weak gel, where *G′* is greater than *G″* at all frequencies. Compared to this fraction, the mechanical spectra of F80 were indicative of a liquid-like character (*G″* values exceeded those of *G′* at all frequencies and both moduli were highly dependent on frequency) rather than of a permanently cross-linked network. Therefore, relatively limited cross-linking appears to take place in the case of F80 as well as F70 and F95 fractions.

For covalently cross-linked networks and hydrogels, such as those examined in the present report, the shear modulus is related mainly to the number (density) of elastically effective (chemical) cross-links (Clark & Ross-Murphy, 1987); the latter would be proportional to the number of functional sites available for covalent cross-linking (feruloyl groups) and polymer concen-

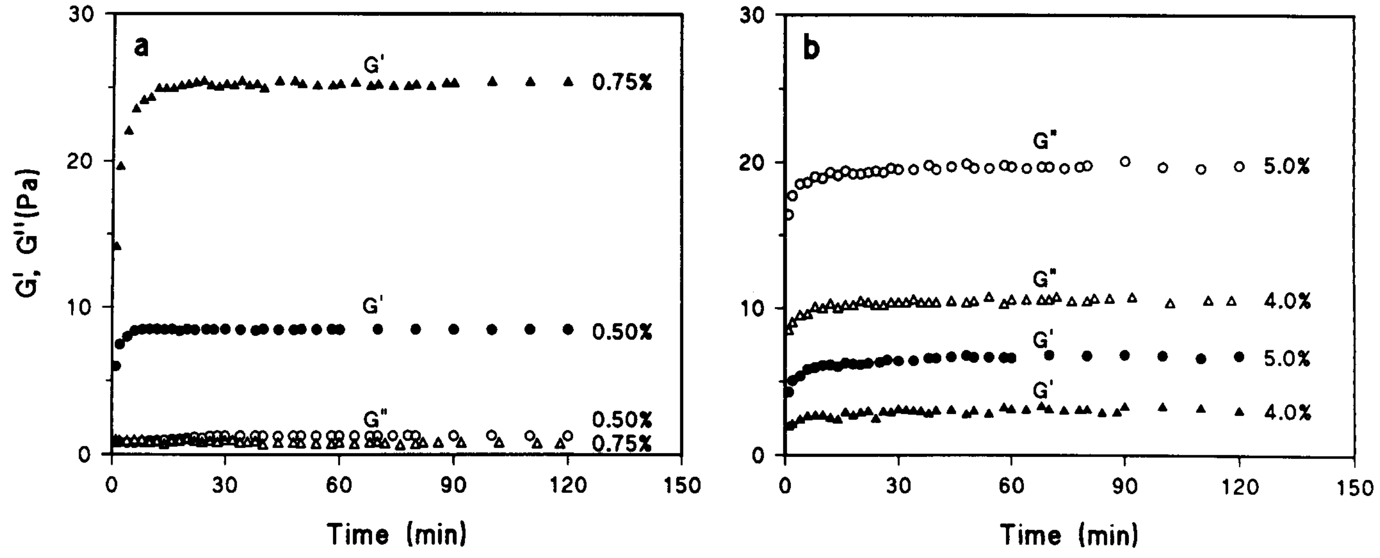

Fig. 6. Storage (*G′*) and loss (*G″*) moduli vs time for aqueous solutions of arabinoxylan fractions treated with peroxidase (0·22 PU/ml) and H_2O_2 (1·5 ppm) at 15°C. Data obtained at 1·0 Hz and 4% strain. (a) F60, (b) F80.

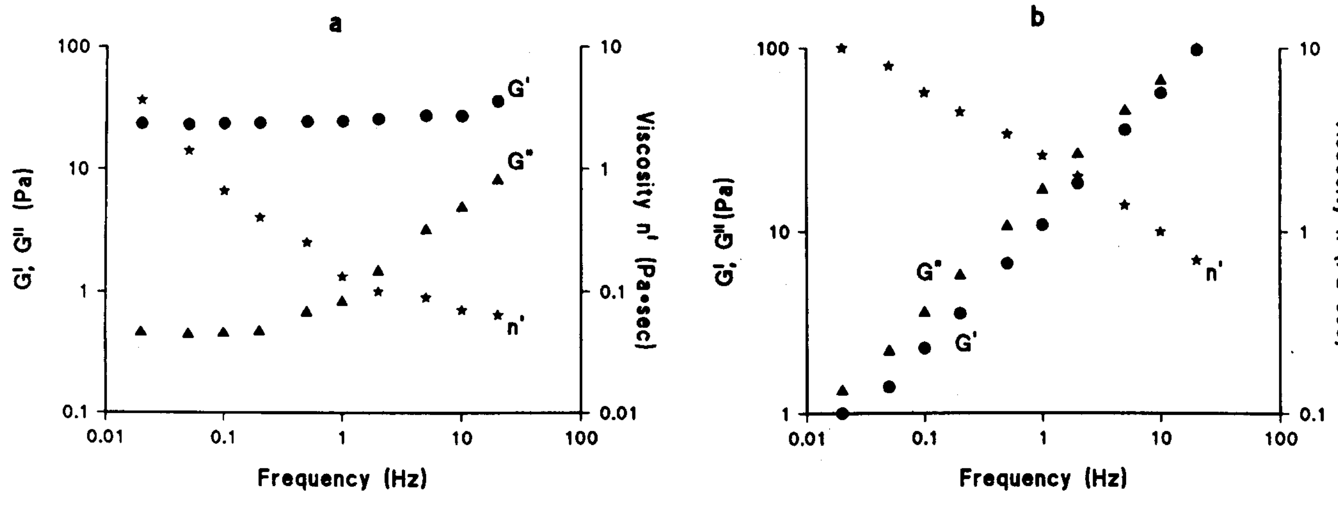

Fig. 7. Frequency dependence of storage (*G′*) and loss (*G″*) moduli, and dynamic viscosity (η') for arabinoxylan fractions after 1 h treatment with peroxidase (0·22 PU/ml) and H_2O_2 (1·5 ppm) at 15°C. (a) F60 (0·75% w/v), (b) F80 (5·0% w/v).

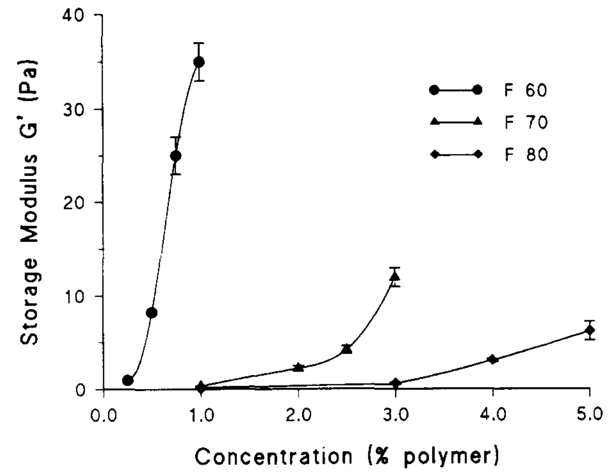

Fig. 8. Concentration dependence of storage modulus (G') for arabinoxylan fractions F60, F70, and F80 after 1 h treatment with peroxidase (0·22 PU/ml) and H_2O_2 (1·5 ppm) at 15°C; frequency 1·0 Hz, strain less than 4%.

tration. The relationships between G' and arabinoxylan concentration for F60, F70, and F80 are shown in Fig. 8, while Table 4 summarizes the rheological parameters of networks formed after treatment of the fractions with the oxidant for one hour. Compared to the high G' values attained by gels of F60 even at low polymer concentrations, all other fractions exhibited a rather poor gelling capacity. Substantial increases in G' for F70 and F80 occurred only above 2·0 and 4·0% (w/v), respectively. Even then, solutions of increased viscosity rather than gels were formed as evidenced by the high tan δ values (Table 4). Tan δ is a parameter which is very sensitive to changes of G' and G'' with respect to each other. A high value of tan δ (>1) implies a liquid-like character of a viscoelastic system, while a low value of tan δ ($<0·1$) points to a more elastic character (Ross-Murphy, 1984). Although the tan δ for F70, F80, and F95 decreased with increasing polymer concentration, it never reached values below 1·0. The data of Fig. 8 also point to differences in the concentration dependence of G' among fractions. Over the range of concentrations examined, the gels of F60 showed a strong linear relationship between the two parameters, while the remaining samples gave exponential modulus–concentration responses.

The observed differences in the gelling potential of arabinoxylan fractions can be explained at least in part by the differences in molecular size of these polymers. It has been previously shown (Izydorczyk *et al.*, 1991b) that the rigidity of cross-linked arabinoxylan gels (as manifested by their G') can be positively correlated with the intrinsic viscosity $[\eta]$ of the native polysaccharide. Indeed, gels of F60 having the highest $[\eta]$ exhibited the highest G' values among all fractions under equivalent polymer concentration. However, it was of interest to note that although the intrinsic viscosities of F70 (4·20 dl/g) and F80 (3·19 dl/g) were not considerably lower than that of F60 (4·70 dl/g), these fractions did not form gel networks in the presence of the oxidant even at high concentrations. It would appear, therefore, that other factors are responsible for the observed rheological behavior of these materials. First, there are differences in the content of feruloyl groups between F60 and the remaining fractions (Table 2). The presence of these groups is pivotal to cross-linking of the arabinoxylan chains and, therefore, their relative amount and distribution along the chain backbone must influence the gelling

Table 4. Storage (G') and loss (G'') moduli, and tan δ for arabinoxylan gels[a]

Fraction	Polymer conc. (%)	G' (Pa)	G'' (Pa)	tan δ
F60	0·25	0·94 ± 0·05[b]	0·62 ± 0·04	0·66
	0·50	8·25 ± 0·40	1·18 ± 0·20	0·14
	0·75	25·00 ± 1·20	0·85 ± 0·05	0·03
	1·0	35·00 ± 3·00	1·18 ± 0·20	0·03
F70	1·0	0·33 ± 0·03	0·60 ± 0·05	1·83
	2·0	2·13 ± 0·50	3·92 ± 0·50	1·84
	2·5	4·20 ± 0·50	7·70 ± 1·00	1·83
	3·0	12·00 ± 1·25	17·30 ± 2·05	1·44
F80	1·0	0·05 ± 0·02	0·22 ± 0·02	4·68
	3·0	0·60 ± 0·05	2·92 ± 0·50	4·86
	4·0	3·04 ± 0·20	10·70 ± 0·90	3·30
	5·0	6·77 ± 1·05	19·60 ± 1·50	2·89
F95	5·0	0·02 ± 0·02	0·20 ± 0·05	10·0

[a] Solutions of arabinoxylan fractions were treated with horseradish peroxidase (0·22 PU/ml) and H_2O_2 (1·5 ppm); the reported values are those obtained after 1 h of reaction.
[b] $n = 3 \pm$ SD.

potential of these polymers. Whether differences in the ratio of *cis* and *trans* isomers of ferulic acid also play a role in the rheological behavior of these polysaccharides remains an open question. In addition to ferulic acid, differences in the structural features among the arabinoxylan fractions, as revealed by NMR and monosaccharide analyses (Figs 2 and 3; Table 1), may also be contributing factors. Andrewartha and co-workers (1979) have demonstrated that arabinosyl side branches enhance the stiffness of arabinoxylan molecules by maintaining the xylan backbones in a fully extended conformation. The least substituted arabinoxylan F60 should therefore be more flexible than F70, F80, and F95. This chain flexibility may allow the establishment of a continuous gel network by facilitating the initial contact between feruloyl residues of neighbouring chains. In the case of the more highly substituted arabinoxylans of F70, F80, and F95, the flexibility and bending of the polymer backbone may be limited and so the contacts between feruloyl groups; this in turn would result in limited cross-linking. Whether the higher ratio of disubstituted/monosubstituted xylose residues in F70, F80, and F95 fractions (NMR data of Figs 2 and 3) also influences feruloyl group reactivity due to chain inflexibility or steric hindrance needs to be further explored.

Interfacial properties

Previous studies on protein foams in which arabinoxylans were incorporated indicated that these polymers can stabilize the foam during heating (Izydorczyk *et al.*, 1991b). In this context, the effect of arabinoxylan fractions on the foam volume of a surface active protein (BSA) before and after heating was assessed. The data of Fig. 9 showed that control solutions of 2% w/v BSA gave rise to high foam volume, which diminished, after 3 min of heating. The addition of arabinoxylans to BSA generally decreased the initial foam volume. Since the initial formation of foam is usually impeded by increase in viscosity of the liquid medium, the most viscous arabinoxylan F60 caused the greatest reduction in the initial foam volume. During heating, however, the presence of arabinoxylans appeared to protect gas cells against thermal disruption. The greatest effect was exerted by F60; F70 and F80 prevented the foam from collapsing to a lesser degree, while F95 had no effect at all. Because of its high viscosity, the F60 fraction seems to contribute the most to the elasticity of the thin film surrounding the gas bubbles thus improving the thermal stability of the foam.

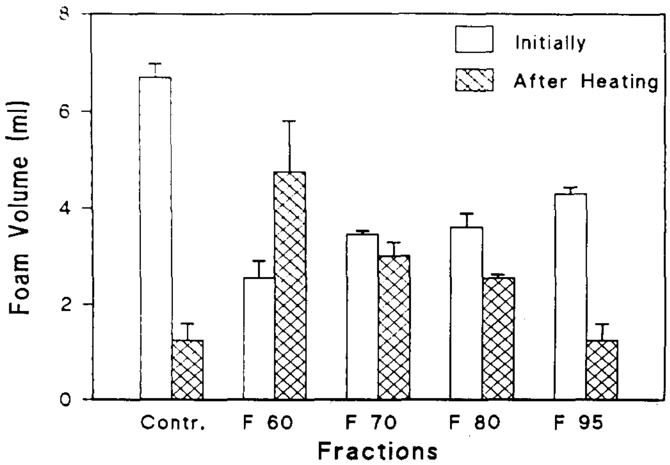

Fig. 9. Effect of arabinoxylan fractions on the foam volume of BSA before and after heating (3 min, 90°C). Arabinoxylan solutions (0·25 ml of 1% w/v) were added to 2% BSA (1 ml) and mixed well.

CONCLUDING REMARKS

In contrast to proteins and other biological molecules synthesized under a strict genetic control, polydispersity is a common characteristic of polysaccharides. Thus, the term homogeneous is difficult to define for these macromolecules since a great deal of variation exists in terms of monosaccharide composition, frequency and type of linkages, extent of branching, and molecular size (Aspinall, 1982). Moreover, it is important to recognize that even relatively small differences in the structure of a polysaccharide can result in substantial changes in its physical properties. In this context, an interesting feature of wheat arabinoxylan, disclosed by the findings of this report, is its structural heterogeneity. Using fractional precipitation by stepwise addition of $(NH_4)_2SO_4$ several fractions of discrete branching patterns were obtained. With increasing concentration of this salt there was an increase in the ratio of arabinose:xylose and in the relative amount of doubly substituted xylose residues as well as a decrease in the molecular size of the isolated polysaccharide. Compared to proteins, selective precipitation by $(NH_4)_2SO_4$ has received very little attention in the area of isolation and fractionation of polysaccharides from aqueous solution. Although, the exact mechanism by which this salt precipitates ('salting out') neutral polysaccharides, like arabinoxylan, remains obscure, the results of the present study indicate that fractional precipitation with this agent is effected not only by the molecular size but also by the fine structure of the polymer. The molecular features of the isolated arabinoxylan fractions lead to functional diversity. Hence, extensive cross-linking (via oxidative coupling of feruloyl groups present in adjacent chains) yielding well-developed three-dimensional hydrated networks, as assessed by dynamic rheological testing, was more prominent with the fraction obtained at a salt concentration corresponding to 60% saturation; this fraction (F60) also had the highest molecular size ($[\eta]$

and gel filtration data) and was the least branched among all fractions. The ability of arabinoxylans to gel diminished with increasing molar ratio of arabinose: xylose and relative amount of double (O → 3) and (O → 2)-linked Ara*f* on the same Xyl*p* residue of the chain backbone; the relative ranking for gelling ability was F60 > F70 > F80 > F95. Among the fractions, F60 was also the most effective in stabilizing protein foams from disruption upon heating, presumably because of its high molecular weight. In conclusion, structural restrictions and chain flexibility, as governed by the primary structure, and feruloyl groups seem to be important determinants of intermolecular interactions between arabinoxylan molecules in aqueous solutions.

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