Chemical Structure, Molecular Size Distributions, and Rheological Properties of Flaxseed Gum†

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Flaxseed gum extracted under optimum conditions of temperature (85–90 °C), pH (6.5–7.0), and a water:seed ratio (13.0) was subjected to dialysis and fractionation by ion exchange chromatography on a high-capacity DEAE-cellulose column. The two fractions obtained [an acidic fraction (AFG) and a neutral fraction (NFG)] were characterized on the basis of molecular size distributions, chemical structure, and rheological properties. The neutral fraction was composed of arabinoxylans and was free of uronic acids. The acidic fraction consisted mainly of pectic-like polysaccharides containing L-rhamnose, D-galactose, and D-galacturonic acid. The higher viscosity of solutions of the neutral fraction can be explained by the presence of a β-D-(1,4)-xylan backbone of the arabinoxylan component, exhibiting a higher hydrodynamic volume than the acidic fraction. In contrast, the weak solution rheological properties of the acidic fraction could be attributed to the smaller molecular size of its constituent polysaccharides.

Keywords: *Mucilage; gel filtration; spectroscopy; polysaccharaides; gums; arabinoxylans; linseed; Linum usitatissimum*

INTRODUCTION

Mucilaginous constituents of flaxseed (*Linum usitatissimum* L.) have been shown to have a considerable potential for use as food gum (BeMiller, 1973; Mazza and Biliaderis, 1989). Previous studies have revealed that flaxseed mucilage consists of two types of polysaccharides: a neutral arabinoxylan and an acidic pectic-like material (Hunt and Jones, 1962; Muralikrishna et al., 1987). Recently, Wannerberger et al. (1991) reported that mucilages extracted from different flax cultivars exhibit different rheological properties. Experiments conducted in our laboratory have shown that extraction conditions also have substantial impact on the yield, chemical composition, and rheological properties of flaxseed gum; therefore, optimum extraction conditions were established using the surface response methodology to maximize the yield, maintain high viscosity of the gum dispersions, and minimize protein contaminants (Cui et al., 1994). The objectives of the present study were to fractionate crude flaxseed gum, obtained by an aqueous process, into a neutral (NFG) and an acidic (AFG) fraction using ion exchange chromatography and to determine the chemical structure, molecular size distributions, and rheological properties of the isolated fractions and crude flaxseed gum.

MATERIALS AND METHODS

Material. Flaxseed cv. Norman, produced commercially in southern Manitoba, Canada, in 1991, was used as the raw material for extraction of gum. All chemicals were of reagent grade unless otherwise specified.

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Extraction and Fractionation of Flaxseed Gum. Flaxseed gum was extracted by an aqueous process and precipitation with 3 volumes of 95% ethanol as described previously (Cui et al., 1994). The crude flaxseed gum (CFG) was dialyzed against distilled water for 3 × 24 h (membrane with a 12,000–14,000 molecular weight cutoff) to obtain dialyzed flaxseed gum (DFG). Flaxseed gum after dialysis was concentrated by vacuum evaporator at 40 °C then freeze-dried. DFG was further separated into an acidic (AFG) and a neutral (NFG) fraction by ion exchange chromatography on a high-capacity DEAE-cellulose (Pierce, Rockford, IL) column (10 × 28 cm) equilibrated with sodium acetate buffer (pH 5.0, 25 mM). The NFG was eluted using the buffer only, while the AFG was eluted with 1.0 M NaCl in the same buffer. The eluates from the ion exchange column were concentrated under vacuum at 40 °C, dialyzed for 3 × 24 h against distilled water, and freeze-dried. The extraction and fractionation procedures of CFG and its fractions are shown in Figure 1.

Chemical Composition and Monosaccharide and Methylation Analyses. Moisture, protein, and mineral contents were determined as described earlier (Mazza and Biliaderis, 1989). Monosaccharides were analyzed following the GLC procedure of Englyst et al. (1982) using a glass capillary column (SP-2330, 30 m × 0.75 mm i.d.). Methylation analysis was carried out according to the method of Ciucanu and Kerek (1984), while the reduction of the uronic acid of AFG after methylation was conducted following a procedure described by O'Neill et al. (1990).

^{13}C NMR Spectroscopy. The proton decoupled ^{13}C NMR spectra of AFG and NFG were recorded on a Bruker AMX 500 FT spectrometer at 65 °C, 2% (w/w) polymer concentration in D_2O. The number of acquisitions was approximately 30 000; pulse repetition time was 1.245 s and radio frequency pulse angle 90° (Cui et al., 1993).

Gel Filtration Chromatography. The molecular size distributions of CFG, DFG, NFG, and AFG were determined by gel filtration chromatography on a Sepharose 2B column (2.5 × 90 cm, Pharmacia Ltd., Montreal, Canada) eluted with 0.1 M NaCl in distilled water (flow rate 30 mL/h, 25 °C). Carbohydrates and uronic acid were monitored according to the procedures of Dubois et al. (1956) and Blumenkrantz and Asboe-Hansen (1973), respectively. A Beckman DU-50 spectrophotometer was used to measure the absorbances at 480 and 520 nm. Molecular weight markers used to calibrate the column were linear dextrans T-500 (MW 466 000) and T-70

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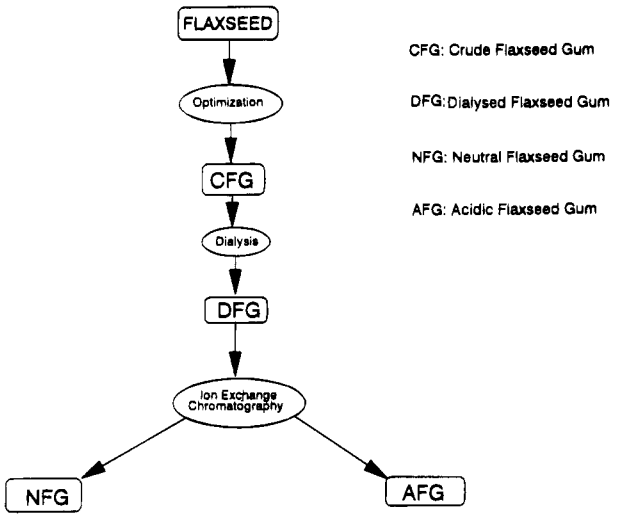

Figure 1. Flow chart of extraction and fractionation of flaxseed gum.

Table 1. Chemical Composition of Crude (CFG) and Dialyzed (DFG) Flaxseed Gums

component	CFG	DFG	component	CFG	DFG
yield (%)	7.9	85.0	sulfur (%)	0.081	0.076
moisture (%)	6.5	7.1	phosphorus (%)	0.11	0.05
protein (%)	9.1	6.3	magnesium (%)	0.31	0.24
carbohydrates[a] (%)	80.3	83.9	potassium (%)	2.53	0.16
			sodium (%)	0.45	0.47
			iron (ppm)	34.5	28.7

[a] Determined by difference.

(MW 69 000). The total volume of the column was determined by the elution volume of D-glucose.

Rheological Measurements. The steady shear flow curves and dynamic rheological measurements of flaxseed gum solutions were carried out as described earlier using a Bohlin VOR rheometer (Cui et al., 1993). A concentric cylinder geometry with height of 63.0 mm, radii of the inner and outer containers of 12.5 and 13.75 mm, respectively, was used for all rheological tests. Polymer solutions (pH 6.5) at 0.3%, 0.5%, 1.0%, and 2.0% (w/w) concentrations were used for the steady shear rheological measurements, while 2.0% (w/w) polymer concentration (pH 6.5) was employed for the small strain oscillatory tests (frequency 0.05–20 Hz, less than 4% strain).

RESULTS AND DISCUSSION

Chemical and Monosaccharide Compositions. The chemical compositions of crude (CFG) and dialyzed (DFG) flaxseed gums are presented in Table 1. The yield of CFG was 7.9% of the seed weight, while the yield of DFG was 85.0% of the crude mucilage. There was little increase in carbohydrates and moisture contents after dialysis, while the total nitrogenous material was decreased from 1.46% to 1.01%. The dialysis process also reduced the potassium level from 2.53% to 0.16%, together with small decreases in sulfur, phosphorus, magnesium, and iron (Table 1). Relative monosaccharide compositions of CFG, DFG, NFG, and AFG are given in Table 2. CFG and DFG had similar neutral monosaccharide ratios, although some slight variances existed. NFG, the neutral fraction of flaxseed gum, contained mainly D-xylose (62.8%) followed by L-arabinose (16.2%), D-glucose (13.6%), and D-galactose (7.4%) with 0% L-rhamnose and L-fucose. In contrast, AFG, the acidic fraction, contained mainly L-rhamnose (54.5%), D-galactose (23.4%), and L-fucose (10.1%) together with small portions of D-xylose (5.5%), L-arabinose (2.0%), and D-glucose (4.5%). The presence of

Table 2. Relative Neutral Monosaccharide Compositions of Crude (CGF) and Dialyzed (DFG) Flaxseed Gums and of Neutral (NFG) and Acidic Fractions (AFG) of Flaxseed Gum

sugar	CFG	DFG	NFG	AFG
L-rhamnose	34.2 ± 2.5[a]	35.5 ± 0.2	0	54.5 ± 0.2
L-fucose	4.5 ± 0.1	5.0 ± 0.0	0	10.1 ± 0.7
L-arabinose	9.8 ± 0.5	8.8 ± 0.2	16.2 ± 0.2	2.0 ± 0.0
D-xylose	32.0 ± 0.5	29.8 ± 0.5	62.8 ± 5.2	5.5 ± 1.2
D-galactose	17.3 ± 1.4	20.9 ± 0.0	7.4 ± 0.0	23.4 ± 1.5
D-glucose	2.2 ± 0.4	0.8 ± 0.4	13.6 ± 1.1	4.5 ± 0.0

[a] Data were determined in duplicate.

Table 3. Molar Ratio[a] of Partially Methylated Acetyl Alditols (PMAA) of Neutral (NFG) and Acidic (AFG) Fractions of Flaxseed Gums

PMAA	NFG	AFG	deduced linkage
2,3,5-Me_3-ara	6.4	0	terminal ara (f)[b]
2,5-Me_2-ara	4.9	0	1,3-linked ara (f)
total ether of ara	11.3	0	
2,3,4-Me_3-xyl	16.2	0	terminal xyl (p)[c]
2,3-Me_2-xyl	21.8	0	1,4-linked xyl (p)
2-Me-xyl	4.1	0	1,3,4-linked xyl (p)
xyl $(acetyl)_5$	14.1	0	1,2,3,4-linked xyl (p)
total ether of xyl	56.2	0	
2,3,4,6-Me_4-gal	17.0	0	terminal gal (p)
2,3,6-Me_3-glc	3.8	tr[d]	1,4-linked glc (p)
3,4-Me_2-rham	0	15.4	1,2-linked rham
4-Me-rham	0	13.1	1,2,3-linked rham
total ether of rham	0	28.5	
2,3-Me_2-gal6D_2	0	10.2	1,4-linked-D-galacturonic acid

[a] Molar ratio was calculated from peak area of GLC. [b] Furanosyl ring. [c] Pyranosyl ring. [d] tr, trace.

D-xylose and L-arabinose in AFG might originate from contamination with the neutral fraction. These findings are comparable with data reported previously (Muralikrishna et al., 1987).

Methylation Analysis and ^{13}C NMR Spectra. Further structural information on NFG and AFG was obtained by methylation analysis and ^{13}C NMR spectra as shown in Table 3 and Figure 2, respectively. The neutral fraction, NFG, was composed mainly of 1,4-linked β-D-xylopyranose backbone chain together with 16.2% terminal D-xylose. Among the backbone chain D-xylose units, 21.8% were nonsubstituted, 4.1% were 3-position-substituted, and 14.1% were doubly (2- and 3-positions) substituted. About 6.4% L-arabinose existed as terminal arabinofuranoses, while 4.9% occurred as 1,3-linked α-L-arabinofuranoses. The D-galactose in NFG was present only as a terminal residue, while the small amount of 1,4-linked β-D-glucose was possibly caused by some soluble cellulose-like material from the cell wall. These results based on methylation analysis are in agreement with the data of Muralikrishna et al. (1987) that the neutral arabinoxylan fraction contained a (1→4) β-D-xylan backbone to which L-arabinose and D-galactose side chains are attached at positions 2 and/or 3. No L-rhamnose or L-fucose residues were observed in NFG, and this is in agreement with the monosaccharide analysis results (Table 2). The methylation analysis of AFG revealed that it mainly contained 1,2-linked (15.4%) and 1,2,3-linked (13.1%) L-rhamnose

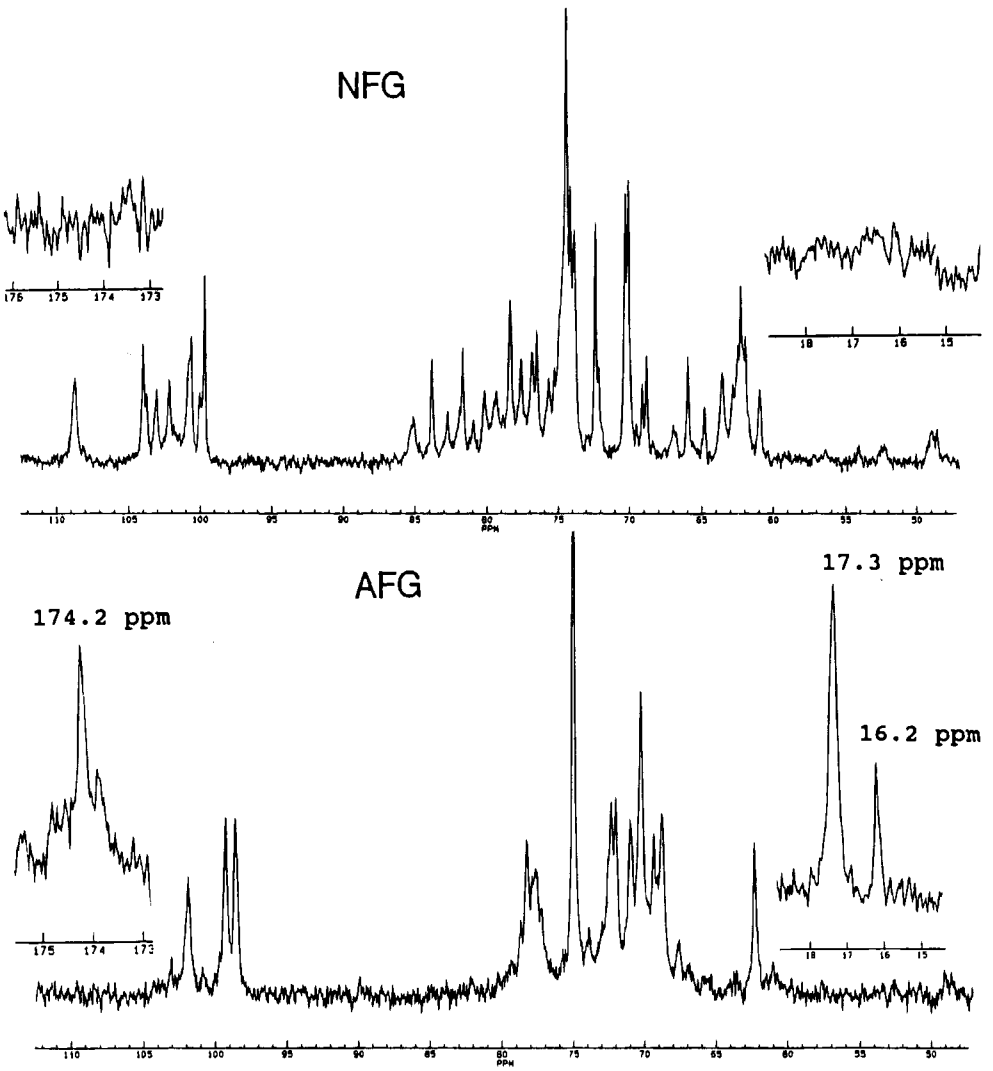

Figure 2. ^{13}C NMR spectra of neutral (NFG) and acidic (AFG) fractions of flaxseed gum [2.0% in D_2O (w/w), 65 °C].

residues with 1,4-linked D-galacturonic acid (10.2%), which is comparable to the data of Muralikrishna et al. (1987) except for the presence of 1,2,3-linked L-rhamnose. The chemical structures of NFG and AFG were further confirmed by ^{13}C NMR spectra as presented in Figure 2. The anomeric resonances at 108 ppm of NFG can be attributed to the α-L-arabinose, while the resonances between 99 and 104 ppm arose from the D-xylose residues (Bock et al., 1984). The lack of resonances at chemical shifts between 170 and 180 ppm and between 15 and 20 ppm supports the conclusion reached from monosaccharide and methylation analyses that there was no acidic component in NFG. The ^{13}C NMR spectrum of AFG is rather simple in the anomeric region, which supports the results of methylation analysis. Resonance at 174.2 ppm can be attributed to the 1,4-linked D-galacturonic acid, while resonances at 17.3 and 16.2 ppm can be attributed to the residues of L-rhamnose and L-fucose, respectively (Cui et al., 1993). The other resonances were not resolved because the spectrum was too complex.

Gel Filtration Chromatography. The molecular size distributions of flaxseed gum and its fractions are presented in Figure 3. Both CFG and DFG consisted mainly of high molecular size species, eluting earlier than the linear dextran T-500. Substantial amounts of uronic acid were detected in both CFG and DFG. Generally, the elution volume of the acidic species appeared later than the peak elution volume of neutral polysaccharides, indicating a smaller hydrodynamic volume for the acidic polysaccharides of flaxseed gum. The dialysis process did not appear to change the molecular size distribution of flaxseed gum significantly since both CFG and DFG revealed similar gel filtration profiles, although a slight shift of the void volume was observed from CFG to DFG. As shown in Figure 3, the gel filtration profile of NFG is similar to those of DFG and CFG, except for the lack of the acidic fraction. These results are in agreement with the monosaccharide and methylation analyses and ^{13}C NMR data which indicate that NFG is a pure neutral polysaccharide fraction. The neutral carbohydrate peak observed near the void volume also suggests that NFG contains mainly high molecular size species, although smaller amounts of neutral carbohydrates were also detected in the low molecular weight region. The molecular size distribution of AFG indicated the presence of carbohydrates with smaller hydrodynamic volume than in NFG, DFG, and CFG.

Rheological Properties. The steady shear flow curves of flaxseed gum and its fractions are shown in Figure 4. CFG exhibited shear thinning behavior (apparent viscosity decreases with increases of shear rate) (Morris et al., 1981) at polymer concentrations above 1.0% over a broad range of shear rates (0.1−1000 s^{-1}). A slight shear thinning behavior for this material

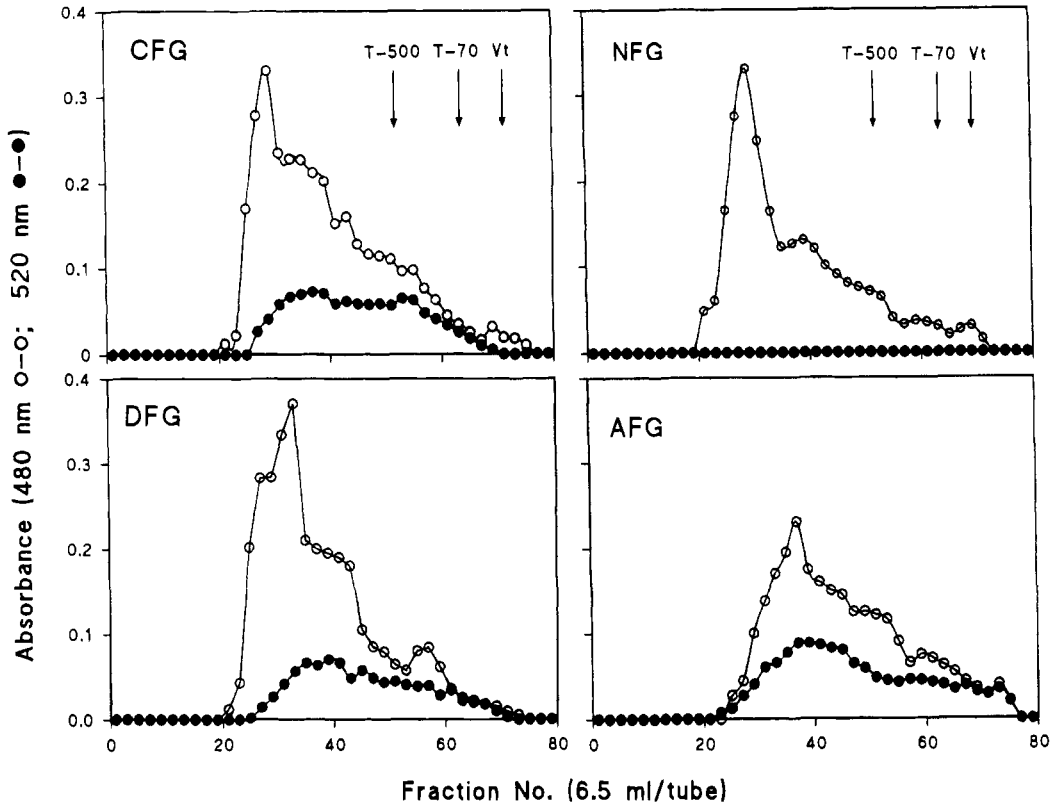

Figure 3. Gel filtration chromatographic profiles of flaxseed gum: crude (CFG), dialyzed (DFG) and neutral (NFG) and acidic (AFG) fractions on Sepharose 2B column (2.5 × 90 cm, flow rate 30 mL/h at 25 °C) eluted with 0.1 M NaCl in distilled water (480 nm, carbohydrates; 520 nm, uronic acids).

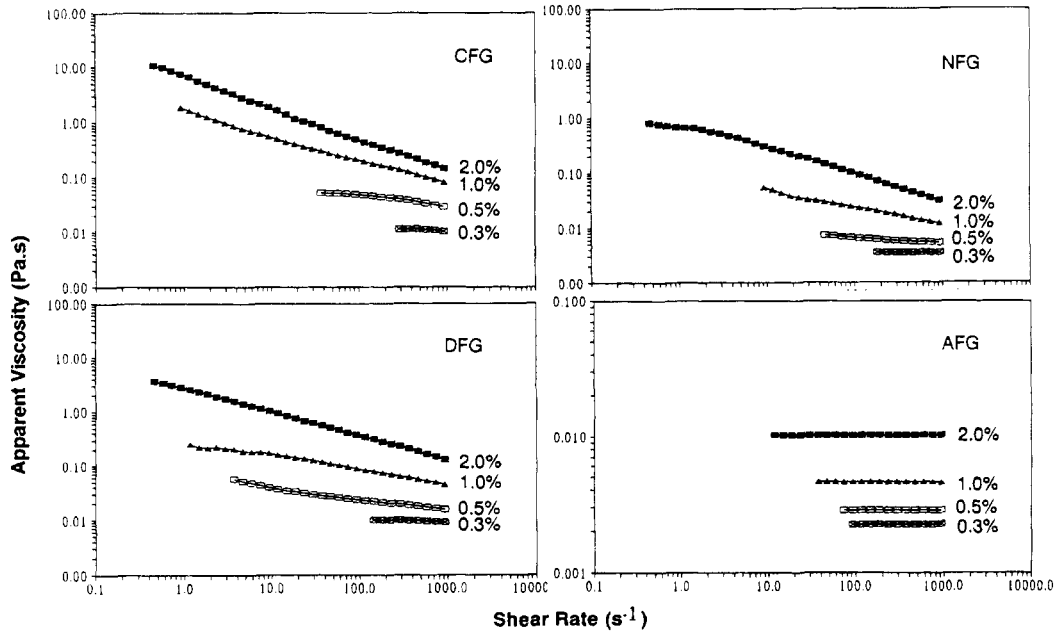

Figure 4. Steady shear rheological flow curves of crude (CFG), dialyzed (DFG), neutral fraction (NFG), and acidic fraction (AFG) solutions (pH 6.5) of flaxseed gum at 25 °C.

was observed at the high shear rate region when polymer concentration was 0.5% or less. Similar shear thinning behavior was also observed for the DFG at all concentrations examined. The similarity in the rheological responses between CFG and DFG parallels those of molecular size distributions (Figure 3) and monosaccharide compositions (Table 2). The NFG in solutions exhibited shear thinning behavior when polymer concentrations were above 0.5%. At 0.3%, the steady shear flow curve of this fraction is basically Newtonian-like, where the apparent viscosity is independent of shear rate. The shear thinning behavior of NFG can be attributed to its high molecular size arabinoxylan component. In contrast, the flow behavior of AFG solutions was different from that of NFG. Newtonian-like flow curves were observed for AFG at all concentrations examined. These rather "weak-solution" rheological properties of AFG can be explained by the much smaller molecular size of its constituent polysaccharides. The overall ranking of apparent viscosity was in the order CFG > DFG > NFG > AFG. It is well documented that polysaccharides with high molecular size

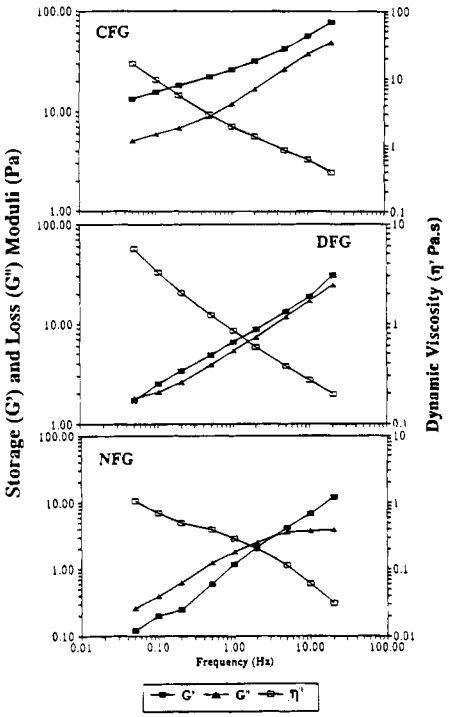

Figure 5. Frequency dependence of storage (G') and loss (G'') moduli and dynamic viscosity (η') of 2.0% (w/w) solutions of crude (CFG), dialyzed (DFG), and neutral fraction (NFG) flaxseed gums (pH 6.5, 25 °C).

and rigid conformation exhibit more pronounced shear thinning rheological behavior (Morris et al., 1981; Izydorczyk and Biliaderis, 1992).

Small deformation oscillatory rheological tests are useful in examining the molecular origin of the rheological properties of hydrocolloid solutions or dispersions. As shown in Figure 5, the CFG dispersions exhibited typical "weak gel" properties with the storage modulus (G') exceeding the loss modulus (G'') over the entire frequency range investigated. The storage modulus (G') reflects the solid-like properties of a viscoelastic material, while the loss modulus reflects its liquid-like character (Whitcomb et al., 1980). The mechanical spectrum of DFG dispersions (2.0% w/w) revealed also a weak gel structure, but much weaker compared to CFG since G' was only slightly higher than G''. The dynamic rheological pattern of NFG was typical of a viscoelastic fluid comparable to that of guar gum solution, as described in a previous study, where G'' is greater than G' at lower frequencies and the reverse is observed at higher frequencies (Cui et al., 1993). It is rather surprising that, following dialysis, DFG exhibited lower apparent viscosity (Figure 4) and much weaker viscoelastic responses compared to CFG (Figure 5). This would suggest that a small molecular weight component (removed by dialysis) in the crude gum might be responsible for the highly viscous character of CFG. The nature of this component is currently under investigation.

Conclusions. Flaxseed gum extracted under optimized aqueous extraction contained over 80.3% carbohydrates. The crude gum was dialyzed and separated by ion exchange chromatography into an acidic (AFG) and a neutral fraction (NFG). The neutral fraction, composed mainly of arabinoxylans with large molecular sizes, exhibited shear thinning behavior at higher concentrations. The acidic fraction contained pectic-like material with much smaller molecular sizes and consequently exhibited Newtonian flow behavior even at concentrations up to 2.0%. Arabinoxylans were the major component responsible for the shear thinning and weak gel properties of flaxseed gum, although possible polymer–polymer interactions may exist.

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