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Flax (*Linum usitatissimum*) Seed Cake: A Potential Source of High Molecular Weight Arabinoxylans?

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Water-soluble polysaccharides were extracted from flaxseed cake and analyzed. Two groups were separated by anion-exchange chromatography. The first one (nonretained) was the major fraction (83%) and possessed a high molecular weight (HMW) arabinoxylan (56%) with an Ara/Xyl ratio of 0.32 and an M_w of 846 000. This polymer was accompanied by a smaller galactoglucan (44%), with an M_w of 6.5×10^4. The latter group (17%), retained by the gel, was further described as a HMW pectin heterogeneous group, with, respectively, 3.1×10^5 and 1.3×10^5. Despite the presence of HMW arabinoxylans, the investigation of rheological flow sweep at the concentration of 2% (*w*/*v*) has shown a slight shear thinning behavior with a small zero-rate viscosity at 9.6 Pa·s.

KEYWORDS: Flaxseed cake; *Linum usitatissimum*; chromatography; arabinoxylan; polysaccharides; multiangle laser light scattering

INTRODUCTION

Flaxseeds are pressed to produce oil for various industrial developments (i.e., paint, linoleum, ...) (*1*−*3*). After the recovery of the oil, the residual cake is primarily used as a protein-rich livestock feed (*4*−*8*). Some attempts have been made to develop other uses for the residual cake, such as recovery of dyes in wastewater (*9*). At this time, no substantial alternatives to animal feed uses have been developed. On the other hand, the flaxseeds have an additional valorizable part in their capability to form a gel around them, called mucilage, when they are wetted (*10*, *11*). Specific applications (such as texturing agents) (*12*−*16*) in the cosmetic industry prove the potential of this compound. Recent studies of the flaxseed mucilage (*17*, *18*) demonstrated the presence of an interesting high molecular weight (HMW) arabinoxylan (AX) as the major component (75%). After the elaboration of a purification process and its enhancement to a large-scale procedure, a structural characterization of this AX has been carried out. It revealed the presence of an AX mixture (polydispersity coefficient = 2.91) with a large average molecular weight of 1.2×10^6 (*18*). Its intrinsic characteristics (monosaccharides, type of linkage, molecular weight) are probably due to the origin of the mucilage pseudo-gel behavior based on intermolecular associations that create a network (*17*). Thus, with the aim of finding new applications for the flaxseed cake in provenance of the oil industry, investigations on the potential presence of those polysaccharides in the water extract of the cake offer particular interest. In this study, the characterization of the polysaccharidic hydrocolloids from flaxseed cake has been undertaken to establish monosaccharide compositions, molecular mass determinations, and rheological data.

MATERIALS AND METHODS

Polysaccharide Extraction from Mucilage and Chromatographic Purification. Flaxseed cake were graciously given by Vandeputte (Ets), Mouscron, Belgium. Pieces of cake were ground finely. Polysaccharides from flax cake mucilage were extracted with stirring in water (1:60 w/v) during 2 h at 40 °C. The ground cake fragments were removed by centrifugation (10000*g*, 7 min, 4 °C). Then, the supernatant was treated by filtration through different mesh sieves (1.5 mm; 160 μm; 40 μm) and concentrated 5 times with a rotative evaporator (40 °C). After an ultracentrifugation (30000*g*, 30 min, 4 °C), the soluble fraction was collected and constitutive polysaccharides were separated at large scale using anion exchange chromatography as described previously (*18*). Purified and freeze-dried polymer extracts were used as raw material to determine their molecular masses and monosaccharide compositions after hydrolysis.

Size Exclusion Chromatography (SEC). To study the molecular mass distribution of neutral polysaccharides, polymers (50 mg) were solubilized in 20 mL of 50 mM ammonium acetate (pH 4.8) and applied on a Sephacryl-400 HR column (XK 2.6 × 100 cm, V_0 = 178 mL, V_T = 530 mL) (Amersham Biosciences) eluted at 76 mL·h^{-1} by 50 mM ammonium acetate. Fractions of 5 mL were collected, and the concentrations of neutral polysaccharides were determined as described below.

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Quantitative Analysis. All analyses were performed at least in triplicate. Concentrations of neutral polysaccharides in the different fractions were determined by a microscale colorimetric assay. Neutral sugars (expressed in D-xylose equivalent) were quantified by absorbance at 480 nm (A_{480}) after the addition of resorcinol in the presence of sulfuric acid (*19*). A microplate spectrophotometer (Opsys MR, Dynex Technologies, Chantilly, VA) was used for absorbance measurements.

Composition Analysis. The constitutive monosaccharide compositions in each polymer have been studied on the fractions separated by chromatography (anion exchanger followed by the Sephacryl elution). Fractions were pooled, dialyzed (14000 Da MWCO) for 24 h against deionized water, and freeze-dried. Their respective hydrolysis has been conducted for 3 h at 100 °C with 1 mL of trifluoroacetic acid (2 M). The composition was determined using high-performance anion-exchange chromatography (HPAEC), on a Carbopac PA-1 analytical column (4 × 250 mm). Detection was performed with a pulsed amperometric ED50 detector (Dionex Corp., Sunnyvale, CA). Twenty-five microliters of sample were injected with an autosampler. Each carbohydrate concentration was established after integration of respective areas [Chromeleon management system (Dionex)] and comparison with standard curves obtained with all relevant monosaccharides standards (Sigma). To investigate the neutral monosaccharides, the elution has been achieved isocratically with 16 mM NaOH at a flow rate of 1 mL·min^{-1}. On the other hand, to elucidate the acidic sugars composition of all samples, a gradient of 160 mM NaOH (solvent A) and 600 mM ammonium acetate in 160 mM NaOH (solvent B) was applied at a flow rate of 1 mL·min^{-1}. The gradient contained four steps (expressed in percent B in A): 0% during 10 min; 0−100% from 10 to 40 min; 100% from 40 to 45 min; 100−0% from 45 to 50 min.

On-line SEC/MALLS. Average molecular weight and molecular weight distribution were determined by high-pressure size exclusion chromatography (HPSEC) with on-line multiangle laser light scattering (MALLS) [DAWN-EOS (Wyatt Technology Inc., Santa Barbara, CA) filled with a K5 cell and a He−Ne laser ($\lambda = 690$ nm)] and differential refractive index (DRI) detectors (*20*). Columns [OHPAK SB-G guard column, OHPAK SB 804 and 806 HQ columns (Shodex)] were eluted with 0.1 M $LiNO_3$ at 0.6 mL·min^{-1}. Solvent was filtered through a 0.1 μm filter unit (Millipore), degassed (ERC-413), and filtered through a 0.45 μm filter upstream column. The samples were injected through a 100 μL full loop. Collected data were analyzed using the Astra V-4-81-05 software package. The concentration of each eluted fraction was determined with the DRI (ERC 7515A) according to the known value of d*n*/d*C* (0.15).

Viscosity Measurements. Rheological determinations were performed at steady shear with an AR 2000 rheometer (TA Instruments, New Castle, DE) comprising a Peltier system and coupled with a circulating bath that maintained the temperature at 25 °C. Flaxseed neutral polysaccharides were obtained as described elsewhere (*18*), whereas the flaxseed cake AEC nonretained fraction was recovered as described above. All of the samples were solubilized at 2% (w/v) in deionized water with stirring until complete solubilization. Shear flow behavior was assessed by measuring the viscosity using a steel cone-and-plate (40 mm radius, 2°) over the shear rates of 0.1−1414 s^{-1}.

RESULTS AND DISCUSSION

As the seed mucilage comprises various constitutive types of polysaccharides, such as rhamnogalacturonans and arabinoxylans (*17*, *18*, *21*, *22*), it is reasonable to consider that the pressure process applied on seeds could have an impact on the polysaccharide distributions. To examine this distribution, structural and physicochemical studies have been undertaken. From then on, the improved method used for the flaxseed mucilage study (*18*), including the anion-exchange chromatography (AEC) at large scale, was used to approach the purification of the cake polysaccharides (**Figure 1**). Due to the cake's heterogeneity, numerous extractions were done with a yield in polysaccharides of 44.6 g per 100 g of cake. In all cases, two peaks appeared constantly. They were named, respectively, F1 (83%) and F2 (17%).

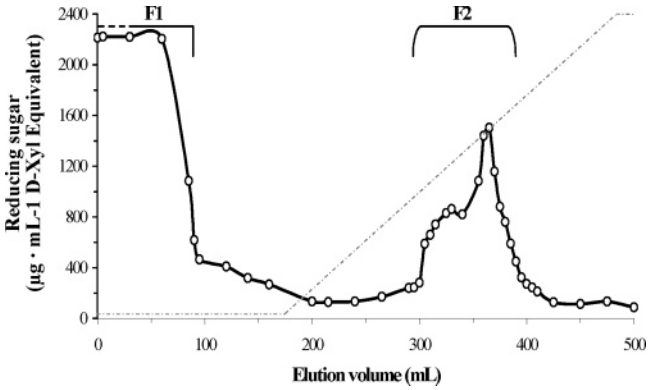

Figure 1. Quantification of neutral (○) polysaccharides, after IEC on Q-Sepharose Big Beads, by A_{480} analysis after specific identification using the method described by Monsigny (*19*). Neutral polymers concentrations are expressed in D-Xyl equivalent (μg·mL^{-1}). (- - -) NaCl gradient from 0 to 1 M.

Table 1. Relative Monosaccharide Composition of the Different Fractions Recovered by Anion Exchange and by Size Exclusion Chromatography Analyzed by HPAEC

	sugar content (molar ratio)							
fraction	D-Xyl	D-Gal	D-Ara	L-Fuc	L-Rha	D-Glc	D-GalA	yield[a]
F1	30.2	19.2	7.9	5.6	6.2	28.3	2.5	83
$F1_A$	41.5	25.8	13.3	7.8	9.1	1.5	1	56
$F1_B$	2.1	15.3	6.6	6.7	4.7	64.6	0	44
F2	4.5	30.9	28.1	2.6	7.1	6.4	20.4	17

[a] Based on total amount of material recovered (% w/w).

F1, the AEC nonretained fraction, constituted the preponderant polysaccharidic fraction with 37% of the cake. The monosaccharide composition was further determined by HPAEC (**Table 1**). The result brought to the fore the presence of xylose in major part (30.2%) and two principal other neutral monomers: glucose (28.3%) and galactose (19.2%). The respective proportions of D-Xyl, D-Gal, and L-Ara did not follow those displayed in previous study for flaxseeds (*17*). The ratio Ara/Xyl of 0.26 appeared to be slightly higher compared to those measured in yellow flaxseeds (0.21) (*18*) and let us foresee the presence of an arabinoxylan. The presence in minor amount of rhamnose and galacturonic acid could be potentially attributed to hairy regions of pectins. In fact, their relative proportions between each other are in accordance with those described by Cui et al. for the pectins of the flaxseeds mucilage (*17*). The nonretention of these fragments portends the towering esterification level on the carboxyl located at C6 of the galacturonic acid by methyl groups, preventing their connection with the chromatographic anion exchanger. With regard to significant rates of galactose and glucose, the hypothesis of the existence of other polymers (in addition to the arabinoxylan) can be proposed.

So as to separate potential F1 polymers, additional investigations were done. Fraction F1 has been recovered and freeze-dried, and 50 mg was eluted on SEC through an S-400 HR column. The heterogeneity of the F1 fraction was illustrated by the detection of two appreciably homogeneous peaks called $F1_A$ and $F1_B$ (**Figure 2**). Then, they were gathered individually in, respectively, 56 and 44% and analyzed (**Table 1**). The respective positions of $F1_A$ and $F1_B$ in the S-400 SEC chromatogram pointed out a smaller molecular weight for the latter.

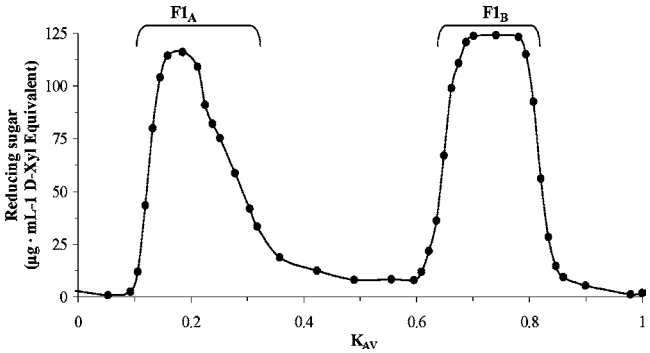

Figure 2. Quantification of neutral (○) polysaccharides during SEC on S400 HR column, by A_{480} analysis after specific identification using the method described Monsigny (*31*). Neutral polymers concentrations are expressed in D-Xyl equivalent ($\mu g \cdot mL^{-1}$).

Table 2. Determination of the Molecular Weight, Polydispersity Coefficient and Relative Proportion of Each Recovered Fraction Analyzed by SEC-MALLS

fraction	M_w[a]	M_w/M_n[a]	yield[b]
$F1_A$	8.46×10^5	1.2	98
$F1_B$	6.5×10^4	1.2	88
global F2	2.0×10^5	1.5	
$F2_A$	3.1×10^5	1.0	45
$F2_B$	1.3×10^5	1.4	55

[a] M_w, weight-average molecular weight; M_w/M_n, polydispersity coefficient. [b] Based on calculated mass recovered (% w/w).

The former, $F1_A$, exhibits a monosaccharide composition globally similar to the entire nonretained AEC fraction (excepted for glucose), characteristic of an arabinoxylan with a high content of xylose (41.5%), an A/X ratio of 0.32, and a wide substitution by galactose. Concerning the $F1_B$ fraction, a significant concentration of glucose (64.6%), accompanied by galactose in a minor amount (15.3%), was observed. These results suggested the presence of glucans and/or galactans, two cell-wall polymers (*23*, *24*). At this stage of the work, the overall nonretained AEC fraction (F1) was presumed to be a polysaccharide mixture consisting preponderantly of arabinoxylans ($F1_A$), glucans and/or galactans ($F1_B$), and, in smaller proportions, pectins.

HPSEC with RI and MALLS detections was used to characterize the polymers by providing realistic values of their molecular weights and molecular weight distributions (**Table 2**). As intended, the SEC-MALLS analysis of the $F1_A$ fraction (**Figure 3**) allowed the recovery of a single polymer (polydispersity coefficient = 1.2) with a M_w of 846 000. In contrast, the observation with RI signal of only one peak in fraction $F1_B$ (**Figure 4**) thwarted the possible involvement of two distinct macromolecules, such as glucans and galactans. However, the hypothesis of their elution in the same time can be considered even it is less probable as the polydispersity coefficient is close to 1 (**Table 2**). Therefore, the polymer could be assimilated to a galactoglucan with a M_w of 6.5×10^4. We underscore, on the chromatogram, the presence of a residual pollution (illustrated by the MALLS signal) of this fraction by the $F1_A$ polymer (8%).

After the description of the nonretained AEC fraction, we analyzed the fraction F2 (**Figure 1**) represented in minor amount (17%). The profile tended to be heterogeneous, implying the coexistence of several species of macromolecules. This was corroborated by the polydispersity coefficient of 1.5 obtained by the SEC-MALLS analysis on the global F2 fraction (**Table 2**). With regard to its proportions in Gal (30.9%), Ara (28.1%),

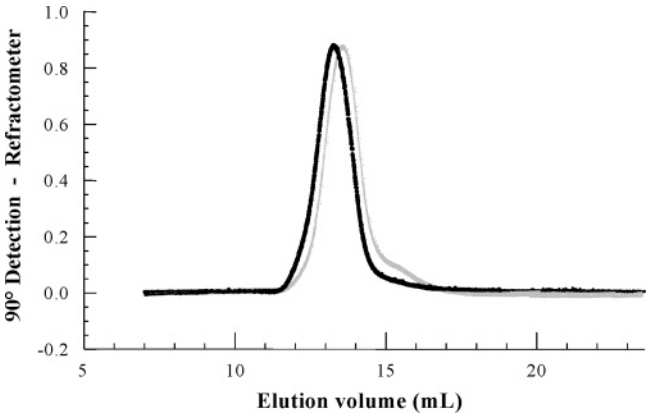

Figure 3. 90° MALLS (—) and refractometer signals (–) of fraction $F1_A$ after chromatography on OHPAK SB 804 and 806 HQ columns.

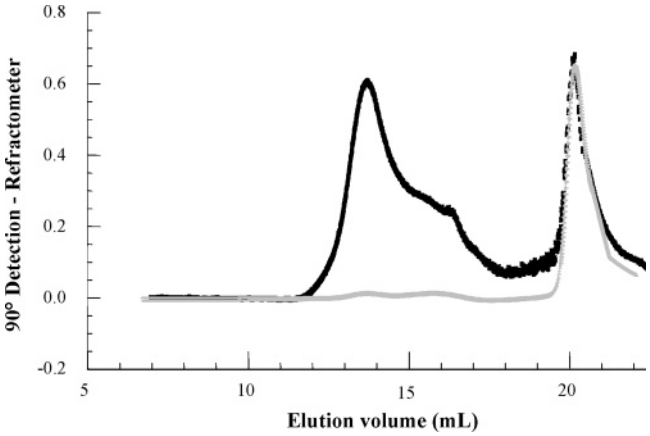

Figure 4. 90° MALLS (—) and refractometer signals (–) of fraction $F1_B$ after chromatography on OHPAK SB 804 and 806 HQ columns.

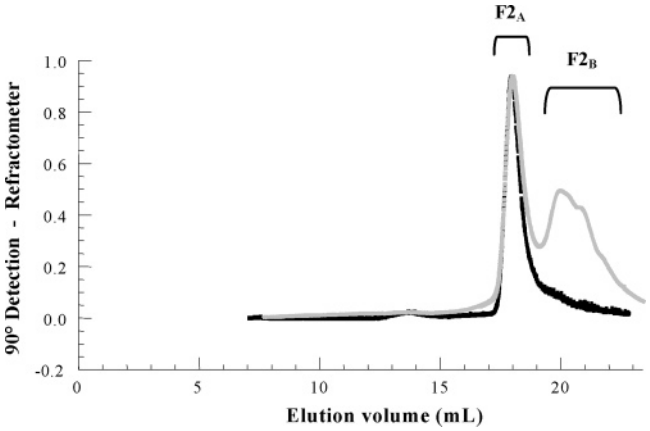

Figure 5. 90° MALLS (—) and refractometer signals (–) of fraction F2 after chromatography on OHPAK SB 804 and 806 HQ columns.

GalA (20.4%), and Rha (7.1%), this fraction may be qualified as a pectin type (*23*, *24*). **Figure 5** displays the presence of two major groups, respectively, $F2_A$ and $F2_B$, in the F2 fraction. The first one presented M_w/M_n close to 1, whereas the latter exhibited a polydispersity (1.4). Thus, $F2_A$ and $F2_B$ displayed, respectively, 3.1×10^5 and 1.3×10^5 molecular weight averages (**Table 2**). This analysis procured the information that fraction F2 contained two separate HMW macromolecular families.

To investigate the possible presence of interesting rheological properties, the comparison of steady shear flow curves between flaxseeds and flaxseed cake AEC nonretained fractions has been carried out (**Figure 6**). They exhibited, at 2% (w/v), a shear

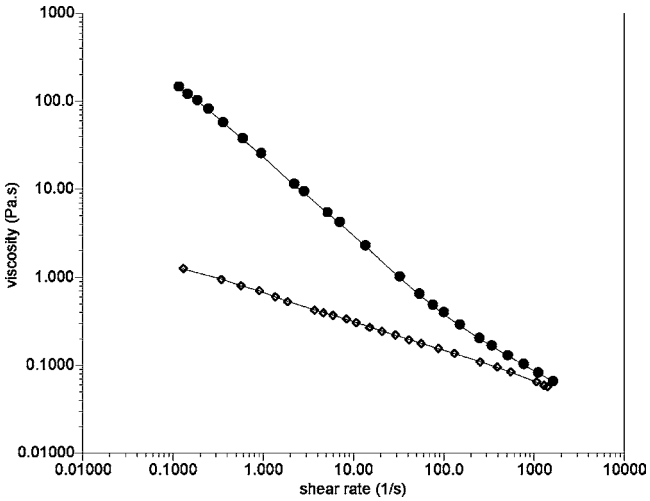

Figure 6. Steady shear rheological flow curves of purified neutral fraction of flaxseeds (●) and flaxseed cake (◇) mucilages at 25 °C.

thinning behavior (apparent viscosity decreases with increases of shear rate) over the broad range of shear rates. A slight shear thinning behavior was observed with the cake material. Despite these similarities of rheological responses, they differed widely in their zero rate viscosity evaluated by fitting the data to a Cross model, with 826.5 and 9.6 Pa•s for, respectively, the seeds and the cake dispersions. This result demonstrates that this flaxseed cake neutral fraction, even if it included HMW arabinoxylans, presents an insignificant viscosity and cannot be used in the future in that condition as texturing agent. Astoundingly, this fraction contains the same type of HMW AX (according to the structural studies) as the flaxseed mucilage neutral fraction previously studied (*18*). The important polydispersity of the latter (2.94) suggests the presence of several families of arabinoxylans. They could interact with themselves to furnish this interesting rheological behavior (gel formation). Hence, the negative impact of the oil extraction process (temperature and high pressure) on the rheological properties could be attributed to the destruction of this putative network. The exploration of this heterogeneity and of possible interactions is actually in progress in our laboratory.

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