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Molecular investigations of flaxseed mucilage polysaccharides

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ABSTRACT

The molecular properties of flaxseed mucilage were determined using a multi-angle laser light scattering (MALLS) detector coupled on-line to size exclusion chromatography (SEC) and asymmetric flow field-flow fractionation (AF4). Water and salt solution were tested as mobile phases. The SEC-MALLS method gave partial information and enabled molecular characterization of disaggregated mucilage molecules. Regardless of the eluent used, the observed M_w ranged from about 1.6×10^6 to more than 10×10^6 g/mol for mucilage polysaccharides. The AF4-MALLS system enabled a complete analysis of mucilage carbohydrate aggregates in water, in which two populations were satisfactorily separated. The molecular weight distribution (MWD) of molecules ranged from 1.5×10^6 to more than 4×10^8 g/mol. Experiments showed that the conformational structure of mucilage molecules was strongly influenced by ionic strength. Mucilage carbohydrates exhibited a spherical and compact structure in NaCl solution while they displayed a random-coil conformation in water.

1. Introduction

Flax (*Linum usitatissimum* L.) contains a great quantity of soluble (9–10%) and insoluble (20–30%) fibers [1]. Soluble fibers, known as mucilage polysaccharides, are located in the mucous epidermis, the outer layer of the hull [2]. Reports on the extraction of flaxseed mucilage are common in the literature and the yield can range from 3.5 to 10.2% of the total seed weight [2,3]. Mucilage yield and its composition depend on the genotype, the environment, and the extraction conditions [4,5].

Different methods can be used to characterize complex polysaccharides. Size-exclusion chromatography or flow field-flow fractionation, both coupled on-line with a multi-angle laser light-scattering detector (SEC-MALLS/AF4-MALLS), or matrix-assisted laser desorption/ionization coupled on-line with time-of-flight mass spectrometry (MALDI–TOF–MS) have been described as very efficient methods to provide useful information about the characterization of a highly disperse polymer matrix. AF4 methods seem to provide a better coverage of particle sizes [3,6–8]. A combination of ion exchange and size exclusion chromatography enabled purification of three distinct polysaccharides from mucilage [9]. The flaxseed mucilage consists of one neutral arabinoxylan fraction and two acidic fractions. The neutral fraction (75%) is an arabinoxylan (AX) with a (1–4)-β-D-xylan backbone substituted by arabinose and D- and L-galactose chains on the *O*-2 and *O*-3 of a xylose position. Terminal glucuronic acid and D-galactose residues were also found to branch out from the xylan chain [6,10,11]. The two other fractions correspond to type I rhamnogalacturonans (RG I), acidic polysaccharides consisting of a backbone of −4)-α-D-galactosyluronic acid-(1–2)-α-L-rhamnose-(1-, in which rhamnose can be substituted with linear α-L-arabinan and β-D-galactan oligosaccharides and type I arabinogalactans [10]. Warrand et al. [9] performed a molecular weight analysis of the mucilage using SEC coupled with a MALLS detector. They revealed that the neutral polymer is disperse and its average molecular weight is 1.2×10^6 g/mol. The two acidic fractions were characterized, the first (15%) being rather homogeneous with an average M_w of 6.5×10^5 g/mol, while the second (85%) was relatively heterogeneous with an average M_w of 1.7×10^4 g/mol. Warrand et al. [6] analyzed the neutral fraction with SEC-MALLS and were able to identify three polymer groups of arabinoxylans with variations in galactose and fucose content. Their molecular weights were 5.7×10^6 g/mol, 9.3×10^5 g/mol and 3.2×10^5 g/mol and they represented 11.1%, 42.4% and 52.4% of the neutral fraction, respectively. Guilloux et al. obtained the same profile with higher molecular weights (4.3×10^6 g/mol, 1.3×10^6 g/mol and 7.8×10^5 g/mol) [3].

The present study concerns the molecular characterization of flaxseed mucilage using both SEC and AF4 coupled on-line with

Abbreviations: AF4, asymmetric flow field-flow fractionation; FFF, field-flow fractionation; MALLS, multi-angle laser light scattering; M_w, weight-average molecular weight; MWD, molecular weight distribution; $\langle r_g^2 \rangle$, root mean square radius; R_z, *z*-average root mean square radius; RI, refractive index; SEC, size exclusion chromatography.

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a MALLS detector. Its main objective was to determine both the qualitative and quantitative characterization of mucilage, as well as other molecular characteristics of this complex polysaccharide such as molecular weight distribution and conformational features. We report a practical application of asymmetric flow field-flow fractionation with multi-angle light scattering and refractive index detection (AF4-MALLS-RI) for the separation and characterization of flaxseed mucilage. AF4 is a sub-type of the larger family of flow-field fractionation techniques [12] and offers the advantage of an open-channel separation without shear degradation and/or column adsorption effects that can arise with SEC. Unlike SEC, resolution can be controlled by varying the cross-flow. Once sufficient resolution is achieved, detection by MALLS-RI enables the calculation of the mass percent of the extracted carbohydrate polymers and determination of the weight-average molecular weight and root mean square radii (R_z) without the need for calibration standards. A higher resolution would enable more reliable molecular characterization and differentiation of samples in which variations may be small.

2. Materials and methods

2.1. Plant materials

Mature oil-type flaxseeds analyzed in this study correspond to brown commercial cultivars grown in 2012 in the North of France. Cultivars were kindly given by GIE Linéa Semences de Lin and Laboulet Semences.

2.2. Mucilage extraction

Extractions were performed using a seed/distilled water ratio of 1:25 (w/v) in preheated water for 1 h at 80 °C with an adjustable temperature-controlled heater. Mechanical stirring was carried out using a paddle? At 140 rpm. The extracts were filtered through a strainer and a 250-μm sieve and precipitated using three volumes of 99% isopropyl alcohol for 1 h at 4 °C. Precipitates were recovered by centrifugation and dispersed in distilled water before being freeze-dried. The extraction yield was expressed as the percentage of the dried mucilage (g) for 100 g of dried seeds.

2.3. Protein and ash content

Mucilage protein content was determined according to the Dumas method (the AOAC method 990.03 [13]). The nitrogen content of the samples was quantified by the flash combustion technique using a LECO FP528 nitrogen analyzer (LECO France, Garges les Gonesse, France). One hundred milligrams of dried mucilage was weighed on tin foil and analyzed in triplicate. A conversion factor of 6.25 was used to calculate the crude protein content from the nitrogen content. The ash content of the mucilage was determined according to the AOAC method 923.03 [14].

2.4. Preparation of mucilage

25 mg of freeze-dried mucilage was solubilized in 25 mL of the carrier liquids. Preparation of mucilage samples required magnetic stirring at 80 °C for 1 h. The samples were filtered through a 0.45-μm pore-size regenerated cellulose syringe filter (Phenomenex, Le Pecq, France) prior to injection.

2.5. Instruments

Both SEC and AF4 fractionations were carried out using the AF4 Eclipse3 F System (Wyatt Technology, Santa Barbara, CA, USA) serially connected to a UV detector tuned at 214 nm (Agilent 1200, Agilent Technologies, Germany), a MALLS detector operating at a wavelength of 658 nm (Dawn multi-angle Heleos TM, Wyatt Technology Corporation, Europe) and an interferometric refractometer measuring at 632.8 nm (Optilab rEX, Wyatt Technology Corporation, Europe). Flows and injections were performed with an Agilent 1100HPLC equipped with an in-line vacuum degasser, isocratic pump and autosampler (Agilent Technologies, Germany). A 0.1-μm in-line filter (Pall Corporation, France) was installed between the main pump and the Eclipse system.

Data were collected and processed with the Astra Software version (v. 5.3.4.20) (Wyatt Technology). The MALLS detector was calibrated with toluene. 2×10^4 g/mol pullulan at a concentration of 6 mg/mL was used to normalize the diodes of the MALLS detector. The RI detector was calibrated with sodium chloride and the temperature was set at 35 °C. A dn/dc value of 0.134 mL/g was determined for the mucilage in our carrier liquid. This was obtained experimentally by direct injection into the RI detector of five different concentrations of mucilage using an external 2-mL loop. The latter enabled all of the injected mass to be recovered.

Table 1
The method parameters of the AF4 system for flaxseed mucilage analysis.

Mode	Time (min)	Duration (min)	X mL start	X mL end
Focus	0	0.6		
Focus + injection	0.6	2		
Focus	2.6	5		
Elution	7.6	15	0.5	0
Elution	22.6	20	0	0

2.6. Size exclusion chromatography

Deionized water or 0.1 M NaCl (w/v) containing 0.02% NaN_3 (w/v) added as a preservative was used as the mobile phase and filtered through a 0.45-μm PTFE membrane (Millipore, France). The carrier solution was maintained at 30 °C. 100 μL of mucilage polymers (1 mg/mL of water) was injected onto an on-line size exclusion chromatography column and eluted at a flow rate of 0.5 mL/min for 60 min. The SEC line consisted of an OHpak SB-G guard column and two OHpak SB-804HQ and SB-806HQ columns in series (300 mm L × 8 mm I.D., Shodex Showa Denko K.K., Japan).

2.7. Asymmetric flow field-flow fractionation

The channel had a trapezoidal geometry and a length of 195 mm. The thickness of the spacer used in this experiment was 350 μm. The ultrafiltration membrane forming the accumulation wall consisted of regenerated cellulose with a molecular weight cut-off of 10^4 g/mol (Millipore PLGC membrane, Wyatt Technology Corporation, Europe).

The AF4 method using deionized water or 0.1 M NaCl containing 0.02% NaN_3 as eluent is described in Table 1. The temperature of the mobile phase was set at 30 °C. The channel flow rate was set at 0.5 mL/min and the focus flow rate at 0.5 mL/min. After 0.6 min of cross-flow adjustment and focus flow equilibration, the sample injection (1 mg/mL) onto the channel was started at a flow rate of 0.20 mL/min for 2 min to rinse the sample loop thoroughly. Then, molecules were relaxed and focused for 5 min at a flow rate of 0.5 mL/min before elution was started. For the elution, an initial cross-flow rate of 0.5 mL/min was set, and then linearly decreased to 0 mL/min over 15 min. The cross-flow rate was finally set at 0.0 mL/min for 20 min.