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Influence of the extraction time on macromolecular parameters of galactomannans

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ABSTRACT

This study evaluated the aqueous extraction of galactomannans from the seeds of *Mimosa scabrella* (GM), *Stryphnodendron adstringens* (GS) and *Schizolobium parahybae* (GG) for 1, 2, 3, 4, 6, 24 and 48 h. The efficiency of extraction processes was assessed in terms of yield, carbohydrate and protein content. The extraction process, as well as the source of the galactomananns generated molecules with differences in molar mass, viscosity and rigidity analyzed by HPSEC-MALLS/RI/VIS. The extraction time results for each species, based on minimum extraction time and HPSEC-MALLS/RI/VIS results, were 4 h (GM4 h), 6 h (GS6 h) and 2 h (GG2 h) for GM, GS and GG, respectively. In most cases, the apparent persistence length, as determined by viscometry, indicated that aggregates remained in galactomannans after centrifugation and filtration. Results suggest an effective extraction time for each plant source of galactomannan based on its performance and its macromolecular behavior in solution.

1. Introduction

Galactomannans exhibit a wide range of commercially useful properties. They are a versatile material with many applications and different physiochemical properties. In simple aqueous systems, they are effective viscosifiers or thickeners, and stabilizers of emulsions. The absence of toxicity also favors their use in the textile, pharmaceutical, biomedical, cosmetics, and food industries. Because they are plant products, they are also a valuable renewable resource. Galactomannans constitute the second largest group of storage polysaccharides in the plant world (Morrison & Karkalas, 1990; Ono et al., 2003). They have been found in endosperm of seeds of different subfamilies of Leguminosae, mainly *Caesalpinioideae*, *Faboideae* and *Mimosoideae* (Dea & Morrison, 1975; Samonte, Mendoza, Ilag, de la Cruz, & Ramirez, 1989).

Seed galactomannans consist of a (1→4)-linked β-D-mannopyranosyl backbone, substituted at O-6 by single units of α-D-galactopyranose. The mannose/galactose ratio (M/G) varies from approximately 10–1, depending on the plant source. α-D-Galactopyranose residues in the side chains have a marked effect on solubility, and a minimum galactose substitution level of ~10% is necessary for water solubility (Dea & Morrison, 1975; Srivastava & Kapoor, 2005).

The three major galactomannans of commercial importance in the food and non-food industries are guar (*Cyamopsis tetragonolobus*, M/G 1.5), tara (*Caesalpinia spinosa*, M/G 3.0) and locust bean gums (*Ceratonia siliqua*, M/G 3.5). Other commercial galactomannans, such as gums of *Cassia tora* (M/G 8.3), *Trigonella foenum-graecum* (M/G 1.0) and *Prosopis juliflora* (M/G 1.2), are also produced (Dakia, Blecker, Robert, Wathlet, & Paquot, 2008; Funami et al., 2008; Pollard, Eder, Fischer, & Windhab, 2010).

Many reports have been published on the composition and physiochemical properties of galactomannans from different plant sources (Doyle, Giannouli, Martin, Brooks, & Morris, 2006; Gallão et al., 2013; Nwokocha & Williams, 2012; Singh, Sethi, & Tiwari, 2009; Souza, Lucyszyn, Ferraz, & Sierakowski, 2010; Vieira, Mendes, Gallão, & Brito, 2007). However, only guar, locust bean, tara and cassia gum are manufactured on a large scale, and they have been investigated extensively by various researchers (Daas, Grolle, Vliet, Schols, & De Jongh, 2002; Dakia et al., 2008; Funami et al., 2008; Pollard et al., 2008; Renou, Petibon, Malhiac, & Grisel, 2013).

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Mimosa scabrella (bracatinga M/G 1.1), *Stryphnodendron adstringens* (barbatimão M/G 1.5), both of *Mimosoideae* subfamily, and *Schizolobium parahybae* (guapuruvu M/G 3.0) of *Caesalpinioideae* subfamily, which are abundant throughout Brazilian forests, could be additional sources of galactomannan for biotechnological applications. The structural, physical and biological properties of the galactomannan from these seeds have been studied previously (Ganter, Cardoso, Kaminski, & Reicher, 1997; Ganter, Sabbi, & Reed, 2001; Noleto et al., 2009; Ughini, Andreazza, Ganter, & Bresolin, 2004; Vendrusculo et al., 2009; Vianna-Filho, Petkowicz, & Silveira, 2013).

The novelty of the present study is the analysis of the physicochemical properties of *M. scabrella* (**GM**), *S. adstringens* (**GS**) and *S. parahybae* (**GG**) galactomannans at different extraction times using high performance size exclusion chromatography (HPSEC) coupled to multi-angle laser light scattering (MALLS), refractive index (RI) and viscometer (VIS) detectors to evaluate various macromolecular and hydrodynamic properties of the galactomannans and determine how to optimize polysaccharide extraction.

2. Experimental

2.1. Seeds

Mimosa scabrella and *Stryphnodendron adstringens* seeds were acquired from the Instituto Ambiental do Paraná. *Schizolobium parahybae* seeds were purchased from the Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA).

2.2. Extraction of galactomannans

The galactomannans were obtained using an extraction process, with different polysaccharide extraction times using fresh milled seeds for each replicate.

After being milled in a Willey Mill Standard Model No. 3 (Arthur H. Thomas Co.), the flours (powders) obtained from seeds (~0.3 cm size each) of *M. scabrella* (100 g) and *S. adstringens* (100 g) were sieved, and the powder with the same apparent size < 430–620 μm (width opening of 1000 μm) were submitted to enzymatic inactivation in boiling water and then cooled to room temperature. The volume was adjusted to 2 L with purified distilled water at 25 °C to start the extraction under agitation at 750 rpm, using a mechanical stirrer.

Differently from *M. scabrella* and *S. adstringens*, the galactomannan from *S. parahybae* was obtained directly from endosperm due to its higher size of seeds (3 cm). *S. parahybae* seeds (300 g) were boiled in water for 30 min. Next, the seed coat was manually removed and the seeds were cooled for 18 h. Each seed was cut, followed by manual separation of endosperm, which was subjected to drying for 3 days at room temperature. After drying, the endosperm was milled and sieved in a Tecnal Willey TE-650 mill. For extraction, 11.5 g of endosperm was used.

Extractions of the three species were performed at 1, 2, 3, 4, 6, 24 and 48 h, and drops of toluene were added as preservative. At each extraction time, the material was centrifuged in a Hitachi Himac CR21E at 15,428 × *g* for 30 min at 10 °C, and the residue was discarded. The extracts obtained after centrifugation were filtered and treated with 10 g NaCl per L of solution to prevent co-precipitation of protein, then ethanol was added to a concentration of 70%, v/v, and the suspension was kept at 4 °C for 24 h. The precipitates were washed twice with ethanol (90% and 96%), centrifuged at 15,428 × *g* for 20 min at 10 °C after each wash, and then dried under vacuum.

2.3. Polysaccharide characterization

Galactomannans extracted at each time point were hydrolyzed with 2 mol L^{-1} TFA (8 h, 100 °C), and the products reduced with $NaBH_4$ (Wolfrom & Thompson, 1963a) to yield alditols, which were acetylated with pyridine–acetic anhydride (1:1, v/v, 16 h at 25 °C) (Wolfrom & Thompson, 1963b). The resulting alditol acetates were analyzed by gas–liquid chromatography (GLC) using a Hewlett Packard 5890A Series II chromatograph with a flame ionization detector (FID), and injector temperature of 250 °C, a DB-210 capillary column (30 m × 0.25 mm i.d.) with a film thickness of 0.25 μm at 220 °C, and nitrogen (2.0 mL min^{-1}) as the carrier gas.

Total carbohydrate and proteins contents of polymers were determined by methods of Dubois, Gilles, Hamilton, Rebers and Smith (1956) and Hartree (1972), respectively.

2.4. Light scattering analysis

2.4.1. Real-time monitoring of galactomannan disaggregation by time-dependent static light scattering (TDSLS)

The effect on the concentration and aggregation of the galactomannans was determined in real time using the Waters Chromatography System with the following detectors: DSP-F Wyatt Technology multi-angle laser light scattering detector (MALLS), WATERS 2410 differential refractive index (RI) detector and a custom built Validyne P55 (VIS) viscometer attached to an Elenco Precision XP-581 voltmeter, according to Freitas, Busato, Mitchell and Silveira (2011).

The samples were passed through the detectors at 1 mL min^{-1} using a WATERS 515 pump. Elution was monitored by multidetectors and using 0.1 mol L^{-1} $NaNO_2$ and 0.5 g L^{-1} NaN_3 as eluent. The data were collected using Wyatt Technology ASTRA software, version 4.70.07.

The samples were analyzed at an initial concentration of 1 mg mL^{-1} and subjected to three purification steps. The samples were previously solubilized for 16 h and centrifuged at 10,000 rpm at 12 °C for 30 min and were sequentially filtered through 1.2, 0.45 and 0.22 μm cellulose ester filters (Millipore). A recirculation system was used to ensure that there was no change in concentration, so that correct concentrations could be obtained. Retained aggregated material was taken in account, and the galactomannan concentrations (after the filtration process) were corrected before HPSEC-MALLS/RI/VIS measurements. Aggregates also could be disassembled using shear process.

2.4.2. High-performance size-exclusion chromatography coupled to multidetector (HPSEC-MALLS/RI/VIS)

High performance size exclusion chromatography (HPSEC-MALLS/RI/VIS) analyses of the samples was performed on a Waters chromatograph equipped with four Ultrahydrogel columns connected in series (2000, 500, 250, 120; molecular weight ranged from 7×10^6 to 1×10^2 g mol^{-1}; Milford, MA, USA) and attached to the multidetection system, which consisted of a Waters 2410 differential refractometer (RI) detector (Milford, MA, USA), a DSP-F Wyatt Technology multiangle laser light scattering (MALLS) detector (Santa Barbara, CA, USA) and a custom built Validyne P55 (VIS) viscometer attached to an Elenco Precision XP-581 voltmeter adapted on-line.

The mobile phase consisted of a 0.1 mol L^{-1} $NaNO_2$ solution containing 0.5 g L^{-1} NaN_3 at a flow rate of 0.6 mL min^{-1}. Samples were solubilized in 0.1 mol L^{-1} $NaNO_2$ solution containing NaN_3 (0.5 g L^{-1}) for 16 h under magnetic stirring at room temperature, centrifuged (30 min/10,000 rpm/12 °C) and filtered sequentially through 1.20, 0.45 and 0.22 μm cellulose acetate filters (Millipore). The samples (1 mg mL^{-1}) were injected into the equipment and

analyzed at 25 °C. HPSEC-MALLS/RI/VIS data were collected and analyzed using Wyatt Technology ASTRA software, version 4.70.07.

The dn/dc of the samples at concentrations from 0.1 to 1 mg mL^{-1} was previously determined at 25 °C in a Waters RI using 0.1 mol L^{-1} $NaNO_2$ as the solvent. Analysis was performed for the homogeneity, weight average molar mass (M_w), polydispersion index (M_w/M_n), reduced viscosity ($\eta_{sp/c}$) and the radius of gyration $\langle S^2\rangle_z$, parameters using the Zimm equation (Eq. (1)). The data were analyzed with the equation:

$$\frac{Kc}{R\theta} = \frac{1}{M_w}\left(1 + \frac{q^2 \cdot \langle S^2\rangle z}{3} + 2A_2 c\right) \quad (1)$$

$R\theta$ is the excess Rayleigh scattering at scattering vector q (cm^{-1}) and polymer concentration c (g cm^{-3}), and A_2 is the second virial coefficient and K the optical factor, which can be determined by the source of vertically polarized incident light, according to Eq. (2) (Zimm, 1948).

$$K = \frac{4\pi^2 n^2 (dn/dc)^2}{N_A \lambda^4} \quad (2)$$

n is the solvent refractive index, λ is the vacuum wavelength of the incident light, dn/dc is the differential refractive index for the polymer in the chosen solvent, and $q = (4\pi n/\lambda)\sin(\theta/2)$, where θ is the scattering angle. Eq. (1) permits direct determination of M_w, A_2 and the z-averaged mean square radius of gyration $\langle S^2\rangle_z$.

Using the $\langle S^2\rangle_z$ chain values, it was possible to determine the persistence length L_p. L_p is a useful measure for gauging polymer stiffness. In the wormlike chain model, it is related to the polymer contour length L and unperturbed mean square radius of gyration $\langle S^2\rangle_0$, which is the value in Θ-solvent conditions (no excluded volume) (Reed & Reed, 1991):

$$\langle S^2\rangle_0 = \frac{LL_P}{3} - L_p^2 + \frac{2L_p^3}{L} - \left(\frac{2L_p^4}{L^2}\right)\left[1 - \exp\left(-\frac{L}{L_p}\right)\right] \quad (3)$$

The residue masses (m_0) used to determine the contour length ($L = (M/m_0) \times 0.52$ nm) were 262 g mol^{-1}, 230–240 g mol^{-1} and 230–236 g mol^{-1} for the GM, GS and GG samples, respectively, based on the substitution degree determined by alditol-acetates GLC analysis.

The scattering measures were the perturbed $\langle S^2\rangle$, consisting of $\langle S^2\rangle_0$ and intra-chain excluded volume. $\langle S^2\rangle_0$ can be replaced by $\langle S^2\rangle$, in Eq. (3), and the 'apparent persistence length' (L'_p) is obtained, which is nearly a mass-independent measure of coil 'expansivity'. L'_p is robust in this sense, it varies weakly with molar mass (M) because the experimental and theoretical limits of the scaling coefficient α in $\langle S^2\rangle^{1/2} = bM^{\alpha}$ fall in the range of >0.5 and ≤0.6 for coil molecules in good solvents. Using light scattering information, $L'_{p,w}$ and $L'_{p,z}$ were determined using M_w and $\langle S^2\rangle_w^{1/2}$ and M_z and $\langle S^2\rangle_Z^{1/2}$, respectively (Freitas, Drenski, Alb, & Reed, 2010).

Of course, the presence of aggregates calls into question the use of the persistence length notion. The apparent persistence length $L'_{p,\eta}$ was also determined from the $\langle S^2\rangle$ determined by viscometric analysis (to reduce the aggregates influence) using the Flory-Fox Eq. (4) (1951).

$$[\eta] = 6^{3/2}\frac{\Phi}{M}\langle S^2\rangle^{3/2} \quad (4)$$

where Φ is the Flory constant 2.56×10^{23} mol^{-1}.

2.4.3. Statistical analysis

The mean values were obtained in triplicate and statistically analyzed with standard deviations. Analysis of variance followed by Tuckey's post hoc test was utilized to determine the significance ($p < 0.05$).

3. Results

3.1. Effect of filtration on the concentration, yield and galactomannan aggregates

Initial solution concentration of 1 mg mL^{-1} could be altered during a long extraction processing of the galactomannan samples (centrifugation and filtration), for HPSEC experiments. We realized that a time-dependent static light scattering (TDSLS) experiment, using a recirculation system with different filter pores, would ensure the exact concentration and that correct dn/dc measurements were performed.

Fig. 1 shows the results obtained during the real-time monitoring by recirculating TDSLS of two GM samples obtained by extraction performed at 2 h (GM2 h – Fig. 1A) and 48 h (GM48 h – Fig. 1B). For GM2 h, the viscosity remained constant with little reduction of RI after sequential filtration (0.45 and 0.22 μm). However, the 90° LS intensity decreased significantly after filtration through 0.22 μm. Almost no decrease in RI and viscosity was observed for GM48 h, following filtration between 1.2 and 0.22 μm.

Likewise, the LS profiles for GG2 h and GG48 h indicate a notable reduction following 0.45 μm filtration (data not shown). This finding indicates that the concentration was exact for the next macromolecular characterization. The same procedure was also performed for GS to correct the concentration after the filtration process and reduce the influence of aggregates (Table 1).

3.2. Galactomannan extraction yields and M/G molar ratio

Table 2 presents the whole seeds GM, GS and GG galactomannan yields for different extraction times. The extraction method used was home-designed and permitted the acquisition of a pure galactomannan – comparable to commercially obtained guar, locust bean and tara gum – from the ground endosperm.

The yield of galactomannan extracted from GM was 23.6% on average, where GM sample obtained by extraction performed at 4 h (GM4 h) had the higher yield (26% – Table 2). For GS, there was an increase in yield from the first to sixth hour by 48.8%. The increased yield up to 6 h reflected the viability of the extraction process at this time. The GM (M/G 1.1) extraction yields were superior to those of GS (M/G 2–2.6) (without statistical analysis, only comparing the yields) using the same extraction times, which may be explained by the higher degree of galactose substitution in the GM backbone. Unlike the GM examined using the whole seed, galactomannans extracted from GG showed a slightly increased yield due to the increased extraction time. However, the increase in the extraction yield at 48 h compared to the first hour was only 12.7%, which does not justify a lengthy extraction time.

A small increase in the total carbohydrate and protein yield as function of the extraction time was observed for all the three seeds (Table 2), unless to carbohydrate content of GS that the major decrease of 12% was obtained for GS4 h in comparison of GS1 h.

The M/G ratios for GM were 1.1 for all extraction times. For GS extractions, the M/G ratio ranged from 2 to 2.6. The M/G ratio for GG extractions remained between 2.2 and 2.6 (Table 3).

3.3. High-performance size-exclusion chromatography with multidetector analysis

The cumulative mass distribution can be observed in Fig. 2. A profile obtained by HPSEC-MALLS/RI/VIS from GS at 6 h of extraction is shown as an insert in Fig. 2. This profile shows the multi-angle laser light scattering detector (MALLS – 90°), the refractive index detector (RI – Volts) and the viscometric detector (VIS – Volts).

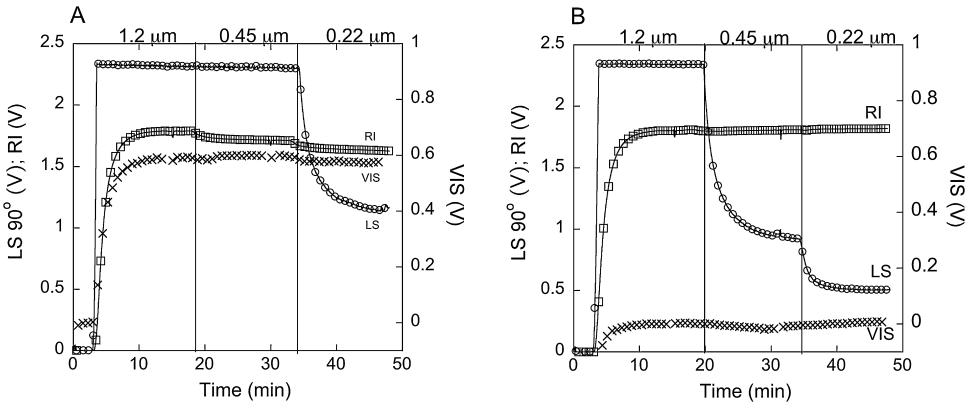

Fig. 1. Profiles selected from triplicates analysis: Light scattering (LS 90°); refraction index (RI); viscometer (VIS) of GM galactomannan samples of (A) 2 and (B) 48 h extractions prepared by centrifugation and 1.2, 0.45 and 0.22 μm sequential filtrations.

Table 1
Concentration of the galactomannan after filtration process at 2 and 48 h of extraction using TDSLS.

Samples	Time of direct extraction (h)	Filtration		
		1.2	0.45	0.22
		Concentration mg mL^{-1}		
GM	2	$0.95 \pm 6 \times 10^{-3}$	$0.92 \pm 1 \times 10^{-3}$	$0.87 \pm 1 \times 10^{-3}$
	48	$0.96 \pm 6 \times 10^{-3}$	$0.96 \pm 6 \times 10^{-3}$	$0.97 \pm 1 \times 10^{-3}$
GS	2	$0.39 \pm 3 \times 10^{-3}$	$0.41 \pm 2 \times 10^{-3}$	$0.24 \pm 2 \times 10^{-3}$
	48	$0.78 \pm 1 \times 10^{-3}$	$0.77 \pm 1 \times 10^{-3}$	$0.73 \pm 2 \times 10^{-3}$
GG	2	$0.94 \pm 9 \times 10^{-3}$	$0.90 \pm 1.5 \times 10^{-3}$	$0.86 \pm 2 \times 10^{-3}$
	48	$0.83 \pm 5 \times 10^{-3}$	$0.85 \pm 2 \times 10^{-3}$	$0.86 \pm 3 \times 10^{-3}$

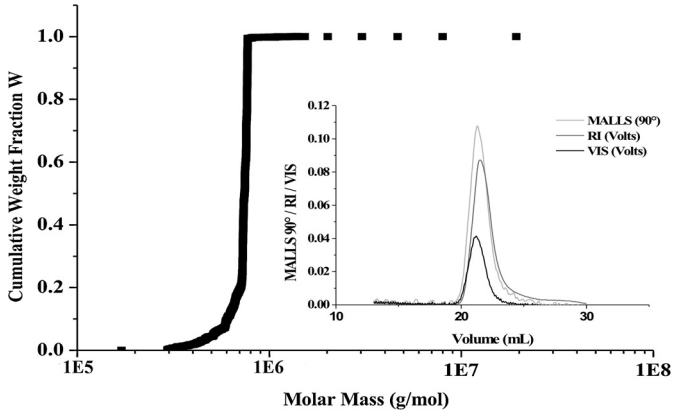

Fig. 2. Profile by HPSEC-MALLS/RI/VIS of *S. adstringens* galactomannan (GS6d) using $NaNO_2$ eluent considering logarithmic function of molar mass and cumulative weight fraction. Insert: Elution profile by HPSEC-MALLS/RI/VIS of *S. adstringens* galactomannan (GS6 h) using $NaNO_2$ eluent and MALLS 90°, RI and VIS detectors.

Table 3 compares the weight average molar mass (M_w), polydispersity index (M_w/M_n), radius of gyration $\langle S^2 \rangle_z^{1/2}$ as a function of molecular mass, the scaling coefficient α, intrinsic viscosity $[\eta]$ and apparent persistence length (L_p') determined from *z* and *w* (average molar mass and radius of gyration by HPSEC-MALLS and HPSEC-MALLS-viscometer) for GM, GS and GG.

Table 3 shows the values of the α obtained and standard deviations, which indicate that the polymer behaved as a random coil in a good solvent, with little polymer chain expansion or contraction.

We observed no significant differences when comparing the values obtained for M_w, M_w/M_n, $\langle S^2 \rangle_z^{1/2}$ variables in the extraction of *M. scabrella* seeds. This observation suggests that the extraction time had no effect on these parameters for this species (GM). However a different $[\eta]$ value was obtained at 48 h, suggesting that the aggregates are still influencing the experiments, mainly due to a variable recovery from HPSEC-MALLS experiments ($p < 0.05$).

For GS analysis, there was a relationship between the M_w and $\langle S^2 \rangle_z^{1/2}$ values and extraction times, but no statistical difference

Table 2
Extraction yields in % mass, % total sugar and % protein from *M. scabrella* (GM), *S. adstringens* (GS) and *S. parahybae* (GG) galactomannans as from the whole seeds.

Time (h)	GM			GS			GG		
	Yield* (%)	Carbohydrate** (%)	Protein*** (%)	Yield* (%)	Carbohydrate** (%)	Protein*** (%)	Yield* (%)	Carbohydrate** (%)	Protein*** (%)
1	22.2	89	5.5	12.3	90	7.8	6.3	89	1.9
2	23.1	86	5.4	10.8	87	7.5	5.2	91	2.0
3	24.5	88	5.7	10.4	86	8.0	5.6	93	2.0
4	26.0	91	6.0	12.0	79	7.9	5.9	92	1.8
6	23.5	93	5.9	18.3	86	8.2	6.1	94	2.1
24	26.0	92	6.2	18.2	87	8.2	6.6	95	2.2
48	20.1	91	6.3	18.6	89	8.4	7.1	94	2.3

* Yield obtained in terms of mass.
** Total carbohydrate according Dubois et al. (1956).
*** Protein determination according Hartree (1972).

Table 3
Physico-chemical parameters determinated by HPSEC-MALLS/RI/VIS of *M. scabrella* (GM), *S. adstringens* (GS) and *S. parahybae* (GG) galactomannans obtained by aqueous extraction.

Sample	M/G ratio	Recovery[a] %	$M_w 10^6$ g mol^{-1}	M_w/M_n	$\langle S^2\rangle_z^{1/2}$ nm	α	$[\eta]$ mL g^{-1}	$L'_{p,\eta}$ nm[*]	$L'_{p,w}$ nm[*]	$L'_{p,z}$ nm[*]
GM1 h	1.1	98.1 ± 5.9a	1.1 ± 0.4a	2.0 ± 0.4a	83 ± 21a	0.5 ± 0.1	434.1 ± 28.1a	4.4 ± 0.5	13.0 ± 6.5	13.0 ± 1.5
GM2 h	1.1	76.8 ± 11.4a	0.8 ± 0.0a	1.5 ± 0.2a	58 ± 4a	0.5 ± 0.1	355.7 ± 28.9a	4.1 ± 0.5	8.1 ± 1.0	9.5 ± 4.6
GM3 h	1.1	83.7 ± 13.2a	1.0 ± 0.4a	1.9 ± 0.3a	76 ± 22a	0.5 ± 0.1	416.8 ± 5.0a	3.2 ± 0.7	11.0 ± 3.0	15.0 ± 6.7
GM4 h	1.1	97.8 ± 12.9a	1.0 ± 0.4a	1.9 ± 0.3a	73 ± 24a	0.6 ± 0.1	435.0 ± 13.0a	3.2 ± 0.5	10.0 ± 2.8	13.4 ± 6.4
GM6 h	1.1	100.0 ± 16.2a	0.9 ± 0.3a	1.9 ± 0.2a	63 ± 20a	0.6 ± 0.2	431.7 ± 50.8a	3.3 ± 0.5	8.6 ± 3.0	12.0 ± 5.8
GM24 h	1.1	86.2 ± 7.1a	1.1 ± 0.4a	2.0 ± 0.3a	83 ± 33a	0.5 ± 0.1	466.1 ± 186.0a	3.9 ± 0.7	11.0 ± 3.0	13.4 ± 7.0
GM48 h	1.1	54.4 ± 61.2a	1.4 ± 0.4a	1.3 ± 0.4a	41 ± 30a	0.6 ± 0.4	143.3 ± 53.8b	2.4 ± 1.2	6.5 ± 5.6	2.52 ± 0.7
GS1 h	2	56.1 ± 4.9a	1.7 ± 0.8a	1.3 ± 0.3abc	108 ± 54a	0.5 ± 0.2	1207.0 ± 6.2a	5.4 ± 0.3	11.0 ± 5.6	13.2 ± 9.4
GS2 h	2	59.8 ± 17.1a	1.3 ± 0.2ab	1.1 ± 0.1a	84 ± 17a	0.5 ± 0.1	1346.8 ± 15.8a	5.5 ± 0.5	11.0 ± 0.8	9.85 ± 4.7
GS3 h	2.3	60.8 ± 6.5a	1.0 ± 0.1ab	1.1 ± 0.1a	73 ± 21a	0.5 ± 0.2	1291.8 ± 32.8a	5.8 ± 0.1	9.1 ± 4.0	9.2 ± 5.4
GS4 h	2.4	63.9 ± 12.5a	1.1 ± 0.1ab	1.2 ± 0.1ab	80 ± 18a	0.6 ± 0.2	1156.1 ± 22.2a	5.3 ± 0.3	10.0 ± 1.6	12.3 ± 5.6
GS6 h	2.4	66.1 ± 14.3a	1.2 ± 0.2ab	1.1 ± 0.0a	75 ± 24a	0.4 ± 0.2	1317.8 ± 1.3a	5.5 ± 0.3	9.0 ± 3.6	10.0 ± 7.7
GS24 h	2.4	72.4 ± 4.0a	0.9 ± 0.3b	1.7 ± 0.5bc	69 ± 36a	0.5 ± 0.0	829.6 ± 36.8a	4.7 ± 0.7	8.2 ± 5.5	7.7 ± 0.4
GS48 h	2.6	71.5 ± 18.3a	0.7 ± 0.1b	1.8 ± 0.4c	64 ± 14a	0.4 ± 0.1	846.5 ± 25.8a	5.5 ± 0.5	8.3 ± 2.9	14.2 ± 7.7
GG1 h	2.2	98.6 ± 7.8a	0.7 ± 0.1abc	1.6 ± 0.3a	75 ± 4ab	0.5 ± 0.2	669.9 ± 18.3a	5.4 ± 0.1	12.0 ± 0.3	21.5 ± 3.0
GG2 h	2.2	99.3 ± 3.8a	0.9 ± 0.4a	1.4 ± 0.3a	98 ± 29a	0.5 ± 0.1	983.2 ± 12.6a	5.4 ± 0.1	13.0 ± 2.1	19.0 ± 6.8
GG3 h	2.4	93.6 ± 0.6a	0.9 ± 0.0ab	1.4 ± 0.3a	91 ± 10a	0.5 ± 0.2	826.0 ± 21.8a	5.3 ± 0.1	14.0 ± 2.3	16.9 ± 4.5
GG4 h	2.6	88.7 ± 20.3a	0.6 ± 0.0bcd	1.3 ± 0.1a	65 ± 11ab	0.6 ± 0.1	868.7 ± 54.8a	5.3 ± 0.1	12.0 ± 1.1	12.1 ± 3.3
GG6 h	2.6	84.1 ± 18.6a	0.6 ± 0.1cd	1.3 ± 0.2a	62 ± 15ab	0.6 ± 0.1	908.7 ± 20.4a	5.3 ± 0.1	11.0 ± 3.2	11.5 ± 4.7
GG24 h	2.6	84.2 ± 18.5a	0.5 ± 0.0cd	1.3 ± 0.1a	53 ± 14b	0.5 ± 0.2	493.3 ± 234.8a	5.1 ± 0.2	9.8 ± 1.6	9.9 ± 4.0
GG48 h	2.6	94.2 ± 11.8a	0.3 ± 0.0d	1.4 ± 0.1a	44 ± 8b	0.6 ± 0.2	380.6 ± 137.4a	4.9 ± 0.5	8.4 ± 2.5	8.6 ± 2.3

Means followed by same letter do not differ at 5% probability.
[a] Recovery calculated from HPSEC-MALLS/RI/VIS.
[*] Determined using a Kratky-Porod worm-like equation.

between the $\langle S^2\rangle_z^{1/2}$ means. The downward trend of the M_w results with increase of extraction time was confirmed statistically for differences in the means. Statistical analysis also showed that increasing the extraction time did not influence the recovery rate.

Statistical differences were observed in the polydispersity index with different extraction times, but there was no change in the polydispersion tendency due to time. For GG, we observed a reduction, after 3 h, in molar mass, $\langle S^2\rangle_z^{1/2}$ and $[\eta]$ as a function of extraction time.

In all of the samples (GM, GS and GG), we observed a significant reduction in L'_p, using the viscometer as compared to the light scattering information. For all of the samples evaluated, $L'_{p,\eta}$ was lower than $L'_{p,w}$ and $L'_{p,z}$ due the presence of aggregates that remained in the solution.

4. Discussion

The extraction process, as well as the source of the galactomananns generated molecules with differences in molar mass, viscosity and rigidity, as well as in the mannose/galactose (M/G) ratio. All of these factors characterize the functional properties of galactomannans in aqueous dispersions and their macromolecular and hydrodynamic behavior (Larizadou, Biliaderis, & Izydorczyk, 2000). Due to their water-soluble characteristics, polysaccharides are extracted in high yield from endosperm, which possesses energy-reserve and hydration functions (Pollard et al., 2010).

The yield of the GM4 h galactomannan (26%) were similar to yields described in the literature for galactomannans obtained from *Cassia javanica* (26%) and *Caesalpinia pulcherrima* (25%) (Azero & Andrade, 2002), as well as commercial sample of locust bean gum (28%) (Bouzouita et al., 2007). However, the results from previous studies were generally obtained by overnight water extraction.

The GM (Mimosoidea subfamily) yielded galactomannans with constant M/G ratio (1.1). However, galactomannans from GS (Mimosoidea subfamily) and GG (Caesalpinioideae subfamily) species showed change in M/G across extraction times (2.0–2.6 for GS and 2.2–2.6 for GG). The Caesalpinioideae subfamily was already known to give rise to galactomannans with increasing values of the M/G ratio and may be modified by different extraction processes. However the extractions from species belonging to the Mimosoideae subfamilies yielded galactomannans with constant M/G ratios (Buckeridge, Dietrich, & Lima, 2000).

For GS and GG the fractions of galactomannan obtained at the start of the extraction process were more soluble in water and less substituted at the end of the process. Possibly due to the content of heterogeneous and polydisperse population of galactomannans with respect to both the degree of substitution and the molar mass.

The effect of the extraction time on recovery, viscosity and light scattering of the samples was determined during sample processing (centrifugation and filtration). Although many studies on light scattering have used concentrated or poorly soluble solutions to eliminate aggregation, the aggregation process is inevitable, making it very difficult to characterize the polymer by light scattering techniques (Freitas et al., 2010, 2011; Kanao, Matsuda, & Sato, 2003).

The presence and removal of aggregates was indicated by decrease in light scattering (MALLS 90°), maintenance of the (RI), and maintenance of the viscosity on membrane filtration for the samples of GS, GM and GG. For these samples the centrifugation and filtration steps contributed to removing aggregates in solution. The maintenance of viscosity and small concentration differences, with a significant reduction in the LS, is strictly related to the aggregate removal during sample preparation. The molecular aggregates have a significant influence in light scattering, mainly at low scattering vectors (q) (data not shown). Specially for GM48 h, a huge LS reduction was observed, suggesting that a large extraction time promotes an increase in the number of aggregated galactomannan in solution.

It is well known that galactomannans have different molecular characteristics, such as differing conformations in solution, a fact often attributed to the molar mass or differences in the length of the polymer chain, as well as the extent of galactose substitution. The intrinsic viscosity can also be influenced by expansion or contraction of the chain due to the influence of the addition of galactosyl units to the mannan chain (Pollard et al., 2010; Semenova, 2007).

The GM sample had a $\langle S^2\rangle_z^{1/2}$ values ranging from ≈ 60 to 80 nm (Table 3), with the lowest value being obtained at 48 h, accompanied by lower recovery rates and viscosity compared to those at the initial extraction times. Intrinsic viscosity was reduced, and this result characterized the exhaustive (complete) extractions of galactomannans (Pollard et al., 2008). For GM, we did not observe any statistically significant differences between the means of the values obtained for M_w, M_w/M_n, $\langle S^2\rangle_z^{1/2}$, $[\eta]$ and recovery rate variables in the various extraction. However, a huge increase in the error for all the analysis was observed at 48 h of extraction. This could be related to aggregates in solution. Our hypothesis is that the shearing process, generated during filtration and in the HPSEC column, can break down aggregates, but they can reform during the sample resting. This could explain the significant variation on recovery, M_w, $\langle S^2\rangle_z^{1/2}$ and $[\eta]$ for GM48 h. The aggregates could specifically increase the M_w and reduce the $\langle S^2\rangle_z^{1/2}$ and $[\eta]$, due to a more compact structure formation (Table 3).

For GS, we observed a relationship between the M_w and $\langle S^2\rangle_z^{1/2}$ values and extraction time, but there was no statistical difference between the $\langle S^2\rangle_z^{1/2}$ means. The downward trend of the M_w results with increased extraction time was confirmed statistically for mean differences. Statistical analysis also showed that increasing the extraction time did not influence the recovery rate from HPSEC columns.

The GS6 h sample exhibited a polydispersity index closer to 1 and an 18.3% yield. These results combined with those presented in Table 1 suggest that this extraction time was best for obtaining GS. Our results for GS6 h were comparable to those obtained previously (Ganter, Heyraud, Petkowicz, Rinaudo, & Reicher, 1995). Except for the yield, the results obtained for the GS6 h sample were similar to those for *S. adstringens*, which indicates the effectiveness of the 6 h extraction.

For GG galactomannan, all of the extractions showed a high recovery rate from the HPSEC-MALLS system (Table 3). We also observed a reduction in M_w and $[\eta]$ and $\langle S^2\rangle_z^{1/2}$, as well as a decrease in GG at 24 h and 48 h. The decrease in the values, M_w and $[\eta]$ and $\langle S^2\rangle_z^{1/2}$ suggests that extended extraction times can result in polymer chain degradation (Pollard et al., 2010). Chain degradation is defined here as either the reduction in molar mass due to breakage of covalent bonds in the main chain or in the branches or branch removal from the main chain. These breaks can occur randomly or sequentially (McCoy & Madras, 1997).

The extraction of GG at 2 h presented the highest values for $\langle S^2\rangle_z^{1/2}$ and $[\eta]$ (98 nm and 983.2 mL g^{-1}, respectively). The 3rd extraction yielded similar results for the same parameters, but an additional hour is required for this extraction, a fact that makes GG extraction with 2 h more favorable. The results for GG at 2 h of extraction, obtained by HPSEC-MALLS/RI/VIS, were comparable to those obtained previously by the authors (Vianna-Filho et al., 2013).

The method reported by Pollard et al. (2008) improved the 2 h extraction process for locust bean gum, whose M/G ratio is similar to that of GG. Those authors obtained a recovery rate of 78.5%, and the polydispersity index ranged from 1.5 to 1.8. These similarities suggest that the extraction process is viable up to 2 h for GG.

Petkowicz et al. (1999) evaluated the apparent persistence length (L'_p) for oligosaccharides from GM and reported an L_p of 9.3 nm, also from the worm-like or Kratky-Porod method and similar to our average results. The $L'_{p,\eta}$ determined by the viscometer $\langle S^2\rangle_\eta^{1/2}$ was similar to the previously published value of 3–4 nm for galactomannans reported by Patel, Picout, Ross-Murphy and Harding (2006), where $[\eta]$ and M_w were obtained using the popular Bushin–Bohdanecky method. This similarity suggests that the influence of entanglements and aggregates in the galactomannans could be reduced using the viscometer without any other treatment processes, such as heating or pressure.

The statistical analyses did not reveal any differences in L'_p in our data across extraction times for the three species. However, in all cases, there was a reduction in L'_p with increasing time. The $L'_{p,\eta}$ was lower than the $L'_{p,w}$ and $L'_{p,z}$ in almost all of the samples, which clearly indicates that some molecular aggregates remained in the system, even after centrifugation and filtration. As the viscometer is less susceptible to the aggregates in the systems, we demonstrated that the viscometric data are best for determining the L'_p of the galactomannans. Similar results have been reported for other polysaccharides (Freitas et al., 2010, 2011) and for guar galactomannans (Lubambo et al., 2013).

The techniques adopted in this work, such as analysis of polymer extraction types and times, purification by centrifugation and filtration, concentration analysis, and light scattering with multidetector (HPSEC-MALLS/RI/VIS), provide an initial assessment of native Brazilian galactomannans sources, including *M. scabrella* (GM), *S. adstringens* (GS) and *S. parahybae* (GG). These methods can serve as a model for analysis of polysaccharides from other species.

The individual assessment of the three species was important because the galactomannans extracted from seeds belonging to the same subfamily showed different yields, monosaccharide compositions and physicochemical properties in solution.

5. Conclusion

It was observed that for *M. scabrella* (GM), extraction time is irrelevant, with similar results for all extraction times. For *S. adstringens* (GS), yield was increased from 1 to 6 h, while the yield of the pure *S. parahybae* (GG) endosperm extraction increased over time. Signs of degradation were verified by HPSEC-MALLS/RI/VIS, especially at 24 and 48 h for *S. adstringens* and *S. parahybae*. According to statistical analysis, there was no statistically significant difference between the results for M_w, M_w/M_n, $\langle S^2\rangle_z^{1/2}$ and recovery rate. The reduction observed in L'_p using the viscometer confirmed the presence of aggregates that remained in the samples after filtration and passage through the column. The best extraction time results for each species, based on minimum extraction time and HPSEC-MALLS/RI/VIS results, were 4 h (GM4 h), 6 h (GS6 h) and 2 h (GG2 h) for *M. scabrella*, *S. adstringens* and *S. parahybae*, respectively.

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