Carbohydrate Polymers 84 (2011) 550–559

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Characterization of galactomannans derived from legume endosperms of genus *Sesbania* (Faboideae)

M.A. Pollard*, P. Fischer, E.J. Windhab

Laboratory of Food Process Engineering, Institute of Food Science, Nutrition, & Health, ETH Zurich, Schmelzbergstr. 9, 8092 Zürich, Switzerland

ARTICLE INFO

Article history:
Received 28 September 2010
Received in revised form
26 November 2010
Accepted 6 December 2010
Available online 13 December 2010

Keywords:
Galactomannan
Guar
Sesbania
Multidetection SEC

ABSTRACT

Galactomannan polysaccharides extracted from seed endosperms of 12 species of the genus *Sesbania* (legume subfamily Faboideae) have been characterized by size-exclusion chromatography, dilute-solution viscometry, and oscillatory-shear rheology to determine their potential as aqueous thickeners. The molecular composition and chain-length distribution were found to be nearly identical, and thus galactomannans within this genus are presumed to share a common molecular structure ($DS_{gal} \approx 0.7$, $M_w \approx 2.5 \times 10^6$, PDI ≈ 2). Solutions at $c > c^*$ exhibited shear-thinning behavior, and strong dependence of viscosity on concentration ($\eta \sim c^5$). Purified samples had Huggins' constants near 0.5, and negligible surface activity based on pendant drop tensiometry. Seed characteristics such as shape, mass, and endosperm content were also assessed, and based on this investigation, some *Sesbania* legume endosperms are advantageous for industrial processing and could be adapted for guar gum replacement.

1. Introduction

Anderson's 1949 screening study of galactomannan seed gums reported that 5 *Sesbaniae* seeds had enlarged endosperms (ca. 5–20% of seed mass) and therefore were potential sources of galactomannan gums (Anderson, 1949). *Sesbaniae* (Faboideae) legumes are primarily bushy plants used for soil enrichment and animal forage, and several species are widely cultivated throughout the tropics (Evans & Rotar, 1987; Wiersema & León, 1999). They comprise at least 10 species in Australia (8 endemic) (Burbidge, 1965), 7 species in Hawaii (all endemic) (Char, 1983), 33 species in Africa (Gillet, 1963), and several New World species that are toxic to livestock (Powell, Plattner, & Suffness, 1990).

Although more intensive research began in 1950s, there is still only limited characterization available on *Sesbaniae* seed gums. Studies have been limited primarily to *S. grandiflora* (L.) Pers., a small tree cultivated in the tropics, and *S. egyptica* (= *S. egyptiaca*), an African species. Rao and Rao (1964) reported that *S. grandiflora* seeds contained water-soluble galactomannan with a chain length of 18–20 hexose units and galactose to mannose (G:M) ratio of 2:3 (degree of galactose substitution, $DS_{gal} = 0.66$). Srivastava, Singh, and Rao (1967) investigated the molecular structure of *S. grandiflora* galactomannan further and suggested that the chemical structure may be same as guar (*C. tetragonoloba*), with G:M ratio of 1:2 ($DS_{gal} = 0.50$). *S. speciosa* was also found to contain a galactomannan with G:M of 1:2.2 ($DS_{gal} = 0.45$) (Rao, Rao, & Rao, 1980). Kapoor and Farooqi (1979) concluded that the viscosity behavior of *S. aculeata* galactomannan (dhaincha gum) was similar to carob (*C. siliqua*) and guar galactomannan seed gums. Wankhede et al. (1995) also studied *S. aculeata* and reported G:M to be 1.2:2.2 ($DS_{gal} = 0.54$). Mathur and Mathur (2005) reported that *S. bispinosa* had G:M of 1:2 ($DS_{gal} = 0.50$), but lower molecular weight than guar.

More recent studies indicate that the chemical composition and structure of *S. egyptica* closely resembles guar. Bhattacharyya, Das, Banerji, and Farooqi (1982) reported that this galactomannan had the classical galactomannan molecular structure, with G:M of 1:1.67 ($DS_{gal} = 0.59$) and degree of polymerization of 35–38. Gupta and Grasdalen (1989) investigated the fine-structure of *S. egyptiaca* galactomannan using NMR methods, and reported that average chain composition by several different analytical methods were in very good agreement ($DS_{gal} = 0.62$–0.65). We also reported that *S. egyptica*, *S. sesban*, and *S. grandiflora* had very high molar masses (≈2–3 million g/mol) based on SEC, with a broad chain-length distribution very similar to guar (Pollard, Eder, Fischer, & Windhab, 2010).

As *Sesbaniae* seed galactomannans appeared to be promising for further development as aqueous thickeners, we have assessed the characteristics of 12 *Sesbaniae* galactomannans which were available from commercial suppliers. Table 1 lists the trade names of these 12 species as well as their probable botanical classification (USDA ARS-GRIN; ILDIS). Note that inconsistent naming of *Sesbania* species within the literature may have led in some instances to misidentification (Evans & Rotar, 1987). For instance, *S. aegyptiaca* (= *S. egyptica*) appears to be an orthographic variant of *S. sesban* (L.)

* Corresponding author. Tel.: +41 44 632 8536; fax: +41 44 632 1155.
E-mail address: michael.pollard@ilw.agrl.ethz.ch (M.A. Pollard).

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doi:10.1016/j.carbpol.2010.12.019

Table 1
Nomenclature and identification of the *Sesbania* species investigated in this study.

Trade name	Identification[a]	Common names[b]
S. aculeata[c]	*S. bispinosa* (Jacq.) W. Wight var. *bispinosa*	Prickly sesban (Eng.) Dhaincha (Hind.)
S. cannabina[c]	*S. cannabina* (Retz.) Pers.	Common sesban (Eng.) Dhaincha (Hind.) Yellow pea bush (Aust.)
S. drummondii[d]	*S. drummondii* (Rydb.) Cory	Poison-bean, rattlebox, rattlebush (Eng.)
S. egyptica[c]	*S. sesban* (L.) Merr. subsp. *sesban* var. *sesban* (= *S. aegyptiaca* (Poir.) Pers.)	Egyptian riverhemp, river bean, African sesbania (Eng.)
S. exaltata[d]	*S. exaltata* (Raf.) Rydb. ex A.W. Hill	Coffeebean, Colorado river-hemp, peatree (Eng.)
S. formosa[c]	*S. formosa* (F. Muell.) N.T. Burb.	Dragon flower-tree, swamp corkwood, white dragon-tree (Eng.)
S. grandiflora[c]	*S. grandiflora* (L.) Pers.	Scarlet wistaria tree, vegetable hummingbird, West Indian pea, agathi (Eng.) Agasti (Hind.)
S. javanica[c]	*S. javanica* Miq.	Marsh sesbania (Eng.)
S. rostrata[c]	*S. rostrata* Bremek. and Oberm.	
S. sesban[c]	*S. sesban* (L.) Merr.	Egyptian riverhemp, river bean, African sesbania (Eng.)
S. speciosa[c]	*S. speciosa* Taub.	Wisteria tree (Eng.)
S. tripetii[c]	*S. punicea* (Cav.) Benth.	Brazilian glory-pea, coffeeweed, rattlebox, rattlepod, red sesbania (Eng.)

[a] USDA GRIN database.
[b] Multilingual Multiscript Plant Name Database.
[c] Sandeman Seeds, Lalongue, France.
[d] B&T World Seeds, Paguignan, Aigues-Vives, France.

Merr., according to the International Legume database & Information Service (ILDIS, 2010).

Our study addresses primarily the question of molecular structure of *Sesbania* galactomannans after extraction from the seed, and therefore we attempted to determine whether the two molecular structure parameters, DS_{gal} and M_w, were similar to guar, as suggested from previous work, or showed some unique features. The primary tools to examine this question were Size Exclusion Chromatography (SEC) with multidetection, which provides access to molecular size as well as chain composition information when employed with the Mark–Houwink–Sakurada (MHS) model, and ^{13}C Nuclear Magnetic Resonance (NMR) to estimate chain composition using peak abundances. Additionally, rheometry and viscometry were employed to assess the flow and interaction behavior of dilute and semidilute solutions. We have also documented the seed size, shape, and internal morphology, which should be beneficial for any future development of these seeds as gum sources, and possibly help to resolve conflicts over botanical identification. Limited sample quantity remains one drawback of the study, as a full comparison cannot be made of all molecular structural parameters or properties.

2. Experimental

2.1.1. Seed origin

Certified guar seeds (Lewis cultivar) were provided by Texas Foundation Seed, Vernon, TX, USA, and purified reference guar was obtained using industrial endosperm splits (Unipektin AG, Eschenz, CH). *S. exaltata* and *S. drummondii* were purchased from B&T World Seeds, Paguignan, Aigues-Vives, France. The remaining seeds were purchased from Sandeman Seeds, Lalongue, France.

2.1.2. Seed characteristics

Size, mass, and component analysis were conducted on as-received seeds; malformed or dried seeds were discarded. Mass frequency distribution was performed by binning of ca. 150 seeds after determining seed mass on an analytical balance. Seed sizes (length, width, thickness) were determined with a digital caliper. Component masses were determined gravimetrically after air-drying the manually separated endosperm, germ, and hull.

2.1.3. Photographic images

Photographs were taken under natural light with a Nikon D700 equipped with a Nikkor AF 60 mm lens. Color correction was based on the 18% gray value. Cropping of digitized images and scale calibration was performed with ImageJ software (version 1.43, http://rsb.info.nih.gov/ij/index.html).

2.2. Swelling tests, endosperm extraction, and powder preparation

Endosperm swelling tests, extraction, and powder preparation were conducted as described previously (Pollard et al., 2010).

2.3. Multidetection SEC

The multidetection SEC instrumentation, method, and protocols were described previously (Pollard et al., 2010). Samples were prepared by dissolving unpurified endosperm powders at room temperature so that $c_{polysaccharide} \approx 0.15$ mg/ml, then tempered at 85 °C for 30 min, filtered through 0.46 PTFE syringe filter, and separated on broad-distribution pore-size columns at 35 °C.

2.4. DS_{gal} determination by ^{13}C NMR

The molar average DS_{gal} was estimated from proton-decoupled ^{13}C NMR spectra of purified galactomannans in D_2O, based on peak integration of resolved peaks using a peak-fitting routine (software iNMR, Mestrelab Research). Peaks were assigned based on previous studies (Bociek, Izzard, Morrison, & Welti, 1981; Grasdalen & Painter, 1980; Gupta & Grasdalen, 1989). DS_{gal} was determined from the following peak ratios: splitting (substituted vs. unsubstituted) of C-5 mannose resonance, splitting of C-6 mannose, and C-1 galactose/C-1 mannose. The best agreement based on a reference guar was obtained from the C-6 splitting and the C-1 signals.

2.5. DS_{gal} determination by SEC and MHS model

The MHS model for galactomannans can be written as follows:

$$[\eta] = K(M_o)^{-(1+a)} \cdot m_{bb} \cdot M^a \quad (1a)$$

where $K \cdot m_{bb} = 18.0$ and $a = 0.61$ were reported in the previous study (Pollard et al., 2010). In this model, M_o is the repeat unit mass, a molar average which varies according to chain composition, m_{bb} is the fixed molar mass of backbone mannose units (162 g/mol), $[\eta]$ has units dL/g, $[\eta]$ and M refer to 'local' values from inline detection, and the exponent is independent of M_o within the measurement error.

Using a substitution $M_o \approx m_{bb} + DS_{gal} \cdot m_{bb}$, the model can be expressed in terms of the molar average degree of substitution,

Fig. 1. Color photographs of *Sesbania* legume seeds investigated in this study (scale bar subdivision = 1 mm).

DS_{gal}, as follows:

$$[\eta] = K(DS_{gal} + 1)^{-(1+a)} \cdot m_{bb}^{-a} \cdot M^{a} \quad (1b)$$

DS_{gal} can now be determined as a fitting parameter, taking $[\eta]_{peak} - M_{peak}$ pairs from SEC data. The peak values refer to those obtained over the chromatographic peak to avoid artifacts.

It is also possible to apply this method more generally across the entire chain-length distribution, to estimate DS_{gal} for every elution increment. The main problem with this method are convolution artifacts from viscometric detection, which generate positive curvature in plots of $\log[\eta]$ vs. $\log M$. To avoid these convolution artifacts without applying an arbitrary data treatment, it is best to use only peak values of $[\eta]$ and M signals. This necessarily means that DS_{gal} refers only to a very narrow high-MW portion of the molecular components. The whole sample average determined by NMR may be different if there is wide composition dispersity within the sample.

2.6. Rheometry

Rheometry was performed on an MCR-300 rheometer using Couette and plate-plate geometries (Anton Paar GmbH, Graz, Austria), equilibrated at 25 °C. Solutions were prepared from endosperm powders by dissolving at room temperature, tempering the solution at 85 °C for 30 min, and storing at 5 °C for no longer than 3 days before measurement. Measurements were performed within the linear viscoelastic regime. Low-frequency oscillatory data satisfied the Cox–Merz empirical rule ($\eta^*(\omega)_{oscill.} = \eta(d\gamma/dt)_{shear}$).

2.7. Surface tension

The home-built apparatus and experimental method for pendant-drop tensiometry measurement were described previously (Gunde, Kumar, Lehnert-Batar, Mäder, & Windhab, 2001). Surface tension of polysaccharide solutions ($c \approx 0.5$ mg/ml) was determined over a period of 8 h equilibration time at 20 °C.

3. Results

3.1. Seed characteristics

Color photographs of the 12 *Sesbaniae* seed types are shown in Fig. 1. Mean seed weight was found to be species-dependent, ranging from ca. 10 mg (*S. sesban*) to 106 mg (*S. drummondii*). Variation around the mean for a given species is well approximated

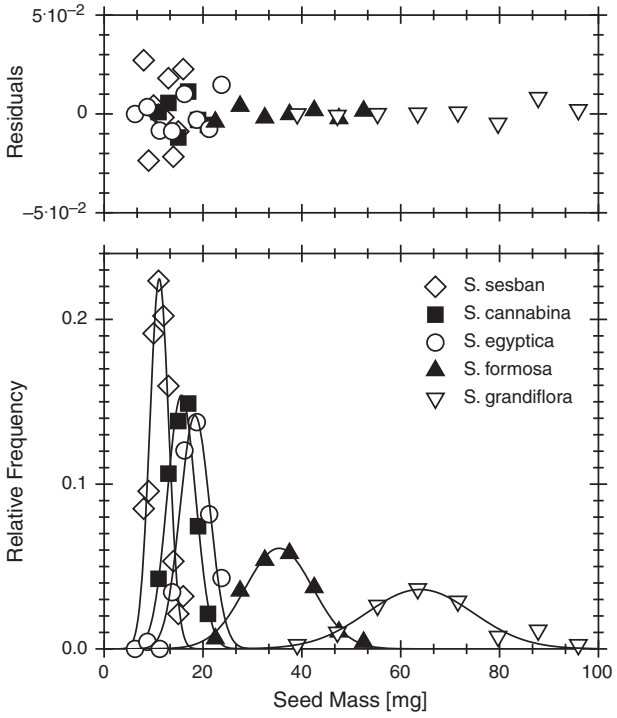

Fig. 2. Seed mass distribution plotted as a histogram. Gaussian fits and residuals are shown.

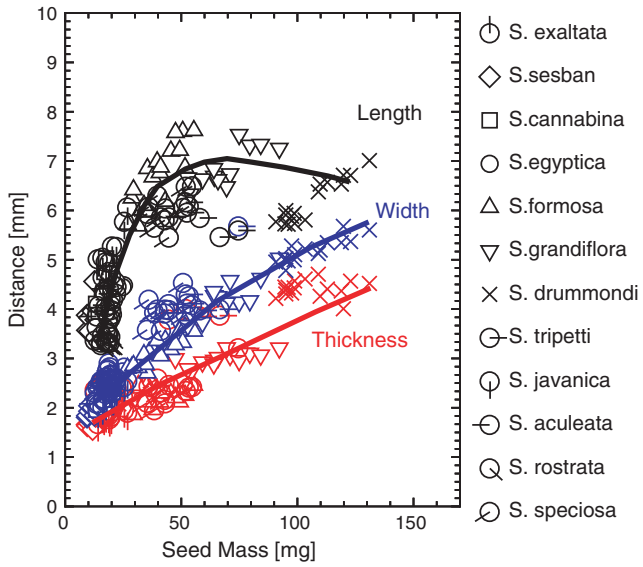

Fig. 4. Seed dimensions plotted as a function of seed mass. Length, width, and thickness refer to major, semi-major and minor axes, respectively. Lines are drawn through the data as an aid to viewing.

by a Gaussian frequency distribution (Fig. 2), with nearly constant relative peak width (mean/st.dev. ≈ 5).

To illustrate seed shape, photographs were taken of seeds set on glass slides, oriented along the major seed axes (Fig. 3). Seed width and thickness appear to follow a consistent growth law when plotted as a function of seed mass (Fig. 4). Seed length is more variable, particularly for the larger seeds, such that the length/width or length/thickness ratios are not constant.

In Fig. 5 are images of seed components as obtained by manual dissection of the seeds after swelling. The seed morphology for each type appears to be largely the same: semi-transparent white endosperm completely surrounding a yellow germ. After drying the percentage of each component was determined on a dry mass basis and for convenience the data are plotted as a function of dry seed mass (Fig. 6). The percentage of hull relative to the dry seed mass was quite constant for all species, but endosperm and germ content were variable. The highest endosperm content, 30–40%, was found in the smallest seeds, whereas the heavier seeds contained closer to 25% endosperm. For a given species, endosperm mass appears to scale proportional to seed mass, and thus the endosperm content is constant for a given species.

S. tripetii seeds showed the strongest departure from the overall trends in Figs. 4 and 6, having larger widths, higher hull content and correspondingly lower germ content. These seeds also required the harshest swelling treatment (>30 min in boiling water).

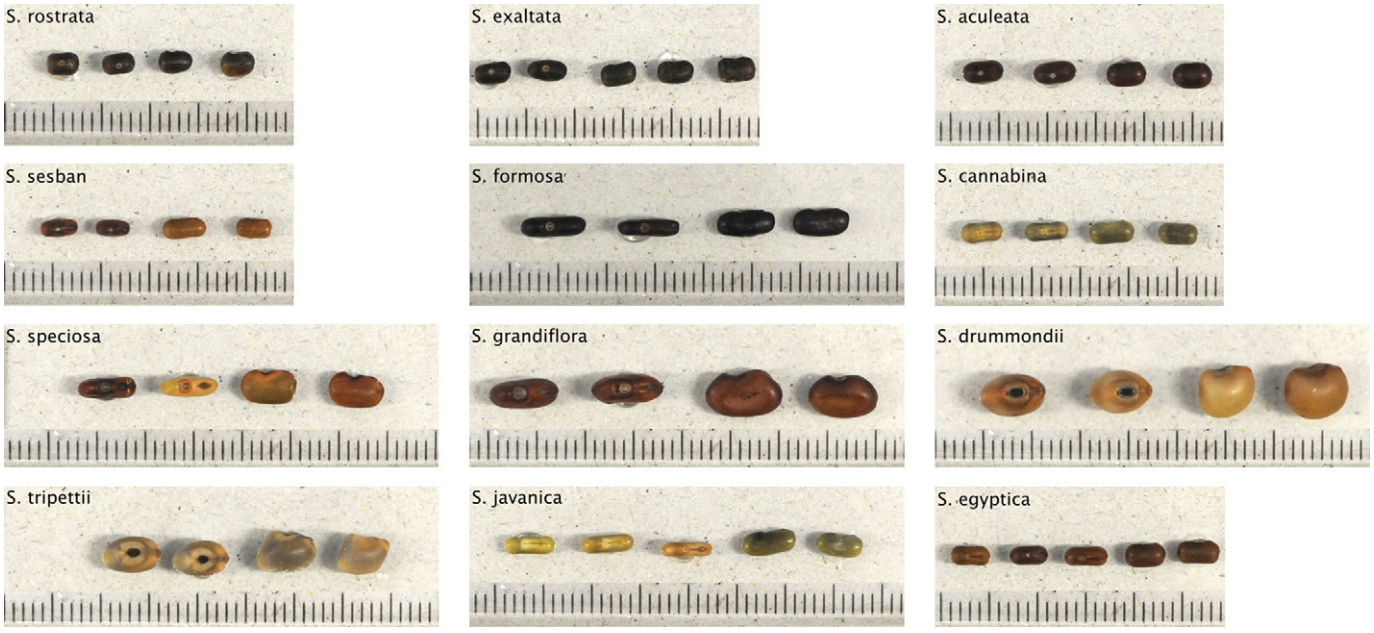

Fig. 3. *Sesbania* seed shape and dimension as viewed along major seed axes (scale bar subdivision = 1 mm).

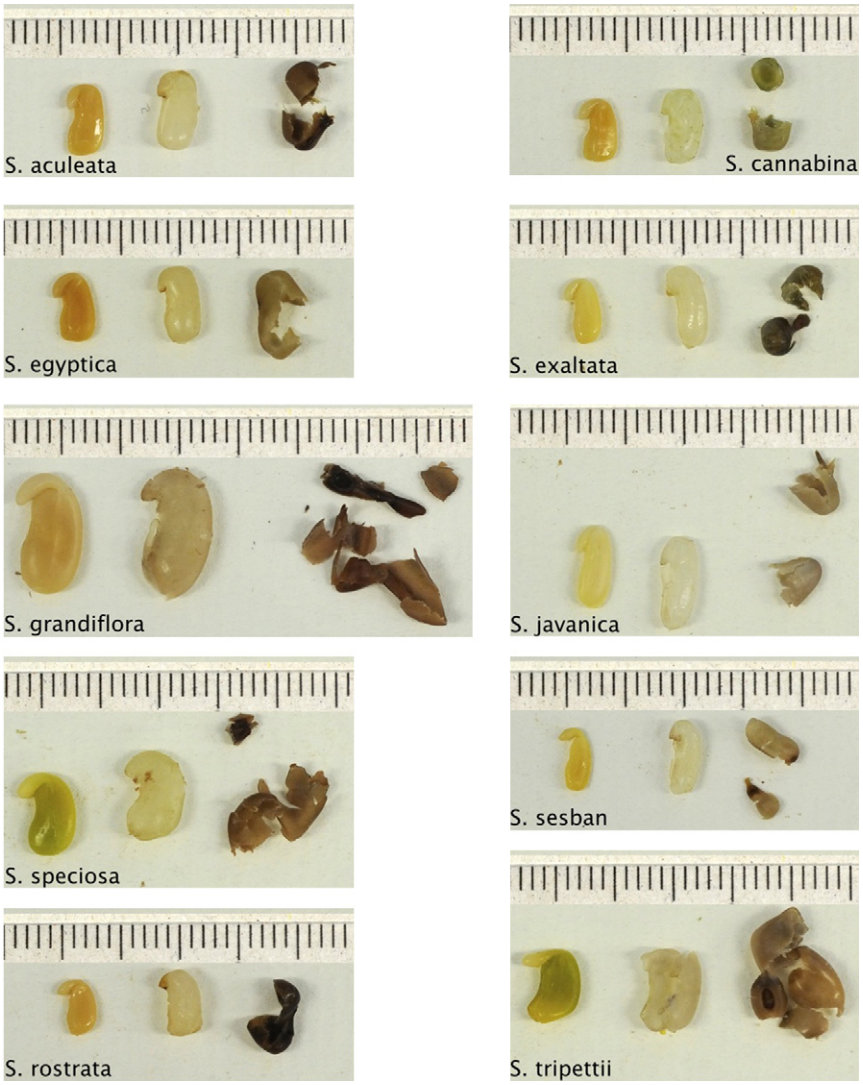

Fig. 5. Morphology of *Sesbaniae* seeds obtained by manual dissection of swollen seeds. From left to right are the inner germ, surrounding endosperm, and outer seed coat (scale bar subdivision = 1 mm).

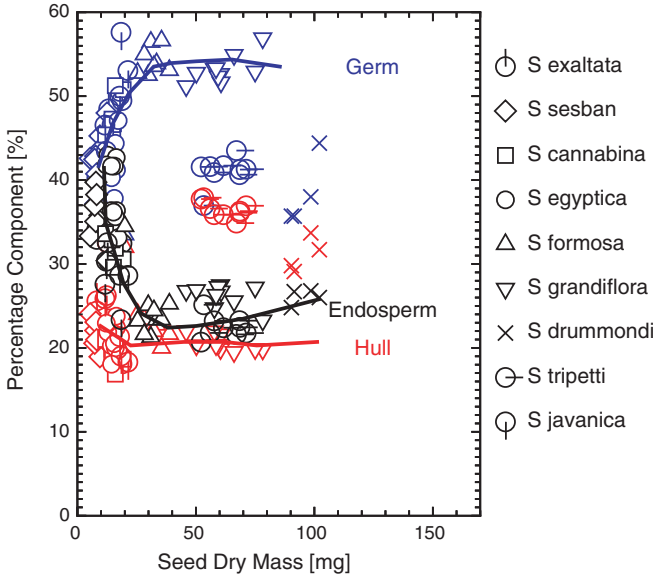

Fig. 6. Proportion of endosperm, germ and hull content plotted as a function of dry seed mass. Lines are drawn as a guide to the eye.

The observations on mass, size, and shape suggests a convenient subdivision of *Sesbaniae* seeds into two categories: (a) small, cylindrical seeds and (b) large pea-shape seeds. The former comprise *S. aculeata*, *S. cannabina*, *S. egyptica*, *S. exaltata*, *S. javanica*, *S. rostrata*, and *S. sesban*, all of which have similar appearance, size (ca. 10–20 mg), and endosperm content (ca. 35%). This category appears to correspond to a botanical classification—the '*S. sesban* complex' (Evans & Rotar, 1987). The pea-shape seeds are more strongly differentiated in terms of appearance and size; these comprise *S. drummondii*, *S. formosa*, *S. grandiflora*, *S. speciosa*, and *S. tripetii*. The endosperm content of this category is lower (ca. 25%), and the germ proportionally larger, than the cylindrical seeds.

3.2. SEC characterization

Six galactomannans were characterized by SEC to determine average molecular weight, intrinsic viscosity, molar mass distribution, size–mass scaling, and provide estimates of the galactomannan content (Table 2). The results were very similar to galactomannans from other species within the Faboideae subfamily (e.g., fenugreek and guar), with following ranges observed: M_w(LALS) = 2.1–2.8 × 10^6 g/mol, $[\eta]_w$ = 15.0–18.6 dL/g. Values of M_w obtained by the Viscotek triple detection method (TD) were

Table 2
SEC characterization of *Sesbaniae* galactomannans prepared from unpurified endosperm powders (LALS = low-angle light scattering, TD = Viscotek triple-detection, UC = universal calibration).

	M_w-LALS/10^6 (g/mol)	PDI	M_w-TD/10^6 (g/mol)	PDI	M_w-UC/10^6 (g/mol)	PDI	$[\eta]$ (dL/g)	Endosperm galactomannan content (est.) (%)	DS_{gal} (est.) (–)
S. cannabina	2.47 ± 0.01[a]	1.7	2.43 ± 0.11[a]	2.6	1.93 ± 0.01[a]	4.1	17.0 ± 0.1[a]	39.2 ± 0.5[a]	0.70
S. egyptica	2.81 ± 0.02	1.5	2.85 ± 0.12	2.5	2.03 ± 0.04	3.5	18.6 ± 0.1	36.9 ± 0.2	0.71
S. exaltata	2.04 ± 0	1.3	2.65 ± 0.01	2.4	1.59 ± 0.02	3.2	15.0 ± 0.1	42.0 ± 0	0.70
S. formosa	2.77 ± 0.08	1.7	2.44 ± 0.11	2.6	1.89 ± 0.01	3.8	17.6 ± 1.3	31.7 ± 0.3	0.75
S. grandiflora	2.32 ± 0.14	2.1	2.55 ± 0.06	3.1	1.75 ± 0.01	5.1	17.4 ± 0.1	24.1 ± 0.3	0.71
S. sesban	2.44 ± 0.04	1.8	2.43 ± 0.03	2.9	1.74 ± 0.01	4.4	16.5 ± 0.1	38.7 ± 0.2	0.71

[a] Standard deviation of duplicate measurement.

in excellent agreement with the standard LALS results; somewhat lower values were obtained by universal calibration method (UC).

The refractive index chromatograms show only unimodal, broad peaks (Fig. 7). Polydispersities were close to or slightly less than 2.0, and the derived molar mass distributions were very similar to that found for guar. Variations in the distribution are only slight would easily be explained by slight hydrolysis of some samples. Over the main chromatographic peak, the intrinisic viscosity and mass signals for each of the 6 galactomannans display the expected dependence on elution volume and overlap to a considerable extent. Such a high degree of overlap of locally detected signals is a strong indication that these elution increments contain identical molecular species. This interpretation is strengthened by noting that M and $[\eta]$ are independent determinations for each eluting fraction. Because the $[\eta]$ signal is very sensitive to chain composition, as reflected in DS_{gal} (Pollard et al., 2010), we can also assume that DS_{gal} for the components eluting near the main peak must be very similar. The size–mass scaling exponents were quite sensitive to the fitting region due to positive curvature found on $\log[\eta] - \log M$ coordinates, but were close to those found in the previous study ($[\eta] \sim M^{0.61}$, $R_v \sim M^{0.54}$).

It is unfortunately not possible to state whether the entire distribution of molecular components is identical across all samples, as the signals are not sufficiently sensitive at very low and very high retention volume. The derived R_v signal indicates that only chains having R_v of 50–150 nm are, to a good approximation, giving identical signals. Outside these limits, inline detection is not reliable, and it cannot be guaranteed in fact that separation exclusively by size has taken place in this part of the chromatogram.

The SEC data as a whole are best explained by assuming that each *Sesbaniae* galactomannan contains a subset, and perhaps a majority, of molecular components in common. That is, average composition and molecular weight distribution are close enough to be regarded as indistinguishable by current methods. The SEC characterization therefore provides high quality data supporting a model of conservation of the molecular structure at least across these 6 galactomannans, and possibly for the entire genus *Sesbaniae*.

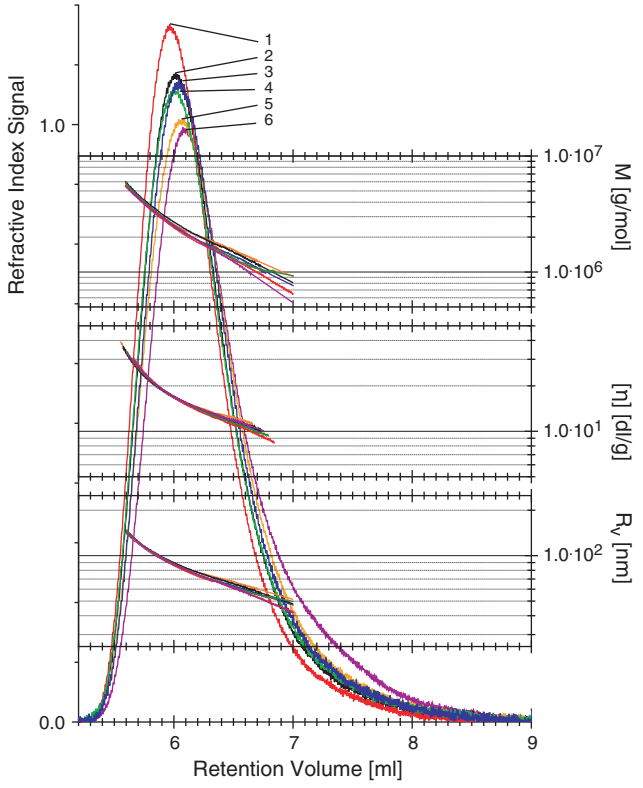

Fig. 7. Multidetection SEC profiles of *Sesbaniae* galactomannans. Refractive index traces are overlaid with inline detector signals: molar mass M, intrinsic viscosity $[\eta]$, and derived viscometric radius R_v. Labels: 1—*S. egyptica*, 2—*S. cannabina*, 3—*S. grandiflora*, 4—*S. formosa*, 5—*S. sesban*, 6—*S. exaltata*.

3.3. DS_{gal} determination by ^{13}C NMR

The molar average degree of galactose substitution, DS_{gal}, was obtained from integration of ^{13}C spectra from three purified samples and found to be very similar to the molecular composition of guar (DS_{gal} = 0.654, Daas, Grolle, Vliet, Schols, & de Jongh, 2002), although the exact values were sensitive to the chosen resonance peak (Table 3). For these three samples, DS_{gal} was 0.59–0.73 using the split C-5 resonance, and 0.59–0.79 using the C-1(Man) and C-1(Gal) resonances. The split C-6 resonance gave lower values, 0.49–0.57, which was also the case for the guar standard. It is possible that C-6 has a longer relaxation time, which would prevent quantitative assessment of ^{13}C abundance under the chosen pulse delay time (10 s). The result for *S. egyptica* is close that found by Gupta and Grasdalen (1989) who reported DS_{gal} = 0.62 by ^{13}C NMR for a hydrolyzed sample from the same seed. Our decision to use nondegraded samples at concentrations above c^* unfortunately resulted in low S/N spectra that required peak-fitting to extract peak areas. Diad information could not be obtained due to the poor

Table 3
Average degree of galactose substitution DS_{gal} of purified galactomannans by ^{13}C NMR, based on mannose carbon 5 splitting, mannose carbon 6 splitting, and the ratio of galactose carbon 1 and mannose carbon 1.

	DS_{gal}		
	M-5 splitting	M-6 splitting	G-1/M-1
C. tetragonoloba	0.68	0.49	0.62
S. egyptica	0.73	0.57	0.79
S. grandiflora	0.59	0.49	0.62
S. sesban	0.63	0.53	0.59

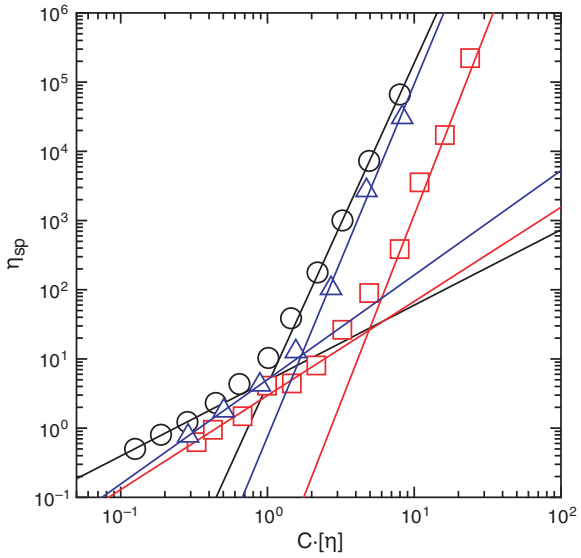

Fig. 8. Concentration dependence of specific viscosity at 25 °C for aqueous solutions of guar (circle), *S. egyptica* (square), and *S. grandiflora* (triangle).

resolution. Under these conditions, it is not meaningful to further interpret the variation of DS_{gal} values across the sample set.

3.4. DS_{gal} determination by SEC and galactomannan model

A more precise albeit indirect determination of DS_{gal} was obtained using the Mark–Houwink–Sakurada (MHS) model for galactomannans, where the prefactor depends on average chain composition (i.e. DS_{gal}), owing to the marked sensitivity of the $[\eta]$ signal to local chain density from galactose substituents. This method yielded $DS_{gal} \approx 0.71$ for 5 of the *Sesbaniae* galactomannans and $DS_{gal} \approx 0.75$ for *S. formosa* (reported in Table 2), far less variation in structure suggested by NMR. This is not surprising considering the high precision in measurement of M and $[\eta]$ by the SEC method and supports our conclusion that the *Sesbaniae* galactomannans have nearly identical molecular structure.

3.5. Rheological characterization

In steady-state shear flow the galactomannan solutions displayed shear-thinning behavior (best-fit to the Cross model) at concentrations above c^*. Specific viscosity-concentration maps were developed for *S. egyptica* and *S. grandiflora* using shear viscosity data at shear rates within the Newtonian regime (Fig. 8). The dilute and entangled regimes are identified by the sharp viscosity increase occurring at $c^* \approx 1/[\eta]$, at which $\eta_{sp} \approx 10$ for galactomannans (Morris, Cutler, Ross-Murphy, & Rees, 1981). The power laws were obtained from a linear regression and are plotted in Fig. 8

$$c < c^*: \quad \eta_{(guar)} \sim C^{1.2}, \quad \eta_{(S.\,egyptica)} \sim C^{1.4}, \quad \eta_{(S.\,grandiflora)} \sim C^{1.5}$$
$$c > c^*: \quad \eta_{(guar)} \sim C^{4.7}, \quad \eta_{(S.\,egyptica)} \sim C^{5.5}, \quad \eta_{(S.\,grandiflora)} \sim C^{5.1}$$

The critical value of the overlap parameter $c[\eta]^*$, obtained from the fits in Fig. 8, were as follows: 1.7 (guar), 6.5 (*S. egyptica*), 3.0 (*S. grandiflora*).

These observation broadly agree with the expected viscosity behavior for high-M galactomannans, despite being obtained on unpurified solutions. The concentration exponents for $c > c^*$ are similar in magnitude to those reported in the literature for purified galactomannans (for instance, Robinson, Ross-Murphy, and Morris (1982) reported $\eta \sim c^{5.1}$ for guar; Nwokocha and Williams (2009) reported $\eta \sim c^{4.6}$ for *Mucuna flagellipes* galactomannan). Because of the low data resolution, the exponents from the *Sesbaniae* galactomannans are not experimentally distinguishable from guar, but we believe $\eta \sim c^5$ for galactomannans is a good working value for galactomannans having $M_w \approx 2$–3 million g/mol. It is interesting to note that the absolute value of this exponent is close to the prediction from Colby's 2-parameter scaling theory (Colby & Rubinstein, 1990), which predicts for the entangled, semi-dilute regime a viscosity exponent of 4.7 in theta solvent and 3.9 in good solvent (Colby, Fetters, Funk, & Graessley, 1991).

As all three example galactomannans in this case have nearly the same intrinsic viscosities, there should be considerable overlap in the masterplot in Fig. 8. The arbitrary shifts along the $c[\eta]$ axis arise mainly from uncertainty in polysaccharide concentration, which has to be estimated from SEC elution data or the even less accurate purification yield. The industrial-manufactured guar sample is reported to contain ca. 80% galactomannan, whereas the homemade *Sesbaniae* powders contain only around 30% (Table 2). Homemade guar samples were in one instance found to be much lower than industrial types (ca. 40%) (Pollard et al., 2010). This effect of sample 'purity' illustrates that the guar gum industry's focus on botanical selection of high-yield cultivars, and optimization of milling methods to produce fully hydrating polysaccharides results in more mass-efficient powders. Similar approaches may be required to improve the mass-efficiency of unpurified *Sesbaniae* galactomannan seed gums.

Excellent superpositions can be achieved in Fig. 8 by including an arbitrary shift parameter to account for this source of error. Most reported values for the critical overlap parameter for galactomannans were determined for purified materials, tending toward ≈2.5, but reported as high as 7.6, depending on the galactomannan type and method of analysis (Kapoor, Milas, Taravel, & Rinaudo, 1994; Morris et al., 1981; Sittikijyothin, Torres, & Gonçalves, 2005; Yoo, Figueiredo, & Rao, 1994).

Classical entanglement response was observed from linear viscoelastic measurements of semidilute solutions of both guar, *S. formosa*, and *S. egyptica* solutions prepared at similar polysaccharide concentration. A linear viscoelastic mastercurve for semidilute guar solutions was first determined, to serve as reference (Fig. 9). Superposition of $G'(\nu)$ and $G''(\nu)$ was achieved using time-concentration superposition (c = 3–24 mg/ml) via a frequency shift factor, $\nu \cdot a_c$, and a modulus shift factor, $G' \cdot b_c$ and $G'' \cdot b_c$. The required shift factors varied as power laws in concentration: $a_c \sim c^{2.9}$, $b_c \sim c^{-2.0}$. For comparison, the linear viscoelastic curve of a *S. formosa* solution at a similar concentration is also shown in the figure, with an added decade vertical shift. Although the full concentration range could not be explored due to the limited sample available, the crossover modulus and frequency lie within the experimental window at the chosen concentration, and are very close to that of the guar solution.

3.6. Dilute solution viscometry

Huggins and Kraemer extrapolations for the three purified *Sesbaniae* galactomannans and reference guar are shown in Fig. 10. The extrapolations are in excellent agreement with each other (Table 4). A significant drop in $[\eta]$ compared to the unpurified galactomannans is probably attributed to some degradation during handling of the purification process, which requires hydration, followed by precipitation, drying, and an additional milling step. Cunha, de Paula, and Feitosa (2007) reported similar reductions in $[\eta]$ using the same basic purification method.

The Huggins' constants k' were within the range 0.49–0.59, very close to the values expected for a polymer in theta solvent. Values of k' near or exceeding 0.5 were also reported in numerous instances for guar in water (Cheng, Brown, & Prud'homme, 2002; Cunha et al., 2007; Doublier & Launay, 1981; Funami et al., 2005; Risica, Dentini, & Crescenzi, 2005). Because the second-virial coefficient and MHS

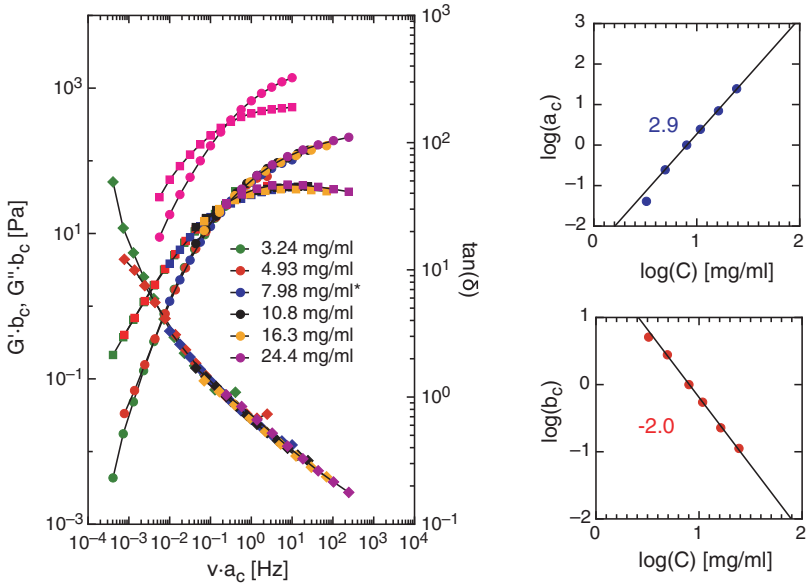

Fig. 9. Mastercurve of linear viscoelastic moduli $G' \cdot b_c$ and $G'' \cdot b_c$ vs. reduced frequency $v \cdot a_c$ for guar, obtained by frequency-concentration superposition over a wide concentration range above c^*. Inset shows concentration dependence of horizontal shift factor a_c and vertical shift factor b_c. Data from *S. formosa* solutions are shown at comparable polysaccharide concentration, shifted up by 1 decade.

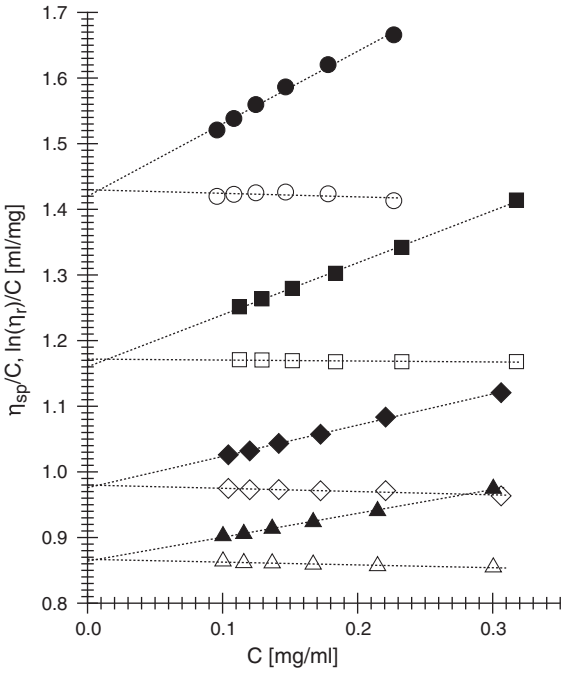

Fig. 10. Huggins' and Kraemer extrapolations obtained by dilute solution viscometry for purified galactomannans at 25 °C: guar (circle) *S. egyptica* (square), *S. sesban* (diamond), and *S. grandiflora* (triangle).

exponent for guar-water suggest at least moderate solvent quality ($a = 0.61$ (Pollard et al., 2010), $A_2 = +4.35 \times 10^{-4}$ cm^3 mol g^{-2} (Simonet et al., 2002)), it is usually argued that hydrogen-bonding or other chain–chain interactions are favorable for these molecules. Similar argument would be applicable for *Sesbaniae* galactomannans.

3.7. Surface tension

Purified galactomannans were found to have low to negligible surface inactivity, based on pendant-drop tensiometry of solutions prepared at ca. 0.5 mg/ml. The interfacial tension was within 1 mN m of pure water for all samples except *S. egyptica*. For this sample there was small initial decay of signal by about 2.5 mN m and thereafter a weak systematic decrease in surface activity occurs over a span of 10 h. Neither effect is considered typical and we hypothesize that protein contamination as well as some solvent evaporation during measurement caused this artifact. Galactomannan polysaccharides are not expected to show preferential interactions at the air–water surface, provided that endogenous proteins and enzymes are eliminated during purification. Such proteins are substantially, but probably not completely removed, by the ethanol purification method (Cunha et al., 2007).

Table 4
Dilute solution viscometry results obtained on purified galactomannans in H_2O at 25 °C.

	Huggins extrapolation		Kraemer extrapolation	
	$[\eta]_H$ (dL/g)	k_H	$[\eta]_K$ (dL/g)	k_K
C. tetragonoloba	14.2	0.548	14.3	−0.026
S. egyptica	11.6	0.589	11.7	−0.011
S. grandiflora	8.6	0.487	8.7	−0.057
S. sesban	9.8	0.500	9.8	−0.052

4. Discussion

Sesbaniae galactomannans appear to share a common molecular structure having $DS_{gal} \approx 0.7$, $M_w \approx 2.5 \times 10^6$, and a broad chain length distribution of the Flory or Schulz-Zimm type. These characteristics are very similar to guar galactomannan, the only major difference being the slightly lower degree of galactose substitution of guar, and therefore the rheological and interaction properties of these galactomannans are also very similar.

The finding of conserved molecular structure across galactomannans of a given taxonomic grouping is consistent with previous studies. DS_{gal} is known to be genetically controlled and more or less constant for a particular legume species, and closely related species also have very similar DS_{gal}. For instance, Reid and Meier (1970) reported that galactomannans from *Medicago*, *Melilotus*, *Trifolium*, and *Trigonella* seeds, all genera within the Faboideae

subfamily, had identical chain composition ($DS_{gal} \approx 0.85$, with 1 exception). More recent studies have concluded that such high galactose-content galactomannans are confined to the taxonomically more-advanced Faboideae subfamily of legumes (Buckeridge, Panegass, Rocha, & Dietrich, 1995), which includes the *Sesbaniae* species as well as guar and fenugreek.

We recently reported that several Faboideae galactomannans had M_w about twice that of galactomannans from the less-advanced Caesalpinioideae subfamily, indicating that M_w may also be a parameter of taxonomic interest. For either subfamily, the chain length distribution fit closely to theoretical models for random polymerization, the only differences occurring therefore in the scale parameter. Thus, the only distinguishing characteristic of a given galactomannan's chain length distribution may be a scale factor (Pollard et al., 2010). Our new data indicate that the chain length distributions of *Sesbaniae* galactomannans are so similar to guar and fenugreek that we might consider this a general pattern for all galactomannans from the larger Faboideae subfamily grouping. If this idea is valid, it would imply a certain homologous character for Faboideae-derived galactomannans; further investigation of this grouping would therefore be expected to find M_w confined to a range of about 2–3 million g/mol due to the conserved distribution scale parameter, whereas DS_{gal} will vary through a wide range about 0.5–1.0. This working hypothesis may be tested with galactomannans derived from other Faboideae tribes and genera.

Although not studied here, the galactose distribution pattern (or fine structure) may be the most relevant parameter for interaction properties, particularly in mixtures with other biopolymers (Dea & Morrison, 1975). Gupta and Grasdalen (1989) rejected block-like and alternating sequence distributions for *S. egyptiaca* as incompatible with diad analysis from NMR, leading to the supposition that side groups are statistically distributed. Mathur and Mathur (2005) found that *S. bispinosa*/xanthan mixtures were synergistic with respect to viscosity but lacked gelling properties, a key indicator that the galactomannan backbone in that case lacks long unsubstituted blocks. Until more sequence information is available, we can only speculate based on these observations that the galactose patterning probably resembles that of guar, and is expected to be very similar across the whole *Sesbaniae* grouping. Nevertheless, it remains a possibility that the statistical pattern can vary to some extent even keeping DS_{gal} constant, based on current models of biosynthesis (Reid, Edwards, Gidley, & Clark, 1995).

To further develop these galactomannans for different applications, it may beneficial to take advantage of characteristic differences in the size, endosperm content, extractability/friability, and/or galactomannan content of the various seeds comprising this genus. One advantage of the cylindrical *Sesbaniae* seeds is that the seed coat can be removed from swollen seeds by friction, which is facilitated by the simpler morphology and seed shape. On the other hand, the larger pea-shaped seeds could possibly be introduced in preexisting dry milling processes. A disadvantage, however, is the lower overall polysaccharide content of *Sesbaniae* seed endosperms, into the range of 20–40%. This can be possibly addressed through botanical selection of suitable cultivars and further horticultural studies.

Acknowledgements

M.P. acknowledges R. Gunde, who performed tensiometry measurements, and M. Ebert, who performed ^{13}C NMR measurements.

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