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Vinal gum, a galactomannan from *Prosopis ruscifolia* seeds: Physicochemical characterization

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ABSTRACT

Physico-chemical and rheological characterization of the gum extracted from the endosperm of *vinal* (*Prosopis ruscifolia*) seeds was performed. The CG-MS analysis revealed that *vinal* gum is a galactomannan with a mannose/galactose ratio of 1.6, with traces of arabinose and glucose residues. The structure was further confirmed by ^{13}C NMR which showed several similarities between *vinal* gum and guar gum spectra. The viscosity molecular weight was $1.43 \pm 0.04 \cdot 10^6$ Da (obtained from Huggins plot) and the average number molecular weight was $0.7 \cdot 10^5$ Da. Shear continuous rheology studies showed a shear thinning behavior at concentrations higher than 0.04% (w/v) of *vinal* gum and an apparent viscosity slightly lower than that of guar gum at the same concentration. Mechanical spectra revealed that *vinal* gum has a typical macromolecular solution behavior with the moduli crossing point that characterized semi-diluted (0.16–0.3% w/v) gum solutions. The present work provides structural, physicochemical and rheological information of a new galactomannan from an abundant and available non-traditional source, being a starting point for food, pharmaceutical or other industrial potential applications of *vinal* gum.

1. Introduction

Many leguminous have galactomannan gums in their seed endosperm. Among them, guar gum and locust bean gum (GG and LBG, extracted from *Cyamopsis tetragonoloba* and *Ceratonia siliqua*, respectively) are the most abundant and easily available polysaccharides. The application of galactomannan gums as thickening, emulsifying, microencapsulating and stabilizing agents is extensively reported (Chaires-Martínez, Salazar-Montoya, & Ramos-Ramírez, 2008; Román-Guerrero et al., 2009). Moreover, the applications of these gums have been expanded and they are currently employed in the petroleum industry (during the drilling process) causing a large rise in their demand and price. There was also an increase in the use of hydrocolloids for the food industry, related to the design of innovative food products with especial health-promoting or taste properties (Douiare & Norton, 2013) and to the demand of fat-reduced products keeping the original texture profile of the product (Bayarri, Chuliá, & Costell, 2010).

Many species from *Prosopis* family contain galactomannan gums in their seeds that have been characterized, and demonstrated to possess functional properties as emulsifier, thickener and stabilizer (*Prosopis pallida* – Chaires-Martínez et al., 2008; *Prosopis chilensis* – Estevez, Escobar, & Sepúlveda, 2012; Matsuhiro, Presle, Saenz, & Urzua, 2006; *Prosopis* spp. – López-Franco, Cervantes-Montaño, Martínez-Robinson, Lizardi-Mendoza, & Robles-Ozuna, 2013; *Prosopis juliflora* – Pinto-Vieira, Pereira-Mendes, Gallão, & Sousa de Brito, 2007; *Prosopis velutina* – Saunders et al., 1986).

Vinal (*Prosopis ruscifolia*) is an extensively growing tree from the North-East of Argentina, South America, covering about 2 million

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ha (Bernardi, Freyre, Sambucetti, & Pirovani, 2004). Besides being able to grow under extreme conditions (temperature, drought, poor soils and high salinity), *vinal* tree develop spontaneously in deforested lands (Bernardi, 2000).

Vinal fruits consist of pods composed by exocarp, pulp, endocarp and seeds. Flour of *vinal* pulp is nowadays used to prepare different foods at a regional level (Bernardi, 2000) and its high protein content (10.5% w/w) makes it suitable for human and livestock consumption as well as to enrich other flours (Freyre et al., 2003). It has been proposed that *vinal* seeds contain an interesting gum in their endosperm that could replace the commercial GG and LBG gums (Freyre et al., 2003). It was recently reported that *vinal* gum can modify lactose crystallization kinetics and crystal morphology (Busch, Santagapita, & Buera, 2013).

However, the gum from *vinal* seeds has not been previously studied. The aim of this work was to characterize the gum extracted from *vinal* seeds and to evaluate its composition, structure and rheology as a starting point for the development of innovative food, cosmetics or pharmaceutical formulations.

2. Materials and methods

2.1. Hydrocolloids

2.1.1. Vinal gum extraction

Vinal pods were collected in Formosa province, Argentina, in 2010. The separation of the seeds from the pods was performed in a rice mill and then passing the product through 3360 and 1410 μm sieves (Zonytest®, Rey y Ronzoni S.R.L., Buenos Aires, Argentina).

The *vinal* gum (VG) extraction was done by an alkaline treatment of the seeds (Chaires-Martínez et al., 2008) and by flocculation in ethanol. Briefly, 20 g of seeds (300 g of pods) were treated at 25 °C in 200 mL of 1 M NaOH for 24 h with continuous stirring. Fig. 1 shows *vinal* pods and their seeds after alkaline treatment revealing the different parts of the seed (dark tegument, endosperm and germ), which were separated manually. VG was extracted from the obtained endosperm by placing it in 100 mL of distilled water under stirring for 24 h. The mixture was centrifuged for 3 min at 2604 rcf (25 °C) and the supernatant solution was poured into 200 mL of absolute ethanol (Biopack, Sistemas Analíticos S.A., Zárate, Argentina). Flocculation of the polymer occurred during storage in the refrigerator (8 °C) for 3 h. Purification was done by solubilization in 50 mL of bidistilled water and reprecipitation in ethanol. The obtained VG was dried in a vacuum oven at 25 °C (300 mbar) to remove the ethanol and then freeze-dried (Heto Holten A/S, cooling trap model CT 110 freeze-dryer, Heto Lab Equipment, Denmark, operating at a condenser plate temperature of −111 °C, a chamber pressure of 30 Pa, and shelf temperature of 25 °C).

2.1.2. Commercial gum

Guar gum (GG) from Cordis S.A. (Villa Luzuriaga, Buenos Aires, Argentina) was used as model galactomannan for comparison purposes.

2.2. Total carbohydrate content

Total carbohydrate content (%CH) was analyzed by the phenol–H_2SO_4 method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956), without previous hydrolysis of the polysaccharide. Briefly, 0.5 mL of a solution containing 5% (w/v) of phenol (Carlo Erba Reagents, Val de Reuil, France) was added to 0.5 mL of polymer solution (10–70 μg of carbohydrates/mL). Then, 2.5 mL of H_2SO_4 98% (Anedra, Research AG S.A., Tigre, Argentina) was added. Absorbance was measured at 490 nm after 30 min of reaction. A standard curve was done with D(+)mannose (Sigma Aldrich Co., St. Louis, MO, USA) in the range of 10–80 μg/mL and results were expressed as total carbohydrate content (%CH).

2.3. Protein content

Protein content was measured by the Bradford method (Bradford, 1976), slightly modified due to the high viscosity and low protein content of the gums. Briefly, 0.5 mL of protein reagent (0.07% (w/v) Coomassie Brilliant Blue (Fluka, Sigma Aldrich Co.), 31% (w/v) ethanol (Biopack, Sistemas Analíticos S.A.), and 56.1% (w/v) phosphoric acid (Anedra, Research AG SA)) was added to 1 mL of a 0.15 M NaCl solution (Biopack, Sistemas Analíticos S.A.) containing 3 mg/mL of *vinal* gum. After 5 min at 25 °C, the absorption at 595 nm was measured. A standard curve was done with bovine serum albumin (BSA, from Sigma Aldrich Co.) in the range of 1–25 mg/mL of BSA.

2.4. Differential refractive index

The refractive index measurements were done with a Schmidt + Haensch DUR-W2 refractometer (Scientific Equipment Source, Oshawa, ON, Canada) at 25.00 ± 0.02 °C controlled by a high precision water bath (model 7320 from Hard Scientific, Fluke Corporation, Everett, Washington, USA). The equipment was tested with MilliQ water at the settled temperature by performing ten measurements, obtaining a value of 1.33249 ± 0.00001, which was

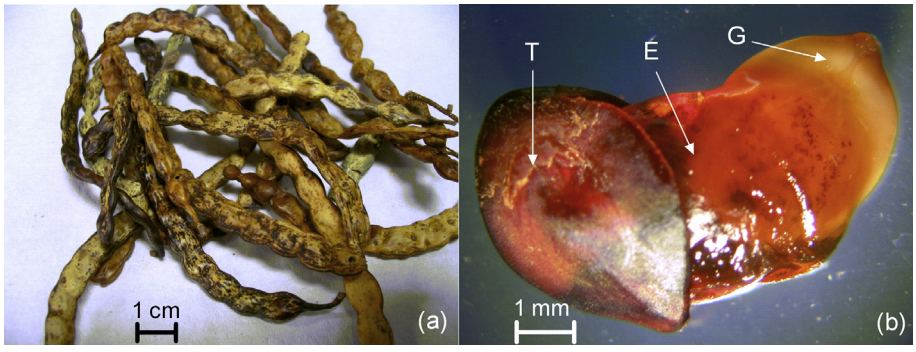

Fig. 1. (a) *Vinal* (*Prosopis ruscifolia*) pods and (b) seeds after alkaline treatment. Germ (G), endosperm (E) and tegument (T) are indicated. The gelly consistency of the endosperm is due to *vinal* gum, from which it is extracted.

coincident with the values found in literature (1.33250, Tilton & Taylor, 1934).

2.5. ^{13}C NMR spectroscopy

For ^{13}C NMR spectroscopy, samples (5–10 mg) were dissolved in D_2O (0.5 mL) in 5-mm tubes (internal diameter). 125.7-MHz ^{13}C NMR ^{1}H-decoupled spectra of gums were recorded at 25 °C on a Bruker AMX 500 spectrometer.

2.6. Monosaccharide composition

Polysaccharides were subjected to acidic hydrolysis of the samples (2 M trifluoroacetic acid at 121 °C for 2 h), reduction with $NaBH_4$ and acetylation (1:1 mixture of acetic anhydride–pyridine). Analysis of the acetylated derivatives by GLC was carried out isothermally at 220 °C on a Shimadzu GC17A gas chromatograph equipped with flame-ionization detector and fitted with a fused-silica capillary column SP-2330 (0.25 mm i.d., 30 m; 0.20 μm). N_2 was used as the carrier gas at a flow rate of 1 mL min^{-1}, and the split ratio was 100:1. The injector and detector temperatures were 250 °C. GLC–MS of the alditol acetates was carried out on a Shimadzu GCMSQP5050A gas chromatograph/mass spectrometer working at 70 eV. Chromatography was performed on the SP-2330 capillary column, with He total flow rate at 33 mL min^{-1}, injector temperature at 240 °C, and the split ratio was 100:1. Mass spectra were recorded over a mass range between 30 and 500 amu.

2.7. Molecular weight determination

2.7.1. Molecular weights by capillary viscosimetry (M_v)

Several dilute solutions of the polymers were prepared between 0.02 and 0.1% (w/v) by dispersing the corresponding polymer amount in bidistilled water, which were stirred for 14 h at 500 rpm at 25 °C. *Vinal* gum was further sonicated (Elma S30H, Elmasonic, Singen, Germany) for 15 min in order to avoid aggregates, which was confirmed by dynamic light scattering, as reported in Fig. S1 of Supplementary File. Relative viscosities (η_{rel}) within 1.2–2.0 were measured with a Cannon-Fenske IVA viscometer series 50 (IVA, CABA, Argentina) at a temperature of 25.0 ± 0.1 °C controlled with a thermostatic bath (model MP-13H from Instrumentalia S.R.L., Mendoza, Argentina). The flow time for bidistilled water was 372 ± 1 s. Three measurements of 8 mL were measured for each concentration. All the measurements were conducted at concentrations below coil overlap concentration (c^*), determined at 0.14 g/dL, as shown in the Fig. S2 of Supplementary File. The intrinsic viscosity [η] was evaluated from both Huggins' and Kraemer plots (equations (1) and (2), respectively).

$$\frac{\eta_{sp}}{C} = [\eta] + k'[\eta]^2 C \tag{1}$$

$$\frac{\ln(\eta_{rel})}{C} = [\eta] + k''[\eta]^2 C \tag{2}$$

where η_{sp} is the specific viscosity, C is the gum concentration % (w/v), k' and k'' are Huggins and Kraemer coefficients, respectively.

The molecular weight by viscosity (M_v) was calculated using the Mark Houwink Sakurada equation given by Doublier and Launay (1981) for guar gum and modified by Gaisford, Harding, Mitchell, and Bradley (1986) to take into account the different M/G values in galactomannans (equations (3) and (4)).

$$[\eta] = 11.55 \times 10^{-6}[(1-\alpha) \times M_v]^{0.98} \tag{3}$$

$$\alpha = \frac{1}{[M/G+1]} \tag{4}$$

where M/G is the mannose/galactose ratio, [η] is the intrinsic viscosity (expressed in dL/g).

2.7.2. Average molecular weights by reducing groups (M_n)

Average molecular weight was calculated by the determination of reducing end-groups, using the colorimetric method described by Park and Johnson (1949). Briefly, 0.5 mL of an aqueous solution of each sample (containing 0.5–1 mg of gum) was added to 0.5 mL of a solution of 1.5 mM potassium ferricyanide (solution A), and 0.5 mL of a solution of 50 mM sodium carbonate and 10 mM potassium cyanide (solution B). The mixture was heated for 15 min at 100 °C. After cooling at room temperature, 2.5 mL of a third solution (C) (1.36 mM ferric ammonium sulfate in 25 mM sulfuric acid and 3 mM sodium dodecyl sulfate (SDS)) was added. The absorbance was measured at 690 nm. A calibration curve was done with D(+) mannose (Sigma Aldrich Co.).

Average molecular weight (M_n) was calculated by equation (5).

$$\overline{M}_n = \frac{m \cdot 180 \cdot d}{P_r} \tag{5}$$

where m is the mass of gum, d is the dilution factor and P_r is the reducing power (expressed as g of mannose/100 g of gum).

2.8. Rheological measurements

VG and GG were suspended in bidistilled water, stirred 24 h at 25 °C and 500 rpm. The concentrations range was adjusted between 0.01 and 0.5% (w/v), in order to make them suitable either for shear continuous or oscillatory assays. 50 ppm azide was added to the samples in order to avoid contamination.

2.8.1. Shear continuous rheology assays

A viscometer Brookfield DV-LVT (Brookfield Engineering Laboratories, Inc., Middleboro, Massachusetts, USA) was used with a cone and plate configuration (CP41) in order to measure the apparent viscosity, defined as the measured viscosity at a specific shear rate. Three measurements were done each one on 10 mL of gum solution in the range of 0.01–0.1% (w/v). Measurements of apparent viscosity and strain were done in a shear rate range between 0.6 and 400 Hz at 25 °C. Flux curves were modeled by the Ostwald power law (equation (6)).

$$\tau = k \cdot \dot{\gamma}^n \tag{6}$$

where τ is the shear stress, $\dot{\gamma}$ is the shear rate, k represents the consistency index and n is the flow index ($n = 1$ referred to Newtonian behavior, n < 1 to pseudoplastic and n > 1 to dilatant flows respectively).

2.8.2. Oscillatory assays

A controlled shear rheometer (AR-G2 TA instruments, New Castle, USA) with a plate-and-plate geometry of 40 mm diameter and 1 mm of gap between the plates. Duplicates were done each one on 1.5 mL of gum solution (in a range of 0.16–0.3% (w/v)) and samples were sonicated (Elma S30H, Elmasonic) for 15 min in order to avoid aggregates. An amplitude sweep was performed with frequency control in order to establish the linear range of viscoelasticity. Dynamic elastic or storage modulus (G′) and dynamic

viscous or loss modulus (G″) were evaluated by varying the frequency from 0.01 to 50 Hz with a 10% constant strain. The temperature was controlled at 20.0 ± 0.1 °C with a peltier plate.

2.9. Statistical analysis

The experimental data were fitted by linear regressions with the corresponding equations, by minimizing the square differences. One-way analysis of variance (ANOVA) with Tukey post test using Prism v5 (GraphPad Software, Inc., San Diego, CA, USA) was used to analyze the differences between mean values for each model. When the analysis of variance indicated differences among means, a student test was performed to differentiate means with 95% confidence ($p < 0.05$).

3. Results and discussion

3.1. General composition of the extracted vinal gum

VG was extracted and purified from the endosperm of *P. ruscifolia* seeds. The endosperm represents the 45% of the total weight of the seed, while the germ/embryo and the tegument represent the 40 and 15%, respectively. The endosperm of GG seeds represents between 34 and 40% of the total weight of the seeds (Mudgil, Barak, & Khatkar, 2014), a value which is near but lower than the obtained for *P. ruscifolia* seeds. The VG yield obtained after extraction and purification processes at laboratory scale was of 19% of the seeds, and 42% of the dried endosperm. These values are in agreement to those reported for other galactomannans (16.5% for *Prosopis glandulosa* – Martínez-Ávila et al., 2014; 13% for *Prosopis flexuosa* and *P. pallida*, Ibañez & Ferrero, 2003, and Chaires-Martínez et al., 2008, respectively; 11.9% for *Gleditsia triacanthos*, Sciarini, Maldonado, Ribotta, Pérez, & León, 2009; and between 17 and 26% for non-*Prosopis* galactomannans – Cerqueira et al., 2009). Table 1 shows the results for total carbohydrate, water-soluble protein content and differential refractive index for the obtained and purified VG, and the commercial GG. The total carbohydrate content for VG and GG were similar between them, and were also similar to the values reported for GG in literature (Mathur, 2012, chap. 2–4; Mudgil et al., 2014). The low protein content (1.9%) of VG indicated the effectiveness of the purification process. The previously reported protein content of GG ranges from 0.6 to 6 %, and it depends extremely on the extraction and purification processes (Chudzikowski, 1971; Martínez-Ávila et al., 2014). Bouzouita et al. (2007) and Haddarah et al. (2014) have reported an important reduction on protein content of gums purified with alcohols (isopropanol and ethanol). Many specifications for food grade additives including GG have a maximum allowed protein content of 10% (Kawamura, 2008, FDA Chemical and Technical assessment).

The refractive index increment (dn/dc) represents the refractive index variation when increasing concentration of a solution. The dn/dc obtained for VG was higher than the one obtained for GG, and was in the range of those obtained for other galactomannans (0.146 mL g^{-1} – Beer, Wood, & Weisz, 1999). Ng et al. (2009) reported a dn/dc value for GG of 0.1399 mL g^{-1}, which was similar to the value obtained in present work. The dn/dc value is essential for using many optical techniques: polymer molecular weight determination and characterization by light scattering (Vinod et al., 2008), correcting data in surface plasmon resonance biosensors (Davis & Wilson, 2000), refractometric measurement of protein concentrations in analytical ultracentrifugation, and for many optical imaging (Zhao, Brown, & Schuck, 2011).

Table 1
Total carbohydrate content, water-soluble protein content and refractive index increment of *vinal* gum (VG) and guar gum (GG).

	VG	GG
Carbohydrate content (g monosaccharide/100 g dry gum)	76 ± 3[a]	71.1 ± 0.5[a]
Protein content (g/100 g dry gum)	1.9 ± 0.2[a]	2.1 ± 0.3[a]
Refractive index increment (mL/g carbohydrate)	0.152 ± 0.003[a]	0.136 ± 0.004[b]

Standard deviation values are included. For a given property in each raw, significant difference between gums is indicated with different letters (a–b; $P < 0.05$).

3.2. Chemical structure of vinal gum

^{13}C NMR spectrum of VG is shown in Fig. 2. The anomeric carbons, assigned to α-D-Gal and β-D-Man residues, were registered at 99.48 and 100.77 ppm, respectively (Table 2). The linkage at C-4 of mannosyl units was determined by the signal at 77.81 ppm. Diagnostic signals for β-D-Man substituted at C-6 were found at 74.06 and 67.27 ppm, assigned to C-5 and C-6, respectively; while the resonances for unsubstituted β-D-Man residues were observed at 75.82 (C-5) and 61.48 ppm (C-6). It has been reported that C-4 of β-D-Man residues is sensitive to diad or triad sequences; the broad resonance centered at 77.81 ppm could indicate that galactose branches are randomly distributed on the backbone. The signals observed and the general pattern of the spectrum are consistent with a backbone constituted by (1 → 4)-linked β-D-mannopyranosyl (β-D-Man*p*) residues with single stubs of α-D-galactopyranosyl (α-D-Gal*p*) units, attached at C-6. This spectrum is in agreement with those reported for galactomannans with the same substitution pattern (Albuquerque et al., 2014; Chaubey & Kapoor, 2001; Cheng & Neiss, 2012; Kapoor et al., 1998; Manzi & Cerezo, 1986; Muschin & Yoshida, 2012).

Monosaccharide composition determined by GC–MS for both VG and GG showed that mannose was the major sugar component, followed by galactose, as reported in Table 3. This fact is consistent with a galactomannan composed by a main chain of mannose residues, substituted with galactosyl side chains in ratios 8:5 and 9:5 for VG and GG, respectively. For VG, traces of arabinose and glucose units were also found. The mannose/galactose ratio (M/G) is an important feature that defines many physicochemical characteristics of galactomannans. The conformation and flexibility of the polymer chain (Wu, Li, Cui, Eskin, & Goff, 2012), the synergism with other polymers to act as thickening agent (Pinheiro et al., 2011), the solubility in water and gel formation ability (Cerqueira et al., 2009), and the rheological and emulsification properties (Wu, Li, Cui, Eskin, & Goff, 2009) are determined by the M/G ratio. The M/Gs were 1.6 (VG) and 1.8 (GG), in agreement with data reported for GG (Azero & Andrade, 2006). The obtained M/G values (slightly smaller for VG) correspond to highly branched galactomannans with high intrinsic viscosity (Wu et al., 2009), thickening power, and water solubility (Doublier & Launay, 1981). Taking into account the ^{13}C NMR spectrum and the M/G ratio, a proposed model structure for the polysaccharide is shown in Fig. 3. It is composed by a backbone of 4-linked β-D-mannopyranosyl units branched with α-D-galactopyranosyl units at C-6, in an approximate mannose–galactose ratio of 8:5.

3.3. Macromolecular characterization and molecular weight determination

Intrinsic viscosity [η] of gums is related to the hydrodynamic volume occupied by chains in a given solvent. It depends on their molecular structure and weight, as well as on the solvent quality (Cerqueira et al., 2009). Fig. 4 shows Huggins and Kraemer plots

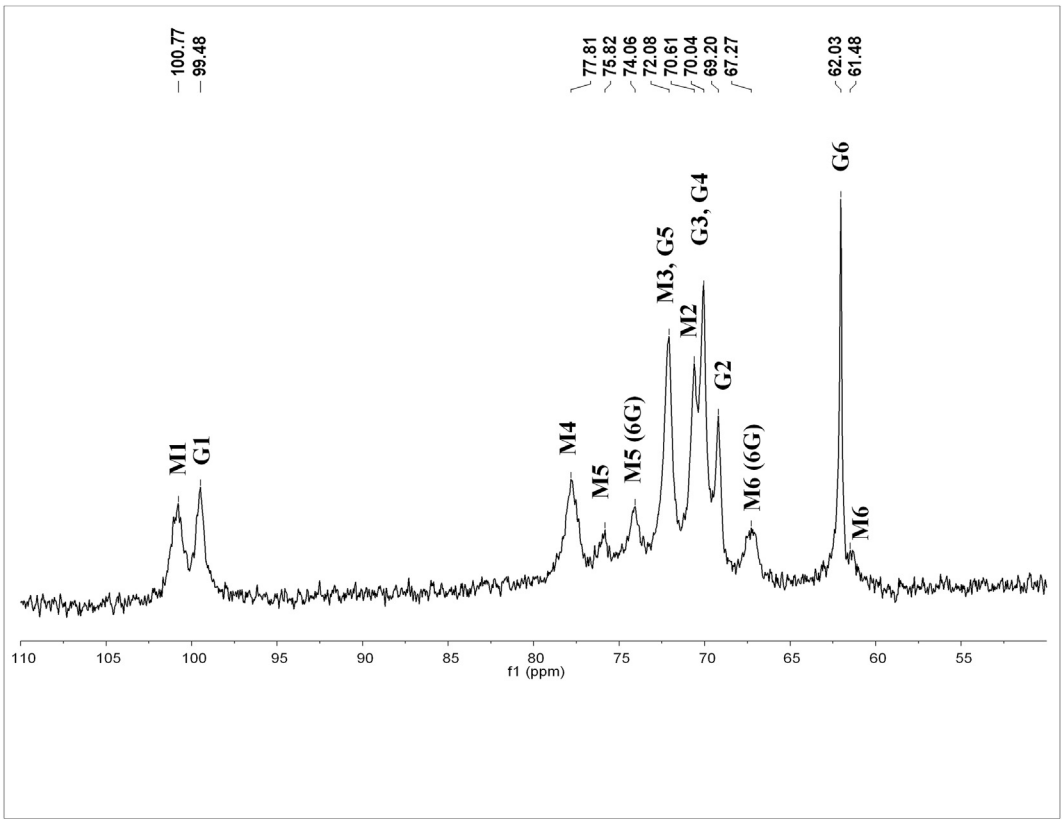

Fig. 2. ^{13}C NMR of the alkali-extracted *vinal* gum. M: mannose; G: galactose; $M_i(6G)$: mannose units branched with galactose residues in C-6.

Table 2
^{13}C NMR signals of *vinal* gum. Chemical shifts are expressed in ppm.

Residue	C-1	C-2	C-3	C-4	C-5	C-6
α-D-Gal*p*	99.48	69.20	70.04	70.04	72.08	62.03
β-D-Man*p*	100.77	70.61	72.08	77.81	75.82	61.48
β-D-Man*p* (branched at O-6)	100.77	70.61	72.08	77.81	74.06	67.27

Table 3
Monosaccharide content (mol %) and mannose/galactose ratio (M/G) of *vinal* gum (VG) and guar gum (GG) determined by GLC.

	VG	GG
Arabinose	Traces[a]	*ND*[b]
Mannose	62 ± 1	64 ± 1
Galactose	38.2 ± 0.6	36.5 ± 0.6
Glucose	Traces[a]	*ND*[b]
M/G	1.6	1.8

[a] Traces: less than 1%.
[b] *ND*: not detectable.

employed for the calculation of [η] of VG and GG. The [η] calculated by Huggins and Kraemer plots were consistent between them, being 7.7 ± 0.1 and 7.6 ± 0.1 dL/g for VG, and 11.6 ± 0.4 and 12.1 ± 0.2 dL/g for GG, respectively. VG showed smaller [η] than GG, which in turn are slightly higher than those reported by Azero and Andrade (2006) (10.3 and 10.8 dL/g from Huggins and Kraemer plots, respectively) and 10.5 dL/g reported by Brummer, Cui, and Wang (2003). The slopes of curves in Fig. 4 are related to the Huggins and Kraemer coefficients (k' and k'', respectively). The value of k' indicates the polymer interactions with solvent and the aggregation state of the polymer (Cerqueira et al., 2009), which is determined by the characteristics of the gum in the suspension (size distribution, conformation and dissolution degree) (Gaisford et al., 1986) as well as by the entanglement, unentanglement and configurational relaxation of the polymeric chains (Thombre & Gide, 2013). In a good solvent and for flexible macromolecules, k' values are close to 0.3, but in case of aggregation, it can be higher than 1 (Sittikijyothin, Torres, & Gonçalves, 2005). The obtained value for the Huggins coefficient for VG ($k' = 0.23 \pm 0.04$) indicated a good dispersion of the gum in the solvent, and were consistent with others previously reported. Huggins coefficient for GG ($k' = 0.7 \pm 0.1$) indicated that even though the gum was not aggregated, the interactions with the solvent were not as good as for VG. Sittikijyothin et al. (2005) reported for another highly industrially used galactomannan as tara gum k' values in the range 0.57–0.79, depending on the purification degree of samples.

Intrinsic viscosities (Fig. 4) were related to the viscometric molecular weight (M_v) through the Mark Houwink Sakurada (MSK) equation modified by Gaisford et al. (1986), and the results are shown in Table 4. The calculated M_v were similar for both gums, but as expected for the results obtained for intrinsic viscosities (Fig. 4) they were higher for GG and for Kraemer plots. The obtained values for GG were also close to the one ($1.81 \cdot 10^6$ Da) reported by Azero and Andrade (2006) using the intrinsic viscosity value obtained from Huggins plot.

It is important to consider that gums are made up of mixtures of chains with different molecular weights (Mathur, 2012, chap. 2–4). The average molecular weight (M_n) (the ordinary arithmetic average of the molecular masses of the individual macromolecules) was calculated by measuring the reducing end-groups by Park and Johnson (1949). Considering the theoretical distribution curve that describes the relationship between the number of

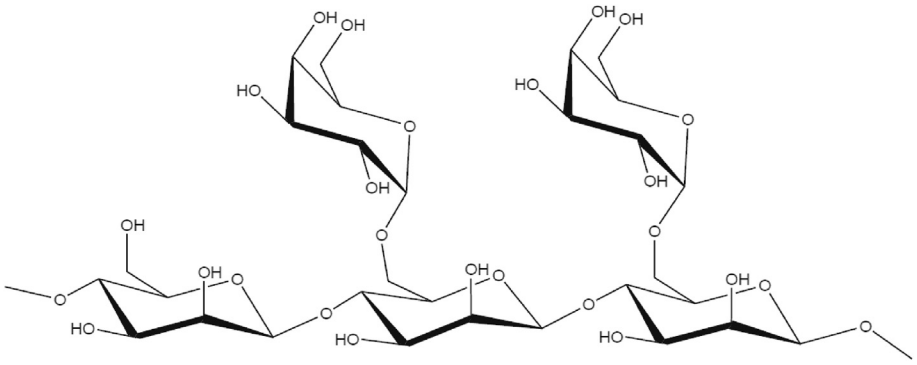

Fig. 3. Structure proposed for *vinal* gum. A main chain composed by 4-linked β-D-mannopyranosyl units with branches of α-D-galactopyranose linked to C-6, with an 8:5 mannose–galactose ratio.

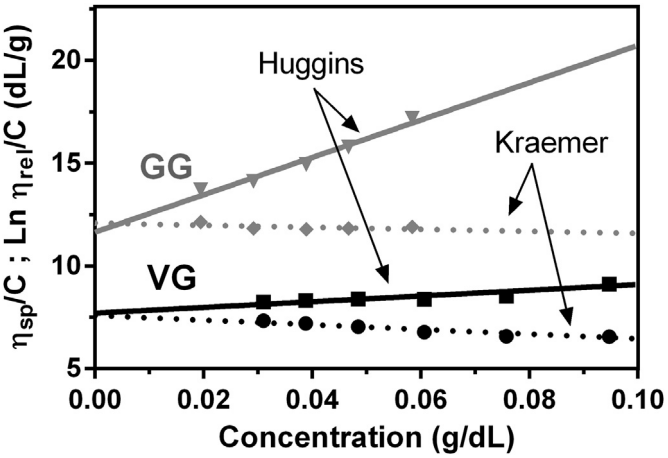

Fig. 4. Huggins (solid lines) and Kraemer (dotted lines) plots for *vinal* gum (VG) and guar gum (GG).

Table 4
Molecular weight by viscosity (M_v) obtained by Huggins and Kraemer equations and average molecular weight (M_n) of *vinal* gum (VG) and guar gum (GG).

	VG	GG
M_v by Huggins (Da·10^6)	1.43 ± 0.04^b	2.07 ± 0.04^a
M_v by Kraemer (Da·10^6)	1.41 ± 0.02^b	2.16 ± 0.02^a
M_n (Da·10^5)	0.73 ± 0.03^b	1.10 ± 0.04^a

Standard deviation values are included. For a molecular weight in each raw, significant difference between gums is indicated with different letters (a–b; $P < 0.05$).

moles of each polymer chain and its molecular mass, M_n is generally lower than M_v, as shown in Table 4 for VG and GG. Besides, GG showed higher both M_v and M_n than VG. Then, the higher intrinsic viscosity of GG in comparison with VG is related to its higher molecular mass. Mudgil et al. (2014) reported for GG M_n values varying in the range of 0.25–5.0·10^6 Da (2011). The obtained M_v and M_n for GG are in the range of the reported values, considering some variation in the raw material and to different methodologies used.

3.4. Rheological characterization: shear continuous and oscillatory assays

3.4.1. Shear continuous rheology

The flow behavior of gums solutions were determined by shear continuous assays. The variation of stress as a function of the shear rate for VG (0.01–01% (w/v)) and GG (0.06% (w/v)) is shown in Fig. 5. The experimental points were successfully fitted by the power law, and the obtained parameters are presented in Table 5. VG solutions at concentrations higher than 0.04% (w/v) presented shear-thinning behavior ($n < 1$). Galactomannans typically appeared with a random coil conformation and have a Newtonian behavior at very low concentrations (Mathur, 2012, chap. 2–4). At concentrations higher than this value, the apparent viscosity decreased as the shear rate increased due to the alignment of polymer chains in the flow direction (pseudoplastic behavior) (Mathur, 2012, chap. 2–4). The apparent viscosity increased as VG concentration increases (as showed in Fig. S3 in the Supplementary File), as well as the consistency index k, as shown in Table 5. This linear increment on viscosity at low concentrations corresponds to extended and hence linear polymer chains, with scarcely flexible monomer linkage in the backbone (Mathur, 2012, chap. 2–4). These macromolecular characteristics are consistent for galactomannans such as GG and VG, in which the mannose units are linked as β(1 → 4). GG at 0.06 and VG at 0.1 (% (w/v)) showed similar flow curves, but a higher concentration of VG is needed to achieve the same thickening power than GG. This is directly related to the above mentioned higher molecular weight of GG.

3.4.2. Oscillatory assays

Dynamic measurements of VG and GG solutions were performed. Loss and storage moduli as a function of frequency are shown in Fig. 6 for a 0.3% (w/v) *vinal* gum suspension. The data for a 0.15% (w/v) guar gum were included in the inset for comparative purposes. Both moduli showed strong dependence on the

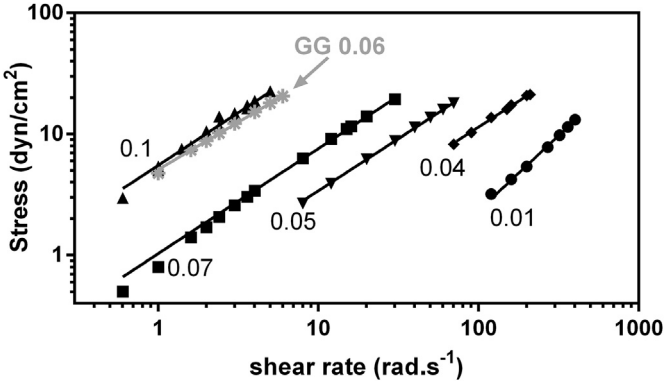

Fig. 5. Stress as a function of the shear rate for different concentrations expressed as % (w/v) of *vinal* gum (VG) and guar gum (GG, in gray) at 0.06% (w/v). Dots are experimental data and lines show the power law model fitting.

Table 5
Power law parameters for *vinal* gum and guar gum rotational assays. *k* indicates consistency index and *n* is the flow index.

	Vinal gum (% (w/v))					Guar gum
	0.01	0.04	0.05	0.07	0.1	0.06 (% (w/v))
k (cm^2 dyn^{-1} s^n)	0.015 ± 0.001^f	0.23 ± 0.01^e	0.469 ± 0.008^d	1.03 ± 0.01^c	5.5 ± 0.1^a	5.097 ± 0.007^b
n	1.13 ± 0.01^a	0.848 ± 0.009^c	0.860 ± 0.004^b	0.867 ± 0.005^b	0.86 ± 0.02^b	0.782 ± 0.007^d
R^2	0.998	0.998	0.999	0.999	0.985	0.999

Standard deviation values are included. For each parameter, significant differences between concentrations and gums are indicated with different letters (a–f; $P < 0.05$).

frequency and the mechanical spectra of *vinal* gum were typical of macromolecular solutions, and were similar to those reported for semi-diluted solutions of other galactomannan gums (guar gum – Torres, Hallmark, & Wilson, 2014; Wientjes, Duits, Jongschaap, & Mellema, 2000; *Cassia grandis* gum – Albuquerque et al., 2014; *Espina corona* gum – Perduca et al., 2013). As reported by Wientjes et al. (2000) for GG, two crossover points of moduli could be observed for *vinal* gum: one at 3 s^{-1} and 0.1 Pa and the other one at much lower frequencies around 0.2 s^{-1} and 0.004 Pa. Between these points, the loss modulus predominates on the storage modulus. The crossover point around 1–10 Hz is typical of macromolecular concentrated solutions, and is a consequence of the viscoelastic behavior of polymers. The concentration and frequency values at which the elastic component predominates over the viscous one, indicates the region where the solution behaves as a solid. The crossover points were observed for other galactomannan gums, such as GG (4.8 s^{-1} and 0.38 Pa for a 0.15% (w/v) solution (inset Fig. 6)), Espina corona gum (from *Gleditsia amorphoides* seeds) at 1 s^{-1} and ~30 Pa, for a concentration of 0.5% (w/v) (Perduca et al., 2013). As already reported for other gums, the crossover point shifts to higher frequency values when the gum concentration decreases (Doublier & Launay, 1981; Wientjes et al., 2000). This behavior is shown in Fig. S4 of the supplementary file for *vinal*, guar, locust bean, tara and Espina corona gums. A complete description of guar gum viscoelastic behavior was reported by Wientjes et al. (2000). Working in an extended frequency range, these authors observed two storage modulus plateau zones at low frequencies with a very broad relaxation spectrum. They attributed the rheological behavior of guar gum to the existence of two or more relaxations mechanisms. A model based on strong and weak associations, according to the number of free backbone units (Goycoolea, Morris, & Gidley, 1995) and other one promoted by star-like structures, caused by strong physical bonds, could explain the observed behavior. Being *vinal* gum a similar type of polymer, some of these associations could also take place.

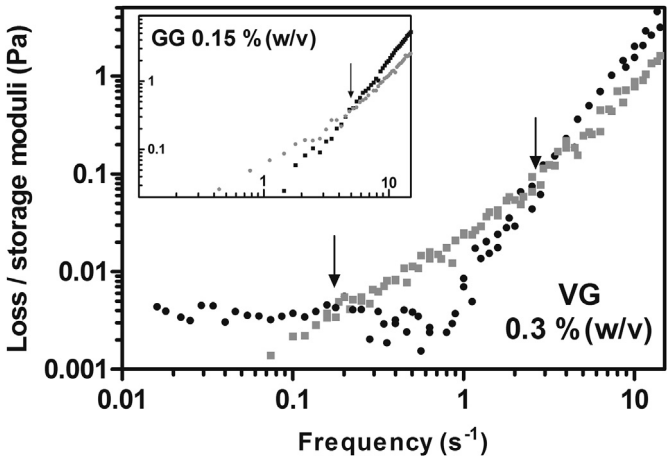

Fig. 6. Loss (gray) and storage (black) moduli for a 0.3% (w/v) *vinal* gum (VG) and a 0.15% (w/v) guar gum (GG, inset) solutions. Arrows indicate moduli crossover points.

4. Conclusions

Present paper confirmed that the gum extracted from *vinal* (*P. ruscifolia*) seeds is a high molecular weight galactomannan with a mannose/galactose ratio of 1.6. *Vinal* gum showed similar physicochemical and rheological characteristics than guar gum, one of the most widely used galactomannans.

Vinal gum showed shear thinning behavior and a thickening power slightly lower than guar gum. Besides, the mechanical spectra of *vinal* gum allowed characterizing it as a macromolecular solution, showing storage and loss moduli dependence on the frequency even at high concentrations, similarly to guar gum. The overall data indicates the thickening characteristics of the *vinal* gum suspensions even at low concentration, revealing it capability as thickener agent.

Galactomannans are broadly used in the industry, and *vinal* gum could be considered a new non-traditional gum with similar performance than guar gum. The better understanding of chemical and rheological characteristics of the studied systems may help to promote the potential applications of *vinal* gum for the development of innovative food, cosmetics or pharmaceutical formulations.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.foodhyd.2015.04.035.

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