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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

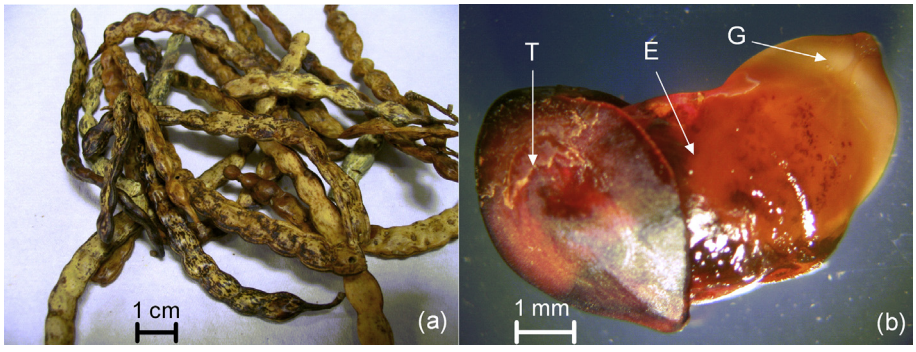

Fig. 1. (a) *Vinal* (*Prosopis ruscifolia*) pods and (b) seeds after alkaline treatment. Germ (G), endosperm (E) and tegument (T) are indicated. The gelly consistency of the endosperm is due to *vinal* gum, from which it is extracted.