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HYDROTHERMAL TECHNIQUE FOR ISOLATION OF GALACTOMANNAN FROM SEEDS OF SONORAN MEZQUITE (*Prosopis spp.*)

TÉCNICA HIDROTERMAL PARA AISLAR GALACTOMANANOS DE SEMILLAS DE MEZQUITE SONORENSE (*Prosopis spp.*)

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Abstract

Mezquite seeds are a good source of polysaccharides called galactomannan. The method for galactomannan extraction has been standardized by using autoclave for time intervals of 5, 10 and 15 minutes at 121°C with different endosperm:water ratios 1:10, 1:20 and 1:30 (w/v). The optimum yield obtained was 17%. Physicochemical characterization was done and structural properties of extracted material have been determined by FTIR and proton NMR. Polysaccharides extracted were assumed to be galactomannans according to the literature. Thermal parameters showed no significant changes on theirs properties. The standardized method can be scalable to industrial process.

Keywords: hydrothermal extraction, galactomannan, *Prosopis spp*., biopolymer, physicochemical and structural characterization.

Resumen

Las semillas de mezquite son una buena fuente de polisacáridos llamados galactomananos. Se estandarizó un método para la extracción de galactomananos usando una autoclave a intervalos de tiempo de 5, 10 y 15 minutos a 121°C con diferentes tasas de endospermo:agua 1:10, 1:20 y 1:30 (p/v). El rendimiento óptimo obtenido fue de 17%. Se realizó caracterización fisicoquímica y las propiedades estructurales del material extraído se determinaron por FTIR y RMN de protón. Se asumió que los polisacáridos extraídos son galactomananos de acuerdo a la literatura. Los parámetros térmicos no muestran cambios significativos en sus propiedades. Este Método estandarizado puede ser escalable a nivel industrial.

Palabras clave: extracción hidrotermal, galactomanano, *Prosopis spp*., biopolímero, caracterización fisicoquímica y estructural.

1 Introduction

The mezquite tree (*Prosopis spp.*), is native from arid and semi-arid zones of the planet, adaptable to extreme drought and heights above sea level. The mezquite belongs to the Leguminoseae family, Minosoidae sub-family and Prosopis gender. The name mezquite comes from the Aztec word "misquitl". Because of his low water requirement it can growth on deserts zone. México has 56 and 23 millions of arid zone and semi-arid zones nearly of 40% of the national territory. Mezquite growths naturally on all the Mexican territory but the mayor density of mezquite is on Sonora (López-Franco *et al*. 2006).

Each part of the plant is used as a source for human and animal food, wood and charcoal, building material, medicine, nectar for apiculture, shadow, and several other uses (Felker *et al*. 1981). In the leguminous, the seeds has three parts: germ that fees the grain, bran that protects the grain and endosperm that provides energy. The galactomannan

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is located in the seed's endosperm and is considered a multifunctional compound that accumulate humidity during their growth; it serves as an energy source during the germination and provided mechanical protection to the seed (Chaires-Martínez *et al*. 2008).

Galactomannan (GM) have a backbone chain of mannose moieties linked by β-1,4 glycosidic linkages, which is substituted with single units of galactose linked α-1,6 to mannose backbone chain. Physicochemical and functional characteristics of galactomannans depend on mannose/galactose (M/G) ratio, which basically is determined by source and method employed for the extraction of this kind of polysaccharides (Cerqueira *et al*. 2009). There are some commercial galactomannans such as guar gum; locust bean gum, tara gum and fenugreek gum which are used in food and pharmaceutical industries. They have high water-binding capacity and generate viscous solutions acting as good emulsifying agents, effective thickeners and stabilizers (Wu *et al*. 2009).

There are studies to extract galactomannans using techniques that uses alkaline medium using NaOH (Ibañez and Ferrero. 2003; Chaires-Martínez *et al*. 2008; Estévez *et al*. 2004), water heated at 100°C in a pot (Martínez-Ávila *et al*. 2014; Pinto-Vieira *et al*. 2007), water at room temperature in a pot (López-Franco *et al*. 2013; Salvalaggio *et al*. 2014), combination of Soxhlet extraction with water at room temperature (Azero and Andrade 2002), combination of maceration in benzene-ethanol solution with water at room temperature (Pawar and Lalitha 2014), giving a pre-treatment with H_2SO_4 and using ethanol at 70°C (Cerqueira *et al*. 2009), using a benzene-ethanol extraction (Jian-Xin *et al*. 2011). Some of this methods may cause environmental problems by producing hazardous contaminants, others are difficult to scale for an industrial application or it is necessary to wait long periods of time. Thus, the present study was designed to development a hydrothermal process that can be used for the commercial production of galactomannans from mezquite seeds using a scalable process in an ecological way.

2 Materials and methods

2.1 Raw materials

The mezquite (*Prosopis spp*.) seeds were acquired from the National Forestry Commission (CONAFOR) at Hermosillo, Sonora México. The seeds source is located 29° north latitude and 110° west longitude.

2.2 Compositional analysis of seeds

The moisture in the seeds was calculated gravimetrically using either the Sand or Gauze methods. Protein content was performed using the Kjeldahl-Gunning method. Fats were measured by the Soxhlet method and ashes were obtained by incineration at 550°C in a furnace oven for 8 hours. The total carbohydrates content was calculated by difference (100 % - moisture % - protein % - fats % - ashes %).

2.3 Extraction of galactomannans

The mezquite seeds were grinded and the germ and hull were discarded. The endosperm was saved for the extraction of the galactomannans. An autoclave with water at 121°C and 15 lb. of pressure was used for the aqueous extraction of galactomannan, different water ratios with a constant quantity of endosperm were added into a flask. The mixture was maintained in the autoclave for different times. A control sample using water at room temperature and extraction time of 5 hours was also prepared. The experimental details are shown in Table 1. After the hydrothermal treatment, the endosperm and the liquid were separated by filtration using gauze, the endosperm was re-dissolved in distilled water and the process was repeated. The liquids are mixed and the galactomannans were recovered using precipitation with ethanol 96% in a 1:2 volume proportion. The precipitate was dried in air oven at 50°C.

Table 1. Experimental details for the galactomannans extraction.

Treatment	Time (minutes)	Endosperm:Water ratio (w/v)
Control (25°C)	300	1:10, 1:20, 1:30
Autoclave (121°C 15 lb pressure)	5, 10, 15	1:10, 1:20, 1:30

2.4 Purification of galactomannans

The powder obtained in the previous step was purified by dissolving it in distilled water at 2.5 g/L with constant stirring at room temperature, centrifuged at 4000 rpm for 10 min and the galactomannans were recovered adding to the supernatant ethanol 96% in a 1:2 volume proportion. The extracted solid phase was dried at 50°C and stored for further use.

2.5 Methods

Intrinsic viscosity and molecular weight of galactomannan was measured at 25°C using a BS/IP/MSL. The viscosity average molecular weight (Mv) was calculated from the intrinsic viscosity using the Mark Houwink Sakurada equation Ec. (1):

$$\eta = K \cdot Mv^{\alpha} \tag{1}$$

with $K = 5.13 \times 10^{-4}$ and $\alpha = 0.72$ which are the constants reported for guar gum (Beer *et al*., 1999).

The solubility of galactomannan was calculated by preparing suspensions (20ml) of galacomannan (1% w/w) in water at 25°C for 30 min with continuous stirring. The suspensions were then centrifuged at 800 rpm for 15 min. Samples of 10 mL of the supernatants were dried in a convection oven at 100°C for 12 h and the solubility was calculated using Ec. (2):

$$\%Sol = [(W_f)(20)/(W_i)(10)] \times 100 \tag{2}$$

Where W_i is the weight of galactomannan used to prepare the suspensions and W_f is the weight of the galactomannan recovered from the solution.

The Mannose/Galactose ratio of galactomannan was calculated from hydrolysed samples using an HPLC system (DIONEX) with a pulsed amperometric detector ED50 (electrochemical detector), an analytic column Dionex-CarboPac PA10 4x250mm and a precolumn Dionex-CarboPac PA10 4x50mm. The samples were compared with a standard of galactose and mannose.

Fourier transform infra-red spectroscopy (FTIR) analyses of the galactomannans were obtained in a Perkin-Elmer spectrometer. For these analyses, 1 mg of material was mixed with KBr to form pellets and the spectra were recorded in transmission mode from 4000 to 400 cm^{-1} at a resolution of 2 cm^{-1}.

Proton NMR (^{1}H NMR) spectra were obtained using a Bruker 400 MHz NMR spectrometer. All spectra were recorded in D_2O at room temperature. Chemical shift was given on the d (ppm) and assignments of the ^{1}H NMR spectra were based on the literature. X-ray photoelectron spectroscopy was performed using a monochromatic Al Kα X-ray source and emission current of 6 mA. X-ray diffraction was performed at room temperature with a commercial diffractometer using Cu (λ = 1.54 Å) X-rays at a scanning rate of 2°/min from 5° to 62° of 2θ.

Thermal degradation of the samples was studied on a Thermo-gravimetric Analyzer (TGA) (Pyris 1 TGA, Perkin Elmer, USA). 5 mg of sample was heated at 10°C per minute from room temperature to 500°C in nitrogen atmosphere. Differential Scanning Calorimetry (DSC) (Diamond DSC Perkin Elmer, USA) was also used to study the thermal properties of the samples. 10 mg of sample was weighed into the sample pan. Temperature was held at 30°C for 1 min then the sample was heated to 200°C at a rate of 10°C per minute under nitrogen atmosphere.

2.6 Statistical analysis

Experiments were performed in triplicate and the results were represented by means and standard deviations. ANOVA and least significant difference test was done using Statgraphics Centurion XVI by using completely randomized design.

3 Results and discussion

3.1 Seeds composition

The data resulting from the compositional analysis of the mezquite seeds is show on Table 2. Mezquite seeds has mainly protein and polysaccharides making them an attractive source of this macromolecules.

Table 2. Composition of mezquite seeds.

Component (%)	Mesquite seeds
Moisture	**0.333 ± 0.036**
Protein	**45.435 ± 0.490**
Fats	**6.881 ± 0.002**
Ashes	**3.696 ± 0.060**
Carbohydrates	**43.653 ± 0.833**

3.2 Extraction of galactomannans

To understand the effect between the independent variables (time and endosperm: water ratio) on the yield extraction, the experiments were done at

Table 3. Galactomannans yield

Experiment	**Variable**		**% Yield**
	Time (minutes)	Endosperm: water ratio	
Control (25°C)	300	1:10	2.967 ± 0.018
		1:20	3.035 ± 0.205
		1:30	3.276 ± 0.089
Autoclave (121°C 15lb Pressure)	5	1:10	7.450 ± 1.009
		1:20	8.408 ± 0.663
		1:30	7.695 ± 0.240
	10	1:10	6.219 ± 0.892
		1:20	9.418 ± 0.722
		1:30	10.027 ± 1.122
	15	1:10	8.599 ± 0.926
		1:20	9.710 ± 0.156
		1:30	9.820 ± 1.028

different combinations of this parameters using an experimental design which results are present on table 3. The yield increase with the augmentation of the ratio and time, this indicate an improvement of the hot water access to the endosperm making the polysaccharide spread easier from the seeds.

ANOVA analysis (supplementary material Table S1) shows a significate differences between variables (time and endosperm:water ratio). To choose the treatment a test of multiple ranges was done; for time (supplementary material Table S2) the best option to maximize is 10 or 15 minutes; for endosperm: water ratio (supplementary material Table S3) the best option to maximize is 1:20 or 1:30. The interaction (supplementary material Figure S1) indicate that to maximize is better use 10 or 15 minutes with a ratio of 1:20 or 1:30. The chosen process was 15 minutes with a ratio of 1:20 because with this relation the quantity of solvent to use for precipitation is lower and the yield is higher.

Only seeds subject to this treatment were used for the second extraction reaching a yield of 17 %, similar to those reported by López-Franco *et al*. 2013 of 14.2 % who use the same seeds, but lower than the yield obtained from other seeds such as *S. tora* 35%, (Pawar and Lalitha 2014) *G. triacanthos* 24.73% and *C. pulcherrima* 25.70% (Cerqueira *et al*. 2009). The differences in yield could be related to biological and compositional factors including mesquite species, source of the seeds or the stage of development of the endosperm. Other factor that may explain the variation in yield is the type of extraction process used.

3.3 Physicochemical characterization

Table 4 shows the physicochemical results for the extracted galactomannan. The somewhat lower values of intrinsic viscosity [η] (1.77 dL/g) and molecular weight (Mr) (82 465 g/mol) can be attributed to the mechanism of extraction that might result in polysaccharide decomposition into smaller molecules due to the increased temperature of extraction (Lu and Saka, 2010; Lu and Saka 2012). The solubility of galactomannan depends basically on its ability to form hydrogen bonds with water (Prajapati *et al*. 2013). The percentage of mannose and galactose in the galactomannan were 58.46 ± 4.89 and 28.5 ± 3.11, respectively. The ratio of 2.05 is close to the ratio obtained by López-Franco *et al*. 2013 of 1.50 using the same type of mesquite seed and the calculated ratio for guar gum (1.43). However, this ratio is low compared to the locust bean (3.7) and tara (3.0) gums (Wu *et al*., 2009).

Table 4. Physicochemical analysis of galactomannans.

Physicochemical analysis	Value
Intrinsic viscosity [η] (dL/g)	1.77
Molecular weight (Mv) (g/mol)	82,465
Solubility at 25°C	12.64%
Galactose: mannose ratio	2.05

3.4 Chemical characterization

Figure 1 shows the FT-IR results of the galactomannan and the position of the main absorption bands are summarized in Table 5 from here the peaks at 868.42 and 811.46 cm^{-1} are related with the presence of anomeric configurations (α and β conformers) and glycosidic linkages, attributed to α-D-galactopyranose units and β-D-mannopyranose units, respectively so this can be related with the "galactomannans" sugar composition (Bravo *et al*., 1994).

The ^{1}H NMR spectrum of the galactomannan is shown in Figure 2. The singlet at 5.01 ppm, arises from H-1(Gal) and is compatible with the expected conformation of the α-D-galactopyranose ring. The signal for 1H (Man) appears at 4.73 ppm, which corresponds to the monomeric β-D-mannopyranose but it is not apparent because appears at the same chemical shift that D_2O, however all the other signal are similar to characteristics structure of galactomannans (Bresolin *et al*. 1998; Pinto-Viera *et al*. 2007; Muschin and Takashi 2012; Joshi and Kapoor 2009; Pawar and Lalitha 2014). Based on data from FT-IR and proton NMR it can be deduct that the polysaccharide extracted from mezquite seed's is a galactomannan with the structure shown in Figure 3.

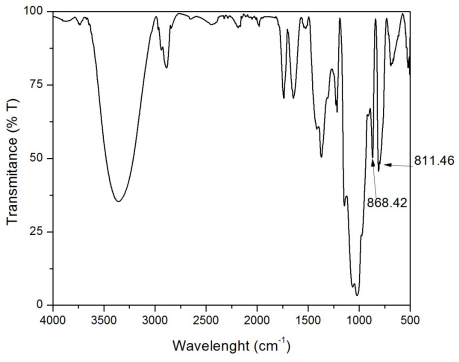

Fig. 1. FT-IR spectra of galactomannan from mezquite seed

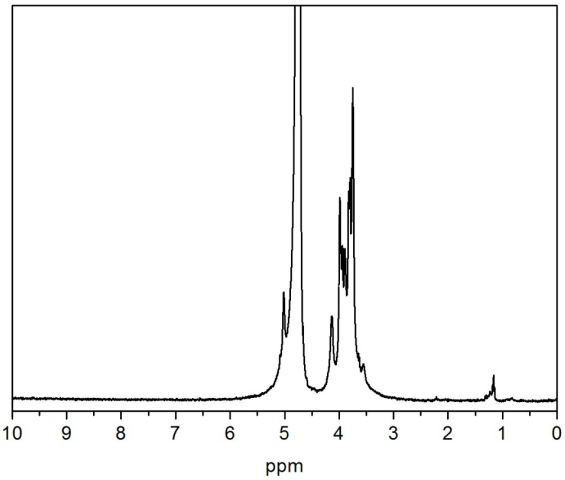

Fig. 2. ^{1}H RMN spectra of galactomannan from mezquite seed.

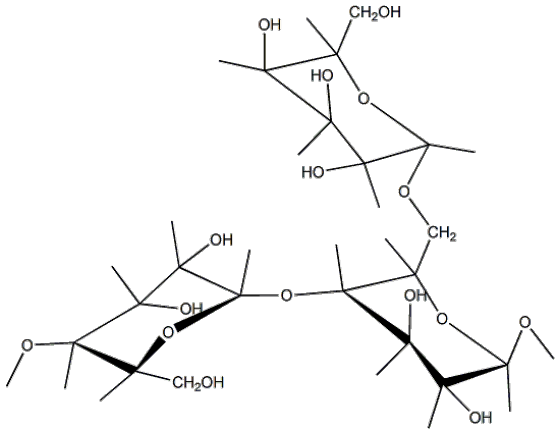

Fig. 3. Structure for a galactomannan from mezquite seed.

The DRX analysis is shown on figure 4 with signal al 20°, 38° and 44° this indicate that the galactomannans had crystallinity due to the orientation of their braches with a syndiotactic or isotactic structure (Carraher, 2003).

Table 5. Characteristic IR absorption bands of galactomannan.

Characteristic group	Wavenumber (cm^{-1})	Reference
Polymer crystallinity. Changes in this region indicates conformational changes	500 - 700	Tulchinsky *et al*. (1976)
Occurrence of β-D-mannopyranose units	811.46	Prado *et al*. (2005)
Ocurrence of α-D-galactopyranose units	868.42	Prado *et al*. (2005)
CH_2 twisting vibration	1021.26	Mudgil *et al*. (2012)
Primary alcoholic CH_2OH stretching mode	1065.43	Mudgil *et al*. (2012)
Associated to the several vibration modes of the C-O groups of carbohydrates.	1145.92	Prado *et al*. (2005)
Symmetrical deformations of CH_2 group and COH	1217.38, 1227.79, 1371.57 and 1541.72	Mudgil *et al*. (2012)
Carbohydrate molecule hydration	1644.82	Mudgil *et al*. (2012)
C-H stretching of CH_2 group	2888.82	Kacurakova *et al*. (1998)
O-H stretching vibration of polymer and water involved in hydrogen bonding	3340.01	Fringant *et al*. (1995)

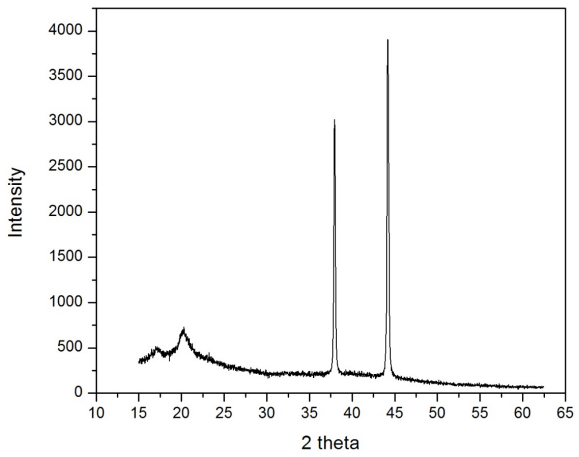

Fig. 4. DRX for a galactomannan from mezquite seed.

3.5 Thermal characteristics

In Figure 5 is shown up the thermogram with the results of the calorimetric analysis by DSC. Likewise, Table 6 shows up the values of calorific capacity, fusion temperature and vitreous transition temperature. Reports from other galactomannans indicates that they have higher vitreous transition temperature (52.92 °C and 52 °C) (Prajapati *et al*. 2013; Chaires-Martínez *et al*. 2008) than that found in this research, this could be related to the chemical structure of these polysaccharides, particularly the Mannose: Galactose ratio and the galactose branch distribution pattern along the mannose chain (Bresolin *et al*. 1998).

Table 6. Thermal analysis of galactomannans.

Thermal parameters	Value
Calorific capacity, Cp (J/g.°C)	0.54
Vitreous transition temperature, Tg (°C)	49.34
Fusion temperature, Tm (°C)	106.19

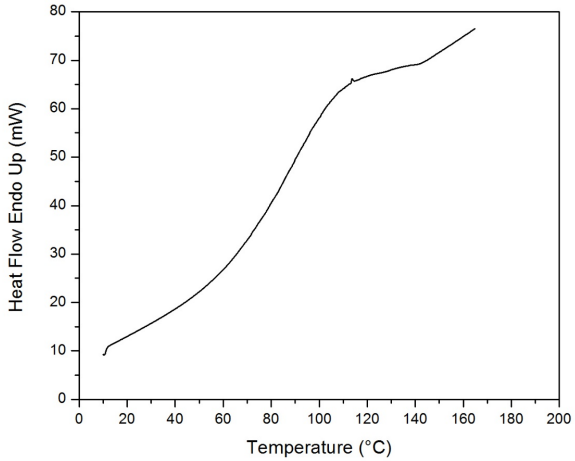

Fig. 5. DSC of galactomannan from mezquite seed.

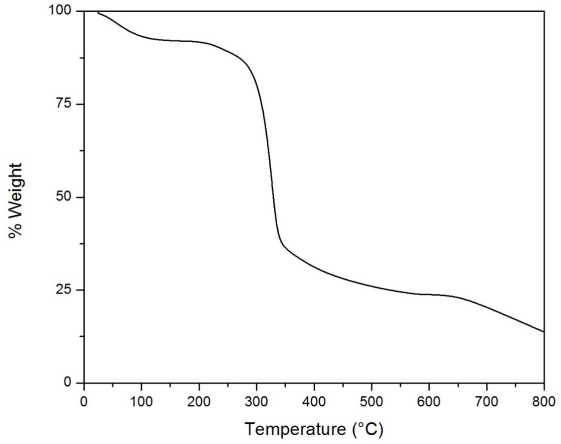

Fig. 6. TGA of galactomannan from mezquite seed.

Thermogravimetry analysis results is illustrated in figure 6 it show two mass loss events for both polymers, being the first near 100 °C, which may be attributed to the loss of adsorbed and structural water of biopolymers. The second mass loss event with a peak temperature of 317.67°C resulted in a weight loss of approximately 55%, which may be attributed to the polysaccharide decomposition. The peak temperature obtained is very similar to guar gum (314°C). (Vendruscolo *et al*., 2009).

Conclusions

The results of the hydrothermal extraction showed that the optimum yield with a smaller use of solvent is obtained using endosperm:water ratio of 1:20 with a treatment in autoclave for 15 minutes. The polysaccharides extracted from mesquite seeds were assumed to be galactomannans according to the literature survey of FT-IR and ^{1}H NMR analysis result. The extraction conditions did not have significant differences in physicochemical properties comparing with other galactomannans. TGA and DSC analysis showed that thermal stabilities were not affected by the hydrothermal extraction. The development of an ecological and scalable process for extraction of galactomannans was accomplished.

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Nomenclature

K	proportionality constant of biopolymer, mL/g
Mv	molecular weight, g/mol
Greek symbols	
	intrinsic viscosity, dL/g
α	expansion factor

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