Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 101 (2007) 70–73
Food Chemistry
www.elsevier.com/locate/foodchem

NMR study of galactomannans from the seeds of mesquite tree (*Prosopis juliflora* (Sw) DC)

Ícaro Gusmão Pinto Vieira [a], Francisca Noélia Pereira Mendes [a], Maria Izabel Gallão [b], Edy Sousa de Brito [c,*]

[a] *Parque de Desenvolvimento Tecnológico (PADETEC), Campus do Pici, CEP 60455 970, Fortaleza, CE, Brazil*
[b] *Department of Biology, Federal University of Ceará, Fortaleza, CE, Brazil*
[c] *Embrapa Tropical Agroindustry, R Dra Sara Mesquita, 2270, CEP 60511 110, Fortaleza, CE, Brazil*

Received 29 August 2005; received in revised form 11 November 2005; accepted 11 November 2005

Abstract

The seed gum from *Prosopis juliflora* is a galactomannan polysaccharide, which is located in the endosperm. Regarding *P. juliflora* galactomannan structural studies, there are reports on galactose substitutional pattern, but supporting data by NMR correlation studies are scarce. In this work, a procedure to obtain *P. juliflora* gum without contamination was employed and the gum was studied by NMR spectroscopy. Overall, results show that mesquite seed has a 1.1:1 Man/Gal ratio.

Keywords: Polysaccharides; Mesquite tree; *Prosopis juliflora*; Galactomannan; NMR

1. Introduction

Seed galactomannans, commonly known as seed gums (Dea & Morrison, 1975), have widespread industrial applications in food paper, textile, petroleum, pharmaceuticals and cosmetics (Whistler, 1973). They are mostly found in the endosperm of leguminous seeds as cell wall storage components and energy reserves. *P. juliflora* seed gum was identified as a galactomannan with a Gal:Man (1:4.2) (Figueiredo, 1983) and with a close similarity to guar and carob polysaccharides (Figueiredo & Price, 1990). Galactomannans have the fundamental structure consisting of a main chain of β-(1-4)-D-mannopyranose units substituted by single α-D-galactopyranose units at O-6, although there are few deviations from this basic structure. They differ from each other in mannose:galactose ratio and fine structure regarding distribution of single galactose branches on the main chain, causing variations in solubility, rheology and other properties. Determination of the distribution of (1-6)-linked, α-D-galactopyranosyl side groups along the (1-4)-linked β-D-mannan chains in legume seed galactomannans has so far been attempted by X-ray diffraction analysis (Dey, 1978; McCleary, Clarck, Dea, & Rees, 1985), degradation by purified 3-D-mannanases (Palmer & Ballantyne, 1950), specific methods of chemical degradation (Courtois & Le Dizet, 1970; McCleary & Matheson, 1975; McCleary, 1978), methylation analysis after periodate oxidation (Baker & Whistler, 1975; Hoffman & Svensson, 1978) and theoretical analysis of periodate-oxidation kinetics (González, 1978; Hoffman, Lindberg, & Painter, 1976; Painter, Gonzalez, & Hemmer, 1979). Agreement among different groups, using different methods, has been poor. Part of the difficulty may be due to variation in structure among different samples of galactomannans isolated from the same species. The aim of this work was to purify *P. juliflora* galactomannan obtained by previous separation of the endosperm from the embryo and seed coating followed by extraction of endosperm with hot water, and to evaluate their structure by NMR.

* Corresponding author.
E-mail address: edy@cnpat.embrapa.br (E.S. de Brito).

0308-8146/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2005.11.052

2. Materials and methods

2.1. Polysaccharide source

Seeds of *P. juliflora* were collected in the semi-arid zone of the Northeastern region of Brazil. In the laboratory the pods were dried, broken and the seeds dehulled.

2.2. Polysaccharide isolation

The seeds were treated with water at 100 °C, for 20 min and then kept at 4 °C until swelling took place for 12 h. Thereafter, the seed coats were removed and each side of the endosperm separated from the embryo. Both sections of the endosperm were isolated and submitted to exhaustive hot (85 °C) aqueous extractions. The water viscous solution was filtered in polyester (5 μm) and the aqueous filtered solution was freeze-dried.

2.3. Nuclear magnetic resonance spectroscopy

For ^{13}C NMR analysis, spectra were obtained on Avance DRX-500 Bruker Spectrometers, equipped with a process controller. A galactomannan sample (10 mg/ml) was dissolved in D_2O at 80–85 °C with continuous stirring for 5 h. Spectra were recorded at 85 °C under conditions of inverse gated decoupling proton decoupled spectrum without NOE. Peak integrals were obtained using the Bruker software and assignments were made comparing the results to references (Ishrud, Zahid, Zhou, & Pan, 2001; Kapoor et al., 1998; Ramesh, Yamaki, Ono, & Tsushida, 2001). For ^{1}H NMR analysis, the galactomannan was dissolved in high quality D_2O (99.96% D). The spectra were obtained at 85 °C using a relaxation delay of 1 s and a pulse width of 90 ° to reach the conditions of quantitative analysis.

3. Results and discussion

3.1. Isolation and purification

The isolated endosperm of the seeds contains the water-soluble galactomannan, while the rest of the seed coat and embryo mainly contains pentoses. Both sides of the endosperm were manually separated from the seed coat and embryo and submitted to hot aqueous (85 °C) extractions. Thus, the main substances present in the seed coat and embryo (proteins, lipids, crude fibers and xylose and pentose polyssacharides) that could contaminate the polysaccharides were separated from the endosperm before the extraction of the galactomannan. Such procedure avoids contamination of the galactomannan with pentose and xylose, which are the main impurities in the galactomannans. To avoid decomposition and hydrolysis of the galactomannan in the aqueous extraction process we used temperatures lower than 85 °C. The galactomannan were obtained in a very pure form after freeze-drying of the filtrated product and the yield of the pure galactomannan was 10–14% by this process.

3.2. ^{1}H NMR spectroscopy

The 500 MHz, ^{1}H NMR spectrum of *P. juliflora* galactomannan and its anomeric region in the spectrum are shown in Fig. 1. The resonances of the anomeric protons are well separated, and their identification is self-evident from the known monomeric compositions of the samples (González, 1978; Hoffman et al., 1976; Painter et al., 1979). The Gal:Man ratio can be obtained directly from the relative areas of the signals for ^{1}H (Gal) and ^{1}H (Man) (Fig. 1) and resulted in a Gal:Man = 1.00:1.13. Such result differs from those obtained in other work with *P. juliflora* polysaccharide, in which a Gal:Man = 1.0:4.2 was observed (Figueiredo, 1983). On the other hand, our results were in accordance with previous works for other galactomannans, including *P. juliflora* gum (Buckeridge, Pavegassi, Rocha, & Dietrich, 1995). The large singlet at 5.52 ppm which is more intense, arises from H-1(Gal) and is compatible with the expected conformation of the α-D-galactopyranose ring. The signal for ^{1}H (Man) was observed at 5.24 ppm, which corresponds to the monomeric β-D-mannopyranose (Grasdalen & Painter, 1980; Kapoor et al., 1998).

3.3. ^{13}C NMR spectroscopy

The 125 MHz, ^{13}C NMR spectrum of *P. juliflora* galactomannan (Fig. 2) illustrates the great promise of ^{13}C NMR for sequential analysis of polysaccharides. All the different carbon lines are resolved, and their chemical shifts are recorded in Table 1. The resonances associated with the D-galactose and D-mannose residues were distinguished by making use of the different monomeric compositions of the samples, as determined by ^{1}H NMR. The spectral regions

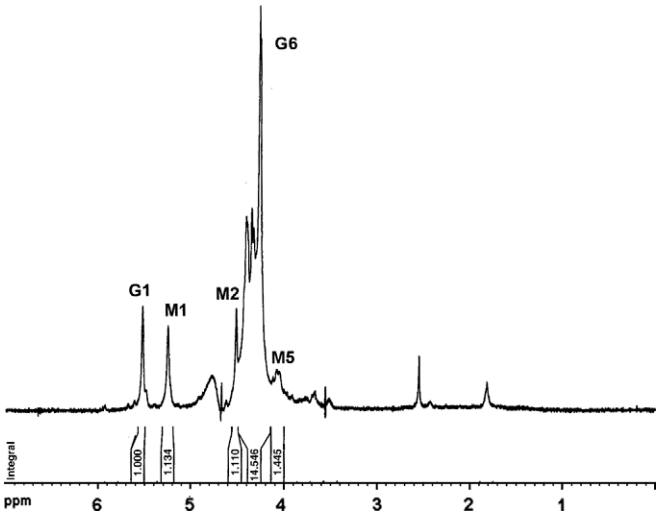

Fig. 1. ^{1}H NMR spectra (500 MHz) of solutions (10 mg/ml) in D_2O of the galactomannans from *Prosopis juliflora*.

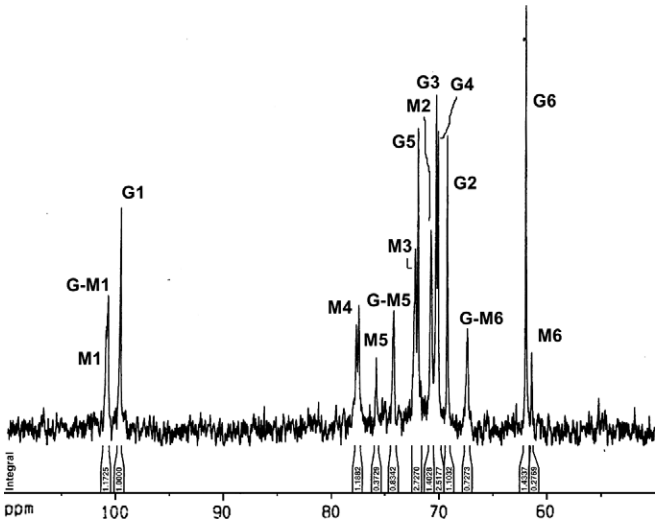

Fig. 2. ^{13}C NMR spectrum (1250 MHz) of a solution (10 mg/ml) in D_2O of the galactomannans from *Prosopis juliflora*.

Table 1
Assignments of peaks in ^{13}C NMR spectra of *Prosopis juliflora* seed galactomannans[a]

Type of unit	C-1	C-2	C-3	C-4	C-5	C-6
α-D-Galactopyranosyl	99.63	69.25	70.29	70.12	71.96	61.94
β-D-Mannopyranosyl, unbranched at HO-6	100.77	70.75	72.21	77.47	75.84	61.40
β-D-Mannopyranosyl, branched at HO-6	100.77	70.75	72.21	77.69	75.84	67.39

[a] Shifts ppm downfield from internal sodium 3-(trimethylsilyl)-propionate.

of the anomeric carbons (100.77 and 99.63 ppm) and the methylene carbons (61.94 and 61.40 ppm) are well depicted (Table 1). During galactomannan ^{13}C NMR studies, all the carbon lines were well resolved and the chemical shifts were found in accordance with those reported by the literature (Bociek, Izzard, Morrison, & Welti, 1981; González, 1978; Hoffman et al., 1976; Painter et al., 1979). In the ^{13}C NMR spectra, the resonances' relative areas were not significantly influenced by the nuclear Overhauser enhancements (Fig. 2). A maximum value of $T1 = 0.5$ s was observed for the C-6(Gal) resonance. The chemical shifts are reported in Table 2. Under the instrumental conditions chosen, the relative abundance of the two monomers was also directly obtained from the relative peak-areas of the corresponding ^{13}C NMR signals (Table 2) and were in accordance with the literature (Bociek et al., 1981; Grasdalen & Painter, 1980; Manzi, Cerezo, & Shoolery, 1986; Noble & Taravel, 1987).

Table 2
Monosaccharide composition in *Prosopis juliflora* seed galactomannans

Monosaccharides	Methods	
	^{13}C NMR	^{1}H NMR
D-Galactose	1.00	1.00
D-Mannose	1.17	1.13

The extraction procedure used resulted in a pure gum and well-defined spectra. The present NMR study, in spite of the low accuracy of the assay (±5% to 10%), allowed to stablish for *P. juliflora* seed polysaccharide a Gal:Man ratio of 1.0:1.1, as determined by the relative areas of the anomeric substituted and non-substituted signals of the ^{1}H and ^{13}C NMR.

Acknowledgement

To Prof. Dr. Edilberto Rocha Silveira (CENAUREM-UFC) for NMR spectra.

References

Baker, C. W., & Whistler, R. L. (1975). Distribution of D-Galactosyl groups in guaran and locust bean gum. *Carbohydrate Research, 45*, 237–243.

Bociek, S. M., Izzard, M. J., Morrison, A., & Welti, D. (1981). The ^{13}C NMR spectra of (1-6)-α-D-galactosyl-(1-4)-β-D-mannans. *Carbohydrate Research, 93*, 279–283.

Buckeridge, M., Pavegassi, V. R., Rocha, D. C., & Dietrich, S. M. (1995). Seed galactomannans in the classification and evolution of the Leguminosae. *Phytochemistry, 38*, 871–875.

Courtois, J. E., & Le Dizet, P. (1970). Studies on galactomannans. 4. Action of some mannases on various galactomannans. *Bulletin de la Societe de Chimie Biologique, 52*, 15–22.

Dea, I. C. M., & Morrison, A. (1975). Chemistry and interactions of seed galactomannans. *Advances in Carbohydrate Chemistry and Biochemistry, 31*, 241–312.

Dey, P. M. (1978). Biochemistry of plant galactomannans. *Advances Carbohydrate Chemistry Biochemistry, 35*, 341–376.

Figueiredo, A. A. (1983). Extração, identificação e características do polissacarído da semente de algaroba (*Prosopis juliflora* DC). *Ciência e Tecnologia de Alimentos, 3*(1), 82–89.

Figueiredo, A. A., & Price, R. L. (1990). Chemical structure of mesquite (Prosopis juliflora DC) seed polysaccharide. *Ciência eTecnologia de Alimentos, 10*(2), 287–300.

González, J. J. (1978). Sequential-analysis by cooperative reactions on copolymers: structure of locust bean gum and guaran. *Macromolecules, 11*, 1074–1085.

Grasdalen, H., & Painter, J. T. (1980). NMR studies of composition and sequence in legume-seed galactomannans. *Carbohydrate Research, 81*, 59–66.

Hoffman, J., & Svensson, S. (1978). Studies of the distribution of the D-galactosyl side-chains in guaran. *Carbohydrate Research, 65*, 65–71.

Hoffman, J., Lindberg, B., & Painter, T. J. (1976). Distribution of D-galactose residues in guaran and locust bean legume. *Acta Chemica Scandinavica Series B, 30*, 365–366.

Ishrud, O., Zahid, M., Zhou, H., & Pan, Y. (2001). A water-soluble galactomannan from the seeds of *Phoenix dactylifera* L. *Carbohydrate Research, 335*, 297–301.

Kapoor, V. P., Taravel, F. R., Joseleau, J. P., Milas, M., Chanzy, H., & Rinaudo, M. (1998). Cassia epectabilis DC seed galactomannan: structural, crystallographical and rheological studies. *Carbohydrate Research, 306*, 231–241.

Manzi, A. E., Cerezo, A. S., & Shoolery, J. N. (1986). High resolution ^{13}C NMR spectroscopy of legume seed galactomannans. *Carbohydrate Research, 148*, 189–197.

McCleary, B. V., Clarck, A. H., Dea, I. C. M., & Rees, A. D. (1985). The fine structure of carob and guar galactomannans. *Carbohydrate Research, 139*, 237–260.

McCleary, B. V., & Matheson, N. K. (1975). Galactomannan structure and β-mannanase and β-mannosidase activity in germinating legume seeds. *Phytochemistry, 14*, 1187–1194.

McCleary, B. V. (1978). Simple assay procedure for β-mannase. *Carbohydrate Research, 67*, 213–221.

Noble, O., & Taravel, R. F. (1987). Nuclear magnetic resonance evidence of the guarborate interaction. *Carbohydrate Research, 166*, 1–11.

Painter, T. J., Gonzalez, J. J., & Hemmer, P. C. (1979). The distribution of D-galactosyl groups in guaran and locust-bean gum: new evidence from periodate oxidation. *Carbohydrate Research, 69*, 217–226.

Palmer, K. J., & Ballantyne, M. (1950). The structure of (I) some pectin esters and (II) guar galactomannan. *Journal of the American Chemical Society, 72*, 736–741.

Ramesh, H. P., Yamaki, K., Ono, H., & Tsushida, T. (2001). Two-dimensional NMR spectroscopy studies of fenugreek (*Trigonella foenum-graecum* L.). Galactomannan without chemical fragmentation. *Carbohydrate Polymers, 45*, 69–77.

Whistler, R. L. (1973). *Industrial gums* (2nd ed.). New York: Academic Press.