

Structural and thermal characterization of galactomannans from non-conventional sources

Miguel A. Cerqueira^a, Bartolomeu W.S. Souza^a, Joana Simões^b, José A. Teixeira^a, M. Rosário M. Domingues^b, Manuel A. Coimbra^b, António A. Vicente^{a,*}

^a IBB – Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal

^b QOPNA - Departamento de Química, Universidade de Aveiro, 3810-193 Aveiro, Portugal

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ABSTRACT

Galactomannans of *Gleditsia triacanthos*, *Caesalpinia pulcherrima* and *Adenantha pavonina* were structurally and thermally characterized. Methylation analyses confirmed that these three galactomannans are composed by a 4-linked mannose polymer with galactose side chains attached at the C6 position. *G. triacanthos* galactomannan had an estimated average degree of polymerization of 224 mannose residues, with a degree of branching of 0.24, *C. pulcherrima* galactomannan had a degree of polymerization of 252 and a degree of branching of 0.30, and *A. pavonina* galactomannan had a degree of polymerization of 475 and a degree of branching of 0.60. Enzymatic hydrolysis with *endo*- β -mannanase and the subsequent analyses of the low molecular weight fraction by ESI-MS/MS indicated the presence of characteristic structural features of galactomannans. Furthermore, acetyl and pentosyl residues were detected in the galactomannan of *G. triacanthos* and analyses of higher molecular weight fractions from *G. triacanthos* were performed, confirming that presence. Differential scanning calorimetry (DSC) showed the presence of two peaks related with water loss, confirmed by thermogravimetric analysis (TGA). The different compositions of galactomannans with different mannose content and different molecular weights influence their thermal behaviour. A higher content of mannose lead to higher values of the enthalpy change (first thermal transition) and glass transition temperature (T_g), while the enthalpy change of the second thermal transition was influenced by the viscosity average molecular weight (M_v) of the samples.

This work provides understanding of structural and thermal properties of three galactomannans from non-conventional sources, thus contributing to a better insight on their possible food, pharmaceutical or biomedical applications.

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1. Introduction

In general, hydrocolloids are hydrophilic molecules with high molecular weight that can be used by food industry to modify texture, rheological characteristics, appearance and conservation of food stuffs due to their ability to stabilize emulsions and dispersions and retain water (Rosell, Rojas, & De Barber, 2001). Currently, consumers' demand for healthier, safer and novel natural foods fostered the introduction of alternative sources of seed gums (Joshi & Kapoor, 2003) and it is therefore important to characterize such materials in view of their possible applications. Biodegradable polymers are interesting for biomedical engineering, featuring two major advantages over non-biodegradable polymers: they are gradually absorbed by the human body, and some of them are able to regenerate tissues through the interaction of their biodegrad-

tion with immunologic cells (Chu, 2003). They may also be used, e.g. for the production of edible and biodegradable films and coating materials (Cerqueira, Lima et al., 2009).

Galactomannans, extracted from the endosperm of numerous plants (particularly the *Leguminosae*) are polysaccharides built up of a β -(1–4)-D-mannan backbone with single D-galactose branches linked α -(1–6). They are water soluble hydrocolloids forming highly viscous, stable aqueous solutions (Neukom, 1989). Galactomannans can often be used in different forms for human consumption. Featuring different physicochemical properties, galactomannans are a versatile material used for many applications: they are excellent stiffeners and stabilizers of emulsions, and the absence of toxicity allows their use in the textile, pharmaceutical, biomedical, cosmetics and food industries (Srivastava & Kapoor, 2005; Vieira, Mendes, Gallão, & de Brito, 2007; Yi & Zhang, 2007).

Commercially available galactomannans such as locust bean gum (*Ceratonia siliqua*), guar gum (*Cyamopsis tetragonolobus*) and tara gum (*Caesalpinia spinosa*) have found applicability as controlled release agents (Baveja, Ranga Rao, Arora, Mathur, & Vinayah,

* Corresponding author. Tel.: +351 253 604419; fax: +351 253 678986.

E-mail address: avicente@deb.uminho.pt (A.A. Vicente).

1991; Krishnaiah, Karthikeyan, Gouri Sankar, & Satyanarayana, 2002; Varshosaz, Tavakoli, & Eram, 2006). In order to guarantee the application of galactomannans in pharmaceutical and biomedical industries a full characterization is necessary (Vendruscolo et al., 2009). In particular, Latin American sources of galactomannans are not well known, in spite of the rich biodiversity of the local flora and of the favourable climate for their production (Azero & Andrade, 2002).

Non-traditional galactomannans were isolated from seeds of *Gleditsia triacanthos*, *Caesalpinia pulcherrima* and *Adenanthera pavonina*. Their yield of extraction, monosaccharide composition, mannose/galactose ratio, intrinsic viscosity and viscosity average molecular weight were previously reported (Cerqueira, Pinheiro et al., 2009). In the present work they were further characterized by methylation analysis and by enzymatic hydrolysis followed by analysis by tandem mass spectrometry (MS/MS) using electrospray ionization (ESI). Their thermal characterization was performed by thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) together with a characterization by Fourier transform infrared (FTIR) spectroscopy.

2. Materials and methods

2.1. Raw material

Galactomannans from *G. triacanthos* (GT), *C. pulcherrima* (CP) and *A. pavonina* (AP) were obtained as previously described in Cerqueira, Pinheiro et al. (2009).

2.2. Methylation and GC/MS analyses

Polysaccharides were activated with powdered NaOH and methylated with CH_3I (Ciucanu & Kerek, 1984; Isogai, Ishizu, & Nakano, 1985) as described by Coimbra et al. (1996). The sample (2–3 mg) was dispersed in 2 mL of dried DMSO and sonicated occasionally until it was fully dispersed. NaOH pellets (100 mg) powdered under argon were added to the solution. The sample was sonicated for 90 min and allowed to stand for an additional 90 min. The methylated material was dissolved in 3 mL of water and extracted with 4 mL of chloroform. The organic layer was washed three times with 3 mL of water, evaporated to dryness, and remethylated to achieve a complete methylation of all free OH groups. The fully methylated material was then hydrolyzed with 2 M trifluoroacetic acid (1 mL) at 121 °C for 1 h, cooled, and rotary evaporated at 35 °C. The partially methylated sugars were then dissolved in 0.3 mL of 2 M NH_3 and 20 mg of NaBD_4 were added. The mixture was allowed to react at 30 °C for 1 h, and the excess of the reducing agent was destroyed by the addition of 0.1 mL of glacial acetic acid. The acetylation of the partially methylated alditols was performed by adding 1-methylimidazole (0.45 mL) and acetic anhydride (3 mL) and allowing to react for 30 min at 30 °C. This solution was treated with water (3 mL) to decompose the excess of acetic anhydride, and the partially methylated alditol acetates (PMAA) were extracted with dichloromethane (3–5 mL). The dichloromethane phase was washed with water and evaporated to dryness. The PMAA were dissolved in dichloromethane (70 μL) and analyzed by GC–MS. GC–MS analysis was performed in a HP series 2 gas chromatograph and Trio-1S VG mass-lab using a DB-1 capillary column (30 m length, 0.32 mm i.d., and 0.25 μm of film thickness). The samples were injected in splitless mode (time of splitless 0.75 min), with the injector and detector operating at 210 and 220 °C, respectively, using the following temperature program: 55 °C for 0.75 min followed by a linear increase of 45 °C min^{-1} until 140 °C, and standing 1 min at this temperature, followed by a linear increase of 2.5 °C min^{-1} until 218 °C, with further 37 min at

218 °C. Linear velocity of the carrier gas (He) was set at 40 cm s^{-1} at 200 °C, with a solvent delay of 4 min. MS scans were performed for GC–MS between 400 and 35 m/z at 70 eV ionization energy.

2.3. Enzymatic hydrolysis

Samples (14 mg) were hydrolyzed with pure *endo*- β -(1–4)-mannanase (1 U) preparation (Megazyme, EC 3.2.1.78) during 48 h at 37 °C with continuous stirring in a 100 mM Na-acetate buffer pH 5.5 containing 0.02% sodium azide. The freeze-dried material was dissolved in pyridine-acetate 100 mM buffer pH 5.25, and loaded on a XK 1.6/100 column containing Biogel P-4 (Bio-Rad) previously equilibrated with the same buffer. Fractions (1 mL) were collected and assayed for sugars by static light scattering (Sedere, France). Results were subsequently confirmed by the phenol- H_2SO_4 method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The appropriate fractions were pooled and rotary evaporated until all buffer was removed by repeated additions of distilled water, and freeze-dried.

2.4. Electrospray ionization mass spectrometry (ESI-MS and ESI-MS/MS)

Freeze-dried fractions obtained after enzymatic hydrolysis and fractionated through Biogel P4 column were dissolved in 200 μL of 1:1 MeOH–water containing 1% (v/v) formic acid. Samples were introduced into the mass spectrometer using a flow rate of 8 $\mu\text{L min}^{-1}$. Positive-ion ESI-MS and MS/MS spectra were acquired using a LXQ linear ion trap mass spectrometer (ThermoFinnigan, San Jose, CA). Typical ESI conditions were: nitrogen sheath gas 30 psi, spray voltage 5 kV, heated capillary temperature 275 °C, capillary voltage 1 V and tube lens voltage 40 V. The precursor ion accumulation time for each scan was controlled by the automatic gain control function of the instrument. ESI-MS/MS experiments were performed on mass-selected precursor ions using standard isolation and excitation procedures (activation q value of 0.25, activation time of 30 ms). The collision energy used was between 20 and 24 (arbitrary units). Data acquisition was carried out with an Xcalibur data system.

2.5. Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC)

TGA and DSC measurements were carried out in N_2 atmosphere using a Shimadzu TGA-50 and a Shimadzu DSC-50 equipment (Shimadzu Corporation, Kyoto, Japan) calibrated with Indium as standard. Analyses were started at 20 °C and continued up to 580 °C, with a linear increase of 10 °C min^{-1} . Samples were weighed (approximately 5–10 mg of dry matter) in Aluminium DSC pans (Al crimp Pan C.201–52943) being the empty pans used as a reference. Data were treated using TASY software (Shimadzu Corporation, Kyoto, Japan). Enthalpy was calculated using the area of the peaks between the onset temperature and the end set temperature. For glass transition temperature (T_g) determinations two heating runs were performed between 20 and 250 °C. After the first heating run the samples were left to cool naturally at room temperature under N_2 atmosphere. The second run was used for T_g determination (Dong, Ruan, Wang, Zhao, & Bi, 2004; Mitsuiki, Yamamoto, Mizuno, & Matoki, 1998; Yi & Zhang, 2007).

2.6. Fourier transform infrared (FTIR) spectroscopy

IR spectra of the polysaccharides were determined using a Fourier transform infrared spectrometer (FTIR) (Perkin-Elmer 16PC spectrometer, Boston, USA). The polysaccharide was ground with spectroscopic grade potassium bromide (KBr) powder and then

Table 1
Glycosidic-linkage analyses of *G. triacanthos* (GT), *C. pulcherrima* (CP) and *A. pavonina* (AP) galactomannans.

Linkage	Relative abundance (%)		
	GT	CP	AP
Terminal-Manp	0.35 ± 0.17	0.30 ± 0.09	0.13 ± 0.11
4-Manp	59.15 ± 4.57	53.18 ± 7.16	24.73 ± 0.48
4,6-Manp	18.93 ± 4.51	22.05 ± 3.81	36.84 ± 9.48
Total Man	78.43 ± 4.07 (66.9) ^a	75.53 ± 2.86 (69.1) ^a	61.70 ± 9.19 (52.8) ^a
DPm	224	252	475
Terminal-Galp	17.23 ± 4.50	20.92 ± 3.62	34.02 ± 7.80
4-Galp	3.45 ± 1.03	2.70 ± 0.05	2.95 ± 0.28
Total Gal	20.69 ± 3.81 (23.7) ^a	26.21 ± 3.68 (24.0) ^a	36.97 ± 7.52 (39.2) ^a
Terminal-Araf	0.55 ± 0.15	0.24 ± 0.22	–
5-Araf	0.34 ± 0.13	0.51 ± 0.35	0.83 ± 0.65
Total Ara	0.89 ± 0.27 (4.5) ^a	0.92 ± 0.06 (1.2) ^a	0.52 ± 0.34 (0.8) ^a

^a Values of monosaccharide composition obtained in Cerqueira, Pinheiro et al. (2009).

pressed into 1 mm pellets for FTIR measurement in the wavenumber range of 400 and 4000 cm⁻¹ using 16 scans. Each spectrum was baseline corrected and the absorbance was normalised between 0 and 1.

3. Results and discussion

3.1. Methylation and GC-MS analyses

Table 1 shows the glycosidic linkage composition of the three galactomannans. Partially methylated alditol acetates analyzed by GC/MS revealed a polysaccharide containing nonreducing terminal units of Manp (0.35%, 0.30% and 0.13%) and Galp (17.23%, 20.92% and 34.02%) as well as 4-Manp (59.15%, 53.18% and 24.73%), 4,6-Manp (18.93%, 22.05% and 36.84%) and 4-Galp (3.45%, 2.70% and 2.95%), for *G. triacanthos*, *C. pulcherrima* and *A. pavonina*, respectively. The results of the methylation analyses confirm the structure of the galactomannans as a 1–4 mannose with galactose side chains attached at the C6 position. The total content of mannosyl and galactosyl residues is in agreement with previous work on monosaccharide composition (Cerqueira, Pinheiro et al., 2009). Results also show the presence of arabinose residues in galactomannan extracts as reported in other works with *G. triacanthos* (Manzi, Ancibor, & Cerezo, 1990; Navarro, Cerezo, & Stortz, 2002).

The degree of polymerization of mannose residues for the three galactomannans are within the range usually found for polysaccharides structures (above 100) (Izydorczyk, 2005), showing in this case the greater value for *A. pavonina* galactomannan. *G. triacanthos* galactomannan had an estimated average degree of polymerization of 224 mannose residues, with a degree of branching of 0.24, *C. pulcherrima* had a degree of polymerization of 252 and a degree of branching of 0.30, and *A. pavonina* had a degree of polymerization of 475 and a degree of branching of 0.60. These values are in line with the values of the viscosity average molecular weight (*M_v*) reported by Cerqueira, Pinheiro et al. (2009), showing a positive correlation between the degree of polymerization and *M_v*, although the values obtained by methylation analysis are much lower (40–80 kDa) than those obtained by viscosity measurements (1.6–1.8 MDa). These differences are possibly due to some polysaccharide depolymerization during methylation, as previously suggested by Nunes and Coimbra (2001).

3.2. Enzymatic hydrolyses and ESI-MS analyses

Galactomannan samples were submitted to *endo*-β-mannanase hydrolyses in order to determine their detailed molecular structure, followed by size-exclusion chromatography (SEC) on a Biogel-P4 column, in order to obtain size-homogeneous oligosaccharide fractions (F₁–F₃). The results in the chromatograms corresponding to

G. triacanthos (GT), *C. pulcherrima* (CP) and *A. pavonina* (AP) samples are shown in Fig. 1a–c, respectively. According to the known enzymatic mechanism of *Aspergillus niger endo*-β-mannanase, the hydrolysis of β-(1–4)-linked mannan backbone is hindered by the presence of Gal residues, e.g. the extent of hydrolysis is higher for low galactose-substituted mannan backbones (Daas, Schols, & Jongh, 2000). The elution profile obtained by SEC after *endo*-β-mannanase hydrolyses showed different abundances of F₃, F₂ and F₁ fractions in each sample. The GT sample showed the highest abundance of low molecular weight fractions, when compared with the other samples, while AP presented the lowest abundance of fractions with lower molecular weight. This pattern can be related with the values of the degree of branching for these polysaccharides. AP galactomannan presents the highest value of degree of

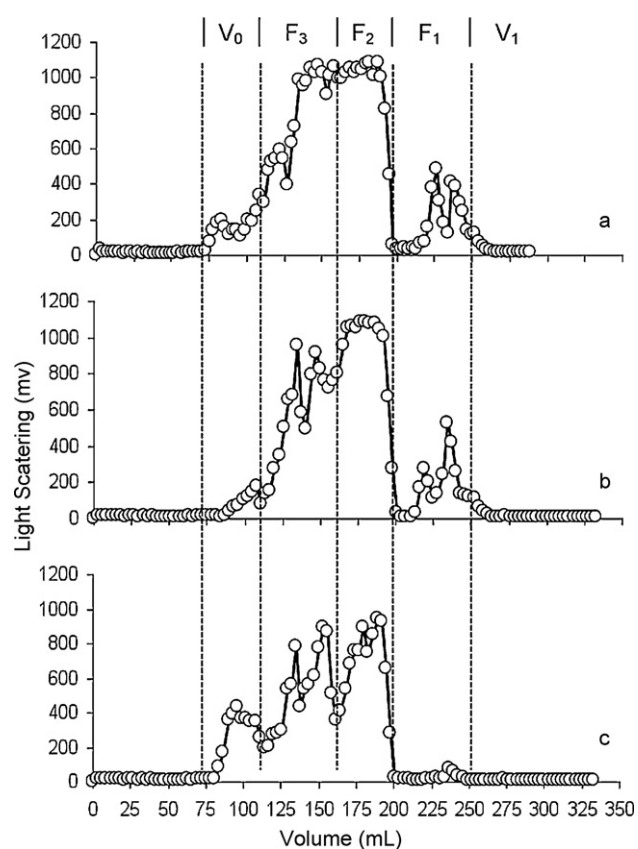


Fig. 1. Size-exclusion chromatography of oligosaccharides obtained after hydrolysis with *endo*-β-mannanase for (a) *G. triacanthos*, (b) *C. pulcherrima* and (c) *A. pavonina*. V₀ – void volume; V₁ – total volume. F₁–F₃ are oligosaccharide fractions with increasing molecular weights.

Table 2

Oligosaccharide m/z ions ($[M+Na]^+$) observed by ESI-MS after hydrolysis with *endo*- β -mannanase of *G. triacanthos* (GT), *C. pulcherrima* (CP) and *A. pavonina* (AP) galactomannans.

Sample, n	GT					
	2	3	4	5	6	7
$[Hex_n + Na]^+$	365	527	689	851	1013	1175
$[AcHex_n + Na]^+$		569		893	1055	
$[Hex_n AcPent + Na]^+$		701				
Sample, n	CP			AP		
	2	3	4	2	3	4
$[Hex_n + Na]^+$	365		527	689		
$[Hex_n + Na]^+$	365		527	689		

branching (lowest abundance of fractions with lower molecular weight) while the GT galactomannan presents the lowest value of degree of branching (higher abundance of fractions with lower molecular weight).

The analysis by ESI-MS allows studying intact oligosaccharides, even when present in mixtures and with low abundance, without any manipulation/derivatization being required (Nunes, Domingues, & Coimbra, 2005). ESI-MS spectra of oligosaccharide F_1 fractions obtained from GT, CP and AP polysaccharides present similar features. Table 2 shows the oligosaccharides identified as $[M+Na]^+$ ions in ESI-MS spectra of galactomannans from GT, CP and AP. The major oligosaccharides of F_1 for each of the three studied galactomannans were identified as the sodium adducts $[M+Na]^+$ of Hex_2 and Hex_3 followed by Hex_4 at m/z 365, m/z 527 and m/z 689, respectively. On GT samples the presence of ions at m/z 569 and m/z 701, attributed to the acetylated tri- and tetrasaccharides Hex_3Ac_1 and $Hex_3Ac_1Pent_1$, respectively, were detected. To confirm the presence of acetyl and pentosyl groups in GT polysaccharide, F_2 fraction was submitted to a second SEC in a Biogel-P4 column, leading to two fractions: F_{21} and F_{22} (Fig. 2). After this purification step, the acetylated groups were still detected, with the presence of ions at m/z 893 and 1055, attributed to the acetylated penta- and hexasaccharides. In these F_{21} and F_{22} fractions, the sodium adducts $[M+Na]^+$ of Hex_5 , Hex_6 and Hex_7 were detected at m/z 851, m/z 1013 and m/z 1175, respectively (Table 2). The presence of acetyl groups was observed in other works that used ESI-MS and MS/MS for the characterization of galactomannans (Nunes et al., 2005). In order to confirm the proposed oligosaccharide structures observed in the ESI-MS spectra, these ions were further analyzed by ESI-MS/MS.

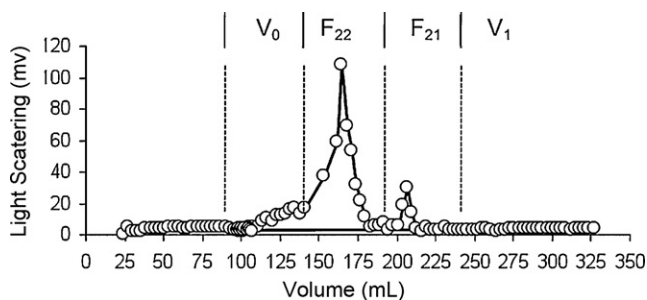


Fig. 2. Size-exclusion chromatography of fraction F_2 from *G. triacanthos* galactomannan. V_0 – void volume; V_1 – total volume. F_{21} and F_{22} are oligosaccharide fractions with increasing molecular weights.

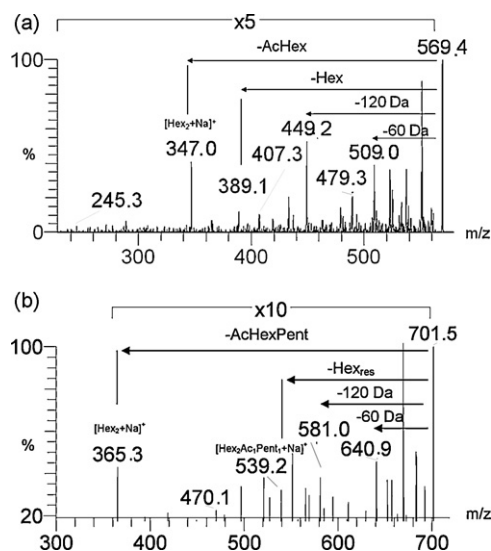


Fig. 3. ESI-MS/MS spectra of $[M+Na]^+$ adducts and schematic fragmentation pathways of Hex_3Ac_1 (a) and $Hex_3Ac_1Pent_1$ (b) for fraction F_1 of *G. triacanthos*.

3.3. ESI-MS/MS spectra of the obtained oligosaccharides

Oligosaccharide cleavages under ESI-MS/MS conditions are the result of glycosidic cleavages between two sugar residues and of cross-ring cleavages (cleavage of two bonds within the sugar ring).

3.3.1. *G. triacanthos*

The ESI-MS/MS spectrum of $[Hex_2 + Na]^+$ (m/z 365) and $[Hex_3 + Na]^+$ (m/z 527) for GT (results not shown) present predominant ions attributed to a glycosidic cleavage. The higher intensity of the loss of a neutral fragment with 60 Da is characteristic of a cross-ring $^{0,2}A_2$ cleavages, related with the (1–4)-linked hexoses. Also the absence of product ions due to losses of 90 Da, are characteristic of the MS/MS spectrum of 4-linked hexoses (Hofmeister, Zhou, & Leary, 1991).

The ESI-MS/MS spectrum of $[Hex_3Ac + Na]^+$ (m/z 569), presented in Fig. 3a, showed the presence of the ions at m/z 389 and m/z 347, corresponding to the loss of a Hex unit (–180 Da) and of the loss of one Hex_{res} plus AcHex (–222 Da), respectively. Although of low intensity, it is observed the ions at m/z 407 and m/z 245 due the loss of one and two Hex_{res} (162 and 324 Da), respectively. Fig. 3a also shows the ions at m/z 509 (loss of 60 Da), m/z 479 (loss of 90 Da) and, with higher abundance, the ion at m/z 449 (loss of 120 Da), corresponding to a 1–6 type linkage (Nunes et al., 2005). These fragmentation pathways allowed to identify this oligosaccharide as being composed by two Man units linked by a (1–4) linkage and an additional Gal residue (1–6)-linked, in accordance with methylation analysis. Also, the loss of AcHex (–222 Da) confirm the presence of an acetylated hexose unit.

The ESI-MS/MS spectrum of $[Hex_3AcPent + Na]^+$ (m/z 701) (Fig. 3b), show the product ions at m/z 539, corresponding to the loss of one Hex_{res} (–162 Da), and at m/z 365, attributed to the loss of one block HexAcPent. To confirm the presence of acetyl groups on this galactomannan structure the oligosaccharides at m/z 893 and m/z 1055 observed in the fraction F_{22} were also analyzed by ESI-MS/MS (Fig. 4).

Fig. 4a shows the ESI-MS/MS spectrum of $[Hex_5Ac + Na]^+$ (m/z 893), that confirms the presence of ions at m/z 731, m/z 569 and m/z 407 due the loss of one, two and three 162 Da (Hex_{res}), respectively. The ion m/z 347 was formed by the combined loss of a Hex unit (180 Da) and of one AcHex (222 Da).

The ESI-MS/MS spectrum of $[Hex_6Ac + Na]^+$ (m/z 1055) (Fig. 4b), shows the presence of ions m/z 893, m/z 731, m/z 569 and m/z 407

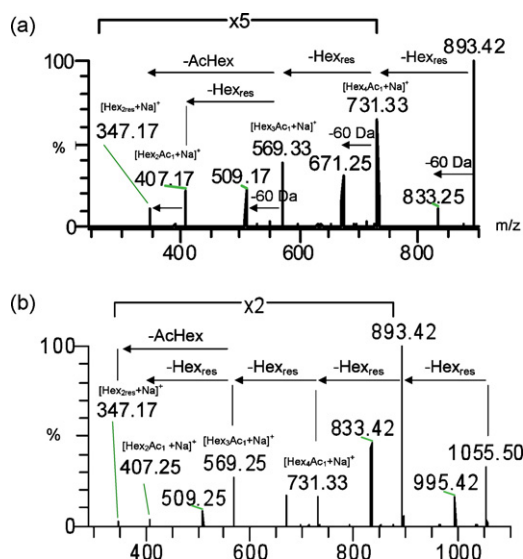


Fig. 4. ESI-MS/MS spectra of $[M + Na]^+$ adducts and schematic fragmentation pathways of Hex_5Ac_1 (a) and Hex_6Ac_1 (b) for fractions F_{22} of *G. triacanthos*.

due the loss of one, two, three and four 162 Da (Hex_{res}), respectively. The ion m/z 347 corresponds to the loss of AcHex (222 Da) plus loss of three hexose residues. These results indicate that mannans isolated from galactomannan of GT contain hexose residues bearing one acetyl group.

3.3.2. *C. pulcherrima* and *A. pavonina*

The ESI-MS/MS spectrum of $[Hex_2 + Na]^+$ (m/z 365) and $[Hex_3 + Na]^+$ (m/z 527) (data not shown), showed the presence of predominant ions attributed to C/Y-type glycosidic bond cleavage.

The ESI-MS/MS spectrum of $[Hex_3 + Na]^+$ and $[Hex_4 + Na]^+$ of AP samples (data not shown), corresponds to tri- and tetrasaccharide $[M + Na]^+$ ions, respectively. ESI-MS/MS spectra of the ions at m/z 527 and m/z 689 show loss of one and two Hex_{res} respectively, confirming that they are oligosaccharides composed by hexose residues. The loss of 60 Da, with higher abundance, in the ESI-MS/MS spectrum of $[Hex_3 + Na]^+$ is a characteristic of the MS/MS spectrum of (1–4)-linked hexoses. Also the ESI-MS/MS spectrum of $[Hex_4 + Na]^+$, presents losses of 60 Da, characteristic of the MS/MS spectra of (1–4)-linked hexoses.

The analyses of lower molecular weight fractions (F_1) of the three galactomannans show similar structures, although some differences such as the presence of acetyl and pentosyl groups in GT galactomannan can be noticed.

3.4. Thermogravimetric analyses (TGA) and differential scanning calorimetry (DSC)

DSC and TGA analyses were performed in order to understand the thermal behaviour of galactomannan and how can it be influenced by polysaccharide structure. DSC was used for studying

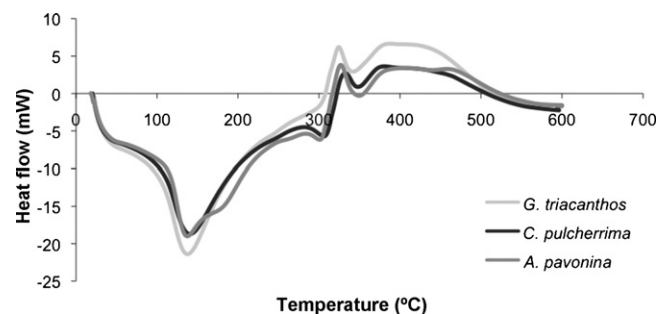


Fig. 5. DSC scans of the three analyzed galactomannans obtained at a heating rate of $10^\circ C\ min^{-1}$ under nitrogen atmosphere.

thermal transitions occurring in the course of heating under an inert atmosphere. DSC thermograms present typical natural polysaccharides DSC plots (Chaires-Martínez et al., 2008; Vendruscolo et al., 2009; Zohuriaan & Shokrolahi, 2004). Peak temperatures for the various thermal effects as well as the associated enthalpy changes for all the polysaccharides studied are given in Table 3. Most of the DSC traces exhibited early endothermic events in the temperature range between 99 and $224^\circ C$, which can be explained by water evaporation. Similar results were observed in other galactomannans: Vendruscolo et al. (2009) reported an endothermic event near $100^\circ C$ and Chaires-Martínez et al. (2008) detected peaks between 117.64 and $119.71^\circ C$. The second event was related to an endothermic peak between 306.59 and $345.56^\circ C$ (Table 3).

Some differences can be observed between samples in the DSC thermograms (Fig. 5). The enthalpy change for the first thermal transition (ΔH_1) increased by 8.7% from AP to GT, being the GT sample the polysaccharide with the highest ΔH_1 value. These increases have a direct relationship with the values of intrinsic viscosity and of the mannose:galactose ratio of the samples, in agreement with mannose molar content of GT, CP and AP samples of 66.9, 69.1 and 52.8 mol%, respectively (Cerqueira, Pinheiro et al., 2009). Na and Lee (1997) showed that the energy required for the thermal transition of lactan gum was related to the steady shear viscosity ranking of gum samples. The increase of mannose content in the sample indicates a lower branching and a higher binding energy between the backbone monosaccharides (Na & Lee, 1997); similar results were reported by Chaires-Martínez, Salazar-Montoya, & Ramos-Ramírez (2008) who compared guar gum with a lower content of Man/Gal ratio with locust bean gum, having guar gum displayed lower values of ΔH_1 .

Table 3 shows the values of enthalpy change for the second thermal transition, ΔH_2 . The values obtained for galactomannans of GT, CP and AP were 33.98 , 34.79 and $47.75\ J\ g^{-1}$, respectively. These values are related to the samples' thermal decomposition and are in agreement with the viscosity-based average molecular weight (M_v) and with the degrees of polymerization and branching of the samples (Riande et al., 2000; Sperling, 2006), which are both higher for AP galactomannan (Cerqueira, Pinheiro et al., 2009).

Glass transition temperature (T_g) is a parameter associated with the system's mobility, and is defined as a physical change from a

Table 3

Peak temperatures (T_1 and T_2) in DSC thermograms, enthalpy changes (ΔH_1 and ΔH_2) and glass transition temperatures (T_g) of galactomannan samples.

Sample	Peak no.	Onset ($^\circ C$)	T_1 ($^\circ C$)	T_2 ($^\circ C$)	Endset ($^\circ C$)	ΔH_1 ($J\ g^{-1}$)	ΔH_2 ($J\ g^{-1}$)	T_g^a ($^\circ C$)
<i>G. triacanthos</i>	1	102.05	137.11	–	205.72	324	–	56.4
	2	311.09	–	322.41	337.13	–	33.98	–
<i>C. pulcherrima</i>	1	101.52	139.18	–	203.56	326	–	66.9
	2	313.65	–	329.83	344.30	–	34.79	–
<i>A. pavonina</i>	1	112.80	151.36	–	208.13	298	–	52.6
	2	306.59	–	328.16	345.56	–	47.75	–

^a Obtained by the second heating run.

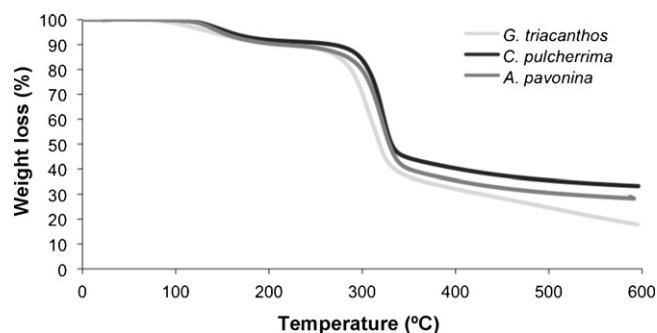


Fig. 6. Thermogravimetric curves for the three analyzed galactomannans obtained at a heating rate of $10^{\circ}\text{C min}^{-1}$ under nitrogen atmosphere.

glassy to a rubbery state in amorphous materials promoted by heat or addition of a plasticizer (Roos & Karel, 1991). T_g values of polymers can be explained by a great number of reasons as molecular weight, crystallinity and intermolecular bonding (Sperling, 2006). Chaires-Martínez et al. (2008) attribute the differences in T_g values of galactomannans to the M/G ratio and to the distribution of galactose units in mannose chains. Table 3 shows T_g values for the galactomannans under consideration; these values show that a higher M/G ratio leads to higher values of T_g . These values are close to those reported in the literature for other galactomannan sources (Chaires-Martínez et al., 2008; Yi & Zhang, 2007).

Also TGA curves (Fig. 6) are in agreement with other works on galactomannans (Varma, Kokane, Pathak, & Pradhan, 1997; Vendruscolo et al., 2009; Zohuriaan & Shokrolahi, 2004), showing two mass loss events for all polymers. The first occurs near 100°C and may be attributed to the loss of adsorbed and structural water, which in turn is associated to the hydrophilic nature of the functional groups of each polysaccharide. The second mass loss event resulted in a weight loss of ca. 45%, due to polysaccharide thermal decomposition, presenting peaks of the derivative of the weight loss curve (DTG) at 309.81 , 321.73 and 320.62°C for GT, CP and AP, respectively (Table 4). These values are in conformity with the values presented by Vendruscolo et al. (2009) for the galactomannan of *Mimosa scabrella*, with values ranging between 299.7 and 311.9°C , depending on the drying temperature. Also Varma et al. (1997) presented a DTG value of 306°C for guar gum.

3.5. Fourier transform infrared (FTIR) spectroscopy

Fig. 7 shows the FTIR spectrum of the analyzed galactomannans. The IR spectra of galactomannans show peaks at 815 and 872 cm^{-1} that 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 (Figueiró, Côes, Moreira, & Sombra, 2004; Prado, Kim, Özen, & Mauer, 2005; Yuen, Choi, Phillips, & Ma, 2009). The broad band between 1198 and 983 cm^{-1} results from the stretching vibration of C–O in C–O–H bonds (e.g. glycosidic bonds) and is related with the galactomannans' sugar composition. The peak

Table 4
Thermogravimetric data for *G. triacanthos* (GT), *C. pulcherrima* (CP) and *A. pavonina* (AP) galactomannan samples.

Sample	Peak	DTG ($^{\circ}\text{C}$)
GT	1	136.89
	2	309.81
CP	1	143.32
	2	321.73
AP	1	141.85
	2	320.62

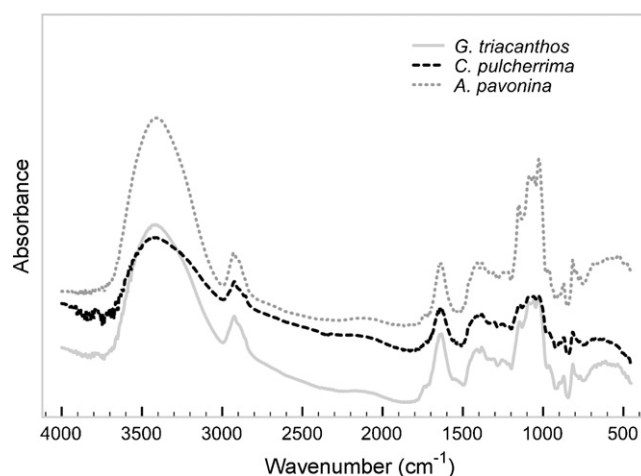


Fig. 7. FTIR spectra of the three analyzed galactomannans in the spectral region between 400 and 4000 cm^{-1} .

at 1152 cm^{-1} corresponds to bending vibrational modes of C–O, present in the pyranose ring, while the broad band between 1134 and 983 cm^{-1} is a characteristic contribution of C–OH bending (Figueiró et al., 2004; Prashanth et al., 2006). The broad bands ranging between 2800 – 3000 and 3100 – 3500 cm^{-1} are attributed to C–H stretching and to O–H stretching vibration, respectively (Yuen, Choi et al. 2009).

4. Conclusions

The structural and thermal properties of three galactomannans from non-traditional sources (seeds of *G. triacanthos*, *C. pulcherrima* and *A. pavonina*) were evaluated. These three galactomannans are characterized by a 1–4-linked mannose polymer with galactose side chains attached at the C6 position. Enzymatic hydrolysis with *endo*- β -mannanase followed by analyses by ESI-MS/MS indicates the presence of acetyl and pentose groups in the galactomannan of *G. triacanthos*. Thermal analyses have shown that the different galactomannan compositions, with different mannose content and different molecular weight, influence their thermal behaviour. A higher content of mannose leads to higher values of enthalpy change and T_g , and the enthalpy change for the second thermal transition is influenced by the viscosity average molecular weight of the samples.

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