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Physico-chemical properties of seed xyloglucans from different sources

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Abstract

A xyloglucan (XG) was extracted from the seeds of *Hymenaea courbaril* (Jatobá) harvested from trees growing at different locations in Brazil. They were from Foz do Chopin Forest Reserve, State of Paraná (HC-I), Cuiaba (HC-II) and Sinope/State of Mato Grosso (HC-III), Natal/State of Rio Grande do Norte (HC-IV), Fortaleza/State of Ceará (HC-V) and *Tamarindus indica* (TM) from Conceição de Almeida/State of Bahia, Brazil. It was submitted to SEC-MALLS analysis and its intrinsic viscosity determined. When a solution of XG was heated to 85 °C for 2 h, there was a reduction of aggregation and the XG from the seeds of *H. courbaril* had a higher value of C_∞ when compared with that of from *T. indica*, probably due to the presence of an unique oligossacharide series present in the *H. courbaril* samples (XXXXG). This gives rise to a more rigid molecule when compared with the XG from *T. indica*.

Keywords: Xyloglucan; Component oligosaccharides; *Hymenaea courbaril*; Aggregation

1. Introduction

Plant xyloglucans (XG) are polysaccharides with potential industrial applications. They are found in the primary cell walls of higher plants (dicotyledons and non-graminaceous monocotyledons) and in the cotyledon of some dicotyledonous seeds, where they function as storage polysaccharides (Reid, 1985). They have a cellulose-like (1→4)-linked β-glucan backbone to which single-unit α-D-Xyl*p* substituents are attached at O-6. Some Xyl*p* residues are further substituted at O-2 by β-D-Gal*p* (Hayashi, 1989).

A xyloglucan was first detected in seeds of *Hymenaea courbaril* by Buckeridge and Dietrich (1990) and Kooiman (1960). Its chemical structure was, subsequently determined by methylation analysis (Lima, Reicher, Corrêa, Ganter, & Sierakowski, 1993) and analysis of derived oligosaccharides (Lima, Rechia, Ganter, Reicher, & Sierakowski, 1995). It was found that the glycosidic linkages are practically the same as those of the XG from seeds of *Tamarindus indica*. In these studies, samples of seeds were collected from isolated populations in the State of São Paulo, Brazil.

Buckeridge, Rocha, Reid, and Dietrich (1992) examined the structure of the xyloglucan (XG) from seeds of *H. courbaril* using a cellulose-limit digest enzyme to give oligosaccharides. In 1997, they carried out a sequential enzymatic digestion with β-glucosidase and oligosaccharide specific α-xylosidase, both from nasturtium, and detected the presence of a new family of xyloglucan oligosaccharides (XXXXG) (Buckeridge, Crombie, Mendes, Reid, Gidley, & Vieira, 1997). This family of oligosaccharides (XXXXG), but with a galactosyl substituent was obtained and identified by Tiné, Lima, and Buckeridge (2003).

Other studies were performed in which the XGs from *H. courbaril* seeds were obtained from different locations in Brazil and their structure and properties determined (De Lima & Buckeridge, 2003; Freitas, Gorin, Neves, & Sierakowski, 2003; Freitas, Martin, Paula, Feitosa, & Sierakowski, 2004; Lima et al., 2003; Martin, Freitas, Obayashi, & Sierakowski, 2003; Martin, Souza-lima, Gorin, Reicher, & Sierakowski, 2000; Tiné, Cortelazzo, & Buckeridge, 2000; Tiné et al., 2003; Vargas-Rechia et al., 1998).

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Studies have also been carried out on xyloglucans isolated from seeds of *T. indica*. Taylor and Atkins (1985) using X-ray diffraction, observed a repeating lattice spacing of 2.06 nm arising from a group of four glucose residues. Gidley et al. (1991) obtained by light scattering measurements the molecular masses, R_g, R_h and C_∞ that were 8.8×10^5 g mol^{-1}, 110, 71 and 110 nm, respectively. Lang and Burchard (1993) used static and dynamic light scattering methodology to study the random side chain aggregation behaviour of single chains. This type of aggregation is to form bundle-shaped particles and causes a remarkable degree of particle stiffness. Such a behaviour was related by Lang and Kajiwara (1993), who observed multi stranded aggregates in aqueous solution. For the xyloglucan from *Detarium senegalense*, Wang Ellis, Ross-Murphy, and Grant Reid (1996) and Wang, Ellis, Ross-Murphy, and Burchard (1997) found similar oligosaccharide sequences as those in *T. indica* xyloglucan and observed the presence of lateral branching. Picout, Ross-Murphy, Errington, and Harding (2003) enhanced the solubilization of the xyloglucans obtained from seeds of *D. senegalense* and *T. indica* and observed that increased pressure and heating substantially reduced molecular aggregation.

Multiangle laser light scattering (MALLS) has been used to observe aggregation in other polysaccharides such as galactomannans (Cheng, Brown, & Prud'Homme, 2002; Robinson, Ross-Murphy, & Morris, 1982), linear α-glucans (Roger, Axelos, & Colona, 2000), and polygalacturonic acid (Alonso-Mougán, Fraga, Meijide, Rodriguez-Núñez & Vázquez-Tato, 2003).

The size exclusion chromatography (SEC) coupled to MALLS has several advantages when compared with the static light scattering detector used alone in the study of macromolecular behaviour, such as faster analyses and the use of only one solution. Errors in measurement are usually considered to be ±3–5% for M_w and ±5–10% for R_{gw}. Another advantage is that complete molecular weight distribution can be obtained (Roger, Axelos & Colonna, 2000). Parallel refractometer detection (RI) is excellent in size exclusion chromatography (SEC). Such a combination of techniques is very sensitive for detection of aggregates in polymer solutions (Wittgren & Porsch, 2002).

Although the chemical structures of seed galactomannans and xyloglucans are well known, studies on their variation in plant material from different sources (or populations) are rare. As examples are the study for galactomannans from *Leucaena leucocephala* (Buckeridge, Panegassi, Rocha, & Dietrich, 1995) and for XG from seeds of *Copaifera langsdorffii* from populations of forest and savanna (Buckeridge et al., 1992). In the latter study, the authors found important differences in the fine structure of the xyloglucans from the two biomes and proposed that these differences might be related to possible environmental effects during polysaccharide biosynthesis.

We now carry out a fine structural analysis on oligosaccharides derived from xyloglucan from seeds obtained from different regions in Brazil, namely: (Cuiabá and Sinope/State of Mato Grosso, Fortaleza/State of Ceará, Natal/State of Rio Grande do Norte, Foz do Chopin Forest Reserve, State of Paraná). We examined the macromolecular behaviour of the xyloglucans from *H. courbaril* and the results were compared with that from *T. indica*.

2. Experimental

2.1. Plant material

Seeds of *H. courbaril* L. were harvested from trees growing in the Foz do Chopin Forest Reserve, State of Paraná (HC-I), Cuiaba (HC-II) and Sinope/State of Mato Grosso (HC-III), Natal/State of Rio Grande do Norte (HC-IV), Fortaleza/State of Ceará (HC-V). Seeds of *T. indica* (TM) were from a commercial source, Conceição de Almeida /State of Bahia, Brazil).

2.2. Extraction of seeds and isolation of xyloglucans

Xyloglucans (XGs) were obtained by exhaustive aqueous extraction at 25 °C of pooled and milled seeds (40 g L^{-1} for each extraction process). Each viscous extract was centrifuged at $10{,}000\times g$ for 20 min and the supernatant passed sequentially through Millipore filter membranes with pore sizes of 3 and 0.8 μm. The purified polymer was obtained after precipitation with two volumes of 96% ethanol and washed with acetone (Freitas et al., 2003).

2.3. Chemical analyses

Total carbohydrate was assayed by the phenol–H_2SO_4 method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956), protein by that of Hartree (1972), and moisture by loss of water in a thermogravimetric analysis from 25 to 200 °C carried out at 10 °C min^{-1}.

Monosaccharide contents of XGs were determined after partial acid hydrolysis with 72% H_2SO_4 (w/w) for 1 h on ice, and complete hydrolysis effected by dilution to 8% and heating at 100 °C for 4 h (Selvendran, March, & Ring, 1979). The solutions were neutralised with $BaCO_3$, filtered and the filtrate from each hydrolysis reduced with $NaBH_4$, followed by acetylation with pyridine–Ac_2O (1:1 v/v) for 12 h at 25 °C (Wolfrom & Thompson, 1963). The resulting alditol–acetates were analysed by GC-MS Varian and detected with a Saturn 2000R mass spectrometer, using a DB-225 capillary column at 220 °C with nitrogen as the carrier gas.

2.4. Size exclusion chromatography (SEC)—multiangle laser-light scattering (MALLS)

To calculate the molecular mass, the ratios of the refractive index and concentration (dn/dc) of the XGs (concentrations of 1.0, 0.5, 0.25 and 0.125 g L^{-1},

filtered through Millipore filter 0.45 μm) a Waters 2410 differential refractometer at wave length of 546 nm was used.

For SEC analysis, aqueous solutions of XG (0.5 g L^{-1}) were filtered through a Millipore filter (0.22 μm) and injected in a series 2000, 500, 250 and 120 Waters' ultrahydrogel columns. Detection was carried out with a Waters 2410 differential refractometer and a light scattering multiangle at 632.8 nm (DAWN DSP-F Wyatt technology model). The eluent used in this system was 0.1 mol L^{-1} sodium nitrite containing 200 ppm of sodium azide at a flux of 0.6 ml min^{-1}.

The analyses mentioned above were also performed with the samples heated at 85 °C for 2 h in a sealed tube, and after cooling to 25 °C the samples were injected in to the SEC. The heating was also maintained for 4, 6, 8 and 24 h for the same sample.

The critical concentration referring to the limit between the semi-diluted and diluted concentrations, was determined by means of an equation, namely

$$c* = M_w/4/3\pi \times N_a \times R_g^3 \quad (1)$$

where

M_w weight average molecular weight (g/mol);
N_a Avogadro number and
R_g gyration radius (nm)

Knowing the radius of gyration and M_w from SEC-MALLS, and working in concentration lower than the c^*, was possible to predict the intrinsic viscosity from the Flory–Fox equation (Flory, 1953)

$$[\eta] = \Phi_0 6^{3/2} R_{gw}^3/M_w \quad (2)$$

where

Φ_0 viscosity parameter
R_{gw} radius of gyration (nm) and
M_w weight average molecular weight (g/mol)

R_{gw} according to Gidley et al. (1991) is influenced by the excluded volume and the expansion factor was calculated using the equation

$$[\eta] = \Phi_{(\varepsilon)} 6^{3/2} R_{gw}^3/M_w \quad (3)$$

$$\Phi_{(\varepsilon)} = \Phi_0(1 - 2.63\varepsilon - 2.86\varepsilon^2) \quad (4)$$

$$\varepsilon = 2\alpha - 1 \quad (5)$$

where α is the angular fit between the R_g versus molecular mass (M_w).

The value of α was also confirmed using the Mark–Houwink–Sakurada exponent (a) (double logarithmic plot of the intrinsic viscosity against M_w). The angular fit (a) gave the exponent of Mark–Houwink–Sakurada and can be used to calculate the α value. Using these different approaches, the values of (α) are identical (data not shown).

To obtain the characteristic ratio (C_∞) and persistence length (L_p) was used a method using the R_g and M_w in Eq. (6) is in accord with Roger et al. (2000), where C_∞ is

$$C_\infty = \lim_{(n\to\infty)} L_p/n_w l^2 \quad (6)$$

where

L_p mean square end to end distance;
L length of the rods and
n_w M_w/m_0 = degree of polymerization

For a random coil molecule the value of L is proportional to:

$$L = 6R_{gw}^2 \quad (7)$$

The characteristic ratio can be obtained using the following equation:

$$C_\infty = 6R_{gw}^2/n_w l^2 = 6R_{gw}^2 m_0/M_w l^2 \quad (8)$$

Roger et al. (2000) proposed a methodology to obtain graphically the value of C_∞ using the relation:**

$$R_g^2/M_w = C_\infty l^2/6m_0(1 + cIM_w^{1/2}) \quad (9)$$

The linear fit of the R_{gw}^2/M_w variation as a function of $M_w^{1/2}$ gives the linear coefficient C_∞ l^2/m_0. Using a monomeric length (l) of 0.52 nm and m_0 of 360 was possible determine the value of C_∞. Another form to obtain the value of the C_∞ is by the use of the Stockmayer–Fixman plot. This approach showed a good correlation with the Roger plot (data not shown).

The values of characteristic ratio were possible to estimate the value of persistence length, by the equation:

$$C_\infty = 2(L_p/l) - 1 \quad (10)$$

l length of rods
L_p persistence length

2.5. Oligosaccharide composition

XG samples were treated with *Trichoderma viride* 'cellulase' from Megazyme, Australia for 32 h at 30 °C at pH 5,0. The products of the enzymolyses were analysed by High Performance Ion Exchange Chromatography (HPAEC) Dionex system DX500, using a Carbopack PA-1 column and an amperometric pulse detector (PAD). The eluent used was NaOH 88 mmol L^{-1} with a gradient of NaOAc 0.5 mol L^{-1} from 7 to 15%.

2.6. Rheology

The XGs (2 g L^{-1}) were solubilised for 16 h in 0.1 mol L^{-1} sodium nitrite containing 200 ppm of azide, at 25 °C. After this, the sample was heated for 2 h at 85 °C, in a sealed tube to avoid evaporation. The analysis was performed before and after heating, at a Brookfield rheometer, model LV DV-III, cone–plate, spindle cp-40, and the temperature was controlled using a Brookfield TC500 batch at 25 °C. Viscosity measurements were also determined using a capillary viscometer.

The Huggins equation showed the intrinsic viscosity $[\eta]$ by the extrapolation of (reduced viscosity) η_{red} to the limit of zero concentration ($c \rightarrow 0$) where the linear coefficient is represented by $[\eta]$.

3. Results and discussion

3.1. Chemical analysis

XG samples obtained from seeds of *H. courbaril* harvested in different locations in Brazil were structurally compared with each other and that prepared from *T. indica* seeds. Such a comparative study is relevant because any difference in its primary structure could possibly give rise to a distinct macromolecular behaviour in solution.

All the XGs were extracted from seeds with cold water, the solution centrifuged, and the supernatant filtrate successively through 3 and 0.8 μm Millipore filters gave an yield of ~17%, related to seeds. Table 1 shows the protein, carbohydrate, moisture content and specific optical rotation of each XG. The results show that the protein and carbohydrate levels are only a little different. However, it is noteworthy that the specific rotation of the XG obtained from seeds in Natal (HC-IV) is significantly more positive, showing a higher proportion of α-D- and/or α-L-linkages.

The monossacharide contents of the XGs are shown in Table 2, each containing significant amounts of glucose, xylose, and galactose and a smaller one of arabinose.

Table 1
Total carbohydrate, protein, and moisture contents and specific rotations of XGs, obtained from seed of *T. indica* and *H. courbaril* grown in different Brazilian locations

Samples	% (m/v)			$[\alpha]_d^{20}$
	Total sugar[a]	Protein[b]	Moisture[c]	
HC-I	85	2.9	10.8	80.1
HC-II	86	2.9	7.3	77.8
HC-III	85	2.2	9.2	75.5
HC-IV	88	2.4	7.0	97.8
HC-V	90	2.6	8.9	78.4
TM	84	3.0	10.5	80,0

[a] Method of Dubois et al. (1956).
[b] Method of Hartree (1972).
[c] Thermogravimetric method.

Table 2
Monosaccharide composition[a] of xyloglucans

Sample	Monosacharides (mol%)					
	Glc	Xyl	Gal	Man	Ara	Fuc
HC-I	43.0	36.3	16.0	–	4.8	–
HC-II	39.0	42.0	16.8		2.3	
HC-III	44.1	38.6	16.1		1.2	
HC-IV	49.0	32.0	14.0	2.1	1.3	1.3
HC-V	45.0	37.3	16.2		1.5	
TM	38.0	35.5	22.0		4.7	

[a] By GC of derived alditol acetates, column DB-225 at 220 °C.

However, in the case of HC-IV, mannose and fucose were also present and these components could be an integral part of the polysaccharide as it is homogeneous by SEC-MALLS (data not shown). The *T. indica* XG and that represented by HC-I have a four-time higher level of arabinose when compared with the other samples. In order to further elaborate the fine structure of the XGs, including that of *T. indica* seeds, we carried out a comparative analysis of oligosaccharides liberated on digestion with a celulase/endoglucanase (Megazyme-Austrália), following the procedure of Buckeridge et al. (1997) and Tiné et al. (2003). The relative proportions of the oligosaccharides were also determined in Fig. 1(a) typical elution profile of HPAEC analysis of the enzymic product is shown (in this case HC-I) is shown.

Apart from the already known differences found between XGs from *H. courbaril* and *T. indica* (Buckeridge et al., 1997), the different samples from the former polymer showed similar structures, when probed by this oligosaccharide analysis. According the nomenclature proposed by Fry et al. (1993), oligosaccharides identified were XXG, and XXXG, XXLG, XLLG, herein named XXXG series together with the apparently exclusive XXXXG series, that correspond to the oligosaccharides XXXXG, XXXLG, XLXXG and XXLXG. In Table 3, it can be observed that the relative proportions among the oligosaccharides from *H. courbaril* and *T. indica* XGs have a difference of 7–13% in the ratio XXLG/XLXG. Another difference was the reduced content of XXG pentasaccharide in the sample

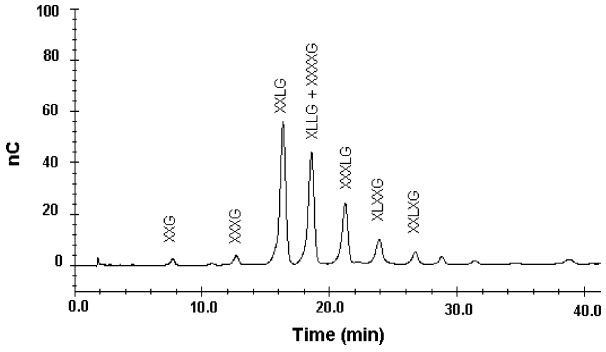

Fig. 1. Elution profile of the limit digest oligosaccharides obtained by cellulase digestion of the xyloglucan obtained from Foz Chopim (HC-I) reservation.

Table 3
Relative proportion of oligosaccharides obtained by enzymatic hydrolysis with cellulase of XGs from seeds of *H. courbaril* and *T. indica*, as determined by High Performance Anion Exchange Chromatography

Oligosaccharides	Samples					
	HC-I	HC-II	HC-III	HC-IV	HC-V	TM[a]
XXG	1.4	1.2	1.5	0.6	1.1	Traces
XXXG	2.1	2.6	1.9	2.3	2.4	24.4
XLXG	Traces	Traces	Traces	Traces	Traces	8.0
XXLG	38.5	41.5	42.7	44.3	41.9	36.0
XLLG+ XXXXG	30.5	30.3	25.8	27.6	29.6	–
XLLG	–	–	–	–	–	31.6
XXXLG	16.7	14.5	14.5	15.5	14.5	–
XLXXG	7.7	7.5	7.4	7.3	8.4	–
XXLXG	3.2	2.3	3.2	2.3	2.2	–

[a] Data from Tine et al. (2003).

Table 4
Critical concentration (c^* g L^{-1}) values for XGs

Sample	Using M_w and R_w	Rheology
HC-I	0.9	0.7
HC-II	0.6	0.7
HC-III	0.9	1.3
HC-IV	1.1	1.3
HC-V	0.8	0.8
TM	0.9	1.0

derived from HC-IV (50%), but with a much higher proportion of XXG found in *H. courbaril* in relation to that in the *T. indica* sample. These observations strongly suggest that the distribution of terminal galactose units in the branches along the main β-glucan chain is quite different in the two polymers. Whereas in Tamarind it appears to be more concentrated to certain regions, denoted by the concentration of galactoses in only four or five oligosaccharides, from the *H. courbaril*, XG, ~10 different oligosaccharides were detected and the proportion of the doubly-branched (XLLG) oligosaccharides was proportionally lower.

As it is likely that these small variations in fine structure could generate changes in the hydrodynamic behaviour of the XGs in solution, they were examined by physicochemical methods. Our hypothesis is that the presence of the XXXXG series in the XG from *H. courbaril* seeds might give rise to distinct properties and these could help us to better understand their behaviour in solution. Thus, comparative rheology studies on solutions of XGs from different sources was performed, by determining their intrinsic viscosity and also show their molar mass by SEC-MALLS/RI.

3.2. Rheological studies

The determination of the critical concentration (c^*) was important due to the possibility of analysing macromolecular behaviour in a dilute solution. This is necessary as polymer–polymer interaction is not significant, so that it does not need to be considered in the system.

Concentration dependence was determined in two different systems. In the semi-diluted concentration, it was $c^{1.56}$, and at the diluted concentrations it was $c^{1.1}$. By plotting log η_{sp} as a function of the coil overlap parameter $c[\eta]$ (data not shown), it was possible also to determine the critical rheological concentration. These values and those obtained from M_w and R_{gw} from Eq. (1) are shown in Table 4.

The critical concentration (c^*) obtained from light scattering and rheological analyses are very similar. These limits between the semi-diluted and diluted solutions were used to carry out GPC and intrinsic viscosity determinations. So, in order to determine intrinsic viscosity by rheology or SEC-MALLS, we only used concentrations of up to c^* (Tables 5 and 6).

The intrinsic viscosity determined by analysis performed using a Brookfield rheometer gave the same absolute value as in the capillary determination (comparative data not shown).

In Table 5, it is possible to observe values for M_w, M_n, M_w/M_n, R_{gw}, R_{gn}, $[\eta]_w$, $[\eta]_n$ and $[\eta]_{Rheology}$ for the six samples of XGs.

The Flory–Fox theory was developed for a monodisperse polymer, and this can generate higher values of intrinsic viscosity when mathematically approached, compared with the rheological data. The discrepancy between the values for $[\eta]_w$ and $[\eta]_{Rheology}$ may be due to polydispersity and excluded volume that influence the radius of gyration and draining effects that affect the parameter Φ (Gidley et al., 1991). Another important observation is that aggregation can generate a lowering influence on the value of intrinsic viscosity determined by rheology (Wang, Wood, Cui, & Ross-Murphy, 2001). So reduction in the aggregation must theoretically approximate the values of $[\eta]$ obtained from SEC-MALLS and rheology (Table 6).

Table 5
Molecular mass (g mol^{-1}), radius of gyration (nm) and the $[\eta]$ (mL g^{-1}) values for XGs

Sample	M_w	M_n	M_w/M_n	R_w	R_n	$[\eta]_w$	$[\eta]_n$	$[\eta]_{rheology}$
HC-I	1.5×10^6	8.7×10^5	1.7	87	69.2	1780	1515	740
HC-II	2.2×10^6	1.5×10^6	1.4	110.4	93.7	2626	2322	1054
HC-III	1.05×10^6	6.2×10^5	1.7	76.4	60.2	1329	1105	680
HC-IV	1.4×10^6	9.0×10^5	1.6	88.8	69.5	1719	1320	710
HC-V	8.5×10^5	5.3×10^6	1.6	67.2	53.6	1346	1090	886
TM	1.1×10^6	7.2×10^5	1.5	68.6	55.4	1233	990	522

Table 6
Molecular mass (g mol^{-1}), radius of gyration (nm) and the [η] (mL g^{-1}) values for XGs after heating solutions at 85 °C for 2 h

Sample	M_w	M_n	M_w/M_n	R_w	R_n	$[\eta]_w$	$[\eta]_n$	$[\eta]_{rheology}$
HC-I	8.7×10^5	5.4×10^5	1.6	60.2	46.7	1001	747	750
HC-II	1.5×10^6	1.0×10^6	1.4	85.4	70.8	1143	947	994
HC-III	8.0×10^5	4.7×10^5	1.7	60.9	44.1	877	573	605
HC-IV	9.4×10^5	5.6×10^5	1.6	65.6	45.9	726	418	487
HC-V	6.0×10^5	3.9×10^5	1.5	52.0	42.0	870	709	860
TM	9.3×10^5	6.2×10^5	1.5	57.4	44.7	825	580	418

The supramolecular aggregation of water-soluble polysaccharides in aqueous solution is a critical barrier to the accurate characterisation of their molecular properties when the on-line light scattering technique is used. To minimise aggregation in water, heating and increased pressure treatments can be used in order to fully disperse the polymer molecules. A prerequisite for these methods is that the polymer does not undergo depolymerisation through covalent bond scission (Wang et al., 2001). To evaluate the aggregation of seed XGs from *H. courbaril* they were studied in terms of their molar mass, R_{gw} and [η] after 16 h of constant agitation at 25 °C and a period of 2 h at 85 °C under constant stirring. In Fig. 2, the results for the sample of HC-I, a change in the elution of peak occurred after the heating, to higher elution volumes, but without altering the lower molar masses in the system. This indicates a reduction in the molar mass of the molecule but without generating new polymers with lowers mass. The same behaviour has been observed by Wang et al. (2001), who studied the XG of *D. senegalense*.

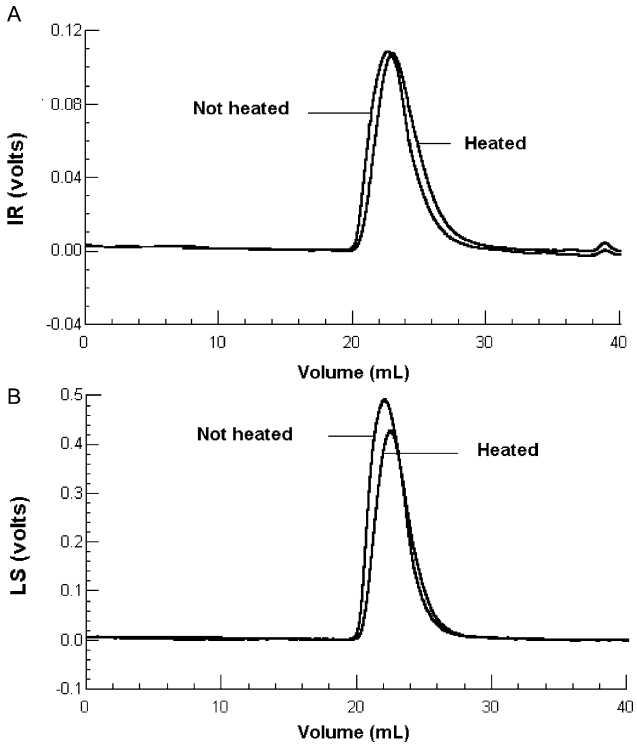

Fig. 2. SEC elution profile of HC-I using detector IR (A) and LS (B) (MALLS) at 90°.

After calculation of its molar mass, we observed that HC-I had a reduction of molar mass of ~41% and a gyration ratio of ~31%, but without change in M_w/M_n.

If the aggregation of stretched chains is considered, it is likely that a stiff rod has been formed and the radius of gyration does not increase with increasing molar mass. So, an increase in M_w occurs, but not in the same proportion as R_{gw}, indicating a stronger aggregation in the central zone than on the ends and side chains (Alonso-Mougán et al., 2003).

We have also observed a large reduction in R_{gw}, which indicates the existence of side-chain aggregation. A similar behaviour was observed for the other XG samples from *H. courbaril*. The intrinsic viscosity was reduced by ~35% for HC-III and HC-IV, and a maximum of 56% reduction was found for HC-II and HC-V. These data indicate that on heating, a dissociation process apparently occurs in solution, giving rise to a structure with a lower molecular mass, R_{gw} and [η]. This confirms the observations of side chain aggregation by Lang and Burchard (1993) and Lang and Kajiwara (1993) for xyloglucan from *T. indica* seeds and of Wang et al. (1997, 2001) for *D. senegalense* seeds.

Using the Roger plot and the values of M_w and R_{gw} of all the XGs solubilised at 25 °C for 16 h, we obtained from the linear intercept in (Fig. 3) the value of $5.15\times10^{-3}\pm0.63\times10^{-3}$ that generates a value to C_∞ and persistence lengths of 41 and 11, respectively. But, after heating at 85 °C for 2 h the samples had a value of C_∞ of 31 and persistence length of 8.4 nm.

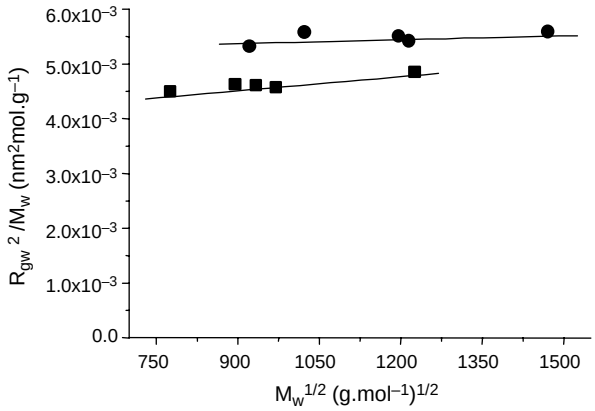

Fig. 3. Plot of R_g^2/M_w as a function of the $M_w^{1/2}$ for the xyloglucans from *H. courbaril* seeds solubilised at 25 °C for 16 h (●) and after solubilisation at 25 °C for 16 h were heated at 85 °C for 2 h (■).

Table 7
Characterisitc ratio (C_∞) and persistence length (L_p) values for XG using the Roger's plot

Sample	Characteristic ratio (C_∞)	Persistence length (L_p) nm
HC-I	33.5±0.9	8.9±0.3
HC-II	41.2±0.8	10.9±0.2
HC-III	37.5±1.0	10.1±0.2
HC-IV	54.4±4.4	14.5±1.0
HC-V	31.1±3.2	8.4±0.8
TM	21.8±1.1	6.2±0.2

Table 8
Characterisitc ratio (C_∞) and persistence length (L_p) value for XG after heating solutions at 85 °C for 2 h, using the Roger's plot

Sample	Characteristic ratio (C_∞)	Persistence length (L_p) nm
HC-I	23.2±1.4	6.5±0.3
HC-II	27.2±1.3	7.5±0.2
HC-III	25.5±3.8	7.1±0.2
HC-IV	43.2±1.3	11.7±1.0
HC-V	24.8±3.0	6.9±0.8
TM	15.2±1.0	4.4±0.8

The characteristic ratio and the persistence length of each XG sample were obtained using a fraction of M_w and R_g obtained from the Zimm linear interpolation ($Kc/R\theta$ by the $\sin^2\theta/2$) at different elution volumes of 19.8, 20.4, 21, 21.6, 22.2, 22.8, 23.4, 24, 24.6 and 25.2 mL (Beer, Wood, & Weisz, 1999; Wang et al., 2001). The aggregation in the samples was determined by measuring the values of C_∞ and persistence length before and after heating the solution to 85 °C for 2 h. If aggregation were present, these values would be theoretically lower than that of the unheated solution. The results obtained are shown in Tables 7 and 8, where it is possible to observe a reduction in the characteristic ratio and persistence length of 34% for the samples of XGs from *H. courbaril* and of 30% for the *T. indica* XG. The decrease in C_∞ after heating treatment indicates dissociation of the molecular aggregates, thus improving the description of molecular characteristics (Wang et al., 2001). The differences between the values of the characteristic ratio and persistence length for XGs, obtained from *H. courbaril* and from *T. indica*, can arise due to the primary structure of these molecules, which gives rise to a more rigid XG molecule of *H. courbaril*. This is supported by the presence of the XXXXG series of derived oligosaccharides. So, the increase of one unit of glucose substituted by xylose increases the persistence length by 1.5–1.6 times, showing that the difference in the primary (fine) structure can alter the hydrodynamic behaviour. Such differences among the XGs from *H. courbaril*, mainly in sample HC-IV, must be due the reduced level of XXG (50%), that would theoretically reduce the number of points at which the structure is mobile (non-substituted glucose unit), forming an even more rigid molecule. The L_p value for tamarind XG after heating was practically the same as that obtained by Picout et al. (2003), showing a good correlation between the Roger and Stockmayer–Fixman plot.

4. Conclusions

We found that XGs of *H. courbaril* seeds, obtained from different sources, did not have significant differences in oligosaccharides formed on enzymolysis, but different from those from tamarind XG. The possible consequences of such variations for biological and industrial applications may be important.

The XG from *H. courbaril* seeds formed aggregates in aqueous solution, but after heating at 85 °C for 2 h, a reduction in molar mass, viscosity and R_{gw} occurred. Calculation of C_∞ and persistence length (L_p) values revealed that heating reduces both parameters, confirming the dissociation of the XG aggregates.

These properties appear to be directly related to the distribution of xylose side chains, linked to the main chain of the polymer, which results in a more widely spaced distribution of galactose nonreducing end-units.

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