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Degalatosylation of xyloglucan: Effect on aggregation and conformation, as determined by time dependent static light scattering, HPSEC–MALLS and viscosimetry

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ABSTRACT

Degalactosylation of xyloglucan from the seeds of *Hymenaea courbaril* by β-galactosidase, which strips the (1 → 2)β-D-galactose side groups, was monitored in real time using time dependent static light scattering (TDSLS), viscosimetry and HPSEC–MALLS. The galactose side-chain stripping rate constant (α) was determined by TDSLS as 1.3×10^{-6} s^{-1} and the scattering at 90° showed a little upwards curvature, indicating a high fraction of mass in the backbone (f_p 0.78). There was an increase in aggregation during degalactosylation, especially during later stages, resulting in a microgel at dilute concentrations. L_p was determined using viscosimetric techniques in order to avoid the influence of aggregates. A value of approximately 4 nm was obtained, both before and after enzymatic hydrolysis. The results confirm that aggregation is the principal phenomenon responsible for gelling of degalactosylated xyloglucan and that the conformation is practically unaffected during the enzymatic treatment.

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1. Introduction

Xyloglucans are the main glycans that interlace cellulose microfibrils in most flowering plants (Carpita & Gibeaut, 1993; Fry, 1989; McNeil, Darvill, & Fry, 1984). Besides being a structural component of primary cell walls, they play important roles in the control of cell expansion and as a reserve of carbon in the seeds of many dicotyledons (Buckeridge, Santos, & Tiné, 2000; Fry, 1989). Seed xyloglucans have a cellulose-like (1 → 4)-linked β-D-glucan main-chain, substituted at *O*-6 by single-unit α-D-xylopyranose (Xyl*p*) side-chains. Some of them are further substituted at *O*-2 by β-D-galactopyranose (Gal*p*). These seed xyloglucans have a large number of commercial and industrial applications, especially those obtained from seeds of *Tamarindus indica* (Gidley et al., 1991; Picout, Ross-Murphy, Errington, & Harding, 2003; Rao & Srivastava, 1973). They are widely used as food and cosmetic additives, where they act as thickeners and stabilizing agents (Maeda, Yamashita, & Morita, 2007; Petkowicz, Vargas-Rechia, Busato, & Richer, 2005; Yamatoya & Shirakawa, 2003).

Although native xyloglucans do not form gels, Miyazaki et al. (1998) demonstrated that the partial degradation of the xyloglucan of the seeds of *T. indica* by β-D-galactosidase led to the formation of a hydrogel that is thermally reversible in dilute aqueous solutions. At high degrees of degalactosylation, the gel has a significantly higher storage modulus than that of other thermo-reversible hydrogels (Brun-Graeppi et al., 2010; Busato, Reicher, Domingues, & Silveira, 2009; Nisbet et al., 2006; Shirakawa, Yamatoya, & Nishinari, 1998), since the (1 → 2)β-D-galactose side groups interfere with the interactions between the gel-forming main chains. As a resulting of this gelling property, xyloglucan has potential biomedical applications as a component of oral, ocular, transdermal and rectal drug delivery systems and as a matrix for hepatocyte attachment (Burgalassi, Chetoni, Panichi, Boldrini, & Saettone, 2000; Coviello, Matricardi, Marianecci, & Alhaique, 2007; Kawasaki et al., 1999; Miyazaki et al., 1998; Miyazaki, Kawasaki, Endo, & Attwood, 2001a; Miyazaki, Kawasaki, Shirakawa, et al., 2001b; Miyazaki et al., 2003; Seo et al., 2004; Strickland et al., 2001; Suisha et al., 1998; Takahashi et al., 2002; Yamatoya & Shirakawa, 2003).

The seeds of *Hymenaea courbaril* (jatobá), which is found abundantly throughout Brazilian forests, could be an additional source of xyloglucan for biotechnological applications. The structural, physical and biological properties of the xyloglucan from these seeds have been extensively studied (Alcântara, Martin, Silva, Dietrich, & Buckeridge, 2006; Buckeridge et al., 1997; dos Santos, Purgatto, Mercier, & Buckeridge, 2004; Freitas, Gorin, Neves, & Sierakowski, 2003; Freitas et al., 2005; Lima & Buckeridge, 2001; Lima, Reicher, Corrêa, Ganter, & Sierakowski, 1993; Lima, Rechia, Ganter, Reicher, & Sierakowski, 1995; Lima-Nishimura et al., 2003;

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Lucyszyn et al., 2009; Martin, Freitas, Obayashi, & Sierakowski, 2003; Martin, Souza-Lima, Gorin, Reicher, and Sierakowski (2000); Ribeiro, Arizaga, Wypych, & Sierakowski, 2009; Tiné, Silva, Lima, Carpita, & Buckeridge, 2006; Vargas-Rechia et al., 1998). In our own work, we used nondestructive oscillatory measurements to show that the partial degalactosylation of xyloglucan from *H. courbaril* seeds with β-D-galactosidase led to the formation of a network structure like a gel (Busato et al., 2009). This contrasts with the native xyloglucan, which forms a viscoelastic solution. Temperature sweeps confirmed that the enzymatically modified xyloglucan is a thermally responsive hydrogel.

In order to characterize better the potential for use of the degalactosylated xyloglucan of *H. courbaril* in industrial applications, it is necessary to determine how the enzymatic treatment influences its molecular weight and chain conformation (Burgalassi et al., 2000). Time dependent static light scattering (TDSLS) is a useful tool for this investigation, since it can be used to monitor changes in light scattering intensity and angular distribution on the time scale of the degalactosylation process. It yields comprehensive, model-independent information about monomer conversion, polymer degradation, molecular weights, intrinsic viscosities, average composition drifts and distributions, conductivity and chemical modification (Alb, Mignard, Drenski, & Reed, 2004; Drenski & Reed, 2004; Sorci & Reed, 2002).

In the current work we undertook a real time analysis, using TDSLS and viscosimetry, in order to evaluate the macromolecular and hydrodynamic properties of the xyloglucan from seeds of *H. courbaril* during enzymatic degalactosylation. The aim was to determine whether the gelling properties of degalactosylated xyloglucan are due to macromolecular aggregation and, if so, whether a conformational change of the xyloglucan, from random coil to rigid rod, is necessary in order for macromolecule aggregation to occur.

2. Experimental

2.1. General methods

Total carbohydrate was determined by the phenol-sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956), using glucose as the standard, and protein content by that of Hartree (1972), using BSA as the standard. Monosaccharide composition was determined by gas–liquid chromatography (GLC) as described by Busato et al. (2009) and confirmed by ^{13}C NMR. ^{13}C NMR spectra were obtained using a 400 MHz Bruker model DRX Avance spectrometer at 100 MHz in the Fourier transform mode, with complete proton decoupling at 70 °C, using DMSO-d_6 as solvent, in a tube of 0.5 cm i.d. The spectral width was 200 ppm, chemical shifts being reported as δ (ppm) relative to the resonance of DDS (sodium 4,4-dimethyl-4-silopentano-1-sulphonate) as the internal standard ($\delta = 0$).

2.2. Plant material and polysaccharide isolation

Seeds from *H. courbaril* (jatobá) were acquired from IPEF (Instituto de Pesquisas e Estudos Florestais), State of São Paulo, Brazil. The seeds were boiled in water for 1 h to inactivate enzymes and then held in the water at 4 °C until swelling took place. They were then dehulled and the cotyledons milled and defatted with toluene:ethanol (2:1, v/v) in a Soxhlet extractor. The material was dried and submitted to aqueous extraction at 25 °C for 1 h. After centrifugation (10,000 × *g*, 20 min), the extract was added to ethanol; the precipitate was washed twice with a gradient of ethanol (80%, 90%, and 100%) and then dried under vacuum at 25 °C. The polysaccharide obtained was named "Fraction JN".

2.3. Enzymatic hydrolysis of xyloglucan

Fraction JN (containing 1 mg/mL xyloglucan from *H. courbaril* seeds) was filtered sequentially through 0.8, 0.45 and 0.22 μm cellulose ester filters (Millipore). β-galactosidase from *Aspergillus niger* (MEGAZYME) was then added at a concentration of 0.8 U/mL and the sample was incubated for 24 h at 50 °C and pH 6.8. The contents of the reactor were recirculated through the TDSLS equipment, as described below. The degalactosylated xyloglucan sample was named fraction JH.

2.4. Real time monitoring of xyloglucan degalactosylation

TDSLS and multi angle laser light scattering (MALLS) measurements were done according to Zimm (1948), whose equation, at low concentrations and for $q^2\langle S^2\rangle_z \ll 1$, is

$$\frac{Kc}{I_R(q,c)} = \frac{1}{M_w}\left(1 + \frac{q^2\langle S^2\rangle_z}{3}\right) + 2A_2c \quad (1)$$

where $I_R(q,c)$ is the excess Rayleigh scattering ratio (cm^{-1}) at scattering vector q (nm^{-1}) and polymer concentration c (g/cm^3). M_w and A_2 are the weight-average molar mass and second virial coefficient, respectively. $\langle S^2\rangle_z$ is the z average of the square of the radius of gyration (R_g). $q = (4\pi n/\lambda)\sin(\theta/2)$, where θ is the scattering angle, n is the index of refraction of the solvent and λ is the vacuum wavelength of the incident light (632 nm). In Eq. (1), K is an optical constant, given for vertically polarized incident light by:

$$K = \frac{4\pi^2 n^2 (dn/dc)^2}{N_A\lambda^4} \quad (2)$$

where N_A is Avogadro's number and dn/dc is the differential refractive index increment of the solvent–solute solution with respect to change in solute concentration. The dn/dc of xyloglucan was determined using a Waters differential refractometer model 2410 at a wavelength of 546 nm, with at least 5 concentrations, between 1 and 0.1 mg/mL (filtered by Millipore filter 0.45 μm). The values of dn/dc were determined as 0.134 and 0.184 mL/g, respectively, for the xyloglucans in fractions JN and JH, using 0.1 M $NaNO_2$ as the solvent at 25 °C. These dn/dc values were used for the weight-average molar mass calculation. Regarding dn/dc, the values cited were referent to the technique mentioned above and different concentration detectors or methods of sample preparation could give a wide variation or results, as previously reported in the literature by Christensen et al. (2001).

In order to calculate the persistence length (L_p), the radius of gyration (R_g) was first obtained from the intrinsic viscosity [η] (determined by viscosimetry), using the Flory-Fox Equation (1953):

$$R_g^3 = \frac{[\eta]M_w}{\phi_o 6^{3/2}} \quad (3)$$

where ϕ_o is the viscosity parameter. The influence of excluded volume and the expansion factor on ϕ_o was calculated according to Gidley et al. (1991). The characteristic ratio (C_∞) and L_p were obtained using the following equation (Freitas et al., 2005; Roger, Axelos, & Colona, 2000):

$$C_\infty = \frac{6R_g^2 m_o}{M_w l^2} = 2\left(\frac{L_p}{l}\right) - 1 \quad (4)$$

where m_o is the monomer molar weight calculated as starting from 360 to 300 g/mol (according to the degree of galactosylation) and l is the monomeric length (0.52 nm), as previously described by Taylor and Atkins (1985).

A custom built TDSLS system was used. The xyloglucan, after filtration (50 mL), was withdrawn from a 100 mL reactor, equilibrated at 50 °C/20 min, by a Shimadzu LC-20AT pump. A pre-pump

filter of 2 μm and a 0.5 μm inline pre-pump filter were used. TDSLS was carried out using a multidetection equipment consisting of a homebuilt single capillary viscometer based on a Valydine DP15 pressure transducer and Valydine CD12 carrier demodulator, a 7 angle static light scattering BI-MwA MALLS (Brookhaven) in flow mode and a refraction index (RID-10A) detector (Shimadzu), used in a loop with the reactor. The data collected from the continuous recirculation at 0.5 mL/min were used to obtain parameters of apparent molar mass, viscosity and concentration in real time.

Heat inactivated JH fractions (96 ± 1 °C for 20 min) removed from the reactor during the experiment were analyzed by dynamic light scattering (DLS). The samples were evaluated on a Brookhaven instruments BI-90 Plus, with 90° detection. The samples were used for intensity autocorrelation computation and analysis by the standard method of moments, to yield average D_z at the higher moments, or cumulant analysis. From D_z, the hydrodynamic radius of the *z*-averaged equivalent sphere, R_h, was determined by the Stokes–Einstein relationship for spherical particles:

$$D_z = \frac{kT}{6\pi\eta R_h} \tag{5}$$

where D_z is the diffusion coefficient and k, T, and η are the Boltzmann constant, the absolute temperature and the solvent viscosity, respectively (Berne & Pecora, 1990).

The same heat inactivated samples were also analyzed on a multidetector high performance size exclusion chromatograph (HPSEC), using a Shodex SB 804 column, coupled to a BI-M_wA MALLS detector, Waters RI detector, and a custom-built viscometer (VIS). The eluent used was 0.1 M $NaNO_2$, containing 0.02 ppm of NaN_3, at a flow rate of 0.8 mL/min. The HPSEC standard used to calculate delay volumes was polyethylene oxide (PEO), with nominal mass 101,200 g/mol and polydispersity of 1.06 (Polymer Lab). The JN and JH fractions were analyzed by ^{13}C RMN to determine the percentage galactose removal (%GR) during this hydrolysis step, which was calculated as follows:

$$\%GR = 100\% \cdot \frac{m_b - m_a}{m_b} \tag{6}$$

where m_b is the molar concentration of galactose before hydrolysis and m_a is the molar concentration of galactose after hydrolysis (Shirakawa et al., 1998).

2.5. Galactose side-chain stripping

The fraction of mass in the backbone (f_p) can be obtained comparing the beginning $Kc/I_r\,(q,0)$ to a plateau of $Kc/I_r\,(q,t_{end})$ after the hydrolysis, so no fitting is required for f_p, which can be obtained from the relation:

$$f_p = \sqrt{\frac{Kc_o/I_r(q,0)}{Kc_o/I_r(q,t_{end})}} \tag{7}$$

The decay rate α can then be obtained by a single parameter fit to $Kc_o/I_r(q,t)$ according to:

$$\frac{Kc_o}{I_r(q,t)} = \frac{[1+u(t)/3]}{M_{t,o}[(f_p+(1-f_p)\exp(-\alpha t)]^2} \tag{8}$$

where $M_{t,o}$ is the total initial polymer mass (equal to $M_p + M_{s,o}$, where $M_{s,o}$ is the initial side-chain mass). The parameter $u(t)$ is defined by:

$$u(t) = q^2 R_{gz}(t) \tag{9}$$

It is assumed that the stripped side chains themselves scatter insignificantly compared to the remaining backbone. Also, when the change in R_{gz} is small during the reaction, the side-chain stripping rate α and the fraction of mass in the backbone f_p can be computed without any explicit knowledge of $M_{t,o}$, $A_{2,o}$ or $R_{gz,o}$. Even

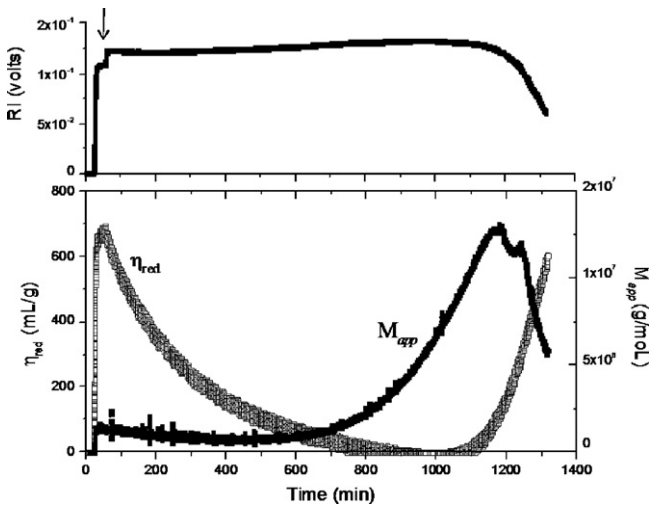

Fig. 1. Degalactosylation of xyloglucan at 1 mg/mL using β-D-galactosidase (0.8 U/mL) from *Aspergillus niger* at 50 °C in water. A loop was created with a flow of 0.5 mL/min and passed through a refractive index (RI) detector, a viscometer and a static light scattering detector (TDSLS).

K and c_o do not need to be known, since it suffices simply to measure the relative solvent subtracted scattering level instead of the absolutely calibrated $I_r(q,t)$, and f_p can even be obtained from a straight line fit to the initial slope of $Kc/I_r\,(q,t)$ (Ganter, Sabbi, & Reed, 2001).

3. Results

3.1. Chemical composition of water-soluble xyloglucan

Milled endosperms of *H. courbaril* seeds were defatted with toluene–ethanol, and extracted with water at 25 °C. The extract was treated with excess ethanol to give a precipitate of xyloglucan (fraction JN, 15.5% yield), which was water-soluble. It contained protein (5%) and carbohydrate (85%). The monosaccharide composition of fraction JN was glucose (52.2%), xylose (34.8%), and galactose (13.0%).

3.2. Real time monitoring of xyloglucan degalactosylation by TDSLS

Fig. 1 shows the results obtained during the real time monitoring by TDSLS of the enzymatic degalactosylation of xyloglucan. After addition of β-D-galactosidase to the reactor (arrow in Fig. 1), the reduced viscosity (η_{red}) began to decrease. It reached a minimum at 1000 min and then increased again. The apparent molecular weight (M_{app}) decreased slightly initially, but after 500 min it began to increase significantly.

The reduction in RI after 1100 min indicates that the polymer was excluded from the detectors (Fig. 1). This exclusion would explain the decrease of the apparent molar mass (M_{app}). Due to the exclusion from the detectors, the total pressure of the system increased and consequently, the differential pressure increased. In turn, this led to an increase in the value calculated for reduced viscosity (η_{red}).

Fig. 2 shows Kc/I_r versus time during enzymatic degalactosylation of the xyloglucan. The scattering at $\theta = 90°$ shows a little initial upwards curvature, and the amount of scattering was consistent with a high value of f_p (0.78, Eq. (7)) and, subsequently, after the first small plateau (~400 min after addition of enzyme), the scattering at $\theta = 90°$ decreases. Linear regression of the part of the curve between 0 and 400 min gave a galactose side-chain stripping-rate constant (α) of $1.3 \times 10^{-6}\ s^{-1}$.

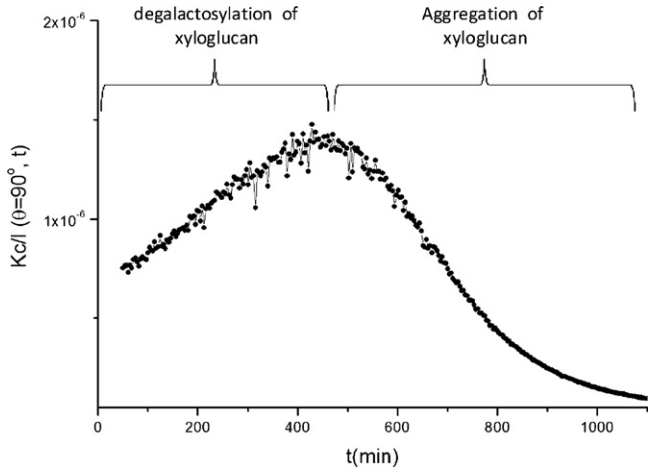

Fig. 2. *Kc*/*I* vs. time (min) for degalactosylation of xyloglucan at 1 mg/mL at $\theta = 90^{\circ}$, using β-D-galactosidase (0.8 U/mL) from *A. niger* at 50 °C in water. A loop was created with a flow of 0.5 mL/min and passed through the static light scattering detector.

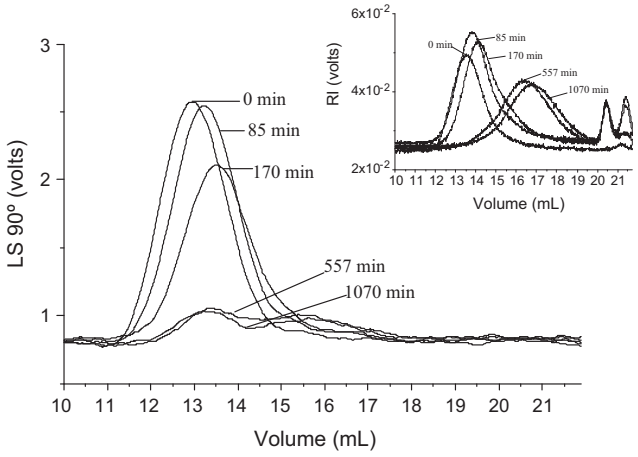

Fig. 3. HPSEC–MALLS of xyloglucan samples obtained at different times of degalactosylation. The eluent used was 0.1 M $NaNO_2$ with 0.02% NaN_3, at 25 °C. Light scattering (LS) at 90°, and insert profile using RI detector.

3.3. HPSEC–MALLS and viscosimetric analysis of xyloglucan

Table 1 presents the weight-average molar mass (M_w), the polydispersity index (M_w/M_n), the radius of gyration (R_g), the radius of gyration calculated using the Flory-Fox equation (R_g, Flory), the hydrodynamic radio (R_h), the intrinsic viscosity [η] and the persistence length (L_p) for the samples of xyloglucan collected at different times during the degalactosylation. L_p was determined using Gaussian-chain modeling (Eq. (4)), and during enzymatic treatment it was practically constant at about 4.0 nm.

Fig. 3 shows HPSEC–MALLS chromatograms of xyloglucan samples obtained at different times of enzymatic degalactosylation. The total light scattering intensity decreased over time. After 450 min, the peak, determined by light scattering and RI, was shifted to larger elution volumes. This shift is due both to degalactosylation and to the fact that the aggregates that formed in the solution were removed by the HPSEC–MALLS pre-filter. This filtration effect also caused the reduced viscosity (η_{red}) after 450 min to fall to very low values. The same behavior was observed during TDSLS analysis.

3.4. Chemical composition of partially degalactosylated xyloglucan

The chemical structures of xyloglucan before (fraction JN) and after side-chain stripping (fraction JH) were compared by ^{13}C NMR spectroscopy (Fig. 4). The well-defined anomeric signals δ 105.3, 103.26, and 99.87 for fraction JN (Fig. 4A) are due to terminal β-galactopyranose, β-glucopyranose and α-xylopyranose residues, respectively. Other signals are those of the *O*-substituted C-2 of the α-xylopyranosyl units (δ 81.20) and the C-5 (δ 75.87) and C-4 (δ 68.81) of the β-galactopyranosyl units. With respect to fraction JH, signals at δ 73.14, 72.25 and 62.04, which correspond to C-3, C-2 and C-6 of β-galactopyranosyl units, respectively, were not detected (Fig. 4B) (Lima et al., 1995; Lima-Nishimura et al., 2003; Martin et al., 2003). The integral of the anomeric signs for JN and JH confirms the GLC data, with 71% of galactose content reduction.

4. Discussion

Native xyloglucans are not able to form gels, but enzymatic degalactosylation leads to the formation of a thermally reversible gel (Busato et al., 2009). The present work provides new insights into the gelling mechanism, the kinetics of enzymatic degalactosylation and the minimum fraction of polymer in the backbone f_p that is necessary for gel formation to occur.

The galactose side-chain stripping-rate (α) is an important parameter that can guide the development of industrial processes for the enzymatic degalactosylation of xyloglucan. Maximization of this parameter can be used as the objective function in studies undertaken to optimize reaction conditions. It is useful to determine how α varies as a function of the temperature and of the concentrations of enzyme and xyloglucan. Ganter et al. (2001) evaluated, using TDSLS, the degalactosylation of galactomannans in real time using α-D-galactosidase. The side-chain stripping rate constant obtained for three galactomannans, with different degrees of galactose substitution on the mannose backbone, varied from 4.03×10^{-5} to $6.36 \times 10^{-5}\ s^{-1}$. These values are over one order of magnitude higher that the value of $1.3 \times 10^{-6}\ s^{-1}$ obtained in the current work.

It is clear that side chain removal is necessary for gel formation, but the mechanism of promotion of gel formation is not clear. There are two possibilities. The gel may form simply because the hinder-

Table 1
Characteristics of partially degalactosylated xyloglucan obtained from *Hymenaea courbaril* seeds and analyzed by HPSEC–MALLS and DLS.

Time (min)	M_w[a] (g/mol)	M_v (g/mol)	M_w/M_n[a]	R_g[a] (nm)	R_g[b] (nm)	R_h[c] (nm)	[η][a] (mL/g)	L_p[d]
0	1.70×10^6	1.48×10^6	1.9	134	62	170	752	3.8
85	1.02×10^6	1.06×10^6	1.8	99	41	90	639	4.0
170	0.76×10^6	0.74×10^6	1.7	89	39	55.6	497	4.2
255	0.44×10^6	0.37×10^6	1.7	56	27	530	348	4.2
425	0.15×10^6	0.09×10^6	1.7	92	13	975	145	3.7
1070	0.17×10^6	–	3.0	129	12	866	708	–

[a] HPSEC–MALLS analysis using Eq. (1).
[b] Flory-Fox Eq. (3).
[c] DLS analysis using Eq. (5).
[d] R_g from Flory-Fox Eq. (3) and L_p from Eq. (4).

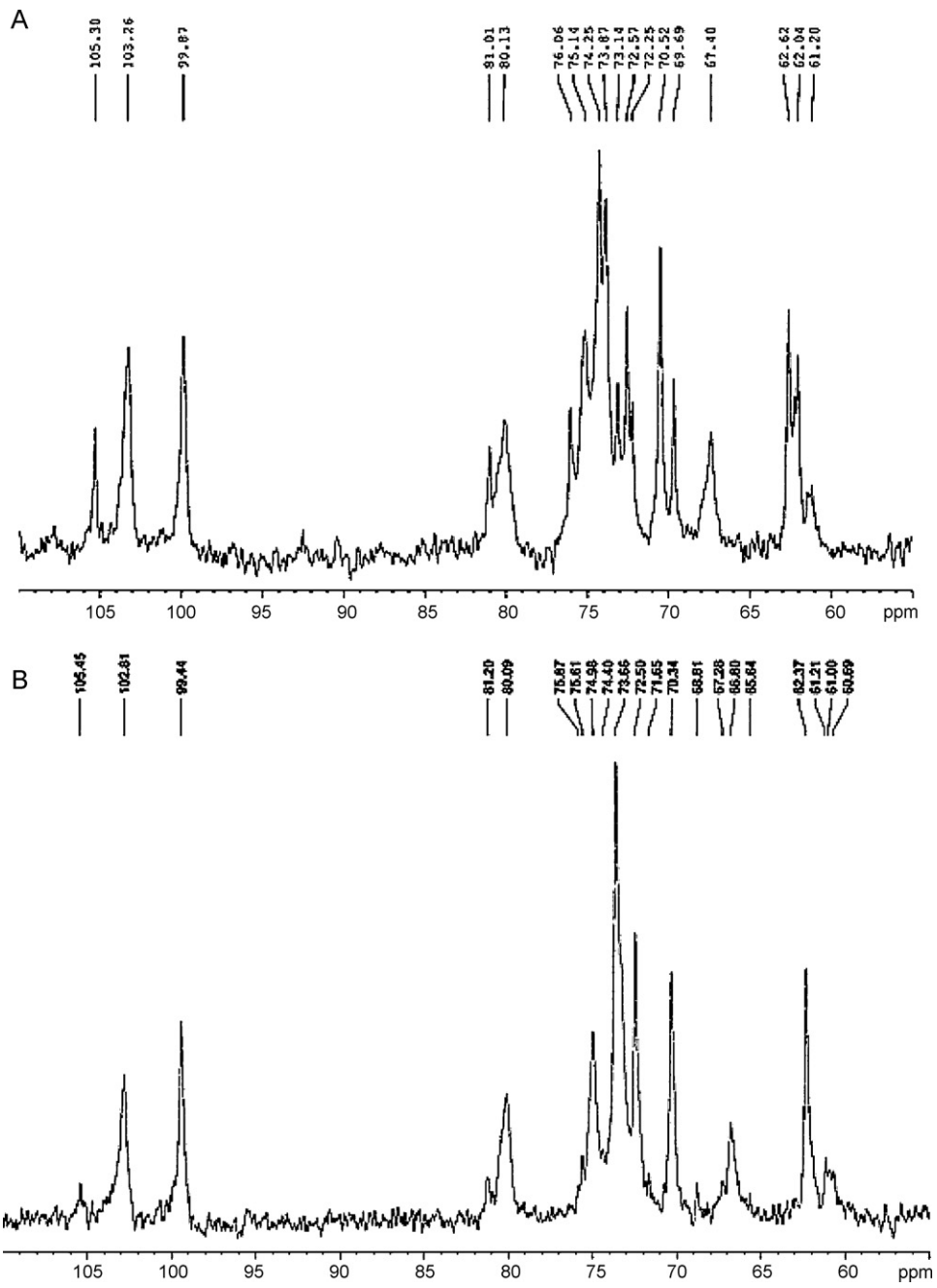

Fig. 4. ^{13}C NMR spectrum of xyloglucan from the seeds of *Hymenaea courbaril* before (A) and after (B) enzymolysis with α-D-galactosidase (0.8 U/mL) from *A. niger* at 50 °C in DMSO-d_6 at 70 °C.

ing side chains are removed, allowing direct interaction between the main chains, or it may form because side chain removal promotes a change in conformation of the molecule from a random coil to a rigid rod. The evidence for each of these phenomena is discussed in the following subsections.

4.1. Evaluation of evidence that side chain removal directly favors aggregation

The TDSLS data in Fig. 1 confirm that aggregates of xyloglucan were formed after degalactosylation and therefore confirm that the removal of the hindering side groups contributes to gel formation. This is consistent with previous reports that have used rheology and scanning electron microscopy analysis in degalactosylated samples of xyloglucan (Busato et al., 2009; Shirakawa et al., 1998; Yamatoya & Shirakawa, 2003).

Ganter et al. (2001) also observed aggregation when galactomannans were treated with β-D-galactosidase. The fact that the removal of the galactose substituents leads to aggregation suggests that these substituents confer solubility in water on both galactomannan and xyloglucan. Additionally, the aggregation may represent the microgelling process of xyloglucan.

The decrease in static light scattering from 400 to 1000 min after addition of enzyme (Fig. 2) represents the aggregation of the galactose-stripped backbone of xyloglucan. The aggregation starts more significantly at an f_p of 0.78, suggesting that the practically complete removal of hindering side groups contributes to aggregation and gel formation. The results obtained by ^{13}C NMR confirm

that in fraction JH the xyloglucan backbone was degalactosylated due to intensity reduction of the chemical shifts related to galactose substitution, 71% of reduction (Fig. 4B).

The aggregation of xyloglucan, after degalactosylation, was also confirmed by DLS with an increase in R_h (Table 1) and average count rate (AVG) with increasing enzymatic treatment time (data not shown), since the measured quantity is a *z*-average of the population, the DLS measurement is very sensitive to small populations of aggregates (Berne & Pecora, 1990).

4.2. Evaluation of evidence that side chain removal promotes conformational changes

The combination of static and dynamic light scattering data obtained in the present work provides qualitative information about the architecture of the macromolecules that cannot be obtained with either method alone and allows an evaluation of the degree to which conformational changes might have contributed to gel formation. For example, this combination of data allows calculation of the structure-sensitive parameter ρ (R_g/R_h) (Burchard, 1994), which depends on the chain structure, conformation and polydispersity (Grimm, Kriigef, & Burchudb, 1995).

Brun-Graeppi et al. (2010) used this approach to characterize the sol–gel transition of xyloglucan hydrogels, and their results for ρ suggest that the structure was more compact after degalactosylation. However, the determination of ρ is not a simple matter when aggregates form. Freitas et al. (2005) observed that when aggregates are present the use of an uncorrected value of R_g, as determined by MALLS, in the calculation of L_p, results in a false rigid rod conformation.

There are two strategies that can be used to reduce the influence of aggregates and therefore allow calculation of the persistence length (L_p). Firstly, the influence of aggregates can be avoided by calculating R_g using the Flory-Fox equation, based on intrinsic viscosity measurements. Secondly, the aggregation itself can be minimized by submitting the samples to high temperature and pressure (Freitas et al., 2005; Picout et al., 2003). The use of the Flory-Fox equation is advantageous, since heat treatment can cause thermal decomposition and may in fact be inefficient in reducing aggregation (Freitas, Drenski, Alb, & Reed, 2010; Freitas et al., 2005). Also the seriousness of the aggregation problem with biopolymers means that it is useful to make viscosity measurements, since these are virtually unaffected by small populations of aggregates (Freitas et al., 2010).

The L_p of xyloglucan after degalactosylation was practically unaffected and similar to the values of ~4.4 nm that were previously reported by Freitas et al. (2005) for the native xyloglucan of *H. courbaril*, on the basis of light scattering data. Patel et al. (2008) observed variation in the estimation of the persistence length of xyloglucan related to the hydrodynamic technique used, but overall our results are compatible with values of L_p of 4–8 nm that have been reported for native xyloglucans from various sources (Patel et al., 2008; Picout et al., 2003; Ren, Picout, Ellis, Ross-Murphy, & Reid, 2005).

The constant L_p suggests that there was no conformational modification of xyloglucan during degalactosylation and that the aggregation of xyloglucan is the main phenomenon responsible for gel formation. The aggregation of xyloglucan in diluted conditions depends on the extent of galactose stripping and the f_p value suggests that aggregation occurred after complete degalactosylation. The aggregates formed after degalactosylation, not the isolated macromolecule, have a rigid rod-like behavior, as previously observed by Brun-Graeppi et al. (2010), and are responsible for the increase of the solid-like behavior in the rheological experiments.

5. Conclusion

The aggregation of xyloglucan is the major factor responsible for gel formation and the conformation was practically unaffected by the enzymatic treatment. The stripping rate constant of the lateral galactose on the xyloglucan ($1.3 \times 10^{-6}\ s^{-1}$ determined by TDSLS), f_p (0.78) and a constant L_p (of 4 nm before and after enzymatic hydrolysis) confirm the proposition. The data presented in the current paper will help to guide future applications using modified xyloglucan from the seeds of *H. courbaril* as a gelling additive.

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