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Effect of the oxidation level on the thermogravimetric kinetics of an oxidized galactoxyloglucan from *Hymenaea courbaril* (Jatobá) seeds

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Abstract

A natural hydrocolloid extracted from *Hymenaea courbaril* seeds contains Glc:Xyl:Gal:Ara in a molar ratio of 51:27:21:1. Selective TEMPO radical oxidation (2,2,6,6-tetramethylpiperidine-1-oxyl) at C-6 of the polysaccharide gave products with 3.0, 5.1 and 9.5% of carboxyl groups as determined colorimetrically. Molecular mass analyses showed a decrease in the M_w of the oxidized galactoxyloglucans and an increase in angular fit from the RMS ratio versus molar mass plot. This indicated that the oxidation process generated a more rigid structure, going from a random coil in the native polymer to a rod conformation in the oxidized samples, probably due to the polyanionic structure. Thermogravimetric kinetics of degradation was obtained using the Arrhenius equation and an increase in the degradation rate, was apparently directly dependent on the oxidation level.

Keywords: *H. courbaril*; Galactoxyloglucan; Polysaccharide; TEMPO oxidation; Thermogravimetry

1. Introduction

Galactoxyloglucans as hydrocolloid model can be present in the primary cell walls of higher plants (dicotyledons and non-graminaceous monocotyledons) and in the cotyledonary cell of some dicotyledonous seeds, where they function as a storage polysaccharide [1]. An example is that obtained from seeds of *Tamaridus indica*, which has a large number of commercial and industrial applications [2]. In Brazil, an abundant font of a galactoxyloglucan is seeds from *Hymenaea courbaril* (Jatobá), which Lima et al. [3,4] analyzed by methylation and found that its glycosidic linkages are practically the same as those of other seed galactoxyloglucans, which have a cellulose-like β-(1,4)-D-glucan backbone to which single-unit α-D-Xyl*p* substituents are attached at O-6. Some Xyl*p* residues are substituted at O-2 by β-D-Gal*p*.

In the Carbohydrate Group of Federal University of Paraná (UFPR) considerable attention has been paid to the galactoxyloglucan from the seeds of *H. courbaril*, which was obtained at different Brazilian locations and whose structure and properties have been determined (Lima et al. [3,4]; Souza-Lima et al. [5]; Vargas-Rechia et al. [6]; Martin et al. [7]; Freitas et al. [8]).

The structure and applications of polysaccharides and their oxidized products have been widely studied. Thermogravimetric analysis has been reported to be a promising and sensitive technique for characterizing structural modifications of natural polysaccharides (Stivala et al. [9]; Varma and Chavan [10] and Varma et al. [11]). As an example, Varma et al. [11] studied the galactomannan of guar gum and its periodate oxidation products, having 1.2, 3.1, 13, 26.7 and 54.9% of oxidation, and showed that the thermal analysis is a sensitive tool for differentiating periodate oxi-

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dized products. In this work, the authors observe that occur an intrinsic relation between molecular composition and oxidative stability.

Using the methodology of de Nooy et al. [12–14], Sierakowski et al. [15] studied the selective oxidation of a galactomannan from seeds of *Leucaena leucocephala* (Man:Gal ratio of 1:3) with TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl) at CH_2OH-6 which gave rise to CO_2H-6 groups. Sierakowski et al. [16] studied the oxidation and characterization of a galactomannan extracted from seeds of *Cassia fastuosa* by the same method and investigated the adsorption behavior of the polyelectrolyte onto amino-terminated surfaces, by ellipsometry and contact angle measurements.

We now investigate the effect of oxidation levels on the structure, conformation and thermal properties of a galactoxyloglucan obtained from seeds of *H. courbaril*.

2. Experimental

2.1. Plant material

Seeds of *H. courbaril* were harvested in the Foz do Chopin Forest Reserve, Paraná State, Brazil. The galactoxyloglucan (HXG) was obtained by exhaustive aqueous extraction at 25 °C from pooled and milled seeds (40 g l^{-1} for each extraction process). The viscous extracts were centrifuged at 10,000 × *g* and the supernatant passed sequentially through Millipore filter membranes with pore sizes of 3 and 0.8 μm and, then precipitated with two volumes of ethanol, washed with acetone and dried at 25 °C to give pure products [8].

2.2. Selective oxidation by TEMPO

Galactoxyloglucan (1 g) was solubilized at 25 °C in 500 ml of water. The solution was treated with TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl) (0.53 mg), NaBr (4.64 mg), and sodium hypochlorite (0.26 ml at 12%), that each 0.1 mmol of primary alcohol was converted to acid. The reaction mixture was maintained at 3 °C, with stirring under nitrogen. At the end of the oxidation process, 16 mg of $NaBH_4$/0.1 mmol of primary alcohol and 20 ml of ethanol was used to reduce the excess of oxidizing agent in the medium.

The alcohol to acid conversion was estimated by titration of the mixture with NaOH (0.0888 mol l^{-1}) during the oxidation process. The initial pH was ∼9.5 and was maintained by titration with NaOH aqueous solution when the pH decreased to 9.2. The formation of carboxyl groups corresponded to the amount of titrated NaOH and then to obtain theoretical oxidation ratios of 3, 6 and 12%, we used 7.0, 14 and 28 ml of the standard NaOH aqueous solution.

The TEMPO (stable radical) (Fig. 1a) can be oxidized by several reagents to give a nitrosonium ion (Fig. 1b). This is a strong oxidant and shows selectivity towards primary hydroxyl functions over secondary ones. Obviously there will be a competing reaction due the undesired oxidation of secondary alcohols by hypochlorite and hypobromite that occur mainly at pH <9. But using higher pH (9.2–9.5) and lower temperature the selectivity was more than 95% [12,13]. The schematic representation of the oxidation process was showed in the Fig. 1.

2.3. Chemical analysis

Total carbohydrate was assayed by the phenol–H_2SO_4 method [17], protein by the method of Hartree [18], ash and moisture by that of AOAC [19]. The uronic acid content was assayed by the *m*-hydroxybiphenyl method [20].

Monosaccharide contents of galactoxyloglucan (HXG) were determined on complete acid hydrolysis with M TFA at 100 °C for 5 h [21]. The solutions were evaporated to a residue that was repeatedly dissolved in H_2O and evaporated. The products from each hydrolysis were reduced with $NaBH_4$ and, then acetylated with pyridine–Ac_2O (1:1 (v/v)), for 12 h at 25 °C [22]. The resulting alditol acetates were analyzed by GC–MS Varian and detection with a Saturn 2000R mass spectrometer, gas chromatography, using a DB-225 capillary column at 220 °C with nitrogen as the carrier gas.

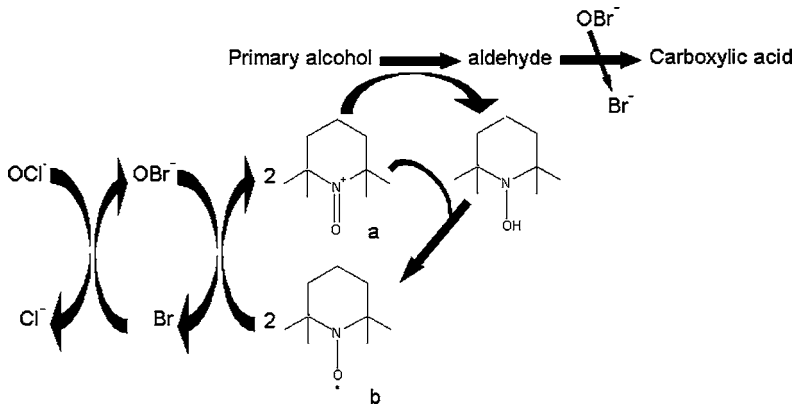

Fig. 1. Schematic representation of TEMPO oxidation process [12].

2.4. Infrared spectroscopy—FTIR

FTIR analysis was carried out using a spectrometer Hartmann and Braum MB-series, with KBr as support, at a range of 400–4000 cm^{-1}, a resolution of 2 cm^{-1} and 19 scans at a rate of 10 scans per minute. Solutions of oxidized samples were passed through a cationic resin (H^+) to give the acid form of the polymer.

2.5. Average molar mass (M_w) and R_g

To calculate the molar mass, the ratios of the refractive index to the concentration (d*n*/d*c*) of the galactoxyloglucan (HXG) and oxidized galactoxyloglucans (HXGOX) were determined using a Waters 2410 differential refractometer at wave length of 546 nm with concentrations of 1.0; 0.5; 0.25 and 0.125 g l^{-1} (filtered through Millipore filter 0.45 μm).

Aqueous solutions of HXG (0.5 g l^{-1}) and HXGOX (4.0 g l^{-1}) were filtered through a Millipore filter (0.22 μm) and injected into a GPC with 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 azide at a flux of 0.6 ml min^{-1}.

2.6. Thermogravimetric analysis

The experiments were conducted using a Shimadzu 50 H Thermogravimetric Analyzer (TGA) with a sample weight of 9.9 ± 0.1 mg. All the experiments were performed under a flux of nitrogen, which as maintained at a constant flow rate of 50 ml min^{-1}. Experiments were conducted at five heating rates of 2.5, 5.0, 10.0, 20.0 and 40.0 °C min^{-1} with the sample being heated from 25 to 600 °C. The sample holder was a platinum pan [23,24]. The samples were kept at 105 °C for 3 h before each experiment (this precaution was taken due the differential water content in the samples, using in this form the same mass of polysaccharides in the analysis) and the weight-loss curves were normalized to 100% of mass at 200 °C, due to the residual moisture present in the sample.

2.7. Kinetic analysis

Various theoretical and empirical models are utilized to represent the observed transition. In each model, the velocity of reaction can be expressed as a function $f(\alpha)$, where α is the conversion value, in this case the degradation. The dependence of the constant rate on temperature can be obtained from the Arrhenius equation (Eq. (1)). To determine the activation energy (E_a), the ln of heating rates (K) was plotted against the reciprocal of the absolute temperature for different weight-losses (α = 5, 10, 20, 30, 40, 50, 60 and 80%). The slope of the line gave $-E_a/R$, where R is the universal gas constant. The intersection of the line with the ordenate gives the pre-exponential factor (A) in the Arrhenius equation [23–25] (Eq. (1)):

$$\ln K = \ln A - \frac{E_a}{RT} \quad (1)$$

For each E_a value corresponding to α, the constant rate at 250 and 350 °C was calculated.

3. Results and discussion

3.1. General physical and chemical analyses

Milled endosperms of *H. courbaril* seeds were submitted to aqueous extraction at 25 °C and, after fractionation using Millipore filters, a water-soluble galactoxyloglucan (HXG) was obtained (18.5% yield) [8].

Chemical analysis showed the presence of carbohydrate (81%), protein (2%), moisture (15%) and ash (0.4%) [8].

In the TEMPO oxidation process the polymer can be oxidized in the C-6 of galactose and also in C-6 of internal glucose units, but we calculate the percentage of oxidation only in relation to the C-6 content of galactose. The HXG in study has a molar relation Gal:Xyl:Glc of ∼1:1.2:2.4 and traces of arabinose units. To generate 3, 6 and 12% of oxidized galactose, 0.166, 0.333 and 0.666 mmol were necessary, respectively.

GC–MS analysis of derived alditol acetates, showed that HXG contained Glc:Xyl:Gal:Ara in a molar ratio of 51:27:21:1. In 9.5% oxidized polysaccharide, carrying out hydrolysis under identical conditions, the values were 58:31:10:1. The level of glucose and xylose, thus increase by ∼6%, but the galactose level decrease by 11.6%, indicating that the oxidation process occurs mainly at this unit, due the formation of the galacturonic acid.

During the oxidation process, the time and the volume of aqueous NaOH added was used to the control of the derivatization process (Fig. 2). The degree of oxidation was confirmed by a colorimetric method for quantification of

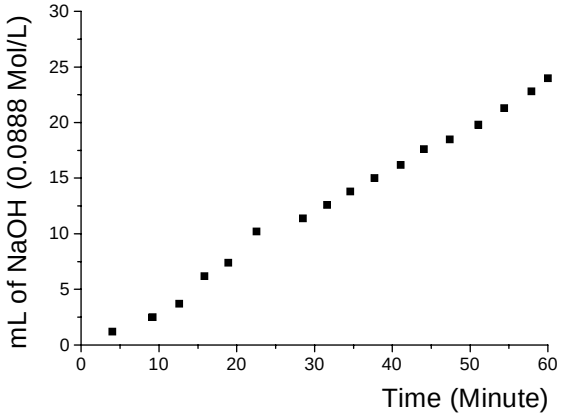

Fig. 2. Volume titrated of NaOH solution (0.0888 mol l^{-1}) as a function of the time during the oxidation process.

Table 1
Determination of oxidation levels of HXGs derived

Sample	Colorimetric method (%)[a]
HXGOX3	2.8 ± 0.4
HXGOX5	5.4 ± 0.5
HXGOX10	9.5 ± 0.15

[a] Uronic acid colorimetric method [20]. Median ± standard derivation of three independent analysis.

uronic acids (Table 1) [20]. The same approach was used by de Nooy et al. [12–14]. The products with 2.8, 5.4 and 9.5% of oxidation are now named HXGOX3, HXGOX5 and HXGOX10, respectively.

The presence of uronic acid was qualitatively confirmed in the products by infrared spectroscopy, where a band was observed at 1735 cm^{-1} arising from the stretching vibration of carbonyl group (C=O) (data not shown).

The d*n*/d*c* values for the HXG and HXGOXs were 0.113 and 0.130 ml g^{-1}, respectively. The molar mass by GPC-light scattering analysis showed that conversion to an oxidized polymer was practically complete, because light scattering detected a molecule with a higher molar mass and with the same elution time as that of the unmodified polymer (HXG: 35–45 min—peak A), but as the concentration in the oxidized polymer was so low it was impossible to detect by the refraction index. The new peak with larger elution time (38.5–56 min—peak B), in the Fig. 3, appears in the oxidized samples and represents the product. M_w values were 1.4×10^6 and 1.0×10^5 g mol^{-1}, respectively for HXG and HXGOXs. In the sample with high degree of oxidation (HXGOX10) the peak A, that represents the unmodified polymer, is smaller than that with 3% oxidation. On analyzing the elution profile with the refractive index detector (data not shown) of the modified polymers, it was only possible to observe peak B, and not the starting material (peak A). This indicated a homogeneous macromolecule, with highly polydisperse values of M_w/M_n from 1.4 for the original polymer, to ~3.0 for the product. Another important observation is that the plot of the RMS radius against molar mass (Fig. 4) gave information concerning conformation in solution. With the increase of the degree of oxidation, it changes from a random coil (angular fit of 0.5) to a rod-like structure (1.0) (Fig. 4).

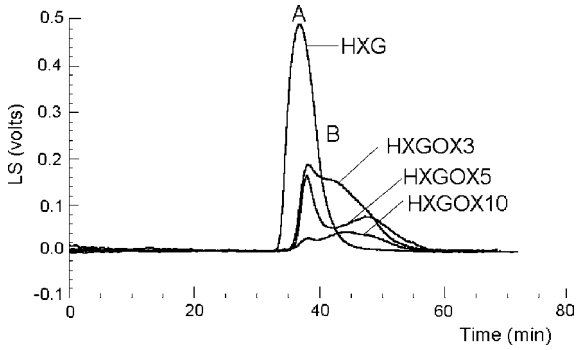

Fig. 3. GPC analysis with light scattering detector at 90° for HXG and HXGOX3, HXGOX5 and HXGOX10. (A) Native and (B) oxidized polysaccharides.

3.2. Thermogravimetric analysis

Fig. 5 shows the thermal degradation of HXG (A), HXGOX3 (B), HXGOX5 (C) and HXGOX10 (D) at heating rates of 2.5, 5.0, 10.0, 20.0 and 40.0 °C min^{-1} under nitrogen, starting from 200 to 600 °C.

Fig. 6 shows an overlapping of the derivative of the weight-loss curves (dTG) at 2.5 °C min^{-1} for HXG, and 3, 5 and 10% HXGOX. It demonstrates that with an increasing degree of oxidation, the temperature at which degradation begins to be observed is lower. This indicates that the oxidation reduced the thermal stability at the beginning of the degradation process. The temperature at the beginning (T_{onset}), end (T_{end}) and middle point (T_m), and the mass loss in each part of the degradation process is shown in Table 2.

Fig. 6 and Table 2 simplify the observation that:

- A new process of degradation appears with the modified polysaccharide at lower temperatures (first event ~230–278 °C), when compared with that of the unmodified one. The second event is referent to the unmodified polymer apparently.

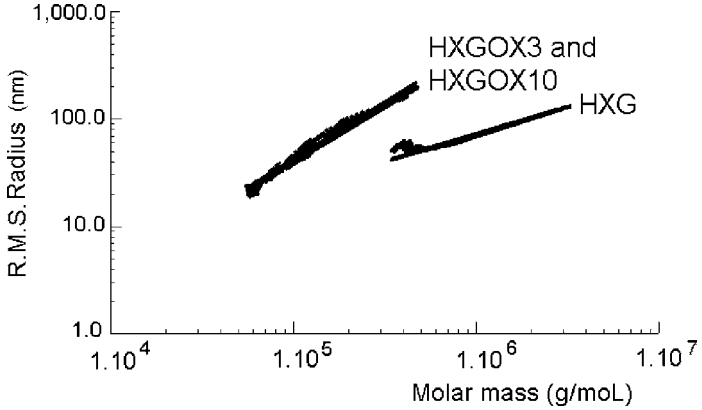

Fig. 4. rms radius (nm) as a function of molar mass (g mol^{-1}).

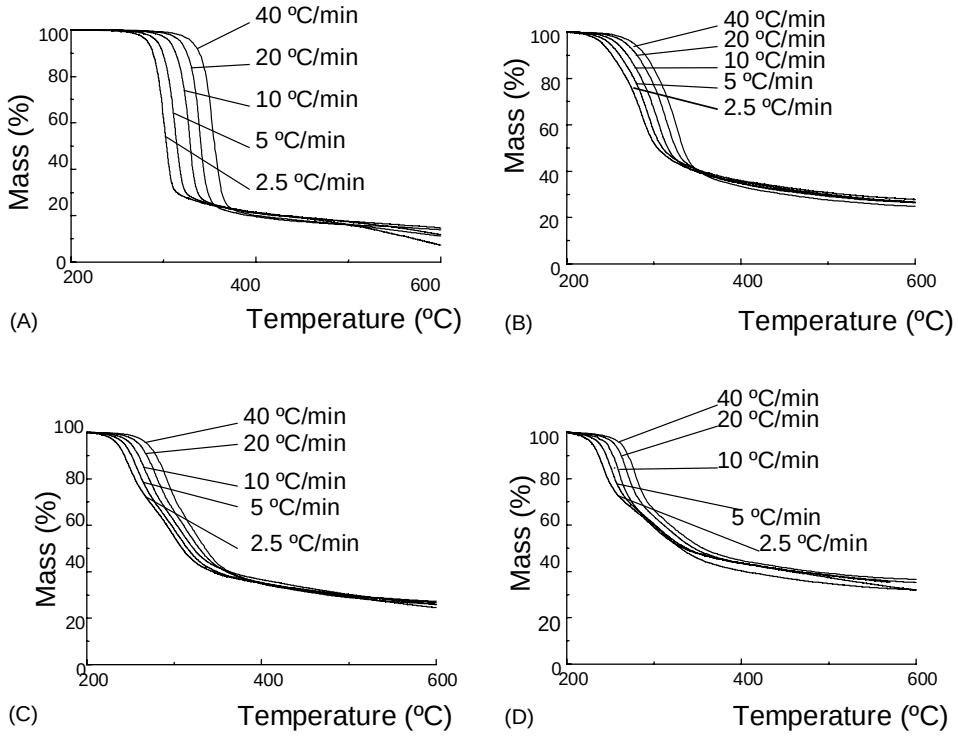

Fig. 5. Weight-loss curves for HXG (A), HXGOX3 (B), HXGOX5 (C) and HXGOX10 (D) at heating rates of 2.5, 5.0, 10.0, 20.0 and 40.0 °C min^{-1} under nitrogen.

Table 2
Degradation temperatures and weight-loss percentages of galactoxyloglucan and that with in 3, 5 and 10% oxidized, at a heating rate of 2.5 °C min^{-1}

Sample	T_{onset} (°C)	T_{end} (°C)	T_m (°C)	Weight-loss (%)
HXG	277	317	300	70.4
HXGOX3 first event	232	260	247	12.9
HXGOX3 second event	264	309	284	42.8
HXGOX5 first event	231	263	248	30.5
HXGOX5 second event	277	327	298	29.9
HXGOX10 first event	226	253	241	28.5
HXGOX10 second event	275	329	299	18.8

- The area of the new event (first event) in the modified polymer showed in Table 2, increases with the degree of oxidation.
- The Table 2 show that the total degree of degradation decreases with the increase of oxidation (first and second event). At the beginning heating process the oxidation level increase the degradation, and at the end generate products more stable to degradation.

3.3. Kinetic analysis

Fig. 7 shows ln of the heating rates (°C min^{-1}) against the reciprocal of the absolute temperature. The slope of this plot was utilized to obtain the values of E_a at different α (%) values.

Fig. 8 shows the relation between the activation energy (E_a) of HXGOXs and HXG obtained at different α values (5, 10, 20, 30, 40 and 50%). The data indicate an increase in the energy of activation in the thermal degradation process is dependent of the oxidation level of the molecule. With HXGOX3, the E_a was up to 1.68 times, HXGOX5 up to 1.94 times, and HXGOX10 up to 2.5 times increase, compared with that of the native polysaccharide. The data show that

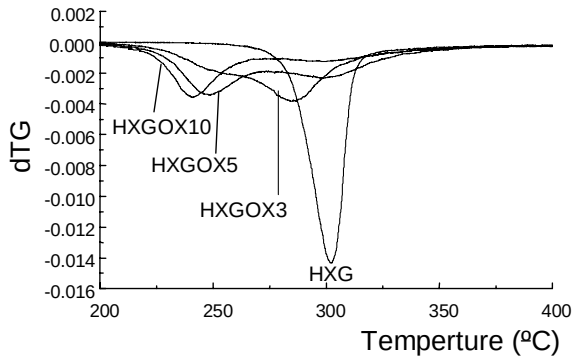

Fig. 6. dTG of weight-loss curves for HXG, HXGOX3, HXGOX5 and HXGOX10 at a heating rate of 2.5 °C min^{-1} under nitrogen.

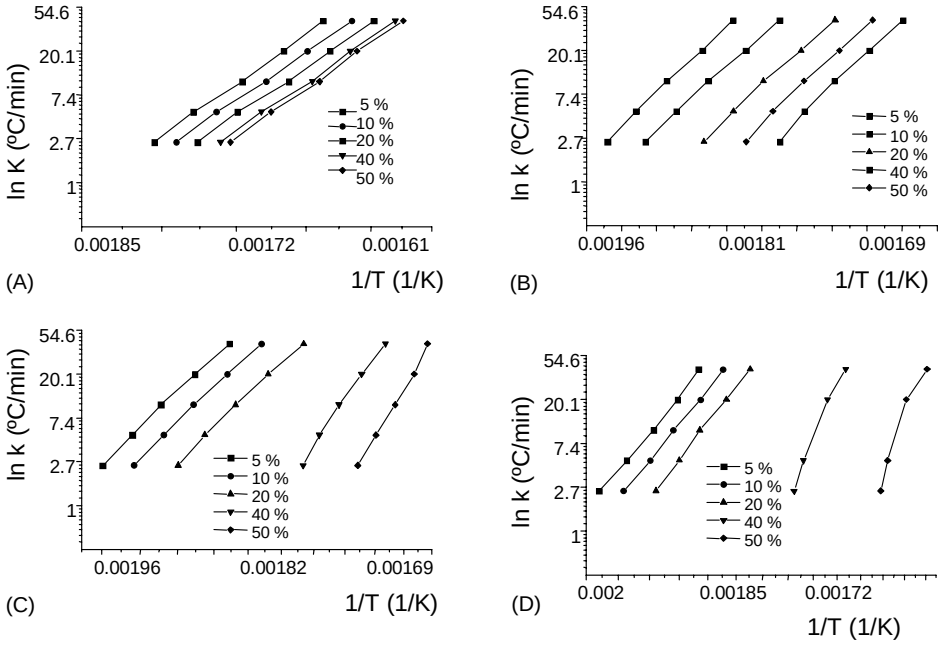

Fig. 7. $\ln K$ against $1/T$ for HXG (A), HXGOX3 (B), HXGOX5 (C), and HXGOX10 (D). The lines represent α (%).

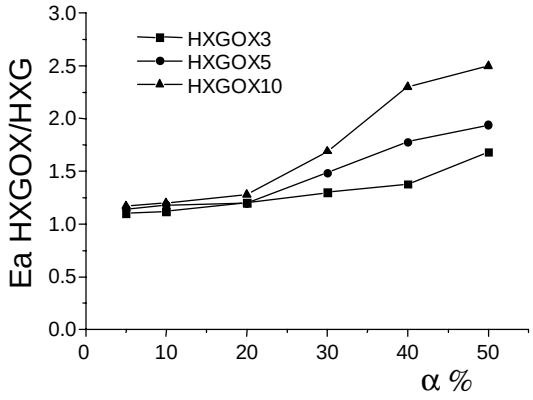

Fig. 8. Ratio between the E_a of HXGOXs to HXG at different α (%) values.

an increase in the oxidation level generates an increase in the energy necessary for thermal degradation. The E_a data also confirm a better thermal stability after 20% polyelectrolyte degradation, because of an increase in the E_a (after this degradation percentage). A mathematical analysis, using the conversion values of 5–20%, it was possible to observe a linear equation fit for these data, without large deviation from the straight line for all the values of samples studied. However, at α of 20–50% a straight line fit was obtained with angular coefficient for the HXGOX/HXG ratio of 0.0152, 0.0252 and 0.0427, respectively for 3, 5 and 10% oxidation. The ratio among these data indicates that the increase in E_a depends on the oxidation level. As an example, if the oxidation increased by 1.7-fold (HXGOX5/HXGOX3), the increase of E_a obtained by the ratio between the angular coefficient also increased in a same proportion of 1.65. This is an indication that the energy used for degradation is directly dependent of the level of oxidation.

Fig. 9 shows the rate constant of HXG and HXGOX obtained at 250 °C (Fig. 9A) and 350 °C (Fig. 9B). A notable effect occurs when the rate was obtained at 250 °C (Fig. 9A); at a low loss mass (α%) the rate constant was larger then that of the unmodified polysaccharide by 19-, 33- and 53-fold, respectively, for samples with 3, 5 and 10%

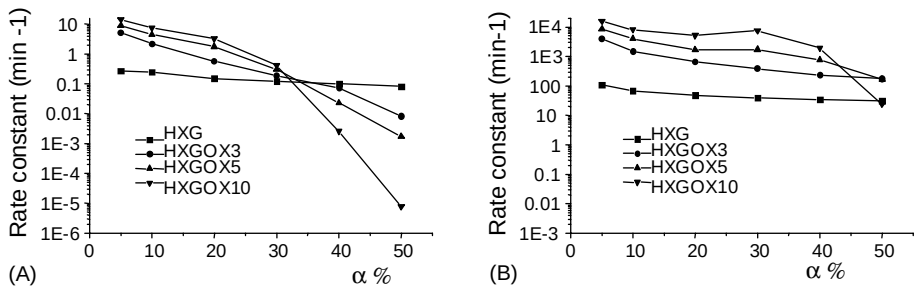

Fig. 9. Rate constant of HXG, HXGOX3, HXGOX5 and HXGOX10 at 250 °C (A) and 350 °C (B).

of oxidation. At higher level of loss mass (50%), the ratio appears to be the opposite from that found for a low mass loss. In this case, the loss of mass of modified in relation to unmodified polymer was 0.1, 0.02 and 9.4×10^{-5} times, respectively, for HXGOX3, HXGOX5 and HXGOX10. This shows a large reduction of the rate constant occurs in this system, depending on the level of oxidation, probably due the formation of a stable degradation product. This was observed when the α (%) value was higher than 40%, when the products appear to be more stable that the native polymer.

Fig. 9B shows that at a weight-loss of 5% for HXGOX3, HXGOX5 and HXGOX10 the ratios of increment are 36, 79 and 146 times at 350 °C, respectively. This increase in the rate at the beginning of the degradation process indicates that the selective oxidation produce uronic acid mainly at the galactose residue (monosaccharide composition by GC–MS and due by the fact that the xylose does not have a free CH_2OH group) generating less stable products to heating.

4. Conclusions

The galactoxyloglucans containing CO_2H-6 groups obtained by selective TEMPO oxidation of HXG contained 2.8, 5.4 and 9.5% uronic acid. The oxidations were confirmed by colorimetric and spectroscopic method (FTIR) which give related results, although the found is more accurate HXGOX3, HXGOX5 and HXGOX10 that suffered a reduction in the molar mass from 1.4×10^6 to $\sim 1.0 \times 10^5\,g\,mol^{-1}$, as observed by refractive index and light scattering detectors. The introduction of uronic acid units also modifies the conformations from a random coil for HXG to a rod-like structure for the products. The kinetics of thermal degradation were directly dependent of the oxidation level, namely that with the increase in the oxidation level, increased proportionally E_a and the rate constant at the beginning of the process. This new application is important since it can be used to better differentiate oxidized polymers, with small differences in the degree of oxidation.

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