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Structure of hardwood glucuronoxylans: modifications and impact on pulp retention during wood kraft pulping

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Abstract

The behaviour of different hardwood glucuronoxylans during the kraft pulping process was investigated. Woods and pulps xylans were isolated and characterized by size exclusion chromatography, methylation (linkage) analysis and ^{1}H NMR. *Eucalyptus globulus* and *Eucalyptus urograndis* showed xylan retention significantly higher than that of *Betula pendula*. The higher retention of *Eucalyptus* xylans was assigned to (i) their higher average molecular weight (31 against 24 KDa in *B. pendula*) and to (ii) the presence of *O*-2 substituted 4-*O*-methyl-α-D-glucuronic acid groups ([→2)-Glc*p*A-(1→]) with galactopyranosyl/glucopyranosyl residues belonging to fragments of galactan/glucan chains that were absent in *B. pendula* xylans. A significant part of uronic acids, particularly [→2)-Glc*p*A-(1→] units, remain in fibres until the end of pulping. The acetylation degree and distribution of acetyl groups between Xyl*p* units, in general terms, was similar in the three types of xylans. Unexpectedly, about 20% of the acetyl groups persisted in pulps xylans till the end of pulping.
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Keywords: Glucuronoxylan; *E. globulus*; Kraft pulping; Kraft pulp; Xylan structure; *O*-acetylation

1. Introduction

Hardwoods are important raw materials used in the production of writing and printing papers. *Betula pendula* (birch) is the dominant species for such applications in Northern Europe, while *Eucalyptus* species (such as *E. globulus*, *E. grandis* and *E. urograndis*) represent the main fibre sources for the pulp and paper industry in the Iberian Peninsula and South America (Hillman, 2002). These woods show significant differences in their chemical compositions (Sjöström, 1993), which affect their industrial processing behaviour and the quality of the obtained fibres and corresponding papers.

Kraft pulping is the process most widely used in the industrial production of wood chemical pulps (Gullichsen & Fogelholm, 1999). Wood chips are treated at high temperatures (150–170 °C) with strongly alkaline solution composed mainly of sodium hydroxide and sodium sulphide. Under such alkaline conditions, lignin is extensively degraded and dissolved (90–95%), liberating the wood fibres, composed essentially by cellulose and hemicelluloses. During the pulping, wood polysaccharides may be also partially dissolved and/or degraded, mainly by alkaline hydrolysis and sequential elimination of the terminal reducing end groups (*peeling*), affecting the performance of the process and quality of fibres (Genco, Busayasakul, Medhora, & Robbins, 1990; Sjöström, 1977). Particularly, the partial degradation and dissolution of hemicelluloses of hardwoods during the kraft process is highly responsible for a significant consumption of pulping chemicals and a decrease of the pulp yield and papermaking properties of pulps (Clark, 1985; Sjöström, 1993; Schild, Muller, & Sixta, 1996; Paavilainen, 1989).

O-acetyl-(4-*O*-methylglucurono)-xylans are the main hemicelluloses present in traditional industrial hardwoods, their content varying between 15 and 30% (wood weight basis) (Sjöström, 1993). However, recently, we have shown that the *E. globulus* wood acetylated xylan shows a chemical structure rather different from that found in other hardwoods: about one third of the 4-*O*-methyl-α-D-glucuronopyranosyl residues are substituted at *O*-2 by D-galactopyranosyl and D-glucopyranosyl units (Evtuguin, Tomás, Silva, & Pascoal Neto, 2003; Shatalov, Evtuguin, & Pascoal

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Neto, 1999). The results obtained so far strongly suggest that the hexose units at the *O*-2 position of the D-glucopyranosyluronic acid residue in *E. globulus* glucuronoxylan may represent linking points between this hemicellulose and other polysaccharides in the cell wall (Evtuguin et al., 2003). In spite of these newly data on *E. globulus* xylan, there is still a lack of knowledge about the behaviour this polysaccharide during the alkaline pulping. Also, it is not known if this peculiar structure is specific to *E. globulus* or is common to different *Eucalyptus* species. This situation prompted us to investigate the structure and kraft pulping behaviour of the xylans from *E. globulus*, *E. urograndis* and *B. pendula*, aiming to understand how the structural features may determine the xylan stability and its retention in fibres, thus affecting the kraft pulping yield.

2. Materials and methods

2.1. Materials

Eucalyptus globulus from Portugal, *Eucalyptus urograndis* from Brazil and *Betula pendula* from Sweden were used in the pulping experiments in the form of industrial chips. Wood chemical analysis and holocellulose isolation were performed using wood sawdust (40–60 mesh). Prior to analysis, woods and pulps were extracted for 6 h in a Soxhlet extractor with 2:1 (v/v) toluene-ethanol and with dichloromethane, respectively.

2.2. Kraft pulping experiments

Kraft pulping experiments were carried out in 5.8 L forced circulation batch digesters (M/K model 409 MII) equipped with an external electric heating system and temperature control.

Reference pulps with similar residual lignin contents (kappa number 18.6 ± 0.3) were prepared using the following conditions: liquor-to-wood ratio (L/Kg), 4:1; sulphidity, 28%; initial temperature, 40 °C; final temperature, 160 °C; temperature rate, 1 °C/min. Active alkali (%, as Na_2O) and pulping time were adjusted in order to attain the same kappa number: 15% and 160 min for *E. globulus*; 18% and 190 min for *E. urograndis*; 18% and 210 min for *B. pendula*. At the end of the pulping, pulps were thoroughly washed with distilled water and air-dried.

Pulping experiments aiming to investigate the behaviour of wood polysaccharides along the kraft pulping time were carried out using the same conditions for the three species: liquor-to-wood ratio (L/Kg), 4:1; active alkali (%, as Na_2O), 15%; sulphidity, 28%; initial temperature, 30 °C; final temperature, 160 °C; temperature rate, 1.44 °C/min. The pulps or partially delignified woods thus obtained were treated on a disc refiner aiming to complete fibre separation, thoroughly washed with distilled water and air-dried.

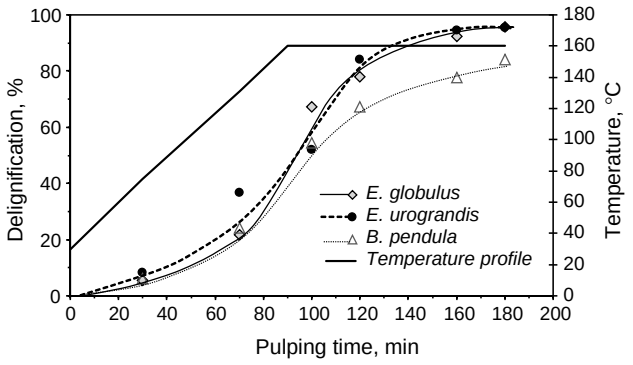

Fig. 1. Delignification curves and temperature profile of kraft pulping of *E. globulus*, *E. urograndis* and *B. pendula* woods.

The evolution of delignification with time for the different species is shown in Fig. 1.

The kappa number and Klason lignin content of pulps were determined by standard TAPPI methods (Tappi, 1996).

2.3. Preparation of holocellulose

Holocelluloses were obtained by delignification of wood and pulps with peracetic acid solution (pH 4.5) at 16% and 10%, respectively, as previously published (Evtuguin et al., 2003). The lignin content in holocelluloses (less than 2%) was monitored by the methodology described elsewhere (Evtuguin, Daniel, & Pascoal Neto, 2002), involving the dissolution in cadoxene solution and measurement of absorbance at 280 nm. The percentage of holocellulose isolated was about 70% (wood basis) for woods and between 92% and 97% for pulps (pulp basis).

2.4. Isolation of xylans

The xylans of woods and pulps were extracted from the corresponding holocelluloses by KOH solution (10%) as described elsewhere (Browning, 1967). The pH of the alkaline extract was adjusted to 2 with formic acid and the xylan was precipitated in an excess of ethanol *p.a.* The supernatant was removed off and the precipitated xylans was centrifuged, washed with methanol several times and dried at room temperature under vacuum. The ash content was quantified for each xylan sample obtained.

Alternatively, woods and pulps xylans were extracted from the corresponding holocelluloses (previously ball milled during 2 h) with Me_2SO (HPLC grade, 1:60 (w/w) holocellulose-to-Me_2SO ratio) at 50 °C, for 20 h, with stirring under nitrogen atmosphere as previously described (Evtuguin at al, 2003). The Me_2SO dissolved xylan was acidified with formic acid to pH 2 and precipitated with an excess of ethanol *p.a.* (about ten times), centrifuged, washed and dried as above.

2.5. Carbohydrate analysis

The woods, pulps and isolated xylans were subjected to treatment with 72% H_2SO_4 at 20 °C for 3 h followed by 1.5 h hydrolysis with 1 M H_2SO_4 at 100 °C (Seaman hydrolysis) and the released neutral monosaccharides were determined as alditol acetate derivatives by gas chromatography (Selvendran, Verne, & Faulks, 1989). The quantitative analysis was carried out on a Varian 3350 gas chromatograph equipped with a FID detector and with a DB-225 J&W column (30 m × 0.25 mm i.d. × 0.15 μm film thickness). The chromatographic conditions were as follows: initial temperature −220 °C (5 min); temperature rate 2 °C/min; final temperature 230 °C (5 min); injector temperature −230 °C; detector temperature −230 °C. The quantification was made using calibration curves with standards.

2.6. Methylation (linkages) analysis

The xylans isolated by aqueous KOH were submitted to linkage analysis using a modification of the method described by Isogai, Ishizu, & Nakano (1985). Sodium hydroxide powder was added to a solution of 10 mg of xylan in 2 mL of Me_2SO followed by CH_3I at room temperature for 4 h. The methylated xylan was recovered by dialysis against distilled water and freeze-dried. The process was repeated to guarantee a complete methylation of xylan. The methylated samples were treated with $LiAlD_4$ in dry THF, as described elsewhere (Ring & Selvendran, 1978; Lindberg & Lönngren, 1978), in order to identify the uronic acid residues. The methylated polysaccharide was hydrolyzed with aqueous 90% formic acid for 1.5 h at 100 °C and then with 0.15 M H_2SO_4 for 6 h at 100 °C. The derivatization of partially methylated sugar units as alditol acetates was carried out by the same method as for carbohydrate analysis of woods and pulps. Products of methylation and hydrolysis were separated and identified by GC-MS (Lindberg, 1972) on a Trace Gas Chromatograph 2000 Series equipped with a Finnigan Trace MS mass spectrometer, using helium as carrier gas (35 cm/s) and a DB-1 J&W capillary column (30 m × 0.32 mm i.d. × 0.25 μm film thickness). The column program temperature was 125–225 °C, 2 °C/min, the injector temperature 230 °C and the detector temperature 250 °C. The quantitative analysis was carried out on a GC Varian 3350 equipped with a FID detector using the same column and program as described above for carbohydrate analysis.

2.7. Size-exclusion chromatography (SEC)

The xylan isolated by Me_2SO extraction were dissolved in a small volume of 10% LiCl solution in *N*,*N*-dimethylacetamide HPLC grade at 70–80 °C and further diluted with DMAC to a xylan concentration of about 4 mg/mL. The SEC analysis was carried out on two PLgel 10 μm MIXED B 300×7.5 mm columns protected by a PLgel 10 μm pre-column (Polymer Laboratories, UK) using a PL-GPC 110 system (Polymer Laboratories). The columns, injector system and the detector (RI) were maintained at 70 °C during the analysis. The eluent (0.1 M LiCl solution in DMAC) was pumped at a flow rate of 0.9 mL/min. The analytical columns were calibrated with pullulan standard (Polymer Laboratories) in the range of 0.8–100 kDa.

2.8. ^1H NMR spectroscopy

The xylans isolated by Me_2SO extraction were dissolved in D_2O and the spectra were recorded at 60 °C on a Bruker AMX 300 spectrometer operating at 300.13 MHz. Relaxation delay was 12 s, r.f. 90° -pulse width of 10.2 μs and about 1000 pulses were collected. The chemical shifts were reported relative to sodium 3-(trimethylsilyl)propionate-d_4 used as internal standard (δ 0.00).

3. Results and discussion

3.1. Composition of woods and kraft pulps

The three hardwoods investigated show significantly different chemical compositions (Table 1). Although lignin content ranges between 21.5% for *B. pendula* and 27.9 for *E. urograndis*, the most significant difference between the three species becomes from polysaccharides composition as suggested from monosaccharide analysis. *B. pendula* is extremely rich in xylans, having the lowest cellulose content. The uronic acid content of the three woods also

Table 1
Woods and unbleached pulp compositions and yields of kraft pulping experiments (kappa number 18.6±0.3)

	E. globulus	*E. urograndis*	*B. pendula*
Wood composition, %			
Extractives (ethanol/toluene)	1.72	1.91	2.24
Lignin (Klason)[a]	22.1	27.9	21.5
Neutral anhydrous monosaccharides			
Glc	53.4	52.1	44.5
Xyl	14.2	11.4	23.6
Rha	0.3	0.2	0.8
Ara	0.4	0.4	0.7
Man	1.1	0.7	2.1
Gal	1.5	1.2	0.8
Unbleached pulp composition, % (wood basis)			
Lignin (Klason)[a]	1.3	1.0	1.3
Neutral anhydrous monosaccharides			
Glc	45.0	40.2	38.8
Xyl	10.6	6.8	12.4
Rha	0.1	0.2	0.1
Ara	0.1	0.1	0.1
Man	0.1	0.1	0.3
Gal	0.4	0.1	0
Pulp yield, %	58.7	49.0	53.1

[a] Uncorrected for polyphenolics content.

indicates that the *Eucalyptus* xylans have a frequency of glucuronic substitution higher than that of *B. pendula* xylan.

When the woods were kraft pulped to similar residual lignin content (kappa number 18.6 ± 0.3, adjusting the alkaline chemical charge and pulping time), pulp yields were noticeably different (Table 1): *E. globulus* and *B. pendula* woods, having similar lignin contents, showed pulp yields of 58.7 and 53.1%, respectively. The main contribution for the lignin-free yield loss in *B. pendula* comes from the degradation of xylan, as suggested from the percentage of dissolved xylose, 11% against 4–5% (wood basis) in *Eucalyptus*. Such a difference cannot be assigned simply to the different alkaline conditions required (15% active alkali for *E. globulus* and 18% for *E. urograndis* and *B. pendula*) and, consequently, denote a higher stability of the *Eucalyptus* xylans, when compared to *B. pendula*. The amount of xylose dissolved during the pulping represents 25%, 40% and 47% of the initial xylose content of *E. globulus*, *E. urograndis* and *B. pendula* woods, respectively. On the other hand, the amount of dissolved glucose is in the range 6–12% (wood basis), representing 16, 23 and 13% of the initial glucose content in *E. globulus*, *E. urograndis* and *B. pendula* woods, respectively. Such a different behaviour prompted us to investigate in more detail the profile of polysaccharides dissolution/retention along the different stages of the kraft cooking process, using exactly the same pulping conditions for the three wood species.

3.2. Polysaccharides retention/dissolution along the kraft pulping

The delignification curves obtained from the pulping experiments using the same conditions for the different woods investigated (Fig. 1), show the typical three distinct consecutive kinetic phases of the kraft pulping process (Sjöström, 1993): (i) the initial phase (0–70 min), corresponding to the reactor heating up period, with a slow delignification rate, where about 20–30% delignification takes place, followed by (ii) the bulk phase (70–120 min), with a much faster lignin removal rate, leading to about 90% delignification and, finally, (iii) the residual phase (>120 min), where the rate of delignification decreases. The delignification kinetic of *B. pendula* in bulk phase is slower and the final degree of delignification is lower than in the case of the *Eucalyptus* species. Such behaviour is assigned to differences in the corresponding lignins structure (Pinto, Evtuguin, & Pascoal Neto, 2004).

The dissolution/retention behaviour of wood polysaccharides was monitored by neutral monosaccharide quantitative analysis. Fig. 2 shows the evolution of the dissolved xylose and glucose (as anhydrous monosaccharides) along the kraft pulping. The pattern of dissolution of these two monosaccharides is significantly different in *Eucalyptus* species and *B. pendula* woods.

Glucose is dissolved in a great extent (~5%, wood basis) in the initial phase of delignification of both the *Eucalyptus*

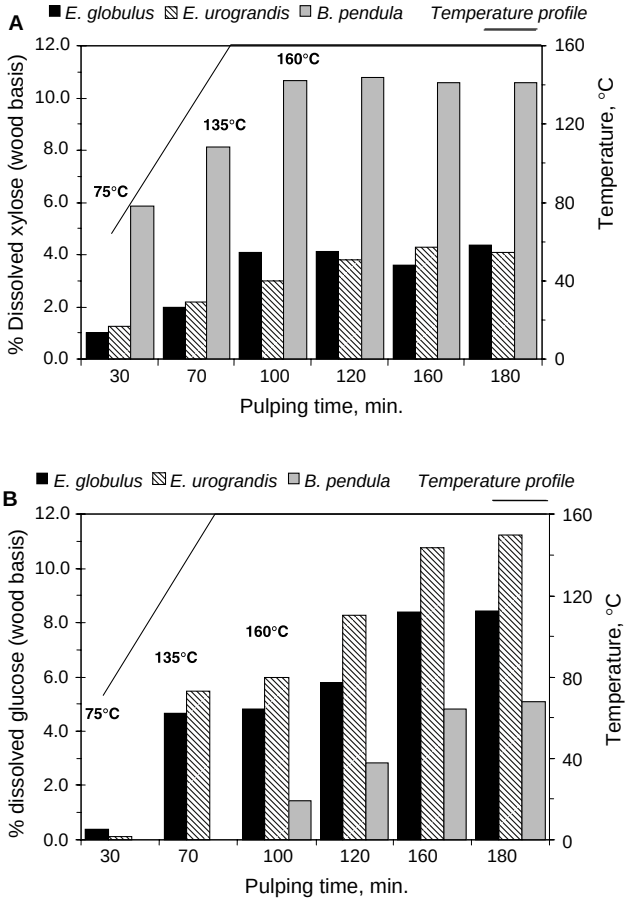

Fig. 2. Percentage of dissolved xylose (A) and glucose (B) (as anhydrous-monosaccharides) during wood kraft pulping under similar conditions (see Fig. 1).

species, while with *B. pendula*, no glucose dissolution was detected (Fig. 2). Although the partial degradation (essentially by peeling reaction) of a fraction of less ordered cellulose and dissolution of glucomannans can contribute to this phenomenon, the observed dissolution of glucose in the case of *Eucalyptus* may be most likely assigned to the dissolution of wood amylopectin. We have shown recently that amilopectin is present in *E. globulus* black liquors since the early stages of delignification, representing about 10% (w/w) of the black liquor dissolved polysaccharides (Lisboa, Evtuguin, Pascoal Neto, & Goodfellow, 2005). On the other hand, X-ray diffraction analysis of celluloses from the three woods did not show significant differences in their supramolecular structure (Pascoal Neto & Evtuguin, 2004).

In the case of *B. pendula*, the glucose dissolution starts between 70 and 100 min, at temperature higher than 130 °C, and may be assigned mainly to peeling and random alkaline hydrolysis of amorphous cellulose (Clayton et al., 1983; Sjöstrom, 1993). In the three woods, the degradation of cellulose pursues along the bulk phase, showing an accentuated decrease of the rate of dissolution in the residual phase (Fig. 2).

The degradation and dissolution of xylans starts since early stages of the delignification (Fig. 2) and is particularly pronounced in the case of *B. pendula*. The amount of xylose dissolved in the first 70 min represents 47%, 54% and 76% of the xylose dissolved during the pulping (180 min) of *E. globulus*, *E. urograndis* and *B. pendula*, respectively. At this stage, besides the initial dissolution of low molecular weight xylans, degradation occurs mainly by the sequential elimination of the reducing end groups. During the bulk phase, after the pulping temperature is reached, the degradation and dissolution of xylans pursues, the random cleavage of glycosidic bonds and secondary peeling prevailing at this stage of the process (Aurell, 1964; Clayton et al., 1983; Sjöstrom, 1993). During the residual phase an enrichment of xylose content in pulp is observed, particularly in the case of *E. globulus* at 160 min, which may be explained by xylan re-precipitation. At the end of the pulping, a significant fraction of xylans dissolved in black liquor (~10 g/L) keep their polymeric nature (Mw 17–19 KDa) (Lisboa et al., 2005). When the alkali concentration in liquor decreases at the later stages of the process, such xylans may precipitate or adsorb onto the surface of fibres. This phenomenon is also favoured by a decrease of the xylan degree of branching, which promotes a higher frequency of hydrogen bounds between xylan chains and between xylan and accessible fractions of cellulose (Clayton, et al., 1983; Sjöstrom, 1993).

Under the similar pulping conditions used in this set of experiments, the extent of xylan degradation and/or dissolution in the three phases of the kraft process is always much higher in *B. pendula* than in *E. globulus and E. urograndis* (Fig. 2), confirming the higher stability of *Eucalyptus* xylans. Aiming to understand such different behaviour, we isolated these polysaccharides from woods and pulps along the kraft pulping and submitted them to a detailed structural characterization.

3.3. Isolation of xylans

Xylans were extracted from peracetic acid holocelluloses with 10% KOH aqueous solution or with Me_2SO. The yields of xylans isolated with the alkaline solution were always higher than 50%. Although a partial alkaline degradation cannot be avoided (e.g. alkaline hydrolysis of acetyl groups), the high extraction yield ensures a good compromise between representativity of native xylans and extent of degradation. These xylans were used in methylation (linkages) analysis. On the other hand, the Me_2SO soft extraction, in spite of the lower yields obtained (30–60%) allow preserving, as much as possible, the original structure of the polysaccharide, namely the original *O*-acetyl moieties. Such xylans were submitted to SEC and NMR analysis.

3.4. Molecular weight assessment

The molecular weight of Me_2SO soluble xylans was assessed using SEC. The elution curves of xylans (not shown) exhibited a unimodal Gaussian molecular weight distribution, indicating the structural homogeneity of the isolated polysaccharide sample. Fig. 3 shows the weight average molecular weights (Mw) of wood xylans and their evolution during the kraft pulping. The molecular weight of *E. globulus* wood xylan (31 kDa) is close to that of *E. urograndis* and higher than that of *B. pendula* wood (24 kD). For the three species, the Mw decreases mainly during the first 120 min (initial phase and first half part of bulk phase), which matches with the temperature increase during kraft pulping (Fig. 1) and with the intense xylose loss occurring during this period (Fig. 2(A)). The lower Mw of native xylan in *B. pendula* wood is certainly contributing to the much higher rate of xylan dissolution when compared to the *Eucalyptus* species (Fig. 2(A)).

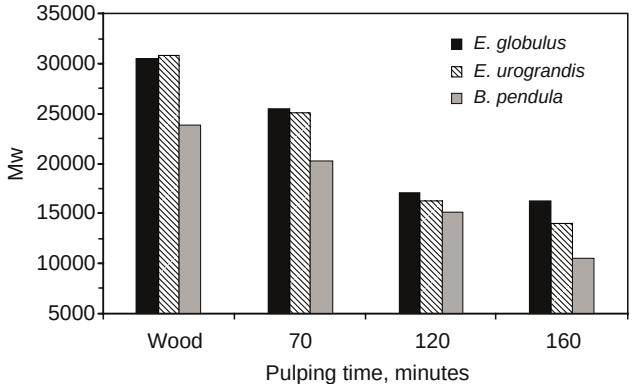

Fig. 3. Xylan average molecular weight (Mw) evolution during wood kraft pulping under similar conditions (see Fig. 1) and in reference pulps with similar residual lignin contents (kappa number 18.6±0.3).

During the residual phase, the Mw of *E. globulus* xylan remains practically constant, while *E. urograndis* and *B. pendula* show a decrease of the Mw of the corresponding xylans. It may be suggested that the observed decrease of Mw is most likely assigned to random alkaline hydrolysis of glycosidic linkages because the loss of xylose is quite insignificant during this stage (Fig. 2(A)).

The xylans of pulps with similar residual lignin contents (kappa number 18.6) show Mw of 16, 14 and 11 kDa for *E. globulus*, *E. urograndis* and *B. pendula*, respectively, corresponding to Mw reductions of 47, 55 and 56%, when compared to wood xylans.

3.5. Linkage analysis

The relative abundance of the most abundant partially methylated products identified in the linkage analysis of woods and pulps xylans is presented in Table 2. The three xylans are essentially composed by a linear backbone constituted by (1→4) linked β-D-xylopyranosyl units

Table 2
Methylation analysis of *E. globulus*, *E. urograndis* and *B. pendula* woods and unbleached kraft pulps xylans (relative abundance of different structural units)

	Structural units	Pulping time, minutes				
		Wood	70	120	160	KN 18.6±0.3
E. globules	Xylp-(1→)	1.0	1.4	1.9	2.3	2.3
	→4)-Xylp-(1→	86.7	87.2	88.0	92.3	92.3
	→2,4)-Xylp-(1→	12.3	11.4	10.1	5.4	5.4
	GlcpA-(1→	7.1	5.5	5.5	1.4	1.4
	→2)-GlcpA-(1→	2.5	2.7	2.3	1.3	1.3
	Galp-(1→	2.0	1.8	2.3	1.7	1.7
E. urograndis	Xylp-(1→)	1.3	2.0	1.4	3.8	2.8
	→4)-Xylp-(1→	84.2	82.9	88.3	89.8	91.4
	→2,4)-Xylp-(1→	14.5	15.1	10.3	6.4	5.8
	GlcpA-(1→	9.8	8.7	6.1	1.9	1.7
	→2)-GlcpA-(1→	2.3	2.9	1.6	0.8	0.7
	Galp-(1→	2.5	1.6	1.3	1.0	0.8
B. pendula	Xylp-(1→)	0.9	1.2	1.7	2.9	1.5
	→4)-Xylp-(1→	90.6	90.0	90.2	92.5	96.0
	→2,4)-Xylp-(1→	8.5	8.8	8.1	4.6	2.5
	GlcpA-(1→	7.1	5.6	5.6	2.7	1.7
	→2)-GlcpA-(1→	0.0	0.0	0.0	0.0	0.0
	Galp-(1→	0.9	0.2	t.	0.0	t.

t., traces.

partially *O*-2 substituted with 4-*O*-methyl-α-D-glucuronosyl units.

The most relevant structural difference in the xylans structure is the presence of *O*-2 substituted 4-*O*-methyl-α-D-glucuronic acid ([→2)-Glc*p*A-(1→]) units in *Eucalyptus* xylans, in addition to terminal MeGlcA ([Glc*p*A-(1→]), linked to the xylan backbone (Table 2). Such structural specificity was previously detected in *E. globulus* xylan (Evtuguin et al., 2003; Shatalov et al., 1999); the results here obtained strongly suggest that this feature is common to other *Eucalyptus* species. The *O*-2 substituted MeGlcA represents 20–30% of the uronic acid moieties in *Eucalyptus* xylans (Table 2). According to our previous results (Evtuguin et al., 2003), the MeGlcA may be *O*-2 substituted either by terminal galactose ([Gal*p*-(1→]) or substituted galactose or glucose ([→4)-Gal*p*A-(1→], [→4)-Glc*p*-(1→]). Roughly, 70% of glucuronosyl units are substituted at *O*-2 with Gal*p* and 30% with Glc*p* units (Evtuguin et al., 2003). As previously suggested (Evtuguin et al., 2003), these hexose units may belong to other polysaccharides, namely rhamnoarabinogalactans and glucans. Hence, the *O*-2 substituted MeGlcA group present in *Eucalyptus* xylans, constituting points of linkage between xylan and other cell wall polysaccharides, may effectively contribute to the high retention of this hemicellulose during kraft pulping. In the case of *B. pendula*, the [→2)-Glc*p*A-(1→] moieties were not detected, the [→2,4)-Xyl*p*-(1→] unit being the only point of branching of the backbone of xylan.

The results from methylation analysis (Table 2) show that *Eucalyptus* xylans have a degree of branching (including both terminal and *O*-2 substituted MeGlcA) higher than that in *B. pendula*, in agreement with results from carbohydrate analysis (Table 1). The number of uronic acid substituents per 100 backbone xylose units in *E. globulus*, *E. urograndis* and *B. pendula* xylans is 9.6, 12.1 and 7.1, respectively. The extent of ramification may have opposite effects in xylan retention during the kraft pulping. A higher degree of branching generally facilitates the solubility of xylans in the pulping solution; on the other hand, the presence of glucuronosyl units linked to *O*-2 in terminal xylopyranosyl units may prevent the isomerization of the reducing xylopyranose to the corresponding 2-ulose derivative, thus retarding the peeling reaction (Clayton et al., 1983). The results from linkage analysis (Table 2) show an apparent misbalance between the frequency of the [→2,4)-Xyl*p*-(1→] structure and the sum of [Glc*p*A-(1→] and [→2)-Glc*p*A-(1→] units. This can be explained by the presence of acetyl groups, difficult to saponify (see ^{1}H NMR results), or hexenuronic acid degradation residues (resulting from the peracetic acid treatment) at *O*-2 of xylan backbone.

The results of linkage analysis of pulps xylans (Table 2) show that the main structural modifications of xylans remaining in the pulp occur after the pulping temperature (160 °C) is reached, between 120 and 160 min. Although the relative abundance of 4-*O*-methyl-α-D-glucuronic acid groups ([Glc*p*A-(1→] and [→2)-Glc*p*A-(1→] units) on xylan backbone decreases since the early stages of the pulping (certainly by alkaline hydrolysis but also by conversion into hexenuronic acid (Daniel, Pascoal Neto, Evtuguin, & Silvestre, 2003)) the most significant decrease in the degree of branching is reached at the end of the bulk phase (120–160 min). The residual xylans in pulps with kappa number 18.6, show 2.7, 2.4 and 1.7 uronic acid substituents per 100 backbone xylose units in *E. globulus*, *E. urograndis* and *B. pendula* xylans, respectively. Interestingly, in the case of *Eucalyptus* xylans, while the frequency of terminal MeGlcA is reduced during the pulping from 7–9/100 Xyl*p* to 1–2/100 Xyl*p*

(80% reduction), the relative abundance of *O*-2 substituted MeGlcA decreases from 2–3/100 Xyl*p* to about 1/100 Xyl*p* (50% and 70% reduction in *E. globulus* and *E. urograndis*, respectively) (Table 2). Hence, the *O*-2 substituted glucuronosyl moieties should be more resistant to the alkaline degradation than the corresponding terminal counterparts, contributing to retard the peeling reaction and the gradual dissolution of xylan in the pulping solution.

The decrease of the relative abundance of [Glc*p*A-(1→] and [→2)-Glc*p*A-(1→] structures is, in general terms consistent with the decrease of frequency of [→2,4)-Xyl*p*-(1→] units. The discrepancy between the frequency of this last structure and [Glc*p*A-(1→] and [→2)-Glc*p*A-(1→] substituents may be assigned to the partial conversion of MeGlcA groups into hexenuronic acid (Daniel et al., 2003) and to the presence of acetyl groups in *O*-2 which resisted to the alkaline conditions used in the pulping and methylation analysis.

3.6. ^{1}H NMR analysis

^{1}H NMR analysis was performed in wood and pulps xylans, isolated by extraction with Me_2SO. The proton resonances were assigned using literature data (Hazendonk, Reinerink, Waard, & Dam, 1996; Teleman, Lundqvist, Tjerneld, Stalbrand, & Dahlman, 2000; Teleman, Tenkanen, Jacobs, & Dahlman, 2002) and recently published data from homonuclear (TOCSY) and heteronuclear (HSQC) correlation NMR experiments (Evtuguin et al., 2003).

The ^{1}H NMR spectra (4.2–5.5 ppm) of woods xylans are shown in Fig. 4. The region of anomeric protons shows terminal MeGlcA groups (tGlcA) with a relative abundance of 7–9 units/100 Xyl*p*, in general agreement with results obtained by linkage analysis. The presence of substituted MeGlcA units (sGlcA) was confirmed in *Eucalyptus* species xylans. Hexenuronic acid groups were not detected since they were degraded by peracetic acid during the preparation of holocellulose. The ^{1}H NMR spectra of pulps xylans (not shown) confirmed, in general terms, the results obtained by methylation analysis on the behaviour of MeGlcA groups along the different phases of the kraft pulping.

The ^{1}H NMR spectra of wood xylans (Fig. 4) in the region 4.6–5.2 ppm is dominated by signals of protons in different acetylated xylopyranose units, allowing their identification and quantification (Fig. 5). The calculated degree of acetylation (number of acetyl groups/100 Xyl*p*, %) was 51% for both *E. globulus* and *E. urograndis* xylans and 48% for *B. pendula* xylan. As expected, xylopyranose units acetylated in *O*-2 or *O*-3 (X-2Ac, X-3Ac) represent the most abundant acetylated moieties (15–17% each). When compared with *B. pendula*, *Eucalyptus* xylans present a higher frequency (10–12%) of occurrence of *O*-3 acetylated Xyl*p* units substituted at *O*-2 with MeGlcA (X-3Ac-2GlcA). Such figure suggests that almost all the MeGlcA groups are linked to *O*-3 acetylated xylopyranose units. Xyl*p*

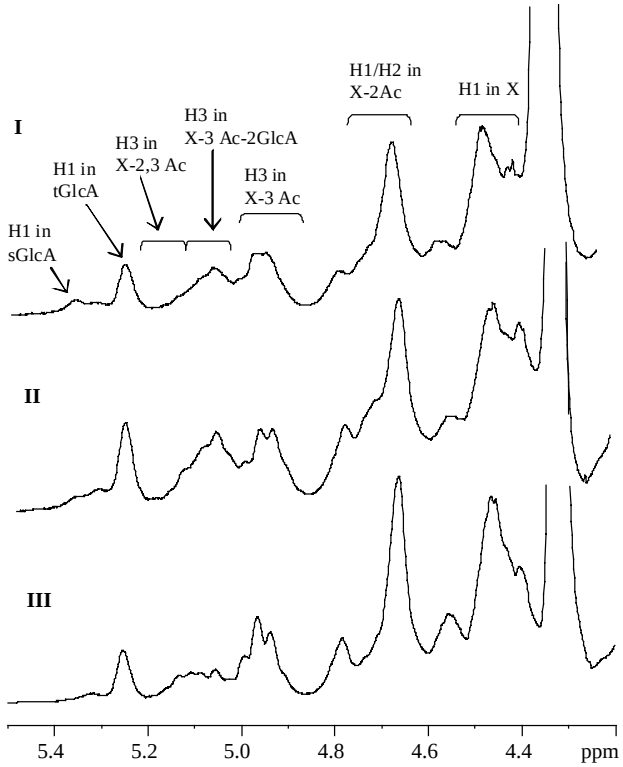

Fig. 4. Expansion of ^{1}H NMR spectra of *O*-acetyl-(4-*O*-methylglucurono) xylan from *E. globulus* (I), *E. urograndis* (II) and *B. pendula* (III) woods. The designations are as follows: X: Acetylated Xyl*p* units; X-2Ac, X-3Ac and X-2,3Ac: Xyl*p* units substituted by OAc at *O*-2, *O*-3, and simultaneously at *O*-2 and *O*-3; X-3Ac-2GlcA: Xyl*p* possessing simultaneous substitution at *O*-3 by OAc and at *O*-2 by MeGlc*A*; tGlcA: Terminal MeGlcA; sGalA: *O*-2 substituted GalA; sGlcA: *O*-2 substituted MeGlcA.

acetylated simultaneously at *O*-2 and *O*-3 (X-2, 3Ac) represents about 5% of the xylopyranose units.

The analysis of ^{1}H NMR spectra of pulps xylans allowed following the behaviour of acetylated moieties along the kraft pulping (Fig. 5). As expected, the degree of acetylation of xylan backbone decreases drastically in the initial phase of pulping (0–70 min) from 48–51% to 14–18%, corresponding to an elimination of acetyl groups of the order of 60–70%. Acetyl groups in *O*-2 of xylapyranose units (X-2Ac) are the most resistant to alkaline hydrolysis. In the following pulping stages (70–160 min) a slight decrease of DS is observed. Surprisingly, at the end of the pulping, in pulps with kappa number 18.6 ± 0.3, a significant amount of acetyl groups remains in final xylan (DS around 9–11%). X-2Ac moieties dominate among the acetylated Xyl*p* units, although significant proportions of X-3Ac, X-2,3Ac and X-3Ac-2GlcA units coexist in the final xylans of all the three species investigated. The presence of such acetyl groups in *O*-2 may determine the discrepancy previously found in methylation analysis between *O*-2 substituted Xyl*p* and the of terminal MeGlcA and *O*-2 substituted MeGlcA, increasing artificially the abundance of the inferred [→2,4)-Xyl*p*-(1→] structure.

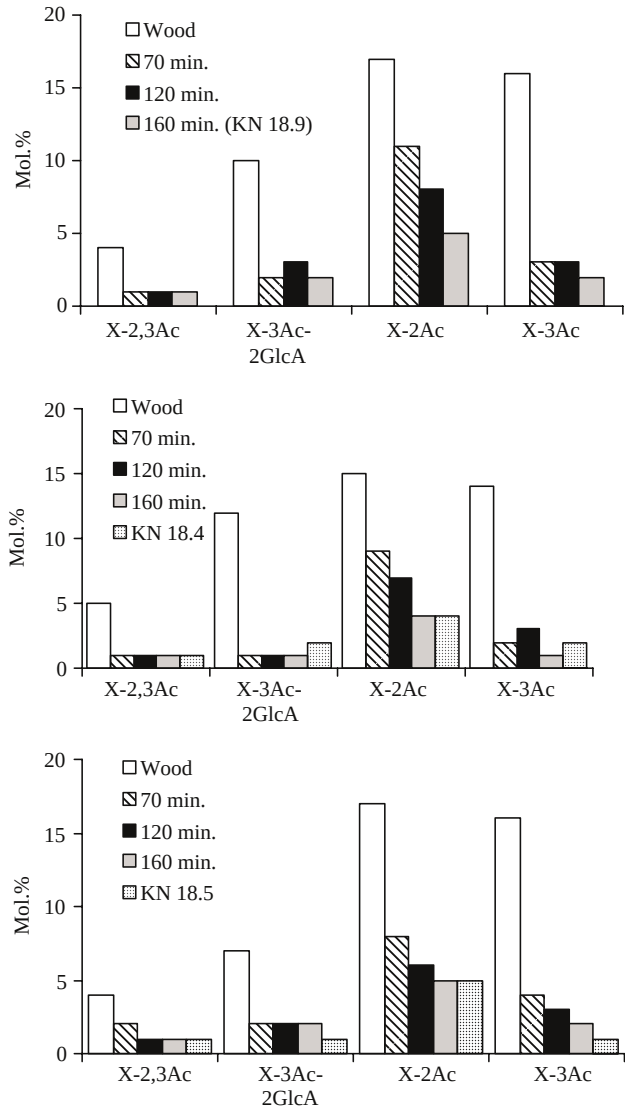

Fig. 5. Distribution of acetyl groups per 100 Xyl*p* units (%) of xylans from wood and pulps, as revealed from ^{1}H NMR. I, *E. globulus*; II, *E. urograndis*; III, *B. pendula*.

The persistency of acetyl groups in pulps xylans after the severe alkaline treatment undergone by wood during kraft pulping treatment was unexpected. Such result may be explained, partially at least, by the heterogeneous localization and lack of accessibility of xylans in wood cell walls, associated, probably, to local pH buffer effects, caused by neutralization of alkali by hydroxyacids issued from alkaline degradation of polysaccharides or by the hydrolysis of part of the acetyl groups.

4. Conclusions

The different hardwoods investigated showed significantly differently profiles of polysaccharides retention on pulp during wood kraft pulping, affecting the final pulp yield. When woods were kraft pulped to similar residual lignin contents (kappa number 18.6 ± 0.3), 4–5% (initial wood basis) of xylose was dissolved in *Eucalyptus globulus* and *Eucalyptus urograndis* against 11% in the case of *Betula pendula*, denoting a higher stability of *Eucalyptus* xylans. On the other hand, the amount of dissolved glucose (8–12%, wood basis) in *Eucalyptus* was higher than in *B. pendula*. Such difference may be assigned to the presence of amylopectin in *Eucalyptus* woods rather than to differences in celluloses structure.

The structural characterization of xylans in wood and pulps obtained along the kraft pulping allowed concluding that the different xylan retention is associated to differences in their composition and structure. *Eucalyptus* woods xylans showed a peculiar structure, containing, in addition to terminal 4-*O*-methyl-α-D-glucuronic acid (MeGlcA) (1→2) linked to the xylan backbone, MeGlcA *O*-2 substituted with galactose or glucose units. Such substituted uronic acid units (absent in *B. pendula* xylan) may constitute linking points between xylan and other cell wall polysaccharides, namely rhamnoarabinogalactans and glucans, contributing to retard xylan degradation and dissolution during kraft pulping. The degree of branching of the *Eucalyptus* xylans is also higher than in *B. pendula* (10–12 against 7 MeGlcA/100 Xyl*p*, respectively). A significant fraction of these units, particularly substituted MeGlcA in the case of *Eucalyptus*, persist in xylan till the end of pulping and, probably, may contribute to retard the sequential elimination of reducing end groups (peeling) in xylan. *Eucalyptus* wood xylans also showed average molecular weight (Mw) higher than that of *B. pendula* xylan, 31 against 24 kDa, respectively, contributing to retard the removal of xylan from fibres. During the pulping, the Mw of *E. globulus*, *E. urograndis* and *B. pendula* xylans is reduced to 16, 14 and 11 KDa, respectively.

The xylans of the woods investigated showed similar degree of acetylation (48–51 AcO/100 Xyl*p*), with the acetyl groups distributed between *O*-2, *O*-3, *O*-2 + *O*-3 of the Xylp units, and in *O*-3 of Xylp units substituted with MeGlcA groups. 60–70% of these acetyl groups are easily eliminated in the early stages of the pulping but, against our expectations, at the end of the pulping around 20% of the acetyl groups remain in pulp xylans.

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