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Isolation and structural characterization of polysaccharides dissolved in *Eucalyptus globulus* kraft black liquors

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Abstract

Polysaccharides dissolved in black liquors from *Eucalyptus globulus* kraft pulping were isolated by acid precipitation, analysed for monosaccharide composition and structurally characterized by methylation analysis and NMR spectroscopy. The precipitated oligo- and polysaccharides, representing 20% of the dissolved and/or degraded wood polysaccharides are essentially composed of xylan and amylopectin. The xylan dissolved in the alkaline black liquor showed a M_w of about 17–19 kDa as revealed by GPC analysis. The major part of the 4-*O*-methylglucuronic residues in the xylan from black liquor were degraded to hexenuronic (4-deoxy-β-L-*threo*-hex-4-enopyranosyluronic acid) units, which was not found to be so pronounced in the case for xylan in the corresponding kraft pulps. The overall structural and molecular weight features of both black liquor and pulp xylans during the kraft pulping are discussed.

Keywords: *Eucalyptus globulus*; Kraft pulping; Black liquor; Xylan; Structural analysis; NMR spectroscopy

1. Introduction

Kraft pulping is the process most widely used in the industrial production of cellulosic chemical pulps from wood. Wood chips are treated at high temperatures (150–170 °C) with an aqueous alkaline solution composed essentially of sodium hydroxide and sodium sulphide. Lignin, an aromatic polymer composed of dehydropolimerized structural units derived from phenylpropane, and primarily responsible for the cohesion between fibres in wood tissues, is degraded and dissolved almost completely (90–95%) in black liquor (the aqueous solution containing the inorganic and organic reaction by-products), allowing fibre separation. In such process, wood polysaccharides, namely cellulose, hemicelluloses and less abundant polysaccharides such as pectins, are also partially degraded to low molecular weight derivatives or dissolved in the black liquor, partially keeping its polymeric nature (Genco, Busayasakul, Medhora, & Robbins, 1990; Sjöström, 1977). The concentration and composition of black liquor dissolved polysaccharides is highly dependent on wood nature and pulping conditions (Simonson, 1963, 1965, 1971; Söderhjelm & Hausalo, 1996).

Part of the dissolved polysaccharides, particularly glucuronoxylan, when the pH of black liquor decreases in the latter stages of the pulping process (alkali depletion), may precipitate or be adsorbed at the surface of fibres, thus increasing the pulp yield and affecting pulp quality (Hansson, 1970; Hansson & Hartler, 1969; Simonson, 1963, 1965, 1971; Yllner & Enström, 1956, 1957). The information available on the detailed structure of the black liquor dissolved polysaccharides is quite scarce (Bikova, Klevinska, & Treimanis, 2000; Engström, Vikkula, Teleman, & Vuorinen, 1995; Simonson, 1963, 1965, 1971). Also, the effect of the composition and structure of the dissolved polysaccharides on the sorption/precipitation process is far from being completely understood.

Eucalypt woods now-a-days represent the major fibre sources in the Iberian Peninsula and South America and have become an attractive raw material for pulp and paper production in other regions of the world (Hillman, 2002). Among the different eucalypt species, *Eucalyptus globulus* is one of the most interesting for such an application.

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This stimulated the detailed chemical studies of the structure of macromolecular components of *E. globulus* (Evtuguin et al., 2001; Evtuguin, Tomás, Silva, & Pascoal Neto, 2003; Shatalov, Evtuguin, & Pascoal Neto, 1999), extractives (Freire, Silvestre, & Pascoal Neto, 2002) as well as their pulping behaviour (Pinto, Evtuguin, Pascoal Neto, & Silvestre, 2002; Pinto, Evtuguin, Pascoal Neto, Silvestre, & Amado, 2002) over the last few years. Particularly, *E. globulus* glucuronoxylan has shown a peculiar structure, quite different from that of other hardwoods such as birch, beech and aspen (Teleman, Lundqvist, Tjerneld, & Stalbrand, 2000; Teleman, Tenkanen, Jacobs, & Dahlman, 2002). About one-third of the 4-*O*-methyl-α-D-glucopyranosyl side residues of the (1→4)-linked β-D-xylanopyranosyl backbone are substituted at *O*-2 by galactosyl and glucosyl units (Evtuguin et al., 2003; Shatalov et al., 1999). It has been suggested that the uronosyl units constitute linkage points between the glucuronoxylan and other polysaccharides in wood cell walls such as glucans and rhamnoarabinogalactans (Evtuguin et al., 2003). The xylopyranosyl units showed an acetylation degree of 0.61 (Evtuguin et al., 2003). Such structural features, together with a high molecular weight (30–36 kDa), contribute to high xylan retention in pulp during kraft pulping. Only about 30% of the initial wood xylan is dissolved, against 40–50% for other industrially important wood species (Pinto, Evtuguin, & Pascoal Neto, 2002). The nature of the black liquor *E. globulus* xylan and its behaviour during the kraft pulping process is almost completely unknown. This lack of knowledge, together with the previously found structural peculiarities of *E. globulus* xylan, prompted us to investigate the composition and structure of black liquor polysaccharides.

In the present work, we have precipitated the polysaccharides dissolved in black liquor at different stages of *E. globulus* laboratory kraft pulping. The composition and structure of the isolated polysaccharides was assessed by monosaccharide and methylation analysis and 1D/2D NMR spectroscopy. The structure of dissolved and pulp retained glucuronoxylans is discussed.

2. Experimental

2.1. Kraft pulping experiments

Industrial chips were cooked by conventional kraft pulping, using a laboratory-scale batch reactor with forced liquor recirculation. The pulping conditions were as follows: liquid-to-wood ratio—4:1, active alkali charge (as Na_2O)—17%, sulfidity—28%, initial temperature—40 °C, final temperature—160 °C, heating rate—1 °C/min. The pulping experiments were interrupted at 100 min (140°, heating up period), 155 min (35 min at 160 °C), 170 min (50 min at 160 °C) and 200 min (80 min at 160 °C). At the end of the cooking, the black liquor (BL) was collected and allowed to cool until ambient temperature. The black liquor was purged by N_2 bubbling during 15 min and stored at 5 °C. The black liquor solids content was determined by drying 5 ml in an oven at 105 °C, until constant weight. The pulp was washed, air dried and weighted for pulp yield determination. The pulp residual lignin content was assessed by kappa number determination (TAPPI, 1996).

2.2. Precipitation and isolation of black liquor dissolved polysaccharides (BLPS)

The black liquor precipitated polysaccharides (BLPS) were isolated following, with minor modifications, a procedure previously described (Engström et al., 1995). About 200 ml of 1,4-dioxane were slowly added with agitation to 100 ml of black liquor, followed by the addition of glacial acetic acid until a pH of between 2 and 3 was reached. The solution and resulting precipitate was kept at 5 °C for 2 days. BLPS were separated by centrifugation and the solution decanted off. The precipitate was sequentially washed up with 150 ml of a 1,4-dioxane–water (2:1) solution, 150 ml of 1,4-dioxane, 150 ml of methanol and 150 ml of acetone and, finally, dried under vacuum with phosphorus pentoxide. The xylan fraction of BLPS was designated as BLX.

2.3. Isolation of pulps xylans (PX)

About 1.5–2 g of pulp were dispersed in water with vigorous mixing during 1 h. The pulp was filtered off and introduced into a three neck flask. Then, 50 ml of 10% aqueous KOH solution containing 0.007 g of $NaBH_4$ was added and left to extract for 2 h at ∼20 °C. The reaction flask was continuously purged with gaseous N_2. At the end of the extraction, the alkaline solution containing the xylan was separated from the pulp by filtration and the pulp was washed with 30 ml of KOH 10% solution and 80 ml of water. The xylan was precipitated, under agitation, by acidification with glacial acetic acid until pH 5–6, followed by the addition of ethanol to a total volume of 1000 ml. The solution containing the precipitated xylan was kept at 5 °C for 2 days and, then, the mixture was centrifuged (after the aspiration of the major part of the clear solution). Finally, the xylan was washed with absolute methanol (three times 150 ml) and dried under vacuum and phosphorus pentoxide. The extraction yields were of the order of 60–80% (w/w).

2.4. Carbohydrate analysis

The *E. globulus* wood (previously ground and sieved to 40–60 mesh), BLPS, PX and corresponding pulps were submitted to Saeman hydrolysis (treatment with H_2SO_4 72% for 3 h at 25 °C, followed by hydrolysis with 1 M H_2SO_4 during 2.5 h at 100 °C). The monosaccharide composition was determined as alditol acetate derivatives by gas chromatography (Selvendran, March, & Ring, 1979).

2.5. Methylation (linkages) analysis

The black liquor precipitated polysaccharides (BLPS) were methylated by two consecutive treatments with methyl iodide in DMSO (NaOH pellets were used to ionise the hydroxyl groups) followed by carboxyl-reduction of the methylated polysaccharides with $LiAlD_4$ in THF to identify the uronic acid derivatives (Lindberg & Lönngren, 1978; Ring & Selvendran, 1978). The methylated BLPS were hydrolysed with 90% HCOOH for 1.5 h at 105 °C, and then with 0.15 M H_2SO_4 for 6 h at 105 °C. After neutralization with NH_3 25%, the methylated BLPS were reduced with $NaBH_4$ and acetylated (Chaplin & Kennedy, 1994). The methylated alditol acetates were identified and quantified by GC-MS (Trace GC 2000 series coupled with Finnigan Trace Ms mass spectrometer) using a DB-1 capillary column (30 m × 0.32 mm i.d., 0.25 μm film thickness) and the following column temperature program: 120 °C (5 min)–225 °C (35 min), heating rate of 2 °C/min; injector temperature: 230 °C, detector temperature: 250 °C.

2.6. GPC Analysis

The GPC analysis of BLPS and PX was performed on two Plgel 10 μm MIXED B 300 mm × 7.5 mm columns protected by a Plgel 10 μm pre-column (Polymer Laboratories Ltd, UK) using a PL-GPC 110 system (Polymer Laboratories). The xylans were dissolved in *N*,*N*-dimethylacetamide (DMA) containing 10% LiCl (w/v) and further diluted with DMA to a xylan concentration of about 0.4% (4 mg/ml). The pre-column and columns, the injector and the detector (RI) were kept constant at 70 °C. DMA with 0.1 M of LiCl (w/v) was used as eluent at a rate of 0.9 ml/min. The calibration of the GPC columns was made with pullulan reference materials (Polymer Laboratories).

2.7. ^1H NMR spectroscopy

The dry precipitated BLPS and PX were dissolved in D_2O and sodium 3-(trimethylsilyl)-propionate-d_4 was added as internal standard. The 1D, ^1H NMR spectra (300 MHz) were recorded at ambient temperature on a Bruker AMX 300 spectrometer. A relaxation delay of 8 s and r.f. angle of 90 °C were used and 600 scans were collected.

The 2D ^1H–^1H TOCSY spectrum ($\tau_{mix} = 0.050$ s) was acquired using the MLEVST pulse program. A spectral width of 2185 Hz was employed in both dimensions. The relaxation delay was 1.5 s. For each FID 128 transients were acquired, the data size was 1024 in $t_1 \times 512$ in t_2. The phase sensitive ^1H-detected HSQC (heteronuclear single quantum coherence) spectrum was acquired over a $F1$ spectral width of 12,000 Hz and a $F2$ width of 2000 Hz with a 2048 × 1024 matrix and 128 transients per increment. The delay between scans was 2 s and the delay for polarization transfer was optimized for $^1J_{CH} = 150$ Hz.

3. Results and discussion

Wood kraft delignification is generally divided into three distinct consecutive kinetic phases (Sjöström, 1993): the initial phase, corresponding to the reactor heating up period, with a slow delignification rate, where about 20–30% delignification takes place, followed by the bulk phase, with a much faster lignin removal rate, leading to about 90% delignification and, finally, the residual phase, where the rate of delignification decreases. The extent of delignification reached at end of the cooking is of the order of 95%. The initial phase is the least selective, the most significant wood polysaccharide dissolution takes place during this period, essentially by direct dissolution and β-elimination of the terminal reducing end groups (*peeling*). In the bulk and residual phases, the elimination of polysaccharides is much less significant, although in the residual phase random alkaline hydrolysis of glycosidic linkages may contribute significantly to the decrease in the degree of polymerization of polysaccharides in the pulp (Sjöström, 1993). In the present work, four *E. globulus* kraft pulps and the respective black liquors were produced with different pulping times (Table 1): 100 min, corresponding to the end of the initial phase (140 °C, heating up period), 155 min in the bulk delignification phase (160 °C) and 170 and 200 min, representing the residual phase (160 °C).

3.1. Isolation and general characterization of black liquor dissolved polysaccharides

The acidification (pH 2–3) of the black liquors (BL), obtained from the different phases of the kraft delignification, in the presence of dioxane lead to the formation of a precipitate which, after purification, is composed essentially of polysaccharides (BLPS), as indicated by the neutral monosaccharide content (about 80–90% of BLPS weight, Table 1) and GPC analysis. It is worth noting that uronic acids (present in our samples, as will be seen later) were not quantified and, thus, the carbohydrate content of BLPS should be even higher than 80–90%, suggesting very low

Table 1
The kappa number and pulp yield of the *E. globulus* kraft pulps investigated and the composition of the corresponding black liquors (BL)

	Pulping time (min)			
	100	155	170	200
Kappa number	n.d.	17.9	13.8	12.8
Pulp yield (%)	79.7	55.2	54.7	54.03
BL solids content, % (w/V)	10.4	14.0	14.5	14.3
BL precipitated polysaccharides (BLPS) concentration				
BL solids (%)	2.9	7.3	6.9	6.0
BL (g/l)	3.0	10.2	10.1	8.6
Neutral anhydro monosaccharide content of BLPS, % (w/w)	83.5	78.2	87.9	81.6

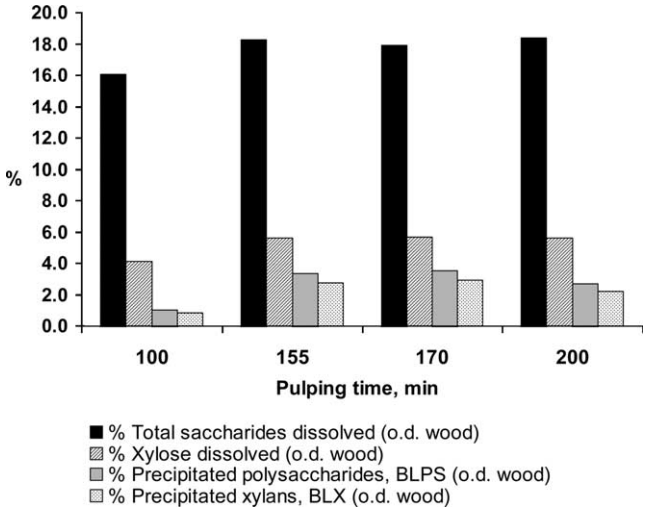

Fig. 1. The balance of total dissolved saccharides (determined by difference from the wood and pulps anhydro monosaccharide composition) and black liquor precipitated polysaccharides, BLPS and BLX (xylan content is estimated as anhydroxylose content of wood, pulps and black liquor precipitated polysaccharides).

lignin contamination. BLPS represent about 20% of the total saccharides removed from wood in the bulk and residual phases of delignification (Fig. 1).

The neutral carbohydrate analysis of BLPS (Table 2) suggests that xylans predominate in the precipitated polysaccharides (xylose represents around 82% of BLPS weight). However, the composition is quite different from that of the xylans, which remained in the pulp (PX) (Table 2). Particularly, high amounts of glucose and galactose were found in BLPS. Part of the galactose units, at least, should be linked at the *O*-2 position to some of the 4-*O*-methylglucuronic units (Evtuguin et al., 2003; Shatalov et al., 1999). The high glucose content (around 10%) could indicate the presence of cellulose oligomers or other glucans, which are resistant to the alkaline degradation.

The profile of the total dissolved saccharides and precipitated polysaccharides is quite different for the different kinetic phases of the kraft pulping process (Fig. 1, Table 1). As expected, polysaccharide dissolution occurs essentially during the initial phase of delignification (Fig. 1). The saccharides dissolved in the black liquor at the end of the initial phase (100 min) represent 16% of the initial wood weight (Fig. 1). This amount increases and stabilizes at 18% in the bulk phase (155 min), it then increases slightly again in the last stages of the residual phase (200 min). The dissolved xylan (determined as anhydroxylose) increases from about 4% (o.d. wood basis) in the initial phase to around 5.6% in the bulk phase. No significant xylan dissolution from pulp takes place after 155 min. Interestingly, the amount of polysaccharides precipitated from black liquor (BLPS) rises from the initial (3 g/l) to the bulk phase (10 g/l), diminishing in the residual phase (Fig. 1, Table 1). Such a decrease may be a result of the intensification of degradation reactions of the dissolved polysaccharides in the alkaline medium ('peeling' and alkaline hydrolysis). This behaviour was previously observed for other wood species (Simonson, 1963, 1965, 1971). The amount of xylan precipitated from black liquor (determined as anhydroxylose) in the bulk phase (155 min) represents about 50% of the xylan removed from wood, decreasing to 40% in the residual phase (200 min) (Fig. 1).

Table 2
Neutral monosaccharide composition (%) of xylans isolated from kraft pulps and polysaccharides isolated from black liquors

	Pulping time (min)	Rha	Fuc	Ara	Xyl	Man	Gal	Glc
Pulp xylans (PX)	100	0.1	0.0	0.3	91.1	0.1	3.0	5.5
	155	0.1	0.0	0.2	97.0	0.0	1.8	0.8
	170	0.1	0.0	0.2	97.4	0.0	1.4	0.8
	200	0.1	0.0	0.2	97.7	0.0	1.3	0.6
Black liquor precipitated polysaccharides (BLPS)	100	0.5	0.0	1.1	82.5	0.9	4.8	10.2
	155	0.2	0.0	1.0	82.0	0.4	6.2	10.2
	170	0.2	0.0	1.3	82.4	0.5	6.3	9.4
	200	0.1	0.0	1.7	81.3	0.5	6.3	10.1

The gel permeation chromatograms of BLPS and PX (Fig. 2) showed the main elution peak between 14 and 18 min, whereas the GPC curve of BLPS additionally revealed a broad shoulder between 12 and 14 min, corresponding to a polysaccharide fraction with a higher average molecular weight (M_w) or to a polysaccharide with a more ramified structure than xylan possessing a higher hydrodynamic molar volume. The major elution peak in BLPS and PX, corresponding to molecular weights around 20–30 kDa, is attributed to xylan (Evtuguin et al., 2003; Shatalov et al., 1999). The M_w of the black liquor precipitated xylan (Table 3) ranges between 18,900 Da in the initial phase and of 17,700 Da in the residual phase. These figures are of the same order of magnitude of those found in industrial black liquors from different wood species

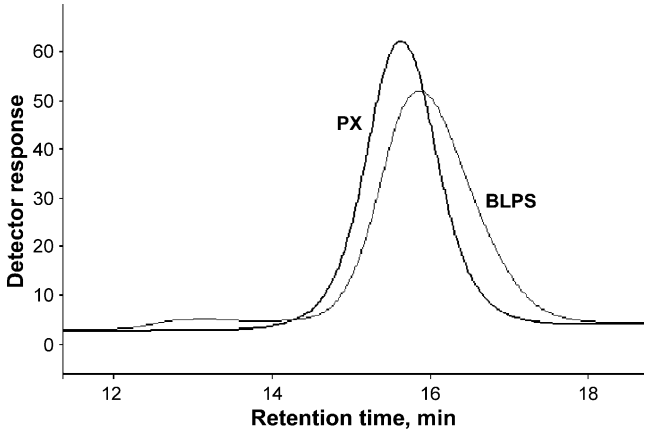

Fig. 2. The GPC elution curve of xylan isolated from pulp (PX) and polysaccharides precipitated from black liquor (BLPS) for the 155 min kraft pulping experiment.

Table 3
Average molecular weight (Da) of xylans isolated from kraft pulps and polysaccharides isolated from black liquors (calculations for BLPS included only the peak eluting between 14 and 18 min assigned to xylan—see Fig. 2)

Pulping time (min)	Pulp xylans (PX)	Black liquor precipitated polysaccharides (BLPS)
100	25,000	18,900
155	27,400	17,600
170	27,000	18,400
200	25,500	17,700

(Bikova et al., 2000; Söderhjelm & Hausalo, 1996). The small M_w variation is a clear evidence of the stability of BLPS in the strongly alkaline conditions prevailing in black liquor. Results from other woods (Bikova et al., 2000; Simonson, 1965) showed that when the pulping is extended, a strong decrease in M_w of dissolved polysaccharides might be observed (Bikova et al., 2000). Again, such a difference may be assigned essentially to the structural specificities of *E. globulus* xylan (Evtuguin et al., 2003; Shatalov et al., 1999). The M_w of BLPS are around 30% lower than those of xylans isolated from the corresponding pulps (Table 3), suggesting that the dissolved xylans have lower molecular weights than those remaining in the pulp. A partial depolymerization of the dissolved polysaccharides may also contribute to lower molecular weight.

Because of their high molecular weight, the black liquor dissolved polysaccharides, precipitated and isolated by acidification from our laboratory black liquor, corresponding to about 3.5% of the initial wood weight at the beginning of the residual phase (170 min, Fig. 1), represent a potential way to increase the yield of industrial pulp production. Under our pulping conditions, the increase of pulp yield at the end of the cooking was not observed (Table 1), probably because the alkaline charge used is higher than typically used in industry. The conditions determining the selective sorption or precipitation of such polysaccharides at fibre surfaces are now under investigation.

3.2. Structural analysis of xylans

The structural changes in xylans remaining in pulp (PX) and black liquor precipitated polysccharides (BLPS) have been monitored using ^{1}H NMR (Fig. 3). The analysis of the anomeric region of the ^{1}H NMR spectra showed the appearance of new signals at 5.36 and at 5.82 ppm both in PX and BLPS. These are normally assigned to H-1 and H-4, respectively, in 4-deoxy-β-L-*threo*-hex-4-enopyranosyluronic acid (HexA) residues formed from 4-*O*-methyl-α-D-glucopyranosyluronic acid (MeGlcA) units by a β-elimination of methoxyl group under alkaline pulping conditions (Teleman et al., 1995). Simultaneously, the resonance of H-1 in MeGlcA residues at 5.29 ppm diminished gradually

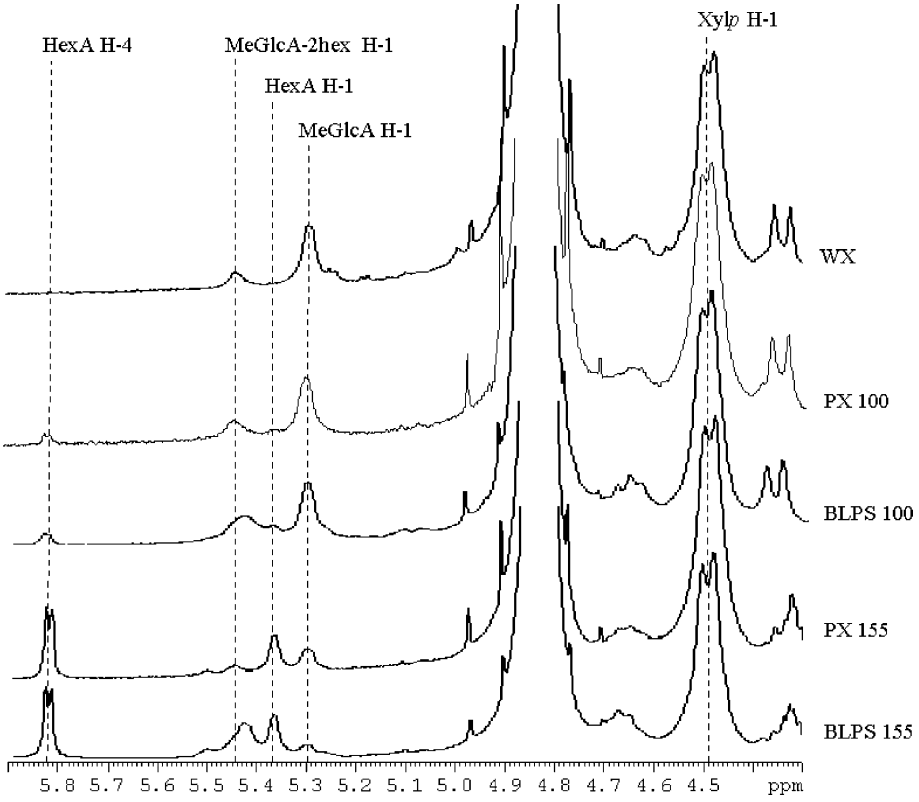

Fig. 3. ^{1}H NMR spectra of wood xylan (WX) and xylans isolated from pulp (PX) and polysccharides from black liquor (BLPS) after the 100 (PX100 and BLPS100) and 155 min (PX155 and BLPS155) kraft pulping of *E. globulus* wood.

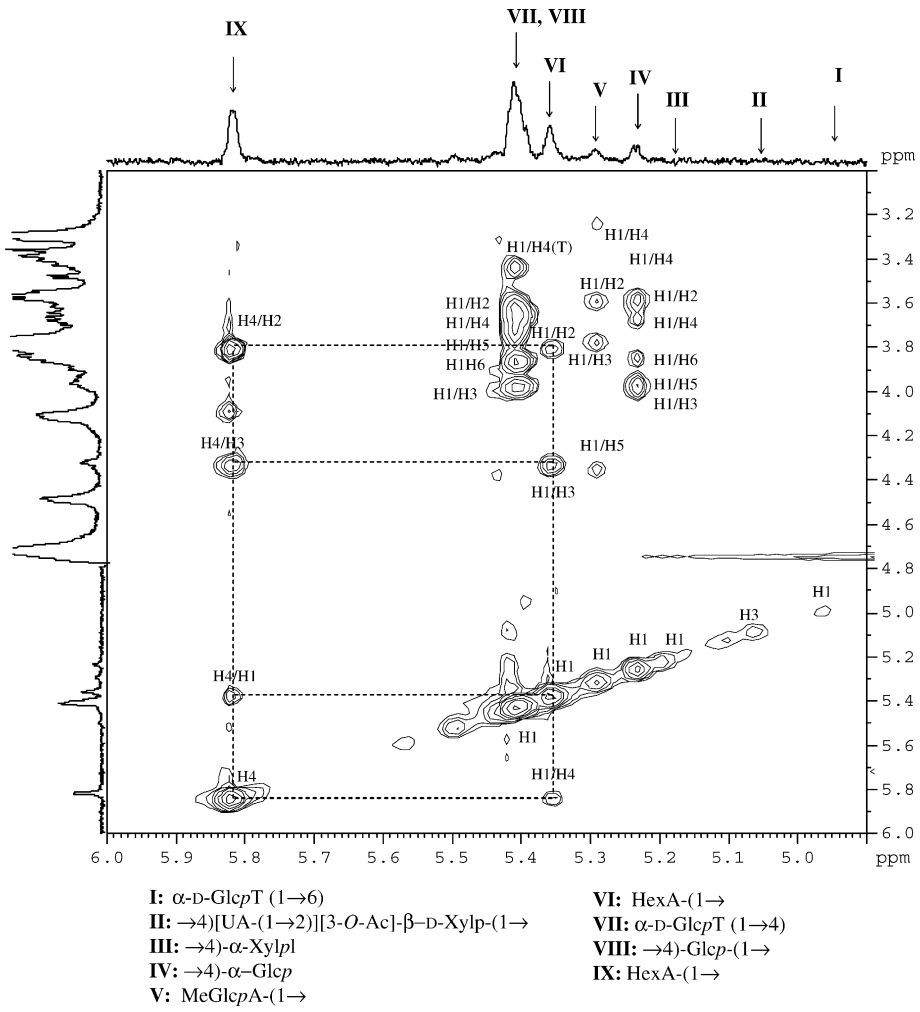

Fig. 4. Anomeric region of the TOCSY spectrum (D_2O, 25 °C) of BLPS obtained after 155 min kraft pulping of *E. globulus* wood.

during the pulping (Fig. 3). According to the analyses of *E. globulus* kraft pulps, more than 60% of MeGlcA residues are transformed to HexA moieties affecting the ECF bleaching because such units contribute to the consumption of chlorine dioxide (Daniel, Pascoal Neto, Evtuguin, & Silvestre, 2003).

The main difference between PX and BLPS is due to the different intensity of the resonance at 5.41 ppm. This signal is close to a resonance at 5.42–5.44 ppm assigned to H-1 in MeGlcA units ramified at *O*-2 with D-Gal*p* or with D-Glc*p* (MeGlcA–2Hex) (Evtuguin et al., 2003; Shatalov et al., 1999) and is rather weak in the spectra of pulp xylans. In the ^{1}H NMR spectra of BLPS the signal at 5.41 is dominant when compared to other anomeric protons. However, a TOCSY spectrum did not confirm, the presence of highly abundant MeGlcA–2Hex residues in BLPS. The typical proton correlations in MeGlcA–2Hex at 3.27 (H-4), at 3.77 (H-2) and at 3.87 (H-3) ppm were absent in TOCSY spectra (Fig. 4). Additionally, in ^{1}H–^{13}C correlation experiments (HSQC) no correlation was detected for the former protons and carbons at 83.1 (C-4), at 75.9 (C-2) and at 73.6 (C-3) ppm, respectively (Fig. 5). The accurate analysis of multiple-bond ^{1}H–^{1}H and single-bond ^{1}H–^{13}C correlation patterns (Figs. 4 and 5) coupled with known literature data (Nilsson, Bergquist, Nilsson, & Gorton, 1996; Teleman, Kruus, Ämmälahti, Buchert, & Nurmi, 1999) suggest that the signals of anomeric protons at 5.41, 5.23 and 4.96 ppm in ^{1}H NMR spectra of BLX belong to ramified α-D-glucan built of α-(1→4)-linked D-glucose residues with α-(1→6)-linked branching points, i.e. amylopectin. This was confirmed by a linkage analysis showing the presence of the structural units →4)-α-D-Glc*p*-(1→ and →4,6)-α-D-Glc*p*-(1→ with a molar proportion of around 10:1 (Table 4). The average degree of polymerization of the linear backbone in amylopectin oligomers, calculated based on molar ratio of the sum of →4)-α-D-Glc*p*-(1→ and →4,6)-α-D-Glc*p*-(1→ units and terminal α-D-Glc*p*-(1→ unit, was around 11. BLPS samples dissolved in water, after the addition of iodine showed the typical violet colour of starch/iodine complex.

Apparently, the α-D-glucan detected in BLPS is not chemically linked to the black liquor dissolved xylan (BLX) because BLPS showed a bimodal molecular weight distribution curve (Fig. 2). The ramified amylopectin oligomers should have a hydrodynamic volume higher

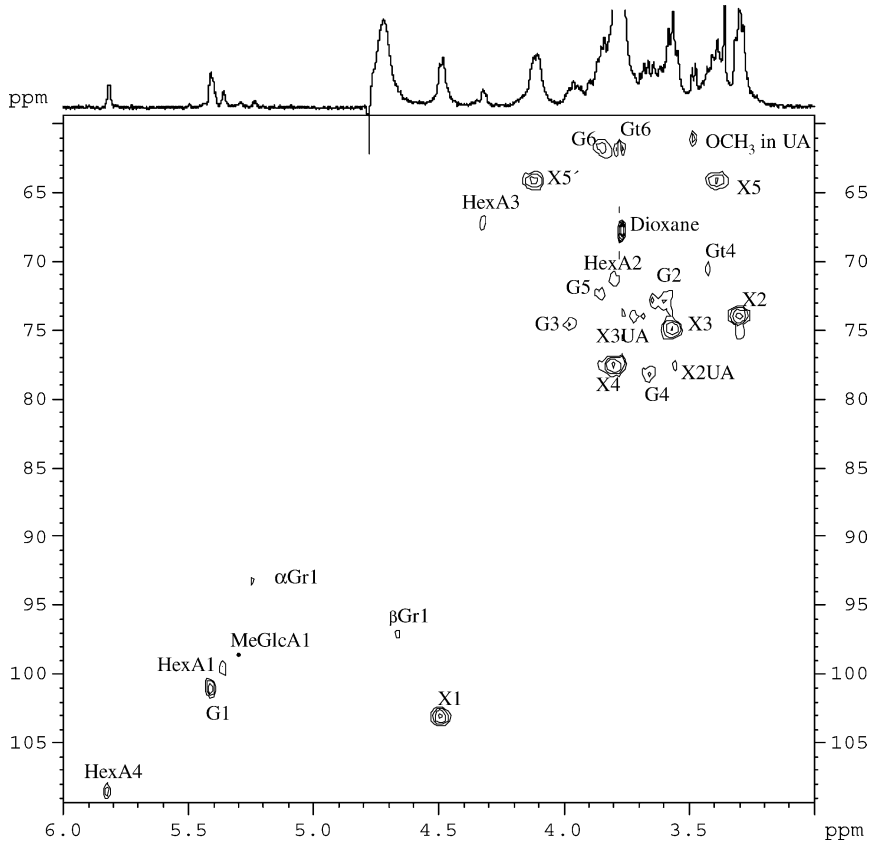

Fig. 5. HSQC (D_2O, 25 °C) spectrum of BLPS isolated from black liquor after the 155 min kraft pulping of *E. globulus* wood. Designations are as follows: X, internal Xyl*p* unit in the heteroxylan backbone; XUA, internal Xyl*p* unit branched at *O*-2 with uronic acid residue (MeGlcA, MeGlcA–Hex or HexA); G, internal (1→4)-linked α-D-Glc*p* unit in the D-glucan backbone; αGr and βGr, α- and β-isomers, respectively, in the terminal reductive D-Glc*p* unit; Gt, terminal non-reductive α-D-Glc*p* unit.

than that of xylan, being eluted faster from the GPC column. The corresponding PX sample, where α-D-glucan was not detected, showed the normal characteristic unimodal molecular weight distribution.

The presence of significant amounts of amylopectin explains the high glucose content in the sugar analysis of BLPS (Table 2) and the inbalance between the weights of BLPS and BLX (Fig. 1). To our knowledge, the presence of the relatively high contents of amylopectin in the *E. globulus* wood is clearly shown here for the first time.

The notable amounts of different galactopyranose, arabinofuranose and minor amounts of rhamnopyranose units, revealed in linkage analysis (Table 4), may be attributed to rhamnoarabinogalactan oligomers linked at *O*-2 of MeGlcA in BLX, as has been previously suggested for the eucalypt wood heteroxylan (Evtuguin et al., 2003). The unbalance between the molar proportion of →2,4)-β-D-Xyl*p*-(1→ and the sum of molar proportions of Glc*p*A-(1→ and of →2)-Glc*p*A-(1→ can be explained by the missing HexA residues contribution, which are inaccessible in the methylation analysis.

^{1}H NMR spectra also enabled the estimation of the relative proportions of different uronosyl groups in PX and BLX during the pulping. Although the PX do not represent the whole xylan in pulp (extraction yields in the range of 50–80%), and have a higher uronic acids content than it would be expected (Evtuguin et al., 2003; Shatalov et al., 1999), some valuable conclusions may

Table 4
Methylation analysis of polysaccharides precipitated from black liquor (BLPS) for the 155 min kraft pulping experiment

Methylated residue	Structural units deduced	Relative mole ratio (mol%)
Xyl-2,3,4	Xyl*p*-(1→	1
Xyl-2,3	→4)-Xyl*p*-(1→	86
Xyl-3	→2,4)-Xyl*p*-(1→	11
Xyl-2	→3,4)-Xyl*p*-(1→	1
Glc-2,3,4 (D_2)	Glc*p* A-(1→	3
Glc-3,4 (D_2)	→2)-Glc*p* A -(1→	1
Gal-2,3,4,6	Gal*p* -(1→	3
Gal-2,3,6	→4)-Gal*p* -(1→	3
Gal-2,3	→4,6)-Gal*p* -(1→	1
Gal-2,4	→3,6)-Gal*p* -(1→	2
Glc-2,3,4,6	Glc*p* -(1→	2
Glc-2,3,6	→4)-Glc*p* -(1→	20
Glc-2,3	→4,6)-Glc*p* -(1→	2
Rha-3	→2,4)-Rha*p*-(1→	<1
Rha-2,4	→3)-Rha*p*-(1→	<1
Ara-2,3,5	Ara*f*-(1→	1

Table 5
^{1}H quantification of uronic acid residues in black liquor precipitated xylans, BLX (number of residues per 100 internal Xyl*p* units)

	Pulping time (min)	MeGlcA	MeGlcA–2Hex	HexA
Pulp xylans (PX)	100	18	8	4
	155	8	4	8
	170	7	3	9
	200	6	3	9
Black liquor precipitated xylan (BLX)	100	18	6	4
	155	4	1	11
	170	3	<1	11
	200	3	<1	10

be drawn from the quantitative ^{1}H NMR analysis. The frequency of the backbone substitutions by different kinds of uronic moieties (MeGlcA, MeGlcA–2Hex and HexA) was calculated per 100 anomeric protons in the internal Xyl*p* units (Fig. 3) and are presented in Table 5. When compared to PX, at the initial phase of the pulping (100 min) BLX showed rather similar abundance of different uronosyl residues. However, in the bulk (155 min) and in the residual phase of delignification (170 and 200 min), BLX revealed a higher relative conversion of MeGlcA and MeGlcA–2Hex to HexA than PX. As a general tendency, the amount of MeGlcA and MeGlcA–2Hex groups decreased gradually both in PX and BLX during the pulping, being, however, almost twice in abundance in PX. At the end of the cooking (170–200 min) the black liquor precipitated xylans showed about 10 HexA and 3 MeGlcA groups per 100 internal Xyl*p* units, while the MeGlcA–2Hex units almost disappeared (Table 5).

4. Conclusions

During the kraft pulping of *E. globulus*, polysaccharides are gradually degraded and/or dissolved in the black liquor to about 18% of initial wood weight. About 20% of such dissolved saccharides may be selectively precipitated as oligo or polysaccharides by black liquor acidification. Xylans degraded and/or dissolved, represent about 5.6% of wood weight and are precipitated from black liquor to an extent of 40–50%. Amylopectin oligosaccharides were found to be the second most abundant fraction of the black liquor precipitated polysaccharides. The black liquor xylans had molecular weights in the range 17–19 kDa, about 30% lower than those remaining in pulp. The small variations observed for the molecular weight of the black liquor xylan along the kraft pulping (even when the cooking is extended) showed the relatively high stability of these polysaccharides to the strongly alkaline medium of the pulping solution. The main structural transformations occur in the xylan side chain moieties. The xylans dissolved in the initial stages of the pulping are rich in uronic acid groups, namely MeGlcA (methyl-α-D-glucopyranosyluronic acid), MeGlcA–2Hex (MeGlcA unit ramified at *O*-2 with D-Gal*p* or with D-Glc*p*) and HexA (4-deoxy-β-L-*threo*-hex-4-enopyranosyluronic acid). During pulping, the black liquor xylans are progressively enriched in HexA, by partial conversion of MeGlcA, while MeGlcA–2Hex units practically disappear.

The investigated oligo- and polysaccharides precipitated from black liquor (representing about 3.5% of the initial wood weight) may be potentially sorbed or precipitated at the surface of fibres, thus increasing the yield of kraft pulp production in an industrial situation. The conditions determining the selective sorption or precipitation of such polysaccharides at fibres surfaces, while keeping lignin in solution, are now under investigation.

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References

Bikova, T., Klevinska, V., & Treimanis, A. (2000). Monitoring of lignin and hemicelluloses in spent cooking liquor during kraft delignification. *Holzforschung*, *54*(1), 66–70.

Chaplin, M. F., & Kennedy, J. F. (Eds.). (1994). *Carbohydrate analysis: A practical approach* (2nd ed.). (pp. 81–87). New York: IRL Press at Oxford University Press.

Daniel, A. I. D., Pascoal Neto, C., Evtuguin, D. V., & Silvestre, A. J. D. (2003). Hexenuronic acid contents of *Eucalyptus globulus* kraft pulps: variation with pulping conditions and effect on ECF bleachability. *Tappi Journal*, *2*(5), 3–8.

Engström, N., Vikkula, A., Teleman, A., & Vuorinen, T. (1995). Structure of hemicellulose in pine kraft cooking liquors. (pp. 195–200). *Proceedings of the Eighth International Symposium on Wood and Pulping Chemistry, 6–9 June, Helsinki, Finland*.

Evtuguin, D. V., Pascoal Neto, C., Silva, A., Domingues, P. M., Amado, F. M. L., Robert, D., & Faix, O. (2001). Comprehensive study on the chemical structure of dioxane lignin from plantation *Eucalyptus globulus* wood. *Journal of Agriculture and Food Chemistry*, *49*, 4252–4261.

Evtuguin, D. V., Tomás, J. L., Silva, A. M. S., & Pascoal Neto, C. (2003). Characterization of an acetylated heteroxylan from *Eucalyptus globulus* labill. *Carbohydrate Research*, *338*, 597–604.

Freire, C. S. R., Silvestre, A. J. D., & Pascoal Neto, C. (2002). Identification of new hydroxy fatty acids and ferulic acid esters in the wood of *Eucalyptus globulus*. *Holforschung*, *56*(2), 143–149.

Genco, J. M., Busayasakul, N., Medhora, H. K., & Robbins, W. (1990). Hemicellulose retention during kraft pulping. *Tappi Journal* , 223–233.

Hansson, J. (1970). Sorption of hemicelluloses on cellulose fibres. Part 3. The temperature dependence on sorption of birch xylan and pine glucomannan at kraft pulping conditions. *Svensk Papperstidning*, *73*(3), 49–53.

Hansson, J., & Hartler, N. (1969). Sorption of hemicelluloses on cellulose fibres. Part 1. Sorption of xylans. *Svensk Papperstidning*, *72*(17), 521–530.

Hillman, D. C. (2002). Single-species pulping. The world's preferred market pulps. *Solutions*, *Nov*, 27–28.

Lindberg, B., & Lönngren, J. (1978). Methylation analysis of complex carbohydrates: general procedure and application for sequence analysis. Methods Enzymology, *50*, 3–33.

Nilsson, G. S., Bergquist, K. E., Nilsson, U., & Gorton, L. (1996). Determination of the degree of branching in normal and amylopectin type potato starch with ^{1}H NMR spectroscopy—improved resolution and two-dimensional spectroscopy. *Starch/Stärke*, *48*, 352–357.

Pinto, P. C., Evtuguin, D. V., & Pascoal Neto, C. (2002). Chemical structure and kraft pulping behaviour of xylans from three different hardwoods: a comparative study. (pp. 313–316). *Proceedings of the Seventh European Workshop on Lignocellulosics and Pulp, 26–29 August, Turku, Finland.*

Pinto, P. C., Evtuguin, D. V., Pascoal Neto, C., & Silvestre, A. J. D. (2002). Behaviour of *Eucalyptus globulus* lignin during kraft pulping. Part 1. Analysis by chemical degradation methods. *Journal of Wood Chemistry and Technology*, *22*(2/3), 93–108.

Pinto, P. C., Evtuguin, D. V., Pascoal Neto, C., Silvestre, A. J. D., & Amado, F. M. L. (2002). Behaviour of *Eucalyptus globulus* lignin during kraft pulping. Part 2. Analysis by NMR, ESI/MS and GPC. *Journal of Wood Chemistry and Technology*, *22*(2/3), 109–125.

Ring, S. G., & Selvendran, R. R. (1978). Purification and methylation analysis of cell-wall materials from *Solanum tuberosum*. *Phytochemistry*, *17*(4), 745–752.

Selvendran, R. R., March, J. F., & Ring, S. G. (1979). Determination of aldoses and uronic acids content of vegetable fiber. *Analytical Biochemistry*, *96*, 282–292.

Shatalov, A. A., Evtuguin, D. V., & Pascoal Neto, C. (1999). (2-*O*-α-D-Galactopyranosyl-4-*O*-methyl-α-D-glucurono)-D-xylan from *Eucalyptus globulus* Labill. *Carbohydrate Research*, *320*, 93–99.

Simonson, R. (1963). The hemicellulose in the sulfate pulping process. Part 1. The isolation of hemicellulose fractions from pine sulfate cooking liquors. *Svensk Papperstidning*, *66*(20), 839–845.

Simonson, R. (1965). The hemicellulose in the sulfate pulping process. Part 3. The isolation of hemicellulose fractions from birch sulfate cooking liquors. *Svensk Papperstidning*, *68*(8), 275–280.

Simonson, R. (1971). The hemicellulose in the sulfate pulping process. *Svensk Papperstidning*, *74*(21), 691–700.

Sjöström, E. (1977). The behaviour of wood polysaccharides during alkaline pulping process. *Tappi Journal*, *60*(9), 151–154.

Sjöström, E. (1993). *Wood pulping* (2nd ed.) *Wood chemistry. Fundamentals and applications*. London, UK: Academic Press Inc. pp. 140–164.

Söderhjelm, L., & Hausalo, T. (1996). Extensive analysis of strong black liquor. *Appita*, *49*(4), 263–268.

TAPPI Test Methods (1996). Atlanta, USA: TAPPI Press.

Teleman, A., Hajunpää, V., Tenkanen, M., Buchert, J., Hausalo, T., Drakenberg, T., & Vuorinen, T. (1995). Characterization of 4-deoxy-β-L-*threo*-hex-4-enopyranosyluronic acid attached to xylan in pine kraft pulp and pulping liquor by ^{1}H and ^{13}C NMR spectroscopy. *Carbohydrate Research*, *272*, 55–71.

Teleman, A., Kruus, K., Ämmälahti, E., Buchert, J., & Nurmi, K. (1999). Structure of dicarboxyl malto-oligomers isolated from hypochlorite-oxidised potato starch studied by ^{1}H and ^{13}C NMR spectroscopy. *Carbohydrate Research*, *315*, 286–292.

Teleman, A., Lundqvist, J., Tjerneld, F., & Stalbrand, H. (2000). Characterization of acetylated 4-*O*-methylglucuronoxylan isolated from aspen employing ^{1}H and ^{13}C NMR spectroscopy. *Carbohydrate Research*, *329*, 807–815.

Teleman, A., Tenkanen, M., Jacobs, A., & Dahlman, O. (2002). Characterization of *O*-acetyl-(4-*O*-methylglucurono)xylan isolated from birch and beech. *Carbohydrate Research*, *337*, 373–377.

Yllner, S., & Enström, B. (1956). Studies of the adsorption of xylan on cellulose fibres during the sulphate cook. Part 1. *Svensk Papperstidning*, *59*(6), 229–232.

Yllner, S., & Enström, B. (1957). Studies of the adsorption of xylan on cellulose fibres during the sulphate cook. Part 2. *Svensk Papperstidning*, *60*(15), 549–554.