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Acetylated heteroxylan from *Agave sisalana* and its behavior in alkaline pulping and TCF/ECF bleaching

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ABSTRACT

The heteroxylan from sisal (*Agave sisalana*), an O-acetyl-(4-*O*-methylglucurono)xylan with a molecular weight (Mw) of 18 kDa, was isolated by extraction of peracetic holocellulose with Me_2SO and thoroughly characterized by wet chemistry, and NMR spectroscopy. The heteroxylan backbone is composed of (1 → 4)-linked β-D-xylopyranosyl units (Xyl*p*) partially branched with terminal (1 → 2)-linked 4-*O*-methyl-α-D-glucuronosyl (MeGlc*p*A, 9 mol%) and a small proportion of α-D-glucuronosyl (Glc*p*A, <1 mol%) residues. Roughly 61 mol% of Xyl*p* residues are acetylated (DS = 0.70). During soda/AQ pulping of sisal fibers, MeGlc*p*A and Glc*p*A are mostly removed or converted to 4-deoxy-β-L-*threo*-hex-4-enopyranosyluronic acid (HexA), although about 15% of the initially present MeGlc*p*A was maintained intact upon cooking. The major part of acetyl groups (95%) was hydrolyzed during pulping. It was proposed that during bleaching, a low molecular weight xylan fraction associated to residual lignin was removed from pulp and small proportion of MeGlc*p*A was additionally converted to HexA. The profiles of uronosyl residues in xylans from TCF and ECF bleached sisal pulps were rather different.

1. Introduction

Sisal fibers are hard fibrous material isolated from the leaves of the sisal plant (*Agave sisalana*), a monocotyledonous plant from the *Agavaceae* family (Gutiérrez, Rodríguez, & del Río, 2008; Li, Mai, & Ye, 2000). Originally from Central America and Mexico, sisal plant is widely cultivated in South America (e.g. Brazil), Australia and Africa (e.g. Kenya) (Gutiérrez et al., 2008; Mwaikambo, 2006). The sisal fibers find out numerous applications in the manufacture of ropes for boats, goods for the agricultural industry and for the reinforcement of polymeric matrices (Gutiérrez et al., 2008; Li et al., 2000; Megiatto, Houreau, Gardrat, Frollini, & Castellan, 2007; Mwaikambo, 2006). Sisal cellulosic pulp possesses such characteristics as high tear resistance, alpha cellulose content, porosity, bulk, moister absorbency and high folding endurance, that offer unique opportunities for the papermaking (Gutiérrez et al., 2008; Hurter, 2001; Idárraga, Ramos, Young, Denes, & Zuñiga, 2001; Megiatto et al., 2007; Mwaikambo, 2006). Easily bleachable sisal chemical pulp is industrially produced by soda pulping in the presence of athroquinone (AQ) as catalyst.

The basic knowledge of the chemical composition of sisal fibers, as well as the behavior of its components during pulping and bleaching, is essential for the better understanding and improving the pulping and bleaching operations and for the assessment of pulp and paper properties. Previous papers have reported the composition of the lipophilic compounds (Gutiérrez et al., 2008) and the structure of the lignin (del Río, Marques, Rencoret, Martínez, & Gutiérrez, 2007; del Río et al., 2008) of sisal fibers, but only limited work has been performed on the structural characterization of the carbohydrate fraction of this fiber (Das Gupta & Mukherjee, 1967; Megiatto et al., 2007; Stewart, Azzini, Hall, & Morrison, 1997). In the present work, we report the structural characterization of a heteroxylan in sisal fibers, as well as in their soda/AQ pulps (unbleached and TCF/ECF bleached). The study of hemicelluloses is of fundamental and practical interest, since their partial degradation and dissolution during pulping is responsible for significant consumption of pulping chemicals, the decrease of pulp yield and the papermaking properties of bleached pulps (Evtuguin, Tomás, Silva, & Pascoal Neto, 2003; Lisboa, Evtuguin, & Pascoal Neto, 2004; Pinto, Evtuguin, & Pascoal Neto, 2005).

Hemicelluloses provide a supporting function to the cell wall being bounded to cellulose fibrils. Hemicelluloses are mainly branched polymers of low molecular weight (DP ≈ 80–200) and are composed by diverse sugar residues (D-xylose, L-arabinose, D-glucose, D-galactose, D-mannose, D-glucuronic acid, 4-*O*-methyl-D-glucuronic acid, D-galacturonic acid, and to a lesser extent, L-rhamnose, L-fucose, and various *O*-methylated neutral sugars) (Ebringerová, Hromádková, & Heinze, 2005; Shimizu, 1991; Sun, Tomkinson, Ma, & Liang, 2000). In particular, glucuronoxylan (GX)

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is the major hemicellulose in such important industrial crops produced by agro-industry as kenaf, bamboo, flax, sisal and jute and is structurally similar to hardwood xylans (Gorshkova et al., 1996; Neto et al., 1996; Rowell, Han, & Rowell, 2000; Stewart et al., 1997; Vignon & Gey, 1997). Among the above-mentioned plants, GX of sisal is one of the less investigated. Previous structural/compositional studies of GX from sisal were carried out mainly with alkali-extracted GX (Das Gupta & Mukherjee, 1967; Stewart et al., 1997). Hence, some essential structural information, such as substitution patterns with acetyl groups, was not assessed. It was suggested, however, that sisal GX is an *O*-acetyl-(4-*O*-methylglucurono)xylan with moderate molecular weight, around 15–20 kDa (Das Gupta & Mukherjee, 1967; Stewart et al., 1997). According to data from methylation analysis of sisal xylan, its backbone is constituted by β-(1–4)-linked D-xylopyranose residues, carrying a low degree of substitution (8–10 mol%) with terminal 4-*O*-methyl-D-glucopyranosyluronic acid residue linked through the *O*-2 position (Das Gupta & Mukherjee, 1967).

In this report, the heteroxylan from sisal fibers was isolated by the extraction of peracetic holocellulose with dimethyl sulfoxide and thoroughly characterized by wet chemistry and NMR spectroscopy. This isolation procedure allowed to obtain a xylan sample with intact *O*-acetyl moieties. Simultaneously, the fate of this heteroxylan structure during soda/anthraquinone pulping and TCF and ECF bleaching was also studied.

2. Experimental

2.1. Samples

Sisal (*A. sisalana*) leaf fibers from Africa (Kenya), their soda/AQ chemical pulps (unbleached and ECF/TCF bleached) and cooking black liquor were supplied by CELESA pulp mill (Tortosa, Spain). The TCF bleaching sequence E(O)P-EP included two hydrogen peroxide stages at 90 °C, the first under oxygen pressure (E(O)P stage) and the second without oxygen (EP stage). The ECF bleaching sequence D-EP included a chlorine dioxide (D) at 60 °C and a hydrogen peroxide (EP) stages at 90 °C.

The samples were air-dried, milled using a knife mill (Janke and Kunkel, Analysenmühle), and extracted with acetone in a Soxhlet apparatus for 8 h. For estimation of the Klason lignin content, the acetone extracted samples were subsequently extracted with hot water (3 h at 100 °C). Klason lignin was estimated as the residue after 72% sulfuric acid hydrolysis of the pre-extracted material according to Tappi standard procedures (Tappi Test Methods, 1993). Ash content was estimated as the residue after 6 h calcination at 575 °C.

2.2. Preparation of holocellulose

The holocelluloses of sisal fibers and their unbleached pulp were obtained from extractives-free sawdust (5.0 g) by delignification with 10% peracetic acid at pH 3.5 for 20 min at 85 °C. After delignification, the holocellulose was filtered off on a porous glass filter, washed with acetone and further with warm water and air-dried. The holocellulose yield was 69.7% and 94.7% for the sisal fibers and unbleached pulp, respectively.

2.3. Isolation of xylans from pulps

The isolation of acidic heteroxylans was carried out by two consecutive extractions with Me_2SO (1.5 g of holocelluloses with 50 mL of Me_2SO in each assay) at 50 °C for 24 h under stirring and further precipitation of the resulted extracts in an excess of 7:2:1 EtOH–MeOH–water acidified by HCOOH. The complete precipitation of the heteroxylan was accomplished in 3 days at 4 °C. The heteroxylan was isolated by centrifugation, washed four times with anhydrous MeOH and quickly dried under vacuum at room temperature. For the ECF and TCF bleached pulps the xylans were extracted directly from the pulp without previous preparation of holocelluloses.

2.4. Isolation of xylan from the black liquor

The xylan of the black liquor was isolated following a procedure previously described, with minor modifications (Engström, Vikkula, Teleman, & Vuorinen, 1995). 200 mL of 1,4-dioxane were slowly added with agitation to 100 mL of diluted with distilled water 1/2 black liquor, followed by the addition of glacial acetic acid until pH between 2–3. The solution and resulting precipitate was kept at 5 °C during 2 days. The black liquor precipitated polysaccharides (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.

2.5. Carbohydrate analysis

The heteroxylan was subjected to hydrolysis with 72% H_2SO_4 at 20 °C for 3 h, followed by 2.5 h hydrolysis with diluted 1 M H_2SO_4 at 100 °C (Saeman hydrolysis) and the released neutral monosaccharides were determined as alditol acetate derivatives by gas chromatography (Selvendran, March, & Ring, 1979). 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 temperature program was started at 220 °C with a 5 min hold, and then raised to a final temperature of 230 °C at 2 °C/min, and held for 5 min. The injector and detector temperatures were set at 230 °C. The quantification was made using calibration curves with standards.

2.6. Acid methanolysis for analysis of sugars and uronic acids

About 4.5 mg of freeze-dried sisal fibers and their pulps (unbleached, ECF and TCF bleached pulps) were subjected to acid methanolysis by the addition of 2 mL, 2 M solution of HCl in anhydrous methanol at 100 °C for 4 h (Sundberg, Sundberg, Lillandt, & Holmbom, 1996). After cooling to room temperature, about 80 μL of pyridine was added to neutralize the acidic solution. Additionally, 1 mL of internal standard solution containing 0.1 mg/mL of sorbitol was added. To avoid fibers silylation, 2 mL of the supernatant reaction solution was separated from fiber suspension and evaporated in a rotary evaporator with a water bath kept at 40–50 °C. The samples were dissolved by addition of 70 μL pyridine. For silylation, 150 μL hexamethyldisiloxane and 80 μL trimethylchlorosilane were added and the samples were shaken well. After 12 h at room temperature, the samples were ready for analysis. GC–MS analysis was performed using a Hewlet-Packard Gas Chromatograph 5890 equipped with a mass selective detector MSD series II, using helium as carrier gas (35 cm/s), equipped with a DB-1 J&W capillary column (30 m × 0.32 mm i.d. 0.25 μm film thickness). The column temperature program was 100 – 4 °C/min – 175 °C followed by 175 – 12 °C/min – 290 °C. The detector (FID) temperature was 290 °C. The different peaks were identified by comparing their mass spectra with mass spectra in Wiley and NIST libraries and that reported in the literature (Bertaud, Sundberg, & Holmbom, 2002; Bleton, Mejanelle, Sansoulet, Goursaud, & Tchapla, 1996; Sundberg et al., 1996).

2.7. Size-exclusion chromatography (SEC)

The xylan samples were dissolved in a small amount of 10% LiCL solution in N,N-dimethylacetamide (DMAC) at 70–80 °C and further diluted with DMAC to a xylan concentration of about 0.5% (5 mg/mL). The SEC analysis has been carried out on two PLgel 10 μm MIXED B 300 mm × 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 pollulan reference standards (Polymer Laboratories) in the range 0.8–100 kDa. The injected volume was 100 μL.

2.8. NMR spectroscopy

One-dimensional ^{1}H NMR spectra of the xylan samples were recorded in D_2O (30 °C) on a Bruker Avance 300 spectrometer operating at 300.13 MHz. Sodium 3-(trimethylsilyl)-propionate-d_4 was used as internal standard (δ 0.00). The relaxation delay was 16 s, r.f. 90°-pulse width of 10.2 μs and about 400 pulses were collected.

All 2D NMR spectra were recorded on a Bruker Avance 300 spectrometer operating at 300.1 MHz for proton and at 75.2 MHz for carbon. 2D ^{1}H–^{1}H COSY spectroscopy was performed at 50 °C using a standard COSY sequence (90° pulse, relaxation delay 2 s). Two-dimensional ^{1}H–^{1}H TOCSY (Total Correlation Spectroscopy) spectra (τ_{mix} = 0.050 s) were acquired at a spectral width of 2185 Hz in both dimensions at 60 °C. The relaxation delay was 2.0 s. For each FID, 128 transients were acquired; the data size was 1024 in t_1 × 512 in t_2. The phase sensitive ^{1}H-detected HSQC (Heteronuclear Single Quantum Coherence) spectrum was acquired at 50 °C over a F1 spectral weight 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 $^1J_{CH}$ = 148 Hz.

2.9. Hexenuronic acid content

The amount of hexenuronic acids (HexA) was determined by acidic hydrolysis in sodium formate buffer at pH 3.0 followed by UV detection of furan derivatives at 245 nm (Vuorinen, Fagerström, Buchert, Tenkanen, & Teleman, 1999).

3. Results and discussion

3.1. Chemical composition of sisal fibers

The chemical composition of sisal (*A. sisalana*) fibers is presented in Table 1. The sugar analysis confirmed the data previously reported by Stewart et al. (1997) indicating that xylan is the principal hemicellulose of sisal fibers and the second most abundant polysaccharide after cellulose. At the same time, taking into account the small amount of lignin (around 6%) and extractives (slightly more than 3%) in sisal fibers, the relatively low yield of peracetic holocellulose (70%) indicates a significant content of easily removable hemicelluloses, other than xylan. These may be pectins and, in particular, glucans that are known to be present in the leaves of the genus *Agave* in noticeable amounts (Nobel, 2003). The misbalance in cellulose content and the amount of glucose detected upon sugars analysis (Table 1) allows suggesting that non-cellulosic glucans may contribute to at least 15% (w/w) of sisal fibers. This fact was further confirmed by analysis of the hemicelluloses dissolved in the black liquor from soda pulping of sisal fibers.

Table 1
Chemical composition of sisal fibers, unbleached soda pulp and ECF and TCF bleached pulps (%, w/w).

Component	Sisal fibers	Unbleached pulp	TCF pulp	ECF pulp
Ash	1.0	1.0	0.4	0.4
Extractives (acetone)	0.8	0.3	0.1	0.1
Extractives (water)	2.3	0.7	0.6	0.4
Klason lignin	5.9	0.7	–	–
Holocellulose	70.0	95.0	–	–
Cellulose[a]	54.5	–	–	–
Neutral sugars				
Rha	0.7	0.7	tr	tr
Ara	1.3	tr	tr	tr
Xyl	20.0	19.0	19.4	20.6
Man	0.8	–	–	–
Gal	1.0	tr	tr	tr
Fuc	<0.5	–	–	–
Glc	75.7	80.4	80.6	79.4

tr: traces.
[a] Kürschner-Hoffer method of determination.

3.2. Isolation and structural characterization of xylan from sisal fibers

The heteroxylan (yield of about 60%, w/w) from sisal fibers was isolated from peracetic holocellulose by two consecutive extractions with Me_2SO followed by precipitation of the extracted polyose in 7:2:1 ethanol/methanol/water. Such procedure guaranteed the isolation of intact and representative xylan sample, which can be structurally characterized including the quantification and distribution of *O*-acetyl moieties (Evtuguin et al., 2003).

The composition of the isolated xylan was assessed by analyses of neutral sugars and easily hydrolyzed sugars after methanolysis (Tables 2 and 3). The high purity of the isolated xylan was confirmed using neutral monosaccharides analysis, that showed the predominance of xylose (Xyl) and only small amounts of glucose (Glc), galactose (Gal), arabinose (Ara) and rhamnose (Rha) (Table 2). The presence of Gal, Ara and Rham may indicate the eventual small contamination of xylan with pectin compounds. This was confirmed by methanolysis studies (Table 3, Fig. 1), which revealed a much higher amount of galacturonic acid than could be expected if arisen only from the terminal structural fragment [→3)-α-L-Rha*p*-(1 → 2)-α-D-Gal*p*A-(1 → 4)-D-Xyl*p*] suggested to be present in xylans (Shimizu, 1991). The presence of D-glucopyranosyluronic acid (Glc*p*A), besides the expected 4-*O*-methyl-D-glucopyranosyluronic acid (MeGlc*p*A), may indicate that, at least part of glucuronosyl moieties attached to xylan backbone, is not methylated. The ratio between internal xylopyranosyl units (Xyl*p*) in the backbone and terminal attached glucuronosyl residues (MeGlc*p*A and Glc*p*A) was estimated to be around 9:1. The molecular weight (M_w) of sisal xylan was about 18 kDa, as revealed by SEC analysis (Fig. 2).

According to previously reported methylation analysis of alkali-extracted sisal xylan, its backbone is constituted by β-(1 → 4)-linked D-xylopyranose residues branched at *O*-2 with

Table 2
Neutral monosaccharide composition (%, w/w) of xylans isolated from sisal fibers, pulps and black liquor.

	Rha	Ara	Xyl	Man	Fuc	Gal	Glc
Sisal fibers	0.9	0.7	93.7	tr	–	1.2	3.5
Unbleached pulp	tr	tr	99.5	–	–	tr	0.5
TCF bleached pulp	tr	tr	99.0	–	–	tr	1.0
ECF bleached pulp	tr	tr	98.7	–	–	tr	1.3
Black liquor precipitated polysaccharides (BLPS)	0.7	3.6	10.9	2.6	1.1	27.1	54.0

tr: traces.

Table 3
Neutral monosaccharide and uronic acids composition (%, w/w) determined by methanolysis of xylans isolated from sisal fibers and their pulps.

	Rha	Ara	Xyl	Man	Gal	Glc	GalA	GlcA	MeGlc*p*A
Sisal fibers	0.8	0.6	83.0	–	0.8	1.3	2.8	0.3	10.5
Unbleached pulp	–	–	98.3	–	–	0.2	–	–	1.5
TCF bleached pulp	–	–	98.0	–	–	0.8	–	–	1.2
ECF bleached pulp	–	–	97.5	–	–	1.2	–	–	1.3

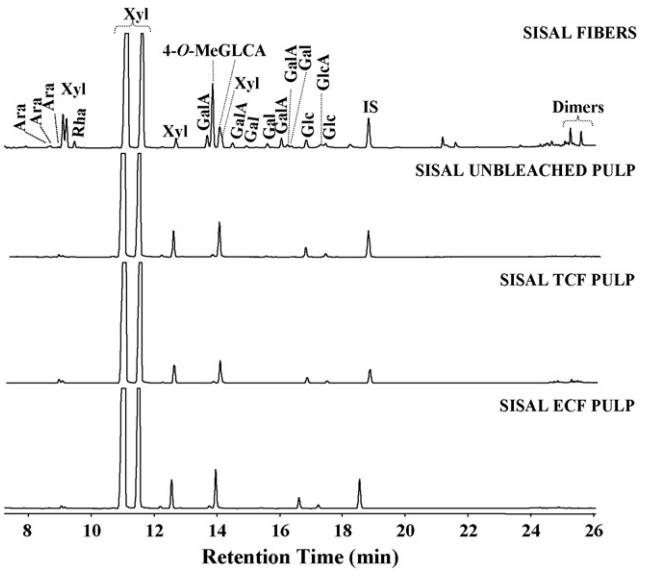

Fig. 1. Gas chromatogram of methylated and silylated sugars obtained by acid methanolysis of xylans isolated from sisal fibers and their pulps. Xyl: xylose, Gal: galactose, Glc: Glucose, Rha: rhamnose, GlcA: glucuronic acid, 4-*O*-MeGlcA: 4-*O*-methylglucuronic acid, and GalA: galacturonic acid.

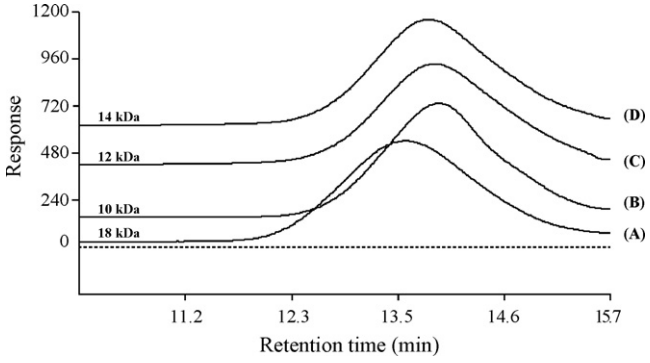

Fig. 2. The GPC elution curves of xylans isolated from sisal fibers and their pulps. (A) Sisal fibers, (B) unbleached pulp, (C) TCF bleached pulp, and (D) ECF bleached pulp.

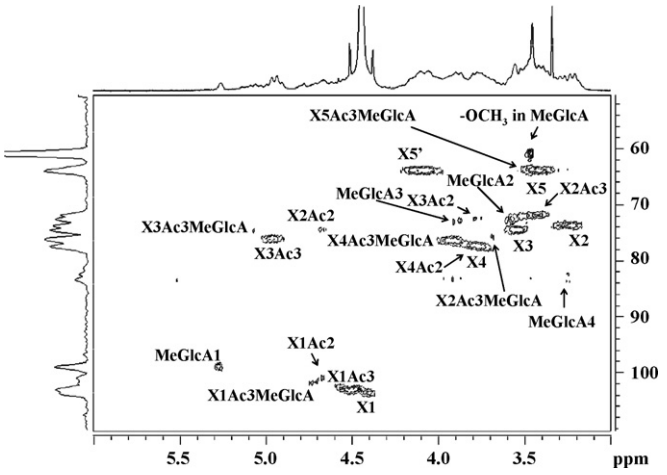

Fig. 3. HSQC spectrum (D_2O, 50 °C) of heteroxylan from sisal fibers.

terminal 4-*O*-methyl-D-glucopyranosyluronic acid residues (Das Gupta & Mukherjee, 1967). These structural features were confirmed by 1D and 2D NMR techniques. Single (COSY) and multiple (TOCSY) bonds ^1H–^1H correlation analyses and ^1H–^{13}C (HSQC) correlations (Fig. 3) allowed assignment of proton and carbon signals in sisal heteroxylan, as summarized in Table 4. Chemical shifts of protons and carbons were practically the same as those reported for acetylated heteroxylans from other plant sources (Evtuguin et al., 2003; Teleman, Tenkanen, Jacobs, & Dahlman, 2002). The anomeric region in the TOCSY spectrum (Fig. 4) revealed the characteristic proton correlations that are normally found in heteroxylans containing attached to backbone non-methylated Glc*p*A residues (Gonçalves, Evtuguin, & Domingues, 2008; Vignon & Gey, 1997). Hence, NMR results corroborates the data obtained by methanolysis (Table 3), evidencing that a small proportion of glucuronic residues in sisal heteroxylan are not methylated. Therefore, it can be suggested that the backbone of sisal heteroxylan is composed of partially acetylated (1 → 4)-linked β-D-Xyl*p* units *O*-2 ramified with terminal (1 → 2)-linked MeGlc*p*A and Glc*p*A.

The acetylation patterns in the heteroxylan backbone were assessed by ^1H NMR spectroscopy based on signal assignment

Table 4
Proton/carbon chemical shifts (δ) of heteroxylan from sisal (30 °C, D_2O).

Structural unit	Assignments					
	H1/C1	H2/C2	H3/C3	H4/C4	H5/C5	
					ax	eq
Xyl (isol)	4.47/102.9	3.28/73.7	3.55/74.5	3.80/77.4	3.40/63.9	4.10/63.9
Xyl-3Ac	4.57/102.4	3.49/71.9	4.98/76.3	3.93/76.5	3.47/63.8	n.d./63.8
Xyl-2Ac	4.68/101.2	4.68/74.6	3.80/72.5	3.87/77.2	3.45/63.8	n.d./63.8
Xyl-2,3Ac	4.80/100.5	4.82/74.6	5.16/74.0	4.06/76.6	3.54/63.9	n.d./63.9
Xyl-3Ac-2GlcA	4.73/102.1	3.70/75.8	5.06/75.8	3.98/77.2	3.50/n.d.	n.d./n.d.
MeGlcA	5.27/98.9	3.57/72.3	3.88/73.7	3.27/83.2	n.d./n.d.	-[a]
GlcA	5.25/n.d.	3.60/n.d.	3.82/n.d.	3.52/n.d.	n.d./n.d.	-[a]

[n.d.] non-determined; [a] not relevant. The following designations were used: Xyl (isol.), non-acetylated Xyl*p* in the backbone isolated from other acetylated Xyl*p* units; Xyl-3Ac, 3-*O*-acetylated Xyl*p*; Xyl-2Ac, 2-*O*-acetylated Xyl*p*; Xyl-2,3Ac, 2,3-di-*O*-acetylated Xyl*p*; Xyl-3Ac-2GlcA, MeGlcA 2-*O*-linked and 3-*O*-acetylated Xyl*p*; MeGlcA, 2-*O*-linked MeGlc*p*A; GlcA, 2-*O*-linked Glc*p*A.

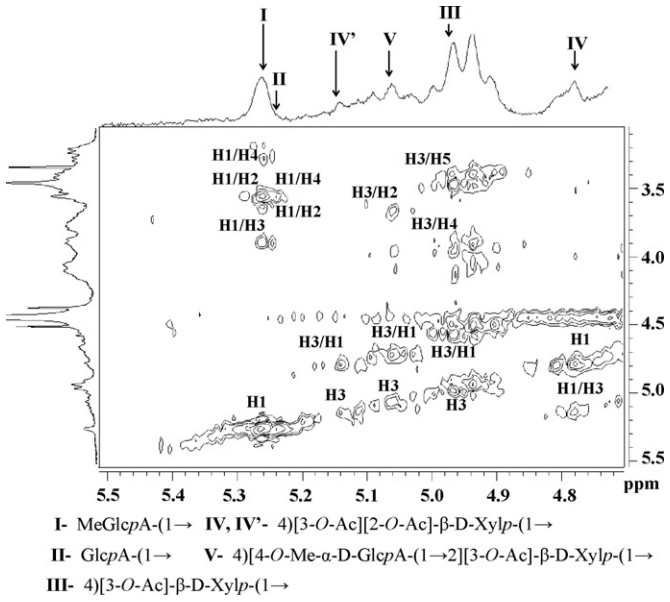

Fig. 4. Anomeric region of the TOCSY spectrum (D_2O, 60 °C) of heteroxylan from sisal fibers.

employing 2D NMR techniques (Table 4). The total ^{1}H NMR spectrum of sisal xylan and its expanded anomeric region with specified groups of protons in particular substructures, are presented in Fig. 5. According to previously published methodology (Evtuguin et al., 2003), internal non-acetylated, 3-*O*- and 2-*O*-acetylated xylose residues, MeGlc*p*A residues were assessed based on their anomeric proton resonances, whereas the amounts of 2,3-di-*O*-acetylated and 3-*O*-acetylated/MeGlc*p*A *O*-2 substituted internal

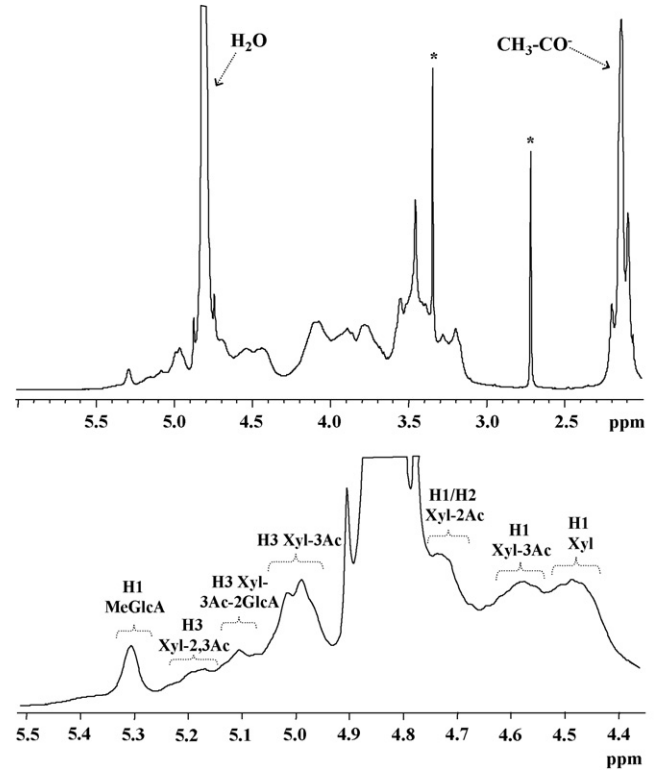

Fig. 5. ^{1}H NMR spectrum (D_2O, 30 °C) of heteroxylans from sisal fibers (top image) and the expanded region of anomeric protons (bottom image). The designations for the structural fragments are the same as in Table 5. *Solvent impurities.

Table 5
Relative content in acetyl groups in structural units of sisal heteroxylan.

Structural fragment and short designation	Relative abundance (per 100 Xyl*p* units)
→4)-β-D-Xyl*p*-(1 → (Xyl)	39
→4)[3-*O*-Ac]-β-D-Xyl*p*-(1 → (Xyl-3Ac)	30
→4)[2-*O*-Ac]-β-D-Xyl*p*-(1 → (Xyl-2Ac)	13
→4)[3-*O*-Ac][2-*O*-Ac]-β-D-Xyl*p*-(1 → (Xyl-2,3Ac)	9
→4)[4-*O*-Me-α-D-Glc*p*A-(1 → 2)][3-*O*-Ac]-β-D-Xyl*p*-(1 → (Xyl-3Ac-2MeGlcA)	9
4-*O*-Me-α-D-Glc*p*A-(1 → (MeGlcA)	9

xylose residues were estimated based on H-3 resonances in corresponding structures (Fig. 5). This allowed the integration of protons from particular structural fragments and their quantification (Table 5). Around 61 mol% of the Xyl*p* residues were acetylated; among them, 39 mol% corresponded to 3-*O*-acetylated, 13 mol% corresponded to 2-*O*-acetylated and 9 mol% corresponded to 2,3-di-*O*-acetylated residues. Accordingly, sisal heteroxylan possessed a substitution degree with acetyl groups of 0.70. Worth notably, Xyl*p* units in backbone of sisal heteroxylan are predominantly 3-*O*-acetylated. The proportion of 3-*O*-acetylated Xyl*p* units in backbone was much higher (almost twice) in sisal xylan than in xylans from woody sources such as birch and beech (Teleman et al., 2002), eucalypt (Evtuguin et al., 2003), paulownia (Gonçalves et al., 2008) and aspen (Teleman, Lundqvist, Tjerneld, Stalbrand, & Dahlman, 2000). Almost all Xyl*p* linked at *O*-2 with MeGlc*p*A (9 mol%) were 3-*O*-acetylated (Table 5).

3.3. Changes in xylan structure during alkaline pulping

During the soda/AQ pulping, around 40% of the xylan was dissolved in the black liquor. This conclusion was made based on the xylose content in delignified unbleached sisal fibers (Table 1) and the pulp yields (*ca.* 60%). The chemical changes in the xylan during pulping were examined comparing the composition and structural features of the xylan from sisal fibers and their soda pulp, both isolated by Me_2SO extraction of the corresponding peracetic holocelluloses (Tables 2 and 3, Fig. 6).

The molecular weight of the xylan remaining in the pulp decreased to 10 kDa, reflecting significant alkali-induced depolymerization (Fig. 2). The xylan suffered also a significant deacetylation (about 95%) and the major part of the uronic moieties (at least 75%) were converted to 4-deoxy-β-L-*threo*-hex-4-enopyranosyluronic acid (hexenuronic acid or HexA), as revealed from ^{1}H NMR spectra (Fig. 6). The presence of HexA, formed under alkaline pulping conditions via β-elimination of methoxyl group, was confirmed applying the total correlation spectroscopy (TOCSY), according to previously published proton chemical shifts (Teleman et al., 1995). The HexA residues may be easily detected in the anomeric region of ^{1}H NMR spectra, which showed the appearance of new signals at 5.36 and at 5.82 ppm that were assigned to H-1 and H-4 in corresponding structures (Fig. 6). The HexA content in sisal soda pulp was 60.6 mmol/kg of pulp as determined after pulp treatment under acidic conditions followed by detection of furoic acid derivatives by UV-spectroscopy at 245 nm (Vuorinen et al., 1999).

The balance of uronic moieties in the initial xylan and in the xylan remaining in the pulp was estimated based on the ratio of anomeric protons in uronic groups at 5.27 ppm and in internal xylopyranose units at 4.47 ppm (pulp xylan) or at 4.40–4.65 ppm (fiber xylan). This analysis indicated a removal of about 30% of all uronic units (MeGlcpA and HexA) from xylan during pulping.

The polysaccharides dissolved in the black liquor (BLPS) during pulping were isolated according to a previously published proce-

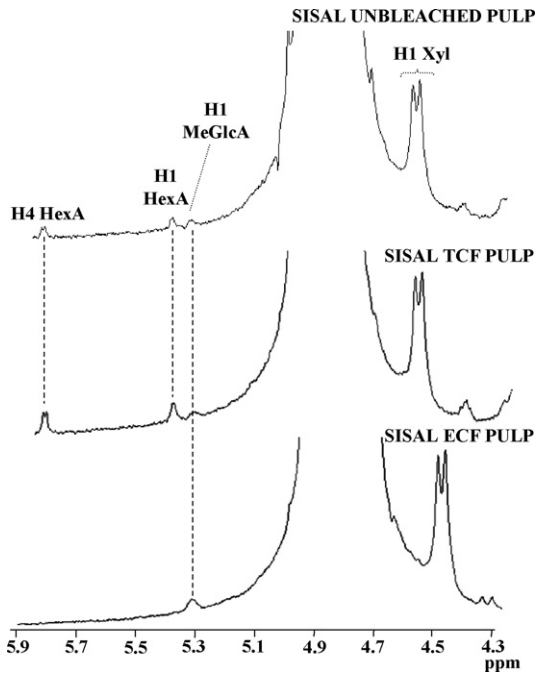

Fig. 6. ^{1}H NMR spectra (D_2O, 30 °C) of heteroxylans isolated from sisal unbleached pulp and TCF and ECF bleached pulps.

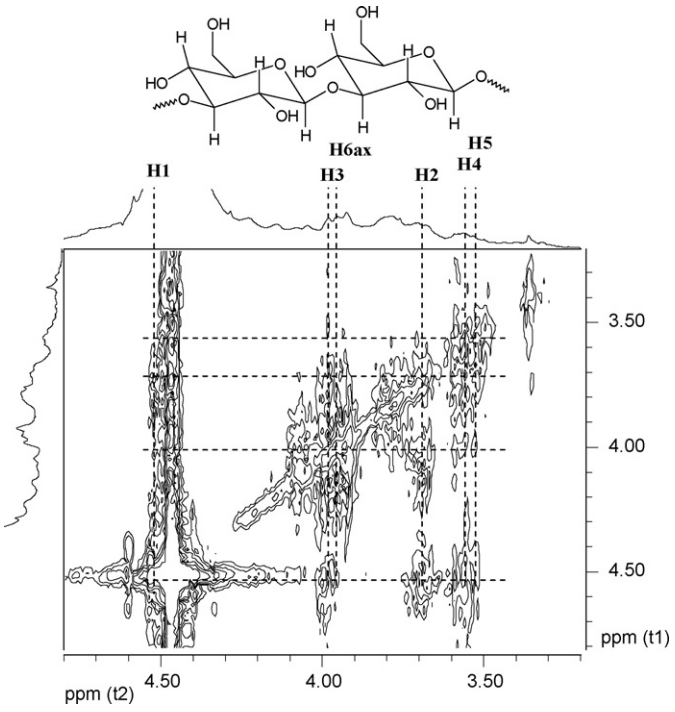

Fig. 7. TOCSY spectrum (D_2O, 60 °C) of BLPS fraction isolated from black liquor. Top image represents the fragment of β-(1-3)-D-glucan backbone.

dure (Engström et al., 1995) and chemically characterized (Table 2). The aim of this study was to compare the structure of the xylan remaining in the pulp with that dissolved in the pulping liquor. Surprisingly, the heteroxylan was a minor polysaccharide dissolved in the liquor and its purification by fractional precipitation failed. At the same time, the analysis of neutral sugars of BLPS revealed glucans as the major precipitated polysaccharides (glucose represents around 54% of BLPS weight) followed by galactans. The preliminary study on BLPS using multiple bonds ^{1}H–^{1}H correlation NMR spectroscopy (TOCSY) gave additional insights into the type of glucans dissolved during pulping from sisal fibers (Fig. 7). These were suggested to be mixture of β-glucans, in particular, β-(1 → 3)-glucans with a low degree of ramification at C6 (callose type), by comparison of the proton-proton correlations with previously published data on proton resonances in β-(1 → 3)-glucan (Torosantucci et al., 2005). However, a more detailed study is required to elucidate the exact structure of the β-glucans in sisal fibers.

3.4. Changes in xylan structure during TCF and ECF bleaching

The structural changes in the heteroxylan from sisal soda pulp during industrial TCF and ECF bleaching were also investigated. The chemical composition and structural features were assessed in xylan samples isolated directly from bleached pulps by Me_2SO extraction. The TCF pulp was obtained by E(O)P-EP bleaching and the ECF pulp was obtained by D-EP sequence. TCF pulp was bleached essentially by hydrogen peroxide under alkaline conditions and included two hydrogen peroxide stages, the first with oxygen and the second without oxygen, at 90 °C, whereas ECF pulp bleaching included a treatment with chlorine dioxide (D) at 60 °C followed by hydrogen peroxide stage under alkaline conditions (EP) at 90 °C.

The chemical analysis of the sugars of TCF and ECF bleached pulps did not show significant changes when compared to the unbleached pulp (Table 1). This indicates that no specific removal of xylan from pulp took place during bleaching. The chemical composition of the xylans isolated from TCF and ECF bleached pulps was also similar to the xylan from unbleached pulp, although a very small decrease in MeGlc*p*A content in pulp xylans after bleaching was detected (Tables 2 and 3). This fact may be explained by a partial degradation of MeGlc*p*A to HexA under alkaline conditions, which are inaccessible for the analysis by methanolysis. This explanation was further supported by ^{1}H NMR analysis that showed a relative increase of the HexA content and a decrease of MeGlc*p*A moieties in the xylan from TCF bleached pulp (Fig. 6). In contrast to the xylan from TCF bleached pulp, the xylan from ECF bleached pulp did not contain HexA residues, which were degraded upon bleaching with chlorine dioxide (Fig. 6). Taking into account that uronic moieties strongly affect the papermaking properties (Lindström, 1992) and brightness stability (Buchert, Bergnor, Lindblad, Viikari, & Ek, 1997) of cellulosic pulps this knowledge may be important to explain the different response of pulps bleached employing TCF and ECF sequences.

The xylans from bleached pulps (either TCF or ECF) did not show any acetyl groups, as revealed by ^{1}H NMR analysis. Hence, alkaline bleaching stages favored the removal of residual acetyl groups from xylan of unbleached pulp. Xylans from bleached pulps possessed slightly higher molecular weight (12 kDa in TCF pulp and 14 kDa in ECF pulp), when compared to this in unbleached pulp (Fig. 2). This fact may be explained by a predominant removal of low molecular weight xylan fractions structurally associated to residual lignin during bleaching.

4. Conclusions

The structure of the heteroxylan isolated from sisal fibers has been characterized and its behavior during soda/AQ pulping and TCF/ECF bleaching has been studied. The data indicates that the heteroxylan backbone is composed by (1 → 4)-linked β-D-xylopyranosyl units (Xyl*p*) partially ramified with terminal (1 → 2)-linked 4-*O*-methyl-α-D-glucuronosyl (MeGlc*p*A, 9 mol%) and a small proportion of α-D-glucuronosyl (Glc*p*A) residues. Around 61 mol% of the Xyl*p* residues are acetylated, the major proportion of acetyl groups being attached at the *O*-3 position of the Xyl*p* residues (39 mol%), followed by acetylation at the *O*-2

position (13 mol%) and diacetylation at both *O*-2 and *O*-3 positions (9 mol%). The molecular weight (M_w) of the heteroxylan was of 18 kDa. Around 40% of xylan was removed during soda pulping. However, the major polysaccharides found in the black liquor were β-glucans rather than xylans. Sisal xylan suffered a significant depolymerisation (M_w decreased to almost half) and deacetylation (95%) during pulping. Terminal MeGlc*p*A residues were partially removed (to about 30%) or converted to HexA in a large extent. HexA revealed to be relatively stable during TCF bleaching with hydrogen peroxide and were predominant among uronic moieties of xylan. Since all HexA were degraded during ECF bleaching with chlorine dioxide, the final pulp contained a xylan with rather small amount of uronosyls (MeGlc*p*A residues). A small proportion of MeGlc*p*A residues (15% from initial amounts), remaining intact during soda pulping, were additionally converted to HexA residues during alkali bleaching stages. After bleaching, the residual acetyl groups were completely removed from the pulp xylan. It was suggested that a low molecular weight fraction of xylan, probably associated to residual lignin, was removed from upon bleaching.

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