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Isolation and characterization of acetylated glucuronoarabinoxylan from sugarcane bagasse and straw

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ABSTRACT

Sugarcane bagasse and straw are generated in large volumes as by-products of agro-industrial production. They are an emerging valuable resource for the generation of hemicellulose-based materials and products, since they contain significant quantities of xylans (often twice as much as in hardwoods). Heteroxylans (yields of ca 20% based on xylose content in sugarcane bagasse and straw) were successfully isolated and purified using mild delignification followed by dimethyl sulfoxide (DMSO) extraction. Delignification with peracetic acid (PAA) was more efficient than traditional sodium chlorite ($NaClO_2$) delignification for xylan extraction from both biomasses, resulting in higher extraction yields and purity. We have shown that the heteroxylans isolated from sugarcane bagasse and straw are acetylated glucuronoarabinoxylans (GAX), with distinct molecular structures. Bagasse GAX had a slightly lower glycosyl substitution molar ratio of Ara*f* to Xyl*p* to (0.5:10) and (4-*O*-Me)Gl*p*A to Xyl*p* (0.1:10) than GAX from straw (0.8:10 and 0.1:10 respectively), but a higher degree of acetylation (0.33 and 0.10, respectively). A higher frequency of acetyl groups substitution at position α-(1 → 3) (Xyl-3Ac) than at position α-(1 → 2) (Xyl-2Ac) was confirmed for both bagasse and straw GAX, with a minor ratio of diacetylation (Xyl-2,3Ac). The size and molecular weight distributions for the acetylated GAX extracted from the sugarcane bagasse and straw were analyzed using multiple-detection size-exclusion chromatography (SEC-DRI-MALLS). Light scattering data provided absolute molar mass values for acetylated GAX with higher average values than did standard calibration. Moreover, the data highlighted differences in the molar mass distributions between the two isolation methods for both types of sugarcane GAX, which can be correlated with the different Ara*f* and acetyl substitution patterns. We have developed an empirical model for the molecular structure of acetylated GAX extracted from sugarcane bagasse and straw with PAA/DMSO through the integration of results obtained from glycosidic linkage analysis, ^{1}H NMR spectroscopy and acetyl quantification. This knowledge of the structure of xylans in sugarcane bagasse and straw will provide a better understanding of the isolation-structure-properties relationship of these biopolymers and, ultimately, create new possibilities for the use of sugarcane xylan in high-value applications, such as biochemicals and bio-based materials.

1. Introduction

A growing demand for the more effective utilization of lignocellulosic biomass has led to greater interest in the use of agro-industrial residues, including sugarcane bagasse and sugarcane straw (Bian, Peng, Xu, Sun, & Kennedy, 2010; Canilha et al., 2012; Carvalho et al., 2015; Pandey, Soccol, Nigam, & Soccol, 2000;

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Sun, Sun, Sun, & Su, 2004; Svärd, Brännvall, & Edlund, 2015). Sugarcane, which is a source of both sugar (sucrose) and ethanol, is one of the most important industrial crops in Brazil. According to the Brazilian Sugarcane Association (UNICA, 2016), the 2015/2016 harvest estimate for South-Central Brazil will result in the production of 618 million tons of sugarcane biomass. Sugarcane bagasse (stalks) and straw (tips and leaves) each represent approximately 14% of the plant and are generated in large amounts as the main agricultural waste from the sugarcane industry (Conab, 2014). Typically, bagasse is burnt to produce steam, while straw is deposited at the harvesting sites. However, being lignocellulosic residues, there is great potential for the use of sugarcane bagasse and straw for the production of pulp and second-generation ethanol, as well as for their conversion into bio-chemicals and bio-based materials (Canilha et al., 2012; Cardona, Quintero, & Paz, 2010; Oliveira et al., 2013; Pandey et al., 2000).

Sugarcane bagasse and straw, similar to other annual and perennial plants, contain large quantities of hemicelluloses – sometimes up to 50% of their chemical composition (Carvalho et al., 2015). This high relative content of hemicelluloses, especially xylan, constitutes an excellent basis for the extraction and valorization of such hemicellulosic fractions (Ebringerová & Heinze, 2000; Ebringerová, Hromádková, & Heinze, 2005). There has been an increasing interest in the exploitation of xylan biopolymers as potential resources for the development of new materials and products (Bosmans et al., 2014; Ebringerová & Heinze, 2000; Höije, Sternemalm, Heikkinen, Tenkanen, & Gatenholm, 2008; Littunen et al., 2015; Peng, Ren, Zhong, & Sun, 2011), which is especially true for xylans that are readily available as by-products of the forest and agriculture industries (Egüés et al., 2014; Svärd et al., 2015). However, the structural heterogeneity of xylans is a strong limiting factor, as they differ in terms of their chemical composition and structural patterns among different biomass sources, and even between different tissues and developmental stages of the same plant (Ebringerová & Heinze, 2000; Stephen, 1983). In order to gain a better understanding of its potential application, a greater knowledge of xylan structure and the isolation-structure-properties relationship is needed (Littunen et al., 2015; Köhnke, Östlund, & Brelid, 2011; Mikkelsen, Flanagan, Wilson, Bacic, & Gidley, 2015).

Typically, the backbone in xylan is formed by β-(1→4)-D-xylopyranosyl (Xyl*p*) units, with possible glycosyl substitutions in positions C-2 and/or C-3, and with a certain number of acetyl groups. The main side groups in the xylan backbone are L-arabinofuranose (Ara*f*), D-glucopyranosyl uronic acid units (GA) and 4-*O*-methyl D-glucuronic acid units (4-*O*-MeGlcA). Other substitutions in xylan can also occur, but they are less abundant (Ebringerová et al., 2005; Evtuguin, Tomás, Silva, & Neto, 2003). Glucuronoarabinoxylan (GAX) and arabinoxylan (AX) are typical for grasses, in which branches of arabinose, GA, 4-*O*-MeGlcA and acetyl groups (Ac) in the backbone of xylose can be observed (Ebringerová & Heinze, 2000; Ebringerová et al., 2005).

The molecular structure of xylans in sugarcane bagasse and straw is expected to be somewhat different, both from each other and from that in other biomasses. Our previous work showed that the amount of xylan found in sugarcane bagasse and straw is at least twice that found in hardwoods cultivate in tropical areas, although it had a lower content of uronic acid units and acetyl substitutions (Alves et al., 2010; Carvalho et al., 2015). The xylans in sugarcane bagasse are considered partially acetylated L-arabino-(4-*O*-methylglucurono)-D-xylans, where glucuronic and arabinose units are linked at *O*-2 and *O*-3, respectively, of internal β-D-xylopyranosyl units in the backbone (Peng, Ren, Xu, Bian, Peng, & Sun, 2009; Shi et al., 2012). However, little information on the substitution patterns of acetyl groups in sugarcane bagasse and straw xylans is currently available, which limits their potential use in the development of xylan-based materials and products.

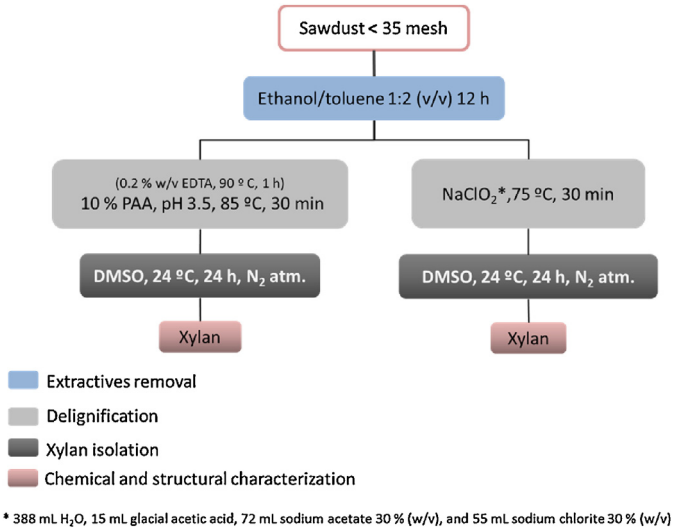

Fig. 1. Working plan for delignification, xylan isolation and chemical and structural characterization of xylan.

Our aim was to investigate the differences in the chemical structure of native acetylated xylans from sugarcane bagasse and straw. We have isolated intact acetylated heteroxylans from sugarcane bagasse and straw by extracting the peracetic acid (PAA) or sodium chlorite ($NaClO_2$) holocelluloses using dimethyl sulfoxide (DMSO). The use of DMSO resulted in partial xylan extraction, but delivered xylan fractions with molecular structures that resembled the structure of the native xylan. The isolated xylans were thoroughly characterized using glycosidic linkage analysis, Fourier transform infrared spectrometry (FTIR), ^{1}H nuclear magnetic resonance spectroscopy (^{1}H NMR) and multiple-detection size-exclusion chromatography (SEC) (using both standard and universal calibration). Based on this information, empirical structural formulas for both types of xylan are proposed. Such knowledge is required for a better understanding of the chemical reactivity of these xylan species during chemical processing and modification and for the creation of new possibilities for the use of sugarcane xylan biopolymers in materials and products.

2. Experimental

2.1. Materials

The raw materials, 5-month old sugarcane (cultivar RB867515) bagasse (stalks after fragmentation and pressing) and straw (leaves and tips), were supplied by the Center for Sugarcane Experimentation (Oratórios, Minas Gerais State, Brazil). The sugarcane bagasse and straw were converted to sawdust (<35 mesh) by using a Wiley mill bench model.

The chemicals used were ethanol 96% (VWR, France), toluene 99.8% (Sigma Aldrich, USA), ethylenediamine tetracetic acid (EDTA) 99% (Sigma Aldrich, USA), peracetic acid (PAA) 39% (Sigma Aldrich, USA), sodium hydroxide (NaOH) pellets analytical grade (Merck Milipore, Germany), acetone 99.5% (VWR, France), acetic acid 100% (VWR, France), sodium acetate 99% (Merck, USA), sodium chlorite ($NaClO_2$) 80% (Alfa Aesar, Germany), dimethyl sulfoxide (DMSO) 99% (VWR, France), formic acid 98/100% (VWR, England) and methanol HPLC grade (Fisher Chemicals, UK).

2.2. Isolation of acetylated heteroxylan

The isolation procedure for acetylated heteroxylan is depicted in Fig. 1. In brief, sugarcane bagasse and straw were converted to saw-

dust (<35 mesh) and then used for extraction. Holocelluloses were prepared from the extractives-free sawdust by delignification with $NaClO_2$ or PAA. Heteroxylan was extracted from the holocelluloses by DMSO, precipitated in ethanol, centrifuged, purified and dried. The xylans yields were ca 19–22% and 3–4% (based on xylose content) from those presented in raw materials for PAA/DMSO and $NaClO_2$/DMSO isolation procedures correspondently. Acetylated heteroxylan was analyzed using monosaccharide and glycosidic linkage analyses, size-exclusion chromatography (SEC), ^{1}H nuclear magnetic resonance spectroscopy (^{1}H NMR) and Fourier transform infrared spectrometry (FTIR).

2.2.1. Extractives removal

Biomass sawdust (<35 mesh) was extracted with ethanol/toluene 1:2 (v/v) for 12 h in a Soxhlex extractor (Shatalov, Evtuguin, & Neto, 1999; Sun et al., 2004). Extractives-free sawdust was air-dried and stored in airtight plastic bags at room temperature prior to use. The moisture of the extractives-free sawdust was determined according to TAPPI T 264 cm-07.

2.2.2. Delignification

2.2.2.1. PAA delignification. Prior to delignification with PAA, the extractives-free bagasse and straw sawdust was treated with 0.2% (w/v) EDTA at 90 °C for 1 h, with constant stirring, in order to remove the metal ions and prevent decomposition of the PAA (Brienzo, Siqueira, & Milagres, 2009). The delignification of the biomasses was performed with 10 g of extractives-free sawdust treated with 500 mL of 10% (v/v) PAA at pH 3.5 (adjusted with sodium hydroxide solution), at 85 °C for 30 min, with constant stirring. After the treatment, the solution was cooled in an ice bath and diluted twice with water. The holocellulose was collected on a porous glass filter P2 (porosity 100), washed with 5 L of warm distilled water and, soon thereafter, with 50 mL of acetone/ethanol 1:1 (v/v) (Evtuguin et al., 2003). The holocellulose was dried at room temperature (24 °C) and stored in airtight containers.

2.2.2.2. Sodium chlorite delignification. 10 g of extractives-free sawdust was treated with 388 mL of water, 15 mL of acetic acid 100%, 72 mL of sodium acetate 30% (w/v) and 55 mL of sodium chlorite 30% (w/v), at 75 °C for 30 min, with constant stirring. After the treatment, the holocellulose was collected on a polystyrene membrane (porosity 60 μm), washed with 5 L of distilled water and, soon thereafter, with 100 mL of acetone (Magaton, Piló-Veloso, & Colodette, 2008). The holocellulose was dried at room temperature (24 °C) and stored in airtight containers.

2.2.3. Isolation of xylans

A sample of 6 g of holocellulose (PAA-holocellulose or $NaClO_2$-holocellulose) was treated with 130 mL of DMSO, at 24 °C for 24 h, under nitrogen atmosphere and with constant stirring (Hägglund, Lindberg, & McPherson, 1956). After the treatment, the suspension was filtered through a polystyrene membrane (porosity 60 μm) and washed with ~20 mL of distilled water. The supernatant liquid was added to 600 mL of ethanol at pH 3.5 (adjusted with formic acid) and left for 12 h at 4 °C (Magaton et al., 2008). The precipitated hemicelluloses were isolated by centrifugation (10 min at 4500 rpm) and washed 5 times with methanol (Evtuguin et al., 2003). The xylans were dried in a vacuum oven for 72 h at 30 °C.

The total yield was estimated gravimetrically based on the amount of starting material (extractives-free biomasses). The xylan yields after DMSO isolation were calculated taking into consideration the amount of xylose in the extractives-free biomasses and the xylan samples, as well as the total yield.

2.3. Compositional and structural characterization

2.3.1. Monosaccharide composition analysis

The monosaccharides composition was determined by acid hydrolysis followed by chromatographic analysis. Total polysaccharide depolymerization of the sugarcane fractions, including glucose from crystalline cellulose, was achieved by sulfuric hydrolysis. Samples (4 mg) were kept in a glass tube with 0.25 mL of 72% sulfuric acid for 3 h at room temperature. Then, deionized water was added to dilute the solution to approx. 1,2-1,3 mol L^{-1} sulfuric acid, and the tubes were incubated at 100 °C for 3 h. Uronic acids in hydrolysates were determined colorimetrically with *m*-phenylphenol using a known procedure published by Selvendran, Verne, and Faulks (1989). The monosaccharide composition of the isolated heteroxylan fractions containing more labile uronic acids (glucuronic and galacturonic acid) was determined by acidic methanolysis (Appeldoorn, Kabel, Van Eylen, Gruppen, & Schols, 2010; Bertaud, Sundber, & Holmbom, 2002). Freeze-dried samples (1 mg) were incubated with 1 mL of 2 mol L^{-1} HCl in dry methanol for 5 h at 100 °C. Subsequently, the samples were neutralized with pyridine, dried under a stream of air, and further hydrolyzed with 2 mol L^{-1} trifluoroacetic acid (TFA) at 121 °C for 1 h. The samples were again dried under a stream of air and dissolved in H_2O.

The hydrolysed monosaccharides by both sulfuric hydrolysis and acidic methanolysis, were analyzed using high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) using an ICS-3000 system (Dionex) equipped with a CarboPac PA1 column (4 × 250 mm, Dionex). Inositol was added to all samples as an internal standard prior to hydrolysis. All experiments were performed in triplicate. Two different gradients were used for the separation and quantification of neutral (Fuc, Ara, Rha, Gal, Glc, Man, and Xyl) and acidic (GalA, GlcA, and 4-*O*-MeGlcA) monosaccharides, as reported in our previous study (McKee et al., 2016). Quantification of 4-*O*-MeGlcA was performed using the analytical response factor for GlcA with the appropriate correction as reported in Chong et al. (2013).

2.3.2. Klason lignin

The Klason lignin content in the holocellulose samples was determined gravimetrically to be insoluble residue after acid hydrolysis with 72% sulfuric acid, according to the TAPPI 222 om-02 standard method (TAPPI, 2011).

2.3.3. Acetyl content and degree of acetylation

The acetyl content of the xylan samples was determined after alkaline hydrolysis with NaOH at 70 °C overnight using high performance liquid chromatography (HPLC) with UV detection (Voragen, Schols, & Pilnik, 1986). The HPLC instrument (Dionex–Thermofisher, CA, USA) was equipped with a UV detector (Rezex, 210 nm) and a ROA-Organic acid column (300 × 7.8 mm; Phenomenex, Torrance, CA, USA). Separations were performed at a flow rate of 0.5 mL min^{-1}, using 2.5 mmol L^{-1} H_2SO_4 as a mobile phase at 50 °C. The degree of acetylation (DA) was determined from the acetyl content in the xylan samples according to Eq. (1)

$$DA = \frac{132 \times \%acetyl}{\left(M_{acetyl} \times 100\right) - \left(M_{acetyl} - 1\right) \times \%acetyl} \quad (1)$$

where: DA is the degree of acetylation, % acetyl is the acetyl content determined by analysis, M_{acetyl} is the acetyl molecular weight (43 g mol^{-1}) and 132 g mol^{-1} is the molecular weight of anhydroxylopyranose (Xu et al., 2010).

2.3.4. Glycosidic linkage analysis

Freeze-dried xylan fractions (1 mg, three technical replicates) were swelled in anhydrous DMSO for 16 h at 60 °C and methylated

in the presence of $NaOH/CH_3I$ five times in order to ensure complete methylation (Ciucanu and Kerek, 1984). The samples were then hydrolyzed with 2 mol L^{-1} TFA at 121 °C for 2 h, reduced with sodium borohydride ($NaBH_4$) and acetylated with acetic anhydride in pyridine (Albersheim, Nevins, English, & Karr, 1967). The obtained permethylated alditol acetates (PMAAs) were separated and analyzed using a gas chromatographer (HP-6890, Agilent Technologies) coupled to an electron-impact mass spectrometer (HP-5973, Agilent Technologies) on a SP-2380 capillary column (30 m × 0.25 mm i.d.; Sigma–Aldrich) with a temperature range increasing from 160 °C to 210 °C at a rate of 1 °C/min using He as carrier gas. The retention times and fragmentation mass spectra from the PMAAs were compared with those of the reference polysaccharides (wheat arabinoxylan, Megazyme, Ireland) and with available data (Carpita & Shea, 1989). The quantification was based on the carbohydrate composition and the relative molar-response factor of each compound (Carpita & Shea, 1989), as detected by GC–MS.

2.3.5. Size-exclusion chromatography

The molar mass distributions of the xylan extracts from the sugarcane bagasse and straw were analyzed using a size-exclusion chromatographer (SECcurity 1260, Polymer Standard Services, Mainz, Germany) coupled in series to a multiple-angle laser light scattering detector (MALLS; BIC-MwA7000, Brookhaven Instrument Corp., New York) and a refractive index detector (SECcurity 1260, Polymer Standard Services, Mainz, Germany). SEC analyses were performed at a flow rate of 0.5 mL min^{-1} using dimethyl sulfoxide (DMSO, HPLC grade, Sigma-Aldrich, Sweden) with 0.5% w/w LiBr (ReagentPlus) as a mobile phase, using a column set consisting of a GRAM PreColumn, 100 and 10000 analytical columns (Polymer Standards Services, Mainz, Germany) thermostatted at 60 °C. Prior to the analyses, the xylans were dissolved directly in the SEC eluent for 16 h at 60 °C. Standard calibration was performed by the injection of pullulan standards of known molar masses provided by Polymer Standards Services (PSS, Mainz, Germany). The SEC elution volumes were then converted into hydrodynamic volumes (V_h) using the Mark-Houwink equation, as reported by Vilaplana and Gilbert (2010). The Mark-Houwink parameters for pullulan in DMSO/LiBr (0.5 wt%) are $K = 2.427 \times 10\text{-}4$ dL g^{-1} and $a = 0.6804$ (Kramer and Kilz, PSS, Mainz, private communication). The differential refractive index increment (dn/dc) for pullulan in DMSO/LiBr 0.5% was considered as 0.0853 mL g^{-1} (Kramer and Kilz, PSS, Mainz, private communication). Data collection and light scattering calibration was performed using the WinGPC software (Polymer Standards Services, Mainz, Germany), with a dn/dc value of 0.0881 mL g^{-1} (calculated for sugarcane xylan in DMSO/LiBr 0.5%; Supplementary material Fig. S3). The SEC weight distribution, $w(\log V_h)$, and the size dependence of the weight-average molecular weight, $\bar{M}_W(V_h)$, were calculated using additional mathematical procedures presented elsewhere (Vilaplana & Gilbert, 2010). The macromolecular size distributions are presented in terms of hydrodynamic radius (R_h), with $V_h = \frac{4}{3} \cdot \pi \cdot R_h^3$. Four different xylan concentrations between 1.0–4.0 g L^{-1} were injected for each sample, which resulted in four replicates for the calculation of the number-average ($\bar{M}_n$) and weigh-average molar mass ($\bar{M}_W$). The molar mass distributions and the average molar mass values for the xylan extracts from sugarcane bagasse have been validated and compared with the injection of a reference arabinoxylan (AX) from wheat endosperm (Megazyme, Ireland).

2.3.6. ^1H NMR spectroscopy

^1H NMR spectra were registered on a Bruker AVANCE 300 spectrometer operating at 300.13 MHz at 298 K. The xylan was dissolved in D_2O (ca 2% w/w) and the sodium 3-(trimethylsilyl)propionate-d_4 (TMSP, δ 0.00) was used as internal standard. The acquisition parameters for the proton spectra were as follows: 12.2 μs pulse width (90°), 18 s relaxation delay, and 300 scans were collected. These conditions guarantee that quantitative information was obtained from the NMR measurements.

2.3.7. Fourier transform infrared spectrometry

The Fourier transform infrared (FTIR) spectra (wavelength 4000–600 cm^{-1}) were recorded using a Perkin-Elmer Spectrum 2000 FTIR spectrometer (Waltham, MA, USA) equipped with an attenuated total reflectance (ATR) system (Spectac MKII Golden Gate Creecstone Ridge, GA, USA). The spectra were obtained from dry samples using 16 scans at a resolution of 4 cm^{-1} and at intervals of 1 cm^{-1} at room temperature. Origin 9.1 software was used for the spectra evaluation.

3. Results and discussions

3.1. Yield and chemical composition of isolated xylan

In our previous work, sugarcane bagasse and straw were chemically characterized (Carvalho et al., 2015). The results thereof, based on the complete mass balance, are set out in the Supplementary material Fig. S1. Bagasse and straw were shown to contain significant quantities of hemicelluloses and pectins, expressed as other sugars (the sum of xylose, galactose, mannose, arabinose, uronic acids and acetyl groups). The high relative content of hemicelluloses in these raw materials constitutes a sound basis for the extraction and valorization of such fractions from sugarcane.

In the present work, delignification methods using PAA and $NaClO_2$ were used prior to the extraction of xylan with DMSO. Using the PAA delignification process, the loss of dry matter (including lignin, ash and, to a lesser extent, polysaccharides) from the initial amount of extractives-free biomasses was 24.8% and 25.7% for bagasse and straw, respectively. The loss of dry matter using the $NaClO_2$ delignification process was 15.4% and 27.7% for bagasse and straw, respectively. The heteroxylans yield (based on the xylose content in sugarcane bagasse and straw) was ca 19–22% for PAA/DMSO isolation procedure, while only ca 3–4% of xylan was extracted from $NaClO_2$ hollocellulose (Table 1).

In a previous study, almost 5 times higher xylan yields were also observed for eucalyptus when using the PAA/DMSO extraction process, in comparison with the $NaClO_2$/DMSO process (Evtuguin et al., 2003). Such result was explained by the higher degree of delignification with peracetic acid and the simultaneous breaking of lignin-xylan ether bonds.

The FTIR spectra obtained for xylan extracted from bagasse and straw were typical for xylans, as shown in Fig. 2: a sharp band at 1039 cm^{-1}, which is due to the C—O, C—C stretching or C—OH bending in the sugar units (Chaikumpollert, Methacanon, & Suchiva, 2004) and the expected bands between 1175 and 1000 cm^{-1} (Sun et al., 2004). The β-glycosidic linkages between the xylose units were evidenced by the presence of a sharp band at 897 cm^{-1} (Gupta, Madan, & Bansal, 1987). The band at 3350–3330 cm^{-1} corresponds to the hydroxyl stretching vibrations of xylans, as well as the water involved in the hydrogen bonding, and the band at 2920 cm^{-1} represents C—H stretching vibrations (Sun et al., 2004). The band at 1160 cm^{-1} indicates the presence of arabinose residues (Egüés et al., 2014). The presence of acetyl groups in the xylan was confirmed by the absorption at 1734 cm^{-1}, which is due to the C=O stretching (Bian et al., 2010). The band at 1241 cm^{-1}, which is also due to the C—O stretching, and the band at 1370 cm^{-1}, which is due to the C—CH_3 stretching, were also confirmed (Xu et al., 2010). The absorption at 1628 cm^{-1} is attributed principally to the water absorbed by xylans (Kačuráková, Belton, Wilson, Hirsch, & Ebringerová, 1998). The weak band at 1510 cm^{-1}, which is due to

Table 1
Isolation yields for the hollocellulose, DMSO extracts and xylan (based on the xylose content in the sugarcane bagasse and straw).

Material	PAA/DMSO			$NaClO_2$/DMSO		
	Holocellulose (%)	DMSO Extract (%)	Xylan (%)	Hollocellulose (%)	DMSO Extract (%)	Xylan (%)
Bagasse	75.2	5.7	21.7	84.6	0.9	3.3
Straw	74.3	7.7	19.3	72.3	1.5	3.8

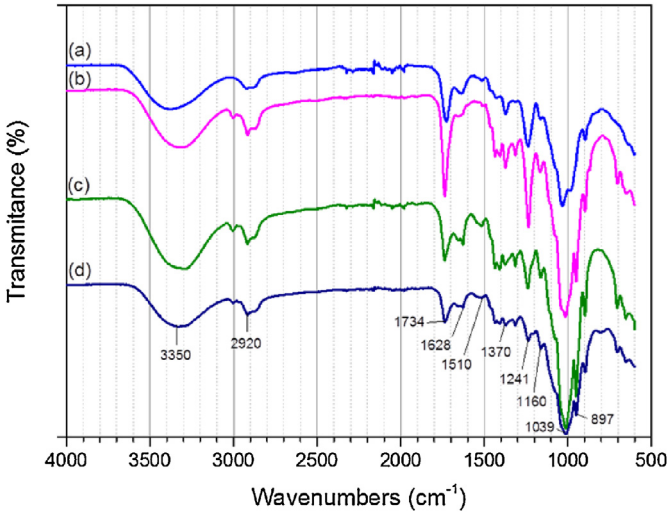

Fig. 2. FTIR spectra for xylan samples extracted from bagasse by PAA/DMSO process (spectrum a), from bagasse by $NaClO_2$/DMSO process from (spectrum b), from straw by PAA/DMSO process (spectrum c) andfrom straw by $NaClO_2$/DMSO process (spectrum d).

the aromatic skeletal vibration, indicates the presence of a small amount of lignin in the xylan samples (Sun et al., 2004) During the isolation procedures, the glycosidic linkages can be disrupted and the hydroxyl groups can be oxidized, resulting in the formation of ketone carbonyl groups, seen as bands at around 1720 cm^{-1} in FTIR spectra (Magaton et al., 2008; Sun & Tomkinson, 2002). The absence of such signals in the spectra we observed in the present work for xylans extracted from bagasse and straw rules out any oxidation reactions during the delignification and isolation procedures.

The evolution of the xylan isolation process was monitored by noting the monosaccharide composition of the extractives-free raw material, the content of hollocelluloses (after delignification with both PAA and $NaClO_2$) and the composition of the DMSO xylan extracts (Fig. 3). As can be observed, xylose is the main component in the DMSO extracts for both investigated materials, which evidences the successful isolation of the xylan using the proposed procedures. The ratio between glucose and xylose is quite similar for the extractives-free biomasses and both types of holocelluloses (delignified by PAA or $NaClO_2$). However, the composition of the DMSO extracts isolated from various types of holocelluloses is quite different. The total amount of uronic moieties in the PAA/DMSO samples were determined calorimetrically in sugars hydrolysates after Saeman hydrolysis (Saeman, 1945) and proved to be fairly similar (1.4% in bagasse and 1.8% in straw). The sugar analysis of the PAA/DMSO samples confirmed the presence of a significant amount of arabinose and 4-*O*-methyl glucuronic acid (MeGlcA), in addition to the xylose, in the bagasse and straw, which was also seen in the FTIR spectra (Fig. 2). Thus heteroxylans in the sugarcane bagasse and straw were typical glucuronoarabinoxylans (GAX). Glucose, galactose and galacturonic acid were also present in the PAA/DMSO samples, although in much smaller amounts. This can be attributed to the minor presence of mixed-linkage β-glucans and pectin polysaccharide components, which is confirmed by the results of the glycosidic linkage analysis.

The $NaClO_2$/DMSO-extracted samples had lower xylose content than those obtained after the PAA/DMSO isolation procedure. The composition of the $NaClO_2$/DMSO-extracted samples, together with the low yields obtained, suggest that this isolation process was less efficient and less selective towards xylan. A relatively high content of glucose, and a considerable increase in the amount of galactose, were found in the $NaClO_2$/DMSO-extracted samples. This further indicates that the isolation of non-xylan hemicelluloses, such as mixed-linkage β-glucans, and other pectic material occurred during the $NaClO_2$/DMSO process. Most likely, the accessibility of DMSO in the cell walls was very low, due to insufficient delignification, and a mixture of such polysaccharides was removed only from the fiber surface.

3.2. Glycosidic linkage analysis in isolated xylans

In order to investigate the substitution patterns of the isolated xylan fractions, linkage (methylation) analysis was performed on the PAA/DMSO and $NaClO_2$/DMSO extracts (Table 2). From the results of linkage analysis, it can be deduced that the general structure of the xylans from both bagasse and straw consists of a linear backbone of (1 → 4)-linked β-D-xylopyranosyl units (Xyl*p*), partially *O*-3 substituted with L-arabinofuranosyl (Ara*f*) units and *O*-2 substituted essentially with 4-*O*-methyl-D-glucuronosyl units (MeGlc*p*A). 4-*O*-methyl-D-glucuronic acid residues were previously detected in sugarcane bagasse and straw by acidic methanolysis (Carvalho et al., 2015). It is worth mentioning that glycosidic linkage analysis is unable to identify the Xyl*p* units modified by acetylation, due to the extreme alkaline conditions applied during the methylation of the samples. The relative ratio of 3,4-Xyl*p* *versus* 2,4-Xyl*p* residues in the linkage analysis evidenced that the substitution at *O*-3 was 3–5 fold higher than substitutions at *O*-2 in the xylan backbone, which as well matches the relative amounts of terminal Ara*f* (t-Ara*f*) and MeGlcA, respectively. Only traces of double substituted 2,3,4-Xyl*p* residues were found in the PAA and $NaClO_2$ treated xylans, in contrast to reports on other grass xylans, such as cereals, where these double substitutions are relatively abundant (Heikkinen et al., 2013). The amounts of GAX present in the different extracts can be calculated directly from the relative abundance of the linkages involved in the structure, *i.e.*, t-Ara*f*, t-Xyl*p*, 4-Xyl*p*, 2-Xyl*p*, 2,4-Xyl*p*. 3,4-Xyl*p*, and 2,3,4-Xyl*p*. These results confirm once again the purity of the xylan extracts obtained after the PAA/DMSO treatment, compared with those obtained after the $NaClO_2$/DMSO procedure. Indeed, when the two isolation treatments were compared, it was evident that the chlorite-extracted xylans contained a significant amount of other contaminating polysaccharides, consistent with the sugar composition results. The GAX content was approximately 85–95% in the PAA/DMSO extracted samples, whereas 75–85% GAX purity was obtained using the $NaClO_2$/DMSO procedure. The presence of 5-Ara*f*, 2-Ara*f*, 3-Ara*f* and 2,5-Ara*f* units, as well as 3-Gal*p* and other branched Gal*p* units, pointed to the presence of arabinan and arabinogalactan, mainly in the $NaClO_2$/DMSO extracts. The extraction of mixed-linkage (1 → 3), (1 → 4)-β-glucan in both the straw and bagasse samples is evidenced by the presence of 3-Glc*p* and 4-Glc*p*. These differences in purity are more pronounced in the straw xylans than in the bagasse samples.

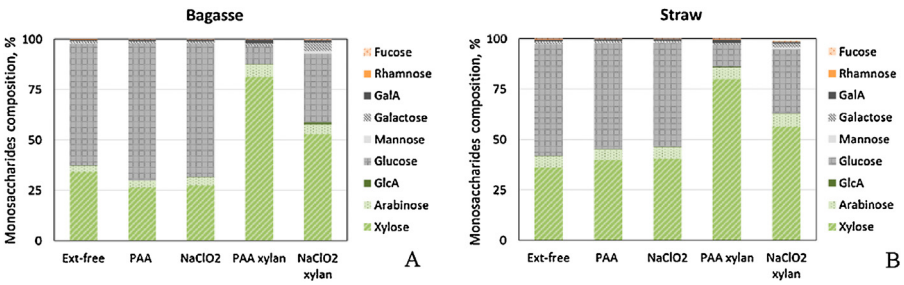

Fig. 3. Sugar composition of bagasse (A) and straw (B) for extractives-free biomasses, PAA-holocellulose, $NaClO_2$-holocellulose and xylan isolated by PAA/DMSO and $NaClO_2$/DMSO.

Table 2
Monosaccharide composition and glycosidic linkage analysis of the xylan extracted from sugarcane bagasse and straw.

Linkage	Structural units deduced	Relative abundance (% mol)[1]			
		Bagasse		Straw	
		PAA/DMSO	$NaClO_2$/DMSO	PAA/DMSO	$NaClO_2$/DMSO
t-Ara*f*	Ara*f*-(1 →	4.5 (0.1)	5.0 (0.4)	5.2 (0.4)	5.7 (0.6)
2-Ara*f*	→ 2) Ara*f*-(1 →	n.d	0.2 (0.2)	0.5 (0.1)	0.8 (0.0)
5-Ara*f*	→ 5) Ara*f*-(1 →	0.2 (0.0)	0.4 (0.1)	0.6 (0.3)	0.7 (0.3)
2,5-Ara*f*	→ 2,5) Ara*f*-(1 →	n.d	0,1 (0,0)	<0,1	0,1 (0,0)
Total Ara[2]		4.7 (0.1)	5.7 (0.1)	6.3 (0.8)	7.2 (1.0)
t-Xyl*p*	Xyl*p*-(1 →	2.5 (0.2)	3.1 (0.2)	2.4 (0.4)	2.3 (0.5)
2-Xyl*p*	→ 2) Xyl*p*-(1 →	n.d	0.3 (0.1)	0.3 (0.1)	0.7 (0.1)
4-Xyl*p*	→ 4)-Xyl*p*-(1 →	80.7 (0.7)	73.0 (2.3)	74.1 (2.3)	53.8 (0.2)
2,4-Xyl*p*	→ 2,4)-Xyl*p*-(1 →	1.1 (0.1)	1.0 (0.1)	0.9 (0.2)	1.1 (0.0)
3,4-Xyl*p*	→ 3,4)-Xyl*p*-(1 →	3.7 (0.1)	4.9 (0.4)	4.2 (0.8)	6.9 (0.7)
2,3,4-Xyl*p*	→ 2,3,4) Xyl*p*-(1 →	<0.1	0.1 (0.0)	0.1 (0.0)	0.2 (0.1)
Total Xyl[2]		88.0 (1.2)	82.4 (2.8)	82.0 (3.8)	65.1 (1.6)
GlcA[2]		0.1 (0.0)	0.7 (0.1)	0.1 (0.0)	0.3 (0.0)
4-*O*-MeGlcA[2]		0.8 (0.1)	0.5 (0.0)	0.5 (0.0)	0.5 (0.0)
Total (4-*O*-Me)GlcA[2]		0.9 (0.1)	1.2 (0.1)	0.6 (0.0)	0.8 (0.0)
t-Glc*p*	Glc*p*(1 →	0.2 (0.0)	0.7 (0.3)	0.2 (0.0)	0,3 (0,0)
3-Glc*p*	→ 3)- Glc*p* A-(1 →	2.6 (0.0)	1.8 (0.3)	3.8 (1.3)	5,5 (0,9)
4-Glc*p*	→ 4) Glc*p*-(1 →	2.2 (0.0)	4.8 (0.8)	4.6 (0.0)	17,0 (0,7)
Total Glc[2]		5.0 (0.1)	7.3 (2.8)	8.6 (1.3)	22.8 (1.6)
4-Man*p*	→ 4) Man*p*-(1 →	n.d.	n.d.	n.d.	n.d.
4,6-Man*p*	→ 4.6) Man*p*-(1 →	n.d.	n.d.	n.d.	n.d.
Total Man[2]		<0.1	1.9 (0.1)	<0.1	0.6 (0.2)
t-Gal*p*	Gal*p*-(1 →	0.5 (0.2)	0.3 (0.1)	0.7 (0.0)	1,8 (0,1)
6-Gal*p*	→ 6) Gal*p*-(1 →	<0.1	<0.1	0.2 (0.1)	0,1 (0,0)
3-Gal*p*	→ 3) Gal*p*-(1 →	<0.1	0.1 (0.0)	0.2 (0.1)	0,1 (0,0)
3,6-Gal*p*	→ 3.6) Gal*p*-(1 →	<0.1	0.1 (0.0)	<0.1	0,2 (0,1)
4,6-Gal*p*	→ 4.6) Gal*p*-(1 →	0.1 (0.0)	0.2 (0.1)	0.4 (0.2)	0,7 (0,0)
Total Gal[2]		1.0 (0.1)	0.7 (0.2)	1.5 (0.5)	3.1 (0.3)
Total Rha[2]		n.d.	<0.1	n.d	<0.1
Total GalA[2]		0.3 (0.0)	0.4 (0.0)	0.8 (0.0)	0.4 (0.0)
Total GAX[3]		92.8	88.5	87.2	73.5
Ara*f*/Xyl*p*[4]		0.05	0.06	0.08	0.11
(4-*O*-Me)Glc*p*A/Xyl*p*[5]		0.01	0.02	0.01	0.01
t:bp[6]		1.14	1.05	1.05	0.80
u:m(Xyl*p*)[7]		16.9	12.3	14.5	6.7

Notes: [1] Relative abundance (%mol) of the different linkages corrected by the results of monosaccharide composition by acid methanolysis; [2]Monosaccharide composition as calculated by acid methanolysis; [3] Total GAX content calculated from the specific linkages (t-Xyl*p* + 4-Xyl*p* + 2-Xyl*p* + 2 × 2,4-Xyl*p* +2 × 3,4-Xyl*p* + 3 × 2,3,4-Xyl*p*); [4] Ara*f*/Xyl*p* ratio calculated based on the monosaccharide composition; [5] (4-*O*-Me)Glc*p*A/Xyl*p* ratio calculated based on the monosaccharide composition; [6]t:bp ratio for GAX calculated based on the amount of terminal sugars (t-Ara*f* and 4-*O*-MeGlcA) *versus* the relative amount of branching points (2,4-Xyl*p* and 3,4-Xyl*p*); [7] u:m(Xyl*p*) ratio for GAX based on the ratio of unsubstituted Xyl*p* units (4-Xyl*p*) and mono-substituted Xyl*p* units (2,4-Xyl*p* and 3,4-Xyl*p*). Standard deviation (. . .); n.d: not determined.

The linkage patterns observed in the straw and bagasse xylans were quite similar, with the straw xylan having a slightly higher degree of Ara*f* branching (Ara*f*/Xyl*p*) than the bagasse xylan. To the best of our knowledge, this is the first time that linkage analysis has been performed on PAA-extracted sugarcane straw or bagasse. Previously reported linkage analysis on alkali-extracted xylan from sugarcane bagasse also showed the absence of double substitutions in this type of xylan, but indicated a 2–3 times greater degree of arabinosyl branching at *O*-3 (Banerjee, Pranovich, Dax, & Willfor, 2014; Mellinger-Silva et al., 2011). These differences might be related either to deacetylation in alkaline conditions, which may have enhanced the extraction of larger GAX populations with a higher Ara*f*/Xyl*p* ratio, or to the higher solubility of acetylated xylan fractions in DMSO, which may have resulted in a smaller degree of arabinosyl branching. The amount of MeGlcA substitutions as

represented by the MeGlcpA//Xylp ratio are very similar for both sugarcane bagasse and straw xylans.

Using the PAA/DMSO process, the formally determined molar ratio between terminal residues (t-Ara*f*) and branch units (2,4-Xyl*p*, 3,4-Xyl*p* and 2,3,4-Xyl*p*), hereinafter referred to as t:bp, was 1.14 for bagasse and 1.05 for straw, which indicates that no under-methylation occurred during the analysis. On the other hand, the $NaClO_2$/DMSO process revealed less conformity in the t:bp ratio for bagasse (1.05) and straw (0.80). This lower ratio in the $NaClO_2$/DMSO xylan samples might indicate insufficient delignification during the chlorite process, which will underestimate the amount of terminal units not reflected in the linkage analysis (Jeffries, 1991).

3.3. Characterization of the structure of acetylated xylan by ^1H NMR

^1H NMR analysis was used to confirm the intramolecular structure of xylan obtained by linkage analysis and to identify and quantify the position of acetyl groups in the xylan backbone. The PAA/DMSO extracted xylans from bagasse and straw were selected for this more detailed structural investigation due to their higher yield and higher purity. The extracted xylans were completely (bagasse) or almost completely (straw) soluble D_2O used for NMR measurements. The ^1H NMR spectra of xylan samples are shown in Fig. 4. The assignments for proton resonances were done using existing databases for glucuronoxylans (Cavagna, Deger, & Puls, 1984; Evtuguin et al., 2003; Hoffmann, Kamerling, & Vliegenthart, 1992; Izydorczyk and Biliaderis, 1992 Magaton et al., 2008; Marques, Gutiérres, del Río, & Evtuguin, 2010; Sun, Cui, Gu, & Zhang, 2011) and are presented in Table 3. The (1 → 4)-linked β-D-xylopyranosyl internal units of the backbone were clearly identified. The integral of protons from acetyl groups ($\underline{CH_3}$—CO-) (δ_H 2.00–2.25) showed good balance to amounts of acetyls in acetylated xylopyranose units identified using characteristic protons in corresponding structures. These acetylated structures were Xyl*p* units acetylated at *O*-3 (anomeric proton integral at δ_H 4.54–4.62), at *O*-2 (anomeric proton and H-2 integrals at δ_H 4.54–4.62) or 2,3-di-*O*-acetylated (H-3 integral at δ_H 5.11–5.15). The Xyl*p* units *O*-3 acetylated and *O*-2 substituted by MeGlcA residues have been also detected (H-3 integral at δ_H 5.05–5.08). Most part of terminal Ara*f* units were α-(1 → 3)-linked to mono-substituted Xyl*p* units as followed from the characteristic anomeric proton resonance at δ_H 5.39 (Izydorczyk & Biliaderis, 1992). This fact, however, not exclude completely the possibility for mono-substituted α-(1 → 2)-Ara*f* and di-substituted Xyl*p* units with α-(1 → 3) and α-(1 → 2)-linked Ara*f* units found previously in wheat arabinoxylan (Izydorczyk & Biliaderis, 1992). However, the results of the glycosidic linkage analysis (Table 2) showed that these di-substituted with Ara*f* Xyl*p* units branches occurred in a very low frequency in xylan from both bagasse and straw.

The presence of α-(1 → 2)-linked MeGlc*p*A to Xyl*p* units was confirmed by the presence of anomeric protons in corresponding structures at δ_H 5.28–5.29 and very narrow signal at ca δ_H 3.45 assigned to protons in methoxyl groups of MeGlc*p*A in heteroxylans (Shatalov et al., 1999; Shi et al., 2012).

3.4. Quantification of content and position of acetyl groups in sugarcane acetylated heteroxylans

The quantification of the total number of released acetyl groups by saponification and subsequent HPLC analysis, together with the assignment of the positions of the acetyl groups by ^1H NMR, allows for the full characterization of the acetyl content (in%), degree of acetylation (D_{Ac}) and acetylation pattern in the isolated acetylated arabinoxylans from sugarcane bagasse and straw (Table 4). The acetyl content in the PAA/DMSO-extracted xylans from bagasse and straw accounts for 8.7% and 2.4%, respectively, which is in agreement with the acetyl contents in extractives-free biomasses reported in our previous work (Carvalho et al., 2015). The acetyl content in straw is significantly lower than in bagasse, which evidences the divergent acetylation pattern in different plant tissues, depending on their developmental stage and function (Gille & Pauly, 2012). The acetyl content and D_{Ac} are markedly lower for the samples extracted after the $NaClO_2$/DMSO process, which again indicates the lower efficiency, and lower selectivity towards acetylated GAX, of this process.

3.5. Average molecular weight and molecular weight distribution

The size and molecular weight distributions for the xylans extracted from the sugarcane bagasse and straw were analyzed using multiple-detection size-exclusion chromatography. The molar mass average values and size distributions, obtained by both standard calibration (using pullulan as a linear calibrant) and absolute calibration (by light scattering), were compared for 4 replicates at different concentrations between 1.0–4.0 g L^{-1}. The number- and weight-average molar masses of the extracted xylan samples are shown in Table 5. The reproducibility of the four replicates is very good as demonstrated by the low standard deviation shown in Table 5, which reinforces the accuracy of the procedure. The SEC weight distribution, $w(\log V_h)$, and the size dependence of the weight-average molecular weight $\bar{M}_W(V_h)$, presented as a function of the hydrodynamic radius (R_h), is shown in Fig. 5. The procedure employed for the calibration of the hydrodynamic size from the SEC, using the Mark-Houwink equation, is presented in the Supplementary material Fig. S2, while the raw SEC chromatograms obtained with a differential refractive index and light scattering (at 90°) are presented in the Supplementary material Fig. S4. The procedure was validated for a reference wheat endosperm arabinoxylan (AX), which exhibited similar molar mass distributions and average molar mass values as those reported by previous publications in a DMSO/LiBr solvent system (Pitkänen et al., 2009; Shelat, Vilaplana, Nicholson, & Gibert, 2010). The molecular solubility of the xylan samples in the SEC eluent (DMSO/LiBr) can be verified by the absence of aggregation spikes in the light scattering signal at 90° (Supplementary material Fig. S3). Aggregation of arabinoxylan has been reported in aqueous conditions (Pitkänen et al., 2009, 2011); however, the use of a polar organic solvent such as DMSO with lithium salts as hydrogen-bond disruptors (such as LiBr) should contribute to an enhanced solubility and the absence of aggregation phenomena during SEC separations, which would bias the molar mass determinations (Shelat et al., 2010; Vilaplana & Gilbert, 2010). The SEC weight distributions, $w(\log V_h)$, for the different sugarcane samples exhibit monomodal size profiles. This confirms the homogeneous macromolecular populations in such extracts, which are related mostly to acetylated GAX macromolecules. The amount of other polysaccharide impurities (mainly mixed-linkage β-glucans and arabinogalactans, as reported by glycosidic linkage analysis in Table 2) should not influence the molar mass distributions and average molar mass values.

The average molar mass values for the extracted xylan samples that were obtained using standard and light scattering calibration show interesting and marked differences. The average molar mass values for the sugarcane bagasse and straw xylans that were obtained using a standard calibration are in the range of 26–40 kDa, which are quite similar to the values previously obtained for sugarcane bagasse xylans (Sun et al., 2004; Peng et al., 2009) and for straw xylans from other grasses (*e.g.*, wheat straw) (Persson, Ren, Joelsson, & Jönsson, 2009). However, the light scattering data provided molar mass values for the sugarcane bagasse and straw that are larger than those obtained from a standard calibration,

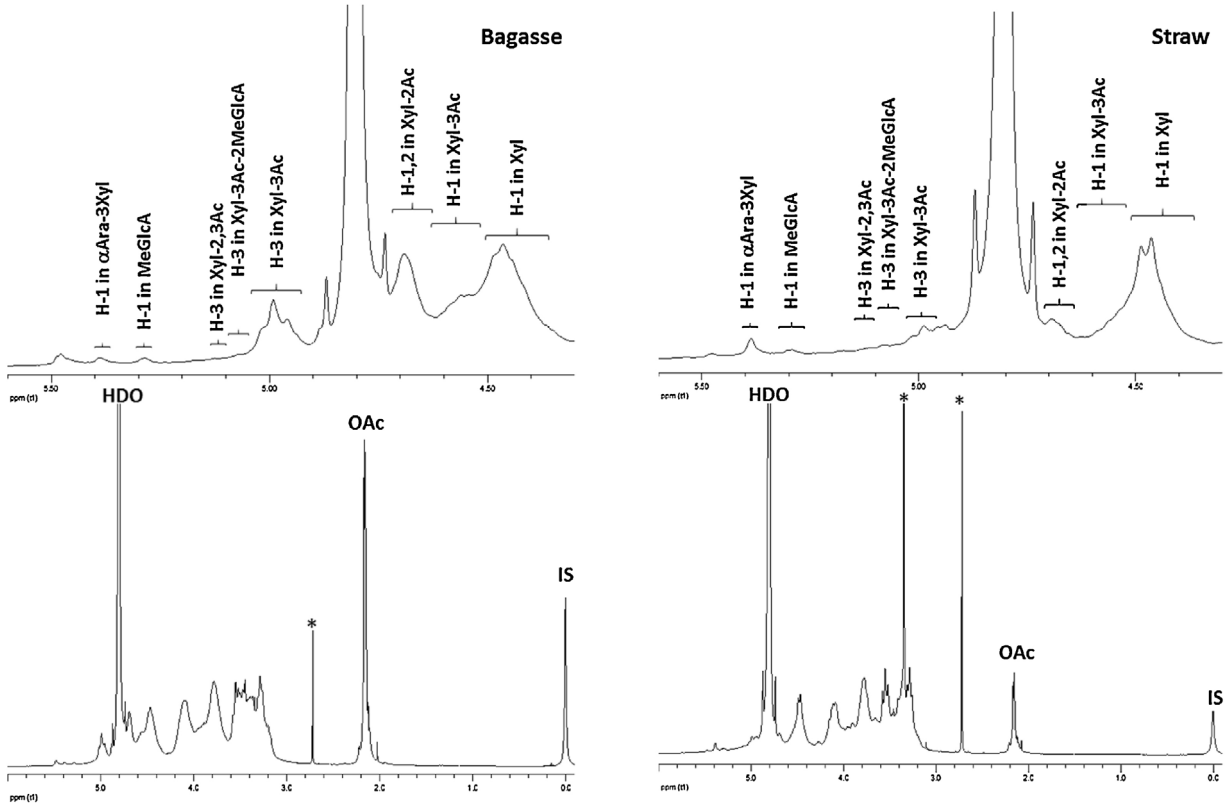

Fig. 4. [1]H NMR spectra of acetylated xylan from bagasse and straw extracted by PAA/DMSO process. Designation for the structural fragments is presented in Table 3. *solvent impurities.

Table 3
[1]H NMR chemical shifts for structural units of acetylated xylan from bagasse and straw obtained by PAA/DMSO process.

Biomass	Structural units	H1	H2	H3	H4	H5	
						ax	eq
Bagasse	Xyl (isol.)	4.46	3.28	3.55	3.78	3.37	n.d.
	Xyl (Xyl-Ac)	4.44	3.22	3.53	n.d	3.36	n.d.
	Xyl-3Ac	4.56	3.47	4.98	n.d.	3.47	n.d.
	Xyl-2Ac	4.68	n.d.	n.d	3.87	3.43	n.d.
	Xyl-2,3Ac	n.d	n.d.	5.13	4.04	3.53	n.d.
	Xyl-3Ac-2MeGlcA	n.d	3.68	5.06	3.98	n.d	n.d
	MeGlcA	5.28	3.57	3.83	3.17	n.d.	–
	αAra-3Xyl	5.39	n.d.	3.93	4.27	n.d	n.d
Straw	Xyl (isol.)	4.48	3.28	3.58	3.77	3.38	n.d.
	Xyl (Xyl-Ac)	4.44	3.20	3.54	n.d	3.35	n.d.
	Xyl-3Ac	4.54	3.46	4.98	3.90	3.46	n.d.
	Xyl-2Ac	4.69	n.d.	n.d.	3.84	n.d.	n.d.
	Xyl-2,3Ac	n.d.	n.d.	5.12	n.d.	n.d.	n.d.
	Xyl-3Ac-2MeGlcA	n.d.	n.d	5.06	3.97	n.d	n.d.
	αAra-3Xyl	5.39	n.d	3.94	4.27	n.d.	n.d
	MeGlcA	5.29	3.57	3.83	3.17	n.d.	–

[n.d] Not detected or non-existent. The designations used were as follows: Xyl (isol.) is non-acetylated Xyl*p* in the backbone isolated from other acetylated Xyl*p* units; Xyl (Xyl-Ac) denotes non-acetylated Xyl*p* linked with neighboring acetylated Xyl*p*; Xyl-3Ac corresponds with 3-*O*-acetylated Xyl*p*; Xyl-2Ac is 2-*O*-acetylated Xyl*p*; Xyl-2,3Ac denotes 2,3-di-*O*-acetylated Xyl*p*; Xyl-3Ac-2GlcA, MeGlcA 2-*O*-linked and 3-*O*-acetylated Xyl*p*; αAra-3Xyl corresponds with terminal arabinose linked to *O-3* of monosubstituted xylose; MeGlcA denotes terminal MeGlcA residue linked to *O-2* in monosubstituted Xyl*p* and also in 3-*O*-acetylated Xyl*p*.

and also indicated some differences between the two extraction methods. Standard calibration provides relative molar masses with respect to a linear macromolecular standard (in our case, pullulan). Due to the fact that SEC separates macromolecules based on size or hydrodynamic volume, V_h (Jones et al., 2009) (which, for SEC, is known to be proportional to the product of the intrinsic viscosity and the number-average molar mass, in accordance with the universal calibration theory established by Hamielec, Ouano, and Nebenzahl (1978) and Kostanski, Keller, and Hamielec (2004), and not molar mass, the average molar mass values and distributions obtained by standard calibration using a linear polymer will underestimate the absolute values for a substituted/branched polymer. During SEC elution, linear macromolecules will have a more elongated hydrodynamic conformation and will elute earlier than macromolecules with a similar molar mass but more substituted and compact structure. In such cases, it is necessary to use light scattering detection to obtain absolute molar mass determinations. The average molar mass values obtained by the light scattering method,

Table 4
Acetyl content, degree of acetylation (D_{Ac}) and acetylation pattern in sugarcane xylans.

Sample	PAA/DMSO					$NaClO_2$/DMSO	
	Acetyl content (%)[a]	D_{Ac}[b]	Acetylation pattern (%)[c]			Acetyl content, (%)[a]	D_{Ac}[b]
			O-2	*O*-3	*O*-2,3		
Bagasse	8.72 (0.37)	0.33 (0.01)	3.38	4.85	0.49	5.39 (0.22)	0.17 (0.01)
Straw	2.42 (0.45)	0.10 (0.01)	0.54	1.75	0.13	1.46 (0.00)	0.05 (0.00)

[a] The acetyl content was obtained by saponification and HPLC analysis of the released acetic acid.
[b] The degree of acetylation was calculated according to Xu et al. (2010).
[c] The relative acetylation pattern (%) was calculated by the integration of the corresponding ^{1}H NMR peak areas for the Xyl-2Ac, Xyl-3Ac and Xyl-2,3Ac signals. Standard deviation (); n.d: not determined.

Table 5
Number-average molar mass ($\bar{M}_n$), weight-average molar mass ($\bar{M}_W$) and dispersity (D) for the extracted xylan samples from sugarcane bagasse and straw using standard and light scattering calibration obtained from SEC-DRI-MALLS. The average molar mass values are compared with a reference AX from wheat endosperm.

Samples	Extraction process	Standard calibration			Light scattering calibration		
		$\bar{M}_n$, kDa	$\bar{M}_W$, kDa	D	$\bar{M}_n$, kDa	$\bar{M}_W$, kDa	D
Bagasse GAX	PAA/DMSO	26.3 (0.5)	40.9 (0.3)	1.56	118.6 (1.8)	122.7 (0.7)	1.03
	$NaClO_2$/DMSO	22.2 (0.5)	35.3 (0.4)	1.59	36.8 (1.0)	45.8 (0.6)	1.06
Straw GAX	PAA/DMSO	18.1 (0.1)	31.6 (0.7)	1.74	93.9 (2.1)	97.1 (2.5)	1.03
	$NaClO_2$/DMSO	16.1 (0.3)	26.3 (0.3)	1.62	37.9 (1.2)	39.8 (1.2)	1.05
Wheat endosperm AX		84.9	255.9	3.01	148.8	289.0	1.94

Standard deviation (. . .).

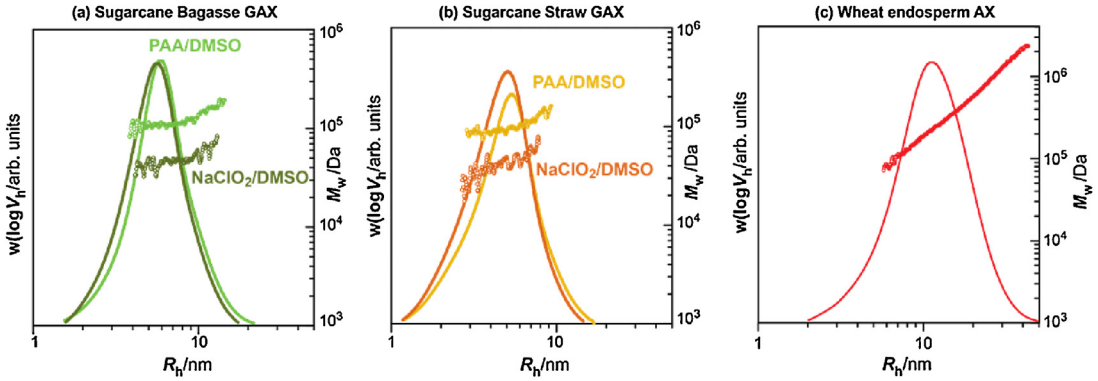

Fig. 5. SEC weight distribution, $w(\log V_h)$, and the size dependence of the weight-average molecular weight, $\bar{M}_W(V_h)$ as a function of the hydrodynamic radius (R_h) obtained after SEC-DRI-MALLS: (a) sugarcane bagasse GAX, (b) sugarcane straw GAX and (c) wheat endosperm AX.

therefore, provide more accurate information on the molecular properties of xylans isolated from sugarcane bagasse and straw. The SEC weight distributions $w(\log V_h)$ for the GAX samples extracted with PAA/DMSO from bagasse and straw show similar profiles, being the distributions for bagasse slightly shifted towards larger macromolecular sizes. The size dependence of the absolute molar mass from lightscattering $\bar{M}_W(V_h)$ shows similarly larger values for bagasse than straw, which corresponds with the reported SEC distributions (Fig. 5). This behavior agrees as well with the larger average molar mass values for the sugarcane bagasse xylans compared to straw using both standard and light scattering calibration (Table 5). These small differences in molar mass between bagasse and straw can be attributed to the larger degree of polymerization in the xylan backbone, in agreement with the results from linkage analysis. A similar trend can be observed using both standard calibration and light scattering data for the samples isolated by $NaClO_2$/DMSO extraction, where the sugarcane bagasse xylans exhibit higher absolute molar mass values than xylans isolated from sugarcane straw.

However, larger differences in the average molar mass values and the size dependence of the weight-average molecular weight $\bar{M}_W(V_h)$ can be observed for both sugarcane bagasse and straw when comparing the samples extracted by the PAA/DMSO method with those extracted by the $NaClO_2$/DMSO method. Both extraction procedures provide xylan samples with similar SEC weight distributions, $w(\log V_h)$, but slightly shifted towards larger macromolecular sizes for the case of PAA/DMSO extracted xylans. However, the absolute molar masses are markedly higher for the xylans extracted by the PAA/DMSO method compared to the $NaClO_2$/DMSO method. These larger molar masses can be attributed to a more selective delignification process by PAA compared to $NaClO_2$, which induces minimized xylan degradation. The different macromolecular conformations for xylans extracted from both methods can be attributed to the larger GAX content in the PAA/DMSO samples, the content of Ara*f* substitutions and the larger degree of acetylation, which results in a more compact conformation in certain sizes (or hydrodynamic volumes) of such macromolecular populations. From these integrated results, it can be inferred that acetylation and glycosyl (Ara*f* and MeGlcA) substitution play a significant role in fine-tuning the macromolecular conformation of the acetylated GAXs extracted from sugarcane bagasse and straw, which, in turn, affects the elution profiles during SEC elution and light scattering detection.

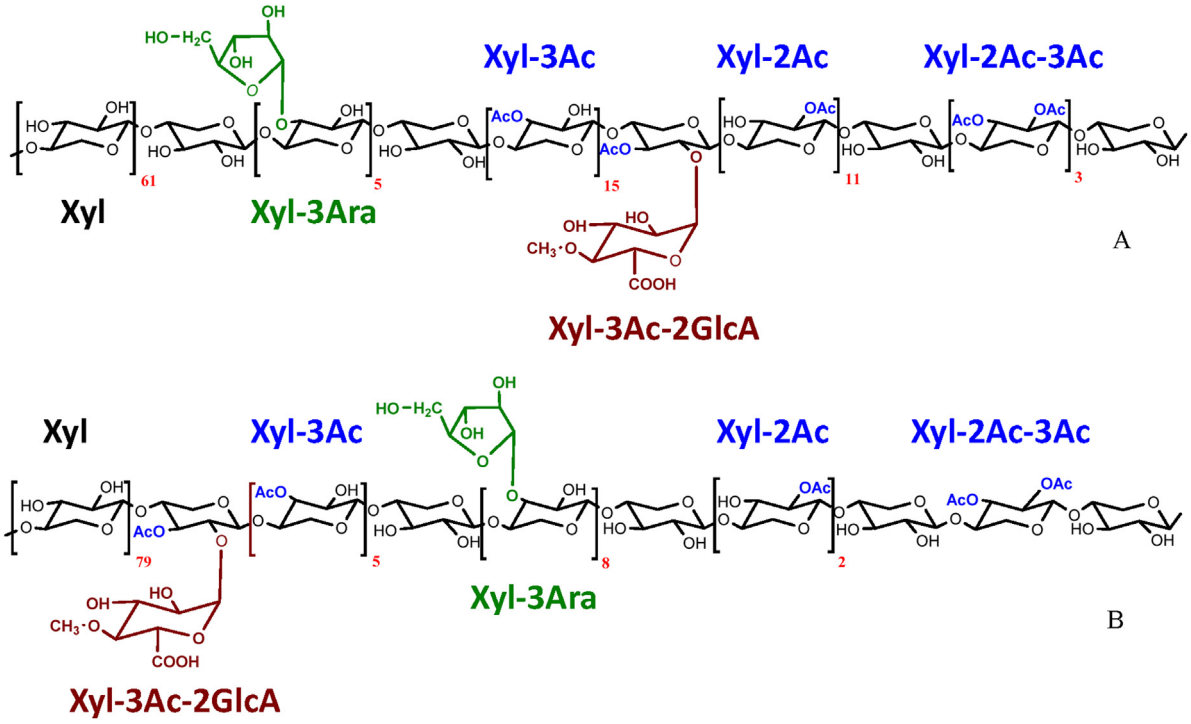

Fig. 6. Empirical structure of xylan isolated by the PAA/DMSO process from bagasse (A) and straw (B).

Table 6
Empirical structure of acetylated GAX from bagasse and straw isolated by PAA/DMSO process. The relative abundance has been calculated integrating the results from glycosidic linkage analysis, acetylation content by HPLC and NMR.

Structural fragments and short designation		Relative abundance (per 100 Xyl*p* units)	
		Bagasse	Straw
→4)-β-D-Xyl*p*-(1→	(Xyl)	65	83
→4)[3-*O*-Ac]-β-D-Xyl*p*-(1→	(Xyl-3Ac)	15	5
→4)[2-*O*-Ac]-β-D-Xyl*p*-(1→	(Xyl-2Ac)	11	2
→4)[3-*O*-Ac] [2-*O*-Ac]-β-D-Xyl*p*-(1→	(Xyl-2,3Ac)	3	1
→4)[α-L-Ara*f*(1→)]-β-D-Xyl*p*-(1→	(Xyl-3Ara)	5	8
→4)[4-*O*-Me-α-D-Glc*p*A-(1→2)][3-*O*-Ac]-β-D-Xyl*p*- (1→	(Xyl-3Ac-2GlcA)	1	1

3.6. Empirical structures of acetylated arabinoxylan from bagasse and straw

The results of the glycosidic linkage analysis, ^{1}H NMR spectroscopy and acetyl quantification were integrated so as to develop, for the first time, an empirical model for the molecular structure of acetylated glucuronoarabinoxylan (GAX) isolated from sugarcane bagasse and straw with PAA/DMSO. These empirical xylan structures, together with nomenclature of the intramolecular motifs, are set out in Fig. 6 and Table 6, respectively.

The acetylated GAX from sugarcane bagasse and sugarcane straw were shown to be structurally different from each other. Acetylated GAX from sugarcane bagasse has a molar ratio between the xylose units, *O*-2 linked glucuronic units and the *O*-3 linked terminal arabinose units of 10:0.1:0.5. Bagasse xylan contained 0.33 acetyl groups per xylose unit, with ca 53% of the acetyl groups being observed at position *O*-3 of the xylose, ca 37% at position *O*-2 and 10% at positions *O*-2 and *O*-3 of the same xylose residue.

Acetylated AGX from sugarcane straw, on the other hand, has a molar ratio between the Xyl*p* units, *O*-2 linked glucuronic units and the *O*-3 linked terminal arabinose units of 10:0.1:0.8, which indicates that straw xylan is slightly more branched than xylan from bagasse, with preference for Ara*f* substitution in position *O*-3. However, straw xylan has significantly lower acetylation (0.10 acetyl groups per xylose unit), with ca 68% of the acetyl groups being observed at position *O*-3 of the xylose, 21% at position O-2 of the xylose and ca 11% at positions *O*-2 and *O*-3 of the same xylose residue.

4. Conclusions

Two different extraction procedures (PAA/DMSO and $NaClO_2$/DMSO) were compared for the isolation of acetylated AGX from sugarcane bagasse and straw. In general, the PAA/DMSO method resulted in greater efficiency and selectivity. This mild isolation methodology, together with detailed structural analyses, provides evidence of the intramolecular Ara and acetyl substitution pattern in sugarcane xylans.

We successfully developed an empirical model for the molecular structure of acetylated glucuronorabinoxylan (GAX) extracted from sugarcane bagasse and straw, integrating the results from methylation glycosidic linkage analysis, H^{1} NMR spectroscopy and acetyl quantification.

We have found that GAX from sugarcane bagasse differs structurally from that of sugarcane straw. Bagasse GAX had a slightly lower glycosyl substitution molar ratio of Ara*f* to Xyl*p* (0.5:10) than xylan from straw (0.8:10), but a higher degree of acetylation (0.33 and 0.10 for bagasse and straw, respectively). The acetyl groups were attached predominantly to positions *O*-3 (53%), *O*-2 (37%) and *O*-2,3 (10%) of the Xyl*p* units in bagasse GAX, and to positions *O*-3

(68%), *O*-2 (21%) and *O*-2,3 (10%) of the Xyl*p* units in straw GAX. These changes in the substitution pattern modulate the conformation of the acetylated GAX, as evidenced by the elution patterns and size distributions under SEC and the absolute molar weight identification by light scattering.

This new knowledge of the structure of xylan in sugarcane bagasse and straw biomasses will provide a better understanding of their behavior during chemical processing and, ultimately, create new possibilities for the use of xylan biopolymers in materials and products.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2016.09.022.

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