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Comparative study of alkali- and acidic organic solvent-soluble hemicellulosic polysaccharides from sugarcane bagasse

F. Xu,[a,b] J. X. Sun,[a] C. F. Liu[b] and R. C. Sun[b,*]

[a] *College of Forestry, North-Western Science and Technology University of Agriculture and Forestry, Yangling 712100, China*
[b] *State Key Laboratory of Pulp and Paper Engineering, South China University of Technology, Guangzhou 510640, China*

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Abstract—Two-stage treatments of sugarcane bagasse with mild alkali and acidic 1,4-dioxane were performed. Pretreatment with 1 M NaOH aqueous solution at 20, 25, 30, 35, and 40 °C for 18 h released 55.5%, 57.3%, 59.1%, 60.9%, and 62.1% of the original hemicelluloses, respectively. Post-treatment of the corresponding alkali-treated residue with 1,4-dioxane–2 M HCl (9:1, v/v) at 87 °C for 2 h, respectively, degraded 11.6%, 11.9%, 11.4%, 10.9%, and 10.6% of hemicelluloses (% dry starting material). It was found that the five alkali-soluble hemicellulosic preparations contained a much higher amounts of xylose (78.0–82.2%) and slightly higher uronic acids (4.8–5.8%), mainly 4-*O*-methyl-α-D-glucopyranosyluronic acid, but were lower in arabinose (9.3–11.7%) and glucose (2.2–4.1%) than those of the corresponding five acidic dioxane-degraded hemicellulosic fractions in which xylose (44.9–46.8%), arabinose (35.9–38.1%), and glucose (13.0–13.7%) were the major sugar constituents. The studies revealed that the five alkali-soluble hemicellulosic preparations were more linear and acidic, and had a large molecular weight (35,200–37,430 g mol^{-1}) than those of the hemicellulosic fractions (12,080–13,320 g mol^{-1}) degraded during the acidic dioxane post-treatment. This demonstrated that the post-treatment with acidic dioxane under the condition used resulted in substantial degradation of the hemicellulosic polymers. The 10 hemicellulosic samples were further characterized by FT-IR and ^{1}H and ^{13}C NMR spectroscopy, GPC and thermal analysis, and the results are reported.

Keywords: Sugarcane bagasse; Hemicelluloses; Extraction; Alkali; Acidic dioxane

1. Introduction

Sugarcane bagasse is a plentiful lignocellulosic waste typically found in countries that process sugarcane such as Brazil, India, Cuba, and China.[1] In general, sugar factories generate approximately 270 kg of bagasse (50% moisture) per metric ton of sugarcane. About 50% of this amount is enough to supply the required energy for the production of sugar and fuel ethanol if there is an adjacent distiller facility.[2] The remaining bagasse is usually stockpiled, constituting an eyesore and an environmental problem, due to the risk of spontaneous combustion of the stored bagasse.[3] On the other hand, in addition to wood, bagasse provides a low-cost feedstock for biological production of fuels and chemicals, which offer economic, environmental, and strategic advantages.[4] Bagasse generally contains 40–45% cellulose and 30–35% hemicelluloses.[5] Primarily in China, bagasse is the second most commonly used non-wood fiber plant material after cereal straws for pulp and paper production, since China does not possess indigenous supplies of wood, but has an abundant supply of such agricultural residues as straw and bagasse.[6] This biomass is, therefore, a renewable feedstock for the production of value-added chemicals from its lignocellulosic constituents, such as hemicelluloses.

Hemicelluloses are a group of complex plant polysaccharides that are biosynthesized in large quantities in most trees and terrestrial plants. An estimated annual production of hemicelluloses on the earth is in the range

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[*] Corresponding author at present address: The BioComposites Centre, University of Wales, Bangor, Gwynedd LL57 2UW, UK. Tel.: +44 1248 382088; e-mail: bcs00a@bangor.ac.uk

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of 60 billion tons. They are after cellulose, the world's second most abundant family of polymers and thus represent an enormous renewable resource that remains almost completely unused. However, in recent years there is emerging interest for the application of hemicelluloses as polymers and other compounds.[7] For example, xylans, obtained from wood or cereal straw, have been tested as gel-forming or thermoplastic materials,[8] as fillers for polypropylene,[9] as a component for paint formulation,[10] and as a coating for cellulosic fibers.[11] In addition to the polymers, xylose, recovered by dilute acid hydrolysis, can be converted to xylitol, a high-valued specialty product, via catalytic or enzymatic hydrogenation.[12] For higher-value products, application in the pharmaceutical industries might be of interest, for instance as tabletting material,[13] for the treatment of wounds,[14] or for preventing blood coagulation.[15]

To the best of our knowledge, there have been no comparative studies on the characterization of alkali- and organic solvent-soluble hemicellulosic polysaccharides present in sugarcane bagasse. In this present work, the bagasse is fractionated by means of a two-stage treatment into cellulosic fibers with low lignin content, lignin fragments, and alkali-soluble hemicellulosic polysaccharides, as well as hemicellulosic oligomers and hemicellulose sugars. Since structure and functions are intimately related, knowledge of the chemistry of this polymer is of crucial importance for its proper utilization. The current work thus aims at fractionally isolating hemicellulosic polysaccharides and their degraded products from the cell walls of bagasse and comparatively determining their structural features.

2. Experimental

2.1. Materials

Sugarcane bagasse (SCB) was obtained from a sugar factory in Guangzhong, China. It was dried in sunlight. The dried bagasse was ground in a Christie laboratory mill to pass a 1.5 mm-size screen and dried again in a cabinet oven with air circulation for 16 h at 60 °C. It was then de-waxed with 2:1 (v/v) toluene–EtOH in a Soxhlet apparatus for 6 h. Lignocellulose is the main composition in SCB, which is a compact structure of cellulose (43.6%), hemicelluloses (33.5%), in close association with lignin (18.1%).[16] All standard chemicals, such as sugars and phenolics, were analytical grade, purchased from Sigma Chemical Company (Beijing).

2.2. Isolation of alkali-soluble hemicelluloses

Wax-free bagasse (2 g) was extracted with 50 mL of 1 M NaOH for 18 h under N_2 with magnetic stirring at 20, 25, 30, 35, and 40 °C, respectively. After each extraction, the residue was filtered off and washed thoroughly with water until the filtrate was neutral, and then dried in an oven at 60 °C for 16 h. The combined supernatant fluid was neutralized to pH 6.0 immediately with dropwise addition of 6 M HCl in an ice bath. All extracts were concentrated and kept at room temperature. The solubilized hemicelluloses were isolated by precipitation of the concentrated filtrates with 3 vol of 95% EtOH. After filtration, the isolated hemicelluloses were thoroughly washed with 70% EtOH and then air dried.

2.3. Isolation of acidic organic solvent-soluble hemicellulosic fragments

Each of the foregoing mild alkali-treated residues were post-treated with 1,4-dioxane–2 M HCl (9:1, v/v) at 87 °C for 2 h with a solvent:solid ratio of 25 mL/g, respectively. After cooling, the residues were filtered off with a nylon cloth and washed with a fresh dioxane–water solution (3 × 30 mL) without HCl. The filtrates and washing liquids were combined and partially concentrated under vacuum. Addition of water followed by evaporation under reduced pressure at 45 °C allowed complete elimination of dioxane. The concentrated filtrates were then neutralized with 2 M NaOH to pH 6.0 in an ice bath. The degraded hemicelluloses were isolated and purified as the method described in the foregoing mild alkaline hydrolysis. The procedure used to isolate alkali- and acidic organic solvent-soluble hemicelluloses is illustrated in Figure 1. All experiments in this study were conducted at least in duplicate.

2.4. Chemical characterization

For the determination of monosaccharide composition, the alkali-soluble hemicellulosic polysaccharides and the acidic organic solvent-degraded hemicellulosic fragments were hydrolyzed using trifluoroacetic acid (2 M, at 120 °C), followed by evaporation to dryness and successive reduction with $NaBH_4$ and acetylation with Ac_2O.[17] The resulting alditol acetates obtained were analyzed by gas chromatography (GC) and identified by their typical retention times. The content of total uronic acids was determined colorometrically by the method of Blumenkrantz and Asboe–Hansen.[18] Method for measurement of the hemicellulosic molecular weights has been described in a previous paper.[19] Klason lignin content in hemicellulosic samples was determined according to Tappi method T 249 cm-85. The chemical composition of phenolics liberated from alkaline nitrobenzene oxidation of the lignins associated in hemicellulosic preparations was determined on a Hichrom H5ODS HPLC column of dimensions 250 × 4.6 mm (Phenomenex Co., Beijing). The identification of the individual compounds was detected at 280 nm by com-

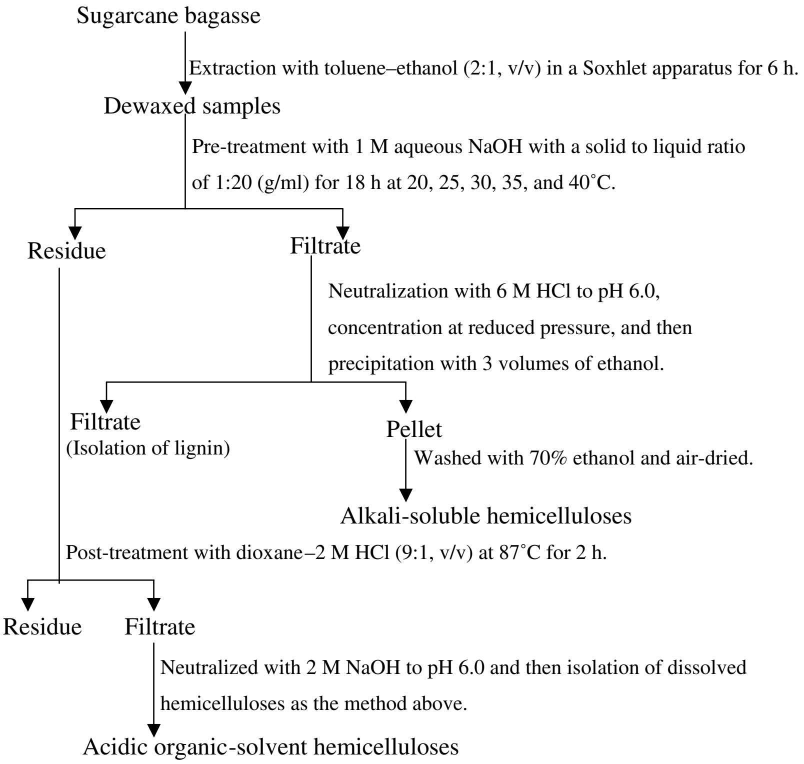

Figure 1. Scheme for isolation of alkali-soluble and acidic organic solvent-soluble hemicelluloses from sugarcane bagasse.

puter comparison of the retention times and peak areas with the authentic phenolics. All analyses were conducted in duplicate, and values were required to be within a 6% reproducibility range.

2.5. Spectroscopic and thermal characterization

FT-IR spectra of the hemicellulosic samples were obtained on an FT-IR spectrophotometer (Nicolet 750) in the range 4000–400 cm^{-1} using a KBr disc containing 1% finely ground samples. The solution-state ^1H NMR spectrum was recorded on a Bruker MSL300 spectrometer at 300 MHz using 15 mg of hemicelluloses in 1.0 mL of D_2O. A ^{13}C NMR spectrum was obtained on a Bruker MSL300 spectrometer at 74.5 MHz. The sample (80 mg) was dissolved in 1 mL D_2O (99.8% D) with overnight stirring at room temperature. The spectrum was recorded at 25 °C after 30,000 scans. Chemical shifts (δ in ppm) are expressed relative to the resonance of Me_4Si ($\delta = 0$). A 60° pulse flipping angle, a 3.9 μs pulse width and a 0.85 s acquisition time were used.

Thermal analysis of the hemicellulosic samples was performed using thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) on a simultaneous thermal analyzer (SDT Q600, TA Instrument). The apparatus was continually flushed with nitrogen. The sample weighed between 9 and 11 mg and heated from room temperature to 600 °C at a rate of 10 °C/min.

3. Results and discussion

3.1. Yield of hemicelluloses

It is known that hydroxyl ions cause swelling of cellulose, hydrolysis of ester linkages, and disruption of intermolecular hydrogen bonds between cellulose and hemicelluloses, bringing a portion of the hemicellulosic material into solution.[20] High concentrations of hydroxide result in higher yields of extraction when performed at room temperature, indicating a disruption of stronger linkages, such as ferulic acid bridges between hemicelluloses and lignin.[21] In the present study, pretreatment of de-waxed bagasse with 1 M NaOH aqueous solution at 20, 25, 30, 35, and 40 °C for 18 h resulted in 55.5%, 57.3%, 59.1%, 60.9%, and 62.1% of the original hemicelluloses release (Table 1), and 54.7%, 58.6%, 64.1%, 70.2%, and 74.0% of the original lignin removal, respectively (data not shown). These results indicated that 1 M NaOH under the conditions used was a mild agent for extracting both hemicelluloses and lignin, and the pretreatment significantly cleaved the α-ether bonds between lignin and hemicelluloses from the cell walls of bagasse, resulting in a substantial dissolution of the polymers of hemicellulosic polysaccharides and lignin macromolecules. An increasing pretreating temperature from 20 to 40 °C led to an increase of total solubilized hemicelluloses by 6.6% and lignin by 19.3%, respectively, suggesting that a large proportion of

Table 1. The yield of hemicelluloses (% dry matter) solubilized or hydrolyzed during the pretreatment of de-waxed SCB with 1 M NaOH aqueous solution at 20, 25, 30, 35, and 40 °C for 18 h, respectively, and post-treatment of the corresponding residue with 1,4-dioxane–2 M HCl (9:1, v/v) at 87 °C for 2 h

Hemicellulosic fraction	Alkali treating temperature (°C)				
	20	25	30	35	40
Total solubilized hemicelluloses during alkali and acidic organic solvent treatments	30.2	31.1	31.2	31.3	31.4
Hemicelluloses solubilized during alkali pretreatment[a]	18.6	19.2	19.8	20.4	20.8
Oligo- and monosaccharides produced in acidic dioxane post-treatment[b]	7.0	8.1	8.2	8.5	8.5
Hemicelluloses recovered during the acidic organic solvent post-treatment[a]	4.6	3.8	3.2	2.4	2.1

[a] Represents the hemicelluloses obtained by precipitation in 3 volumes of ethanol.

[b] Represents the hemicellulosic degradation products which are still solubilized in the supernatant after precipitation of the hemicelluloses in 3 volumes of ethanol, calculated by total hemicelluloses (33.5%)–(hemicellulosic polymers solubilized during the alkali and acidic organic solvent treatments obtained by precipitation in ethanol + associated hemicelluloses in the residues).

hemicelluloses and lignin can be solubilized with 1 M aqueous sodium hydroxide at relatively high temperatures. Up to now, the alkaline extraction is one of the most important tools in the structural characterization of the cell-wall polymers from lignocellulosic materials.

Treatment with organic solvents has proved to be a promising process to achieve complete utilization of lignocellulosics without a negative impact on the environment.[22] In this case, the organic solvent acts primarily to impregnatie the plant tissue and solubilize the lignin and hemicellulosic fragments produced.[23] In particular, in acidic organic solvent treatment, lignin is dissolved or degraded by the acid-catalyzed cleavage of such bonds as α-aryl ether and arylglycerol–β-aryl ether in the lignin macromolecule,[24] leading to release or degradation of hemicelluloses. The results showed that the post-treatment of the corresponding alkali pretreated residue with 1,4-dioxane–2 M HCl (9:1, v/v) at 87 °C for 2 h degraded 11.6%, 11.9%, 11.4%, 10.9%, and 10.6% of hemicelluloses (% dry starting material), in which the yield of the degraded hemicelluloses recovered by precipitation in EtOH during the acidic dioxane post-treatment accounted for 4.6%, 3.8%, 3.2%, 2.4%, and 2.1% (% dry starting material), respectively. Meanwhile, the post-treatment also dissolved or degraded 7.0%, 6.3%, 5.6%, 4.5%, and 4.1% lignin (% dry starting material), corresponding to dissolution of 38.7%, 34.8%, 30.9%, 24.9%, and 22.7% of the original lignin, respectively. The two-stage treatments together degraded 90.1%, 92.8%, 93.1%, 93.4%, and 93.7% of the original hemicelluloses and 93.3%, 93.3%, 95.0%, 95.0%, and 96.7% of the original lignin from de-waxed bagasse when the alkali pretreatment was performed at 20, 25, 30, 35, and 40 °C for 18 h, respectively, revealing that a significant proportion of the hemicelluloses and lignin was efficiently solubilized or degraded by sequential alkaline pretreatment and acidic dioxane post-treatment under the stated conditions.

3.2. Sugar composition

Table 2. The content of neutral sugars (relative % dry hemicelluloses, w/w) and uronic acids (% dry hemicelluloses, w/w) in the hemicelluloses released during the pretreatment of de-waxed bagasse with 1 M NaOH aqueous solution at 20, 25, 30, 35, and 40 °C for 18 h

Sugar/uronic acids	Treating temperature (°C)				
	20	25	30	35	40
Rhamnose	3.0	3.1	4.9	6.0	6.5
Arabinose	9.3	9.8	9.8	11.7	11.6
Xylose	82.2	82.1	81.2	78.4	78.0
Mannose	1.0	1.1	1.1	1.2	1.4
Galactose	0.7	0.7	0.5	0.3	0.3
Glucose	4.1	3.0	2.6	2.5	2.2
Uronic acids	4.8	5.1	5.8	5.6	5.6

The neutral sugar composition and content of uronic acids of five alkali-soluble hemicellulosic preparations are given in Table 2. As shown, xylose is the predominant sugar component, comprising 78.0–82.2% of the total sugars, and arabinose (9.3–11.7%) and uronic acids (4.8–5.8%), mainly 4-*O*-methyl-α-D-glucopyranosyluronic acid (MeGlcA), appeared as the second and third major sugar constituents, indicating the presence of arabino(4-*O*-methyl-D-glucurono)xylans. Rhamnose (3.0–6.5%), and glucose (2.2–4.1%) were observed in small amounts. Mannose (1.0–1.4%) and galactose (0.3–0.7%) occurred as minor quantities, and may have originated from contaminant polysaccharides.[25] Although arabinoxylans from various cereal straws share the same basic chemical structure, they differ in the manner of substitution of the xylan backbone. The main differences were found in the ratio of arabinose to xylose (Ara/Xyl), in the relative proportions and sequence of the various linkages between these two sugars, and in the presence of other substituents.[26] The ratio of Ara/Xyl in these five hemicellulosic fractions were found to be rather low, 0.11–0.15, indicating a more linear structure of the hemicelluloses. In addition, the content of rhamnose, arabinose, mannose, and uronic acids increased with the alkali pretreatment temperature, while that of xylose, galactose, and glucose decreased slightly. These data suggest that polymers bound to the cell wall with lesser branches were released by mild alkaline extraction under low temperature, and they are more susceptible to alkaline degradation, whereas the more branched xylans are preferably released by mild alkali under relatively high temperature.

Table 3. The content of neutral sugars (relative % dry hemicelluloses, w/w) and uronic acids (% dry hemicelluloses, w/w) in the hemicelluloses released during the post-treatment with 1,4-dioxane–2 M HCl (9:1, v/v) at 87 °C for 2 h from the corresponding alkali pretreated bagasse

Sugar/uronic acids	Alkali treating temperature (°C)				
	20	25	30	35	40
Rhamnose	0.7	0.5	0.5	0.5	0.5
Arabinose	38.1	36.2	35.9	36.0	37.9
Xylose	45.1	46.8	46.8	46.7	44.9
Mannose	1.4	1.3	1.3	1.5	1.2
Galactose	1.8	1.9	1.8	2.2	2.0
Glucose	13.0	13.3	13.7	13.4	13.6
Uronic acids	4.6	4.2	3.9	3.7	3.5

Table 3 shows some slight differences in terms of the monosaccharide composition of the degraded hemicelluloses, obtained during the sequential post-treatment with 1,4-dioxane–2 M HCl (9:1, v/v) at 87 °C for 2 h from the corresponding alkali pretreated bagasse. The major monosaccharide in the five degraded polysaccharides was xylose (44.9–46.8%), followed closely by arabinose (35.9–38.1%), indicating that the acidic dioxane-soluble hemicelluloses were mainly arabinoxylans. The Ara/Xyl ratio was much higher (0.77–0.84) than that of the corresponding five hemicellulosic preparations solubilized during the alkali pretreatment, indicating that the five acidic dioxane-soluble hemicelluloses were more highly branched than the alkali-soluble ones. The presence of higher branching of the hemicelluloses in acidic dioxane-soluble fraction verified that the main chain of the polysaccharides was substantially degraded by acidic dioxane under the conditions used. In addition to xylose and arabinose, glucose appeared in significant (13.0–13.7%), presumably due to degradation of the cellulosic polymer, because cellulose microfibrils would have been susceptible to acid hydrolysis. On the other hand, since hemicelluloses are very firmly bound to the cellulosic microfibrils, and cellulose is normally resistant to hydrolysis under these conditions, this glucose probably may also have been derived from a hemicellulosic glucan that is also firmly bound to cellulose. Uronic acids were also found (3.5–4.6%) in the acidic dioxane-soluble fractions, which were lower than in the alkali-soluble hemicellulosic preparations (4.8–5.8%).

3.3. Content of bound lignin

Table 4. The yield (% dry hemicellulose, w/w) of phenolic acids and aldehydes from alkaline nitrobenzene oxidation of bound lignins in the alkali-soluble hemicelluloses released during the pretreatment of dewaxed bagasse with 1 M NaOH aqueous solution at 20, 25, 30, 35, and 40 °C for 18 h

Phenolic acids and aldehydes	Treating temperature (°C)				
	20	25	30	35	40
p-Hydroxybenzoic acid	0.066	0.061	0.068	0.067	0.052
p-Hydroxybenzaldehyde	0.22	0.20	0.11	0.11	0.10
Vanillic acid	0.076	0.075	0.071	0.067	0.060
Vanillin	0.57	0.49	0.52	0.51	0.47
Syringic acid	0.009	0.007	0.010	0.011	0.10
Syringaldehyde	0.66	0.62	0.59	0.51	0.49
p-Coumaric acid	0.033	0.025	0.022	0.020	0.015
Ferulic acid	0.016	0.011	0.009	0.007	0.006
Total	1.65	1.49	1.40	1.30	1.29
Content of Klason lignin	3.18	3.06	2.71	2.63	2.48

To verify the bound lignin in the five alkali-soluble hemicellulosic preparations and five acidic dioxane-soluble hemicellulosic fragments, all hemicellulosic samples were oxidized by alkaline nitrobenzene to provide indication of the lignin composition. Table 4 gives the yield (% dry hemicellulose, w/w) of phenolic acids and aldehydes from alkaline nitrobenzene oxidation of bound lignins in the five alkali-soluble hemicelluloses. The five hemicellulosic preparations contained relatively low amounts of associated lignin, ranging between 2.48% and 3.18%. This relatively lower content of bound lignin in the alkali-soluble hemicelluloses revealed that the α-benzyl ether linkages between lignin and hemicelluloses were significantly cleaved during the mild alkali pretreatment. This is particularly true as the alkali pretreatment was performed at relatively high temperatures. The major products obtained from the alkaline nitrobenzene oxidation, comprised syringaldehyde (0.49–0.66%) and vanillin (0.47–0.57%). A noticeable amount of *p*-hydroxybenzaldehyde (0.10–0.22%) was also found to be present in the oxidation mixtures. *p*-Hydroxybenzoic acid, vanillic acid, syringic acid, *p*-coumaric acid, and ferulic acid were identified in trace amounts. Similar results were found in the corresponding five acidic dioxane-soluble hemicellulosic fractions, as shown in Table 5. The content of bound lignin ranged between 3.96% and 5.12%, which was higher than that of the five alkali-soluble hemicellulosic preparations. This indicated that the degraded hemicelluloses, obtained during the

Table 5. The yield (% dry hemicelluloses, w/w) of phenolic acids and aldehydes from alkaline nitrobenzene oxidation of bound lignins in the hemicelluloses released during the post-treatment of the corresponding alkali pretreated bagasse (at different temperatures) with 1,4-dioxane–2 M HCl (9:1, v/v) at 87 °C for 2 h

Phenolic acids and aldehydes	Alkali treating temperature (°C)				
	20	25	30	35	40
p-Hydroxybenzoic acid	0.25	0.20	0.18	0.17	0.18
p-Hydroxybenzaldehyde	0.36	0.26	0.24	0.18	0.21
Vanillic acid	0.15	0.12	0.12	0.11	0.12
Vanillin	0.97	0.86	0.80	0.66	0.65
Syringic acid	0.075	0.065	0.062	0.062	0.067
Syringaldehyde	0.80	0.78	0.66	0.72	0.78
p-Coumaric acid	0.054	0.046	0.049	0.035	0.026
Ferulic acid	0.029	0.010	0.010	ND[a]	ND
Total	2.69	2.44	2.12	1.94	2.03
Content of Klason lignin	5.12	5.03	4.56	3.78	3.96

[a] ND, not detectable.

acidic dioxane post-treatment, are more tightly associated with lignin than the hemicelluloses, solubilized during the alkali pretreatment. Vanillin (0.65–0.97%) and syringaldehyde (0.66–0.80%) were the dominant phenolic constituents, which together represented for 65.8–71.1% of the total phenolic acids and aldehydes. *p*-Hydroxybenzaldehyde (0.18–0.36%), *p*-hydroxybenzoic acid (0.17–0.25%), and vanillic acid (0.11–0.15%) were present in detectable amounts.

3.4. Molecular weight

Weight-average ($\overline{M}_{\mathrm{w}}$) and number-average ($\overline{M}_{\mathrm{n}}$) molecular weights and polydispersity ($\overline{M}_{\mathrm{w}}/\overline{M}_{\mathrm{n}}$) of the alkali-soluble hemicellulosic preparations and the acidic organic solvent-soluble hemicellulosic fractions were determined by gel-permeation chromatography, and the results are listed in Tables 6 and 7. As expected, the five hemicellulosic preparations, solubilized during the alkali pretreatment showed a much higher degree of polymerisation, with $\overline{M}_{\mathrm{w}}$ values between 35,200 and 37,430 g mol^{-1} than those of the hemicellulosic fractions (12,080–13,320 g mol^{-1}), degraded during the acidic dioxane post-treatment. The results show that the post-treatment of the alkali pretreated residue with acidic dioxane in the acidic medium significantly degrades the hemicellulosic macromolecules into low molecular fragments. Additionally, as the data in Tables 6 and 7 show the alkali-soluble hemicellulosic preparations were broader in their molecular-weight distribution and contained a large population of polymers having molecular weights much higher than that of the values of acidic organic solvent-soluble hemicellulosic fractions. Furthermore, the analysis showed that the five polymeric hemicelluloses released during the alkali pretreatment had polydispersity indexes between 5.4 and 5.9, whereas the five corresponding hemicellulosic fractions, degraded during the acidic dioxane post-treatment, gave more narrow molar mass distributions, corresponding to polydispersity indexes from 2.1 to 3.0.

Table 6. Weight-average ($\overline{M}_{\mathrm{w}}$) and number-average ($\overline{M}_{\mathrm{n}}$) molecular weights and polydispersity ($\overline{M}_{\mathrm{w}}/\overline{M}_{\mathrm{n}}$) of the hemicellulosic preparations released during the pretreatment of de-waxed bagasse with 1 M NaOH aqueous solution at 20, 25, 30, 35, and 40 °C for 18 h

	Alkali treating temperature (°C)				
	20	25	30	35	40
$\overline{M}_{\mathrm{w}}$	35,200	35,450	35,820	36,760	37,430
$\overline{M}_{\mathrm{n}}$	6020	6120	6310	6790	6910
$\overline{M}_{\mathrm{w}}/\overline{M}_{\mathrm{n}}$	5.9	5.8	5.7	5.4	5.4

Table 7. Weight-average ($\overline{M}_{\mathrm{w}}$) and number-average ($\overline{M}_{\mathrm{n}}$) molecular weights and polydispersity ($\overline{M}_{\mathrm{w}}/\overline{M}_{\mathrm{n}}$) of the hemicellulosic preparations released during the post-treatment with 1,4-dioxane–2 M HCl (9:1, v/v) at 87 °C for 2 h from the corresponding alkali pretreated bagasse

	Alkali treating temperature (°C)				
	20	25	30	35	40
$\overline{M}_{\mathrm{w}}$	12,080	12,700	12,880	13,320	12,650
$\overline{M}_{\mathrm{n}}$	4470	4360	4280	5590	5990
$\overline{M}_{\mathrm{w}}/\overline{M}_{\mathrm{n}}$	2.7	2.9	3.0	2.4	2.1

3.5. FT-IR spectra

In this study, Fourier transform infrared (FT-IR) spectroscopy was used to evaluate the structural differences between the hemicellulosic fractions. Figure 2 illustrates the FT-IR spectra of the hemicelluloses released during the pretreatment of de-waxed bagasse with 1 M NaOH aqueous solution at 20 °C (spectrum 1), 30 °C (spectrum 2), and 40 °C (spectrum 3) for 18 h. All the spectra showed the same pattern and were similar to those of typical hemicelluloses from cereal straw and grass, indicating a similar structure of the polymers. The attributions of the main absorptions are characteristic of glycosidic structures and are related to OH stretching at 3440 cm^{-1} and C–O–C stretching at 1044 cm^{-1}. Two low-intensity shoulders at 987 and 1092 cm^{-1} are assigned to arabinose substitution at C-3 of the xylose residues, a characteristic typical of substituted arabinoxylans.[17] A sharp band at 895 cm^{-1} is characteristic of β-glycosidic linkages between the sugars.[27] The signal at 1636 cm^{-1} can be attributed to the absorbed water, since hemicelluloses usually have a strong affinity for water.[28] The occurrence of a shoulder at 1511 cm^{-1} is undoubtedly due to the presence of small amounts of associated lignin in the hemicelluloses, and corresponded to the results obtained by alkaline nitrobenzene oxidation. The remaining signals in the spectra, at 1471, 1426, 1386, and 1257 cm^{-1}, relate to C–H and O–H bending and C–C stretching.

The FT-IR spectra of the hemicelluloses recovered during the acidic dioxane post-treatment are shown in Figure 3. The absorbances at 1462, 1430, 1377, 1320, 1265, 1204, 1164, 1111, 1060, 1032, and 897 cm^{-1} are associated with hemicelluloses. In comparison with the spectra of alkali-soluble hemicellulosic fractions, a much weaker band at 897 cm^{-1} in Figure 3 demonstrated that the post-treatment with dioxane under the acidic conditions substantially cleaves the β-glycosidic linkages between the sugar units from the backbone of hemicelluloses, resulting in degraded hemicellulosic

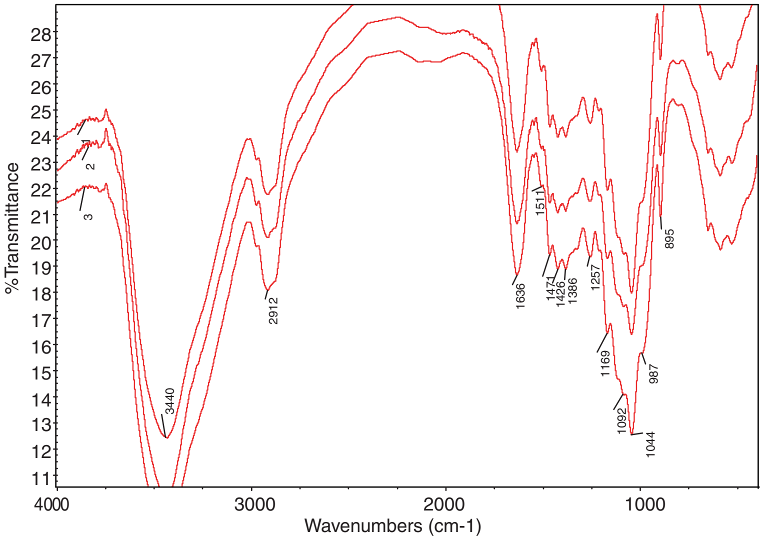

Figure 2. FT-IR spectra of the hemicelluloses released during the pretreatment of de-waxed bagasse with 1 M NaOH aqueous solution at 20 °C (spectrum 1), 30 °C (spectrum 2), and 40 °C (spectrum 3) for 18 h.

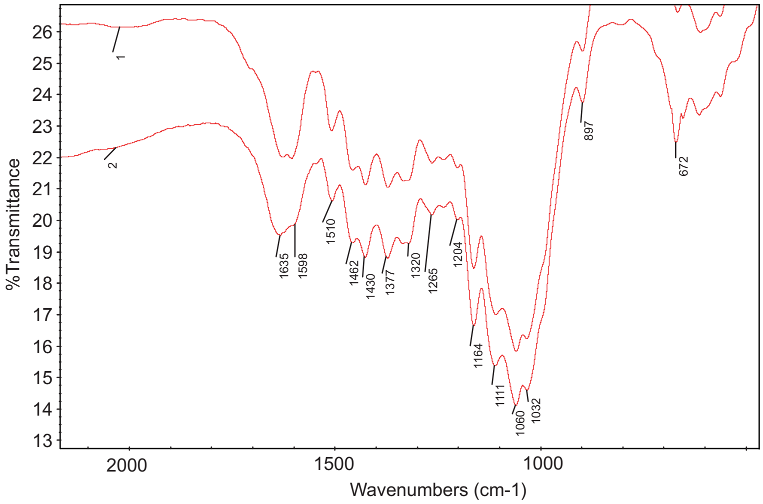

Figure 3. FT-IR spectra of the hemicelluloses isolated with 1,4-dioxane–2 M HCl (9:1, v/v) at 87 °C for 2 h from the corresponding alkali pretreated bagasse (alkali pretreatment at 20 °C for spectrum 1 and 40 °C for spectrum 2).

fragments. On the other hand, a noticeable band at 1510 cm^{-1} relating to the bound lignin corresponded to the content of associated lignin in the degraded hemicellulosic preparation listed in Table 5.

3.6. ^{1}H and ^{13}C NMR spectra

The ^{1}H NMR spectrum of the degraded hemicellulosic fraction isolated with acidic dioxane from 1 M NaOH pretreated (20 °C) bagasse residue shows two signals at 5.2 and 5.0 ppm, for the α-anomeric proton of arabinose substituted at C-3 and C-2 (disubstituted) of the xylose backbone (Fig. 4), indicating a significant amount of substituted xylose residues. Disubstituted β-D-xylopyranose residues having α-L-arabinofuranose substituents at C-2 and C-3 are thus also present. A signal at 4.2 ppm is due to the anomeric protons of β-D-xylose substituted at C-3 (monosubstituted) residues, since the region between 4.1 and 4.5 ppm corresponds to the β-configuration and the region 4.9–5.6 ppm corresponds to the α-configuration.[29] The signals for other protons of arabinose and xylose were observed in the region of 3.0–3.5 and centered at 3.3 ppm.[30] Resonance signals originating from phenolic compounds (6.7 ppm) were clearly visible in the spectrum.[20]

In order to obtain deeper insight into the branched structure of the hemicelluloses, a ^{13}C NMR spectrum of the hemicellulosic preparation isolated with 1 M NaOH at 30 °C for 18 h was recorded, and is shown in Figure 5. The main (1→4)-linked β-D-Xyl*p* units were evidenced by five strong signals at 101.3, 78.0, 77.5, 75.0, and 62.7 ppm, which are, respectively attributed to C-1,

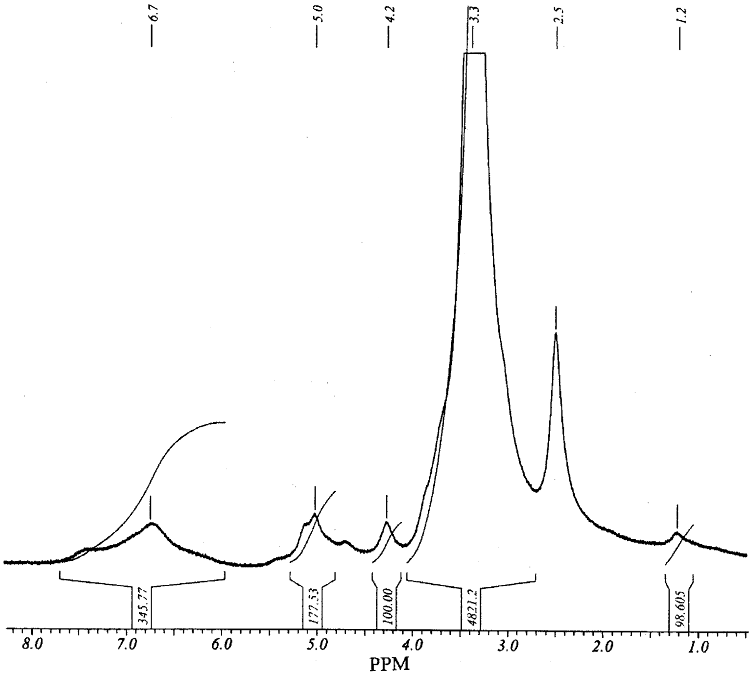

Figure 4. ^{1}H NMR spectrum of the degraded hemicellulosic fraction isolated with acidic dioxane from 1 M NaOH pretreated (20 °C) bagasse residue.

C-4, C-3, C-2, and C-5 of the β-D-Xyl*p* units. The signals at 82.6 and 61.6 ppm belong to C-2 and C-5 of the α-L-Ara*f* residues, respectively. These data revealed that the anomeric configuration of the D-xylopyranose residues is β while the L-arabinofuranose residue is α. A weak signal at 58.3 ppm is originated from the *O*-methoxyl group of the glucuronic acid residue in the xylan. The signals at 18.1 ppm was most likely due to CH_3 from acetyl groups in hemicelluloses, indicating that pretreatment of de-waxed bagasse with a mild alkali (1 M NaOH) under the conditions used cleaved only partially the esterified linkages between acetyl groups and hemicelluloses. The signal at 176.9 ppm is indicative of the carbonyl signal of the esterified ferulic or *p*-coumaric acids in the hemicelluloses. These hydroxycinnamic acids were found to be linked at C-5 of the L-arabinofuranosyl residue of the straw hemicelluloses.[31] Two signals at 150.8 and 131.8 ppm represent the associated lignin in the hemicellulosic fraction, corresponding to the lignin content shown in Table 4.

3.7. Thermal analysis

The thermal properties of the alkali- and acidic dioxane-soluble hemicelluloses were investigated by thermogravimetric analysis and differential scanning calorimetry. The results showed that all of the alkali-soluble hemicellulosic preparations began to decompose at 225 °C, and their maximum rate of weight loss was observed between 280 and 320 °C with a maximum of the exothermic peak at 293–300 °C in DSC thermograms. At 50% weight loss, the degradation temperature was observed

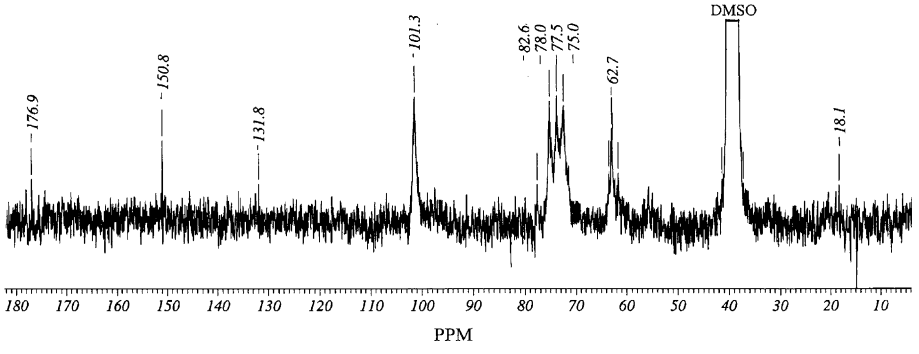

Figure 5. ^{13}C NMR spectrum of hemicellulosic preparation isolated with 1 M NaOH at 30 °C for 18 h.

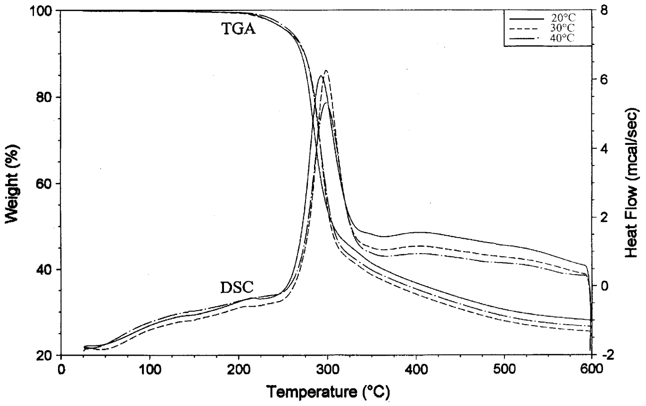

Figure 6. Thermogram of hemicellulosic preparations released during the pretreatment of de-waxed bagasse with 1 M NaOH aqueous solution for 18 h at 20, 30, and 40 °C.

to be 310 °C (Fig. 6). In comparison, the five hemicellulosic fractions, degraded during the acidic dioxane post-treatment, started to decompose at 195 °C, and their maximum rate of weight loss shifted to a much lower temperature, 248–308 °C. These data indicate that all of the alkali-soluble hemicelluloses have higher thermal stability than the acidic dioxane-soluble hemicellulosic fragments, corresponding to their molecular weights in Tables 6 and 7. In other words, the thermal stability of the hemicelluloses increased with an increase in their molecular weight.

The foregoing above results, show that the hemicellulosic preparations, solubilized during the mild alkali pretreatment, are more linear and have higher molecular weights, whereas the hemicellulosic fractions, degraded during the acidic organic solvent post-treatment, are more branched and have lower molecular weight. These hemicellulosic polymers from sugarcane bagasse had a classical structure, with a backbone of β-(1→4)-linked xylosyl residues substituted with arabinose and 4-*O*-methyl-D-glucuronic acid at C-2 and/or C-3 of the main chain. In addition, both alkali-soluble and acidic organic solvent-degraded hemicelluloses contained minor quantities of bound lignin and hydroxycinnamic acids, such as ferulic and *p*-coumaric acids. Furthermore, this study also demonstrated the alkali-soluble hemicelluloses had a higher molecular weight and a higher thermal stability than that of the acidic organic solvent-soluble hemicellulosic fragments.

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