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Effect of Ionic Liquid Pretreatment on the Structure of Hemicelluloses from Corncob

Shao-Ni Sun,[†] Ming-Fei Li,[†] Tong-Qi Yuan,[†] Feng Xu,*[,†] and Run-Cang Sun*[,†,‡]

[†]Institute of Biomass Chemistry and Technology, College of Material Science and Technology, Beijing Forestry University, Beijing 100083, People's Republic of China

[‡]State Key Laboratory of Pulp and Paper Engineering, South China University of Technology, Guangzhou 510640, People's Republic of China

ABSTRACT: Pretreatment is the key to unlock the recalcitrance of lignocellulosic biomass for the productions of biofuels. Ionic liquid pretreatment has drawn increased attention because of its numerous advantages over conventional methods. In this study, corncob was submitted to pretreatments with 1-ethyl-3-methylimadazolium acetate (EMIMAc) and/or H_2O/dimethyl sulfoxide (DMSO) followed by alkaline extraction to isolate hemicelluloses. The hemicellulosic fractions obtained were comprehensively characterized with a series of chemical and spectroscopic technologies, including gel permeation chromatography (GPC), thermogravimetric analysis (TGA), high-performance anion-exchange chromatography (HPAEC), Fourier transform infrared (FTIR) spectroscopy, and one- and two-dimensional nuclear magnetic resonance (NMR). The results showed that the fractions prepared with ionic liquid pretreatments exhibited relatively higher average molecular weights (196 230−349 480 g/mol) than the fraction prepared without pretreatment (M_w, 96 260 g/mol). Furthermore, the pretreated fractions demonstrated higher thermal stability compared to the fractions without pretreatment. Structural characterization indicated that all of the fractions had similar structures, which are composed of a (1 → 4)-linked β-D-xylopyranosyl backbone substituted with arabinofuranosyls attached to O-2 and O-3 and with 4-*O*-methyl-α-D-glucuronic acid also linked to O-2.

KEYWORDS: *Corncob, 1-ethyl-3-methylimadazolium acetate, pretreatment, hemicelluloses, HSQC*

INTRODUCTION

Petroleum is currently used as a major source for chemicals, materials, and fuels but poses major concerns in terms of its future use because of resource limitation, increasing prices, and associated environmental issues. An alternative and sustainable source for the production of chemicals and biofuels is lignocellulosic biomass. Corn is one of the most widely distributed herbaceous crops in the world. It was estimated that the total yield of corn in 2001 was 6.1×10^8 megatons; only in China, the yield of corn has reached 1.2×10^8 megatons.[1] Because the ratio between corn grain and corncob may reach 100:18, a large quantity of corncob can be generated.[1] Corncob has a complex structure, mainly composed of cellulose, hemicelluloses, and lignin. Among these components, hemicelluloses have emerged as an immense renewable biopolymer resource. Hemicelluloses have been reported for a variety of applications, such as gelling agents, viscosity modifiers, and tablet binders. In addition, hemicelluloses can also be converted to chemicals, such as 5-hydroxymethylfurfural, furfural, xylitol, levulinic acid, and succinic acid.[2,3]

Hemicelluloses are heterogeneous polysaccharides in the plant cell walls bound to the other cell wall components in a variety of ways, which restrict their liberation from the cell wall matrix. Hemicelluloses form covalent bonds with lignin, mainly in the form of α-benzyl ether linkages, hydrogen bonds with cellulose, and ester linkages with acetyl groups and hydroxycinnamic acids. Therefore, a pretreatment strategy is thought to be an effective way for enhancing the extraction of hemicellulosic polymers. Ionic liquids (ILs) are salts comprised of large organic cations and inorganic or organic anions with low melting points.[4] ILs have low vapor pressure, tunable physicochemical properties, high thermal stability, and efficient dissolution power.[5] Recently, ILs have received growing attention as promising green solvents for pretreating lignocellulosic biomass and have been widely used in the dissolution of cellulose,[6,7] lignin,[8] and wood chips.[9,10] Pretreatment with ILs have previously been performed at ambient temperatures and pressures and have resulted in low rates of carbohydrate degradation.[11,12] The IL-pretreated biomass was reported to have a reduced content of lignin and lower degree of crystallinity of cellulose and then can be enzymatically hydrolyzed at a faster rate.[5,10,13,14] Despite extensive literature on the use of ILs for the pretreatment of lignocelluloses, there are limited reports on the use of ILs for the isolation of hemicelluloses. Switchable ionic liquid (SIL), prepared by budding CO_2 through a mixture of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and alcohol, was applied for the selective extraction of hemicelluloses from spruce wood.[15] The result revealed that the content of hemicelluloses was reduced by 38 wt % for spruce treated with butanol SIL and by 29 wt % for spruce treated with hexanol SIL.[15]

IL 1-ethyl-3-methylimadazolium acetate (EMIMAc), which is known to be one of the best solvents for lignocellulosic biomass among the ILs,[16] was employed as a medium for dissolving or swelling lignocellulosic biomass. When the dissolved material was precipitated by the addition of water as an anti-solvent, segmental hemicelluloses are present in the recovered solid phase.

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The hemicelluloses can then be separated after the dissolution via regeneration with aqueous alkaline solution. It should be noted that two major constraints prevent ILs from becoming commercially viable. The first constraint is that large amounts of expensive ILs are required, and the second constraint is that the solution becomes extremely toxic during pretreatment, making it difficult to handle. To mitigate these constraints, a novel pretreatment method with IL co-solvents has been developed in the present study. As we known, most ILs are affable to water, and the content of water shows a significant effect on the dissolving of lignocellulosic materials in ILs; however, the anhydrous conditions are difficult to operate in a practical process. Therefore, we tried to study the effect of the pretreatment process using ILs with a high content of water on the structural changes of the lignocellulosic materials in one sample. Dimethyl sulfoxide (DMSO) is widely used as the co-solvent of the ILs to reduce the viscosity of the mixture and also reduced the addition of expensive ILs. Furthermore, many studies about the use of the mixture of EMIMAc and water/DMSO solvent to prepare lignocellulosic biomass have been reported.[17,18] It has been reported that the IL pretreatment can break apart the lignin−carbohydrate complex, increase the surface accessibility of the polysaccharides to the hydrolytic enzymes, and then enhance the efficiency of the enzymatic conversion of biomass. In this work, we focused on the effect of the IL pretreatment on the structure of hemicelluloses. In addition, for comparative analysis, a hemicellulosic fraction was also extracted by alkali without pretreatment. The isolated hemicellulosic fractions were comparatively investigated by high-performance anion-exchange chromatography (HPAEC), gel permeation chromatography (GPC), thermogravimetric analysis (TGA), Fourier transform infrared (FTIR) spectroscopy, ^{1}H and ^{13}C nuclear magnetic resonance (NMR), and heteronuclear single-quantum coherence (HSQC) NMR spectroscopy.

MATERIALS AND METHODS

Materials. Corncob was obtained from Sichuan province, China. After air-drying in the sunlight, the corncob was cut into small pieces. The corncob chips were ground and then sieved to 80−120 mesh. The sieved material was dewaxed with methylbenzene/ethanol (2:1, v/v) in a Soxhlet apparatus for 6 h, and the sample was then dried in an oven for 16 h at 60 °C. The composition of the dewaxed corncob was determined by the standard analytical procedure of the National Renewable Energy Laboratory (NREL)[19] to be 38.61% cellulose, 36.52% hemicelluloses, and 13.54% lignin. EMIMAc (purity > 98.5%) was purchased from Chemer, Hangzhou, China. All chemicals used were of analytical or reagent grade and directly used as purchased without further purification. In the ^{13}C NMR spectra, the chemical shifts reported were calibrated relative to the signal from C_1 (101.9 ppm) of the (1 → 4)-linked *β*-D-Xyl*p* units, which was used as the reference standard.

Isolation and Purification of Hemicelluloses. The dewaxed corncob was submitted to pretreatments in three different systems prior to hemicellulose extraction, as shown below (Figure 1). (1) EMIMAc system: 1.00 g of the dewaxed corncob and 20.00 g of IL EMIMAc were combined in a 100 mL dried three-neck flask. (2) EMIMAc/DMSO system: 1.00 g of corncob, 6.00 g of EMIMAc, 14 mL of DMSO, and 0.20 g of LiCl were combined in a 100 mL three-neck flask. (3) EMIMAc/H_2O system: 1.00 g of corncob, 6.00 g of EMIMAc, and 14 mL of H_2O were combined in a 100 mL three-neck flask. Each treatment was then placed in an oil bath and heated on a hot plate at 110 °C for 5 h under a N_2 atmosphere with magnetic stirring at 600 rpm. The selected temperature for dissolution was monitored by a thermometer placed in the oil bath. After dissolution, the samples were allowed to cool to 80 °C, and then 60 mL of distilled water at 80 °C was added to the flask kept at 80 °C for 3 h. The insoluble residue of each pretreatment was collected by filtration through Buchner funnels and subsequently washed with 60 mL of distilled water. The residues were then treated with 30 mL of 5% NaOH at 80 °C for 3 h, to isolate hemicelluloses. All of the experiments were performed in triplicate.

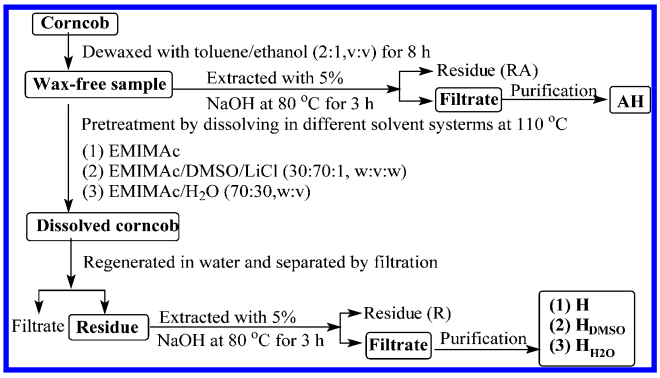

Figure 1. Schematic process of the corncob hemicellulosic fraction based on extraction with 5% NaOH (AH). Hemicellulosic fractions were based on pretreatment with EMIMAc, EMIMAc/H_2O (30:70, w/v), and EMIMAc/DMSO/LiCl (30:70:1, w/v/w), followed by extraction with 5% NaOH.

Table 1. Yield of Hemicelluloses and Content of Neutral Sugars and Uronic Acids in the Hemicellulosic Fractions Obtained from Corncob

	hemicellulosic fraction[a]			
	H	H_{DMSO}	H_{H_2O}	AH
arabinose	18.13	18.22	18.66	17.80
galactose	5.71	5.43	5.34	6.34
glucose	4.35	4.26	4.66	5.84
xylose	71.46	71.77	70.79	69.53
4-*O*-methyl-D-galacturonic acid	0.25	0.22	0.45	0.49
galacturonic acid	0.11	0.10	0.10	ND[b]
Xyl/Ara	3.94	3.94	3.79	3.91
yield (%)[c]	20.13	25.47	26.78	23.25

[a]Corresponding to the hemicellulosic fractions in Figure 1. [b]ND = not detectable. [c]Represent the yield of the hemicellulosic fractions (% dry dewaxed sample, w/w).

In addition, alkaline hemicellulosic fraction (AH) was also isolated by the treatment of corncob using 5% NaOH with a solid/liquid ratio of 1:30 (g/mL) at 80 °C for 3 h. It should be noted that the purification procedure of all of the hemicellulosic fractions obtained was performed according to previous literature.[20]

Chemical and Spectroscopic Characterizations of Hemicellulosic Fractions. The composition of neutral sugars and uronic acids of all of the hemicellulosic fractions was determined by HPAEC. The neutral sugars and uronic acids in the fractions were liberated by hydrolysis with 1.475 mL of 6% sulfuric acid at 105 °C for 2.5 h. After hydrolysis, the samples were diluted 50-fold with ultrapure water and injected into a HPAEC system (Dionex ICS 3000, Sunnyvale, CA) with an amperometric detector, a Carbopac TMPA-20 column (4 × 250 mm, Dionex), and a guard PA-20 column (3 × 30 mm, Dionex). Neutral sugars and uronic acids were separated in carbonate-free 18 mM NaOH under a N_2 atmosphere with post-column addition of 0.3 M NaOH at a rate of 0.5 mL/min. The run time was 45 min, followed by 10 min of elution with 0.2 M NaOH to wash the column, and then 15 min of elution with 18 mM NaOH to re-equilibrate the column. Calibration was performed with a standard solution of L-rhamnose, L-arabinose, D-glucose, D-xylose, D-mannose, D-galactose, glucuronic acid, and galacturonic acid. Results of the yields and sugar compositions of the samples are presented as mean values of three parallels, and the relative standard deviation was below 0.5%.

The molecular weights and molecular weight distributions of all of the hemicellulosic fractions were examined by GPC, using a PL aquagel-OH 50 column (300 × 7.7 mm, Polymer Laboratories, Ltd.). The column oven was controlled at 30 °C. The system was calibrated with PL

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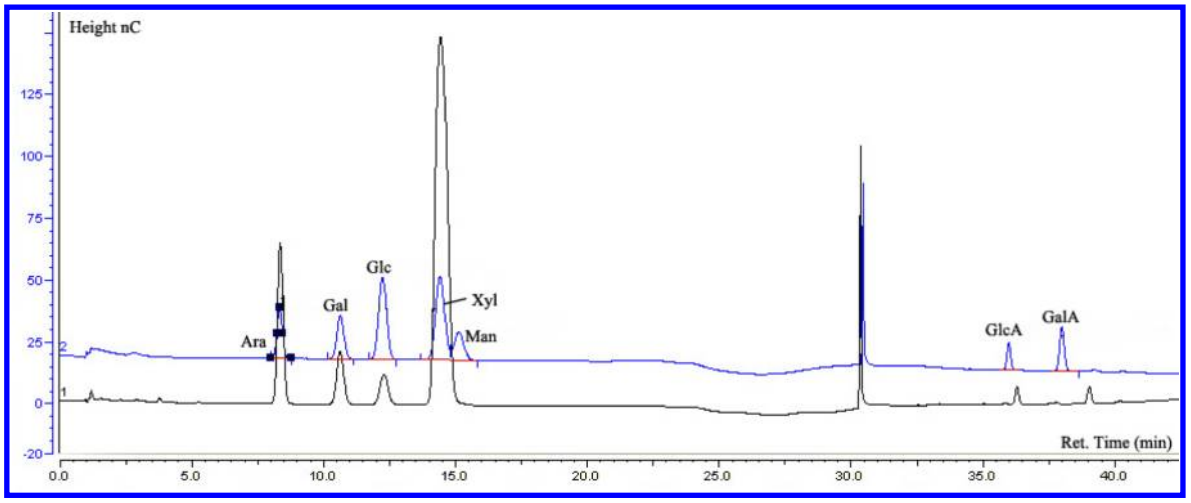

Figure 2. Ion chromatograms of the (1) hemicellulosic fraction H and (2) standard sample.

pullulan polysaccharide standards (peak average molecular weights of 783, 12 200, 100 000, and 1 600 000, Polymer Laboratories, Ltd.). The detector was a differential refractive index detector (RID). The eluent was 0.02 N NaCl in 0.005 M sodium phosphate buffer (pH 7.5), and the flow rate was 0.5 mL/min. All fractions were prepared at a concentration of 0.1% before measurement. The measurement was conducted with three parallels, and the relative standard deviation was below 5%.

Thermal analysis was performed using thermogravimetry (TG) and differential thermogravimetry (DTG) on a simultaneous thermal analyzer (TGA Q500, TA Instruments, New Castle, DE). Between 8 and 10 mg of samples was heated in a platinum crucible from room temperature to 700 °C at a heating rate of 10 °C/min under a N_2 atmosphere.

FTIR spectra were recorded on a Thermo Scientific Nicolet iN10 FTIR microscope (Thermo Nicolet Corporation, Madison, WI) equipped with a liquid-nitrogen-cooled mercury cadmium telluride (MCT) detector. Dried samples were ground and pelletized with BaF_2, and the spectra were recorded in the range of 4000−650 cm^{-1} at 4 cm^{-1} resolution with 128 scans per sample.

The soluble-state ^1H and ^{13}C NMR and two-dimensional (2D) (HSQC) spectra were recorded on a Bruker AV III 400 MHz spectrometer operating in the FT mode at 100.6 MHz. The purified hemicelluloses (15 mg/mL in D_2O for ^1H and 80 mg/mL in D_2O for ^{13}C) were placed in the sample probe, and the resonance spectra were obtained. The chemical shifts of ^1H NMR spectra were calibrated with reference to D_2O, used as an internal standard at 4.70 ppm. The acquisition and relaxation times were 3.9 and 1.0 s, respectively. For ^{13}C NMR spectra, the spectra were recorded at 25 °C after 30 000 scans. A 30° pulse flipping angle, a 9.2 μs pulse width, a 1.36 s acquisition time, and a 1.89 s relaxation delay time were used. The spectral widths were 2200 and 15 400 Hz for the ^1H and ^{13}C dimensions, respectively. The HSQC NMR experiment was conducted with 20 mg of sample dissolved in 1 mL of D_2O after 128 scans. The number of collected complex points was 1024 for the ^1H dimension with a relaxation of 1.5 s. The number of scans was 128, and 256 time increments were recorded in the ^{13}C dimension. The $^1J_{C-H}$ used was 146 Hz. Prior to Fourier transformation, the data matrices were zero-filled up to 1024 points in the ^{13}C dimension. For these signals, the $^1J_{C-H}$ coupling value was relatively similar and used to semi-quantitatively estimate the relative abundance of the different constituents.

RESULTS AND DISCUSSION

Fractional Yield of Hemicelluloses. Hemicelluloses are usually associated with the other cell wall components, such as cellulose, lignin, and other phenolic compounds, by covalent and hydrogen linkages, restricting their extraction from the cell wall matrix. In this study, IL EMIMAc and/or H_2O/DMSO pretreatments followed by alkaline extraction were used to isolate hemicelluloses from corncob. The yields of all of the hemicellulosic fractions (%, oven-dried material) are given in Table 1. As seen, the hemicellulosic fractions H, H_{DMSO}, and H_{H_2O}, prepared with EMIMAc, EMIMAc/DMSO, and EMIMAc/H_2O pretreatments, comprised 20.13, 25.47, and 26.78% of the dewaxed material, corresponding to the dissolution of 55.12, 69.74, and 73.33% of the original hemicelluloses during the extraction process, respectively. On the other hand, the yield of the fraction AH, extracted with 5% NaOH without pretreatment, was 23.25% of the initial amount of dewaxed material, corresponding to the dissolution of 63.66% of the original hemicelluloses. The pretreatments with EMIMAc/DMSO and EMIMAc/H_2O were conducive to the swelling of the cell walls and, thus, slightly improved the dissolution of hemicelluloses in the following alkali extraction processes. The increasing dissolution of hemicelluloses is indicative of a relatively higher accessibility of components and more successful fractionation. However, a relatively low yield of hemicelluloses was obtained by the EMIMAc pretreatment. This was probably due to the fact that a small amount of hemicelluloses was dissolved or degraded in the IL during the EMIMAc pretreatment; hence, a relatively small percentage of hemicelluloses remained in the regenerated residue available to be extracted with sodium hydroxide. Overall, more than 50% of the original hemicelluloses was released during the alkaline extractions with and without pretreatments. This high solubility of hemicelluloses was presumed to be due to the cleavage of the ester bonds between hydroxycinnamic acids (such as *p*-coumaric and ferulic acids) and hemicelluloses or lignin and the cleavage of the α-benzyl ether linkages between lignin and hemicelluloses from the cell walls of corncob by alkali.[21] Taken together, the results above revealed that the

Table 2. Weight-Average (M_w) and Number-Average (M_n) Molecular Weights and Polydispersity (M_w/M_n) of the Hemicellulosic Fractions

	hemicellulosic fraction[a]			
	H	H_{DMSO}	H_{H_2O}	AH
M_w	349480	276810	196230	96260
M_n	57970	58820	51540	29810
M_w/M_n	6.03	4.71	3.81	3.23

[a]Corresponding to the hemicellulosic fractions in Figure 1.

extractions with IL pretreatments did not result in a significant increase of the yield of the hemicelluloses obtained in comparison to the isolation without pretreatment and only a slight increase in the EMIMAc/DMSO and EMIMAc/H_2O pretreatments.

Hemicellulosic Composition. The neutral and acidic sugar compositions of the fractions are given in Table 1 and are expressed as a relative percentage of the total sugars. Two chromatograms of IC analysis for sugars and uronic acid are given in Figure 2. The first and second chromatograms showed the standard sample and the hemicellulosic fraction H of IC analysis for sugars and uronic acids, respectively. As seen from Table 1, xylose was the predominant neutral sugar component in all of the hemicellulosic fractions, comprising 69.53−71.77% of the total sugars. Small amounts of arabinose (17.80−18.66%), galactose (5.34−6.34%), and glucose (4.26−5.84%) were detected in the four hemicellulosic fractions. Additionally, minor quantities of 4-*O*-methyl-D-galacturonic acid (0.22−0.49%) and galacturonic acid (0.10−0.11%) were also detected. These results indicated that the hemicelluloses from corncob may be composed of arabinoxylan. In arabinoxylan, the linear β-(1,4)-D-xylopyranose backbone is substituted by α-L-arabinofuranosyl units in the positions 2-O and/or 3-O.[22] The presence of glucose in small amounts may be coming from glucuronoarabinoxylans and xyloglucans.[23] The galactose detected might be due to galactoarabinoxylans and arabinogalactans.[24] All of the sugar contents of the hemicellulosic fractions prepared with the three pretreatments were almost equal compared to the hemicellulosic fraction AH obtained without pretreatment. The ratios of xylose/arabinose (Xyl/Ara) are indicative of the degree of linearity or branching of hemicellulosic polymers.[20] A high Xyl/Ara ratio should suggest a long-chain polymer having small amounts of branching with other monosaccharide compositions. The Xyl/Ara ratios were approximately 3.79−3.94 in all of the hemicellulosic fractions. No significant differences were observed in the Xyl/Ara ratios, indicating a similar degree of branching of the hemicellulosic polymers.

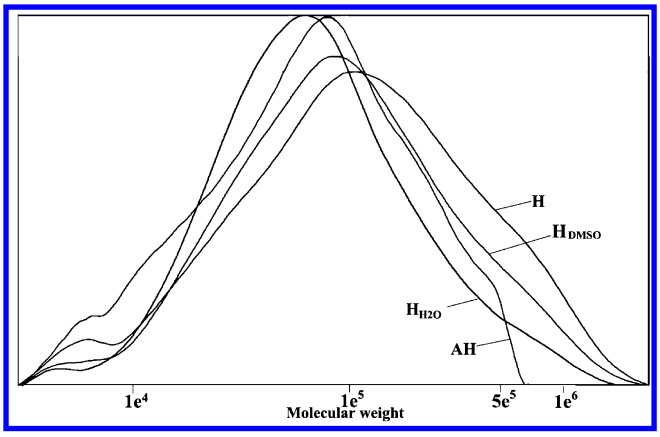

Figure 3. Molecular weight distributions of the four hemicellulosic fractions.

Molecular Weight Analysis. The weight-average (M_w) and number-average (M_n) molecular weights and polydispersity (M_w/M_n) of the hemicellulosic fractions obtained are shown in Table 2, and the molecular weight distributions are illustrated in Figure 3. All of the fractions exhibited weight-average molecular weights varying from 96 260 to 349 480 g/mol. Evidently, the hemicellulosic fractions prepared with EMIMAc, EMIMAc/H_2O, and EMIMAc/DMSO pretreatments showed relatively higher molecular weight averages (M_w, 196 230−349 480 g/mol) than that of the fraction AH prepared without pretreatment (M_w, 96 260 g/mol). This suggested that the pretreatments with EMIMAc were conducive to the swelling of the cell walls, resulting in the release of hemicelluloses with high molecular weights in the following alkaline extraction. As seen in Table 2, the highest M_w (349 480 g/mol) in the fraction H, prepared with EMIMAc pretreatment, implied that IL EMIMAc pretreatment favored the solubilization of macromolecular hemicelluloses

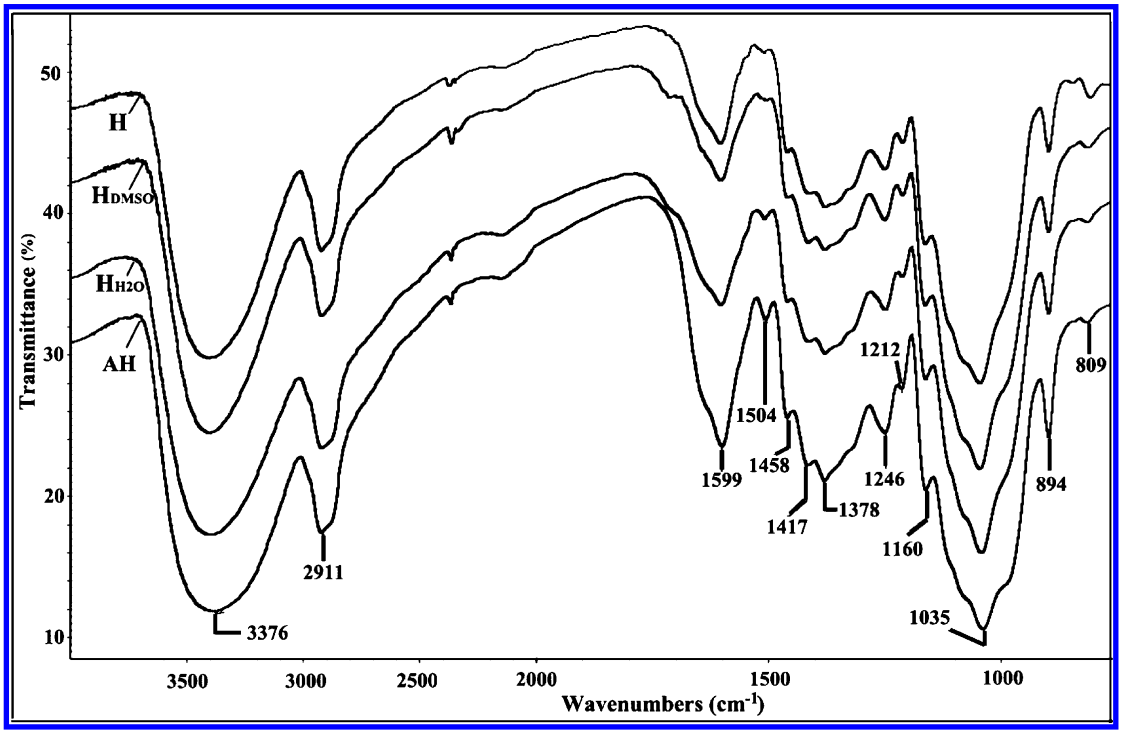

Figure 4. FTIR spectra of the hemicellulosic fractions from corncob.

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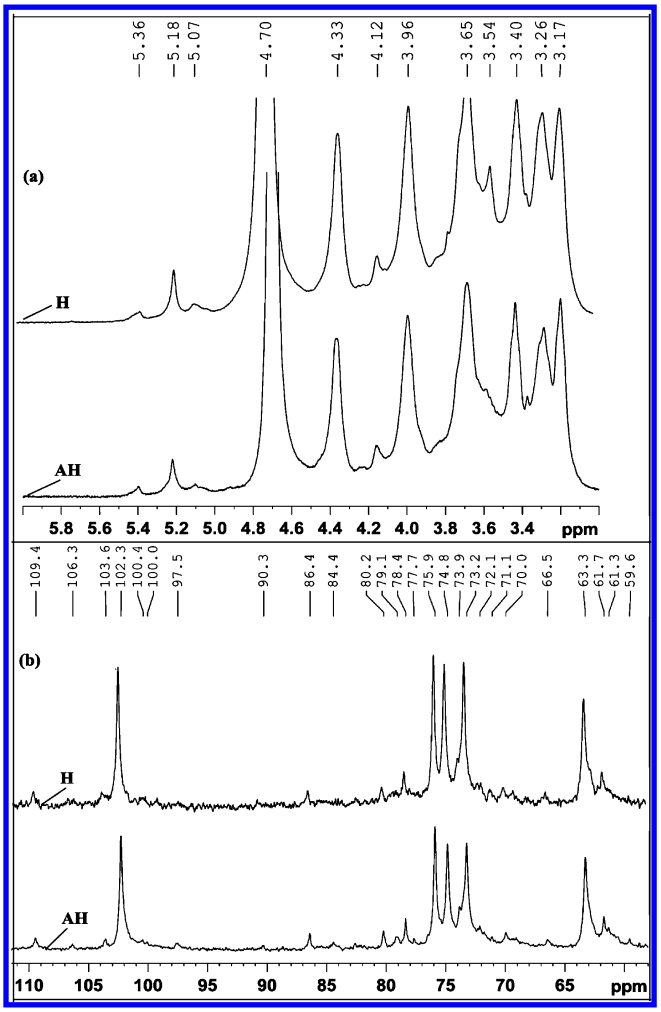

Figure 5. (a) ^{1}H and (b) ^{13}C NMR spectra of hemicellulosic fractions H and AH.

during the alkali extraction. The higher molecular weight average of the hemicellulosic fraction from the EMIMAc pretreatment also suggested that EMIMAc pretreatment did not degrade hemicelluloses to a noticeable extent. However, the pretreatment with EMIMAc/DMSO released hemicellulosic polymers with a lower M_w (276 810 g/mol), which suggested that a slight depolymerization of the native hemicelluloses may occur during the EMIMAc/DMSO pretreatment. The hemicelluloses could be substantially degraded and resulted in fragmentation under the EMIMAc/H_2O pretreatment, indicating that the lower swelling capacity in the EMIMAc/H_2O pretreatment resulted in a lower M_w of hemicellulosic polymers. Additionally, the analysis showed that the hemicellulosic fraction AH obtained without IL pretreatment had a relatively lower polydispersity (M_w/M_n, 3.23) compared to the other three hemicellulosic fractions prepared with IL pretreatments (M_w/M_n, 3.81−6.03). The high M_w/M_n indicated either a heterogeneous nature of the hemicelluloses or possibly some intermolecular interactions.[25]

FTIR Analysis. The FTIR spectra of all four hemicellulosic fractions are illustrated in Figure 4, which clearly showed the typical absorbance bands expected for hemicellulosic polymers, and the peak assignments were conducted according to the literature.[20,26,27] The spectra profiles and relative intensities of the signals appeared to be rather similar, indicating the analogous structure of the hemicelluloses. The bands at 3376 and 2911 cm^{-1} are due to the stretching vibrations of OH and CH, respectively. The specific signal maximum at 1035 cm^{-1} for hemicelluloses is attributed to the stretching and bending vibrations of C−O, C−C, and C−OH and the glycosidic C−O−C, indicating a dominant xylan of the alkali-soluble hemicelluloses, which is supported by the results obtained by sugar analysis. An intensive band at 894 cm^{-1} is assigned to the C_1 group frequency or ring frequency, suggesting the presence of dominant β-glycosidic linkages between xylopyranose units in the main xylan chains. The band at 1599 cm^{-1} is attributed to uronic acid carboxylate, and a signal at 1417 cm^{-1} is from the symmetric stretching vibration in the glucuronic acid groups as side chains. The band at 1458 cm^{-1} is assigned to CH_2 symmetric bending, while the bands at 1378 and 1246 cm^{-1} arise from C−H stretching and OH or C−O bending vibration. The absorption band at 1165 cm^{-1} is presumed to be due to the presence of the arabinosyl side chains, corresponding to the C−O−C vibration in the hemicellulosic polymers. The band at 1730−1750 cm^{-1}, corresponding to acetyl groups, was not observed, hence verifying that the alkaline extraction with or without IL pretreatment under the conditions used completely cleaved the ester bonds in the hemicelluloses. Furthermore, the appearance of a small signal at 1504 cm^{-1} for C═C stretching of the aromatic ring in all of the hemicellulosic fractions revealed that small amounts of associated lignin were present in the hemicelluloses. It can be observed that hemicelluloses prepared with IL pretreatment showed relatively weaker signals at 1504 cm^{-1} compared to hemicelluloses prepared without pretreatment. This was probably due to the fact that the linkages of covalent bonds (mainly α-benzyl ether) and ester bonds were disrupted with a relatively strong alkali in the hemicellulosic fractions prepared with pretreatment than the fraction obtained without pretreatment.

One- and Two-Dimensional NMR Analyses. NMR spectroscopy can be used to obtain more structural information on the building blocks of hemicelluloses. Therefore, the hemicellulosic fractions H and AH were investigated using 1D (^{1}H and ^{13}C) NMR spectroscopy. In addition, all of the hemicellulosic fractions H, H_{DMSO}, H_{H_2O}, and AH were analyzed by 2D (HSQC) NMR spectroscopy. Most of the resonances were assigned by referring to the previous reports.[28−30] The main features of the spectra are almost identical, indicating a similar structure of these hemicellulosic polymers.

The ^{1}H and ^{13}C NMR spectra of hemicelluloses are shown in panels a and b of Figure 5, respectively. In the ^{1}H spectra, the relevant signals occurred in two regions, the anomeric region (5.60−4.90 ppm for α anomers and 4.90−4.30 ppm for β anomers) and the ring proton region (4.50−3.00 ppm). The signals at 3.2−5.6 ppm are caused by the protons of the arabinose and xylose residues, except for the strong signal at 4.70 ppm, which is residual water. The spectra confirmed that D-xylopyranosyl (Xyl*p*) units were the backbone, and these units were linked β-glycosidically, which is in good agreement with the results obtained from sugar and FTIR analyses. The main signals at 4.33 (H-1), 3.96 (H-5eq), 3.65 (H-4), 3.40 (H-3), 3.26 (H-5ax), and 3.17 (H-2) ppm are assigned to Xyl*p* units. The signal at 5.18 ppm was assigned to anomeric protons of terminal α-D-arabinofuranosyl residues, indicating a significant amount of arabinose substituted at O-2 and/or O-3 of the xylan backbone.

In the ^{13}C NMR spectra, the main (1 → 4)-linked β-D-Xyl*p* units were characterized by five strong signals at 102.3, 75.9, 74.8, 73.2, and 63.3 ppm, corresponded to C-1, C-4, C-3, C-2, and C-5, respectively. Signals of the arabinofuranosyl (Ara*f*) units at 109.4,

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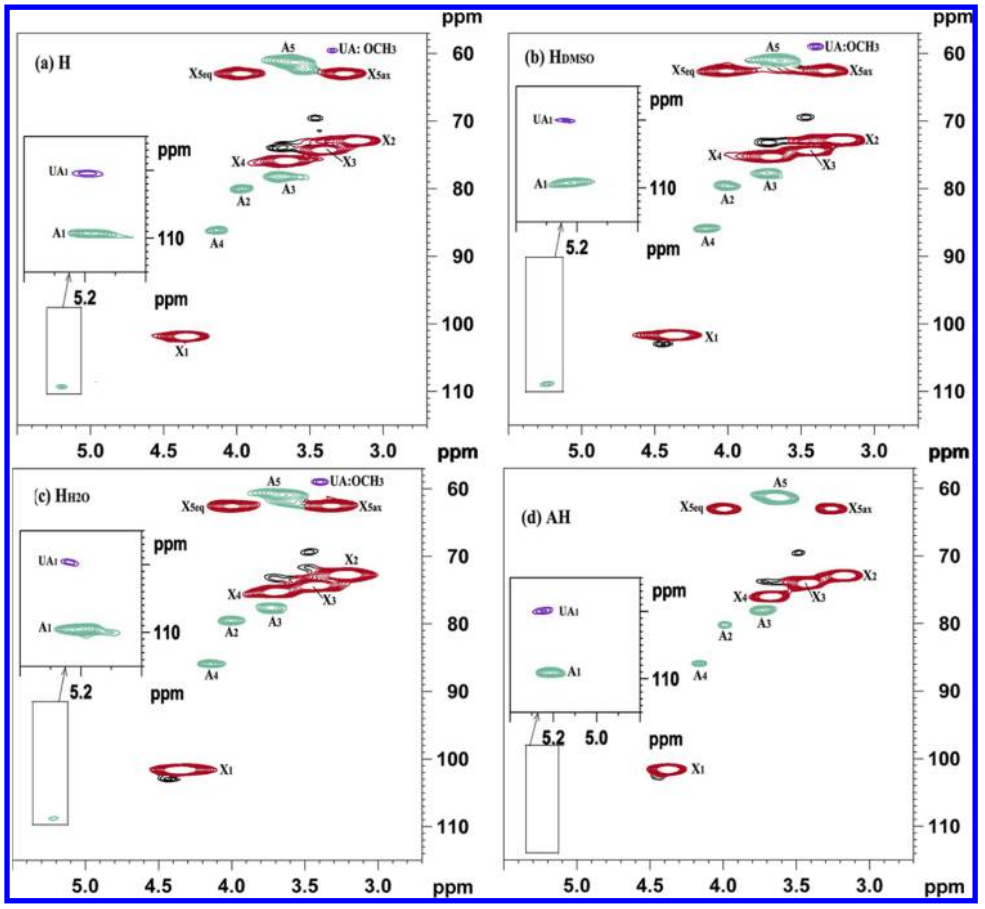

Figure 6. HSQC spectra of the hemicellulosic fractions (a) H, (b) H_{DMSO}, (c) H_{H_2O}, and (d) AH. X, (1 → 4)-*β*-D-Xyl*p*; UA, 4-*O*-Me-*α*-D-GlcpA; and A, *α*-L-Ara*f*.

86.4, 80.2, 78.4, and 61.3 ppm for C-1, C-4, C-2, C-3, and C-5, respectively, were observed in these spectra. Signals at 79.1, 73.9, 72.1, and 59.6 ppm corresponded to C-4, C-3, C-2, and OCH_3 of 4-*O*-methy-*α*-D-glucuronic acid (4-*O*-Me-*α*-D-Glc*p*A). The signal at 97.5 ppm attributed to the anomeric carbons of 4-*O*-Me-*α*-D-Glc*p*A was also observed.

Two-dimensional NMR is a powerful tool for hemicellulose analysis, because signals overlapping in the ^1H and ^{13}C NMR spectra can be resolved. The HSQC spectra of all of the hemicellulosic fractions are illustrated in Figure 6. The chemical shifts reported have been calibrated relative to the signal from C_1-H_1 (101.9/4.34 ppm) of the (1 → 4)-linked *β*-D-Xyl*p* units, which was used as the reference standard. Therefore, the signals at 101.9/4.34 ppm were attributed to the anomeric carbons and protons of Xyl*p* units in all of the hemicellulosic fractions. In addition, the signals at 109.3/5.19 and 97.5/5.18, 109.14/5.20 and 96.93/5.22, 109.1/5.20 and 96.96/5.22, and 109.3/5.20 and 97.65/5.19 ppm were assigned to carbons and anomeric protons of Ara*f* and 4-*O*-Me-*α*-D-Glc*p*A units in H, H_{DMSO}, H_{H_2O}, and AH, respectively. In the HSQC spectrum of the fraction H (Figure 5a) prepared with EMIMAc pretreatment, the five dominant cross-peaks at 101.9/4.34, 75.9/3.66, 74.3/3.41, 73.0/3.18, and 63.1/3.27 and 3.96 ppm, are assigned to C_1-H_1, C_4-H_4, C_3-H_3, C_2-H_2, and C_5-H_5 of the (1 → 4)-linked *β*-D-Xyl*p* units, respectively. The signals of Ara*f* units with less intensity were detected at 109.3/5.19, 86.2/4.13, 80.1/3.96, 78.2/3.70, and 61.4/3.60 ppm, which are characteristic of C_1-H_1, C_4-H_4, C_2-H_2, C_3-H_3, and C_5-H_5, respectively. Furthermore, cross-signals at 103.5/4.28 ppm existed in all HSQC spectra, which were assigned to the C_1-H_1 of (1 → 4)-*β*-D-glucopyranosyl oligosaccharides. In addition, small weak cross-peaks, such as 97.5/5.18 and 59.6/3.35 ppm, which represent C_1-H_1 and OCH_3 of 4-*O*-Me-*α*-D-Glc*p*A units, were also observed. Overall, the HSQC spectra analysis revealed that the hemicellulosic fractions were composed of the (1 → 4)-linked *β*-D-Xyl*p* backbone with Ara*f* attached to O-2 and O-3 and 4-*O*-Me-*α*-D-Glc*p*A linked to O-2 of the Xyl*p* units.

The distribution pattern of side groups in arabinoxylan is important for the solubility, interaction with other polymeric cell wall substances, degradability by enzymes, and other functional properties. The substituted xylose residues may be randomly distributed along the chain, but they are more likely gathered in highly substituted regions, leaving longer sections with no arabinose residues. This phenomenon is related to the function of the polymer in the plant cell walls. Additionally, the presence of larger unsubstituted regions in the xylan chains can cause strong hydrogen bonds, resulting in interchain aggregation, and also make the isolated material partly crystalline.[31] Therefore, the arabinoxylan structure affects so many functional factors.

Thermal Analysis. The thermal properties of the four fractions were investigated by TG and DTG, and the TG/DTG curves are illustrated in Figure 7. As seen, the TG curves showed that the thermal decomposition of hemicelluloses takes place in three major stages. In the first stage, the absorbed water was lost below 100 °C. In the second stage, initial decomposition took place up to 400 °C, which involved the fragmentation of the main and side chains of hemicellulosic polymers. The main products

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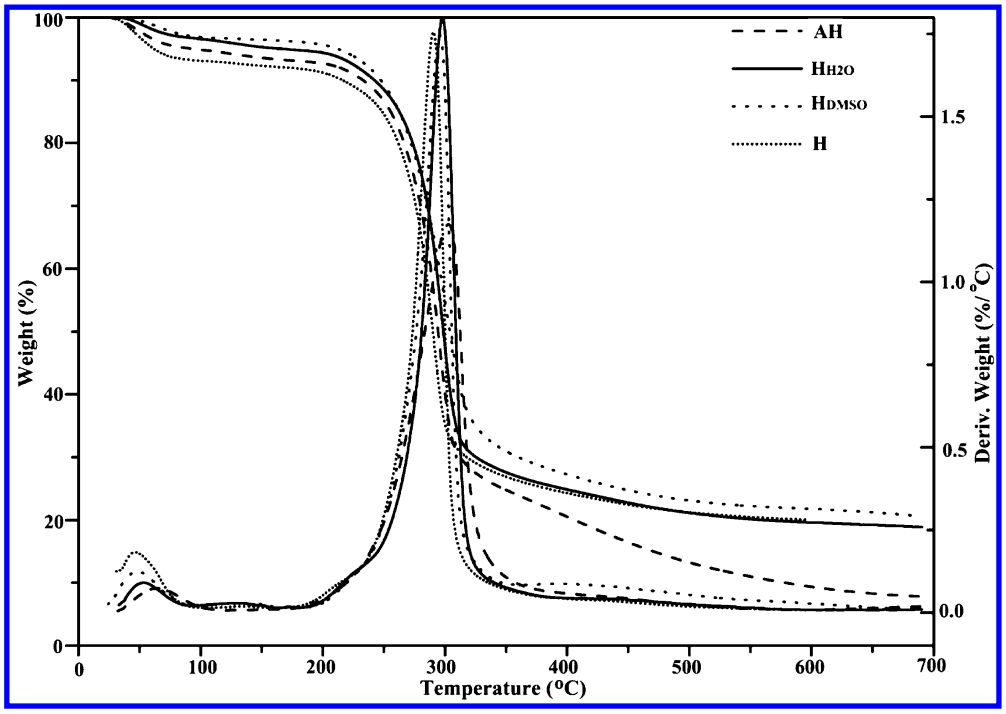

Figure 7. TG and DTG curves of the hemicellulosic fractions.

during the second stage were CO, CO_2, CH_4, CH_3COOH, and HCOOH.[32] As seen from the DTG curves, the temperature of the maximum decomposition rates (V_m) in this stage was observed at ~280 °C in all of the hemicellulosic fractions. However, the maximum decomposition rates of the pretreated hemicellulose fractions (~1.78%/°C) were higher than the maximum decomposition rate of the fraction obtained without pretreatment (~1.20%/°C). In the third stage (more than 400 °C), the char residues at 700 °C were ~20% for H, H_{DMSO}, and H_{H_2O} and 8% for AH. The fractions (H, H_{DMSO}, and H_{H_2O}) prepared with IL pretreatments showed a relatively higher weight of residue than the AH fraction at the same temperature in this decomposition stage. It was due to the fact that the structure of the char residues obtained at the higher heating rate is more stable and inhibits the cracking of the cross-linked molecules.[33,34] A higher molecular weight of the hemicellulosic polymers resulted in more thermal energy needed for the bond cleavage. The remaining char residues at 700 °C for all of the hemicellulosic polymers were presumed to be due to the end products of the decomposition of hemicelluloses. Overall, the thermal stability of the hemicellulosic fractions prepared with IL pretreatments was higher than that of the fraction AH prepared without pretreatment, being attributed to their higher molecular weights.

In summary, there were no significant differences in the contents of sugar components of the extracted hemicelluloses. The hemicellulosic fractions obtained had a similar structure, which had a backbone of (1 → 4)-linked β-D-xylopyranosyl with arabinofuranosyl attached to O-2 and O-3 and 4-*O*-methyl-α-D-glucuronic acid linked to O-2. The average molecular weights of all of the hemicellulosic fractions increased in the order of AH < H_{H_2O} < H_{DMSO} < H. In addition, the fractions prepared with EMIMAc, EMIMAc/H_2O, and EMIMAc/DMSO pretreatments exhibited higher thermal stability compared to the fraction prepared without pretreatment, being attributed to their higher molecular weights.

AUTHOR INFORMATION

Corresponding Author

*Telephone/Fax: +86-10-62336903. E-mail: xfx315@bjfu.edu.cn (F.X.); rcsun3@bjfu.edu.cn (R.-C.S.).

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Notes

The authors declare no competing financial interest.

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