Journal of Applied Polymer Science

Characteristics of hemicelluloses obtained from sweet sorghum based on successive extractions

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ABSTRACT: Hemicelluloses were successively extracted from sweet sorghum by hot water, dioxane, DMSO, and different concentrations of NaOH between 0.5% and 6.0%. The yields of the seven fractions together accounted for 88.6% of the original hemicelluloses. The obtained hemicellulosic subfractions were comprehensively investigated by both destructive methods such as alkaline nitrobenzene oxidation and acid hydrolysis and nondestructive techniques such as gel permeation chromatography, Fourier-transform infrared, ^{13}C-nuclear magnetic resonance, and 2D-heteronuclear singular quantum correlation. Sugar composition studies showed that the water-soluble polysaccharides consisted mainly of glucose, while xylose, arabinose, and glucuronic acid were the major sugars in other hemicellulosic fractions. It was found that the hemicelluloses from sweet sorghum were L-arabino-(4-*O*-methyl-D-glucurono)-xylans. Comparison with the hemicellulosic fractions dissolved by the alkali treatment, the hemicellulosic fraction extracted by DMSO had lower molecular weight. In addition, it was also found that the hemicelluloses prepared by dioxane and DMSO were more branched since that they had higher nonxylose/xylose ratios than those extracted by the alkali treatment, which were more linear and contained higher amounts xylose. © 2015 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 42790.

KEYWORDS: crosslinking; separation techniques; spectroscopy

Received 8 February 2015; accepted 28 July 2015
DOI: 10.1002/app.42790

INTRODUCTION

Nowadays, a mass of materials and energies in our daily life are based on fossil fuels. However, many countries are facing with the challenges of growing fossil fuels crisis. It was reported that the Middle East will be the only major reservoir of abundant crude oil within 20 years.[1] As the resources and environmental problems caused by fossil fuels are extensively recognized as a threat to development, researchers found that industrial crops are a renewable and potentially sustainable alternative to fossil reserves.[2] Sweet sorghum (*Sorghum bicolor* L. Moench) is one of the main industrial crops and is produced in large quantities world-wide every year. It is known for its strong tolerance of harsh environmental conditions, such as drought, arid climate, and alkaline soil; it also incorporate the advantages of corn, sugar cane, and switchgrass.[3,4] As we attempt to use this renewable biomass to produce various chemicals, the development of effective techniques for the study of straw structural characterization is considered to be both important and significant.

Hemicelluloses are natural polysaccharides associated to cellulose and lignin in lignocellulosic biomass and constitute about 15–35% of the total mass of annual and perennial plants.[5] A large amount of hemicelluloses produced from annually renewable biomass feedstock allows them possible to be a potential source for biomaterials, biofuels, and chemicals.[6] The bio-based polymer products have several other advantages such as availability from replenishable agricultural, low cost, biocompatibility, and biodegradability, thereby leading to eco-efficient and the possibility of preparing a variety of chemically or enzymatically modified derivatives for specific end uses.[7–10] In general, hemicelluloses have a heterogeneous composition of various sugar units including D-xylose, D-mannose, D-galactose, D-glucose, L-arabinose, 4-*O*-methylglucuronicacid, and D-galacturonic acid. Among these sugar residues of hemicelluloses, D-xylose, L-arabinose, D-glucose, and D-galactose are the most common sugar units.[11] Not like wood hemicelluloses, grass hemicelluloses contain less proportion of uronic acids, but contain more L-arabinofuranosyl units and branches. Glucuronoarabinoxylans consist of a main chain of

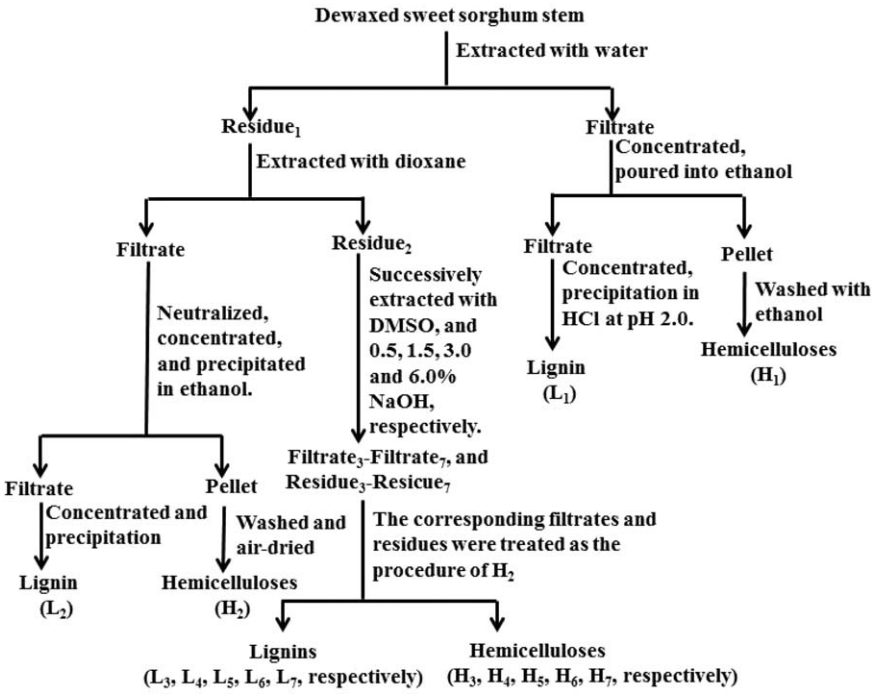

Figure 1. Scheme for isolation of hemicelluloses from sweet sorghum stem.

β-(1→4)-linked xylopyranose residues, which are attached by α-L-arabinofuranose and D-glucuronic acid units at positions 3 and 2 of the xylose residue, respectively.[12,13] In addition, the fine structure of arabinoxylans greatly varies among plant species and even among organs or tissues.

The yield and structure of hemicelluloses are depended on the extraction process. For example, the acetyl groups linked to xylans are usually removed during the extraction of hemicelluloses using alkali. Uronic and phenolic acids in hemicelluloses of graminaceous plants are linked by ester and ether linkages, which are accessible to break during the extraction of hemicelluloses with dilute acids and alkalis.[11] In the last decade, Sun *et al.* showed that alkaline peroxide was an effective agent for delignification and solubilization of hemicelluloses.[10] To avoid the degradation of hemicellulosic substituents, it has been reported that the material was subjected to successive extractions with dioxane and dimethyl sulfoxide (DMSO) and the obtained hemicelluloses showed unchanged structural features.[14] However, only a small part of the hemicelluloses could be obtained by one step extraction with organic solvents or dilute alkalis and the single step tended to liberate heterogeneous hemicellulosic fractions.[15] Therefore, it is necessary to apply successive methods including hot water, organic solvents, and alkali on the isolation of hemicelluloses. By using this process, hemicelluloses from sweet sorghum could be recovered by fractional precipitation and the subsequent physicochemical characterization would be available for the further study of hemicellulosic fractions.

Up to now, the fractionation and characterization of hemicelluloses from sweet sorghum are still lacking to be studied extensively.[16] In this article, the successive extractions of the hemicelluloses from sweet sorghum stems were carried out. The influence of different solvents on the chemical structure of hemicellulosic fractions was also investigated. The physicochemical and structural characterizations of the hemicellulosic fractions were comparatively studied by both the degradation methods such as acid hydrolysis, and nondestructive techniques, *e.g.*, Fourier-transform infrared (FT-IR), gel permeation chromatography (GPC), ^{1}H, ^{13}C, and 2D-heteronuclear singular quantum correlation nuclear magnetic resonance (2D-HSQC NMR).

EXPERIMENTAL

Materials

Sweet sorghum was obtained from the farm of the North-Western University of Agricultural and Forest Sciences and Technology (Yangling, China). The roots and leaves were removed, and the stalks were chipped into small pieces. The main composition (%, w/w) of the stem was cellulose 38.1%, hemicelluloses 35.2%, lignin 16.3%, ash 2.1%, and wax 3.8% on a dry weight basis.[17] After drying at 60°C for 16 h in an oven, the chips were then ground to pass a 0.8 mm size screen, and the powder was further dried in a cabinet oven with air circulation at 60°C for 16 h and stored in a desiccator. Prior to treatment, the dried powder of the sweet sorghum stem was first dewaxed with toluene-ethanol (2 : 1, v/v) in a Soxhlet extractor for 6 h and then air-dried.

Fractional Extraction of Hemicelluloses

Hemicellulosic fractions were isolated by sequential extraction according to the scheme in Figure 1. The dewaxed sweet sorghum stem was extracted with water at 80°C for 3 h with a solid to liquid ratio of 1 : 25 (g mL^{-1}). The insoluble residue after filtration was washed with distilled water. The combined filtrate and washing water were evaporated to 50 mL at reduced pressure. The water-soluble hemicellulosic fraction labeled as H_1 was recovered by precipitation of the concentrated filtrate in three volumes of 95% ethanol. The water-insoluble residue was

Table I. The Yield (% Initial Dry Sweet Sorghum Stem, w/w) of Hemicelluloses, Lignin, and Cellulose-Rich Residue

	Yield (%)							
	F_1[a]	F_2[a]	F_3[a]	F_4[a]	F_5[a]	F_6[a]	F_7[a]	Total
Hemicelluloses	7.0	2.1	1.9	3.9	5.1	5.7	5.5	31.2
Lignin	0.5	0.7	0.2	1.3	1.6	0.8	0.5	5.6
Residue	80.3	77.0	73.5	66.1	57.8	49.5	41.3	

[a] F_1, F_2, F_3, F_4, F_5, F_6, and F_7 represent the preparations of hemicelluloses, lignin and residue obtained by successive treatment of dewaxed sweet sorghum stem with water at 80°C for 2 h, 50% dioxane and DMSO at 75°C for 3 h, and 0.5, 1.5, 3.0 and 6.0% NaOH at 30°C for 3 h.

then sequentially extracted with 50% dioxane at 30°C for 3 h. The slurry was filtered and the insoluble residue was washed with distilled water. The combined filtrate and washing water were neutralized with 6*M* HCl to pH 5.5, and then concentrated to about 30–50 mL under reduced pressure. The dioxane-soluble hemicelluloses were precipitated by pouring the concentrated supernatant fluid into three volumes of 95% ethanol, which was labeled as H_2. The residue was then successively extracted with DMSO at 75°C, and 0.5%, 1.5%, 3.0%, and 6.0% NaOH at 30°C for 3 h, respectively. The solubilized hemicelluloses were then obtained from the corresponding supernatants by neutralizing, precipitating, and filtrating as the procedure of H_2. Note that the sequential treatments with DMSO, 0.5%, 1.5%, 3.0%, and 6.0% NaOH were for the hemicellulosic preparations H_3, H_4, H_5, H_6, H_7, respectively. Meanwhile, the lignin fractions were also isolated during the above mentioned extraction process. After filtration of hemicelluloses, the ethanol solution containing lignin fractions was concentrated by evaporation of ethanol under a reduced pressure. Then the lignin was precipitated at pH 2.0 by adding 1*M* HCl dropwise and centrifuged at the maximum achievable velocity. Then the obtained lignin was purified by washing with acid water (pH 2.0) and then centrifuged. The lignin preparations successively fractionated with distilled water, dioxane, DMSO, 0.5%, 1.5%, 3.0%, and 6.0% NaOH were freeze-dried and labeled as L_1, L_2, L_3, L_4, L_5, L_6, and L_7, respectively. The yields of hemicelluloses and lignins are given on a dry-weight basis related to the dewaxed sweet sorghum stems (Table I).

Characterization of the Hemicellulosic Fractions

The molecular weights of hemicelluloses were determined by GPC (Agilent 1200, USA) on a PL aquagel-OH 50 column (300 × 7.7 mm, Polymer Laboratories) calibrated with PL pullulan polysaccharide standards (peak average molecular weights 738, 12,200, 100,000, and 1,600,000 g mol^{-1}, Polymer Laboratories). The column oven was maintained at 30°C. Detection was achieved with a differential refractive index detector (RID), which was eluted with 5 m*M* sodium phosphate buffer (pH 7.5) containing 0.02*M* NaCl and 0.1% hemicelluloses at a flow rate of 0.5 mL min^{-1}.[18] The composition of monosaccharides in the hemicellulosic subfractions was determined by high-performance anion exchange chromatography (HPAEC). The neutral sugars in the hemicellulosic fractions were liberated by hydrolysis with 10% H_2SO_4 for 2.5 h at 105°C.[19] After hydrolysis, the sample was diluted to 30-fold, filtered, and injected into a HPAEC system (Dionex ISC 3000) with an amperometric detector, an AS50 autosampler, and a Carbopac PA1 column (4 × 250 mm, Dionex). The phenolic aldehydes and acids from alkaline nitrobenzene oxidation were analyzed by high-performance liquid chromatography (HPLC) as previously reported.[20]

The FT-IR spectra of all hemicellulosic fractions were obtained on a spectrophotometer (Nicolet, 750) using KBr discs containing 1% finely ground samples (32 scans, 4 cm^{-1} resolution). The solution-state ^1H NMR spectra were recorded on a Bruker AVIII NMR spectrometer at 400 MHz using 20 mg hemicelluloses in 1.0 mL D_2O. ^{13}C NMR spectra were obtained on a Bruker spectrometer at 100 MHz. The sample (80 mg) was dissolved in 1 mL of D_2O (99.8% D) overnight at room temperature. The ^{13}C NMR spectra were recorded at 25°C after 30,000 scans. The HSQC spectra were acquired by HSQCETGP experiment mode, over a t_1 spectral width of 10,000 Hz and a t_2 width of 1800 Hz and the AQ was 0.13 s. The number of scanning was 64. Data processing was performed using a standard Bruker Topspin-NMR software.

RESULTS AND DISCUSSION

Yield of Hemicelluloses

In the present study, a sequential extraction of hemicelluloses with hot water, 50% dioxane, DMSO, and alkali solution was performed according to the scheme in Figure 1 and the yields of hemicelluloses are given in Table I. As can be seen, the sequential treatment of the dewaxed sweet sorghum stem with distilled water at 80°C for 2 h, followed by 50% dioxane at 30°C, DMSO at 75°C and 0.5, 1.5, 3.0, 6.0% NaOH at 30°C for 3 h released 7.0%, 2.1%, 1.9%, 3.9%, 5.1%, 5.7%, and 5.5% hemicelluloses (% dry starting material), corresponding to the dissolution of 19.9%, 6.0%, 5.4%, 11.1%, 14.5%, 16.2%, and 15.6% of the original hemicelluloses, respectively. Meanwhile, the successive treatments also released 3.1%, 4.3%, 1.2%, 8.0%, 9.8%, 5.0%, and 3.1% of the original lignin, respectively. An increase in NaOH concentration resulted in a sustained increment of total degraded hemicelluloses, indicating that the relatively higher concentrations of NaOH were more efficient to break the lignin-hemicellulose linkage. Concerning the whole process, this successive treatment released 88.7% of the original hemicelluloses in the cell walls of sweet sorghum stem, indicating that substantial amounts of hemicelluloses along with little lignin were extracted sequentially (Table III). This high yield of hemicelluloses may be explained by the extensive cleavage of the ester bonds between hydroxycinnamic acids and hemicelluloses or lignin, and the α-benzyl ether linkages between lignin and hemicelluloses from the cell walls of sweet sorghum under the conditions used.[21] Interestingly, the treatment with hot

Table II. The Content of Neutral Sugars (Relative % of Hemicelluloses Sample, w/w) in Isolated Hemicelluloses Fractions

	Hemicellulose fractions[a]						
Neutral sugars	H_1	H_2	H_3	H_4	H_5	H_6	H_7
Rhamnose	1.11	0.99	0.78	ND[b]	T[c]	T	ND
Arabinose	6.62	9.49	9.10	15.57	10.91	11.99	10.75
Galactose	6.72	4.05	2.38	3.52	1.27	1.59	1.44
Glucose	56.45	29.05	24.95	11.94	6.53	7.17	9.25
Xylose	26.83	51.33	58.50	61.81	76.00	74.25	74.80
Glucuronic acid	1.81	5.06	4.21	7.08	5.24	4.95	3.73
Galacturonic acid	0.46	0.03	0.09	0.08	0.05	0.05	0.03

[a] H_1, H_2, H_3, H_4, H_5, H_6, and H_7 represent hemicellulosic fractions extracted with water at 80°C for 2 h, 50% dioxane at 30°C for 3 h, DMSO at 75°C for 3 h, and 0.5, 1.5, 3.0 and 6.0% NaOH at 30°C for 3 h.
[b] ND = not detected.
[c] T = trace.

water resulted the highest extraction yield, implying that the initial treatment of the dewaxed sweet sorghum with water at 80°C for 3 h librated 22.4% of the total available hemicelluloses. On the other hand, the 50% dioxane and DMSO treatments resulted in a yield of only 6.8% and 6.1% of the total available hemicelluloses, respectively. The four steps of successive extraction with alkali solvents released 64.7% of the total available hemicelluloses in total. Up to now, the alkaline extraction is even one of the most important tools in the structural characterization of the cell-wall polymers from lignocellulosic materials.[17] Moreover, these results illustrated that a portion of the hemicelluloses is loosely attached while a substantial part of the hemicelluloses is embedded firmly in the cell wall of the sweet sorghum. This difference in bound level of these polysaccharides may be because of the various functions of the hemicelluloses in sweet sorghum cell walls.[22]

Sugar Composition of Hemicelluloses

Hemicelluloses are consisted of many different monosaccharides, in which the sugar composition can vary depending on the method of isolation.[23] The monosaccharide composition and the content of uronic acids in the seven hemicellulosic fractions are reported in Table II. Xylose, glucose, and arabinose are the major components, but the proportion of these three sugar components was different between the seven hemicellulosic fractions. The water-soluble hemicellulosic fraction (H_1) was mainly comprised of glucose (56.45%), xylose (26.83%), galactose (6.72%), and arabinose (6.62%). The high percentage of glucose indicated correspondingly more glucans, which was prone to be dissolved in hot water. However, xylose was the major monosaccharide in the hemicellulosic fractions H_2 (51.33%) and H_3 (58.50%), which were sequentially extracted with 50% dioxane solution at 30°C and DMSO at 75°C for 3 h from dewaxed and water-treated sweet sorghum, respectively. Glucose and arabinose appeared to be the other major sugar constituents, suggesting that these hemicellulosic fractions were rich in glucurono- and arabino-xylan. Rhamnose and galacturonic acid were detected in trace amounts. These data showed that the dioxane- and DMSO-soluble hemicellulosic preparations were probably similar in the composition. Xylose (61.8–76.0%) was the predominant sugar in the other four hemicellulosic fractions, which were extracted with 0.5–6.0% NaOH at 30°C for 3 h from the DMSO-treated residue. In addition, arabinose and glucose appeared as noticeable amounts, while galactose and glucuronic acid were observed as minor constituents. The major monosaccharide in all the alkali treatment fractions was xylose (61.2–76.0%) followed by arabinose. An increase of NaOH concentration from 0.5% to 1.5% raised the contents of xylose from 61.8% to 76.0%, whereas a decrement of glucose and arabinose was observed in the hemicellulosic fractions. This result suggested that in sweet sorghum cell walls, more branched hemicelluloses were released from the treatment with lower NaOH concentration, while higher NaOH concentration treatment favored to the extraction of linear hemicelluloses. As the NaOH concentration was further increased to 3.0% and 6.0%, the content of xylose decreased to 74.3% and 74.8%, respectively. However, no further decrease of arabinose and glucose was observed. The content of neutral sugars in H_4-H_7 indicated that the treatments with higher NaOH concentration released hemicelluloses (H_5, H_6, H_7) with lower degree of branching, which was illustrated by the lower Ara/Xyl ratios.[24] This result implied that higher NaOH concentration led to higher solubility of linear hemicelluloses, which were difficult to be released by the former treatment.[25,26] Interestingly, the content of glucose increased to 7.17% and 9.25% when the NaOH concentration rose to 3.0% and 6.0%. This phenomenon may be explained by the fact that higher NaOH concentration degraded more amorphous cellulose from the cell wall of sweet sorghum. In addition, the amount of glucuronic acids in all the hemicellulosic fractions, ranging between 1.8% and 7.1%, was slightly lower than that in the hemicellulosic fractions isolated by aqueous alkali from wheat straw.[27] However, the content of uronic acid was corresponded well to another previous report, in which 1.8–7.0% uronic acid was found in the sugarcane bagasse hemicellulosic fractions extracted by alkaline solution.[20] These distinctions may be explained by the fact that hemicellulose structures and contents varies between different experimental materials.

Contents and Compositions of Phenolic Acids and Aldehydes

It is known that lignin boundes to hemicelluloses by various linkages, including ether linkage between the phenylpropanol and the hydroxyl from polysaccharides, and ester linkage between the cinnamic acid in lignin and the hydroxyl of polysaccharides.[28,29] Moreover, it was reported that most of lignins were directly linked to arabinose through ether bonds, which

Table III. The Yield (% Hemicellulosic Sample, w/w) of Phenolic Acids and Aldehydes from Alkaline Nitrobenzene Oxidation of the Hemicellulosic Fractions

	Hemicellulosic fractions[a]						
Phenolic acidsand aldehydes	H_1	H_2	H_3	H_4	H_5	H_6	H_7
Syringaldehyde	0.030	0.201	0.209	0.121	0.064	0.027	0.056
Syringic acid	0.077	0.425	0.309	0.298	0.184	0.139	0.200
Vanillin	0.072	0.277	0.270	0.211	0.169	0.125	0.126
p-coumaric acid	0.049	0.282	0.291	0.169	0.060	T[b]	ND[c]
Ferulic acid	ND	0.491	0.560	0.402	0.210	0.115	T
Total	0.23	1.68	1.64	1.20	0.69	0.41	0.38

[a] Corresponding to the hemicelluloses fractions in Table II.
[b] T = trace.
[c] ND= Not detected.

was the side chain of the xylan backbone in cereal straw cell walls.[30] To further verify the content of the lignin and its phenolic composition, alkaline nitrobenzene oxidation of the seven hemicellulosic subfractions was performed. Results about the associated lignin are shown by the phenolic acids and aldehydes, which are summarized in Table III. Obviously, all the hemicellulosic fractions contained little associated lignin (0.23–1.68%), implying that a significant of α-ether linkages between lignin and hemicelluloses were cleaved under the conditions given.[21] This was particularly true when the alkali treatment was applied at a relatively higher concentration, since an increase in concentration of NaOH from 0.5% to 6.0% led to a decrease in lignin content from 1.2% to less than 0.4%. Interestingly, compared to the water-soluble hemicellulosic preparation (0.23%), the other six hemicellulosic fractions involved relatively higher contents of associated lignin (0.38–1.68%). The reason for this phenomenon was probably because of the different mechanisms of isolating lignin by various solvents. Generally, hot water only dissolves water-soluble lignin, which is loosely attached in the cell wall. Dioxane and DMSO can both liberate lignin and hemicelluloses. Remarkably, aqueous alkali cleaved effectively the possible lignin-carbohydrates complexes, releasing most hemicelluloses and lignin together.[31]

The content of bound lignin was minimized in the hemicellulosic fraction (H_1) isolated with hot water from dewaxed sweet sorghum stem (0.23%) and maximized in the fraction of H_2 (1.68%) extracted with 50% dioxane. Syringic acid and vanillin were the most products obtained from the oxidation of the hemicellulosic fractions, ranging from 18.9% to 52.6% and 16.4% to 33.2% of the total phenolic monomers, respectively. The two oxidation productions were derived from the syringyl (S) and guaiacyl (G) units, respectively, which were involved in the noncondensed structures of lignin. This result indicated that the associated lignins in the seven hemicellulosic fractions were mainly composed of noncondensed guaiacyl and syringyl units. Higher contents of vanillin and ferulic acid than those of syringaldehyde and syringric acid revealed that the residual lignins in the hemicellulosic preparations contained more guaiacyl units. In addition, small amounts of *p*-coumaric acid and syringaldehyde were found to be presented in the nitrobenzene oxidation products.

It has been reported that the amount of ferulic and *p*-coumaric acids in the cell walls of straw and grass has a significant influence on the formation of the plant cell wall and its mechanical performance and biodegradability.[32] Further studies indicated that *p*-coumaric acid was esterified to lignin and polysaccharides, while ferulic acid was esterified to hemicelluloses and etherified to lignin.[33] Certain amount of ferulic and *p*-coumaric acids in the oxidation productions of hemicellulosic fractions revealed that the treatment with NaOH may result in a significant cleavage of the esterified bonds in sweet sorghum stem, such as the linkages between ferulic acid and hemicelluloses or between *p*-coumaric acid and lignin or hemicelluloses. Meanwhile, the alkaline treatments had a significant effect on the cleavage of the ether linkages between hemicelluloses and lignin, which was illustrated by the release of 68.7% of the original lignin during the successive treatments with aqueous NaOH.

Molecular Weight Distribution

The weight-average (M_w), number-average (M_n) molecular weights, and polydispersity (M_w/M_n) of the seven hemicellulosic fractions are listed in Table IV. Obviously, the water- and DMSO-soluble hemicellulosic fractions H_1 and H_3 showed distinct M_w values of 5800 and 5290 g mol^{-1}, which were much lower than those of the other five hemicellulosic preparations (ranging from 18,830 to 67,270 g mol^{-1}). This results revealed that the treatment with water and DMSO solubilized more small molecular of hemicelluloses from dewaxed sweet sorghum, whereas the dioxane and NaOH-soluble fractions contained more large molecular of hemicelluloses.[34] These results were entirely consistent with our earlier research, in which hemicelluloses were isolated by hot water, $NaCl_2O_3$, and KOH from sweet sorghum stem.[15] It should be noted that the isolation method, solvent species, and chain aggregation had a significant influence on the estimation of polymers molecular weight.[35] Therefore, the solvent species, the treatment time and chain aggregation may be partially responsible for such a wide variation in the estimates of molecular weight of the seven hemicellulosic fractions. Furthermore, the lower polydispersity of H_1-H_3 (1.24–2.30) indicated that the molecular weight distribution of the three hemicellulosic fractions extracted with water and organic solvents was narrower than those of the hemicellulosic subfractions obtained from alkali solution. This may be because

Table IV. Weight-Average ($\bar{M}_w$) and Number-Average ($\bar{M}_n$) Molecular Weights and Polydispersity ($\bar{M}_w/\bar{M}_n$) of the Hemicellulosic Fractions

	Hemicellulosic fractions[a]						
	H_1	H_2	H_3	H_4	H_5	H_6	H_7
M_w	5800	28,260	5290	18,830	65,890	46,070	67,270
M_n	13,350	48,470	6560	91,270	254,340	186,930	430,260
M_w/M_n	2.30	1.72	1.24	4.85	3.86	3.98	6.40

[a] Corresponding to the hemicellulosic fractions in Table II.

of the fact that hot water, dioxane and DMSO did not simultaneously lead to the dissociation of small molecule and large molecule of hemicelluloses.

FTIR Spectra

In order to confirm the typical bands of hemicelluloses and the diverse structures between the different fractions obtained by the successive processes, FT-IR analysis was performed on the seven hemicellulosic preparations. The FT-IR spectra of the three hemicellulosic preparations, extracted with water at 80°C for 2 h (spectrum 1), 50% dioxane at 30°C (spectrum 2), and DMSO at 75°C (spectrum 3) for 3 h from the dewaxed sweet sorghum stem are illustrated in Figure 2. As expected, the spectral profiles and relative intensities of the bands among the three spectra were rather similar, indicating the similar structures of the hemicelluloses. The absorption at 3426 cm^{-1} is attributed to the stretching of —OH groups and that at 2925 cm^{-1} is attributed to C—H stretching. The band at 1634 cm^{-1} was principally associated with absorbed water, since the disordered structures in these macromolecules may easily be hydrated.[36] Obviously, all the three hemicellulosic fractions showed a typical signal pattern for the hemicellulosic moiety, including the specific band maximum in the region from 1200 to 1000 cm^{-1}, which was dominated by ring vibrations overlapped with stretching vibrations of side groups (C—OH) and glycosidic bond vibration (C—O—C).[36] For example, the high absorbance at 1045 cm^{-1} is attributed to the C—O, C—C stretching, or C—OH bending typical of xylans.[37] In the carbonyl stretching region, a intensified band at 1742 cm^{-1} of H_2 and H_3 may attribute to the ester linkages of the carboxyl group of ferulic and/or *p*-coumaric acids, corresponding to the result of alkaline nitrobenzene oxidation, which revealed the higher contents of ferulic and *p*-coumaric acids in H_2 and H_3. As expected, the absence of the signal at 1720 cm^{-1} for carbonyl stretching in all the three spectra implied that the treatments with water and organic solvents under the conditions given did not significantly attack or oxidize the glycosidic linkages and hydroxyl groups in hemicelluloses. The band at 1082 cm^{-1} represents the C—OH bending, which is strongly influenced by degree of branching, indicating more side chains in fraction H_1. A vibration at 903 cm^{-1} corresponding to the C-1 group frequency or ring frequency was characteristic of β-glycosidic linkages between the xylose units in the hemicelluloses.[38] The absence of this band in spectrum 1 implied that the water-soluble hemicelluloses are mainly composed of α-glucan. The small bands at 1424, 1378, 1327, and 1255 cm^{-1} represent C—H stretching and C—O or OH bending vibration in hemicelluloses. Furthermore, the band at 1506 cm^{-1} implied the lignin associated to the hemicelluloses in the fractions, which corresponds to the results in Table II.

Figure 3 illustrated the FT-IR spectra of the alkali-soluble hemicellulosic fraction extracted with 0.5% NaOH (spectrum 1), 1.5% NaOH (spectrum 2), 3.0% NaOH (spectrum 3), and 6.0% NaOH (spectrum 4) from sweet sorghum. The resemble spectra indicated a similar structure of the four hemicellulosic fractions,

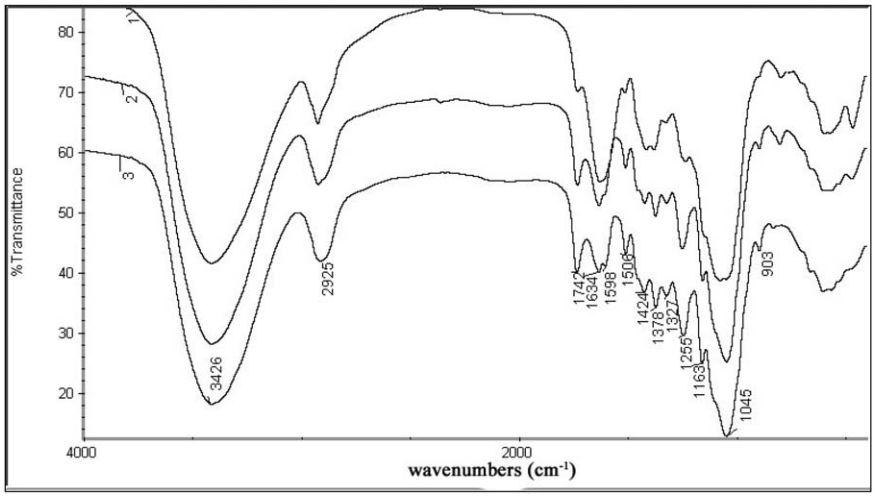

Figure 2. FT-IR spectra of sweet sorghum stem hemicellulosic fractions isolated with water at 80°C for 2 h (spectrum 1), 50% dioxane (spectrum 2) at 30°C, and DMSO (spectrum 3) at 75°C for 3 h.

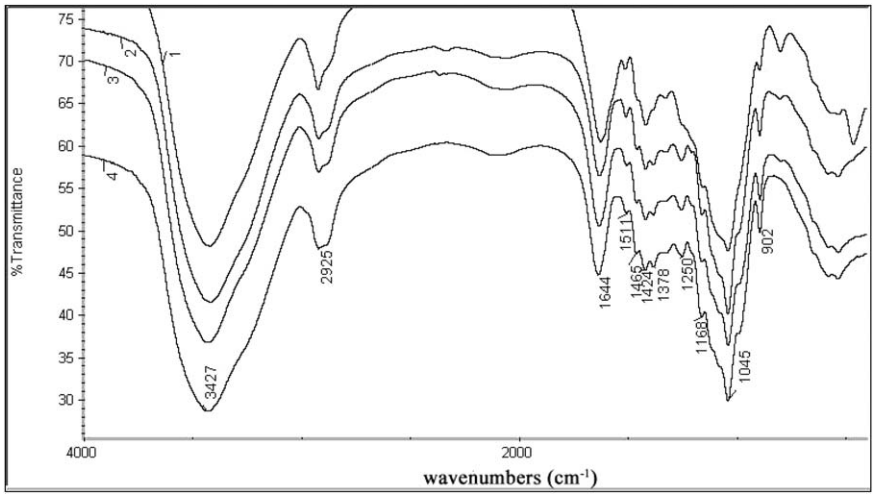

Figure 3. FT-IR spectra of sweet sorghum stem hemicelluloses fractions isolated with 0.5 (spectrum 1), 1.5 (spectrum 2), 3.0 (spectrum 3), and 6.0% (spectrum 4) NaOH at 30°C for 3 h.

which corresponded to their sugar composition. The bands at 1644, 1465, 1250, 1168, 1045, and 902 cm^{-1} are associated with hemicelluloses, in which the two bands at 1168 and 1045 cm^{-1} are typical of arabinoxylans. In spectra 1 and 2, an intensive absorption at 1511 cm^{-1} is characterized by aromatic skeleton vibrations in bound lignin or ferulic and *p*-coumaric acids. This phenomenon indicated that the treatment with 0.5% and 1.5% NaOH released hemicelluloses with more ferulic and *p*-coumaric acids and/or lignin than those from the treatments with higher concentration NaOH, corresponding to the results of alkaline nitrobenzene oxidation. Obviously, the peak around 460 cm^{-1} was probably attributed to Si-O-Si stretching in samples L_1, L_2 and L_4, which indicated that these hemicellulosic fractions contained ash residues. However, the low content of ash (much less than 2.1%) may not significantly influence the structural characterization of the hemicelluloses. Moreover, the former extracting procedure liberates more impurities such as starch and ash, which would facilitate the subsequent extraction of hemicelluloses.

1D and 2D NMR Spectra

Figure 4 shows the ^{1}H NMR spectra of the hemicellulosic subfraction H_5 obtained by treatment with 1.5% NaOH at 30°C for 3 h from sweet sorghum. The signals at δ 3.1–5.4 ppm characterize the protons of arabinose and xylose residue. Specially, the strong signal at δ 4.7 indicates the residual solvent.[39] The chemical shifts of δ 3.15–4.31 are originated from the equatorial protons of anhydroxylose units and 4-*O*-Me-α-D-GlcpA units of hemicelluloses polymers. Anomeric protons of terminal α-D-arabinofuranosyl residue occurs at δ 5.16, which revealed a large amount of substitution at C-2 and C-3 (disubstituted) of the xylose back-bone. Meanwhile, the anomeric protons were also distinguished at δ 4.31, which indicated the substituted at C-3 (monosubstitued) residues of β-D-xylose. This phenomenon can be explained by the fact that the region δ 4.1–4.5 corresponds to the β-configuration and the region between δ 4.9 and 5.6 corresponds to the α-configuration.[40]

In order to get a deeper insight into the branched structure of the hemicelluloses, the ^{13}C NMR spectroscopic analysis was performed on the hemicellulosic subfractions H_5 (Figure 5). Most of the major resonances were assigned by referencing to the data in the previous literatures.[30,41,42] The five signals at δ 102.33, 75.93, 73.30, 72.13, and 61.77 were respectively assigned to C-1, C-4, C-3, C-2, and C-5 of β-D-xyl*p* units, which implied the main (1→4)-linked β-D-xyl*p*

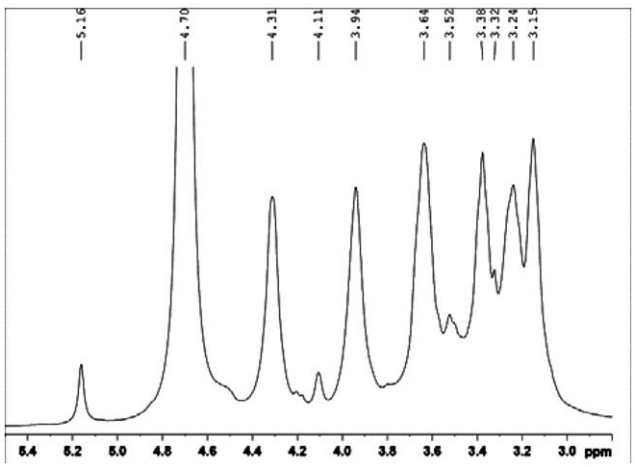

Figure 4. ^{1}H-NMR spectrum of hemicellulosic fraction H_5 isolated with 1.5% NaOH at 30°C for 3 h.

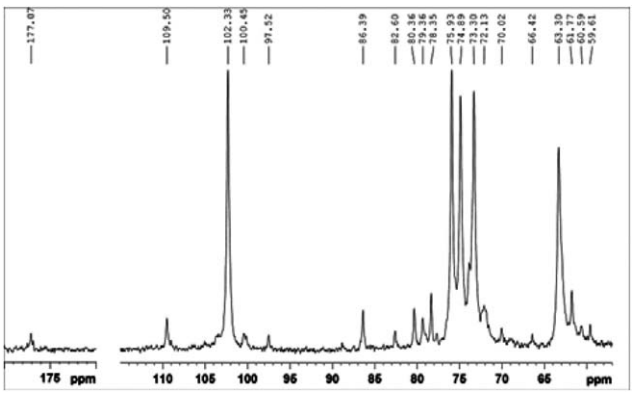

Figure 5. ^{13}C-NMR spectrum of hemicellulosic fraction H_5 isolated with 1.5% NaOH at 30°C for 3 h.

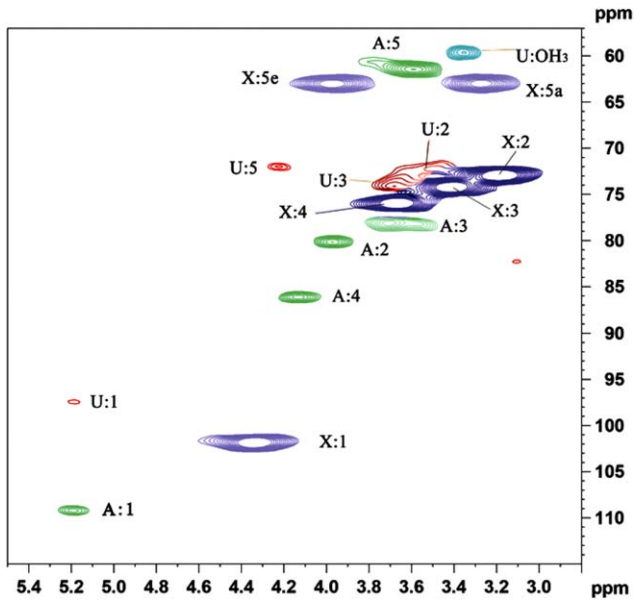

Figure 6. $^{1}H/^{13}C$ NMR (HSQC) spectrum of hemicellulosic fraction H_5 isolated with 1.5% NaOH at 30°C for 3 h. Designations are as follows: X, Xyl*p* units; A, Ara*f* unit; U, 4-*O*-Me-α-D-Glc*p*A unit. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

units of the hemicelluloses in H_5.[43,44] Meanwhile, the signals at δ 109.50, 86.39, 80.36, 78.35, and 61.77 indicated the occurrence of C-1, C-4, C-2, C-3, and C-5 of arabinofuranosyl residues linked to β-D-xylans, respectively. The small signals at δ 177.07, 72.13, 73.76, 73.30, and 59.61 are characteristic of COOH, C-2, C-3, C-5 and 4-*O*-CH_3 of 4-*O*-methy-α-D-glucuronic acid residues, respectively.[45] Two signals at δ 82.3 (data not shown) and 63.4 (overlapped with C-5 of *a*-L-Araf residues) are because of C-5 in the β-Glc*p*-(1→4)- and C-6 in β-Glc*p*-(1→3)-linkages of β-glucans. A weak signal at δ 59.61 was assigned to the *O*-methoxyl group of the glucuronic acid residue in the xylan. The structure of hemicelluloses extracted with 1.5% NaOH can be defined as L-arabino-(4-*O*-methyl-D-glucurono) xylan. These typical signals for L-arabino-(4-*O*-methyl-D-glucurono) xylan revealed that alkaline treatment under the conditions used did not affect the overall structure of the macromolecular hemicelluloses.

The HSQC spectra of the hemicelluloses fraction H_5 are shown in Figure 6. Obviously, the marked five $^{1}H/^{13}C$ cross-peaks at δ_C/δ_H 102.3/4.38, 73.0/3.15, 74.0/3.40, 76.8/3.68, 63.3/4.02, and 3.31 were assigned to C_1-H_1, C_2-H_2, C_3-H_3, C_4-H_4, and C_5-H_5 of the (1→4)-linked-β-D-Xylp units, respectively.[34] The distinct cross-peak at δ_C/δ_H 102.52/4.32 correspond to the C_1-H_1 atoms of glucose units overlapped with the signal from xylose units. In addition, the presence of the *O*-3 group from Ara*f* residues was confirmed by a corresponding small but distinguishable cross-peak at 86.3/4.16 ppm.[9] Furthermore, the C_1-H_1, C_2-H_2, C_3-H_3, C_4-H_4, C_5-H_5 of L-arabinose and OCH_3 of 4-*O*-methyl-D-glucuronic acid were also observed, which were revealed by the weak cross-peaks at δ_C/δ_H 109.3/5.20, 80.3/3.97, 78.36/3.70, 86.5/4.15, 61.8/3.72 + 3.63, and 59.5/3.31, respectively.[44] Based on the above discussions, the hemicellulosic fraction H_5, which was extracted with 1.5% NaOH at 30°C for 3 h, can be characterized as L-arabino-(4-*O*-methyl-α-D-glucurono)-D-xylans backbone decorated with branches at *O*-3 of arabinofuranosyl.

CONCLUSIONS

The sequential treatments of dewaxed sweet sorghum stem with hot water, 50% dioxane, DMSO, and alkaline solutions were effective on the fractionation of hemicelluloses, in which 88.6% of the original hemicelluloses in the cell walls of sweet sorghum were released. Rather low lignin contents (0.2–1.7%) of the seven isolated hemicellulosic fractions were determined. The M_w values of the four residual hemicellulosic preparations obtained by the aqueous alkali solutions were much higher than those of preparations extracted with hot water and DMSO. In addition, it was also found that the hemicelluloses prepared by DMSO were more branched since that they contained more nonxylose such as glucose than those extracted by the alkali treatment, which were more linear and contained higher amounts xylose. There were no significant differences in the structural features of the seven hemicellulosic fractions, which were composed mainly of L-arabino-(4-*O*-methyl-D-glucurono)-D-xylans. Hemicellulosic preparations obtained from 50% dioxane, DMSO, 3.0%, and 6.0% NaOH contained more ferulic and *p*-coumaric acids and/or lignin than other fractions.

ACKNOWLEDGMENTS

The authors are grateful to the financial support from the National Natural Science Foundation of China (31370368, 31170557), West Light Foundation of The Chinese Academy of Sciences.

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