Carbohydrate Polymers 92 (2013) 2224–2231

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Successive alkali extraction and structural characterization of hemicelluloses from sweet sorghum stem

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ARTICLE INFO

Article history:
Received 12 October 2012
Received in revised form 5 November 2012
Accepted 30 November 2012
Available online 10 December 2012

Keywords:
Sweet sorghum stem
Hemicelluloses
Structural characterization
NMR
L-Arabino-4-*O*-methyl-D-glucurono-D-xylan

ABSTRACT

Sweet sorghum stem was successively extracted with water at 90 °C, 0.3, 0.6, 1.0, 1.5, and 2.0% KOH aqueous solution, and 60% ethanol containing 2.5% KOH at 75 °C for 3 h, yielding 76.3% of the original hemicelluloses. Chemical composition and structural characterization of the seven hemicellulosic fractions obtained were comparatively investigated by a combination of HPAEC, GPC, FT-IR, ^{1}H-, ^{13}C-, HSQC NMR and TGA techniques. According to the spectral analysis, hemicelluloses from sweet sorghum stem are assumed to L-arabino-4-*O*-methyl-D-glucurono-D-xylan. In addition, the higher molecular weights of hemicelluloses resulted in a higher thermal stability of the samples. The present study suggests that successive alkali extraction is a promising approach for fractionation of hemicelluloses from sweet sorghum stem and to prepare hemicellulosic polymers with different branching and molecular weights.

1. Introduction

Due to the depletion of non-renewable petroleum-based resources and the environmental pollution issues, the exploration of energy from renewable lignocellulosic resources has attracted increasing attention. Lignocellulosic resources include various agricultural and forest residues such as straws, stems, coniferous and deciduous woods (Saha, 2003). Sweet sorghum has a high biomass yield due to its high photosynthetic efficiency and the stem is usually discarded in the field (Billa, Koullas, Monties, & Koukios, 1997). Moreover, this species is one of the energy crops due to its high sugar content. For instance, sweet sorghum stem has been recognized as one of the most promising ethanol crops in China since it is a renewable, cheap and widely available resource (Wang et al., 2009).

Heretofore, many studies focused on the production of bioethanol from sweet sorghum stem (Gnansounou, Dauriat, & Wyman, 2005; Mamma et al., 1995; Nguyen & Prince, 1996). Sweet sorghum stem is mainly composed of carbohydrate polymers (cellulose and hemicelluloses) and lignin. However, the chemical composition and structural feature of hemicelluloses in sweet sorghum stem has not been intensively studied. Hemicelluloses are heterogeneous polysaccharides in the plant cell wall, which interconnected with cellulose by hydrogen bonds and linked to lignin by covalent bonds (mainly α-benzyl ether linkages). Moreover, hemicelluloses also form ester linkages with acetyl units and hydroxycinnamic acids in Gramineae cell walls (Ebringerová & Heinze, 2000). These bonds restrict the release of hemicelluloses from the cell wall matrix. Therefore, it is necessary to develop a method for extracting hemicelluloses with a high purity and yield. Many approaches have been investigated on the extraction of hemicelluloses, including alkali extraction (Gabrielii, Gatenholm, Glasser, Jain, & Kenne, 2000), steam explosion treatment (Cara, Ruiz, Ballesteros, Negro, & Castro, 2006), ultrasonication, microwave, and organosolv extraction (Sun, Sun, & Ma, 2002). Among these approaches, alkali treatment is well known to disrupt the cell wall and cleave the hydrogen bonds, covalent bonds and ester linkages in the cell wall matrix. Generally, an increase in the strength of alkali can result in a more release of hemicelluloses embedded compactly in the lignocellulosic matrix. This is mainly because the concentration of alkali can effectively reduce the molecular size of hemicellulosic polymers and disrupt the bonds between carbohydrates and lignin (Sun, Sun, & Tomkinson, 2003). In order to achieve an effective extraction of hemicelluloses from lignocellulosic resources, a series of primary studies have been conducted in our laboratory and optimized condition has been developed. The treatment of *Carex meyeriana* Kunth and *Phyllostachys incarnate* Wen with sodium hydroxide solution at

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http://dx.doi.org/10.1016/j.carbpol.2012.11.098

different alkali concentrations for 3 h at 80 and 60 °C, respectively, resulted in a good solubility of original hemicelluloses (>80%) (Mao, Ma, Zhang, & Xu, 2011; Peng et al., 2011). Moreover, the extraction with solutions of alkali in ethanol is effective for disrupting the recalcitrant nature of the plant cell wall. For instance, the treatment of *Caragana sinica* with 70% ethanol containing 1% sodium hydroxide released 35% original hemicellulosic polymers (Xiao, Xu, & Sun, 2011). It should be noted that aqueous solutions of potassium, sodium, and lithium hydroxide are appropriate for extraction hemicelluloses from lignocellulosic resources, but the preferred alkali was potassium hydroxide because potassium acetate formed during the neutralization of the alkali extract is more soluble in ethanol used for precipitation than other acetates (Lawther, Sun, & Banks, 1996).

The aim of the current study was to investigate the effect of successive extraction with water, gradual concentration KOH, and alkaline ethanol solution on the yield and structural features of the hemicellulosic fractions obtained under the given conditions. The extracted hemicellulosic fractions were comparatively investigated by high performance anion exchange chromatography (HPAEC), gel permeation chromatography (GPC), Fourier transform infrared (FT-IR) spectroscopy, ^{1}H- and ^{13}C-nuclear magnetic resonance (NMR), and heteronuclear single quantum coherence (HSQC) NMR spectroscopy. Additionally, thermal stability of the hemicellulosic fractions obtained was also investigated by thermogravimetric analysis (TGA).

2. Experimental

2.1. Materials

Sweet sorghum stem was obtained from the experimental farm of the North-Western University of Agricultural and Forest Sciences and Technology (Yangling, P.R. China). It was dried in sunlight and then cut into small pieces. The pieces were ground and screened to prepare 20–40 mesh size particles. Sweet sorghum stem was first extracted with 2:1 (v/v) toluene–ethanol in a Soxhlet apparatus for 6 h, and the dewaxed particles were allowed to dry in an oven at 60 °C for 3 h. The principal composition (%, w/w) of the dried dewaxed sweet sorghum stem was cellulose 42.3%, hemicelluloses 21.8%, and lignin 18.0%, which was determined by National Renewable Energy laboratory's (NREL) standard analytical procedure (Sluiter et al., 2011), and ash 5.9%, which was measured after heated at 600 °C for 6 h. All chemicals used were of analytical or reagent grade and directly used as purchased without further purification.

2.2. Extraction of hemicelluloses

The hemicellulosic fractions were sequentially extracted with water, alkaline solutions at different concentrations, and alkali ethanol solution, as illustrated in Fig. 1. Specifically, the dewaxed particles (20 g) were successively extracted with distilled water at 90 °C, 0.3, 0.6, 1.0, 1.5 and 2.0% KOH aqueous solutions, and 60% ethanol containing 2.5% KOH at 75 °C for 3 h under a solid to liquid ratio of 1:20 (g/ml). The residues in each step were filtered off with a Buchner funnel, washed thoroughly with distilled water, and further dried in a cabinet oven with air circulation at 60 °C for 16 h. The water-extractable solution was directly concentrated under reduced pressure to about 30 ml, whereas the six alkali-extractable filtrates were acidified to pH 5.5 with glacial acetic acid before they were concentrated in the same way. Subsequently, the concentrated solution was poured into 3 volumes of ethanol with vigorously stirring. The precipitations were centrifuged, freeze-dried, and then labeled as H_{H_2O}, $H_{0.3}$, $H_{0.6}$, $H_{1.0}$, $H_{1.5}$, $H_{2.0}$, and $H_{2.5}$ according to the order of sequential extractions. All the experiments were carried out at least in duplicate.

2.3. Composition analysis

The chemical composition of the hemicellulosic fractions was analyzed by high performance anion exchange chromatography (HPAEC). Each of the fractions (5 mg) was hydrolyzd with 10% sulphuric acid at 105 °C for 2.5 h. Then the hydrolysate was filtrated, diluted 50-fold, and injected into a HPAEC system (Dionex ICS 3000) with an amperometric detector, an AS50 autosample, a CarbopacTM PA-20 column (4 mm × 250 mm, Dionex) and a guard PA-20 column (3 mm × 30 mm, Dionex). Neutral sugars and uronic acids were separated in a 5 mM NaOH isocratic (carbonate free and purged with nitrogen) for 20 min, followed by a 0–75 mM NaAc gradient in a 5 mM NaOH for 15 min. Then the columns were washed with 200 mM NaOH for 10 min to remove carbonate, and followed by a 5 min elution with 5 mM NaOH to re-equilibrate the column before the next injection. The total analysis time was 50 min, and the flow rate was 0.4 ml/min. Calibration was performed with standard solutions of L-rhamnose, L-arabinose, D-galactose, D-glucose, D-xylose, D-mannose, glucuronic acid, and galacturonic acid. Measurements were conducted with two parallels, and reproducibility of the values was found within the range of 5%.

2.4. Molecular weight determination by GPC

The molecular-average weights and molecular weight distributions of all the hemicellulosic fractions were determined by gel permeation chromatograph (GPC), using a PL aquagel-OH 50 column (300 mm × 7.7 mm, Polymer Laboratories Ltd.). The data were calibrated with PL pullulan polysaccharide standards (peak average molecular weights of 783, 12,200, 100,000, 1,600,000, Polymer Laboratories Ltd.). The detector used was a differential refractive index detector (RID). The eluent was 0.02 M NaCl in 0.005 M sodium phosphate buffer (pH 7.5), and the flow rate was 0.5 ml/min. All hemicellulosic samples were prepared at a concentration of 0.1% before measurement. The experiments were analyzed in triplicate. The deviations or standard errors were observed to be lower than 10%.

2.5. FT-IR spectroscopy

FT-IR measurements were performed on a Thermo Scientific Nicolet iN10 FT-IR Microscope (Thermo Nicolet Corporation, Madison, WI, USA) equipped with a liquid nitrogen cooled 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 hemicellulosic sample.

2.6. NMR spectroscopy

Soluble-state ^{1}H- and ^{13}C NMR spectra were recorded on a Bruker AV III 400 MHz spectrometer operating in FT mode at 100.6 MHz. The hemicelluloses (15 mg/ml in D_2O for ^{1}H, 80 mg/ml in D_2O for ^{13}C) were taken in sample probe and the resonance spectra were obtained. The chemical shifts of ^{1}H NMR spectrum were calibrated with reference to D_2O, used as an internal standard, at 4.70 ppm. The acquisition time was 3.9 s, and relaxation time was 1.0 s. For ^{13}C NMR, the spectrum was recorded at 25 °C after 30,000 scans. A 30° pulse flipping angle, a 9.2 μs pulse width, 1.36 s acquisition time, and 1.89 s relaxation delay time were used. The spectral widths were 2200 and 15,400 Hz for the ^{1}H- and ^{13}C NMR, respectively. Heteronuclear single quantum coherence (HSQC) NMR experiment was conducted with 20 mg sample dissolved in 1 ml D_2O. The number of collected complex points was

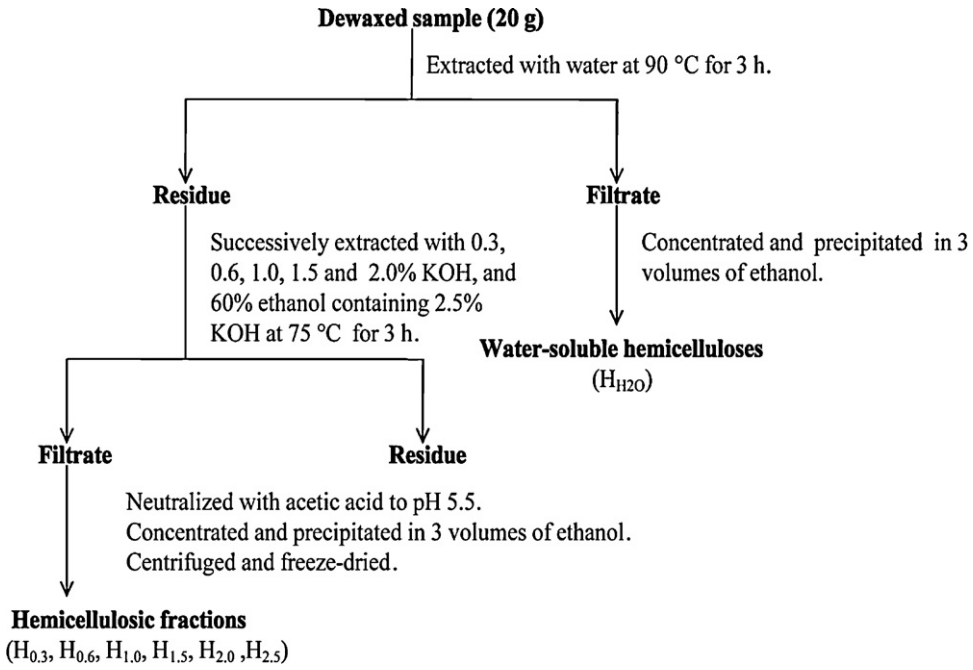

Fig. 1. Scheme for successive isolation of hemicellulosic fractions from sweet sorghum stem.

1024 for the ^{1}H-dimension with a relaxation of 1.5 s. The number of scans was 128, and 256 time increments were recorded in ^{13}C-dimension. The $^{1}J_{C-H}$ used was 146 Hz. Prior to Fourier transformation, the data matrixes were zero filled up to 1024 points in the ^{13}C-dimension.

2.7. Thermal analysis

Thermal behavior of the samples was investigated by using thermogravimetric (TGA) and differential thermogravimetric (DTG) analysis on a simultaneous thermal analyzer (TGA Q500, USA). 8–10 mg of samples were heated in a platinum crucible from room temperature to 700 °C at a heating rate of 20 °C/min under nitrogen atmosphere.

3. Results and discussion

3.1. Yield of hemicelluloses

In the present study, sequential extractions of dewaxed sweet sorghum stem with distilled water at 90 °C, 0.3, 0.6, 1.0, 1.5 and 2.0% KOH aqueous solutions, and 60% ethanol containing 2.5% KOH at 75 °C for 3 h, released 6.5, 1.2, 1.6, 1.9, 2.8, 1.7 and 1.0% of the initial amount of the dried dewaxed sample, corresponding to the dissolution of 29.8, 5.3, 7.4, 8.5, 12.8, 7.9, and 4.6% of the original hemicelluloses, respectively. Obviously, the total yield of the seven hemicellulosic fractions accounted for 76.3% of the original hemicelluloses in the cell walls of the dewaxed sample, indicating that most of the hemicelluloses can be extracted under the given conditions. This high solubility of hemicelluloses was assumed 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 (Sun, Tomkinson, Ma, & Liang, 2000). In all the alkali-extractable hemicelluloses, the yield of the hemicellulosic fractions first increased from 5.3 to 12.8% with the increment of KOH concentrations from 0.3 to 1.5% and then decreased with 1.5–2.5% alkali concentrations. Moreover, the highest yield of hemicellulosic fraction (12.8%) was obtained at the alkali concentration of 1.5% in the extractions.

3.2. Content of neutral sugars and uronic acids

The neutral monosaccharide composition and content of uronic acids of the seven precipitated hemicellulosic fractions are given in Table 1. The water-soluble hemicellulosic fraction (H_{H_2O}) contained a significant amount of glucose (81.4%) together with small amounts of arabinose (5.2%), galactose (6.4%) and xylose (5.0%) as well as a trace amount of mannose (0.7%). The high content of glucose in H_{H_2O} was probably related to the existence of starch as well as α-glucan in plant cell walls of sweet sorghum stem, and similar phenomenon has been reported in literature (Peng, Peng, Bian, Xu, & Sun, 2011; Sun et al., 2000). In comparison, xylose (36.1–73.0%), arabinose (11.4–18.6%), and glucose (6.0–34.3%) were the predominant sugar components of the six alkali-extractable hemicellulosic fractions, and small amounts of galactose (1.1–6.0%) and traces of mannose (≤0.4%) were also detected. Furthermore, the hemicelluloses also contained small amounts of glucuronic acid (4.1–8.4%) and traces of D-galacturonic acid (≤0.1%). The result indicated that

Table 1
The contents of neutral sugars and uronic acids (relative %, w/w) of the hemicellulosic fractions obtained from sweet sorghum stem.

Sugars (%)	Hemicellulosic fractions[a]						
	H_{H2O}	$H_{0.3}$	$H_{0.6}$	$H_{1.0}$	$H_{1.5}$	$H_{2.0}$	$H_{2.5}$
Arabinose	5.2	18.1	18.6	13.1	12.3	11.4	14.1
Galactose	6.4	6.0	3.8	1.8	1.9	1.1	2.2
Glucose	81.4	34.3	15.1	12.8	12.9	6.0	8.6
Xylose	5.0	36.1	54.2	66.9	68.8	73.0	70.8
Mannose	0.7	0.4	ND[b]	ND	ND	ND	ND
Glucuronic acid	1.2	5.0	8.2	5.3	4.1	8.4	4.3
Galacturonic acid	0.2	0.1	0.1	0.1	0.1	0.1	0.1
Xyl/Ara[c]	1.0	2.0	3.0	5.1	5.6	6.4	5.0

[a] Cotton H_{H_2O}, $H_{0.3}$, $H_{0.6}$, $H_{1.0}$, $H_{1.5}$, $H_{2.0}$ and $H_{2.5}$ represent the hemicellulosic fractions isolated by successive extractions with water at 90 °C, 0.3, 0.6, 1.0, 1.5, and 2.0% KOH aqueous solution, and 60% ethanol containing 2.5% KOH at 75 °C for 3 h.
[b] ND, not detectable.
[c] Represent xylose to arabinose ratio.

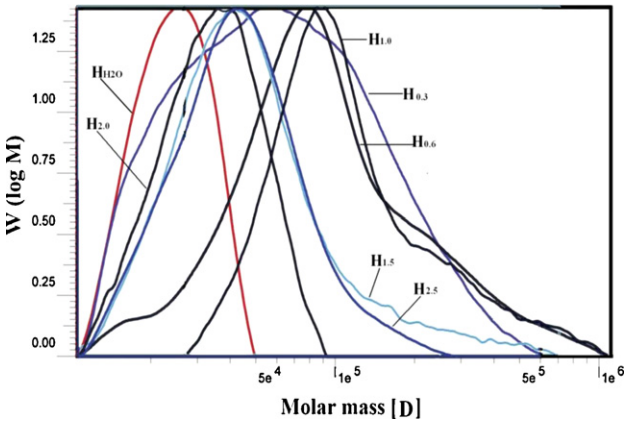

Fig. 2. Molecular weight distribution curves of the seven hemicellulosic fractions.

the two predominant monosaccharides in sweet sorghum stem hemicelluloses were xylose and arabinose, and they were thus termed as arabinoxylans (Maes & Delcour, 2001), whereas small amounts of galactose might be due to galactoarabinoxylans and arabinogalactans (Nandini & Salimath, 2001). It should be noted that as the concentration of KOH increased from 0.3 to 2.0%, the xylose content increased from 36.1 to 73.0%, while the contents of arabinose and glucose decreased from 18.1 to 11.4% and from 34.3 to 6.0%, respectively. However, the xylose content decreased while the contents of arabinose, glucose and galactose showed an increasing trend, when the alkali concentration increased from 2.0 to 2.5%.

Generally, the ratio of xylose to arabinose (Xyl/Ara) is indicative of the degree of linearity or branching of hemicellulosic polymers (Wedig, Jaster, & Moore, 1987). Table 1 shows that with the increment of KOH concentrations from 0.3 to 2.0%, the Xyl/Ara ratios gradually increased from 2.0 to 6.4, implying that the hemicellulosic fractions released in the higher alkali concentration solution had more linear structure, although the difference may occur in the distribution of branches along the xylan backbone and the molecular weight.

3.3. Molecular weight analysis

Molecular weight distribution curves of all the hemicellulosic fractions are illustrated in Fig. 2. It was observed that all the curves showed unimodal distribution. The curve of the water-soluble hemicellulosic fraction H_{H_2O} showed a low molar mass region, whereas those of the six alkali-soluble hemicellulosic fractions exhibited relatively high molar mass regions. The weight-average (M_w) and number-average (M_n) molecular weights along with polydispersity (M_w/M_n) of all the hemicellulosic fractions were calculated based on the curves in Fig. 2 and the results are listed in Table 2. All of the hemicelluloses exhibited M_w ranging between 5910 and 97,810 g/mol. Evidently, the water-soluble hemicellulosic fraction H_{H_2O} showed a relatively low M_w value (5910 g/mol), comparing with the six alkali-soluble hemicellulosic fractions (M_w, 22,750–97,810 g/mol). Meanwhile, an increment of KOH concentrations from 0.3 to 1.0% resulted in an increase of M_w value from 65,290 to 97,810 g/mol. In contrast, as the KOH concentration was further increased from 1.0 to 2.5%, the M_w value decreased from 97,810 to 22,750 g/mol, suggesting that a prominent degradation occurred during the treatment with over a KOH concentration of 1.0%. The result suggested that molecular weights of hemicelluloses vary depending on the method, solvent quality, and chain aggregation for their estimation (Izydorczyk & Biliaderis, 1995). Clearly, the data of the ratio of the polydispersity index (M_w/M_n) revealed that the six alkali-extractable hemicellulosic fractions (M_w/M_n, 1.20–3.06) had a relatively higher polydispersity as compared to the water-soluble hemicelluloses (M_w/M_n, 1.06). By combining with the sugar analysis aforementioned, the hemicelluloses showed a diversity distribution of molecular weight and branching structure.

3.4. FT-IR spectra analysis

Infrared spectroscopy has been proven to be useful for studying physicochemical and conformational properties of carbohydrates. The FT-IR spectra of the hemicellulosic fractions are shown in Fig. 3. As shown in Fig. 3a, H_{H_2O} and $H_{0.3}$ display very similar spectra. The band at 3371 cm^{-1} corresponds to the —OH stretching vibrations of the hemicelluloses, the absorption at 2893 cm^{-1} is attributed to the C—H stretching, and an intensive signal at 1647 cm^{-1} is originated from absorbed water, since hemicelluloses have a strong affinity for water, and in the solid state these macromolecules may have disordered structures that can be easily hydrated (Kačuráková, Belton, Wilson, Hirsch, & Ebringerová, 1998). In addition, the bands at 1415 and 1458 cm^{-1} are attributed to —COO^- symmetric stretching of uronic acid carboxylate and —CH_2 stretching, respectively. Furthermore, a small peak at 1381 cm^{-1} is due to C—H bending, whereas a prominent absorption at 1037 cm^{-1} is assigned to the C—O—C stretching of glycosidic linkages.

The rest five hemicellulosic fractions ($H_{0.6}$, $H_{1.0}$, $H_{1.5}$, $H_{2.0}$ and $H_{2.5}$) also show rather similar absorptions (Fig. 3b), indicating similar structure of these hemicelluloses. The bands at 1592 and 1410 cm^{-1} are related to the symmetric stretching of —COO salt in 4-*O*-methyl-α-D-gulcuronic acid. Moreover, the bands at 1456 cm^{-1} and 1381 cm^{-1} correspond to —CH_2 stretching and C—H bending, respectively. Meanwhile, the absorption band at 1244 cm^{-1} is related to the C—O linkage in the acetyl group, suggesting the acetylation of sweet sorghum stem hemicelluloses. The peaks at 1160 and 980 cm^{-1} are an index of arabinofuranosyl (Ara*f*) contribution (Kačuráková, Ebringerová, Hirsch, & Hromádková, 1994), and the signal absorption at 1038 cm^{-1} is assigned to the C—O—C stretching of glycosidic linkages, which is representative of xylans. The result revealed that arabinoxylan was the dominant alkali-soluble hemicelluloses, corresponding to the sugar analysis. Moreover, a sharp band at 898 cm^{-1} is indicative of the β-configuration of the 1 → 4 glycosidic bond between xylopyranose (Xyl*p*) units of the main xylan linkages (Gupta, Madan, & Bansal, 1987).

Table 2
Weight-average (M_w) and number-average (M_n) molecular weights and polydispersity (M_w/M_n) of the hemicelluloses fractions isolated from sweet sorghum stem.

	Hemicellulosic fractions[a]						
	H_{H_2O}	$H_{0.3}$	$H_{0.6}$	$H_{1.0}$	$H_{1.5}$	$H_{2.0}$	$H_{2.5}$
M_w	5910	65,290	90,740	97,810	66,330	43,720	22,750
M_n	5570	31,600	29,640	47,240	39,630	36,400	13,570
M_w/M_n	1.06	2.06	3.06	2.07	1.67	1.20	1.68

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

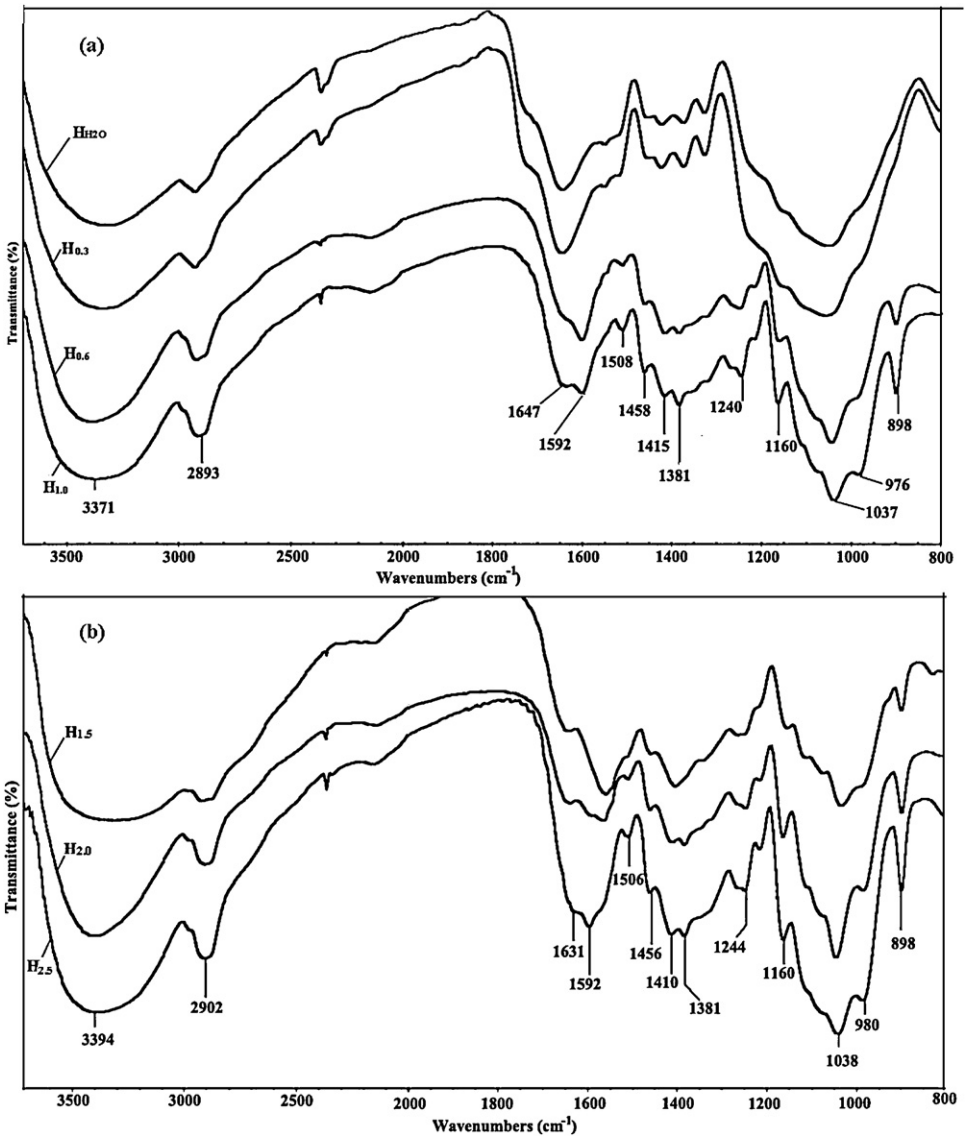

Fig. 3. (a) FT-IR spectra of water-soluble (H_{H_2O}), and 0.3 ($H_{0.3}$), 0.6 ($H_{0.6}$) and 1.0% ($H_{1.0}$) KOH-soluble hemicellulosic fractions and (b) FT-IR spectra of the hemicellulosic fractions isolated with 1.5 ($H_{1.5}$) and 2.0% ($H_{2.0}$) KOH aqueous solution, and 60% ethanol containing 2.5% ($H_{2.5}$) KOH at 75 °C for 3 h.

3.5. NMR analysis

NMR spectra are used to investigate the polymer backbone and the type of side-chain branching along the backbone of hemicelluloses. The ^{1}H-, ^{13}C-, and HSQC NMR spectra of the fraction $H_{2.0}$ were recorded in D_2O as shown in Figs. 4a, b and 5, respectively. The signals were assigned according to the literature (Billa et al., 1997; Teleman, Lundqvist, Tjerneld, Stålbrand, & Dahlman, 2000; Vignon & Gey, 1998).

As can be seen from Fig. 4a, the main signals at 4.28 (H-1), 3.92 (H-5eq), 3.61 (H-4), 3.34 (H-3), 3.22 (H-5ax) and 3.13 (H-2) ppm are originated from β-D-xylopyranosyl units, while the minor signals at 5.13 (H-1), 4.15 (H-5), 3.49 (H-2) and 3.34 (—OCH_3) ppm are assigned to 4-*O*-methyl-α-glucuronic acid units. In general, the chemical shifts of 3.00–4.28 ppm are attributed to the equatorial protons of anhydroxylose units, and 4-*O*-Me-α-D-Glc*p*A units of hemicelluloses polymers. Moreover, a signal of anomeric protons of terminal arabinofuranosyl units was observed at 5.13 ppm, and a strong signal at 4.70 ppm is ascribed to the residual solvent (D_2O).

The ^{13}C NMR spectrum of $H_{2.0}$ (Fig. 4b) shows five strong signals at 102.45, 75.91, 75.10, 73.42, and 63.32 ppm, which are assigned to C-1, C-4, C-3, C-2, and C-5 position of β-D-xylopranosyl units. Other weaker signals at 109.51, 86.46, 82.70, 80.41, and 61.81 ppm, correspond to C-1, C-4, C-3, C-2, and C-5 of the α-L-arabinofuranosyl units. Meanwhile, a small characteristic signal at 59.44 (—OCH_3) ppm is originated from 4-*O*-Me-α-D-Glc*p*A units.

More specific information about $H_{2.0}$ was obtained by 2D-HSQC NMR spectrum (Fig. 5). Clearly, five dominant cross-peaks of (1 → 4)-linked β-D-Xyl*p* units were detected at 102.0/4.31, 73.1/3.15, 74.6/3.67, 75.9/3.64, and 63.1/3.25 ppm, which are characteristic of C_1—H_1, C_2—H_2, C_3—H_3, C_4—H_4, and C_5—H_5, respectively. Moreover, the signals of Ara*f* units with less intensity at 109.3/5.17, 80.1/3.95, 78.3/3.68, 75.9/3.64, 61.6/3.58 ppm are assigned to C_1—H_1, C_2—H_2, C_3—H_3, C_4—H_4, and C_5—H_5 of α-L-arabinofuranosyl units, respectively. In addition, some weak cross-peaks at 97.4/5.17, 71.5/3.42, 73.8/3.65, 82.5/3.07, 72.1/4.21, 59.6/3.33 ppm, corresponding to the C_1—H_1, C_2—H_2, C_3—H_3, C_4—H_4, C_5—H_5 and —OCH_3 of the 4-*O*-Me-α-D-Glc*p*A units, were also detected in the

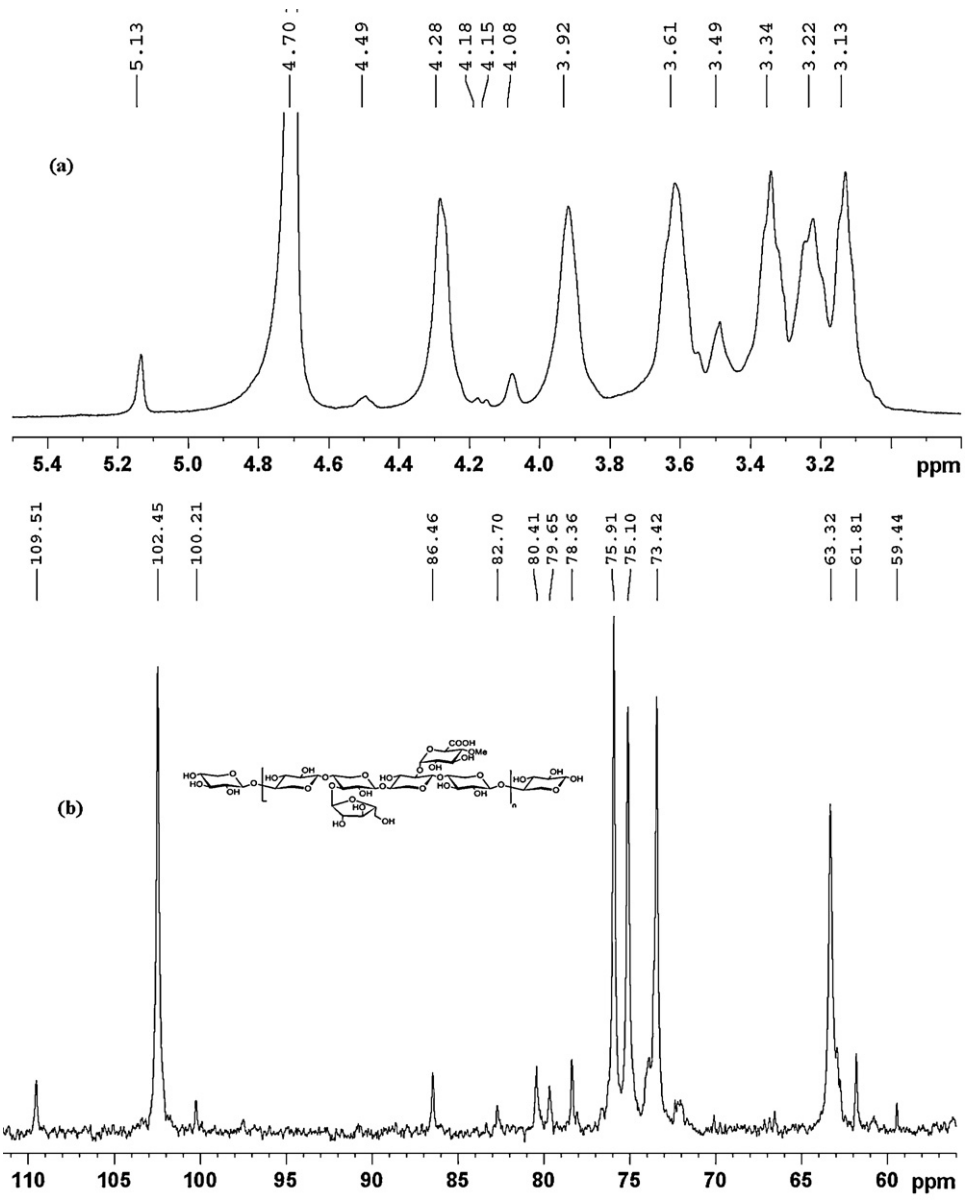

Fig. 4. ^{1}H (a) and ^{13}C (b) NMR spectra of hemicellulosic fraction $H_{2.0}$.

HSQC spectrum. Based on the results of FT-IR and NMR spectra analysis, the structure of hemicelluloses extracted with alkali solutions from sweet sorghum stem was assumed to L-arabino-4-*O*-methyl-D-glucurono-D-xylan.

3.6. Thermal analysis

Thermogravimetric analysis (TGA) is one of the most common techniques for the investigation of thermal properties and the associated kinetics during pyrolysis of biomass. It provides a measurement of weight loss of the sample as a function of time and temperature (Sricharoenchaikul & Atong, 2009). Differential thermogravimetric (DTG) analysis is the first derivative of the former curve (Kozłowski & Władyka-Przybylak, 2008). TGA–DTG curves of the fractions $H_{0.6}$ and $H_{1.5}$ are shown in Fig. 6. As can be seen, the thermal decomposition of hemicelluloses can be divided into three phases. At the first phase, the weight loss observed below 100 °C was ascribed to moisture evaporation. At the second phase, $H_{1.5}$ began to decompose, and the maximum rate of weight loss was observed from 150 to 350 °C. The maximum mass loss rate (0.68%/°C) was observed at 276.34 °C. Compared to $H_{1.5}$, the weight loss for $H_{0.6}$ mainly occurred between 200 and 375 °C with the maximum decomposition rate (1.15%/°C) at 298.23 °C. It was concluded that $H_{0.6}$ was thermally more stable than $H_{1.5}$, being attributed to their higher molecular weights. Importantly, this thermal decomposition process can be attributed to the cracking and abscission of C—C and C—O bonds connected with the main branch of hemicelluloses, and the decarboxylation and decarbonylation may also occur, hence resulting in the high pyrogenic decomposition reactivity of hemicelluloses. Finally, hemicelluloses polymers decomposed into CO, CO_2, and some light hydrocarbon (such as CH_4 and C_2H_4) in this phase (Yang, Yan, Chen, Lee, & Zheng, 2007). The last phase, when the temperature is over 400 °C, the weight loss is a very slight, and devolatilization occurred until temperature reached 700 °C. The solid residues of $H_{0.6}$ and $H_{1.5}$ were 26% and 33% at 700 °C, respectively. The residues are probably attributed to the gasification of some hemicelluloses fractions into ash (Kirubakaran et al., 2009) as well as the end-products of the decomposition of

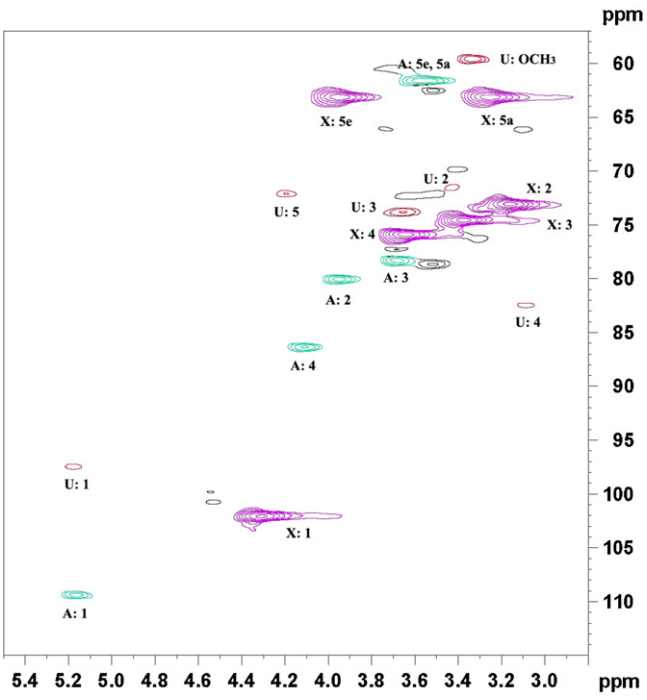

Fig. 5. HSQC spectrum of hemicellulosic fraction $H_{2.0}$.

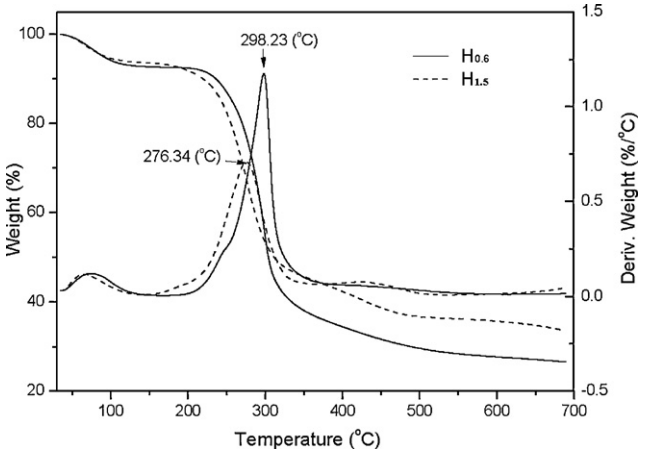

Fig. 6. Thermogram of hemicellulosic fractions $H_{0.6}$ and $H_{1.5}$.

hemicelluloses structure, which are carbonaceous residues formed in an inert atmosphere.

4. Conclusions

Sequential extractions with water and alkalis under increasing concentrations were an effective method to extract hemicelluloses from sweet sorghum stem. The water-soluble hemicelluloses rich in glucose (81.4%) showed a low molecular weight (M_w, 5910 g/mol) and polydispersity (1.06), probably being from starch and α-glucan. In comparison, the alkali-soluble hemicelluloses primarily consisted of xylose (36.1–73.0%) and had a linear structure and larger macromolecules. The hemicellulosic fraction with a high molecular weigh was released by a high concentration of the alkali, whereas a further increase of the alkaline concentration resulted in a decrease of the molecular weight of the hemicelluloses due to the degradation of the macromolecules. The fraction $H_{0.6}$ extracted with 0.6% KOH exhibited a relatively higher thermal stability as compared to $H_{1.5}$ extracted with 1.5% KOH. In addition, the structure of hemicelluloses extracted with alkaline solutions from sweet sorghum stem was assumed to L-arabino-4-*O*-methyl-D-glucurono-D-xylan.

Acknowledgements

The authors are extremely grateful to financial support from the National Natural Science Foundation of China (30930073), the Major State Basic Research Projects of China (973-2010CB732204), and Beijing Forestry University Project (HJ2010-13).

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