FRACTIONAL SEPARATION AND STRUCTURAL FEATURES OF HEMICELLULOSES FROM SWEET SORGHUM LEAVES

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Six hemicellulosic samples were isolated from cell wall material of dewaxed sweet sorghum (*Sorghum bicolor* (L.) Moench) leaves by sequential extractions with distilled water, alkali, and organic alkali solvent. The samples were treated with water, 1% NaOH, and 60% ethanol. The organic alkali samples were treated with 1%, 3%, 5%, and 8% NaOH, which yielded 8.3%, 5.4%, 1.0%, 5.6%, 2.5%, and 4.9% hemicelluloses based on the dry initial sweet sorghum leaves, respectively, and resulted in a total release of 81% of all hemicelluloses originally present in the cell wall. The results indicated that water-soluble hemicelluloses contained noticeable amounts of glucose, arabinose, galactose, and xylose, and had a relatively lower molecular weight (17300 g/mol). The four alkali-soluble hemicellulosic fractions, rich in xylose, were more linear, and had higher molecular weights (48500-128000 g/mol) than those of the alkali organic-soluble hemicellulosic fraction. With an increase of NaOH concentration from 1% to 8%, the ratio of arabinose to xylose decreased from 0.29 to 0.01, which implied that the hemicelluloses obtained by the higher concentration of alkali appeared to be more linear. Based on the sugar analysis, Fourier transform infrared (FT-IR), and nuclear magnetic resonance (NMR) results, 4-*O*-methylglucuronoarabinoxylans were the major constituents of the hemicellulosic polymers.

Keywords: Sweet sorghum leaves; Hemicelluloses; Extraction; Structure

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INTRODUCTION

Lignocellulosic biomass provides a low-cost and uniquely sustainable resource for the production of polymeric materials, fuels, and chemicals, which can reduce greenhouse gas emissions, enhance energy, dispose of problematic solid waste, and improve air quality. Lignocellulosic biomass mainly consists of three renewable polymers, cellulose, hemicelluloses, and lignin. The composition of these constituents can vary from one plant species to another. In general, cellulose, hemicelluloses, and lignin account for 40 to 60, 20 to 40, and 10 to 25 wt.% of biomass materials on a dry basis, respectively (Lin *et al.* 2010). Hemicelluloses are heterogeneous polymers of pentoses (xylose, arabinose), hexoses (mannose, glucose, galactose), and sugar acids. An estimated annual production of hemicelluloses on the earth is in the range of 60 billion tons (Naik *et al.* 2010). In hardwoods, the principal hemicelluloses are *O*-acetyl-4-*O*-methylglucurono-xylans (15 to 30%) and small portions of glucomannan (2 to 5%) (Girio *et al.* 2010). The main hemicelluloses of softwoods are an *O*-acetyl-galactoglucomannan (10-25%) and arabino-4-*O*-methyl-glucuronoxylan (5 to 10%). In species of Gramineae

such as cereal straws, the main hemicelluloses are arabinoxylans (Scheller and Ulvskov 2010). Hemicelluloses are one of the most abundant organic materials in the world and have the potential to be integrated in a wide variety of applications, such as film-forming substances, thickeners, emulsifiers, stabilizers, binders in food industry, pharmaceutical, cosmetic industries, and wet-end additives in papermaking (Spiridon and Popa 2008). For these applications, hemicelluloses have recently been attracting more attention as possible precursors to different value-added materials, fuels, and chemicals. However, hemicelluloses, together with pectins and lignins, form the complex cell wall matrix between the cellulose microfibrils by covalent hydrogen bonding and by ionic and hydrophobic interactions (Sun *et al.* 2000). Because of the tightly interwoven structure, the utilization of hemicelluloses has been quite limited in processes such as the conventional kraft pulping process, as well as ethanol or butanol fermentation process. Therefore, many studies have been undertaken to investigate the possible isolation and fractionation of hemicelluloses in a biorefinery manner.

Sweet sorghum (*Sorghum bicolor* (L.) Moench) is a high-yielding sugar crop characterized by its adaptability to harsh growth conditions, such as its comparatively high tolerance to drought, water logging, salinity, and alkalinity (Corredor *et al.* 2009; Li *et al.* 2010). As a complementary feedstock to sugarcane, sweet sorghum offers the existing sugar-based biofuels industry a flexible, low-cost crop that can be collected from existing harvests and can be processed with the current infrastructure, or with new conversion technologies, to other liquid transportation fuels or bioproducts (Spencer 2009). Sweet sorghum, therefore, is considered to be one of the most promising bio-energy crops (Li and Liao 1992). In general, sweet sorghum stalk is used for the production of sugar-based fuels, chemicals, and materials. The leaves are often burnt as firewood, used as livestock feed, or discarded. Therefore, it is very important for investigators to increase the value of these agricultural residues in response to the increasing cost of manufacturing and uncertain future availability of wood fiber. The hemicelluloses of sorghum husk were characterized as a 1,4-linked linear structure of D-xylopyranose units with highly branched L-arabinose residues by Woolard *et al.* (1976). Verbruggen *et al.* (1995) also extracted highly substituted arabinoxylans (arabinose/xylose = 0.94) from sorghum endosperm, which were also composed of uronic acids, acetyl and feruloyl substituents (Verbruggen *et al.* 1993). However, no research has been dedicated toward the isolation and characterization of water-, alkali organic- and alkali-soluble hemicelluloses from sweet sorghum leaves.

In plant cell walls, there are large amounts of hemicelluloses with a wide variation in content and chemical structure. Hemicelluloses generally consist of several populations of polysaccharide molecules that vary in structural characteristics and properties. In general, one-step dilute alkali treatments extract only part of the hemicelluloses from both holocellulose and lignified materials. Successive treatments with alkali of initially and then higher concentration avoid unnecessary exposure of hemicellulosic material to alkali that are more concentrated than that required for the extraction (Buchala *et al.* 1971). In this case, the hemicellulosic materials from plant cell walls are frequently fractionated to give polysaccharides having different structural features. More importantly, studies of such fractionated materials have led to much structural information in hemicellulosic molecules recovered by the most commonly used procedures (Wilkie 1979). In order to explore structure-property relationships for the hemicellulosic polymers, the current study was to comparatively investigate some

properties and structural features of hemicelluloses obtained by successively extractions of water, alkali, and alkali organic solvents from the sweet sorghum leaves.

EXPERIMENTAL

Materials

Sweet sorghum leaves were obtained from the experimental farm of the Northwest Agriculture and Forestry University (Yangling, China). The leaves were dried by sunlight and then cut into small pieces. The cut leaves were ground to pass a 0.8 mm size screen. After being further dried at 60 °C for 16 h, the powder was dewaxed with 2:1 (v/v) toluene-ethanol in a Soxhlet apparatus for 6 h. The wax-free samples were further dried in a cabinet oven with air circulation at 60 °C for 16 h. The composition (%, w/w) of sweet sorghum leaves was cellulose 37.5%, hemicelluloses 34.2%, lignin 17.1%, which was determined by the method for measuring the chemical composition of wheat straw described by Lawther *et al.* (1995). All standard chemicals, such as sugars and phenolics, were analytical grade, purchased from Sigma Chemical Company (Beijing).

Fractional Separation of Hemicelluloses

Sequential extractions of the wax-free sweet sorghum leaves were carried out according to the scheme in Fig. 1, and the extractions were conducted with two parallels. The wax-free powder (15.0 g) was treated with distilled water at 80 °C for 3 h at a solid-to-liquor ratio of 1:20 (w/v) under stirring.

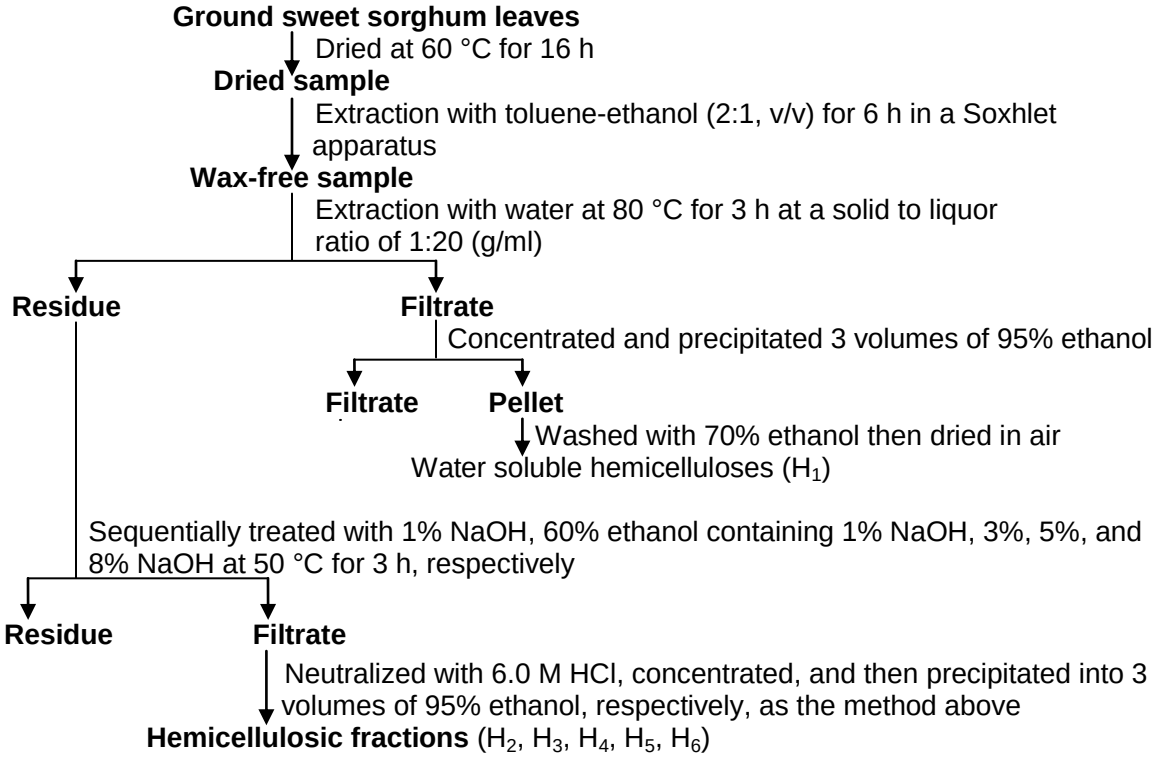

Fig. 1. Scheme for fractional isolation of hemicelluloses from sweet sorghum leaves

After filtration, the filtrates were concentrated to about 50 mL and then mixed with three volumes of 95% ethanol for isolating water-soluble hemicelluloses (H_1). The water-insoluble residue was sequentially treated with 1% NaOH, 60% ethanol containing 1%, 3%, 5%, and 8% NaOH at a solid to liquid ratio of 1:20 (w/v) at 50 °C for 3 h. The filtrate was neutralized with 6.0 M HCl to pH 5.5, and concentrated to about 150 mL. Three volumes of 95% ethanol were added to each concentrated filtrate with continuous stirring, and then the flocculent precipitate appeared. The precipitated hemicellulosic fractions were obtained by centrifugation (3500 g, 15 min), and then redissolved in distilled H_2O, dialyzed (3.5 kDa cutoff) until free of NaCl, and lastly freeze-dried. Note that H_2, H_3, H_4, H_5, and H_6 represent the hemicellulosic fractions isolated by the treatments with 1% NaOH, 60% ethanol containing 1%, 3%, 5%, and 8% NaOH, respectively.

Analytical Method

The constituents of neutral sugars in the isolated hemicellulosic fractions were determined by high performance anion exchange chromatography (HPAEC) according to the reference, Peng *et al.* 2009. The molecular weights of the hemicellulosic fractions were determined by gel permeation chromatography (GPC) on a PL aquagel-OH 50 column (300×7.7 mm, polymer laboratories Ltd), calibrated with PL pullulan polysaccharide standards (peak average molecular weights 783, 12 200, 100 000, 1 600 000, polymer Laboratories Ltd) (Peng *et al.* 2009).

The chemical composition of phenolic acids and aldehydes liberated from alkaline nitrobenzene oxidation of the lignin associated in the hemicellulosic fractions was determined by high-performance liquid chromatography (HPLC, Agilent1200, USA). The individual compounds were detected at 280 nm and identified by computer comparison of the retention times and peak areas with the authentic phenolics. The measurements were conducted with two parallels, and the reproducibility of the values was kept within the range of 6%.

FT-IR spectra of hemicellulosic samples were obtained on an FT-IR spectrophotometer (Nicolet 510) using a KBr disc containing 1% finely ground samples. Thirty-two scans were taken of each sample recorded from 4000 to 400 cm^{-1} at a resolution of 2 cm^{-1} in the transmission mode. The solution-state ^1H NMR spectrum was recorded on a Bruker MSL300 at 300 MHz using 15 mg of hemicelluloses in 1.0 mL of D_2O. ^{13}C NMR spectrum was obtained on the same spectrometer at 74.5 MHz. The sample (80 mg) was dissolved in 1.0 mL D_2O (99.8% D) with overnight stirring at room temperature. The spectrum was recorded at 25 °C after 30 000 scans. Chemical shifts (δ in ppm) are expressed relative to the resonance of Me_4Si ($\delta = 0$). A 60^o pulse flipping angle, a 3.9 μs pulse width and a 0.85 s delay time between scans were used.

Thermal behavior of the hemicelluloses was performed using thermogravimetric analysis (TGA) and differential thermal analysis (DTA) on a simultaneous thermal analyzer (DTG-60, Shimadizu, Japan).

The apparatus was continually flushed with a nitrogen flow of 30 mL/min. The samples weighed between 9.0 and 11.0 mg were heated from room temperature to 550 °C at a rate of 10 °C/min.

RESULTS AND DISCUSSION

Yield and Sugar Composition of Hemicelluloses

The yield of hemicelluloses released by the successive treatments is shown in Table 1. As shown, the treatment with water, 1% NaOH 60% ethanol containing 1%, 3%, 5%, and 8% NaOH under the conditions given yielded 8.3%, 5.4%, 1.0%, 5.6%, 2.5%, and 4.9% hemicelluloses of the dry initial sweet sorghum leaves, respectively. The total yield of the solubilized hemicelluloses accounted for 80.9% of the original hemicelluloses. It should be noted that the amounts of water-soluble hemicelluloses were much higher than those of the alkali organic- and alkali-soluble hemicelluloses (H_2-H_6), which was due to the co-existing of some amount of associated lignin, protein, starch, and pectic substances in H_1. As shown by the data in Table 1, the extraction with 60% ethanol containing 1% NaOH resulted in a lower yield (1.0%) of the hemicelluloses, whereas the treatment with 3% NaOH gave rise to a higher yield (5.6%) of hemicelluloses. The reason is that lignin was also dissolved or degraded by the cleavage of bonds such as α-aryl ether and arylglycerol-β-aryl ether under alkaline conditions, and a large proportion of hemicelluloses were exposed at the surface of residue, which resulted in an increase in the release of the hemicellulosic polymers in aqueous 3% NaOH. These results indicate that organic and alkali solutions under the conditions used significantly cleaved the α-ether bonds between lignin and hemicelluloses, caused cellulose to swell, and disrupted the hydrogen bonds between hemicelluloses and cellulose from the cell wall of sweet sorghum leaves, resulting in a substantial dissolution of hemicellulosic polysaccharides.

Table 1. Yield of Hemicelluloses (% Dry Weight, w/w) Obtained by Sequential Extractions of Sweet Sorghum Leaves

Fractions	Extractant	Yield (% dry weight)
H_1	Distilled water	8.30
H_2	1%NaOH	5.40
H_3	60% ethanol +1% NaOH	0.96
H_4	3% NaOH	5.60
H_5	5% NaOH	2.50
H_6	8% NaOH	4.90
Total		27.66

The neutral sugar composition and content of uronic acids of the six hemicellulosic fractions are given in Table 2. Obviously, the water-soluble hemicelluloses contained noticeable amounts of glucose (50.1%), arabinose (16.9%), galactose (19.8%), and xylose (8.4%). Only minor amounts of rhamnose, mannose, and galacturonic acid were present in this run. The previous result indicated that this run probably contained β-glucan, starch, arabinogalactan, highly substituted arabinoxylans, and the pectic polysaccharides. Similar results have been found in the previous studies on the low molecular weight, highly substituted polysaccharides obtained by extraction with distilled water from wheat straw, and rye leaves (Lawther *et al.* 1995; Xu *et al.* 2007). As shown in Table 2, the monosaccharide of the alkali-organic and alkali-soluble hemicelluloses (H_2-H_6) were mainly composed of xylose, arabinose, glucuronic acid, and glucose, which suggested that substantial proportions of the hemicelluloses in the cell

walls of sweet sorghum probably consisted of glucuronoarabinoxylans and β-(1→3)(1→4)-glucans, which need to be further confirmed by FT-IR and NMR spectra. The 1% NaOH extraction released a higher content of glucose (35.1%), suggesting that glucans were easily released at the relatively low concentration of alkali. Moreover, it should be noted that H_3 obtained by the treatment with alkali organic solvent contained higher amounts of arabinose (43.2%) and galactose (19.9%), and a lower content of xylose (12.5%) than those of the other alkali-soluble hemicelluloses, indicating that the alkali organic solvent under the given conditions presumably promoted the release of arabinogalactan and highly substituted glucuronoarabinoxylan. The four alkali-soluble hemicelluloses yielded a high content of xylose (42.1 to 87.2%) and relatively low amounts of arabinose (0.7 to 12.0%), indicating that alkali-soluble hemicelluloses consisted of a low-substituted population. In general, the arabinose to xylose ratio (Ara/Xyl) is indicative of the degree of linearity or branching of hemicelluloses. Evidently, with the increment of NaOH concentration from 1% to 8%, the Ara/Xyl ratio decreased from 0.29 (H_1) to 0.01(H_6), which were lower than that of arabinoxylan from sorghum endosperm cell walls (Ara/Xyl = 0.94) (Verbruggen *et al.* 1993). This result implied that the hemicelluloses obtained by the higher concentration of alkali appeared to be more linear. This observation was due to the fact that the chains of the unsubstituted hemicelluloses were prone to forming strong hydrogen bonds, causing inter-chain aggregation, and increased difficulty in the isolation process, and thus a higher concentration of alkali was needed (Hoije *et al.* 2005). The residue left after the sequential extraction contained mainly glucose (81.2%), and arabinose (17.6%) as shown by sugar composition analysis in Table 2. This result verified that the hemicelluloses are strongly bound to the cell wall component, cellulose.

Table 2. The Content of Neutral Sugars (Relative % Dry Hemicelluloses, w/w) Uronic Acids (% Dry Hemicelluloses, w/w) in the Isolated Hemicellulosic Fractions

Sugars (%)	Hemicellulosic Fraction						
	H_1	H_2	H_3	H_4	H_5	H_6	Res*
Rhamnose	2.15	1.81	8.88	1.25	0.05	Tr[c]	ND
Arabinose	16.94	12.01	43.16	10.38	1.30	0.65	0.94
Galactose	19.79	9.03	19.91	5.20	1.73	1.51	0.15
Glucose	50.07	35.09	15.56	19.06	8.42	9.11	81.15
Mannose	2.67	Tr[*]	ND[**]	ND	ND	1.54	0.16
Xylose	8.39	42.06	12.48	64.11	88.51	87.18	17.60
Galacturonic acid	2.18	Tr	ND	ND	ND	ND	Tr
Glucuronic acid	ND	0.15	ND	2.40	2.75	0.85	Tr
Arabinose/Xylose	2.02	0.29	3.45	0.16	0.02	0.01	0.05

* Res, Residue; ** Tr, trace; *** ND, not detectable.

Content of Associated Lignin

It is well known that lignin is tightly cross-linked to different polysaccharides in the cell walls by various linkage types, such as the ether linkage of the hydroxyl group at the α-position of lignin side chain with alcoholic hydroxyl of sugar residue and the ester linkage of carboxylic group of the cinnamic acid residue in lignin with alcoholic OH of polysaccharides, which may be important for the recalcitrance of lignocellulosic biomass (Freudenberg and Harkin 1960). To verify the presence of lignin as a contaminant in the

hemicellulosic fractions and the residue, the six runs were oxidized by alkaline nitrobenzene at 170 °C for 3 h. The alkaline nitrobenzene oxidation provided an estimation of the amount of bounded lignin and an indication of its composition. Results concerning the content of the oxidation products are shown in Table 3. As shown, the yields of the total phenolic monomers released from the associated lignin during the alkaline nitrobenzene ranged between 0.24% and 2.12%. Interestingly, in comparison with the lignin content in the water-soluble hemicelluloses (2.12%), the alkali and alkali-organic soluble hemicellulosic runs had a much lower content of associated lignin (0.24 to 1.26%). This result implied that the alkali and alkali organic solvent treatments significantly broke the α-ether bonds between lignin and hemicelluloses from the sweet sorghum leaves. In addition, an increase in the concentration of NaOH from 1% to 8% led to a decrease in lignin content from 1.26% to 0.24%, which further indicated that the relatively higher concentration of alkali had a positive effect on delignification of the bonded lignin in the hemicelluloses during the extraction process. On the other hand, the measurable amount of residual lignin (1.74%) in the six-stage process of treated residue also implied that the polysaccharides in the cell walls of the sweet sorghum leaves were tightly associated with lignin.

Table 3. Yield (% Dry Sample, w/w) of Phenolic Acids and Aldehydes Obtained from Alkaline Nitrobenzene Oxidation of the Lignin Bounded in the Hemicellulosic Fractions and the Residue

Phenolic acids and aldehydes	Hemicellulosic Fraction						
	H_1	H_2	H_3	H_4	H_5	H_6	Res*
p-Hydroxybenzoic acid	0.36	0.55	0.23	0.31	0.14	0.38	0.69
p-Hydroxybenzaldehyde	0.11	0.15	0.20	0.025	0.010	0.007	0.052
Vanillic acid	0.044	0.012	0.038	0.004	0.004	ND**	0.018
Syringic acid	0.054	0.006	0.063	0.006	0.001	0.010	0.011
Vanillin	0.40	0.15	0.19	0.053	0.021	0.034	0.41
Syringaldehyde	1.02	0.32	0.59	0.17	0.060	0.030	0.50
p-Coumaric acid	0.052	0.013	0.062	0.007	ND	0.003	0.003
Acetovanillone	0.006	0.003	0.004	0.001	ND	0.001	0.031
Acetosyringone	0.098	0.025	0.023	0.013	0.005	0.002	0.021
Ferulic acid	0.044	0.015	0.045	ND	ND	ND	ND
Total	2.12	1.26	1.45	0.60	0.24	0.47	1.74
Molar ratio (G:S:H)***	0.83: 1.82:1	0.21: 0.37:1	0.45: 1.11:1	0.15: 0.42:1	0.15: 0.33:1	0.08: 0.08:1	0.22: 0.53:1

* Res, Residue; ** ND, not detectable; *** G represents the sum of total moles of vanillin, vanillic acid, and acetovanillone; S represents the sum of total moles of syringaldehyde, syringic acid, and acetosyringone; and H represents the sum of total moles of *p*-hydroxybenzoic acid and *p*-hydroxybenzaldehyde.

In the case of alkaline nitrobenzene oxidative degradation, the three constitutive monomeric lignin units *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) produced the corresponding products: *p*-hydroxybenzaldehyde and *p*-hydroxybenzoic acid (derived from H); vanillin, vanillic acid, and acetovanillone (derived from G); syringaldehyde, syringic acid, and acetosyringone (derived from S) (Billa *et al.* 1996). In addition, small amounts of *p*-coumaric and ferulic acids were also found to be present in the nitro-

benzene oxidation mixtures. This is in agreement with the previous results reported on the existence of chemical linkages among xylan, lignin, and hydroxycinnamic acids derivatives (Sharma *et al.* 1986; He and Terashima 1991; Sun and Tomkinson 1999; Kato *et al.* 1987). Hydroxycinnamic acids such as *p*-coumaric and ferulic acids occur widely in the cell wall of monocotyledonous plants (Sharma *et al.* 1986; He and Terashima 1991). It has been reported that *p*-coumaric acid is mostly esterified to lignin or polysaccharides, while ferulic acid appeared almost equally in esterified bonds to arabinose in hemicelluloses and in etherified linkage with lignin (Sun and Tomkinson 1999; Kato *et al.* 1987). As can be seen from Table 3, the predominant products in the six hemicellulosic fractions were found to be different from each other, as shown by the various G/S/H ratios. Interestingly, the water and alkali-organic soluble hemicellulosic fractions (H_1 and H_3) were more linked to noncondensed syringyl lignin than to guaiacyl and *p*-hydroxyphenyl lignin, while the four alkali-soluble hemicelluloses were more linked to noncondensed *p*-hydroxyphenyl lignin. The high content of *p*-hydroxybenzoic acid and *p*-hydroxybenzaldehyde was probably derived from *p*-coumaric acid, since most of *p*-coumaric acid was quantitatively oxidized to *p*-hydroxybenzaldehyde by nitrobenzene under the given conditions given (170 °C, 3 h).

Molecular Weight

Gel permeation chromatography (GPC) is an effective method in estimating the molecular weight of unknown polymers or identical chemical structures to those used to calibrate a column (Himmel *et al.* 1989). Table 4 shows weight-average (M_w) and number-average (M_n) molecular weights and polydispersity (M_w/M_n) of the hemicellulosic fractions isolated from the sweet sorghum leaves by GPC. Obviously, the alkali organic- and alkali-soluble hemicelluloses (38700 to 128000 g/mol) had higher molecular weights than water-extractable hemicelluloses (17300 g/mol), implying that alkali extraction is an important tool for obtaining the high-molecular-weight hemicelluloses. Similar results were observed for wheat endosperm arabinxylans and bagasse hemicelluloses (Gruppen *et al.* 1991; Peng *et al.* 2009). In comparison, the M_w of the alkali organic soluble hemicelluloses (H_3, 38700 g/mol) was lower than those of the four alkali-soluble hemicelluloses. The reason was probably due to the fact that the extraction with the 60% ethanol containing 1% NaOH released the branched arabinogalactan and highly substituted glucuronoarabinoxylan, which had relatively low molecular weight.

As shown in Table 4, an increase in alkali concentration from 1% NaOH to 8% NaOH led to a reduction of M_w from 128000 to 48500 g/mol, suggesting that a degradation of hemicelluloses occurred as a consequence of treatment with alkali solution under the given condition.

Additionally, as the concentration of alkali increased, the polydispersity (M_w/M_n) of the hemicellulosic fractions decreased from 12.4 to 2.5. The water and 1% NaOH-soluble hemicelluloses gave a much broader molecular weight distribution, corresponding to the polydispersity indexes of 12.4 and 8.1, respectively. This observation could be explained based on the fact that the hemicellulosic subfractions contained several kinds of polysaccharides, such as β-glucan, and highly substituted arabinoxylans, which were in good agreement with the results of the sugar analysis (Table 2).

Table 4. Weight-average (M_w) and Number-average (M_n) Molecular Weights and Polydispersity (M_w/M_n) of the Hemicellulosic Fractions Isolated from Sweet Sorghum Leaves

	Hemicellulosic Fraction					
	H_1	H_2	H_3	H_4	H_5	H_6
M_w	17300	128000	38700	72600	57600	48500
M_n	1400	15800	9200	19100	20100	19600
M_w/M_n	12.4	8.1	4.2	3.8	2.9	2.5

FT-IR

Infrared spectroscopy is a very useful tool for obtaining rapid information about the structure of wood constituents and chemical changes that occur in wood due to various treatments. Since the advent of the FT-IR spectrometer, this equipment has been used for wood characterization and especially for estimating the lignin and carbohydrate contents in lignocellulosic materials. Figure 2 shows the FT-IR spectra of hemicellulosic runs H_1, H_2, and H_3, prepared by the extraction with distilled water, 1% NaOH, and 60% ethanol containing 1% NaOH, respectively.

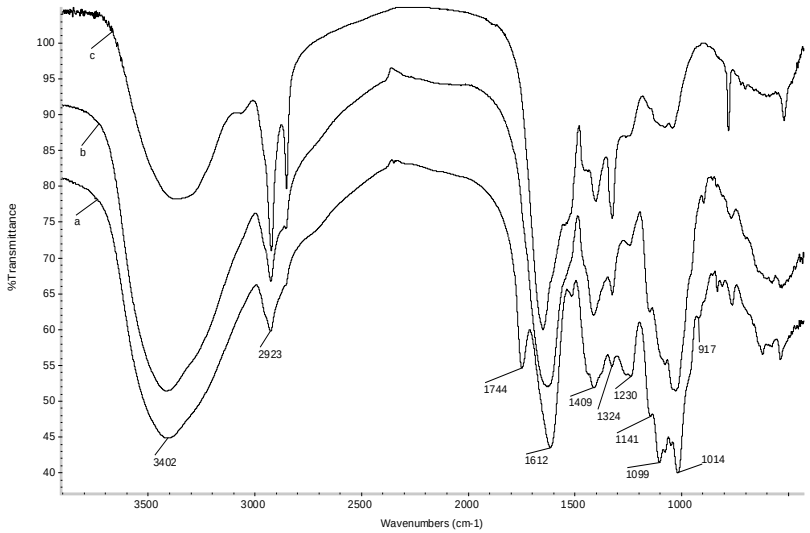

Fig. 2. Spectra of hemicellulosic fractions H_1 (spectrum a), H_2 (spectrum b), and H_3 (spectrum c)

The band at 3403 cm^{-1} is assigned to the stretching of the -OH groups. The C-H stretching vibration gives signals at 2923 and 2850 cm^{-1}. In the carbonyl stretching region, a strong signal at 1612 cm^{-1} is due to the absorbed water, since hemicelluloses usually have a strong affinity for water, and in the solid state these macromolecules may have disordered structures which can easily be hydrated (Chaikumpollert *et al.* 2004). A small peak at 1744 cm^{-1} in the spectrum *a* of water-soluble hemicelluloses (H_1) could be attributed to the acetyl groups of the hemicelluloses or to the ester linkage of carboxylic stretching group of ferulic acid (Komiyama *et al.* 2009), whereas the absence of this signal in spectra *b* (H_2) and *c* (H_3) imply that the alkali and alkali organic solvent under the given conditions completely cleaved the ester bond of hemicelluloses. Therefore, during the isolation process of hemicelluloses, the *O*-acetyl groups located at the main-chain of hemicelluloses could be easily removed by alkali. The adsorption peaks in the 1200 to 800 cm^{-1} region are originated from the C-O stretching about the main

polysaccharides (Kacurakova *et al.* 1994). The peaks at 1409, 1324, and 1230 cm^{-1} represent C-H bending and O-H or C-O bending vibration in hemicelluloses. The occurrence of a peak at 1509 cm^{-1} in the spectrum *a* was attributed to the aromatic skeletal vibrations of the associated lignin in the hemicelluloses (Pandey 1999), which indicated the water-soluble hemicelluloses had relatively high content of associated lignin, corresponding to the data obtained from alkaline nitrobenzene oxidation (see Table 3).

Figure 3 shows the spectra of hemicellulosic fractions H_4 (spectrum *a*), H_5 (spectrum *b*), and H_6 (spectrum *c*). As expected, the spectral profiles and relative intensities of most bands of the three alkali-soluble hemicellulosic polymers were rather similar, corresponding to their similar sugar composition (Table 2). The small band at 890 cm^{-1} is due to the C-1 group frequency or ring frequency, which is characteristic of β-glycosidic linkages between the xylose units, in particular the intensity of the band increase from spectrum *a* to *c*. This result was in accordance with the relative content of xylose, which increased from 64.1% to 87.2% (see Table 3). The strong signal at 1044 cm^{-1} is assigned to the C-O, C-C stretching, or C-OH bending in xylans, indicating a dominant xylan of the fractionated hemicelluloses. The low intensity of the signal at 1160 cm^{-1} indicates the presence of arabinosyl units, which have been reported to be attached to xylopyranosyl constituents (Kacurakova *et al.* 1994).

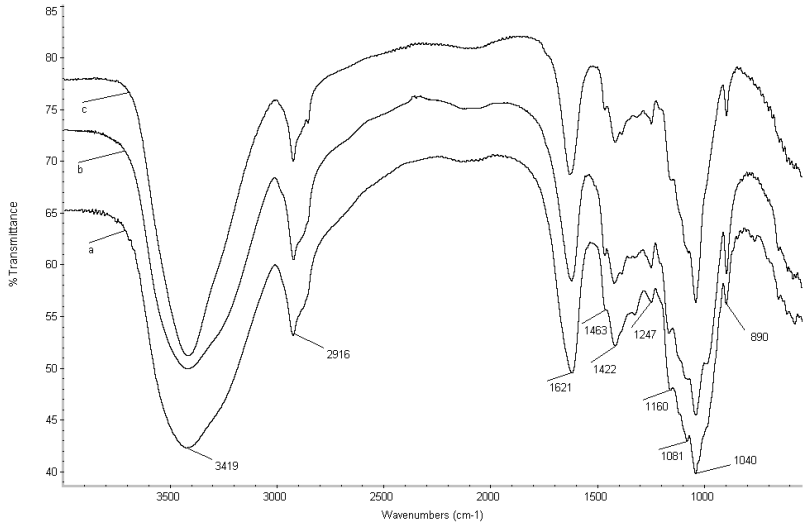

Fig. 3. Spectra of hemicellulosic fractions H_4 (spectrum a), H_5 (spectrum b), and H_6 (spectrum c)

NMR Spectra

In this study, the NMR spectra of the hemicellulosic fraction H_4 obtained by the treatment with 3% NaOH were recorded, and the ^1H and ^{13}C NMR spectra are given in Figs. 4 and 5, respectively. As can be seen from Fig. 4, the ^1H NMR spectrum gave the typical signal pattern expected for a hemicellulosic moiety, the relevant signals occurred in two regions, namely, the anomeric region (5.6 to 4.9 ppm for α-anomers and 4.9 to 4.3 for β-anomers) and the ring proton region at 4.5 to 3.0 ppm (Gonzaga *et al.* 2005). The main two anomeric protons are assigned at 5.2 and 4.3 ppm, which correspond to the arabinofuranosyl and (1→4)-β-D-xylpyranosyl residues. This result confirmed that the xylose is linked by glycosidical linkage, which was supported by the presence of the

small sharp signal at 890 cm^{-1} in the FT-IR spectrum (see Fig. 3). The major signals at 4.3 (H-1), 4.1 (H-5eq), 3.9 (H-4), 3.6 (H-3), 3.3 (H-5ax), and 3.2 (H-2) ppm are attributed to the non-substituted (1→4)-β-D-xylopyranosyl units. The absence of signal at 2.1 ppm indicated that acetyl groups were completely cleaved in the hemicellulosic subfraction (H_4) during the treatment under the given condition, which is consistent with the results of FT-IR spectrum. A strong signal at 4.70 ppm corresponds to the residual solvent (HDO).

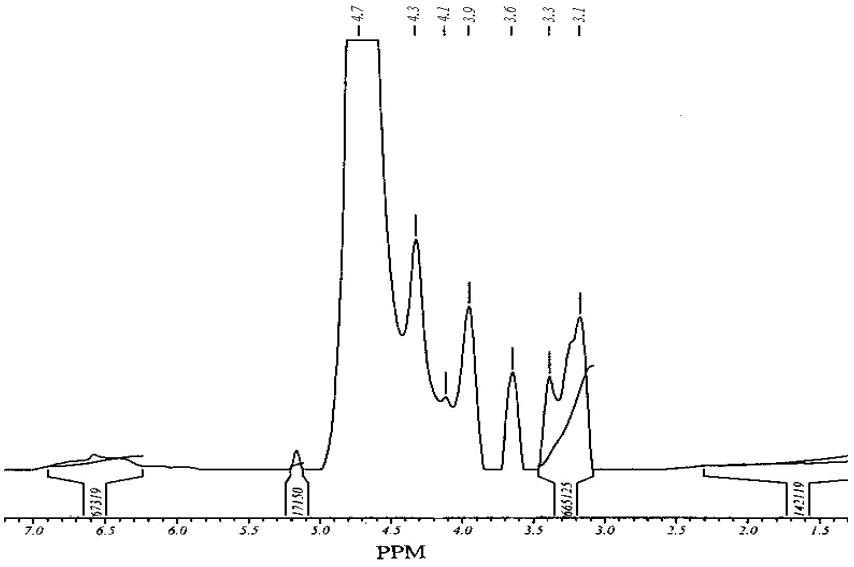

Fig. 4. ^{1}H-NMR spectrum of hemicellulosic fraction H_4

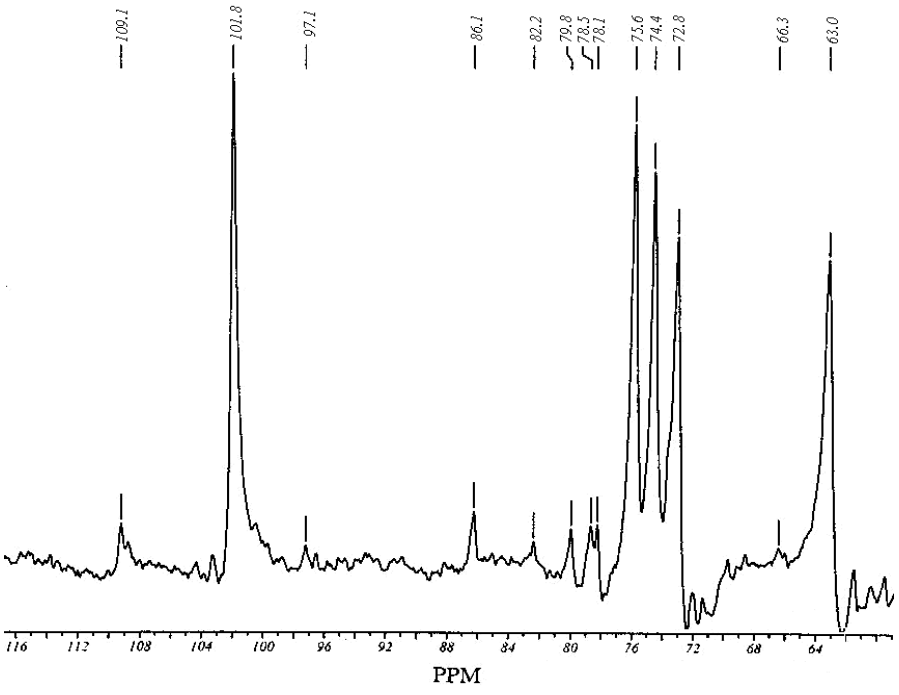

Fig. 5. ^{13}C-NMR spectrum of hemicellulosic fraction H_4

The ^{13}C NMR spectrum contained five major signals corresponding to that of (1→4)-linked-β-xylan. The signal at 101.8 ppm is attributed to the anomeric region in β-configuration, as confirmed by ^{1}H NMR spectrum, while the chemical shifts at 75.6, 74.4, and 72.8, as well as 63.0 ppm correspond to C-4, C-3, C-2, and C-5 of (1→4)-linked-β-xylan, respectively (Habibi and Vignon 2005). The signal at 109.1 ppm is originated from the anomeric carbons of arabinofuranosyl units, and the signals at 86.1, 82.2, 79.8, and 62.2 ppm arise from C-4, C-2, C-3, and C-5 of the arabinofuranosyl residues, respectively. Concerning the glucuronic acid units, the anomeric carbons appeared at 97.1 ppm, and the carboxyl signal of the methoxyl group was found at 60.5 ppm. Two weak signals at 103.5 and 104.1 ppm (data not shown) are assigned to the C-1 of β-(1→3)(1→4)-glucans (Lazaridou *et al.* 2004). The reason for the low intensity of the signals from β-D-glucans was most likely due to its low solubility in D_2O at ambient temperature (20 °C) (Wen *et al.* 2010). Signals of β-D-glucans are overlapped with those of xylan-type hemicelluloses, and their chemical assignment need to be further investigated.

Thermal Analysis

Thermal analysis is a simple, convenient, fast, and effective method for the study of pyrolysis and fire-retardants (Yunchu *et al.* 2000). It has been reported that hemicelluloses are less thermally stable than cellulose and lignin, and that its active degradation takes place more readily at a relatively lower temperature (Demirbas 2000). Figure 6 shows the thermogravimetric analysis (TGA) and derivative thermogravimetry (DTG) curves for the hemicellulosic subfractions H_2 (Curve **a**), H_4 (Curve **b**), and H_5 (Curve **c**) under nitrogen at a heating rate of 10 °C/min. As can be seen, three stages in weight-loss rate were apparent in the TGA curves of the three samples. The TGA-DTG curves showed at 50 to 180 °C, which was due to the evaporation of absorbed water. The initial low temperature mass loss corresponds to loss of humidity, and it is a common feature observed from the wood and wood components (Roman and Winter 2004).

In the second stage, hemicellulosic fractions started their decomposition easily, with a severe weight loss occurring at 204 to 339 °C for H_2, 191 to 338 °C for H_4, and 203 to 345 °C for H_5, respectively. This was caused by the concurrent hemicelluloses degradation processes such as depolymerization, dehydration, and decomposition of glycosyl units, followed by the formation of a charred residue. A release of gas products, such as CO_2, CH_4, CO, and some organics (a mixture of acids, aldehydes, alkanes, and ethers, *etc*.) with some H_2O (Yang *et al.* 2007) was observed during the experiment. In the third stage above 350 °C, the three weight loss TGA curves were found to decay smoothly. The observed weight loss could be attributed to the oxidation and breakdown of the charred residue to lower molecular weight gaseous products (Yang *et al.* 2007). In addition, the maximum weight loss rate reached 0.87 mg min^{-1} at 261 °C for H_2, 1.22 mg min^{-1} at 273 °C for H_4, 0.79 at 252 °C and 0.44 mg min^{-1} at 293 °C for H_5. Two mass loss rate peaks at the second stage could be observed at the DTG curve **c**, which was also observed in those previously reported (d'Almeida *et al.* 2008; Shen *et al.* 2010). Such a phenomenon was due to the different thermal stability of the hemicellulosic fractions, which depended on many factors such as structural inhomogeneity, crystalline and amorphous regions, molecular weight, linear and branched structure of hemicelluloses.

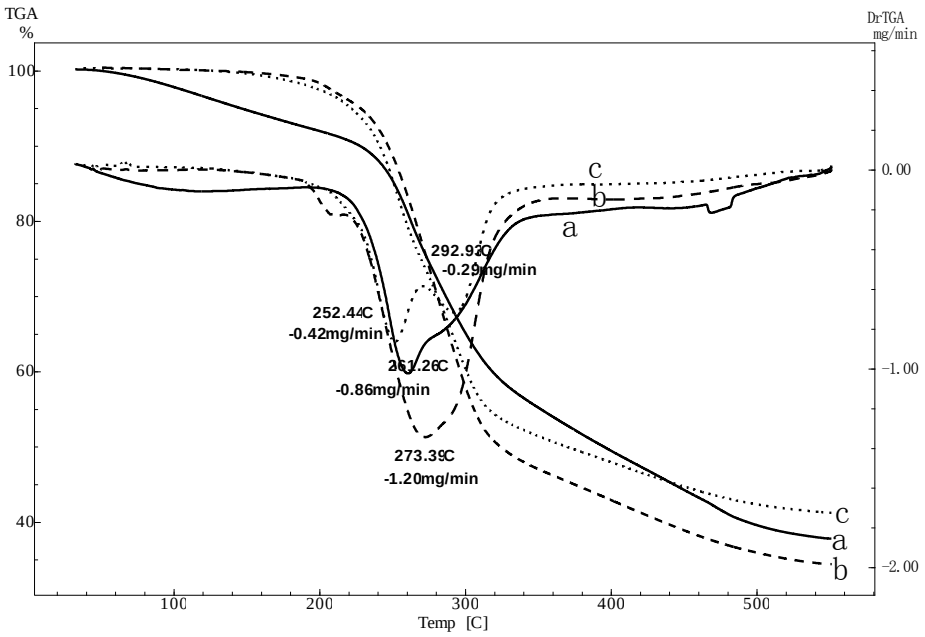

Fig. 6. TGA/DTG of hemicellulosic fractions H_2 (curve a), H_4 (curve b), and H_5 (curve c)

CONCLUSIONS

Under the successive alkali extractions, over 80% of the hemicelluloses in the sweet sorghum cell wall materials were solubilised. The water-soluble hemicelluloses contained noticeable amounts of glucose arabinose, galactose, and xylose, indicating that this run probably contained β-glucan, starch, arabinogalactan, highly substituted arabino-xylans, and pectic polysaccharides. The organic-alkali and alkali-soluble hemicelluloses were mainly composed of xylose, arabinose, glucuronic acid, and glucose, which suggested that a substantial proportion of the hemicelluloses in the cell walls of sweet sorghum consists of glucuronoarabinoxylans and β-(1→3)(1→4)-glucans. In addition, the water and organic-alkali soluble hemicelluloses had lower molecular weights than those of the alkali-soluble hemicelluloses. Moreover, the hemicelluloses isolated with a higher alkali concentration had the features of more linear structure and higher molecular weights. The result of alkaline nitrobenzene oxidation showed that there were strong linkages between polysaccharides and lignin. The treatment with organic alkali and alkali solvents broke the linkages to some extent, leading to a relatively low amount of associated lignin in the hemicellulosic polymers.

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