Successive Fractionations of Hemicelluloses and Lignin from Sorghum Stem by Sodium Hydroxide Aqueous Solutions with Increased Concentrations

Ji-Dong Xu, Ming-Fei Li,* and Run-Cang Sun *

Sorghum stem, an agricultural solid waste discarded in large amounts, was effectively fractionated into its chemical components to achieve value-added utilization. The stem was successively extracted using water at 80 °C and alkali aqueous solutions with increased concentrations (1% NaOH; 60% ethanol containing 1% NaOH, 3% NaOH, 5% NaOH, and 8% NaOH) at 50 °C, which yielded hemicellulose and lignin fractions as well as a cellulose-rich residue. The hemicellulose and lignin fractions were characterized in terms of yield, sugar components, alkaline nitrobenzene, and oxidation analysis. In addition, the molecular weights were determined by gel permeation chromatography and the structures were further identified by Fourier transform infrared spectroscopy and nuclear magnetic resonance spectroscopy. The results indicated that the hemicelluloses yielded from the alkali aqueous solution had a linear xylan structure. The alkali lignin had a typical guaiacyl/syringyl/*p*-hydroxypheny structure and low amounts of contaminating sugars (less than 2%). A high concentration of alkali aqueous solution led to the release of lignin with a large molecular weight, whereas increasing the alkali concentration resulted in lignin degradation. The residual stem after the successive extractions was rich in cellulose and had a low crystallinity. In sum, mild successive extractions are a promising way to fractionate sorghum stem waste for further conversion.

Keywords: Agricultural solid waste; Extraction; Hemicelluloses; Lignin; Sodium hydroxide; Sorghum stem

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INTRODUCTION

Sorghum is a food and energy crop that has a high photosynthetic efficiency and can be planted in drought areas. Among the cereal crops produced globally, sorghum is the fifth most abundant, and its annual production is around 58 million metric tons (Rooney *et al.* 2007). The starches in the grain are good feedstocks for fermentation by amylase. The stem is an agricultural solid waste discarded in large amounts, and it is composed of cellulose, hemicelluloses, and lignin (She *et al.* 2010). This agricultural waste is a good feedstock for conversion into energy, chemicals, and materials. The cellulose in sorghum bagasse is mainly used for the production of paper (Belayachi and Delmas 1997). Recently, bioethanol and other chemicals such as butanol and acetone have been produced using sorghum bagasse as the feedstock (Jafari *et al.* 2016; Yu *et al.* 2016; Banerji *et al.* 2017).

In contrast to the extensive research on the production of energy from sorghum bagasse, relatively less attention has been paid to the isolation of hemicelluloses and lignin from the feedstock. Hemicelluloses are macromolecules composed of approximately 70-200 structural monomers (mainly D-mannose, D-xylose, D-glucose, L-arabinose, D-

galactose, and 4-*O*-methyl-α-D-glucuronic acid, depending on the species) (Scheller and Ulvskov 2010).

Hemicelluloses have wide applications in chemicals, foods, and pharmaceuticals (Peng and She 2014). Lignin is a natural aromatic polymer consisting of phenylpropanoid units linked by aryl–ether, alkyl–ether, and carbon–carbon covalent bonds into a cross-linked polymer network, in which guaiacyl- (G), syringyl- (S), and p-hydroxyphenyl (H) are the main monomers primarily produced from the dehydrogenative radical polymerization (Dashtban *et al.* 2009). The composition of lignin varies between species and vascular plants. Lignin is a waste stream in most pulp mills and biorefinery processes, and it is usually burned to generate heat. However, it is a good feedstock for the production of alternative fuels, renewable chemicals, value added materials, and platform compounds (Upton and Kasko 2016; Wang *et al.* 2016; Chen and Wan 2017).

In the plant cell wall, hemicelluloses are covalently linked with lignin and form hydrogen bonds with cellulose, which is a major barrier to valorization. Many approaches, including physical, chemical, physico-chemical, and biological methods, have been developed to treat lignocelluloses to separate their chemical components, in which bioethanol is the major target product (Mutschlechner *et al.* 2015; Narron *et al.* 2016; Rabemanolontsoa and Saka 2016; Chng *et al.* 2017). Of these methods, chemical pretreatment is a promising process to achieve industrialization due to its efficiency. Organic solvent pretreatment separates cellulose with a high-purity and minor degradation, as well as its ability to produce lignin with superior properties and convertible hemicelluloses degraded products such as furfural and xylitol. However, the organic solvents used are easy to burn, and the recovery cost of the organic solvents is relatively high (Zhang *et al.* 2016). The hydrothermal pretreatment is usually operated at a relatively high temperature in a complicated equipment, producing degraded hemicelluloses, as well as a series of inhibitor products such as furfural, phenolic compounds (Gabhane *et al.* 2014; Rabemanolontsoa and Saka 2016; Shen *et al.* 2016). The alkali method has outstanding delignification capacity and hemicellulose removal because it causes lignocellulose to swell. Sodium, potassium, calcium, and ammonium hydroxides are commonly used for pretreatment; sodium hydroxide is preferred due to its safety, cost, and reusability. NaOH pretreatment at various temperatures (15 °C, 25 °C, 60 °C, and 121 °C) has been examined by many researchers (Zhao *et al.* 2008; Park and Kim 2012; Bali *et al.* 2015; Gabhane *et al.* 2015), but the major focus of these studies is the production of digestible cellulose for bioethanol. The extraction of hemicelluloses and lignin using NaOH and an appropriate method to achieve effective utilization is worth exploring. Because the swelling capacity of NaOH increases with increased concentration, successive extractions using NaOH aqueous solutions with increased concentrations would yield dissolved fractions having distinct properties; thus it will provide useful feedstock for application.

In the present study, sorghum stem was subjected to successive extractions using water and alkali aqueous solutions with increased concentrations at low temperatures, yielding hemicellulose and lignin fractions and a cellulose-rich residue after a further purification step. The hemicellulose and lignin fractions were characterized in terms of yield, sugar components, alkaline nitrobenzene oxidation analysis, and molecular weights. The structures were further identified by Fourier transform infrared (FTIR) spectroscopy and nuclear magnetic resonance (NMR) spectroscopy. The residual stem was also analyzed chemically and structurally. This study provides comprehensive knowledge on the chemical composition and structure of sorghum stem after a mild separation process, which is meaningful for the valorization of this agricultural waste.

EXPERIMENTAL

Material

The sweet sorghum stems were collected from a firm in Yangling, Shaanxi province, China. They were ground and dried at 60 °C for 16 h. The samples sized at 20- to 60-mesh were extracted with toluene-ethanol (2:1, v/v) for 6 h in a Soxhlet apparatus to remove wax. The de-waxed samples were the raw material for the experiment. The samples contained 42.3% cellulose, 21.8% hemicelluloses, and 18.0% lignin (Sun *et al.* 2013a), determined according to the procedure proposed by the National Renewable Energy Laboratory.

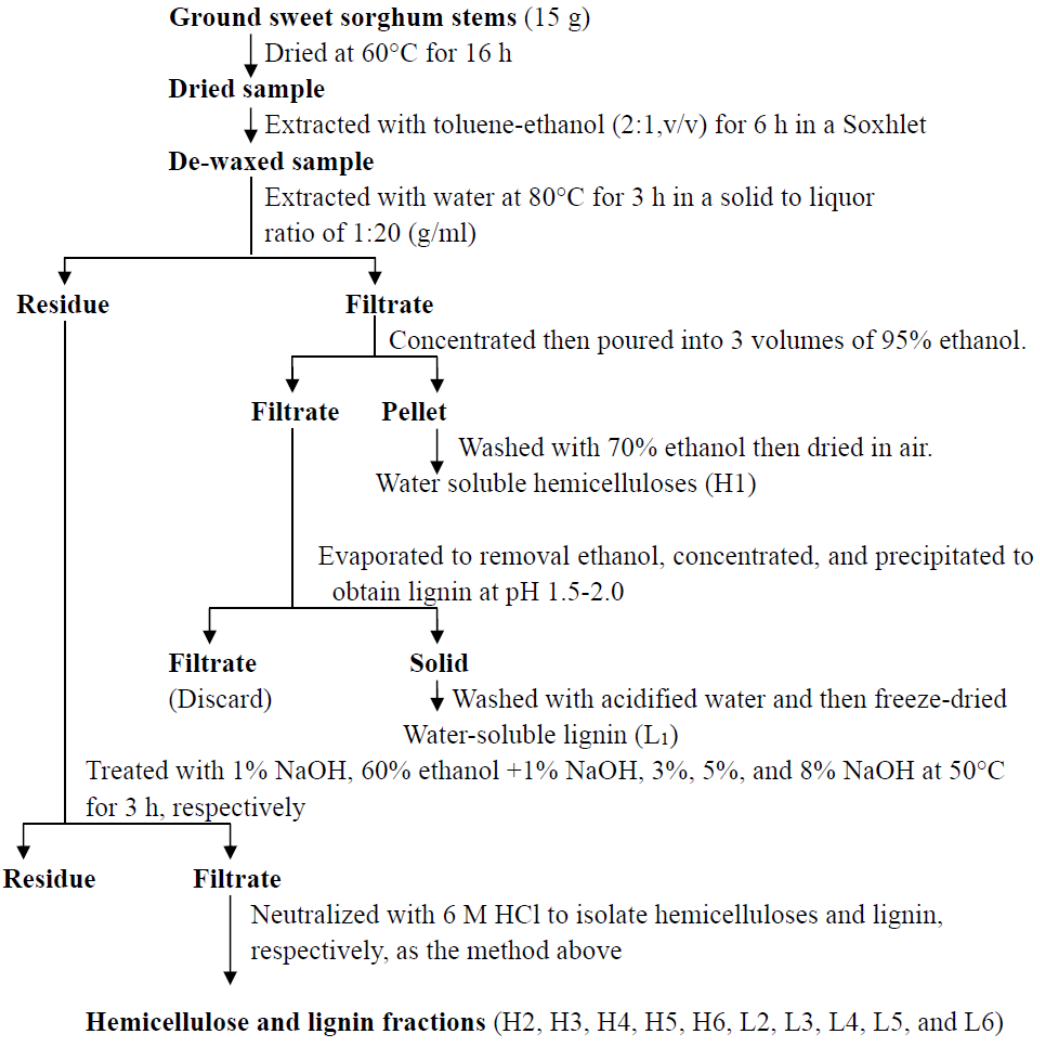


Fig. 1. Scheme for the fractionation of hemicelluloses and lignin from sweet sorghum stem

The Successive Extraction Process

The procedure for the successive extractions of lignin and hemicelluloses from sweet sorghum stems is illustrated in Fig. 1. The de-waxed sorghum stem was extracted with water at 80 °C for 3 h in a solid-to-liquor ratio of 1:20 (g/mL) and then filtered in a Buchner funnel. The filtrate was concentrated and poured into 3 volumes of 95% ethanol. The pellet was washed with 70% ethanol and then air-dried to obtain water soluble

hemicelluloses (H1). The filtrate was evaporated, concentrated, and then precipitated with acid water (pH 1.5 to 2.0, adjusted with HCl). Next, the precipitate was washed with acid water and freeze-dried to obtain water soluble lignin (sample L1). The residue obtained from the water extraction was successively treated with 1% NaOH, 60% ethanol containing 1% NaOH, 3% NaOH, 5% NaOH, and 8% NaOH at 50 °C for 3 h in each step. The filtrate in each step was poured into 3 volumes of 95% ethanol for the extraction of hemicelluloses (H2, H3, H4, H5, and H6), and the supernatants were neutralized with 6 M HCl to isolate the lignin (L2, L3, L4, L5, and L6) according to the method above. The experiments were conducted in triplicate, and the average values are given.

Characterization of Hemicelluloses, Lignin, and Residual Stem

The chemical component (cellulose, hemicelluloses, and lignin) analysis of the stem before and after the treatments was conducted according to the National Renewable Energy Laboratory (NREL) standard procedure (Sluiter *et al.* 2008). Hydrolysis of the stem was achieved using 72% H_2SO_4 for 60 min at 30 °C followed by a further hydrolysis at 121 °C for 1 h after dilution to 4% H_2SO_4. The neutral sugar compositions of the hemicellulose and lignin fractions were analyzed by hydrolysis of the specimen with 6% H_2SO_4 for 2.5 h at 100 °C. The sugars released were characterized by a high-performance anion exchange chromatography (HPAEC) system (Dionex ISC 3000, Sunnyvale, CA, USA) with an ampere detector, an AS50 auto-sample, a Carbopac PA-20 column (4 × 250 mm), and a PA-20 guard column (3 × 30 mm), according to a previous report (Bian *et al.* 2010). Alkaline nitrobenzene oxidation and the liberated phenolic acids and aldehydes were analyzed by high-performance liquid chromatography (HPLC) following a previously reported procedure (Xu *et al.* 2006). The molecular-average weights of the hemicellulose fractions were determined by gel permeation chromatograph (GPC) with a differential refractive index detector using a PL aquagel-OH 50 column (300 mm×7.7 mm, Polymer Laboratories Ltd., Shropshire, UK). The data were calibrated with standard PL pullulan polysaccharides with peak average molecular weights of 783, 12200, 100000, and 1600000 (Polymer Laboratories Ltd.). The eluate was 0.02 M NaCl in 0.005 M sodium phosphate buffer (pH 7.5) with a flow rate of 0.5 mL/min. The molecular weights of the lignin samples were determined by the same system on a PL-gel 10 μm mixed-B 7.5 mm i.d. column, calibrated with polystyrene standards with molecular weights of 1320, 9200, 66000, and 435500, (Polymer Laboratories Ltd.). The column was eluted with tetrahydrofuran at a flow rate of 1.0 mL/min.

FTIR spectra of the samples were collected on a Bruker spectrophotometer (Ettlingen, German) in the range of 4000 to 400 cm $^{-1}$ with a resolution of 4 cm^{-1} after preparing a KBr disk containing 1% ground samples. The solution-state ^{1}H and ^{13}C NMR spectra of the hemicelluloses and lignin were recorded on a Bruker AVIII 400 MHz spectrometer at 25 °C after dissolving the samples in DMSO-d6 using a previously described procedure (Xu *et al.* 2008; Wen *et al.* 2013). A cross polarization/magic angle spinning (CP/MAS) ^{13}C NMR spectrum of the residual stem was acquired on the same NMR spectrometer with a 4 mm magic angle spinning probe. Thermogravimetric (TGA) and differential thermal analysis (DTA) curves of the hemicelluloses were recorded by a thermogravimetric analyzer (SDT Q600, TA Instruments, Leatherhead, UK). A total of 10 mg of the sample was placed in a crucible, and nitrogen was flowed at 100 mL/min during the determination. The sample was heated from 30 °C to 550 °C at a heating rate of 10 °C/min. X-ray diffractograms were recorded by an X-ray diffract meter (XRD-6000, Shimadzu, Kyoto, Japan) with a scanning speed of 5°/min after placing the residual stem

in an aluminum holder. All experiments were conducted in triplicate, and the average values are reported.

RESULTS AND DISCUSSION

Extraction Yield of Hemicelluloses and Lignin

The successive extractions with increased alkali concentrations are a promising process to fractionate lignocellulose as well as to obtain hemicellulose specimens with different characteristics and lignin samples with distinct properties. The yields (based on the initial dry sweet sorghum stem) of the hemicellulose and lignin fractions obtained in the present study are given in Tables 1 and 2. The successive extractions of the de-waxed sweet sorghum stem with water at 80 °C for 3 h with 1% NaOH, 60% ethanol containing 1% NaOH, 3% NaOH, 5% NaOH, and 8% NaOH at 50 °C for 3 h yielded 2.0%, 2.3%, 1.3%, 4.0%, 3.9%, and 1.2% hemicelluloses, respectively, together with 0.4%, 1.8%, 2.1%, 0.9%, 0.8%, and 0.8% lignin. The highest yield of hemicelluloses was obtained at the extraction with 3% NaOH solution. Altogether, the yields of the released hemicelluloses and lignin were 14.7% and 6.8%, corresponding to 67.4% and 37.7% of the original hemicelluloses and lignin in the sorghum stem, respectively. Overall, the successive process removed a large proportion of hemicelluloses from the lignocellulose. However, the extraction with water released only small amounts of lignin. The application of alkali caused the lignocellulose to swell and cleaved ester bonds between hydroxycinnamic acids and lignin/hemicelluloses, as well as the α-benzyl ether linkages between lignin and hemicelluloses (Sun *et al.* 2000). The addition of ethanol promoted the extraction of lignin from the lignocelluloses due to the good solubility of the alkaline ethanol aqueous solution.

Structural Characterization of Hemicelluloses

Characterization of the structure of the extracted samples was conductive to the further applications in industry. The hemicellulose samples were subjected to analyses of sugars, alkaline nitrobenzene oxidation products, and molecular weights (Table 1). For H1, glucose was the major sugar generated from starch, which is in agreement with a previous report (Peng *et al.* 2011). This was attributed to the dissolution of the non-crystalline and hydrophilic starch in hot water. H2 and H3 also exhibited a high glucose content. In H4, H5, and H6, the predominance of xylose together with glucose suggested that these fractions consisted mainly of xylan. In addition, the mass ratio of arabinose to xylose, which is an indicative of the degree of branching, showed a decreasing trend, suggesting that a high concentration of alkali resulted in the release of hemicelluloses with a more liner structure, supporting the previous extractions of hemicelluloses with increased concentrations of KOH (Sun *et al.* 2013b). Lignin is attached to hemicelluloses in the plant cell wall through various linkages. To verify the associated lignin in the hemicellulose fractions, these samples were analyzed using the alkaline nitrobenzene oxidation method to identify the degraded products. The total yields of the products were not higher than 2% and generally decreased from H1 to H6, indicating that the linkages between lignin and hemicelluloses were effectively cleaved. The weight average molecular weight (M_w), number average molecular weight (M_n), and polydispersity index (M_w/M_n) of the samples were calculated. The data were comparable to results reported on hemicelluloses extracted with KOH solution (M_w 22750 to 65290 g/mol) (Sun *et al*. 2013b).

Table 1. Yield, Sugar Analysis, Alkaline Nitrobenzene Oxidation Products, and Molecular Weight of the Hemicellulose Fractions and the Residual Sorghum Stem [a]

Sample	H1	H2	H3	H4	H5	H6	Residue
Yield (%)	2.0	2.3	1.3	4.0	3.9	1.2	44.5
Sugars (%)							
Rhamnose	1.55	2.52	2.68	0.00	0.00	0.00	0.00
Arabinose	11.70	20.23	27.49	1.19	0.41	0.37	0.80
Galactose	0.00	12.11	14.65	0.90	0.69	0.91	0.20
Glucose	78.33	25.30	34.67	16.37	8.08	5.76	81.97
Mannose	1.52	0.00	0.00	0.00	0.00	0.00	0.00
Xylose	6.91	39.85	20.51	81.53	90.82	92.70	17.02
Disaccharides	0.10	0.00	0.00	0.00	0.21	0.00	0.00
Galacturonic acid	0.47	0.10	0.00	0.00	0.00	0.00	0.00
GA[b] and/or 4-*O*-methyl-D-GA	0.00	0.00	0.00	0.36	0.00	0.22	0.00
Phenolic acids and aldehydes (%)							
p-Hydroxybenzoic acid	0.56	0.54	0.36	0.44	0.46	0.49	0.77
p-Hydroxybenzaldehyde	0.08	0.04	0.02	0.25	0.02	0.03	0.10
Vanillic acid	0.05	0.03	0.01	0.01	0.01	0.01	0.13
Syringic acid	0.04	0.06	0.01	0.00	0.01	0.02	0.06
Vanillin	0.36	0.29	0.13	0.08	0.11	0.05	0.60
Syringaldehyde	0.83	0.54	0.27	0.10	0.18	0.10	0.88
p-Coumaric acid	0.01	0.02	0.02	0.02	0.02	0.02	0.08
Acetovanillone	0.01	0.00	0.00	0.00	0.00	0.00	0.04
Acetosyringone	0.05	0.03	0.02	0.01	0.01	0.01	0.07
Ferulic acid	0.01	0.03	0.00	0.01	0.00	0.01	0.01
Total	2.00	1.58	0.84	0.92	0.82	0.74	2.74
Molecular weight							
M_w (g/mol)	123220	216740	57530	59340	31680	58480	
M_n (g/mol)	20560	23310	16340	17440	17670	15160	
M_w/M_n	6.0	9.3	3.5	3.4	1.8	3.9	

[a] The standard errors for yield, sugars, phenolic acids and aldehyde, M_w and M_n were less than 2.2%, 3.5%, 8.2%, 4.5% and 5.6%, respectively.
[b] GA, glucuronic acid.

The FTIR spectra of the hemicellulose fractions are presented in Fig. 2. The band at 3414 cm^{-1} is related to an OH group, and the peak at 2920 cm^{-1} represents CH_2 and CH_3. H1 revealed a more distinct spectrum than the other fractions. The signal at 1746 cm^{-1} is due to acetyl and uronic ester groups, and the small signal at 915 cm^{-1} is due to the α-glycosidic linkages between the sugar units (Huang and Zhang 2009; Li *et al.* 2011). This result suggested that this fraction contained starch, which is expected due to the higher amount of glucose. A very small signal at 1510 cm^{-1} indicated the bound lignin in the fraction. For the other fractions, the typical signal for hemicelluloses was observed at 1036 cm^{-1}, corresponding to the glycoside bond stretching (C-O-C) from xylan. The absorption at 895 cm^{-1} is ascribed to β-glycoside linkages between the sugar units. The signal at 1621 cm^{-1} is due to the absorbed water in the hemicellulose fractions. The bands due to C–H vibrations of polysaccharides were observed at 1467 and 1380 cm^{-1}.

Table 2. Yield, Sugar Analysis, Alkaline Nitrobenzene Oxidation Products, and Molecular Weight of the Lignin Fractions [a]

Sample	L1	L2	L3	L4	L5	L6
Yield (%)	0.4	1.8	2.1	0.9	0.8	0.8
Sugars (%)						
Rhamnose	0.14	0.00	0.36	0.00	0.00	0.00
Arabinose	0.76	0.08	0.11	0.12	0.10	0.13
Galactose	0.54	0.27	0.13	0.61	0.73	0.10
Glucose	9.04	0.01	0.00	0.00	0.00	0.00
Xylose	0.22	0.24	0.23	0.26	0.20	1.65
Disaccharides	0.10	0.34	0.00	0.00	0.00	0.00
Galacturonic acid	0.00	0.78	0.00	0.00	0.00	0.00
Total	10.8	1.72	0.83	0.99	1.03	1.88
Phenolic acids and aldehydes (%)[b]						
p-Hydroxybenzoic acid	5.01	4.12	18.50	55.62	74.72	74.43
p-Hydroxybenzaldehyde	10.32	3.14	6.73	9.05	5.71	3.30
Vanillic acid	2.80	1.72	3.13	1.44	0.00	1.31
Syringic acid	0.24	4.01	10.11	0.72	0.00	1.42
Vanillin	27.61	22.14	16.93	11.61	7.61	10.33
Syringaldehyde	47.13	54.82	40.34	20.54	11.32	8.91
p-Coumaric acid	3.12	3.73	0.22	0.00	0.00	0.00
Acetovanillone	0.54	0.45	0.71	0.57	0.44	0.21
Acetosyringone	2.83	4.73	3.14	0.67	0.24	0.20
Ferulic acid	0.38	1.40	0.55	0.00	0.00	0.00
Molar ratio (S:G:H)	3:2:1	9:2:1	3:1:1	2:1:5	1:1:10	1:1:7
Molecular weight						
M_w (g/mol)	1270	1880	1870	2440	1830	1650
M_n (g/mol)	1050	1170	1050	1510	1050	990
M_w/M_n	1.2	1.6	1.8	1.6	1.7	1.7

[a] The standard errors for yield, sugars, phenolic acids and aldehyde, M_w and M_n were less than 2.4%, 3.4%, 8.3%, 4.7%, and 5.2%, respectively.
[b] based on the total oxidated products.

To investigate the structure of the hemicelluloses in depth, the hemicellulose fraction H6 was further studied with ^{1}H and ^{13} C NMR after being dissolved in DMSO-d6 (Fig. 3). The signal assignment was conducted according to published data on xylan (Teleman *et al.* 2000). In the ^{1}H NMR spectrum, the signal for β-anomeric protons was observed at 4.4 to 4.6 ppm. The signals at 4.30 (C1H), 3.21 (C2H), 3.57 (C3H), and 3.78 (C4H) ppm were assigned to (1→4) linked β-D-xylan, whereas the signals at 3.35 and 4.10 ppm were due to axial C5-H and equatorial C5-H, respectively. In the ^{13}C NMR spectrum, the strong signals for 1,4-linked β-D-xylan units were observed at 102.2 (C1), 73.2 (C2), 74.4 (C3), 76.6 (C4), and 63.4 ppm (C5).

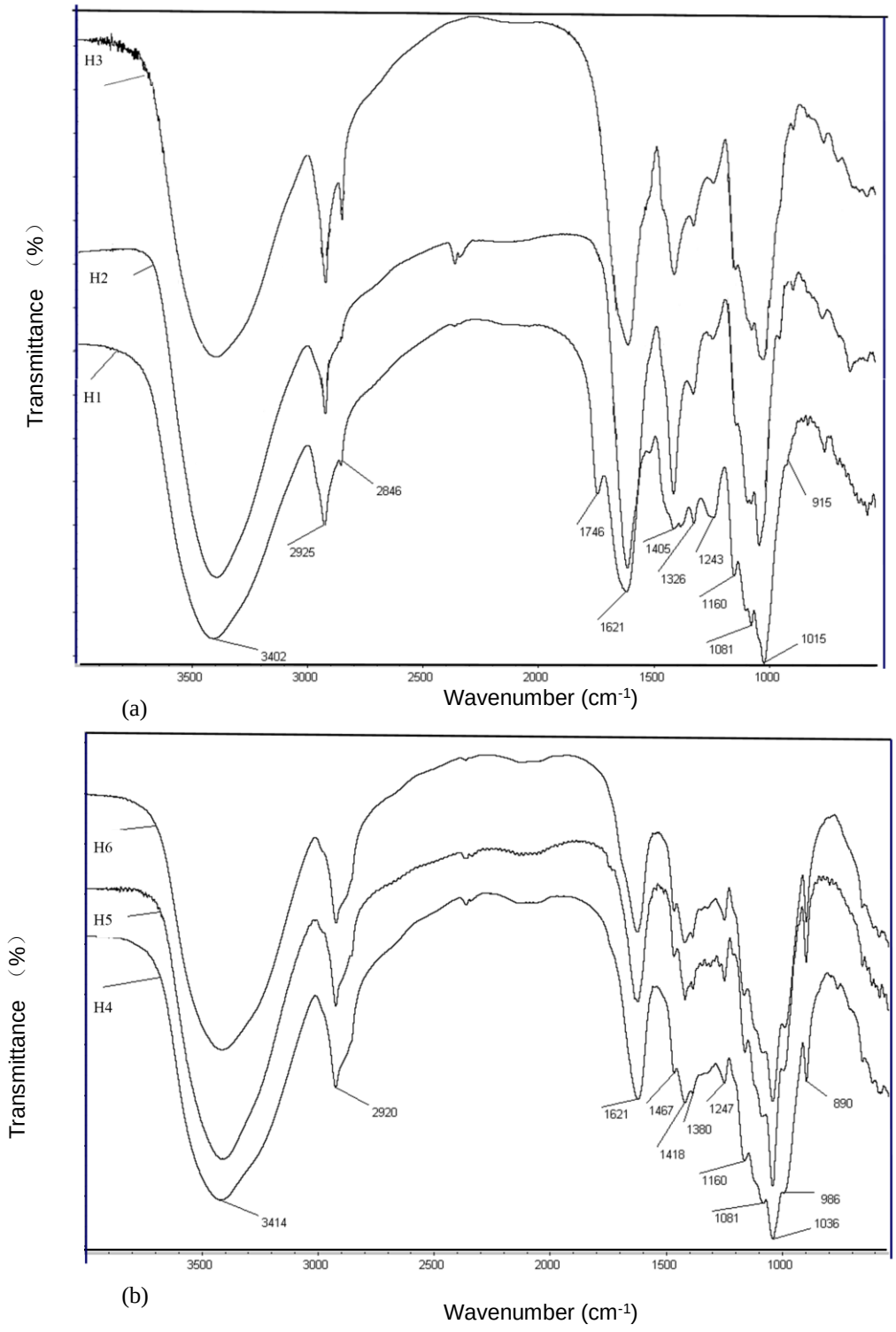
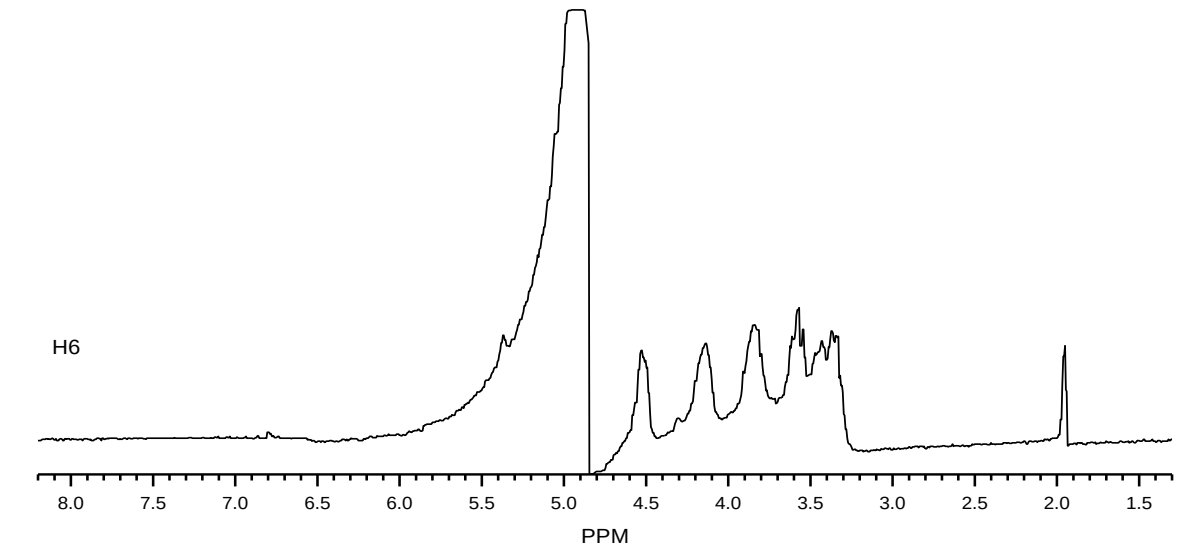
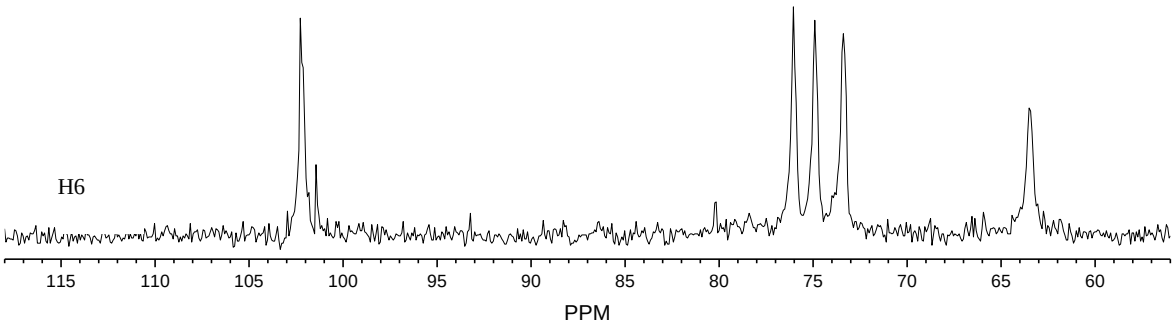


Fig. 2. FTIR spectra of extracted hemicellulose fractions (a: H1, H2, and H3; b:H4, H5 and H6)



(a)



(b)

Fig. 3. ^{1}H (a) and ^{13}CNMR (b) spectra of the hemicellulose fraction H6

The thermal behaviors of the extracted hemicellulose samples H2, H4, and H6 were compared using thermogravimetric analysis; the TGA and DTA curves are presented in Fig. 4. The degradation of the hemicelluloses was divided into three stages, *i.e.*, slow (less than 220 °C), rapid (220 to 340 °C), and residual (higher than 340 °C). In the slow stage, the total weight loss was less than 10%, which was mainly due to the loss of absorbed water (at around 100 °C) and pyrolysis of some hemicelluloses with low molecular weights. In the rapid stage, the maximum degradation rates of weight loss were observed at around 270 °C. After this stage, the degradation slowed, and the residual weights of the sample were 42.1%, 34.8%, and 34.9% for H2, H4, and H6, respectively. The residues produced during this stage were mainly due to the pyrolysis of hemicelluloses and the formation of carbonaceous materials under a nitrogen atmosphere (Kirubakaran *et al*. 2009). In the DTA curves, the thermal degradation of the samples was an exothermal process. In the second stage, all samples exhibited large positive peaks due to rapid degradation. Unlike H2 and H6, H4 had a negative peak at around 220 °C, probably due to its distinct structure.

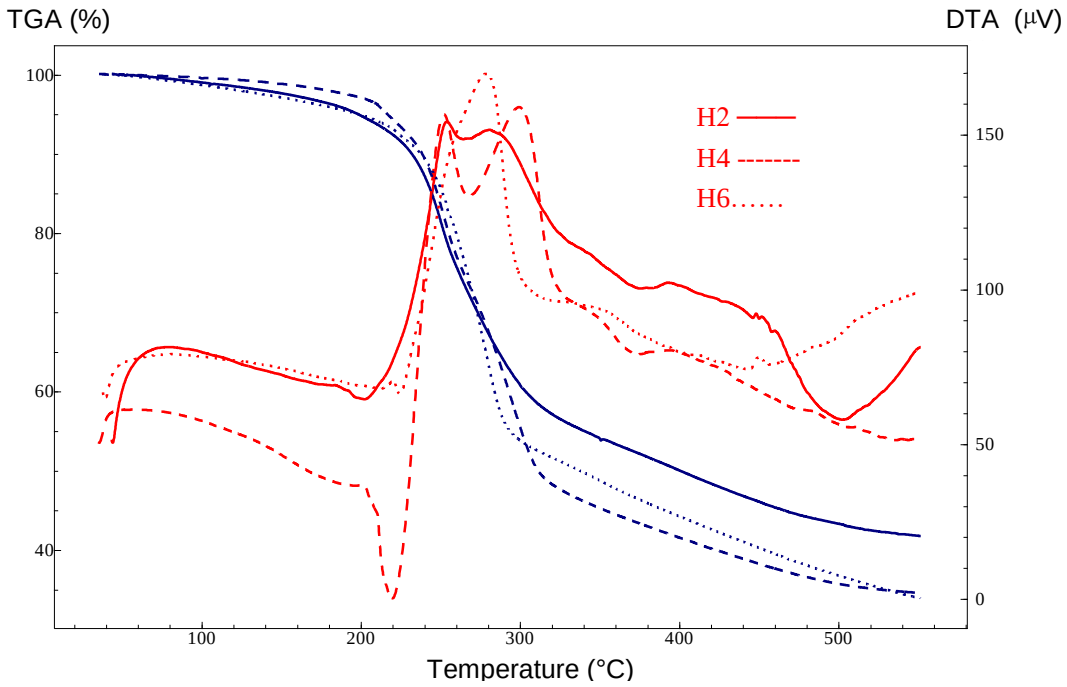
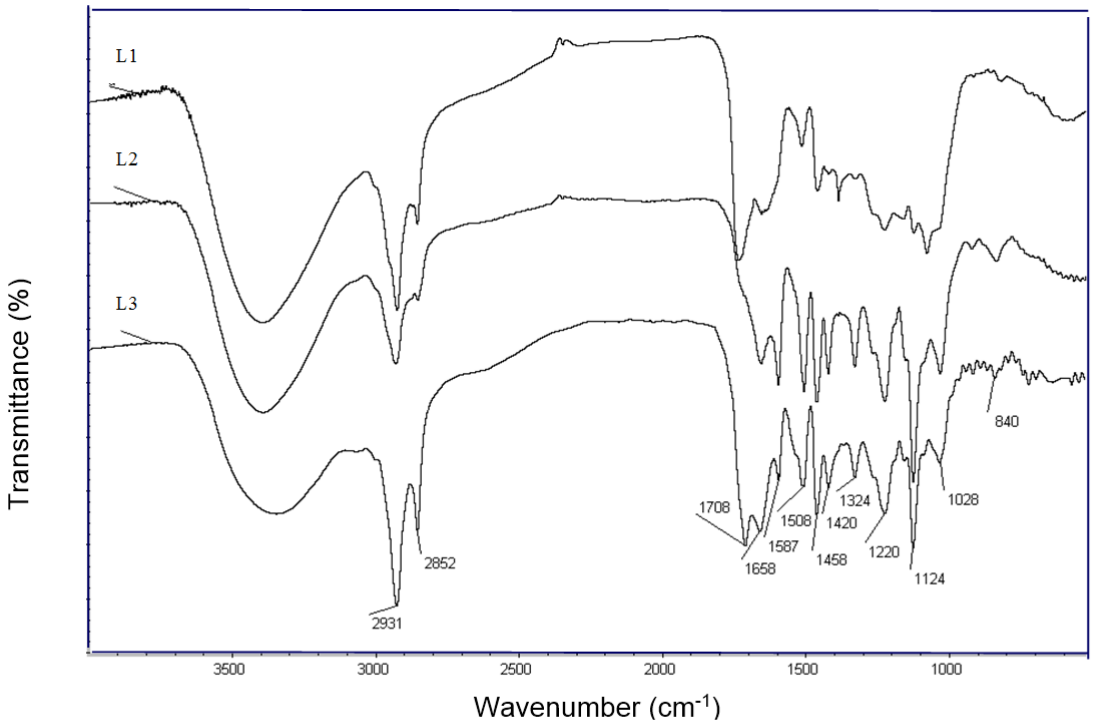


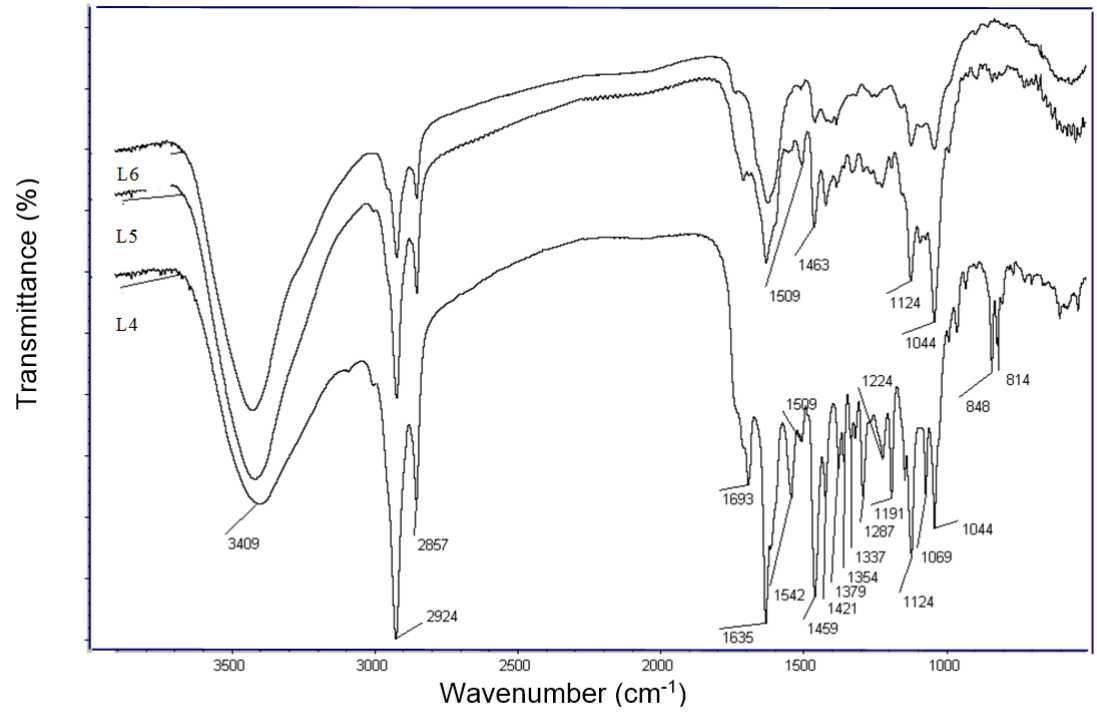
Fig. 4. TGA/DTA curves of hemicellulose fractions

Structure Characterization of the Lignin Fractions

Because there is a lignin-carbohydrate complex in lignocellulose, the isolated lignin usually contains sugars. Therefore, the lignin fractions were submitted to sugar analysis, and the results are shown in Table 2. The bound sugars included glucose (9.04%) followed by arabinose (0.76%) in L1 and galacturonic acid (0.78%) and disaccharides (0.34%) in L2. The fractions L3, L4, L5, and L6 contained minor amounts of xylose and other monosaccharides. The data indicated that the lignin had heterogeneous linkages bound to hemicelluloses. Overall, the content of the contaminated sugars was less than 2% for the fractions from L2 to L6, indicating that alkali extraction can produce lignin with a high purity. This result was similar to the case of the extraction of lignin from softwood with 1% NaOH aqueous solutions at 100 °C, in which the lignin obtained contained less than 4% sugars (Xue *et al.* 2012). The lignin samples were subjected to alkaline nitrobenzene oxidation, and the degraded products were identified. The major products for the lignin samples were vanillin and syringaldehyde for L1 and L2 and *p*-hydroxybenzoic acid and syringaldehyde for L3, L4, L5, and L6. The presence of high amounts of syringaldehyde and *p*-hydroxybenzoic acid indicated that the six lignin samples were GSH types. Clearly, L1, L2, and L3 were rich in S units, whereas L4, L5, and L6 had abundant amounts of H units. Minor amounts of *p*-coumaric and ferulic acids were observed in L1, L2, and L3, supported by the observation that the two hydroxycinnamic acids are strongly bound to lignin in the samples. With increasing alkali concentration, the mass average molecular weight of the lignin first increased from 1270 g/mol in L1 to 2440 g/mol in L4 and then decreased to 1650 g/mol in L6. This result suggested that the alkali solution with a high concentration promoted the release of lignin with a large molecule, while more drastic alkali caused lignin degradation.

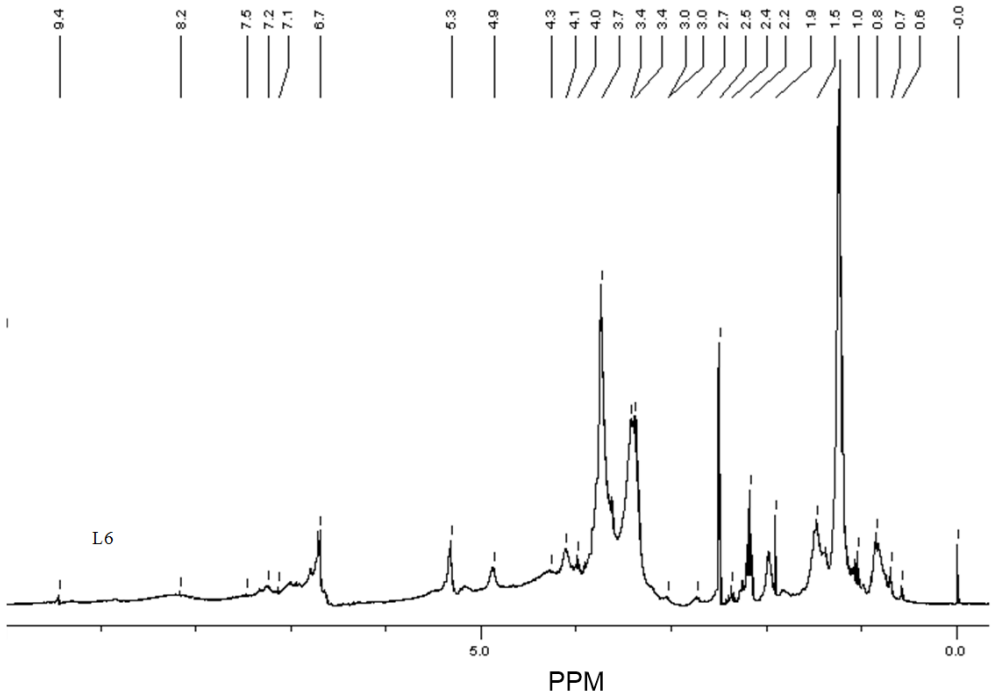


(a)

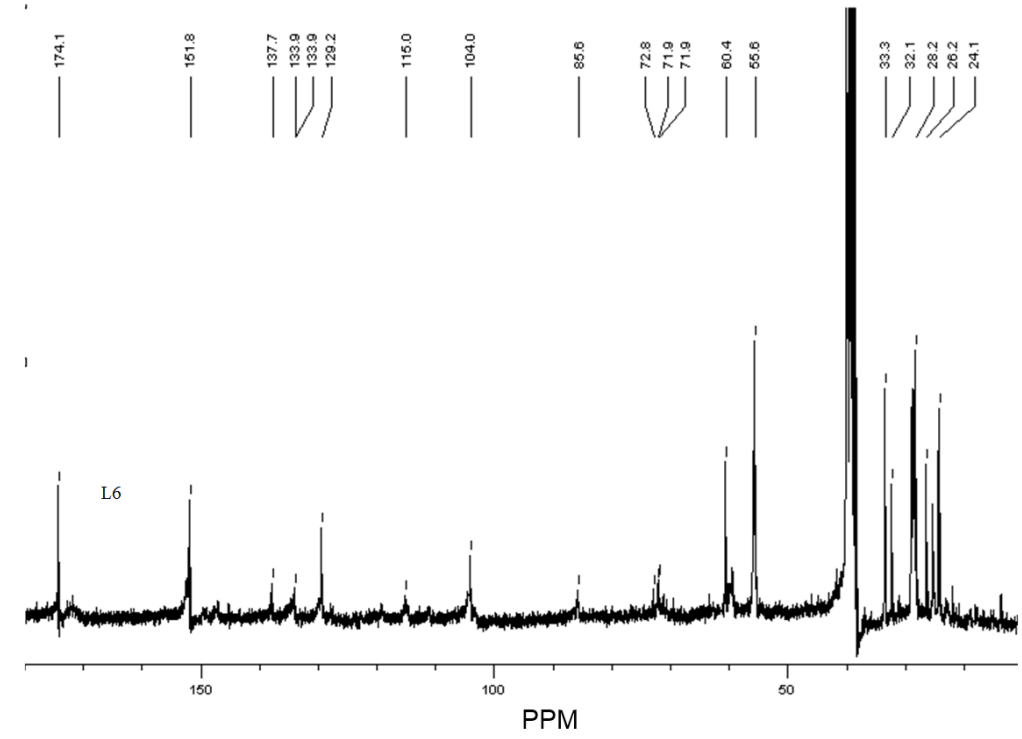


(b)

Fig. 5. FTIR spectra of extracted lignin fractions (a: L1, L2 and L3; b: L4, L5 and L6)



(a)



(b)

Fig. 6. ^{1}H (a) and ^{13}C NMR (b) spectra of the lignin fraction L6

The FTIR spectra of the isolated lignin fractions are presented in Fig. 5. A broad brand at around 3400 cm^{-1} was due to the -OH groups in the phenolic and aliphatic structures. The signals at 2920 and 2855 cm^{-1} were assigned to C-H stretching in CH_3 and CH_2. The main signals of lignin were detected in the range of 1880 to 820 cm^{-1}. The signals at 1740 to 1680 cm^{-1} are related to C=O stretching in unconjugated ketone, carbonyl, and ester groups, while those at 1670 to 1640 cm^{-1} correspond to C=O stretching in conjugated *p*-substituted aryl ketones. Typical signals for aromatic skeleton vibrations were found at 1590, 1508, and 1458 cm^{-1}. The absorption at 1470 to 1450 cm^{-1} is related to C-H deformation, and those at 1370 to 1350 cm^{-1} are due to aliphatic C-H stretching in CH_3 (not -OCH_3) and phenolic –OH. The band at 1287 cm^{-1} is due to the C-H stretching of G units. The absorption at 1230 to 1210 cm^{-1} is related to C-C plus C-O plus C=O stretching. The signal at 1191 cm^{-1} is typical for H, G, and S units of lignin. The signals at 1120 to 1115 cm^{-1} and 1044 cm^{-1} are related to C-H in plane deformation. The peak at around 840 cm^{-1} indicated the *p*-substituted phenolic group. The intensity of the signal at 1738 cm^{-1} in L1 was relatively strong, suggesting C=O stretching in unconjugated ketones and carbonyl groups in lignin (Yan *et al.* 2009). The signal was weaker from L2 to L6, which can be attributed to the cleavage of the linkages in following alkali extraction processes.

The NMR spectra of the lignin fraction L6 obtained by extraction with 8% NaOH at 50 °C for 3 h are presented in Fig. 6. In the ^1H NMR spectrum, typical resonances for lignin were observed. The signals at 6.7 to 6.8 and 7.0 ppm are due to aromatic protons in S and G units in lignin (Faix 1991; An *et al.* 2017). The presence of H units, Cα=O groups, and hydroxycinnamic acids in the lignin fraction was confirmed by the signals at 7.2 and 7.5 ppm (Seca *et al.* 2000). H_β in β-*O*-4 exhibits a signal at 4.8 ppm. The methoxyl proton (–OCH_3) exhibits a sharp signal at 3.7 ppm. The signal at 3.4 ppm is due to the protons in water dissolved in DMSO, and the strong signals at 2.5 ppm correspond to protons in DMSO. Methyl and methylene in saturated aliphatic side chains give signals at 0.8 to 1.2 ppm. With respect to the ^{13}C NMR spectrum, signals at 57 to 103 ppm have relatively low intensity (Xu *et al*. 2008), revealing that the lignin obtained had a low content of contaminated sugars, which is in agreement with the aforementioned sugar analysis. Aromatic signals of lignin were observed in the range of 104 to 168 ppm. The S units exhibit signals at 151.8 (C-3/C-5, S etherified), 146.7 (C-3/C-5, S non-etherified), 137.7 (C-4, S etherified), 133.9 (C-1, S etherified), and 104.7 and 104.1 ppm (C-2/C-6, S non-etherified). The signals at 149.0 (C-3, G etherified), 146.9 (C-4, G etherified), 145.0 (C-4, G non-etherified), 133.9 (C-1, G etherified), 119.0 (C-6, G), and 110.8 ppm (C-2, G) are related to the G units. The signals for H units were observed at 129.2 ppm (C-2/C-6, H). The above results indicated that the lignin fraction had a GSH structure. More intense signals were observed at 151.8 ppm than at 146.9 ppm, implying that S units were more involved in ether linkages than G units. In addition, small signals for etherified ferulic acid were found at 122.5 ppm (C-6, etherified), and those for esterified *p*-coumaric acid at 115.0 ppm (C-3/C-5). These findings were in accordance with the results of alkaline nitrobenzene oxidation of the sample. β-*O*-aryl ether linkages of the sample was observed at 85.6, 72.8, and 60.4 ppm, corresponding to C-β, C-α, and C-γ in β-*O*-4, respectively. The intense signals of resonance indicate that the extraction with strong alkali did not break the β-aryl ether linkages of lignin to a significant extent. The signal at 55.6 ppm arises from –OCH_3. The signals at 14-29 ppm are related to γ-methyl and α- and β-methylene groups inside chains of lignin.

From the analysis above, the hemicelluloses and lignin extracted showed typical structures, which can be used in many fields. The application of hemicelluloses includes

the possibilities such as films, hydrogels, additives for food, and fillers for drug. Lignin may be used for the preparation of dispersants, polyurethane foams, epoxy resins, and antioxidants, *etc*.

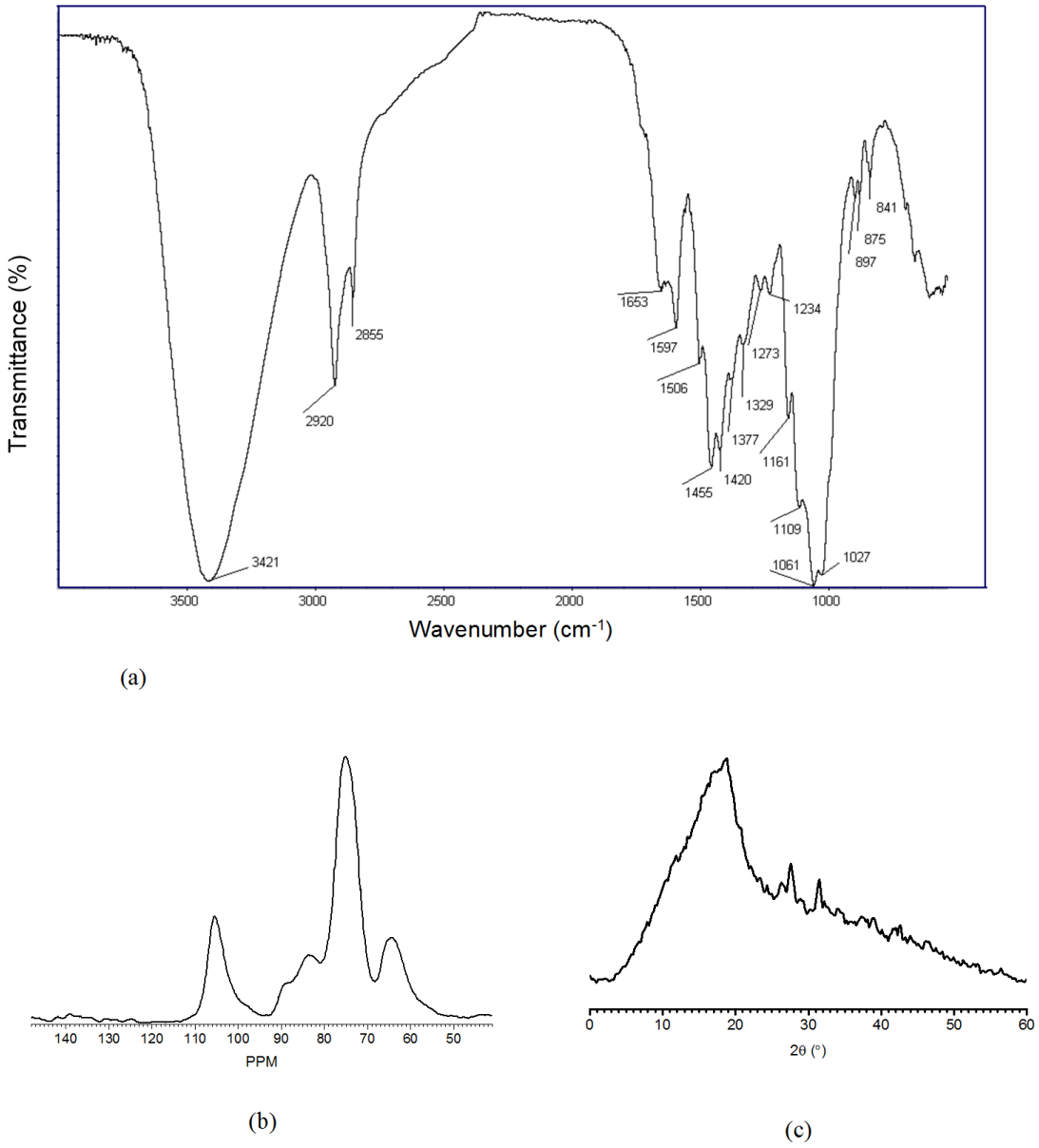


Fig. 7. Structure characterization of the residue after the successive extractions. (a) FTIR spectrum, (b) CP/MAS ^{13}C NMR spectrum, and (c) X-ray diffractogram

Analysis of the Residue

After the successive extractions with water and alkali solutions, the chemical components of the residual sorghum stem changed. Chemical component analysis showed that the residue had a high proportion of cellulose (73.0%) and lower contents of hemicelluloses (15.2%) and lignin (11.0%). The residue produced a very low yield of oxidized products during the alkaline nitrobenzene oxidation, supporting the low content of lignin (Table 1). The structure of the residue was characterized by FTIR, NMR, and X-

ray diffraction (Fig. 7). In the FTIR spectrum, the signals corresponding to lignin at 1597, 1510, and 1420 cm^{-1} were rather weak, indicating effective lignin removal. Strong signals for β-glycoside linkages between sugar units were observed at 897 cm^{-1}. The disappearance of the signal at 1740 cm^{-1} indicated the removal of acetyl groups after alkali treatments. In the CP/MAS ^{13}C NMR spectrum, the signal at 106 ppm was assigned to the C-1 of cellulose and xylan (from hemicelluloses). The resonances at 65 and 89 ppm are due to the C-6 and C-4 of crystalline cellulose, respectively. The overlapped intense peaks at 73 to 75 ppm result from the C-2, C-3, and C-5 of cellulose and xylan. The signal at 62 ppm and the resonance at 84 ppm were related to the C-6 and C-4 of mixed amorphous cellulose together with xylan. X-ray analysis of the residue indicated that it was a mixture of crystal cellulose and amorphous compounds. The calculated crystallinity index was 23.8%, revealing the low crystallinity of the sample. Residue with a relatively low crystallinity index is considered a promising feedstock for further conversion to bioethanol (Li *et al.* 2010; Nitsos *et al.* 2013) .

CONCLUSIONS

1. Successive extractions with water and NaOH aqueous solutions with increased concentrations enabled the effective removal of hemicelluloses (67.4%) and lignin (37.7 %) from sorghum stem at relatively low temperatures.
2. The hemicelluloses obtained from the alkali aqueous solution showed a linear xylan structure, had medium molecular weights (M_w 31700 to 217,000 g/mol), and had minor amounts of bound lignin as evidenced by the low alkaline nitrobenzene oxidation products (less than 2%).
3. The lignin obtained from the alkali solution extractions showed a typical GSH structure and had low amounts of contaminated sugars (less than 2%). The alkali solution with a high concentration led to the release of lignin with a large molecule, while more drastic alkali resulted in the degradation of lignin.
4. The residual stem after the successive extraction was rich in cellulose (73.0%) and had less hemicelluloses and lignin and a low crystallinity, making it an ideal feedstock for further conversion into biofuels.

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