Effects of Ethanol Pretreatment on Dissolution and Structural Changes of Lignin from Steam-exploded Wheat Straw

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Pretreatment of steam-exploded wheat straw by ethanol has been used to dissolve lignin. Different pretreatment conditions were explored by varying reaction temperature, time, and the ratio of solid to liquid to determine that the rate of dissolution was approximately 51.6%. The examination of structural changes in L_P (lignin from ethanol pretreatment pulp) showed that the conjugated carbonyl/carboxyl of lignin was partly destroyed during ethanol pretreatment and alkali extraction process. The content of total hydroxyl groups was increased with increasing ethanol pretreatment. Higher intensities of the aromatic ring in L_P and L_L (lignin from black liquor) fractions compared to that of weak pretreatment conditions indicated that some extent of lignin condensation occurred during the ethanol pretreatment. The ratios of S/G in L_P were higher than in L_L and removal of the methoxyl groups happened during the ethanol pretreatment process, and this led to changes in proportions of lignin structural units.

Keywords: Steam exploded wheat straw; Lignin characterization; Ethanol pretreatment; Delignification

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INTRODUCTION

Wheat straw belongs to the Gramineae family. It is distributed in northern China, which has large areas of wheat planting. The individual separation of its three main components is the basis of biological refining. Hemicellulose can undergo auto-hydrolysis, generating a hydrolysis product mainly in wood sugars by steam explosion pretreatment (Shevchenko *et al.* 2000). The main components of steam-exploded wheat straw are cellulose and lignin, with a very low content of hemicellulose, which can reduce the difficulty of the dissolution of lignin (Zeng *et al.* 2014).

There are many pretreatment methods, such as rice straw pretreated by green liquor that showed excellent performance in delignification selectivity; 92.5% of glucan, 82.4% of xylan, and 81.6% of arabinan in rice straw were recovered with a delignification of 39.4%, which improved hydrolysis efficiency for the production of bioethanol (Gu *et al.* 2012, 2013). Giummarella *et al.* (2016) separated lignin fractions from the black liquor by ultrafiltration to obtain lignin with excellent low viscosity and solubility, which were able to be used in the synthesis of several high value-added products. Kim *et al.* (2011) used ionic liquid for the extraction of the macromolecule lignin and indicated that the G-type lignin was more extractable than the S-type lignin during the treatment of biomass with ionic liquid.

Among various biomass pretreatment methods, organosolv pretreatment is a wellknown delignification method using various organic solvents. The beneficial effect of organosolv pretreatment on the subsequent enzymatic hydrolysis stage has been illustrated in several studies (Hallac *et al*. 2010; Jang *et al*. 2016). The research adopts the way of organic solvent extraction to achieve the purpose of separating lignin. The experiment chooses ethanol as a preprocessing reaction solvent due to its recyclability and non-toxicity (Arato *et al.* 2005). The β-*O*-4 ether bonds together with β-β, β-5, and 5-5 carbon-carbon linkages are identified to be present in lignin substructures (Xu *et al.* 2006). Based on previous research work, with the view to study a pretreatment method (steam explosion and ethanol) of wheat straw, which was a benefit for the making process of bioethanol, ethanol pretreatment was carried out on steam-exploded wheat straw to achieve dissolution of lignin under different reaction conditions. Structural changes of lignin samples from steam-exploded wheat straw by the ethanol pretreatment process were studied under the conditions of different reaction temperature, time, and ratio of solid to liquid. Structural features of L_P and L_L were characterized by spectroscopic analyses including FT-IR, UV, ^{1}H, and ^{13}C NMR. Based on these results, the chemical characteristics of L_P and L_L were extensively compared.

EXPERIMENTAL

Materials

Wheat straw was steam-exploded at a pressure of 1.75 MPa for 3.5 min. Compositional analysis of steam exploded wheat straw on a dry basis was carried out by NREL procedures (Xu *et al.* 2006) to determine the cellulose (66.4%), hemicellulose (0.92%), and lignin (23.4%). All standards and chemicals including xylose, glucose, and arabinose were chromatography grade and procured from Sigma-Aldrich (Shanghai, China); sulfuric acid and ethanol were analytical grade procured from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

Ethanol Pretreatment Process

The 80 g of steam exploded wheat straw on dry basis was introduced into the air bath digester with 55% (*v/v*) ethanol solution and 0.5% (*w/w*) sulfuric acid under the condition of the three factors: reaction temperature was 150 °C, 160 °C, or 170 °C; reaction time was 10 min, 20 min, or 30 min; and the ratio of solid to liquid was 1:4, 1:5, or 1:6 (Fig. 1). After ethanol pretreatment, the pretreated wheat straw was recovered from material collection device and separated into two fractions by filtration to black liquor with lignin and ethanol-pretreated pulp with cellulose-rich residue.

Lignin Extraction Process

Ethanol-pretreated pulp was milled and screened to pass a 40 mesh and to be retained on a 60 mesh screen. The powders were extracted with 60% (*v/v*) alkaline-ethanol (5% (*w/w*) NaOH) at 80 °C for 3 h. Experiments were done in beakers soaked in an electric-heated thermostatic water bath. The supernatant liquid from the extraction was adjusted to pH 5.5 by 4% (*v/v*) HCl, supplied with 3 volumes of anhydrous alcohol, and centrifuged. The filtrate was concentrated under vacuum and neutralized to pH 2, and the precipitate was washed with acidic solution and freeze-dried. Black liquor was supplied with 3 volumes of anhydrous alcohol, with subsequent processing the same as pulp.

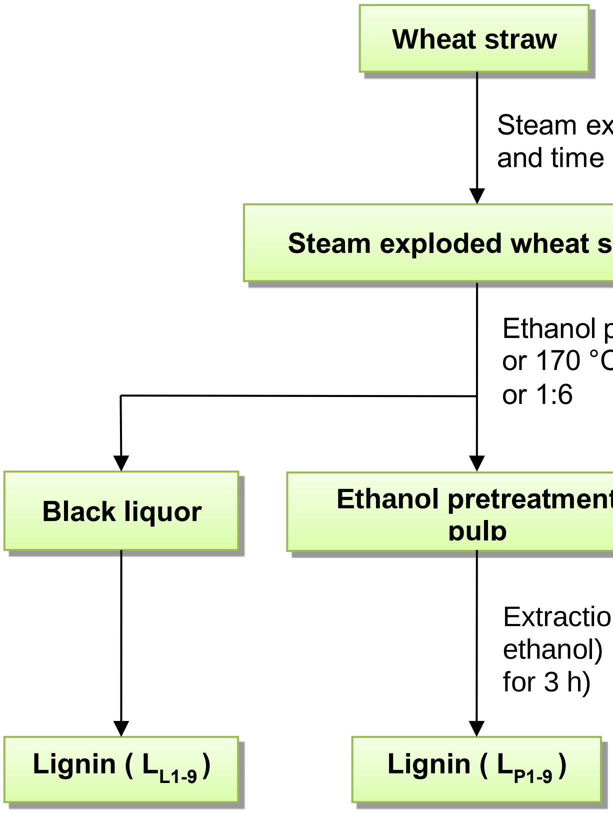

Fig. 1. Pretreatment and lignin extraction process

Analysis Methods

The glucan, xylan, arabinose, cellulose, hemicellulose, and lignin content in ethanol pretreatment pulp were measured by an NREL procedure. Moreover, the cellulose and hemicellulose composition of product in the form of glucan, xylan, and araban were measured by high performance liquid chromatography (HPLC) (Chen *et al.* 2014). Aliquots from the homogenized ethanol-pretreated pulp that was extracted by using anhydrous alcohol were subjected to moisture determination (drying at 105 °C to constant weight) and quantitative acid hydrolysis with 3 mL of 72% sulfuric acid for 1 h. Next, 84 mL of water was added in order to quantify post-hydrolysis as 4% sulfuric acid at 121 °C and maintained for 60 min in order to ensure quantitative conversion of oligomers into monomers. Before HPLC analysis, the solid residue from post-hydrolysis was recovered by filtration and considered as Klason lignin.

Lignin Characterization

The FT-IR measurements were carried out with a Fourier transform infrared spectrometer (Bruker, Karlsruhe, Germany) that was operated in the wavelength range of 4000 cm^{-1} to 400 cm^{-1}. Lignin extracted from ethanol pretreatment pulp and black liquor was milled and roughly mixed with KBr (1:100, *w/w*), and then pressed into a wafer for FT-IR measurement.

The UV-vis spectra were tested on an ultraviolet/visible spectrophotometer (Bruker). Lignin (5 mg) was dissolved in 95% dioxane solution (10 mL). The dissolved solution (1 mL) was diluted to 10 mL with 50% dioxane solution, and the absorbance between 350 nm and 250 nm was tested (Berlin *et al*. 2006).

The solution-state ^{1}H and ^{13}C NMR spectra were tested on a Bruker NMR spectrometer at 400 MHz using 50 mg of lignin dissolved in DMSO-d6. The chemical shifts reported were calibrated relative to the signals from DMSO-d6, used as an internal standard, at 2.5 ppm for the ^{1}H NMR spectra and 39.96 ppm for the ^{13}C NMR spectra.

RESULTS AND DISCUSSION

Yield of Sugar and Composition of Ethanol Pretreatment Pulp

Table 1 shows the effect of reaction temperature, time, and ratio of solid to liquid on dissolution of lignin during the ethanol pretreatment process. From set No.1 to No. 3 and No. 7 to No. 9, the dissolution extent of lignin increased from 44.02% to 51.63%, and 37.65% to 41.31%, respectively. This may be due to an increase in a heat treatment condition resulting into more lignin solubility. This provides additional demonstration that temperature is proportional to solubility of lignin. Glucan content was 2.37 mg/g, and the dissolution extent of lignin was 41.31% under the most severe processing conditions. High processing conditions can cause degradation of cellulose and a condensation reaction of lignin, which are not conducive to the dissolution of lignin. Therefore, the strength of the ethanol pretreatment process (reaction temperature 170 °C, reaction time 20 min, and the ratio of solid to liquid 1:6) should ensure that it maximized the dissolution of lignin on the premise of the reduced degradation of cellulose and minimization of the lignin condensation reaction.

Table 1. Ethanol Pretreatment Operating Parameters, Analysis of Ethanol Pretreatment Pulp and Black Liquor, and Chemical Composition of Ethanol Pretreatment Pulp

Sample ID*	Ethanol-pretreated pulp				
	Glucan (mg/g)	Xylan (mg/g)	Arabinose (mg/g)	Klason lignin (%)	Dissolution rate (%)
20-150-1:4	2.43	0.044	0.00076	13.11(L_{P1})	44.02
20-160-1:5	2.67	0.047	0.00086	14.20(L_{P2})	39.41
20-170-1:6	2.62	0.043	0.00069	11.33(L_{P3})	51.63
10-160-1:4	2.70	0.046	0.00025	11.95(L_{P4})	49.00
20-160-1:5	2.67	0.047	0.00068	14.20(L_{P5})	39.41
30-160-1:6	2.78	0.044	0.00021	11.46(L_{P6})	51.07
10-150-1:5	2.38	0.041	0.00075	14.61(L_{P7})	37.65
20-160-1:5	2.67	0.047	0.00086	14.20(L_{P8})	39.41
30-170-1:5	2.37	0.043	0.00025	13.75(L_{P9})	41.31

* In sample ID, first numerical is reaction time in min, mid numerical is reaction temperature in °C, and last numerical indicates the ratio of solid to liquid.

FT-IR and UV Analysis

The FT-IR spectra of lignin fractions (L_P) and (L_L) separated from ethanol pretreatment pulp and black liquor are shown in Figs. 2 and 3, respectively. The FT-IR spectra show a strong absorption at 3423 cm^{-1}, which was due to the hydroxyl groups of phenolic and aliphatic structures stretching; C-H stretching absorption in aromatic methoxyl groups and in the methyl and methylene groups of the side chains that appear at 2935 cm^{-1} (Yuan *et al.* 2009). Two strong absorption peaks at 1608 cm^{-1} and 1510 cm^{-1} were derived from the skeletal and stretching vibrations of benzene rings, which are characteristic peaks of lignin. The absorption peaks at 1459 cm^{-1} and 1423 cm^{-1} were found in both the spectra, which was derived from the vibration of the benzene rings. As a result, the peaks could act as a standard for detecting the variation of other absorption bands.

The relative intensities of various cross peaks, calculated as the ratio of the intensity of the signal to that at 1510 cm^{-1} (Guo *et al.* 2014), are shown in Tables 2 and 3. The content of total hydroxyl groups was increased with increasing of the ethanol pretreatment, suggesting that more hydroxyl groups were formed due to the cleavage of various ether linkages (Pan *et al.* 2006). Higher intensities of the aromatic ring in L_P and L_L fractions were compared to that of the weak pretreatment conditions, which indicated that some extent of lignin condensation occurred during the ethanol pretreatment (Lan *et al*. 2018). There were differences between the L_P and L_L spectra. The strong absorption peak at 1685 cm^{-1} associated with the conjugated carbonyl/carboxyl stretching was discovered in the spectrogram of L_P, which decreased in the spectrogram of L_L. At the same time, the absorption intensities at approximately 1685 cm^{-1} decreased with increased time, temperature, and ratio of solid to liquid. This respectively corresponded to that from L_{P1} to L_{P3}, L_{P4} to L_{P6}, and L_{P7} to L_{P9} in the spectra of L_P, which was inconspicuous in the spectra of L_L. These findings indicated that the conjugated carbonyl/carboxyl of lignin was partly destroyed due to the acid catalyzed cleavage of various ether linkages with intensified condition in the ethanol pretreatment and alkali extraction process (Sun *et al.* 2000; Pan *et al.* 2006; Lan *et al.* 2018). This phenomenon coincided well with the results shown in the UV-spectra. The characteristic peak of guaiacyl (G) rings could be found in the spectra at 1284 cm^{-1}and 1220 cm^{-1}. The signals at the wavenumbers of 1332 cm^{-1} and 1124 cm^{-1} were assigned to the syringyl units (Lan *et al.* 2018). The ratios of S/G in L_P were higher than in L_L and mean that the removal of the methoxy reaction happened during the ethanol pretreatment process.

The peak of the aromatic C-H deformation appeared at 1033 cm^{-1}. The absorption at 881 cm^{-1} was derived from the C-H out-of-plane vibrations in the aliphatic double bond.

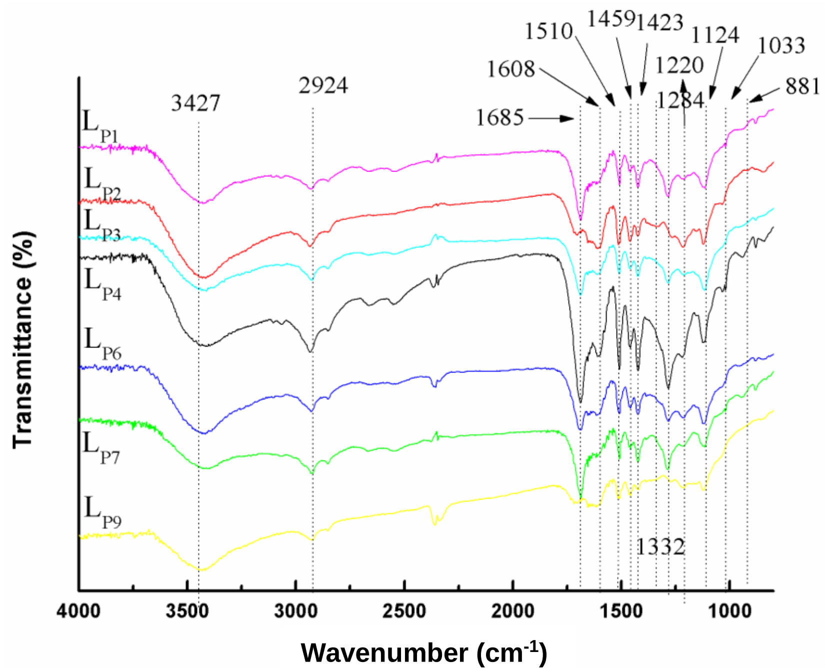

Fig. 2. FT-IR spectra of lignin fractions from ethanol pretreatment pulp

Table 2. Signal Assignment and Relative Intensities in FTIR Spectra of Lignin Samples from Ethanol Pretreatment Pulp*

Assignment	Wavenumber (cm^{-1})	L_{P1}	L_{P2}	L_{P3}	L_{P4}	L_{P5}	L_{P6}	L_{P7}	L_{P8}	L_{P9}
Hydroxyl groups	3423	1.07	1.19	1.08	0.92	1.19	1.13	1.04	1.19	1.36
C-H stretching	2935	1.02	1.02	1.02	0.94	1.02	0.98	1.05	1.02	1.21
Conjugated carbonyl/carboxyl stretching	1685	1.17	0.94	1.10	1.12	0.94	1.11	1.17	0.94	1.02
Benzene rings	1608	0.99	1.03	1.00	0.95	1.03	1.01	0.98	1.03	1.04
Benzene rings	1510	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Benzene rings	1459	0.94	0.99	0.97	0.92	0.99	0.97	0.96	0.99	0.99
Benzene rings	1423	1.01	0.96	1.01	1.01	0.96	1.01	1.01	0.96	0.96
Syringyl rings	1332	0.92	0.91	0.96	0.89	0.91	0.93	0.94	0.91	0.91
Guaiacyl rings	1284	1.05	0.93	1.04	1.07	0.93	1.05	1.04	0.93	0.91
Guaiacyl rings	1220	0.97	1.02	1.00	0.96	1.02	1.03	0.94	1.02	0.95
Syringyl rings	1124	1.07	1.00	1.07	0.90	1.00	0.94	0.94	1.00	1.07
Aromatic C-H deformation	1033	0.82	0.78	0.86	0.72	0.78	0.83	0.80	0.78	0.79
C-H out-of-plane vibrations	881	0.71	0.59	0.73	0.56	0.59	0.66	0.70	0.59	0.62

* The relative intensity was calculated as the ratio of the intensity of the signal to the intensity of the band at 1510 cm^{-1}

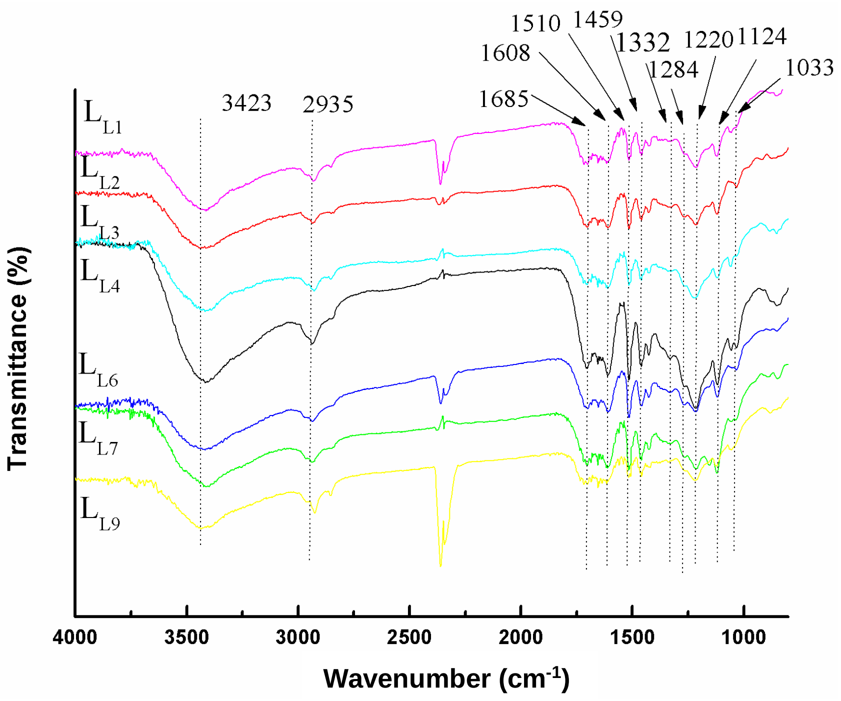

Fig. 3. FT-IR spectra of lignin fractions from black liquor

Table 3. Signal Assignment and Relative Intensities in FTIR Spectra of Lignin Samples from Black Liquor*

Assignment	Wavenumber (cm^{-1})	L_{L1}	L_{L2}	L_{L3}	L_{L4}	L_{L5}	L_{L6}	L_{L7}	L_{L8}	L_{L9}
Hydroxyl groups	3423	1.26	1.12	1.17	1.06	1.12	1.17	1.13	1.12	1.27
C-H stretching	2935	1.12	1.00	1.04	0.84	1.00	1.05	0.98	1.00	1.17
Conjugated carbonyl/carboxyl stretching	1685	1.02	1.00	1.00	0.94	1.00	0.97	1.11	1.00	1.04
Benzene rings	1608	1.03	1.02	1.03	1.02	1.02	1.01	1.01	1.02	1.04
Benzene rings	1510	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Benzene rings	1459	0.99	0.98	0.99	0.97	0.98	0.98	0.98	0.98	1.00
Benzene rings	1423	0.95	0.94	0.94	0.90	0.94	0.94	0.94	0.94	0.96
Syringyl rings	1332	0.93	0.92	0.93	0.93	0.92	0.92	0.91	0.92	0.91
Guaiacyl rings	1284	0.95	0.93	0.97	1.00	0.93	0.94	0.93	0.93	0.95
Guaiacyl rings	1220	1.05	1.00	1.09	1.21	1.00	1.00	1.01	1.00	1.03
Syringyl rings	1124	1.00	1.06	0.96	0.94	1.06	0.93	1.03	1.06	0.96
Aromatic C-H deformation	1033	0.86	0.81	0.83	0.86	0.81	0.82	0.82	0.81	0.84
C-H out-of-plane vibrations	881	0.70	0.67	0.68	0.59	0.67	0.65	0.64	0.67	0.69

* The relative intensity was calculated as the ratio of the intensity of the signal to the intensity of the band at 1510 cm^{-1}

UV spectroscopy has been used to measure the content of acid soluble lignin in hydrolysate. In this study, UV-vis absorption measurements of lignin samples were tested under wavelengths from 350 nm to 250 nm. Figure 4 shows the UV absorption spectra of the lignin samples of ethanol pretreatment pulp. The absorption maxima of the lignin sample appeared at 275 nm, derived from non-conjugated phenolic groups, which were substituted by the oxygen-containing groups in lignin.

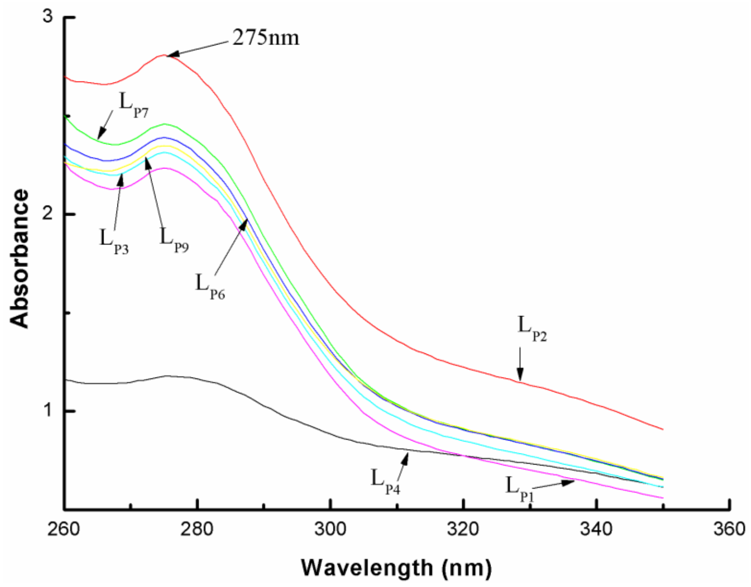

Fig. 4. UV spectra of lignin fractions from ethanol pretreatment pulp

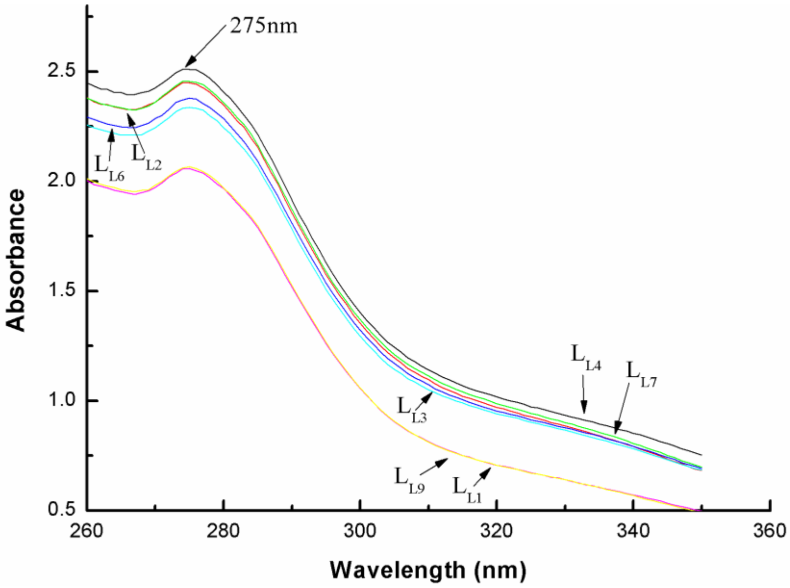

Fig. 5. UV spectra of lignin fractions from black liquor

All the lignin fractions were rich in the syringyl units, as indicated by the fact that syringyl units exhibit bands at somewhat shorter wavelengths, specifically from 268 nm to 276 nm (Yuan *et al.* 2009). The weak absorption at 320 nm in all spectra was attributed to conjugated phenolic groups (Sun *et al.* 2000), its reason lies in the groups linked with benzene rings *via* the oxygen were partly destroyed during the ethanol pretreatment process and alkali extraction process, which was similar to spectra in the L_L of Fig. 5. The segmental conjugated structure of the lignin was destroyed, resulting in a decrease of the absorption at 320 nm. The trend of change in the weak peak in FT-IR spectra at 1685 cm^{-1} conformed to UV spectroscopy results and were related to the conjugated carbonyl/carboxyl stretching.

^{1}H and ^{13}C NMR Spectra Analysis

The ^{1}H NMR spectrum of lignin fraction separated from ethanol pretreatment pulp (L_{P2}) and black liquor (L_{L2}) are given in Fig. 6. The signals of aromatic protons in lignin structural units appear in the 6.0 ppm to 7.5 ppm region, and the signals at 1.24 ppm are due to the protons of aliphatic -OH of the high-shielding effect in the lignin (Guo *et al.* 2014). The signals at 7.11 ppm and 6.72 ppm are attributed to the aromatic protons in the G and S units, respectively (Xu *et al.* 2008). The weak signals between 4.0 ppm and 6.0 ppm are attributed to protons (β-5, β-1, β-β, and β-*O*-4) in the aromatic ring on the side chain (Wang *et al.* 2012). The dominant two signals at 3.74 ppm and 3.37 ppm are attributed to methoxyl protons (-OCH_3) units (Cao *et al.* 2012). Moreover, the signals at 2.20 ppm and 1.98 ppm confirmed presence of acetyl groups of links on the benzene ring from phenolic hydroxyl and alcoholic hydroxyl in the lignin and protons of aliphatic side chain, respectively.

In the spectra of L_{P2} (Fig. 6), the peaks between 7.11 ppm and 6.72 ppm presented in the spectrum of L_{L2} became chaotic and ambiguous, and the ratios of S/G may be changed when compared with L_{P2}. This was due to the mutual transformation of lignin structural units. Compared with L_{L2}, the signal at 3.37 ppm derived from methoxyl protons disappeared in the spectrum of L_{P2}, which demonstrated that demethoxylation occurred during ethanol pretreatment (Hage *et al.* 2010). Meanwhile, the absorption at 2.20 ppm increased in the spectrum of L_{P2}, compared with that of L_{L2}. It was due to the increased content of acetyl groups from phenolic hydroxyl and alcoholic hydroxyl in the lignin of L_{P2}.

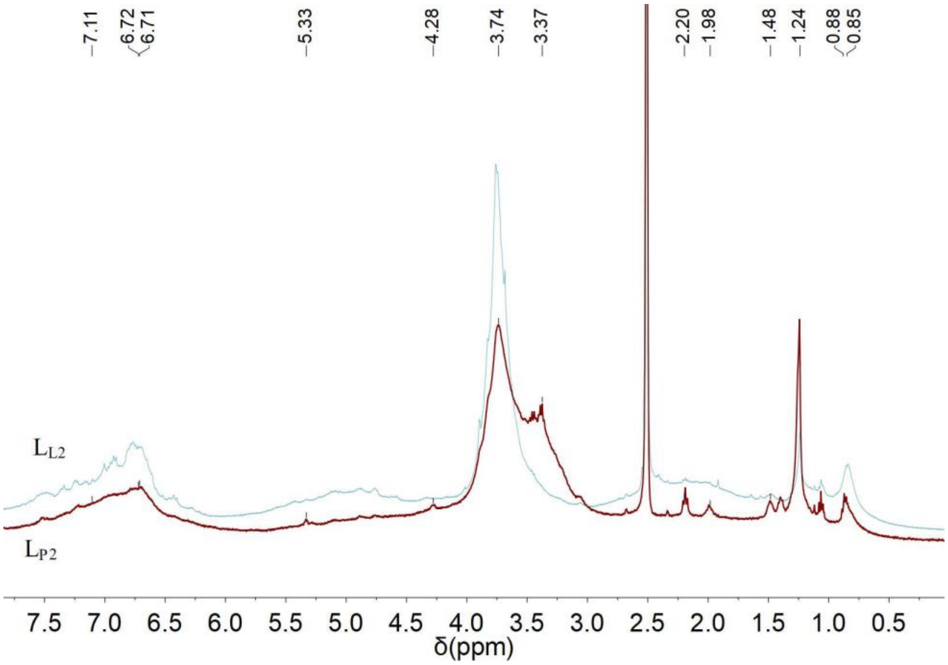

Fig. 6. ^{1}H NMR spectra (in DMSO-d6) of lignin fractions

The qualitative ^{13}C NMR spectra of the lignin fractions were obtained and shown in Fig. 7. The signals of the DMSO showed the strongest absorption at 39.96 ppm (Xu *et al*. 2008). The strong absorption at 172.48 ppm indicated an abundance of carbonyls, which may originate from aliphatic carboxyl carbons in the lignin samples of L_{L2}. The region from 103 ppm to 160 ppm was originated from aromatic carbons (Guo *et al.* 2014). The S unit with α-C=O was at 106.70 (C-2/C-6) ppm. The signals appearing in the ^{13}C NMR spectra at 149.25 (C-3) ppm was originated from the G unit (Wang *et al.* 2012). Moreover, the signal observed at 126.57 ppm belonged to the H unit. The distinction of the absorption peak intensities between L_{L2} and L_{P2} indicated that the proportion of the three-unit basic structural units of wheat straw (H, G, and S) differed during the ethanol pretreatment process (Wen *et al.* 2013), which was in accordance with the FTIR analysis.

The signals below 104 ppm reflected the resonance signals of the aliphatic carbon region. The significant adsorption at 21.0 ppm (CH_3- in acetyl groups) indicated that phenolic hydroxyl and alcoholic hydroxyl in the lignin were destroyed during the ethanol pretreatment process. The peaks of γ-methyl in the *n*-propyl side chain appeared between 15.59 ppm and 29.48 ppm in all spectra (Guo *et al.* 2014). The signal of the side chain carbons of C-β in the β-5 substructures was observed at 62.38 ppm, and the signal of the 5-5 in non-etherified aromatic C-C substructures was observed at approximately 123.0 ppm to 127.0 ppm. The signal of C-γ in the β-*O*-4 was observed at 61.65 ppm (Roland *et al.* 2009). The clear signal in this absorption band implies that the C-C structures are the main type of connections in lignin fractions, rather than β-*O*-4 links. The signal of the β-*O*-4 substructures that appeared in L_{L2} and L_{P2} indicated that a condensation reaction occurred during the ethanol pretreatment (Bu *et al.* 2011). The signal located at 56.58 ppm represented a -OCH_3 group in the S and G units, which disappeared in L_{P2}, indicating that a demethoxyl reaction occurred during the ethanol pretreatment process.

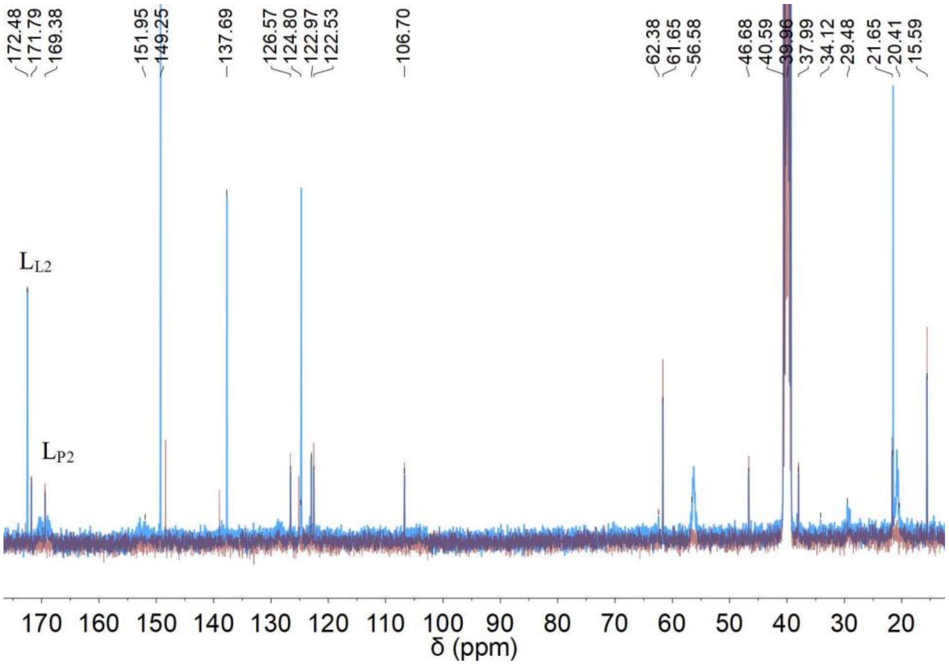

Fig. 7. ^{13}C NMR spectra (in DMSO-d6) of lignin fractions

CONCLUSIONS

1. The chemical and thermal characteristics of the lignin from ethanol pretreatment pulp and black liquor were investigated. The content of total hydroxyl groups was increased with increasing of the ethanol pretreatment, suggesting that more hydroxyl groups were formed due to the cleavage of various ether linkages. Higher intensities of the aromatic ring in L_P and L_L fractions compared to that of weak pretreatment conditions, which indicated that some extent of lignin condensation occurred during the ethanol pretreatment.
2. The conjugated carbonyl/carboxyl of lignin was destroyed partly, and all the lignin fractions were rich in syringyl units. The intensity ratios of S/G in L_P were higher than in L_L. Removal of the methoxyl groups happened during the ethanol pretreatment process, leading to changes in the proportion of lignin structural units. This fact can be confirmed by ^{1}H and ^{13}C spectra.
3. The experimental results indicated that time and temperature were more important than ratio of solid to liquid in the process of separation of lignin by ethanol pretreatment.

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