Structural Changes of Lignin from Wheat Straw by Steam Explosion and Ethanol Pretreatments

Cheng Pan, Zhong Liu,* Xiaoxiao Bai, and Lanfeng Hui

Effects of the pretreatment of wheat straw by steam explosion and ethanol were evaluated relative to the structural changes of lignin from the pretreated pulp. The lignin from steam explosion pulp (L_S), lignin from steam blasting residual liquid (L_L), lignin from ethanol pretreatment pulp (L_E), lignin from black liquor (L_B), and lignin from wheat straw (L_W) were separated, and the structural characteristics of the lignin fractions were compared based on analyses of Fourier transform-infrared, ultraviolet, thermogravimetric, and ^{1}H and ^{13}C nuclear magnetic resonance spectra. The proportions of the three structural units in all lignin fractions clearly changed during the pretreatment process because of inter-conversion reactions. The conjugated structure of lignin was destroyed in the pretreatment process and was also affected by the alkali extraction process. The alcoholic hydroxyl links on the aliphatic side chain were partly transformed into carbonyl groups during ethanol pretreatment. Demethoxylation occurred in all lignin fractions during the ethanol pretreatment and steam explosion process. The thermal stability of the L_B fraction was relatively high because of the condensation reaction.

Keywords: Wheat straw: Steam explosion; Ethanol pretreatment; Lignin

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INTRODUCTION

With the development of biomass resource utilization technology, non-wood resources must be used in a scientific and rational manner in the paper industry. Cheap, readily available, and non-staple food biomass sources that are rich in cellulose and hemicellulose can be an abundant and renewable source of energy chemicals. Wheat belongs to the Gramineae family and is abundantly distributed in Northern China, which has large areas of wheat cultivation.

The biorefinery concept is based on the selective separation of the major lignocellulosic components (cellulose, hemicellulose, and lignin). These components vary in their biodegradability and in their tolerance to the chemical and thermal conditions applied in industrial processing. Hemicellulose is the most easily degraded of these components, whereas lignin is resistant to degradation (Feria *et al.* 2012). Therefore, establishing new methods for the pre-extraction and isolation of hemicelluloses will improve the efficient utilization of biomass components and increase revenue streams for lignocellulosic biorefineries to maximize the utilization of biomass in the production of fuels, chemicals, and materials. Nevertheless, challenges remain in the dissolution of lignin from steam-exploded wheat straw. Several methods have been proposed to separate lignin in wheat straw material, residual lignin in the pulp, and dissolved lignin in the liquid, such as the use of green liquor (Gu *et al.* 2012, 2013), ultrafiltration and selective precipitation (Toledano *et al.* 2010), and ionic liquids (Kim *et al.* 2011). In the first step, hemicellulose that can undergo an autolysis reaction can be used to generate the hydrolysis products. This is commonly applied with steam explosion pretreatment to obtain xylan, which is in turn effectively converted into useful products. Steam explosion pretreatment can improve the economic benefit of the cellulose microbial transformation process and reduce secondary pollution. The main constituents of steam-exploded wheat straw are cellulose and lignin, with a very low hemicellulose content, which can therefore reduce the difficulties associated with the dissolution of lignin. And in the second step, organic solvent extraction can be used to effectively separate lignin. In the present study, we evaluated a method for lignin separation from steam-exploded wheat straw using ethanol as a pre-processing reaction solvent because of its recyclability and non-toxic, harmless nature.

The structure of lignin may be changed by pretreatments such as steam explosion. Robert *et al.* (1988) observed that the condensation reaction and demethylation took place in aspen lignin during steam explosion treatment. Montane *et al.* (1997) found that the methoxyl and aliphatic alcohol contents of lignin of wheat straw decreased during steam explosion. Li *et al.* (2007) reported that depolymerization and repolymerization take place in the lignin due to the acidity created during the treatment. When organic solvents are used in pretreatment, the lignin preparations contain almost equal amounts of non-condensed guaiacyl and syringyl units with fewer *p*-hydroxyphenyl units. 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), and the lignin extracted by ethanol contain *p*-coumaric acid and ferulic acid structure linked by ester bonds (Chen and Liu 2007). Based on previous research work, the structural changes of lignin samples from wheat straw pretreated with steam explosion and ethanol preprocessing were evaluated.

EXPERIMENTAL

Materials

Wheat straw was collected in 2014 from the Dezhou City, Shandong province in Northern China. Wheat straw was air-dried and shredded into 0.3- to 0.5-cm-long pieces using a knife mill and was stored in sealed plastic bags to maintain a stable moisture content which was 6.93%. All standards and chemicals (*e.g.*, xylose, glucose, and arabinose) were of chromatographic grade and obtained from Sigma-Aldrich (Shanghai, China), and sulfuric acid and ethanol were of analytical grade and procured from Sinopharm Chemical Reagent (China).

Pretreatment of Wheat Straw

Steam-exploded wheat straw was formed using a pressure of 1.75 MPa and time of 3.5 min by steam explosion. The treated wheat straw was recovered from the material collection device and separated into the following two fractions by filtration: steam-blasting residual liquid, with xylose-rich hydrolyzate and steam explosion pulp, with cellulose-rich residue. The steam explosion pulp was air-dried and stored until an equilibrium moisture content was attained, which was 5.48%.

Steam explosion pulp on a dry basis was introduced to an air bath digester with 55% (*v/v*) ethanol solution under the following conditions: a reaction temperature of 160 °C, a reaction time of 20 min, and a solid to liquid ratio of 1:5. After ethanol pretreatment, the treated wheat straw was recovered from the material collection device and separated into two fractions by filtration to black liquor with lignin and ethanol pretreatment pulp with cellulose-rich residue.

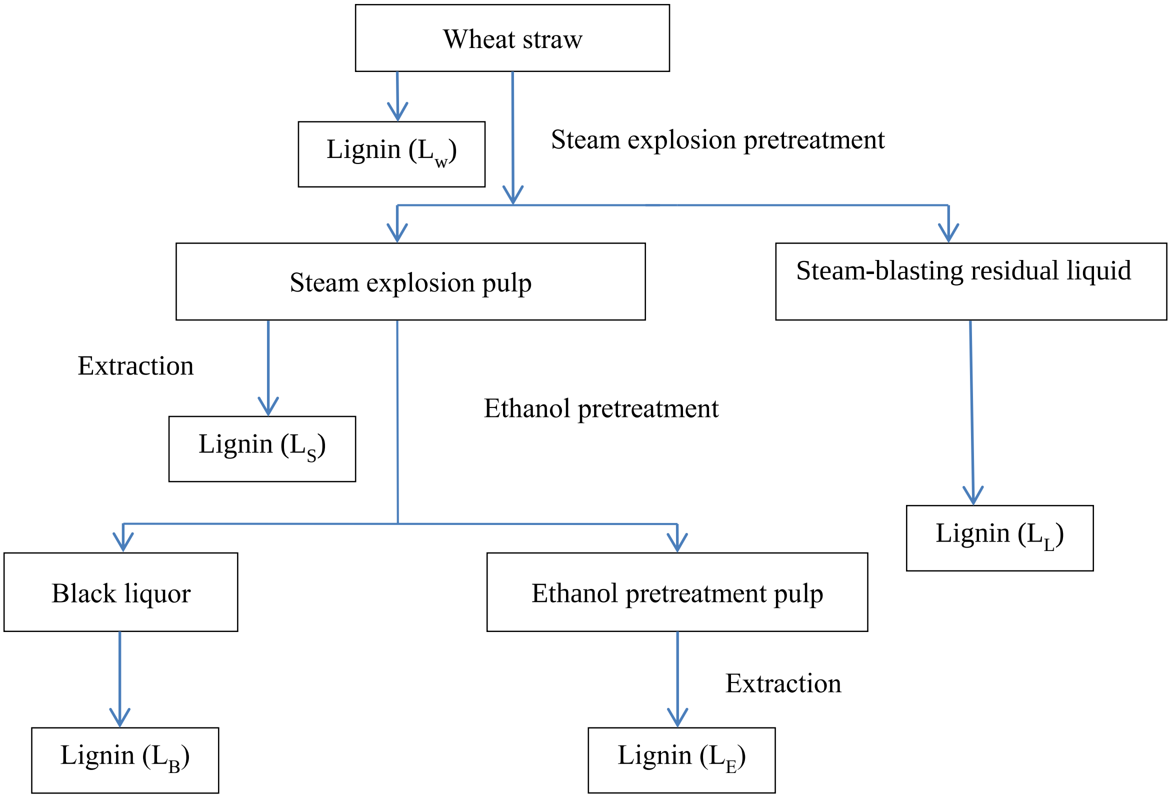


Fig. 1. Operational flow of wheat straw pretreatment. Here, "L_W" is lignin from wheat straw, "L_S" is lignin from steam explosion pulp, "L_L" is lignin from steam blasting residual liquid, "L_E" is lignin from ethanol pretreatment pulp, and "L_B" is lignin from black liquor.

Lignin Extraction Process

Wheat straw, steam explosion pulp, and ethanol pretreatment pulp were milled. The fraction that passed through a 40-mesh screen and was collected on a 60-mesh screen was used for further study. Lignin was extracted from the powders thus obtained with 60% (*v/v*) alkaline-ethanol (5% (*w/w*) NaOH) at 80 °C for 3 h. Experiments were conducted in beakers soaking in an electrically heated thermostatic water bath. The supernatant liquid from the extraction which was alkalinity was adjusted to pH 5.5 by 4% (*v/v*) HCl, supplied with three volumes of anhydrous alcohol, and centrifuged. Then, the filtrate was concentrated and the pH was adjusted to 2, and the precipitate was washed with acidic solution of pH 2 and then freeze-dried (Montane *et. al.* 1997).

The processes for obtaining lignin from black liquor and the steam-blasting residual liquid were relatively simple. The liquor was supplied with three volumes of anhydrous alcohol, and the subsequent processing steps were the same as those described for obtaining the pulp.

Analysis Methods

Fourier transform-infrared (FT-IR) measurements were obtained with an FT-IR spectrometer (Bruker, Germany) using pressed KBr wafers. A total of 10 scans were taken for each sample between 400 cm^{-1} and 4000 cm^{-1}, with a resolution of 4 cm^{-1}. Lignin was milled, roughly mixed with KBr (1:100, w/w), and then pressed into a wafer for FT-IR measurement.

Ultraviolet-visible (UV-vis) spectra were obtained with use of a UV-vis spectrophotometer (Bruker, Germany). Lignin (5 mg) was dissolved in a 95% dioxane solution (10 mL). The dissolved solution (1 mL) was diluted to 10 mL with a 50% dioxane solution, and the absorbance was measured between 250 and 400 nm (Berlin *et al.* 2006).

Thermogravimetric analysis (TGA) was conducted in a thermogravimetric simultaneous thermal analyzer (Hu *et al.* 2015). The samples of lignin were heated from room temperature to 600 °C at 10 °C/min in a nitrogen atmosphere. The initial quantity of all samples was 5 to 10 mg.

The prepared lignin samples were acetylated to boost the signal strength (Mansouri and Salvadó 2006). The solution-state ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded using a Bruker NMR spectrometer at 400 MHz with 100 mg of lignin dissolved in deuterated DMSO. The chemical shifts observed were calibrated relative to the signals obtained from the DMSO sample only, used as an internal standard, at 2.5 ppm for the ^{1}H NMR spectra and at 39.8 ppm for the ^{13}C NMR spectra.

RESULTS AND DISCUSSION

FT-IR and UV Spectra Analysis

Infrared spectroscopy is one of the most commonly applied spectroscopic tools for the study of polymers. The FT-IR spectra of the lignin fractions obtained at 4000 to 400 cm^{-1} are shown in Fig. 2. The FT-IR spectra showed a clearly strong absorption peak at 3399 cm^{-1} caused by stretching of the hydroxyl groups of the phenolic and aliphatic structures. Furthermore, C-H stretching absorption peaks in the aromatic methoxyl groups and in the methyl and methylene groups of the side chains appeared at 2923 cm^{-1}. Strong absorption peaks were observed between 1600 and 1508 cm^{-1} derived from the skeletal and stretching vibrations of the benzene rings. The peak multiplicity at 1267 cm^{-1} is a representative signal of the *p*-hydroxyphenyl structures of herbage lignin (Sun *et al.* 2000).

An absorption peak at 1708 cm^{-1}, reflecting the non-conjugated carbonyl/carboxyl stretching, was observed in the spectra of L_B, L_S, and L_L, and virtually disappeared in the spectra of L_W and L_E. These findings indicated that the non-conjugated hydroxyl groups of lignin were replaced by oxygen-containing groups in the process of ethanol pretreatment, and that the lignin structure was also affected by the alkali extraction process. However, the displacement reaction did not occur in the steam explosion process.

The strong absorption peak at 1654 cm^{-1}, reflecting the conjugated carbonyl/ carboxyl stretching, was observed in all the spectrograms. Thus, the conjugated structure of lignin was destroyed in the reaction process, and it was also affected by the alkali extraction. The absorption peak in the spectrogram of L_E was weak because L_E was the final product of the entire pretreatment process, and therefore showed the highest level of destruction.

The characteristic peak of the syringyl (S) ring at 1326 cm^{-1} was apparent in all spectrograms except for that of L_E, indicating that a demethoxylation reaction occurred in lignin during the ethanol pretreatment process and not in the steam explosion process. The characteristic peak of guaiacyl (G) rings was found in the spectra at 1216 cm^{-1}. Vibration of the carbohydrates appeared at 1000 to 1170 cm^{-1}, and the purity of the sample was in contrast to the amount of absorption peaks within this range.

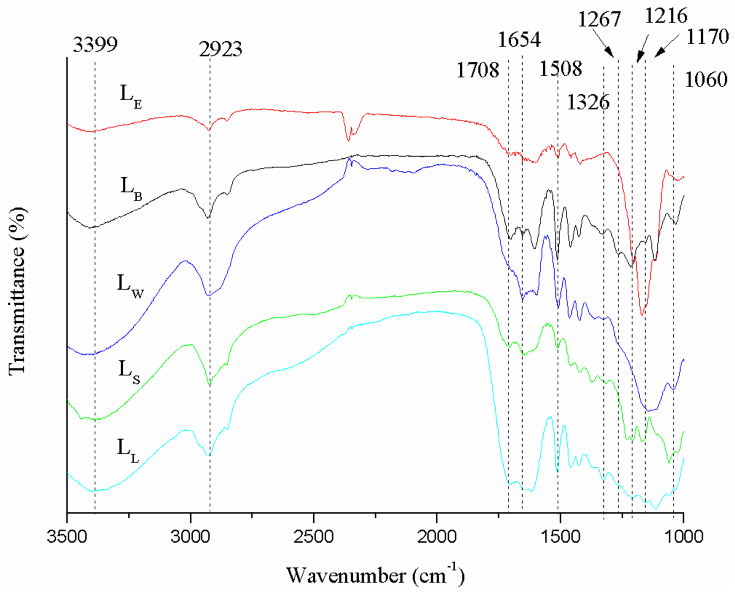


Fig. 2. FT-IR spectra of lignin fractions. “L_W” is lignin from wheat straw, “L_S” is lignin from steam explosion pulp, “L_L” is lignin from steam-blasting residual liquid, “L_E” is lignin from ethanol pretreatment pulp, and “L_B” is lignin from black liquor.

The UV-vis absorption measurements of lignin samples were taken between 250 and 400 nm. Figure 3 shows the UV absorption spectra of the lignin samples. The absorption of lignin samples appeared around 275 nm, which was derived from non-conjugated phenolic groups, and the absorption peak at 320 nm was attributed to conjugated phenolic groups such as *p*-coumaric and ferulic acids (Xu *et al.* 2006), which could be found around 7.44 ppm in the spectrum of Fig. 5 as the aromatic protons in *p*-coumaric and ferulic acids in the lignin fraction (Seca *et al.* 2000).

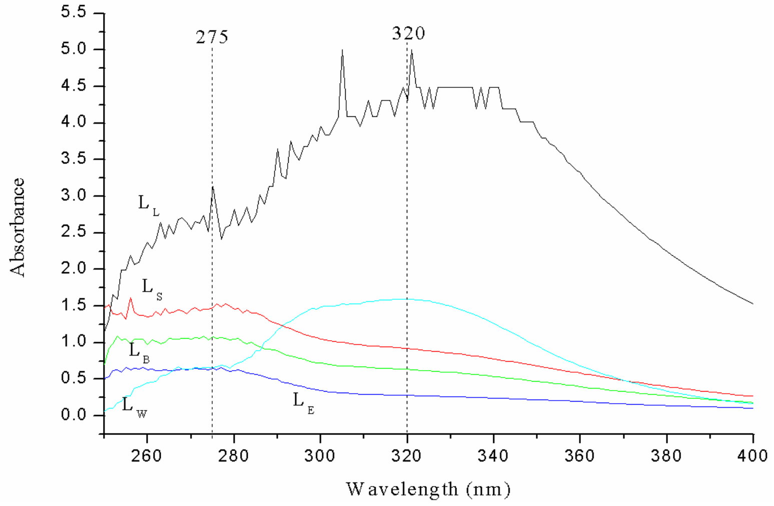
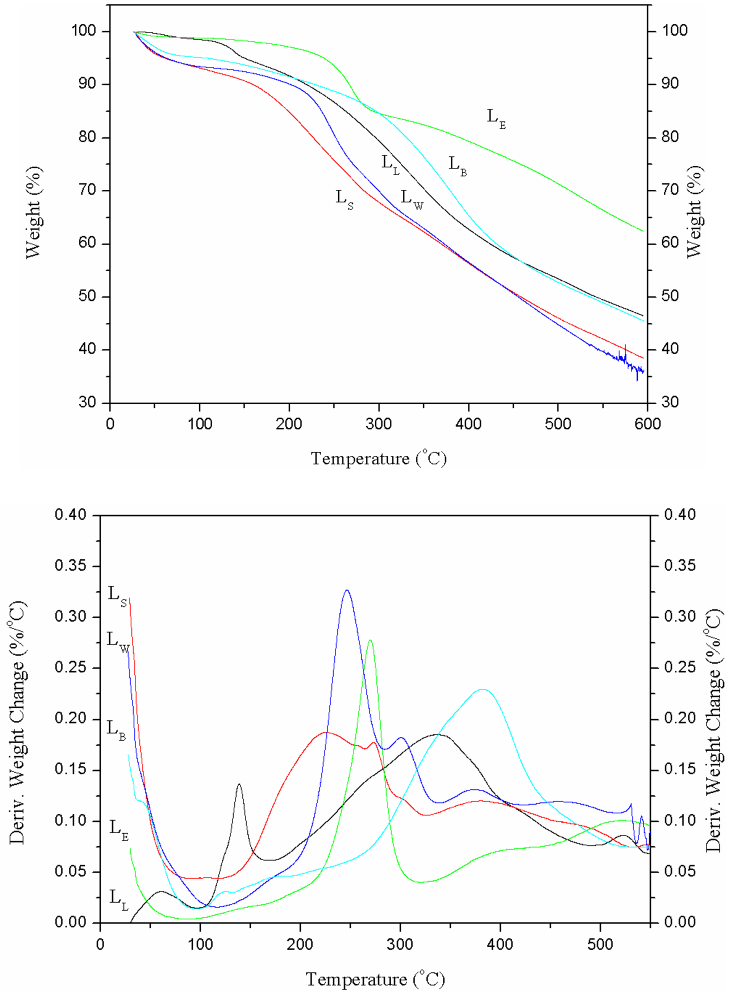


Fig. 3. UV spectra of lignin fractions. “L_W” is lignin from wheat straw, “L_S” is lignin from steam explosion pulp, “L_L” is lignin from steam-blasting residual liquid, “L_E” is lignin from ethanol pretreatment pulp, and “L_B” is lignin from black liquor.

The absorbance values of LW (with a high dilution factor) and LL were higher than those of the other samples at 275 and 320 nm. This suggests that the non-conjugated phenolic groups and the conjugated phenolic groups of LW and LL were not substituted by the oxygen-containing groups in lignin, and were slightly damaged. At the same time, the much lower absorption at 320 nm LS, LE, and LB revealed that the conditions given substantially cleaved the ester or ether bonds between *p*-coumaric acid and ferulic acid, and lignin during ethanol pretreatments (Sun *et al.* 2000). This finding also echoed the variation observed in the weak bands in the FT-IR spectra at 1708 and 1654 cm^{-1}, which were associated with the non-conjugated and conjugated carbonyl/carboxyl stretching, respectively.

TGA

To further characterize the lignin products, TGA was conducted at a heating rate of 10 °C/min (Savy and Piccolo 2014; Zhang *et al.* 2014), and the results are shown in Fig. 4.



(a)

(b)

Fig. 4. TG and DTG curves of lignin fractions. (a) TG curves of the lignin fraction; (b) DTG curves of the lignin fraction. “LW” is lignin from wheat straw, “LS” is lignin from steam explosion pulp, “LL” is lignin from steam-blasting residual liquid, “LE” is lignin from ethanol pretreatment pulp, and “LB” is lignin from black liquor.

When the temperature reached 600 °C, the residual weight of L_W was 36.45%, compared with 38.44% for L_S, 46.45% for L_L, 45.47% for L_B, and 62.37% for L_E. This variation can be attributed to the condensation reaction of the lignin of the ethanol pretreatment pulp during the ethanol pretreatment process, resulting in relatively high thermal stability (McDonough *et al.* 1993; Hage *et al.* 2010; Martin-Sampedro *et al.* 2011). Through the steam explosion pretreatment, the residual weight of L_S was close to that of L_W, indicating that there was no excessive condensation reaction.

Figure 4b shows that the highest loss rate of L_W was 0.325% per °C, compared with 0.187% per °C for L_S, 0.185% per °C for L_L, 0.230% per °C for L_B, and 0.278% per °C for L_E. The loss rate of L_W, which was not pretreated, increased to approximately 0.33% per °C, which was higher than other samples. The structure of lignin changed during the pretreatment process with partial degradation and re-condensation, which was different from the results for the native lignin. The degradation of L_W, L_E, and L_S, which were extracted by alkali, occurred earlier and were higher than that of L_L and L_B, which were obtained with weak acid extraction. This suggests that the lignin structure was changed during the pretreatment process after a certain degree of degradation and condensation (Martin-Sampedro *et al.* 2011).

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

The ^{1}H NMR spectra of the lignin fractions are shown in Fig. 5. The signals of the aromatic protons in three lignin units appeared in the 6.0 to 8.0 ppm region. The peak around 9 to 12 ppm was assigned to carboxylic acids and aldehydes. The signals observed between 0.8 and 1.5 ppm were caused by the protons of the aliphatic side chain in the lignin, and the signals observed at 7.03 and 6.73 ppm can be attributed to the aromatic protons in the G- and S- units, respectively (Xu *et al.* 2008), and the signal at 7.6 ppm can be attributed to *p*-hydroxybenzene units. There were weak signals observed between 4.0 and 6.0 ppm, which can be assigned to protons of the aromatic ring on the side chain. The dominant two signals at 3.75 and 3.37 ppm are ascribed to methoxyl proton ($-OCH_3$) units. Moreover, the signals at 2.23 and 1.96 ppm confirmed the presence of acetyl groups from the phenolic hydroxyl link on the benzene ring and an alcoholic hydroxyl link on the aliphatic side chain, respectively.

The spectra of L_W and L_S were similar, indicating that the lignin structure of the pulp was slightly changed from the steam explosion pretreatment. In the spectrum of L_L, the signal strength at 1.96 ppm decreased obviously, suggesting a partial reaction of the alcoholic hydroxyl links on the aliphatic side chain of L_L. The spectra of L_E and L_B, which were preprocessed with ethanol, were distinct from the others. The peaks between 6.0 and 8.0 ppm observed in the spectra of L_E and L_B became more vague and diverse compared with those of L_W, L_S, and L_L. This difference is attributed to a reaction on the benzene ring, and a mutual change between the three types of aromatic structures (Xu *et al.* 2008; Wen *et al.* 2013). The strong signal at 3.75 ppm and disappearance of the signal at 3.37 ppm in the spectra of L_E and L_B were derived from methoxyl protons, confirming the demethoxylation reaction during the ethanol pretreatment process, which was not observed in the L_W, L_S, and L_L spectra. Furthermore, the absorption at 2.20 ppm increased obviously in the L_B spectrum, compared with those of L_W, L_S, L_E, and L_L, which reflects an increase in the content of acetyl groups from phenolic hydroxyl in the lignin of the L_B fraction. These results coincide well with the analyses of the FT-IR and UV spectra.

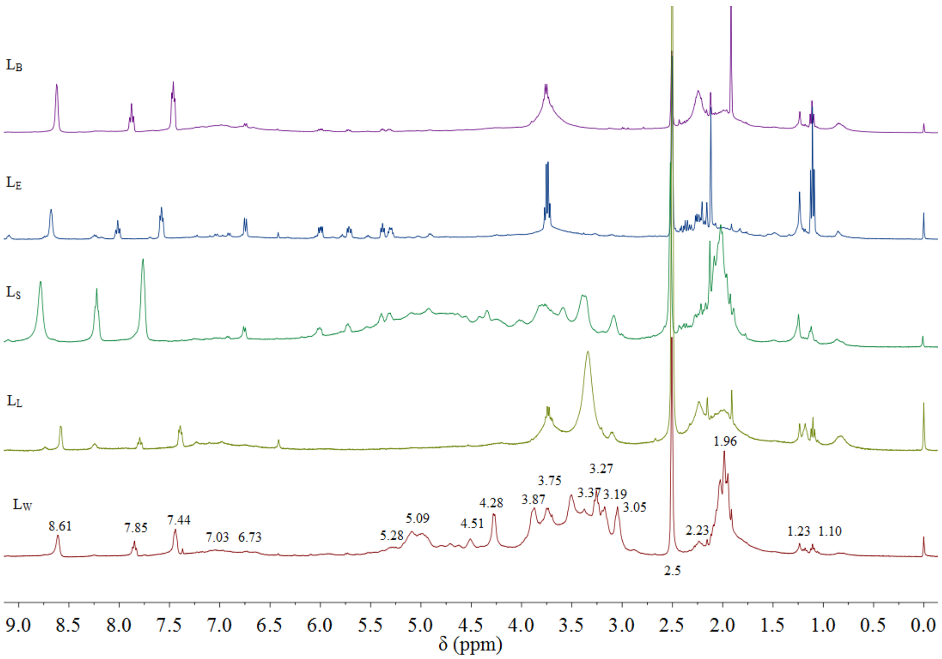


Fig. 5. ^{1}H NMR spectrum (in d6-DMSO) of lignin fractions. "LW" is lignin from wheat straw, "LS" is lignin from steam explosion pulp, "LL" is lignin from steam-blasting residual liquid, "LE" is lignin from ethanol pretreatment pulp, and "LB" is lignin from black liquor.

To gain an overall understanding of the change in the structures of lignin during the pretreatment process, the qualitative ^{13}C NMR spectra of the lignin fractions were obtained and are shown in Fig. 6. The strong absorption at 172.48 ppm, which originated from the carbon in the carbonyl groups, indicated an abundance of carbonyls in the lignin samples of LB relative to the LW, LS, LE, and LL fractions. The likely reason for this is that olefin reacted with ethanol, and then olefin-etherified oxygen-containing groups converted into unstable enol, which oxidizes into ketones (Bu *et al.* 2011).

The region from 103 to 160 ppm originated from the aromatic part of the lignin. The S unit was indicated by signals at 137.7 and 106.5 ppm. The signals appearing in the ^{13}C NMR spectra at 149.26 and 122.53 ppm originated from the G unit. Moreover, the signal observed at 126.58 ppm belongs to the *p*-hydroxyphenyl (H) unit. The distinction between the absorption peak intensities among the samples indicates that the proportion of the three-unit basic structural units of wheat straw (H, G, and S) differed between the LW, LS, LE, LB, and LL fractions. This variation can be attributed to a reaction on the benzene ring, and mutual changes between the three types of aromatic structures (Xu *et al*. 2008; Wen *et al.* 2013), which is similar to the results of the ^{1}H NMR spectra of the lignin fractions. The absorption peak intensities of LB with a two-step pretreatment process were strongest and multiform, and the peaks of the three basic structural units of lignin became increasingly apparent, and were most obvious in samples subjected to the ethanol pretreatment process.

The signals below 104 ppm reflect the resonance signals of aliphatic carbons. The stronger absorption at 20.6 ppm is the characteristic absorption peak of the carbons in the acetyls that are introduced during the acetylation of lignin. The absorption peak intensities of LB and LE increased significantly, which indicated that phenolic hydroxyl and alcoholic hydroxyl in the lignin were destroyed during the ethanol pretreatment process, which was not observed in the spectra of the LL, LW, and LS fractions; this fact is consistent with the

^{1}H NMR spectrum. The peaks of the γ-methyl and α- and β-methylene groups in propyl side chains appeared between 15.59 and 30 ppm in all spectra.

The signal of the side chain carbons of C_β in the β-5 substructures was observed at 63.0 ppm, and the signal of the 5-5 in non-etherified aromatic C-C substructures was observed around 123.0-127.0 ppm. The C_γ signal of the β-*O*-4 substructures 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 at 63.0 ppm disappeared in the spectra of L_S, L_E, L_B, and L_L fractions, demonstrating that the β-5 substructures were destroyed in the pretreatment process; however, the 5-5 substructures were consistently present. The signal of the β-*O*-4 substructures appeared in L_E and L_B and disappeared in L_S, indicating that a condensation reaction occurred during the ethanol pretreatment (Bu *et al.* 2011).

The signal at 56.24 ppm was caused by the -OCH_3 group in the S and G structures, which appeared in L_W, L_L, and L_B, and disappeared in L_S and L_E, indicating that a demethoxyl reaction occurred during the pretreatment process, and that alkaline extraction also likely exerted an influence. The signals of the DMSO (the solvent of the lignin used in ^{13}C NMR detection) showed the strongest absorption at 39.8 ppm. The signals between 65 ppm and 79 ppm reflect the resonance signals of polysaccharides. The resonance signals of L_B and L_L were less than L_W, which suggested that the partial cleavage of linkages between hemicelluloses and lignin during the pretreatment, and the relatively low amount of polysaccharides indicated that the purity of lignin extracted from pulp was higher.

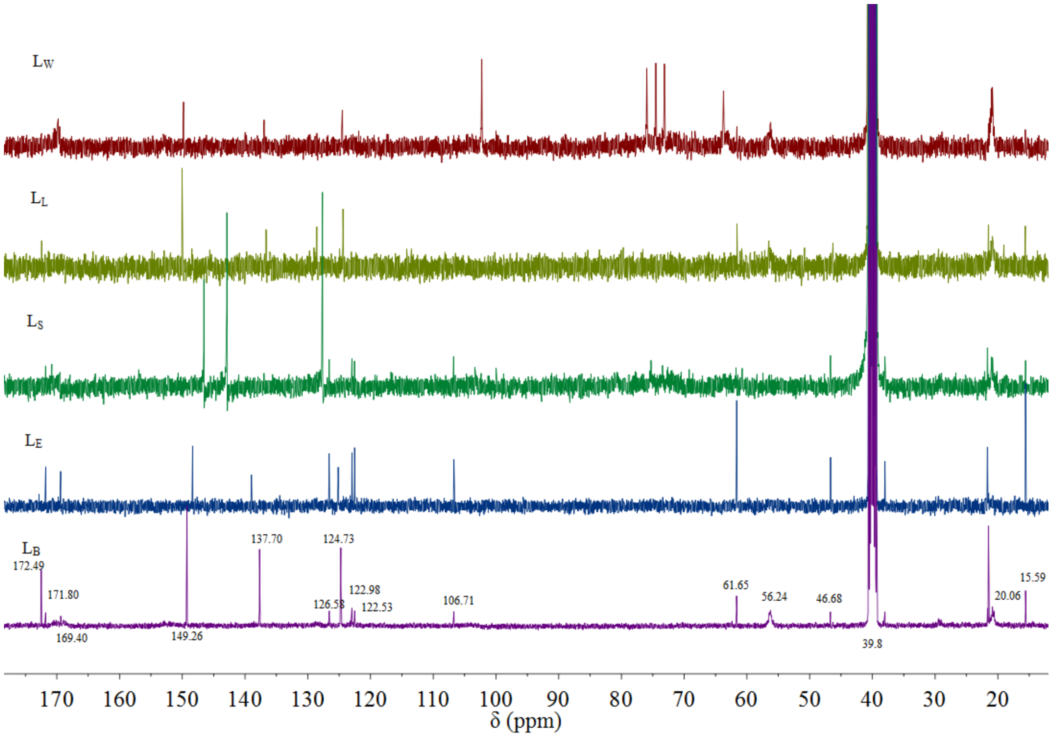


Fig. 6. ^{13}C NMR spectrum (in d6-DMSO) of lignin fractions. "L_W" is lignin from wheat straw, "L_S" is lignin from steam explosion pulp, "L_L" is lignin from steam-blasting residual liquid, "L_E" is lignin from ethanol pretreatment pulp, and "L_B" is lignin from black liquor.

CONCLUSIONS

1. The signals of aromatic protons in three types of lignin units appeared at approximately 6.0 to 8.0 ppm in the ^{1}H NMR spectra, and at 103 to 160 ppm in the ^{13}C NMR spectra. The absorption peak intensities of the LW, LE, LS, LB, and LL fractions were different and multiform, indicating that the proportion of the three structural units in lignin changed during the pretreatment process because of interconversion reactions. This finding also echoed the variation of bands observed in the FT-IR spectra at 1326, 1267 and 1216 cm^{-1}, which are associated with the syringyl (S) ring, the *p*-hydroxyphenyl, and guaiacyl (G) rings, respectively. The ratio of the intensities could be used to estimate the relative contents of *p*-hydroxyphenyl, guaiacyl, and syringyl units in lignin obtained with two-step process.
2. The absorbance values of LE, LS, and LB were lower than that of LW at 275 and 320 nm, indicating that non-conjugated phenolic groups and conjugated phenolic groups were substituted by the oxygen-containing groups in lignin, and the ester or ether bonds between *p*-coumaric acid and ferulic acid, and lignin were substantially cleaved during the pretreatment process. This finding also echoed the variation of bands observed in the FT-IR spectra at 1708 and 1654 cm^{-1}, which are associated with non-conjugated and conjugated carbonyl/carboxyl stretching, respectively. However the absorbance value of LL separated by steam explosion was high, which implied relatively complete structure of the lignin, and the fact was useful for separation of lignin with two-step process.
3. The signals at 3.75 and 56.24 ppm increased from LE to LB and those at 3.37 and 56.24 ppm increased from LS to LL in the ^{1}H and ^{13}C spectra, respectively, which reflected the demethoxylation reaction during the ethanol pretreatment and the steam explosion process.
4. The signals at 1.92 and 20.6 ppm decreased from LS to LE in the ^{1}H and ^{13}C spectra, respectively, which confirmed that the alcoholic hydroxyl link on the aliphatic side chain had partly been transformed into carbonyl during ethanol pretreatment.
5. The signals between 65 ppm and 79 ppm reflect the resonance signals of polysaccharides. The resonance signals of LL and LB obtained by two-step process were less than others, which implied that the separation efficiency from wheat straw become higher with step by step pretreatment, indicating that the purity of lignin extracted from pulp was higher.
6. The residual weight of LW was 36.45%, which was lower than that of LS (38.44%), LL (46.45%), LB (45.47%), and LE (62.37%). Therefore, these results show that the residual weight of lignin increases with pretreatment because of a condensation reaction of lignin, with relatively high thermal stability.

ACKNOWLEDGMENT

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