OPTIMIZING EXTRACTION AND STRUCTURAL CHARACTERIZATION OF ORGANOSOLV LIGNIN FROM WHEAT STRAW

HE-LONG LI, DIAO SHE,[*,**] PAIPENG,[***] QIANG XU,[****] JIA-KUN LIU,[*****] XUE-MING ZHANG,[*****] andZENG-CHAOGENG

College of Resources and Environment, Northwest A&F University, Yangling 712100, China
[*]State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Institute ofSoil and Water Conservation, Northwest A&F University, Yangling 712100,China
[**]Institute of Soil and Water Conservation, CAS&MWR, Yangling 712100, China
[***]College of Forestry, Northwest A&F University, Yangling 712100, China
[****]Research Center of Soil and Water Conservation and Ecological Environment, Chinese Academy of Sciences and Ministry of Education, Yangling 712100, China
[*****]Beijing Key Laboratory of Lignocellulosic Chemistry, Beijing Forestry University, Beijing 100083, China
✉ *Correspondingauthor: Diao She,diaoshe@ms.iswc.ac.cn*

Received July 6, 2015

Organosolv lignin from wheat straw was isolated with 1,3-butanediol at different temperatures (100, 120, 140, 160 and 180 ºC) for 3 h and at 180 ºC for different time periods (1, 1.5, 2, 2.5 and 5 h), respectively. The yield of the lignin fractions was generally high, the highest yield could reach 76.6% of the original lignin content. The most striking characteristics of these lignin fractions were the almost complete absence of neutral sugars (0.87%) and their low molecular weights (1500-2520 g/mol) with narrow polydispersities. Results showed that the organosolv lignin fractions contained a small amount of non-condensed guaiacyl, syringyl and *p*-hydroxyphenyl units, and a higher proportion of syringyl units was detected in the lignin fraction with a prolongation of the treatment time. Moreover, ^{13}C-NMRand 2D HSQC spectra showed that the process did not significantly change the typical structure of the lignin.

Keywords: organsolv lignin, wheat straw, 2D-HSQC, structural characterization

INTRODUCTION

Nowadays, the world's economy is highly dependent on natural fossil resources, such as oil or coal, for the production of fuel, electricity and chemicals.[1] However, with the scarcity of fossil fuel and the concerns about environmental protection, the utilization of biomass resources has attracted increasing worldwide interest. Wheat straw is one of the most important agricultural wastes due to its abundant and cheap source and could be valorized at an industrial level. It is estimated that wheat straw production reaches as much as 950 million tons each year all over the world, which means that a great amount of biomass possesses a high environmental and economic potential.[2,3] However, its use as a raw material for biorefinery constitutes a considerable technological challenge, particularly because of its chemical complexity and recalcitrance. Lignin accounts for 15-40% of the lignocellulosic biomass and has an important influence on some properties of biomass, such as chemical and physical changes during storage.[4,5] Thus, to maximize the potential value of lignin, it is necessary to extensively understand its properties and investigate its potential utilizations to achieve an optimized isolation of the polymer.

Lignin consists mainly of substituted phenylpropane units that are linked together to form a polymer lacking regularity, crystallinity, or optical activity.[6] Generally, they are linked by ether and carbon-carbon bonds, such as *β-O*-4, 4-*O*-5, *β-β*, *β*-5, and 5-5. As a typicalgrass lignin, it is associated with *p*-hydroxycinnamic acids (PHCAs), such as ferulic acid (FA) and *p*-coumaric

acid (PCA), through ethera nd ester linkages.[7] In addition, PHCAs are covalently linked to polysaccharides forming a lignin-hemicelluloses network, which is made up of benzyl-ether, benzyl-ester, and phenyl-glycoside bonds.[8] Due to the complex nature of lignin, better knowledge is required for the removal of lignin, for efficient utilization of lignin and carbohydrates.[9] Nevertheless, a large obstacle for the requirement is the lack of effective methods for the separation of the lignin, while minimizing the extent of chemical modification.[10] Traditionally, Björkman's procedure is a preferred method for isolating relatively pure lignin from wood samples. However, it is not suitable for isolation of lignin from straw and grass due to their lower contents of lignin.[11] Then, another option for isolating lignin involves the use of aqueous alkaline solutions or the use of enzyme to remove most of the carbohydrate fractions prior to aqueous dioxane extraction of ball-milled wood meal.[12,13] In recent years, further improvements in the yield and purity of lignin have been achieved by the use of organic solvents. Many previous reports have proved the applicability of organosolv in various materials.[14-16] The organosolv processes have some obvious advantages, such as economy at small and medium scale, efficient recovery of solvents and by-products, reduced water, energy and reagent consumption, as well as applicability to wood and non-wood raw material.[17] Among the various organic solvents reported in the literature, pulping with high-boiling solvent (HBS) has received particular attention due to its ability to achieve extensive and selective delignification in a single-step operation. The utilization of HBS for isolation of lignin from softwoods and hardwoods has been investigated at 200 °C and 220 °C, respectively.[18] However, less attention has been paid to the isolation of organosolv lignin from cereal straw by HBS without catalysts. The aim of this study was to develop an effective method for isolation and structural characterization of organosolv lignin from wheat straw.

In the present study, organosolv lignin from wheat straw was treated with 1,3-butanediol under different conditions(different temperatures and time). The obtained lignin fractions were characterized using both degradation methods (alkaline nitrobenzene oxidation and acid hydrolysis), and non-destructive techniques, *e.g.* Fourier transform infrared (FT-IR) spectroscopy, gel permeation chromatography (GPC), and state-of-the-art NMR(^{13}C and 2D-HSQC NMR).

EXPERIMENTAL

Materials

Wheat straw was collected from a farm at Northwest A&F University (Yangling, China) and ground to pass a 1 mm size screen. The chemical composition of wheat straw (% dry weight, w/w) is given as the following: cellulose 40.1%, hemicelluloses 32.8%, lignin 14.1%, ash 7.6%, extractives 1.9%, protein 1.8%, and water soluble polysaccharides 1.9%.[19] The dried powder was dewaxed with toluene-ethanol (2:1, v/v) in a Soxhlet for 6 h and dried in an oven for 16 h at 50 °C before use. All chemicals used were of analytical or reagent grade.

Isolation of organosolv lignin from wheat straw

The dewaxed wheat straw was firstly submitted to 1,3-butanediol under a nitrogen atmosphere with a solid-to-liquid ratio of 1:20 (g/mL) and placed into an oil bath under the conditions of 100, 120, 140, 160, and 180 °C for 3 h and for 1, 1.5, 2, 2.5, and 5 h at 180 °C, respectively. After the reaction, the insoluble residue was collected by filtration, washed with distilled water until the pH of the filtrate was neutral, and then dried in an oven at 60 °C. The filtrate was evaporated to dry and then about 70 mL distilled water was added. After neutralizing it to pH 5.5-6.0 with 6 M HCl, the solution was concentrated by rotary evaporation at reduced pressure to about 50 mL. The solubilized hemicelluloses were recovered by precipitation of the concentrated solution in 3 volumes of 95% ethanol. After filtration, the pellets of the hemicelluloses were washed with 70% ethanol and air-dried. Furthermore, the filtrate was concentrated to about 20-30 mL, then acidized with 6 M hydrochloric acid to pH 1.5-2.0, and centrifuged to obtain lignins. Then the lignin fractions were washed with acidified water (pH 2.0) and centrifuged. The samples (L_1-L_{10}) were then freeze-dried and kept in a refrigerator at 5 °C for analysis. The scheme for fractionation of organosolv lignin from wheat straw is illustrated in Figure 1.

Characterization of the organosolv lignin

The molecular-average weights of lignin factions were determined by gel permeation chromatography on PL gel 5 μm Mixed-D columns. The samples were dissolved in terahydrofuran at a concentration of 0.2%, and a 200 μL solution was injected. The column was operated at 40 °C and eluted with tetrahydrofuran at a flow rate of 1 mL/min. The column was calibrated using polystyrene standards.

Alkaline nitrobenzene oxidation of lignin was performed at 170 °C for 3 h. The degradation products were determined by HPLC on a ZORBAX Eclipse XDB-C18 column as described previously.[20] The

compositions of neutral sugars and uronic acids in the isolated lignin fractions were determined by HPAEC. The hemicellulosic moieties associated with lignin fractions were hydrolyzed by 10% H_2SO_4 at 105 °C for 2.5 h. The acid hydrolysis samples were diluted 30-fold, filtered and injected into the HPAEC system (Dionex ISC 3000,U.S.) with an amperometric detector, an AS50 autosampler and a CarbopacTM PA1 column (4×250 mm, Dionex).[21]

FT-IR spectra were obtained on an FT-IR spectrophotometer (Nicolet, 750) using a KBr disc containing 1% finely ground samples. The solution-state ^{13}C-NMR spectra were obtained on a Bruker 250 AC spectrometer operating in the FT mode at 62.4 MHz under total proton decoupled conditions. Samples (250 mg) were dissolved in 1 mL DMSO-d_6 and ^{13}C NMR spectra were obtained at 25 °C after 30,000 scans. A 60 °C pulse flipping angle, a 3.9 μs pulse width, and 0.85 s acquisition time were used. For HSQC experiments, the lignin (80 mg) was dissolved in 0.5 mL of DMSO-d_6. A pulse program with spectral widths of 5000 and 20,000 Hz was used for the ^{13}C dimensions, respectively. The number of transients was 128, and 256 time increments were always recorded in the ^{13}C dimension. Prior to Fourier transformation, the data matrices were zero filled to 1024 points in the ^{13}C dimension. The central solvent (DMSO-d_6) peak was used as an internal reference (δ_C/δ_H 39.5/2.49). A semiquantitative analysis of the intensities of the HSQC cross-signal was performed using Bruker Topspin-NMR processing software.

RESULTSAND DISCUSSION

Yield of the organosolv lignin

As can be calculated from Table 1, after the dewaxed wheat straw was treated with 1,3-butanediol under a nitrogen atmosphere, the yield of organosolv lignin was6.4, 8.5, 17.0, 25.5, 61.7, 37.6, 41.1, 47.5, 50.4 and 76.6% of the original lignin at 100, 120, 140, 160 ,180 °C for 3 h and for 1, 1.5, 2, 2.5, 5 h at 180 °C, respectively. Meanwhile, the treatment also released 7.6, 7.0, 5.8, 6.1, 6.1, 5.8, 5.5, 4.9, 4.0 and 7.9% of the original hemicelluloses, respectively. As expected, the increasing time from 1 to 5 h at 180 °C led to an increase in the yield of lignin from 5.3 to 10.8%.

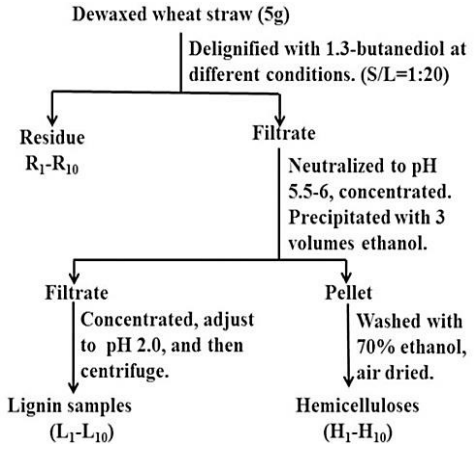

Figure 1: Scheme for isolation of lignins from dewaxed wheat straw

Table 1
Yield (% initial dry wheat straw, w/w) of hemicelluloses, lignin and cellulose-rich residue

	Yield (%)									
	F_1[a]	F_2[a]	F_3[a]	F_4[a]	F_5[a]	F_6[a]	F_7[a]	F_8[a]	F_9[a]	F_{10}[a]
Hemicelluloses	2.5	2.3	1.9	2.0	2.0	1.9	1.8	1.6	1.3	2.6
Lignin	0.9	1.2	2.4	3.6	8.7	5.3	5.8	6.7	7.1	10.8
Residue	91.8	90.8	90.0	90.2	81.0	87.9	85.3	83.3	82.7	76.6

[a]F_1, F_2, F_3, F_4, F_5, F_6, F_7, F_8, F_9, and F_{10} represent degraded polymeric preparations of hemicelluloses, lignin and residue obtained by treatment of dewaxed wheat straw with 1,3-butanediol at 100, 120, 140, 160 and 180 ºC for 3 h and at 180 ºC for 1, 1.5, 2, 2.5 and 5 h, respectively

Table 2

Content of neutral sugars (relative % of lignin sample, w/w) in isolated lignin fractions

Neutral sugars	Lignin fractions[a]									
	L_1	L_2	L_3	L_4	L_5	L_6	L_7	L_8	L_9	L_{10}
Rhamnose	0.09	0.05	0.05	0.03	ND[b]	0.02	0.01	0.03	0.02	ND[b]
Arabinose	0.45	0.35	0.40	0.44	0.36	0.48	0.45	0.38	0.39	0.32
Galactose	0.39	0.40	0.26	0.21	0.08	0.17	0.16	0.12	0.14	0.06
Glucose	1.84	1.28	1.05	0.64	0.39	0.73	0.45	0.40	0.38	0.26
Xylose	0.49	0.41	0.33	0.37	0.26	0.44	0.30	0.25	0.25	0.24
Total	3.26	2.49	2.10	1.70	1.09	1.84	1.37	1.18	1.18	0.87

[a]L_1, L_2, L_3, L_4, L_5, L_6, L_7, L_8, L_9, and L_{10} represent the degraded lignin preparations obtained by treatment of dewaxed wheat straw with 1,3-butanediol at 100, 120,140,160 and 180 ºC for 3 h and at 180 ºC for 1, 1.5, 2, 2.5, and 5 h, respectively; [b]ND = not detected

The fact suggested that more lignin was dissolved in 1,3-butanediol with the increase of time. Similar trends for yields of lignin were also found by increasing the temperature from 100 to 180 °C for 3 h, which raised lignin yield from0.9 to 8.7%. The reason for this was probably that more linkages (*e.g.*, ester and ether bonds) between lignin and hemicelluloses were cleaved with increasing temperature.

Contents of neutral sugars in lignin

The composition of associated hemicelluloses in all lignin fractions was determined by HPAEC after hydrolysis and the results are given in Table 2. As can be seen, all the lignin fractions contained rather low amounts of associated hemicelluloses (0.87-3.26%). Apparently, with the temperature increasing from 100 to 180 °C, the total sugar content decreased from 3.26% to 1.09%, which implies that the higher temperature cleaved more linkages between lignin and polysaccharides in the cell walls of wheat straw, such as ester bonds between ferulic acid and hemicelluloses or between *p*-coumaric acid and lignin and *α*-aryl ether linkages between lignin and hemicelluloses.[10] Moreover, prolonging the treatment time from 1 to 5 h resulted ina decrease in the contents of neutral sugars in lignin between 2.01 and 0.87%. Extending the 1,3-butanediol treatment time from 1 to 2 h showed a slight effect on the yield of lignin (Table 1), but resulted in decreasing neutral sugars content from 1.84 to 1.18%. Therefore, the condition of 180 °C for 2 h may be a good choice for isolation of organosolv lignin with 1,3-butanediol from wheat straw when yields and energy are considered. Specifically, glucose, arabinose, and xylose were identified as the major sugar components, while rhamnose occurred in minor quantities.

Composition of phenolic acids and aldehydes

Table 3 gives the results concerning the analysis of phenolic acids and aldehydes from ten lignin fractions, which were obtained by nitrobenzene oxidation at 170 °C for 3 h. The low yields (7.24-17.83%) of non-condensed phenolic compounds suggested that organosolvlignin fractions isolated with the 1,3-butanediol treatment from wheat straw had a relatively high degree of condensation. The presence of *p*-hydroxylbenzaldehyde (0.70-1.96%) and *p*-hydroxybenzoic acid (0.79-2.88%) was considered to be most probably indicative of non-condensed *p*-hydroxyphenyl (H) units, indicating the incorporation of *p*-hydroxyphenyl or *p*-coumaryl alcohol in the lignin preparation. The appearance of vanillin (0.87-2.50%) and syringaldehyde (0.28-1.57%) resulted from the degradation of non-condensed *β*-aryl ether linked guaiacyl and syringyl units, respectively. Acetovanillone (0.08-0.69%), *p*-coumaric acid (0-0.40%), and acetosyringone (0-0.66%) occurred in small amounts. Clearly, the increase of the treatment temperature from 100 °C to 180 °C for 3 h resulted in a decrease of the total yield of oxidation products from 10.84% to 8.20% (L_1-L_5).By contrast, when the time was increased from 1 to 5 h at 180 °C, the total yield of oxidation products varied from 7.24% to 8.92% (L_5-L_{10}). In addition, although considerable amounts of *p*-coumaric acid and ferulic acid were converted to *p*-hydroxybenzaldehyde and vanillin, respectively, in the nitrobenzene oxidation, the occurrence of small amounts of *p*-coumaric and ferulic acids in the lignin fractions demonstrated that the two hydroxycinnamic acids were tightly bounded to the lignin. It was reported that ferulic acid was linked at the *β*-position of coniferyl alcohol by an

ether bond, while *p*-coumaric acid is known to be extensively esterified at the *γ*-position on the side chain of lignin monomers.[22,23] In comparison, the lower contents of ferulic acid in the lignin fractions L_4-L_{10} (0-0.61%) suggested that more *β*-aryl ether bond was cleaved at higher temperatures. Meanwhile, the low contents of *p*-coumaric acid (0-0.30%) indicated that the ester bond between the side chain of lignin and *p*-coumaric was not influenced significantly by temperature and time under the given conditions.

The relative molar ratios of S (the relatively total moles of syringaldehyde, syringic acid and acetosyringone) to G (the relatively total moles of vanillin, vanillic acid and acetovanillin) to H (the relatively total moles of *p*-hydroxybenzaldehyde and *p*-hydroxybenzoic acid) in the ten lignin oxidation products were 0.8:1.3:1, 0.5:0.8:1, 0.9:1.3:1, 0.9:0.8:1, 0.7:0.4:1, 0.8:1.1:1, 0.8:0.7:1, 0.6:0.6:1, 0.6:0.5:1, and 0.9:0.5:1, respectively. The result showed that organosolv lignin extracted with 1,3-butanediol from wheat straw was SGH type. It is well known that lignins in the primary lamella (P), secondary wall (S2), and compound middle lamellas (CML) have different structures.[24] The relatively lower monomeric ratios of S to G in lignin fractions L_1-L_3 (0.62-0.69) implied that lower temperature released lignins from the middle lamella, which contained more G-lignin.[25] In contrast, the relatively higher monomeric ratios of S to G in L_{10} (1.8) suggested that this lignin fraction was released mainly from the secondary walls, since the secondary wall contains more syringyl units than the middle lamella.[26]

Molecular weight distribution

The question whether the 1,3-butanediol treatment caused some lignin depolymerization was addressed by investigating the gel permeation chromatographic elution for the ten lignin samples. Table 4 gives the weight-average (M_w) and number-average (M_n) molecular weights and polydispersity (M_w/M_n) of the ten lignin fractions. Clearly, most of the lignin fractions showed no significant difference in their molecular-average weights, which ranged from 1500 to 2520g/mol. These data indicated that, under the conditions given, the organosolv had no substantial influence on the M_w of the lignins from wheat straw. An increase in treatment temperature from 100 to 180 °C led to growth of M_w from 1500 to 2520 g/mol, indicating that higher temperature between 100 and 180°C at least, in part, enhanced the solubilization of lignin with higher molecular weights from wheat straw using 1,3-butanediol.Similarly, the increase of treatment time from 1 to 3 h at 180 °C led a growth of M_w from 1680 to 2520 g/mol. On the contrary, further increase of treatment time to 5 h leads to a decrease of M_w to 2080 g/mol. This phenomenon suggested that the lignin fraction with higher molecular weight was dissolved with the extension of treatment time at 180 °C. However, the decrease of M_w was probably due to the minimal degradation of the lignin as a higher amount of *β*-*O*-4 linkage was cleaved.[19,27] The polydispersity index (M_w/M_n) of the lignin fractions prepared with 1,3-butanediol at 100 °C was 1.06, which was lower than that prepared at 180 °C (1.10). These results indicated that the lignin fractions prepared at higher temperature had a relatively lower uniformity of fragment size and percentage of condensed linkages as compared to those obtained at lower temperature.[28] Furthermore, the relatively low values of the polydispersity of these fractions indicated the relatively narrow molecular distributions of the lignin fractions.

FT-IR spectra

The functional groups and structural fragments of lignin fractions were investigated by FT-IR. Figure2illustrates the spectra of the four lignin fractions isolated by1,3-butanediol for 3 h at 100 °C (L_1), 120 °C (L_2), 140 °C (L_3), and 160 °C (L_4). As can be seen from the diagram, all the spectra showed few changes in peak intensities, confirming that the 'core' of the lignin structure did not change significantly under different temperatures. The absence of the band at 1745 cm^{-1} revealed that the labile ester bonds were almost cleaved during 1,3-butanediol treatment under the conditions given.[29] The shoulders at 1716 and 1653$cm^{-1}$are originated from the carbonyl stretching in unconjugated ketone and conjugated carboxylic groups, respectively. This is probably attributable to the occurrence of hydroxycinnamic acids.

Table 3
Yield (% lignin sample, w/w) of phenolic acids and aldehydes from alkaline nitrobenzene oxidation of lignin fractions

Phenolic acids and aldehydes	Lignin fractions[a]									
	L_1	L_2	L_3	L_4	L_5	L_6	L_7	L_8	L_9	L_{10}
p-hydroxybenzoic acid	1.63	2.88	2.47	1.15	1.60	0.79	1.46	1.84	1.67	1.53
Syringaldehyde	0.61	0.69	1.03	0.65	1.00	0.51	0.60	0.53	0.28	1.57
p-hydroxybenzadehyde	0.70	0.74	1.37	1.25	1.96	1.06	1.19	1.07	1.70	1.00
Vanillic acid	1.98	1.95	2.96	0.65	0.50	1.22	0.62	0.43	0.28	0.41
Syringic acid	1.95	1.83	3.32	2.43	1.80	1.55	2.47	2.02	1.89	1.65
Vanillin	1.55	1.38	2.50	1.60	1.04	1.12	1.51	1.29	1.46	0.87
Acetovanillone	0.23	0.20	0.69	0.15	0.09	0.17	0.21	0.25	0.12	0.08
p-coumaric acid	0.07	0.30	0.22	0.00	0.21	0.15	0.40	0.21	0.07	0.22
Acetosyringone	0.30	0.32	0.66	0.12	0.00	0.13	0.00	0.00	0.00	0.00
Ferulic acid	1.83	1.80	2.61	0.61	0.00	0.55	0.45	0.19	0.06	0.00
Total	10.84	12.09	17.83	8.60	8.20	7.24	8.92	7.83	7.53	7.33
Molar ratio(G:S:H)[b]	1.3:0.8:1	0.8:0.5:1	1.3:0.9:1	0.8:0.9:1	0.4:0.7:1	1.1:0.8:1	0.7:0.8:1	0.6:0.6:1	0.5:0.6:1	0.5:0.9:1

[a]Corresponding to the lignin fractions in Table 2; [b]G represents the sum of total moles of vanillin, vanillic acid, and acetovanillone; S represents the sum of total moles of syringaldehyde and syringic acid; and H represents the sum of total moles of *p*-hydroxybenzoic acid and *p*-hydroxybenzaldehyde

Table 4
Weight-average (M_w), number-average (M_n) molecular weights and polydispersity (M_w/M_n) of acid-insoluble lignin fractions

	Lignin fractions[a]									
	L_1	L_2	L_3	L_4	L_5	L_6	L_7	L_8	L_9	L_{10}
M_w	1.500	1.980	2.190	2.400	2.520	1.680	2.090	2.100	2.310	2.080
M_n	1.420	1.820	1.930	2.230	2.300	1.600	2.030	2.020	2.100	1.780
M_w/M_n	1.06	1.09	1.13	1.07	1.10	1.05	1.03	1.04	1.10	1.17

[a] Corresponding to the lignin fractions in Table 2

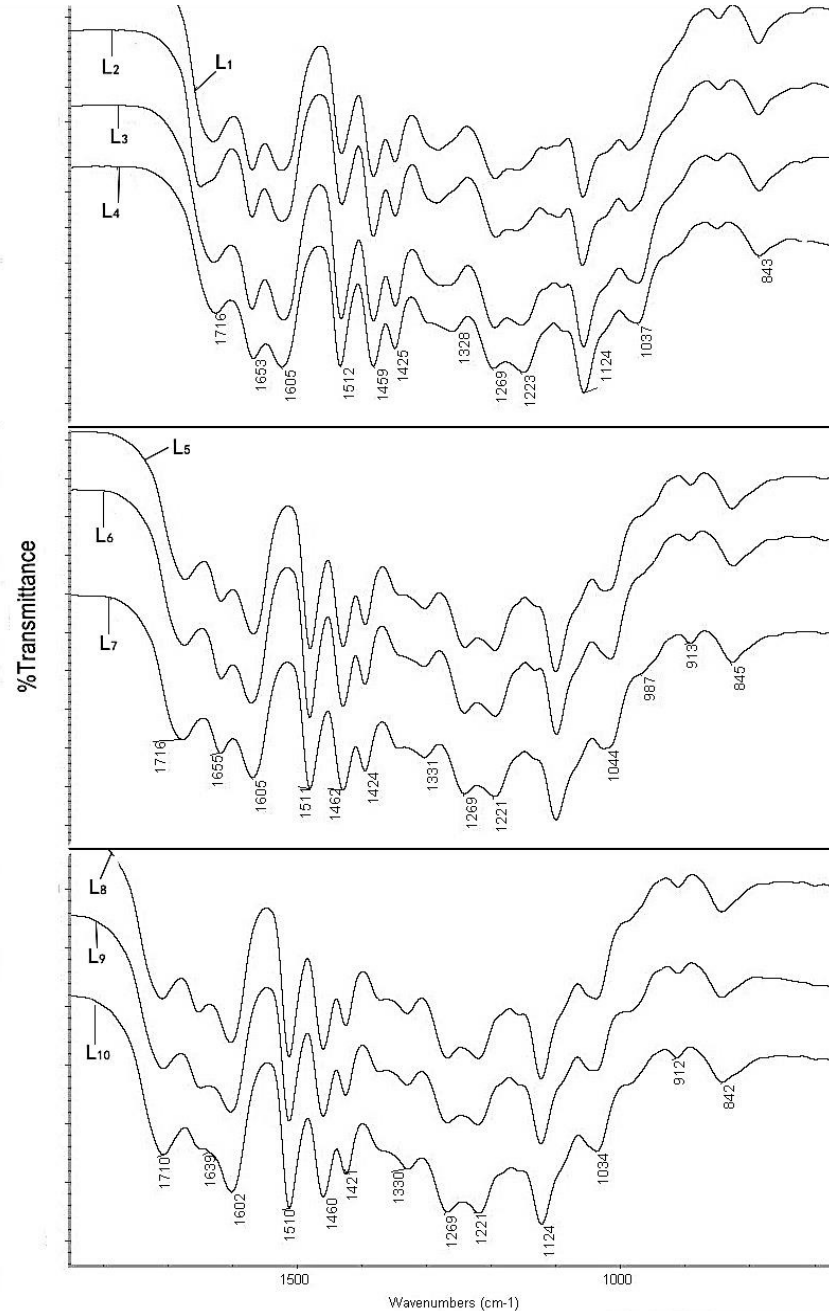

Figure 2: FT-IR spectra of lignin preparations obtained from wheat straw under various processing conditions

Aromatic skeleton vibrations give three strong peaks at 1605, 1512, and 1425 cm^{-1},indicating a primary structure of the four lignin fractions.[30] Moreover, characteristic peaks located at1459 (asymmertric C-H deformations), 1328 (C-O stretching of syringyl), 1269 (C-O stretching of guaiacyl), 1223 (C-O and C=O stretching of aromatic ring), 1124 (aromatic C-H in-plane deformation, syringyl type), 1037 (aromatic C-H in-plane deformation plus C-O in primary alcohols, guaiacyl type), and 843 cm^{-1}(aromatic C-H out of plane deformation) are observed.[31] Besides, the

weak signal at 1156 cm^{-1} shows the presence of a *p*-coumaric ester group, typical for GSH lignins.[32] In addition, the spectra of L_1, L_2, and L_3demonstrated that these lignins are G-rich lignins according to the lignin classification system by Faix *et al*. (1991) because the ratios of 1512 cm^{-1}/1459 cm^{-1} and 1269 cm^{-1}/1223 cm^{-1} are higher in L_1, L_2, and L_3 than those in the L_4 fraction.

For the L_4 lignin fraction, which has a relatively higher content of S units, the band intensities of 1512, 1269, 1124, and 1037 cm^{-1} decrease, while the band intensity at 1328 cm^{-1} increases. In general, G-lignin has two bands at 858 and 820 cm^{-1} and exhibits a maximum at 1140 cm^{-1}. A few percent S units in lignin is enough to change the two absorption bands (858 and 820 cm^{-1}) into a singular band at 843 cm^{-1}, and the maximum peak shifts from 1140 cm^{-1} to a wave number below 1128 cm^{-1}.[33] Therefore, it is clear that the lignin fractions of L_1, L_2, and L_3 were rich in G units, while the L_4 lignin preparation contained more amounts of S units, corresponding to the results obtained by nitrobenzene oxidation in Table 2. Moreover, it was noted that the small band at 1163 cm^{-1} observed in the spectra of lignin fractions L_1 and L_2, while this band disappeared in the lignin samples L_3 and L_4, suggesting lignin fractions L_1 and L_2 contain tiny ester bands, while the ester bonds in the lignin fractions (L_3 and L_4) were fundamentally cleaved at the higher temperatures.

Figure 2 also illustrates FT-IR spectra of the lignin fractions L_5, L_6, and L_7.The similar spectral profiles indicated similar structures of the lignin fractions. Interestingly, on a close comparison of the spectra, a small difference was observed. The absorption intensities at 1512 and 1269 cm^{-1} were lower than those at 1459 and 1223 cm^{-1}, respectively, and a maximum band occurred at 1124 cm^{-1}. This phenomenon indicated that the lignin fractions L_5 and L_7 contained higher amounts of S units than G units, which was paralleled to the results of G:S:H in Table 2. The bottom part of Figure 2 shows a similar increasing trend of S units in the samples L_8-L_{10}, providing evidence for the fact that longer treatment time accelerated higher yield of S units.

^{13}C-NMR spectrum

To obtain further knowledge about the composition and structural features of the lignin fractions, L_9 isolated with 1,3-butanediol at 180°C for 2.5 h was investigated by ^{13}C-NMR spectroscopy (Figure 3). Table 5 lists an extensive compilation of signal assignments of a typical lignin according to the previous studies.[34-36] As expected, the most striking characteristic of the ^{13}C-NMR spectrum is the near disappearance of typical polysaccharide signals between 57 and 103 ppm.[35,37] The spectrum shows signals at 63.0 (C-5, xyl internal unit), 65.8 (C-5, xyl non-reducing end unit), and 69.5 ppm (C-4, xyl non-reducing end unit) for polysaccharides, but the peak intensities were rather weak.[38] A signal at 174.7 ppm is presumed due to C-6 in methyl uronates (C=O in aliphatic acids or esters) of the glucuronic acid residue, which were esterified to the side chains of lignin.

The region from 104.4 to 160.0 ppm is assigned to the aromatic moiety of lignin. The syringyl (S) lignin units are identified by the signals at 152.2 (C-3/C-5, S), 138.2 (C-4, S etherified), 134.9 (C-1, S etherified), 104.3 ppm (C-2/C-6, S). Guaiacyl (G) lignin units give signals at 149.4 (C-4, G etherified), 147.7-148.0 (C-3,G), 144.9 (C-4, G non-etherified), 134.9 (C-1, G etherified), 130.3 (C-1, G non-etherified), 120.0-119.4 (C-6, G), and 111.2 ppm (C-2, G), respectively. In addition, the *p*-hydroxyphenyl (H) residues were observed at 128.0 ppm (C-2/C-6, H). These signals revealed that the lignin fractions could be justified as SGH-lignin, corresponding to the results obtained by nitrobenzene oxidation and FT-IR spectra. A very strong signal at 56.0 ppm belongs to the OCH_3 in syringyl and guaiacyl units. The ^{13}C-NMR spectrum over the range 100-160 ppm is more informative for both the distribution of linkages and substitutions.[24] It is evident that the signals at 168.1 (C-γ, PC ester), 130.3 (C-2/C-6, PC ester), 125.7 (C-1, PC ester), 115.6 and 115.8 ppm (C-3/C-5, PC ester) are originated from esterified *p*-coumaric acid. Etherified ferulic acid was observed with signals at 167.7 (C-γ, FE ether) and 144.9 ppm (C-α, FE ether). A weak signal at 122.9 ppm (C-6, FE ester) implied the minimal esterified ferulic acid.[39] Therefore, these observations suggested that *p*-coumaric acid was associated to lignin by ester bonds at side chains, while ferulic acid was linked to lignin by ether bonds at side chains in the cell wall of wheat straw. The lignin fraction linkages β-*O*-4 ether bonds (C-α, 72.4 ppm; C-β, 86.0-84.0 ppm; C-γ, 60.2 ppm), less amounts of β-β (C-2/C-6, 104.4 ppm; C-γ, 71.7 ppm) and β-5 (C-α, 87.0 ppm; C-γ, 65.3

ppm) carbon-carbon linkages were present between the lignin structural units.

The signals suggested that the linkages in L_9 are composed mainly of *β-O*-4 ether bonds with small amounts of *β-β* and *β*-5 carbon-carbon linkages, and the *β*-aryl ether linkages between the lignin structural units were not substantially broken by 1,3-butanediol under the conditions given.

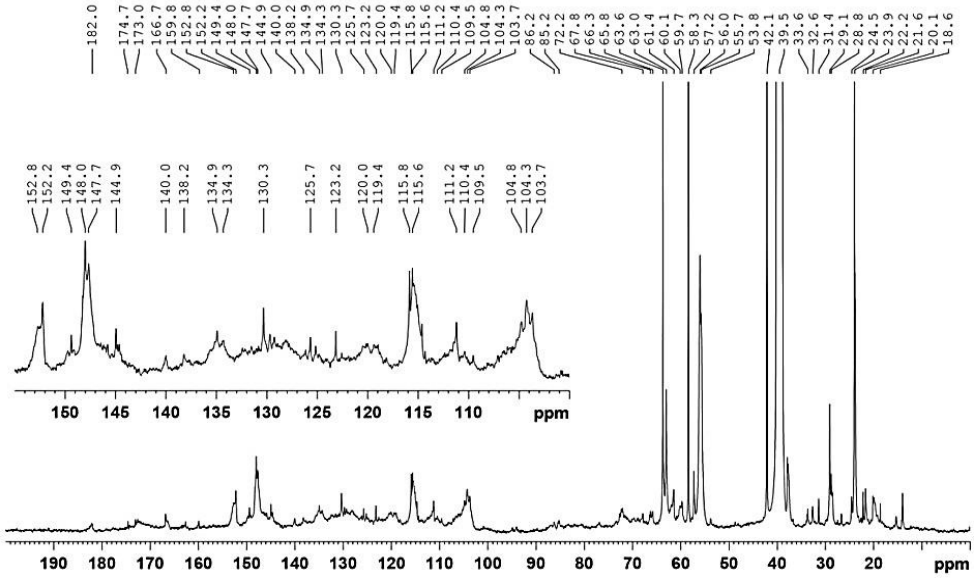

Figure 3: NMR spectra of lignin fraction extracted with 1,3-butanediol at 180 ºC for 2.5 h from dewaxed wheat straw

Table 5
Carbon chemical shifts (δ, ppm) and assignment of the lignin preparation L_9 in ^{13}C NMR spectrum

δ (ppm)	Assignments[a]	δ (ppm)	Assignments[a]
166.7	C-γ, PC ester	115.6	C-3/C-5, PC ester
152.2	C-3/C-5, S etherified	111.2	C-2, G
149.4	C-4, G etherified	104.3	C-2/C-6,Setherified
147.7-148.0	C-3, G	86.2	C-β, in β-O-4 units
144.9	C-4, G non-etherified	72.2	C-α, in β-O-4 units
138.2	C-4, S etherified	69.5	C-4, xylnonreducing end unit
134.9	C-1, S etherified and C-1, G etherified	65.8	C-5, xylnonreducing end unit
130.3	C-2/C-6, PC ester	63.0	C-5, xyl internal unit
128.0	C-2/C-6, H	60.1	C-γ, in β-O-4 units
125.7	C-1, PC ester	56.0	$–OCH_3$, S and G
122.9	C-6, FE ester	25.8-33.6	*α/β*-methylenein*n*-propyl side chains
119.4-120.0	C-6, G	14.1	*γ*–methylin*n*-propyl side chains
115.8	C-3/C-5, PC ester		

[a] Abbreviations: G, guaiacyl unit; S, syringyl unit; H, p-hydroxylphenyl unit; PC, p-coumaric acid; FE, ferulic acid; Xyl, xylose

2D HSQC ^{13}C-^{1}H correlation NMR analysis

2D HSQC NMR technique is a powerful tool for the detailed understanding of the lignin structure and has been widely applied to lignin characterization.[40] To obtain the detailed molecular structures of these lignins, L_9 isolated with 1,3-butanediol at 180°C for 2.5 hwas analyzed by solution 2D HSQC NMR. The spectrum shows three regions corresponding to aliphatic, side-chain, and aromatic ^{13}C-^{1}H correlations. The aliphatic (non-oxygenated) region shows signals with little structural

information. The aliphatic-oxygenated (side chain, δ_C/δ_H 50-90/3.0-5.5) and aromatic (δ_C/δ_H 100-150/6.0-8.0) regions of the spectra of the lignin are shown in Figure 4. The main cross-signals of L_9 in the HSQC spectra are assigned by comparison with the published literature and listed in Table 6.[40-44] Additionally, the main substructures of L_9 are depicted in Figure 5.

The side-chain region (δ_C/δ_H 50-90/2.5-6.0) of the 2D-HSQC NMR spectra provides useful information about the inter-coupling bonds present in lignin (*β-O*-4, *β-β*, *β*-5, *etc.*). The prominent correlating signals observed in the spectra are methoxyl groups (OCH_3, δ_C/δ_H 55.9/3.72) and *β-O*-4 (A) aryl ether linkages, corresponding to the results obtained by ^{13}C-NMR spectrum. The correlations at δ_C/δ_H 71.8/4.82, δ_C/δ_H 85.8-83.4/4.42-4.12 and δ_C/δ_H 59.5/3.75-3.35 belong to the C_α-H_α, C_β-H_β and C_γ-H_γ correlations of the *β-O*-4 ether substructures, respectively. Interestingly, the C_α-H_α correlation in *β-O*-4 substructure of S units ($A_{\alpha(S)}$) was detected at δ_C/δ_H 71.8/4.82, which was somewhat overlapped with the signal of C_α-H_α in *β-O*-4substructure of G units ($A_{\alpha(G)}$ δ_C/δ_H 71.0/4.74). Moreover, resinol (*β-β*) (B) substructures with strong signals were also observed in the HSQC spectra. The signals were detected with their C_α-H_α, C_β-H_β, and double C_γ-H_γ correlations at δ_C/δ_H 85.0/4.63, 53.8/3.05, 70.9/4.18, and 70.9/3.85, respectively.

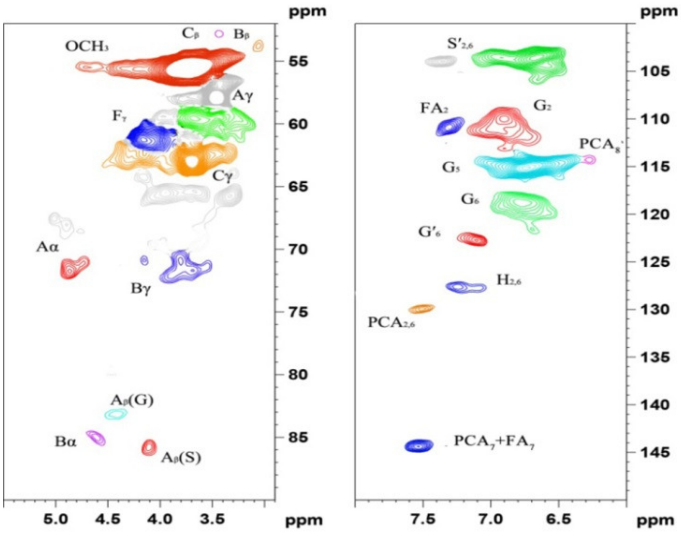

Figure 4: 2D-HSQC NMR spectra of L_9(treated with 1,3-butanediol at 180 ºC for 2.5 h); guaiacyl unit linked with a carbonyl group at C_α (phenolic); (S) syringyl unit; (S′) oxidized syringyl unit linked with a carbonyl group at C_α (phenolic); (FA) ferulate; (PCA) *p*-coumarate

Table 6
Assignment of main lignin ^{1}H-^{13}C cross-signals in the HSQC spectra of the lignin fraction L_9

Labels	δC/δH (ppm)	Assignments
$PCA_7$and FA_7	144.5/7.54	C_α-H_α in *p*-coumarate (PCA) and ferulate (FA)
PCA_8	114.2/6.28	C_β-H $_\beta$in *p*-coumarate (PCA)
$PCA_{2,6}$	129.6/7.48	$C_{2,6}$-$H_{2,6}$ in *p*-coumarate (PCA)
$H_{2,6}$	127.8/7.25	$C_{2,6}$-$H_{2,6}$ in H units (H)
G'_6	122.8/7.12	C_6-H_6 in oxidized (C=O) guaiacyl units (G')
G_6	118.8/6.75	C_6-H_6 in guaiacyl units (G)
D_2	114.0/6.25	C_2-H_2 in spirodienone substructures (D)
G_5	115.2/6.75	C_5-H_5 in guaiacyl units (G)
FA_2	110.8/7.28	C_2-H_2 in ferulate (FA)
G_2	110.1/6.92	C_2-H_2 in guaiacyl units (G)
$T_{2,6}$	103.8/7.38	$C'_{2,6}$-$H'_{2,6}$ in tricin (T)

$S'_{2,6}$	105.5/7.30	$C_{2,6}$-$H_{2,6}$ in oxidized S units (S')
$S_{2,6}$	103.8/6.60 and 6.88	$C_{2,6}$-$H_{2,6}$ in syringyl units (S)
A_β(S)	85.8/4.12	C_β-H_β in β-O-4 linked to S (A, *Erythro*)
B_α	85.0/4.63	C_α-H_α in β-βresinol (B)
A_β(G)	83.4/4.42	C_β-H_β in β-O-4 linked to G (A)
A_α	71.8/4.82	C_α-H_α in β-O-4 unit (A)
B_γ	70.9/4.18 and 3.85	C_γ-H_γ in β-βresinol (B)
A'_γ/A''_γ	63.8/3.62	H_γ-C_γ in γ-acylatedβ-O-4 substructures (A' and A'')
C_γ	62.7/3.78 and 4.30	C_γ-H_γ in phenylcoumaran (C)
F_γ	61.2/4.15	C_γ-H_γ in cinnamyl alcohol end-groups (F)
A_γ	59.5/3.35-3.75	C_γ-H_γ in β-O-4 substructures (A)
OCH_3	55.9/3.72	C-H in methoxyls
B_β	53.8/3.05	C_β-H_β in β-β (resinol) (B)
C_β	52.8/3.41	C_β-H_β in phenylcoumaran (C)

[a]Abbreviations: G, guaiacyl unit; S, syringyl unit; H, *p*-hydroxylphenyl unit; PC, *p*-coumaric acid; FE, ferulic acid; Xyl, xylose; [b] ND = not detected

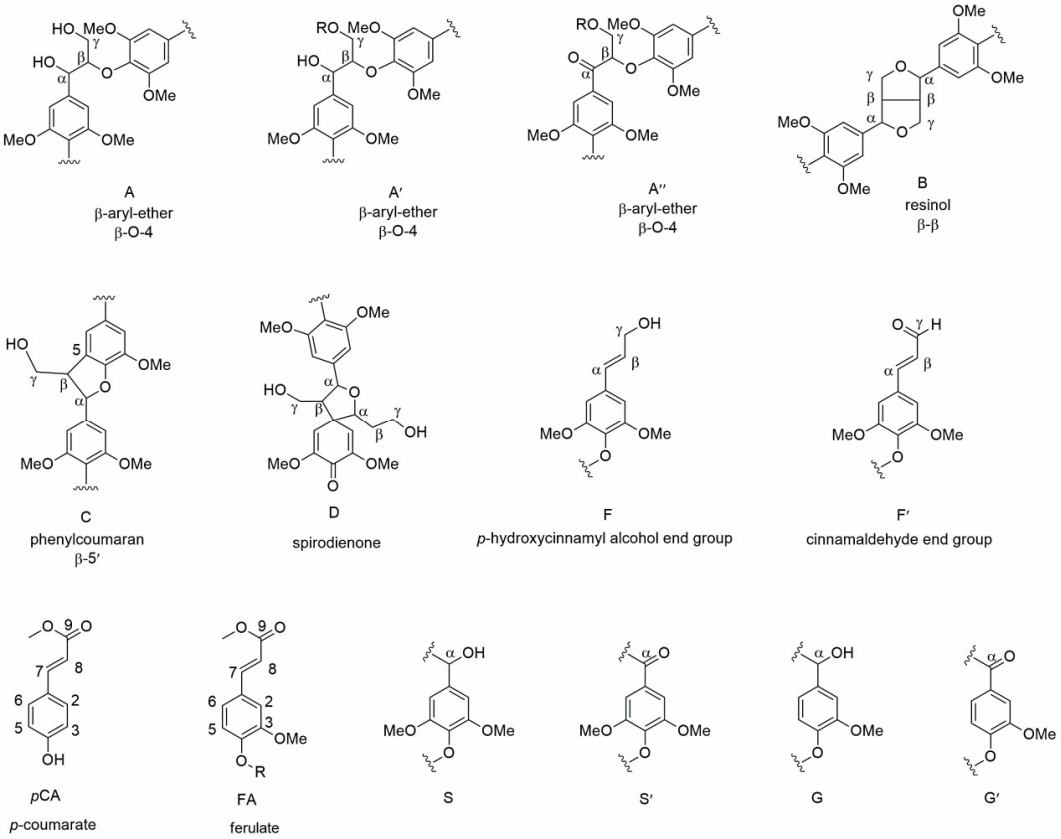

Figure 5: Main structures of lignin fractions isolated with 1,3-butanediol from wheat straw, involving side-chain linkages and aromatic units identified by 2D HSQC: (A) β-*O*-4 linkages; (A′) β-*O*-4 linkages with acetylated γ-carbon; (A″) β-*O*-4 linkages with *p*-coumaroylated γ-carbon; (B) resinol structures formed by β-β, α-*O*-γ, and γ-*O*-αlinkages; (C) phenylcoumarance structures formed by β-5 and α-*O*-4 linkages; (D) spirodienone structures formed by β-1 and α-*O*-α linkages; (F) cinnamyl alcohol end-groups; (F′) cinnamyl aldehyde end-groups; (G) guaiacyl unit; oxidized

The main cross-signals in the aromatic region of the HSQC spectrum corresponded to the aromatic rings of L_9. Signals from syringyl (S), guaiacyl (G) and *p*-hydroxyphenyl (H) units were observed. The S-type lignin units showed a prominent signal for the $C_{2,6}$-$H_{2,6}$ correlations at

δ_C/δ_H 103.8/6.60+6.88, whereas the G-type lignin showed different correlations for C_2-H_2, C_5-H_5, and C_6-H_6 at δ_C/δ_H 110.1/6.92, 115.2/6.75, and 118.8/6.75, respectively. In addition, signals corresponding to $C_{2,6}$-$H_{2,6}$ correlations in C_α-oxidized S units (S′, δ_C/δ_H 105.5/7.30) were also present in the spectra with a lower amount.Two signals in H units were assigned to $C_{3,5}$-$H_{3,5}$ and $C_{2,6}$-$H_{2,6}$ correlations at δ_C/δ_H 115.4/6.63 and 127.8/7.25, respectively. Apparently, the 2D-HSQC spectra clearly revealed that more syringyl units existed in L_9, which was also reflected by the results of G:S:H in Table 3.

In addition to the typical substructures, the esterified *p*-coumaric acid structure (A") was also identified. The signals corresponding to correlations PCA_8 and PCA_7 were revealed by δ_C/δ_H 114.0/6.25 and 144.5/7.54, respectively, implying that most of wheat straw lignins isolated with 1,3-butanediol were esterified by *p*-coumaric acid to the lignin polymers at C_γ position, which is in agreement with previous studies.[45-47] Moreover, signals for ferulate (FA), which was believed to be responsible for cell wall cross-linking in gramineous biomass, are also well resolved.[48] The signals corresponding to correlations FA_2 and FA_6 were observed at δ_C/δ_H 110.8/7.28 and 123.1/7.17 (the later one was overlapped with G'_6), respectively. Meanwhile, FA_7 correlation coincided with that of PCA_7 at δ_C/δ_H 144.5/7.54.

CONCLUSION

Lignins from wheat straw were successfully extracted by1,3-butanediolunder different conditions and were comprehensively characterized. The results demonstrated that the HBS pretreatment conditions had significant effects on the yields and chemical compositionof the lignin. The yield of the extracted lignin increased with an increase in reaction temperature and time. In addition, the results reflected that the major *β-O*-4 linkages and the carbon-carbon linkages (*β-β* and *β*-5) in lignin structures were essentially stable to 1,3-butanediol treatment. Therefore, this proposed procedure is promising for isolating high-purity lignin from wheat straw.

ACKNOWLEDGEMENTS: The authors are grateful for the financial support from the National Natural Science Foundation of China (31370368, 31170557) and West Light Foundation of The Chinese Academy of Science.

REFERENCES

[1] F. Zikeli, T. Ters, K. Fackler, E. Srebotnik and J. B. Li, *Ind. Crop. Prod*., **61**, 249 (2014).
[2] J. C. del Río, J. Rencoret, P. Prinsen, Á. T. Martínez, J. Ralph *et al*., *J. Agric. Food Chem.* ,**60**, 5922 (2012).
[3] M. B. Roncero, A. L. Torres, J. F. Colom and T. Vidal, *Bioresour. Technol*., **87**, 305 (2003).
[4] Q.Y ang, S. B. Wu, R. Louand G. J. Lv, *Wood Sci. Technol.* ,**45**, 419 (2011).
[5] K. V. Sarkanen and H. L. Hergert, in "Lignins: Occurrence, Formation, Structure and Reactions", edited by K. V. Sarkanen and C. H. Ludwig, John Wiley & Sons, Inc., 1971, pp. 43-94.
[6] Q. Lu, W. Z. Li and X. F. Zhu, *Energ. Convers. Manag*., **50**, 1376 (2009).
[7] A. Scalbert, B. Monties, J. Y. Lallemand, E. Guittet and C. Rolando, *Phytochemistry*, **24**, 1359 (1985).
[8] F. Xu, R. C. Sun, M. Z. Zhai, J. X. Sun, J. X. Jiang *et al*., *J. Appl. Polym. Sci*., **108**, 1158 (2008).
[9] K. Yamagishi, T. Kimura and T. Watanabe, *Bioresour. Technol*., **102**, 6937 (2011).
[10] M.Jahan, Z. Lee and Y. Jin,*Cellulose Chem. Technol*., **39**, 85 (2005).
[11] D. E.Kim and X. J. Pan,*Ind. Eng. Chem. Res*., **49**, 12156 (2010).
[12] R. Sanchez, A. Rodriguez, J. C.Garcia, A. Rosal and L. Jimenez, *Bioresour. Technol*., **102**, 1308 (2011).
[13] C. G. Boeriu, F. I. Fitigau, R. J. A. Gosselink, A. E. Frissen, J. Stoutjesdijk *et al*., *Ind. Crop. Prod*., **62**, 481 (2014).
[14] A. Rodríguez, L. Serrano, A. Moral, A. Pérez and L. Jiménez, *Bioresour. Technol*., **99**, 1743 (2008).
[15] G. Wegener and D. Fengel, *Tappi J*.,**62**,97 (1979).
[16] N. Curreli, M. B. Fadda, A. Rescigno, A. C. Rinaldi, G. Soddu *et al*., *Process Biochem*., **32**, 665 (1997).
[17] A. Johansson, O. Aaltonen and P. Ylinen, *Biomass*, **13**, 45 (1987).
[18] T.Kinshimonto and Y. Sano, *Holzforschung*, **56**, 623 (2002).
[19] R. C. Sun, J. Tomkinson, S. Q. Wang and W. Zhu, *Polym. Degrad. Stabil*., **67**, 101 (2000).
[20] P. Peng, F. Peng, J. Bian, F. Xu, R. C. Sun *et al*., *Carbohyd. Polym*., **86**, 883 (2011).
[21] F. Peng, J. L. Ren, F. Xu, J. Bian, P. Peng *et al*., *J. Agric. Food Chem*., **57**, 6305 (2009).
[22] G. J acquet, B. Pollet, C. Lapierre, F. Mhamdi and C. Rolando, *J. Agric. Food Chem*., **43**, 2746 (1995).
[23] T. Kondo, T. Watanabe, T. Ohshita and T. Kyuma, *J. Sci. Food Agric*., **68**, 383 (1995).
[24] Z. Hu, T. F. Yeh, H. Chang, Y. Matsumoto and J. F. Kadla, *Holzforschung*, **60**, 389 (2006).
[25] F. Xu, Q. A.Zhou, J. X. Sun, C. F. Liu, J. L. Ren *et al*., *Process Biochem*., **42**, 913 (2007).
[26] H. Önnerued and G. Gellerstedt, *Holzforschung*, **57**, 255 (2003).

[27] S. L. Sun, J. L. Wen, M. G. Ma and R. C. Sun, *J. Agric. Food Chem.*, **62**, 8120 (2014).
[28] S. S. Y. Tan, D. R. MacFarlane, J. Upfal, L. A. Edye, W. O. S. Doherty *et al.*, *Green Chem.*, **11**, 339 (2009).
[29] D. She, X. N. Nie and F. Xu, *Cellulose Chem. Technol.*, **46**, 207 (2012).
[30] J. G. Buta, F. Zadraziland G. C. Galletti, *J. Agric. Food Chem.*, **37**, 1382 (1989).
[31] A. Tejado, C. Pena, J. Labidi, J. M. Echeverria and I. Mondragon, *Bioresour. Technol.*, **98**, 1655 (2007).
[32] S. Galkin, E. Ammalahti, I. Kilpelainen, G. Brunow and A. Hatakka, *Holzforschung*, **51**, 130 (1997).
[33] A.V. Marques, H. Pereira, J. Rodrigues, D. Meier and O. Faix, *J. Anal. Appl. Pyrol.*, **77**, 169 (2006).
[34] J. L. Wen, S. L. Sun, B. L. Xue and R. C. Sun, *Materials*, **6**, 359 (2013).
[35] E. Billa, D. P. Koullas, B. Monties and E. G. Koukios, *Ind. Crop. Prod.*, **6**, 297 (1997).
[36] H. H. Nimz, D. Robert, O. Faix and M. Nemr, *Holzforschung*, **35**, 16 (1981).
[37] R. C. Sun, B. Xiao and J. M. Lawther, *J. Appl. Polym. Sci.*, **68**, 1633 (1998).
[38] R. C. Sun, X. F. Sun and X. P. Xu, *J. Appl. Polym. Sci.*, **84**, 2512 (2002).
[39] D. S. Himmesbach and F. E. Barton, *J. Agric. Food Chem.*, **28**, 1203 (1980).
[40] A. T. Martínez, J. Rencoret, G. Marques, A. Gutiérrez, D. Ibarra *et al.*, *Phytochemistry*, **69**, 2831 (2008).
[41] K. Cheng, H. Sorek, H. Zimmermann, D. E. Wemmer and M. Pauly, *Anal. Chem.*, **85**, 3213 (2013).
[42] Y. C. Sun, J. K. Xu, F. Xu and R. C. Sun, *Ind. Crop. Prod.*, **47**, 277 (2013).
[43] J. L.Wen, S. L. Sun, B. L. Xue and R. C. Sun, *J. Agric. Food Chem.*, **61**, 635 (2013).
[44] F. Lu and J. Ralph, *Org. Biomol. Chem.*, **6**, 3681 (2008).
[45] M. Shimada, T. Fukuzukaand T. Higuchi, *Tappi J.*, **54**, 72 (1971).
[46] Y. Nakamura and T. Higuchi, *Holzforschung*, **30**,187 (1976).
[47] H. Kim and J. Ralph, *Org. Biomol. Chem.*, **8**, 576 (2010).
[48] J. L.Wen, B. L. Xue, F. Xu and R. C. Sun, *Bioenerg. Res.*, **5**, 886 (2012).