SPECTROSCOPIC COMPARISON OF ORGANOSOLV LIGNINS ISOLATED FROM WHEAT STRAW

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Glycerol lignin (LIG-Gly), dimethylformamide lignin (LIG-DMF) and mixed solvents (glycerol and dimethylformamide) lignin (LIG-Gly, DMF) were isolated from wheat straw by organosolv pulping. The physical characterization of three different lignins isolated under laboratory conditions from wheat straw was performed. The structures of lignins were studied by three non-destructive (FT-IR, ^{1}H and ^{13}C NMR spectroscopy) methods. The examination of FT-IR and ^{1}H NMR spectra did not show changes in the main structure of lignins, but based on ^{13}C NMR spectra of lignin obtained from glycerol (LIG-Gly), C-α, C-β and C-γ in β-O-4 and carbon–carbon bonds, such as β-β, β-5, were observed. In the case of lignin that was obtained from mixed solvents (LIG-Gly, DMF), the signals for C-β and C-γ in β-O-4 and β-1, β-5 and 5-5 type structures were detected. In the ^{13}C NMR spectra, signals relating to the ester groups obtained from the linkage of *p*-coumaric acids, ferulic acids and an ether linkage from ferulic acid and lignin were observed. In the lignin obtained from DMF (LIG-D), no fractures occurred.

Keywords: organosolv, wheat straw, lignin, FT-IR, NMR

INTRODUCTION

Lignin is one of the important chemical constituents of lignocellulosic materials like wood, straws, bagasse, bamboo and grasses.[1] The estimated amount of lignin on earth is 300 billion metric tons with an annual biosynthetic production rate of 20 billion metric tons.[2] Sulphur-free lignin, as a renewable raw material, is of increasing interest because of ecological and economical aspects. Lignins can enter the application process as a reagent and often react with other components of the product,[3,4] such as carbon fiber, activated carbon fibers, adhesives[5] and surfactants.[4]

The heterogenity of lignin is caused by variations in the polymer composition, size, crosslinking and functional groups. Differences exist in molecular composition and linkage type between the phenylpropane monomers.[4] Lignin is an amorphous polymer consisting of phenyl-propane units, and their precursors are three aromatic alcohols (monolignols), namely *p*-coumaryl, coniferyl and sinapyl alcohols (Figure 1 (1, 2, 3, respectively)). The respective aromatic constituents of these alcohols in the polymer are called *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) moieties.[2,6,7] Lignin is one of the major polymers occurring in the plant kingdom. But, despite extensive investigation, the complex and irregular structure of lignin is not fully understood.[1] Wood or straw lignin can be described as a three-dimensional macromolecule with high molecular weight in the range of 100 KD.[8]

The most common linkages formed during lignin biosynthesis are the β-O-4 ether linkages, followed by other types of ether and C–C linkages, such as α-O-4, β–β, β-5, and 5-5.[9] The major chemical functional groups in lignin

include hydroxy, methoxy, carbonyl and carboxylic groups in various amounts and proportions, depending on genetic origin and applied extraction processes.[4]

The spectroscopic techniques, such as infrared (IR) and ^{13}C nuclear magnetic resonance (^{13}C NMR) spectroscopy provide information on the whole structure of the polymer and avoid the possibility of degradation artifacts.[10]

Spectroscopic techniques, such as FT-IR and ^{13}C NMR can be used to investigate "polymeric" lignin either extracted from straw or *in situ* within the native straw substrate.[11] There have been several fractionation studies of wheat straw lignin.[11-16] The structures of straw and grass lignins are not yet fully understood, nor is their precise interrelationship with other cell-wall components known.[10,16]

The lignin from wheat straw was studied in comparison with lignin preparations of alkali lignin (LA), ball-milled straw lignin (LM), enzyme lignin (LE),[17,18] ultrasound-assisted alkali extractions,[19] organosolv lignin,[5] electrolysis and acid precipitation lignin of soda black liquor.[1]
In this research, we investigated the changes of lignin structure during the organosolv treatment processes. Three spectroscopic techniques, FT-IR, ^{1}H and ^{13}C NMR, were used for characterization of lignin structure.

EXPERIMENTAL

Raw material

The wheat straw used in this study was obtained from the local wheat field in Ardebil, Iran. Before pulping, the raw material was cleaned, cut into sample pieces of approximately 3 cm in length and dried at 105 °C for 24 h. The chemical composition of wheat straw was determined as follows: 43.1% Kurschner cellulose (), 19.31% lignin, 72.12% holocellulose, 6.8% ash, and 1.64% ethanol/dichloromethane extractable, on an oven-dry weight basis (moisture content 7.8%).

Analysis of raw materials

The starting materials and the products obtained from them were characterized according to the following standard methods: holocellulose,[20] Klason lignin (TAPPI T 222 om-98), cellulose (Kurschner–Hoffner),[21] ash (TAPPI T211 om-93), ethanol/dichloromethane extractable (TAPPI T 204 cm-97).[22]

Organosolv lignin extraction

Isolation of glycerol lignin

Organosolv glycerol treatment was carried out in a 2000 mL flask placed under reflux condition. The scheme for isolation of the organosolv lignin fraction is illustrated in Figure 2. In this experiment, 70 g oven-dried wheat straw (moisture content 7.8%) was weighed, charged into the flask and refluxed with glycerol as cooking liquor, at reflux cooking temperatures of 195-205 °C and cooking time of 90 min. The liquid/dry straw ratio (L/S) in cooking was set at 13/1. Then the flask was loaded with wheat straw and the cooking liquor and the mixture under stirring was mantel-heated to the reflux temperature. After the cooking process, the samples were filtered through a 400 mesh screen to remove any suspended matter. The filtrates were combined and 3 volumes of water were added to precipitate the glycerol organosolv lignin, which was collected by centrifugation. The obtained precipitate was centrifuged for 20 min at 3000 rpm, washed with distilled water to eliminate soluble carbohydrates, centrifuged again for 20 min at 3000 rpm, and then dried at 25 °C.

Isolation of DMF lignin

Organosolv dimethylformamide treatment was carried out in a 21 L batch cylindrical mini-digester (stainless steel 321). The mini-digester included an electrical heater, a motor actuator and required instruments for measurement and control of pressure and temperature. In this experiment, 100 g oven-dried wheat straw (moisture content 7.8%) was treated with dimethylformamide as cooking liquor, at a cooking temperature of 200 °C and cooking time of 90 min. The liquid/dry straw ratio (L/S) in cooking was set to 13/1. Then the mini-digester was loaded with wheat straw, the cooking liquor and the mixture under circulation. After the cooking process, the samples were filtered through a 400 mesh screen to remove any suspended matter. The solubilized lignin was obtained from the corresponding supernatants by reprecipitation after evaporation of all the organic solvents. Then DMF lignin was washed with distilled water to eliminate soluble carbohydrates, and dried at 25 °C for 24 h.

Isolation of mixed glycerol and DMF lignin

The treatment with glycerol and dimethylformamide mixed in a weight ratio of 50:50 was carried out in a 21 L batch cylindrical mini-digester. In this experiment, 100 g oven-dried wheat straw (moisture content 7.8%) was treated with 50:50 weight ratio of the two solvents as cooking liquor, at a cooking temperature of 200 °C and cooking time of 90 min. The liquid/dry straw ratio (L/S) in cooking was fixed (13/1). Then the mini-digester was loaded with wheat straw and the cooking liquor and the mixture under circulation. After the cooking process, the sample was filtered through a 400 mesh screen to remove any suspended matter. The washes were combined and 3 volumes of water were added to precipitate the mixed glycerol and DMF lignin, which was collected by centrifugation. The obtained

precipitate was centrifuged for 20 min at 3000 rpm, washed with distilled water to eliminate soluble carbohydrates, centrifuged again for 20 min at 3000 rpm, and then dried at 25 °C for 24 h.

Acetylation of lignin

200 mg of lignin was dissolved in a 1:1 acetic anhydride/pyridine mixture (2 mL) and stirred for 72 h at room temperature. Ethanol (25 mL) was added to the reaction mixture, left for 30 min, and then removed with a rotary evaporator. The addition and removal of ethanol was repeated several times to ensure complete removal of acetic acid and pyridine from the sample. Afterwards, the acetylated lignin was dissolved in chloroform (2 mL) and added drop-wise to diethyl ether (100 mL), followed by centrifugation. The precipitate was washed three times with diethyl ether and dried at 25 °C for 24 h.[23]

Structural characterization by spectroscopic techniques

FT-IR analysis was performed in a Shimadzu Fourier Transform Spectrometer (Japan). Pellets for IR analysis were prepared by mixing approximately 5 mg of sample with about 250 mg of potassium bromide (KBr), and then pressing the mixture into a pellet. The pellets were scanned between 4000 and 500 cm^{-1}. The solution-state ^{1}H NMR spectra were obtained on a Bruker DRX-Avance-500 spectrometer at 500 MHz. ^{1}H NMR spectra were recorded at 25 °C for 25 mg of sample dissolved in 1.0 ml $CDCl_3$. For each sample, 16 scans were collected. A 30° pulse flipping angle and a 13 ms acquisition time were used. All ^{13}C NMR experiments were performed on a Bruker Avance-400 spectrometer operating at 400 MHz, under total proton decoupled conditions. The spectra were recorded at 25 °C for 20 mg of sample dissolved in 1.0 mL DMSO-d_6 after 18,000 scans. A 90° pulse flipping angle (pulse program zgpg) and a 16.2 ms acquisition time were used.

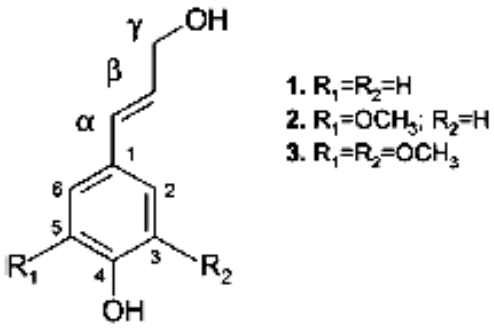


Figure 1: Lignin monolignols

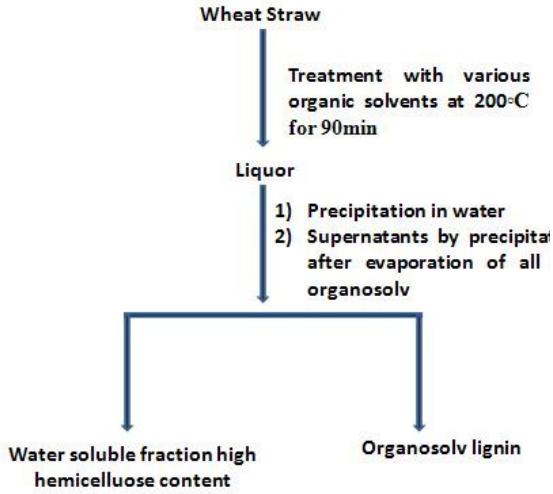


Figure 2: Scheme for isolation of lignin preparations obtained by treatment of wheat straw with organosolv

RESULTS AND DISCUSSION

Comparison of FT-IR spectra

Of the analysis techniques described in the literature, FT-IR spectroscopy presents interesting characteristics, such as high sensitivity and selectivity, high signal-to-noise ratio, accuracy, data handling facility, mechanical simplicity and short time and small amount of sample required for the analysis. In addition, the spectrum of a lignin sample gives an overall view of its chemical structure.[5]

Table 1 gives the assignment of FT-IR bands of the three organosolv lignins. The bands around 830 and 1120 cm^{-1} and a shoulder at 1159 cm^{-1} indicate the presence of guaiacyl (G), *p*-hydroxyphenyl (H) and syringyl (S) units.[8,23] All lignins showed a broad band within the range 3348 to 3425, which is attributed to the hydroxy groups in phenolic and aliphatic structure, and the bands around 2924 cm^{-1}, assigned to C-H stretching in methyl and methylene groups of the side chains. A band at 1710 cm^{-1} has been assigned to carbonyl stretching in unconjugated ketone and carboxyl groups.

A broad absorption region with sharp and distinct bands in the range of 900-1300 cm^{-1}

shows that this area is the area known as the fingerprint. The absence of any prominent bands at 1740 and 1750 cm^{-1} shows that the bonds between lignin and carbohydrates were broken, there resulting pure lignin free of carbohydrates.

The frequency 1425 cm^{-1} can be assigned to aromatic skeletal vibrations and O-CH_3 bending (G, S), R-O aryl ether. The prominent band at 1265 cm^{-1} corresponds to methoxy stretching[24] and absorption region 1212-1221 cm^{-1}, assigned to ring breathing with CO stretching. The band at 1121 cm^{-1} (aromatic C–H in-plane deformation, typical for S unit) confirmed the presence of S units in all lignins. The band at 1146 cm^{-1} was assigned to C-H in-plane deformation in guaiacyl[25] and the bands at 837 and 847 cm^{-1} to aromatic C-H out-of-plane bending. The bands at 837-847 cm^{-1} showed the external link of C-H 2 at 6 positions in syringyl units, the position of all the units indicated para-hydroxy phenyl.[5,25,26]

The bands at 1600, 1510 and 1425 cm^{-1} corresponded to aromatic ring vibrations of phenyl-propane (C9) skeleton,[9] in which the aromatic semicircle vibration (a vibration involving both C-C stretching and a change of the H-C-C bond angle) was assigned at 1510 cm^{-1}.[5]

The two bands at 1713 and 1656 cm^{-1} are due to carbonyls. The band at 1713 cm^{-1} was assigned to carbonyl stretching in unconjugated ketone and carboxyl groups. The 1656 cm^{-1} band is also a carbonyl stretching band due to para-substituted ketones or aryl aldehydes. The carbonyl band for the *p*-coumaric acid should appear at 1666 cm^{-1} and that for the methyl ester at 1689 cm^{-1}.[9,27]

The FT-IR spectra of acetylated lignins are shown in Fig. 3. The absorption band related to OH stretching at 3431 cm^{-1} disappeared, and bands related to phenolic ester compounds appeared at 1765 and 1370 cm^{-1}. The band at 1740 cm^{-1} indicates the stretching vibration of aliphatic carboxylic ester C=O group. Also, the band at 1084 cm^{-1} shows a C-O bond bending in secondary alcohol and aliphatic ethers, only the FT-IR spectrum of acetylated lignin was obtained from glycerol.[25]

Comparison of ^{1}H NMR spectra

Table 2 gives the ^{1}H NMR signal assignment of three organosolv lignins. ^{1}H NMR signal intensity in the range of 6.2-7.75 ppm provides the level of substitution on the aromatic ring of lignins.[1,5] The H_β in β-0-4 structures exhibits two weak signals between 5.2 and 4.5 ppm.

Methoxy protons (-OCH_3) give a sharp signal at 3.7 ppm.[1,5] The peak at δ 3.08 ppm of lignin was attributed to H_β in β-β structures, and the peak at δ 5.44 ppm was assigned to H_α in β-5 structures or noncyclic benzyl aryl ethers were present in LIG-Gly and LIG-Gly, DMF and absent in LIG-DMF. The peaks at δ 4.60 (H_β) and 6.01 ppm (H_α) were essentially attributed to β-0-4 structures. The signal at δ 2.62 ppm was attributed to H_α in β-β structures.[25]

The aromatic and aliphatic acetoxy groups were assigned at 2.3 and 2.0 ppm, respectively.[4] Protons in aliphatic groups in xylanes produced signals between 2.0 and 0.8 ppm.[5] The presence of highly shielded protons in the range δ 0.38-1.58 ppm is noticeable. These protons are clearly due to methyl or methylene groups that are not attached directly to oxygen functions, carbonyl groups, aromatic systems, or other deshielding groups, but their structural origin seems to be hydrocarbon contaminant, because lignin does not include these groups.[1,25]

Comparison of ^{13}C NMR spectra

The ^{13}C NMR signal assignment for the three mentioned acetylated lignins that were obtained in DMSO-d_6 solvent are shown in Table 3. The spectra can be subdivided into regions in order to obtain information about lignin structures, particularly about monomer unit linkage.[9]

The signals in the region from δ 102 to δ 160 ppm originate from aromatic carbons.[1,5,8,9,29,30,31] Based on ^{13}C NMR spectra shown in Figure 4, the syringyl units in acetylated LIG-Gly were detected by signals at δ 153 ppm (etherified C-3, C-5), and δ 105-104 ppm (C-2, C-6), in acetylated LIG-DMF was detected by a signal at 153 ppm (etherified C-3, C-5) and in acetylated LIG-Gly, DMF were detected by signals at δ 153 ppm (etherified C-3, C-5), 145 ppm (C-5, C-3), 135.5 ppm (etherified C-1), 104.7 ppm (C-6, C-2).[5,8,9,23,31,32] The guaiacyl units in acetylated LIG-Gly and acetylated LIG-Gly, DMF produce signals at δ 112.1 ppm (C-2) and 145 ppm (non-etherified C-4), 135.5 (etherified C-1),[1,23] respectively, but the signal related to guaiacyl units in acetylated LIG-DMF was not observed.

The signals in acetylated LIG-Gly and LIG-Gly, DMF at about δ 128.5 ppm, δ 127.1 ppm, respectively,[5,23] were assigned to *p*-hydroxylphenyl (H) units and were not observed in LIG-DMF.

The hydroxycinnamic acids, mainly *p*-coumaric acid (4-hydroxy-trans-cinnamic) and ferulic acid (4-hydroxy-3-methoxy-trans-cinnaic) have been found to crosslink between lignins and polysaccharides in wheat straw. Because of this chemical nature of the lignin, it is practically impossible to extract lignins in pure form. *p*-coumaric acid and lignin fractions in wheat straw were mainly connected with ester linkages. Ferulic acid was associated with wheat straw lignin fractions through ether bonds and with polysaccharides through ester bonds (Figures 5, 6).[2]

In ^{13}C NMR spectra of lignins, the signals at δ 131.1 ppm were assigned to C-2, C-6, those at δ 124.3, 124.6 to C-1 and those at 145, 160.1 ppm to C-4, esterified *p*-coumaric acid in acetylated LIG-Gly and LIG-Gly, DMF,[5,8,9,26] respectively. Etherified ferulic acids exhibited signals at δ 168.7 ppm, esterified ferulic acid was detected with a signal at 123 ppm[5] in both acetylated LIG-Gly and LIG-Gly, DMF, and was absent in acetylated LIG-DMF.

Table 1
Assignment of FT-IR spectra of lignins from wheat straw

Band, cm^{-1}			Assignment
Gly	Gly+DMF	DMF	
3348	3425	3425	OH stretching
2848-2917	2851-2924	2852-2924	CH stretching in methyl & metylene group
1710	1709	1709	C=O stretching in unconjugated carbonyl compounds with aromatic ring
1684	1666	1666	C=O stretching in conjugated p-substitued aryl ketone groups
1645	1649	1647	Carbonyl stretching in γ-lactone
1597	1603	1614	C=C vibrations in aromatic rings
1510	1512	1512	C=C vibrations in aromatic rings
1462	1460	1460	C-H deformations in CH_3, CH_2
1425	1427	1425	-O-CH_3 bending (G, S) and R-O-aryl ether
1269	1263	1265	Guaiacyl ring breathing with C-O stretching
1212	1219	1221	Ring breathing with CO stretching
1146	1156	1156	C-H in-plane deformation in guaiacyl
1121	1121	1121	Aromatic C-H in-plane deformation for syringyl type
1033	1034	1030	Aromatic C-H in-plane deformation for guaiacyl type & C-O deformation in primary alcohol & C-O and C-H bending
847	841	837	Substituted aromatic rings & aromatic C-H out-of-plane bending

Table 2
^{1}H NMR signal assignment for three mentioned acetylated lignins obtained in chloroform-d solvent

δ, units/ppm			Assignment
Gly	GLy+DMF	DMF	
1.26	1.26	1.13-1.8	Hydrocarbon contaminant
2.05-2.11	2.04-2.11	2.04	Aliphatic acetate
2.303	2.30-2.07	2.33-2.38	Aromatic acetate
-	2.91	2.93	Hβ in β-β structures
3.79	3.78	3.83	Protons in methoxyl groups
4.16-4.27	-	4.19	Hγ in several structures
-	4.33	4.33	Hγ primarily in β-O-4 (erythro) and β-*5* structures
4.91-5.04	4.60-5.04	4.5-4.6	Hβ in β-O-4 structures
5.40	5.39-5.49	-	Hα in β-*5* structures and noncyclic benzyl aryl ethers
5.8	5.79-6.25	-	Hα with α-O-AC in β-O-4 and β-l structures, and vinyl protons
6.59-7.15	6.59-7.7	6.64-7.14	Ar-H in Ar-R, H-α in Ar-CH=CH-CHO, H-β in Ar-CH=CH-CHO H-α in Ar-CH = CH-CH_2OAC
7.38-7.75	7.29-7.55	7.29-7.38	Ar-H in Ar–COR

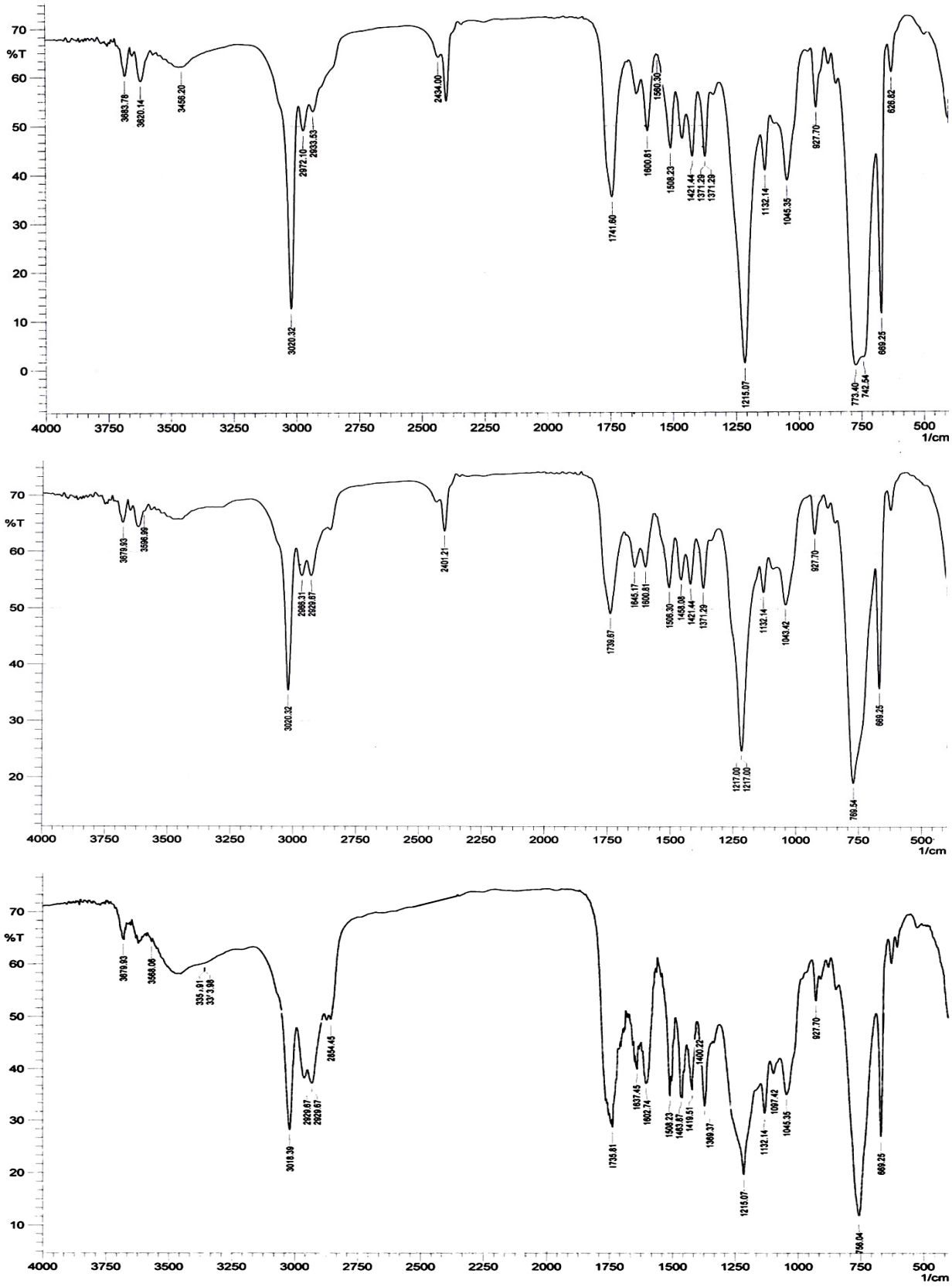
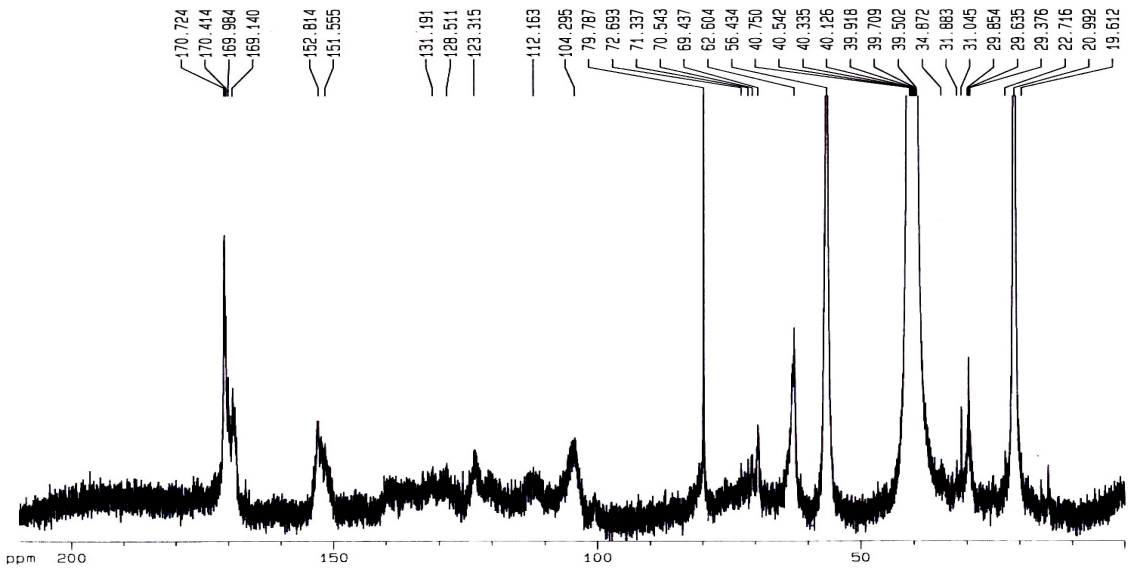


Figure 3: FT-IR spectra of acetylated lignins: a) LIG-Gly, b) LIG-DMF, c) LIG-Gly, DMF

Table 3
^{13}C NMR signal assignment for three mentioned acetylated lignins obtained in DMSO-d_6 solvent

Units/ppm			Assignment
Gly	Gly+DMF	DMF	
-	180	-	C=O in dienone or quinine
170.6	170.6	170.6	-C=O in acetyl group, –COOH, aliphatic
169	168.7	169	-C=O in acetyl group, CO in aromatic acetoxyl
-	160	-	C-4, *p*-coumaric acid ester, C-4 in *p*-hydroxylphenyl unit
153	153	153	C-3, C-5 in S etherified, C_α in cinnamaldehyde
151.5		-	C-3 G etherified
-	145	-	C-4 in G (nonetherified), *p*-coumaric acid
-	135.5	-	C-1 in S etherified; C-1, G etherified
131.	-	-	C-2, C-6, *p*-coumaric acid ester
128.5	127	-	C-2, C-6 in *p*-hydroxylphenyl unit
-	124.5	-	C-1, *p*-coumaric acid ester
123	123	-	C-6 in esterified ferulic acid
-	121	-	C-6, G
112		-	C-2, G
104.3	104.7	-	C-2, C-6 in S
79.8	79.8	79.8	C-β in β-O-4, $C_{\alpha-\beta}$ in α, β-diarylether, C-3 in arabinfuranose, C-α in β-β, β-5
72.7	-	-	C-γ in β-β, C-α in β-O-4
70.5-69.4	-	-	C-4, Xyl non-reducing end unit
-	-	67.8	C-5, Xyl non-reducing end unit
62.6	62.6		C-5, Xyl internal unit, C-γ in β-5, C-γ in β-O-4
56.4	56.4	56.4	OCH_3 in S and G units
-	51	-	C-β in β-1 and β-5
24-34.8	24-34		α,β-Methylene groups



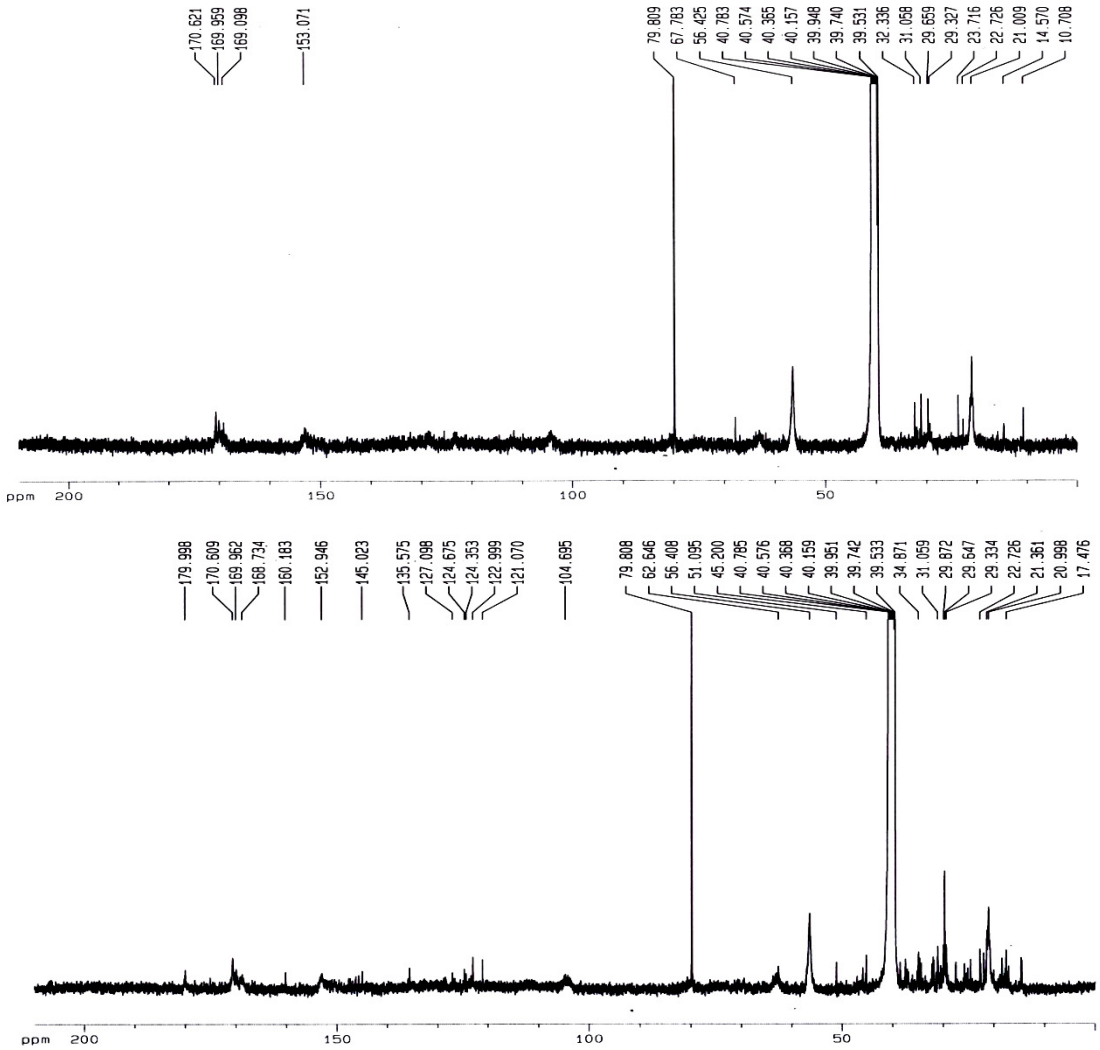


Figure 4: ^{13}C NMR spectra of acetylated lignins obtained by mentioned solvent delignifications: a) LIG-Gly, b) LIG-DMF, c) LIG-Gly, DMF

During the organosolv and aqueous ethanol delignification of wheat straw, the cleavage of α-aryl and β-aryl ether linkages in lignin precursors is a major factor in lignin breakdown.[2] The signal appearing in the region δ 79.8 ppm shows that a dissociation has taken place for all the three lignins obtained by cooking. Figure 7 shows the linkage of α-aryl ether and β-aryl ether in wheat straw lignin.

In wheat straw cell walls, the majority of wheat straw lignin is directly linked to arabinose side chains of xylan by ether bonds without hydroxycinnamic acids (Figure 8).[2] The presence of C-3 in arabinfuranose[5,25] was identified by the signal at δ 79.8 ppm, in all three lignins. β-O-4 linkages, which constitute the main intermonomeric linkage in lignin, were detected by signals at δ 72.69 ppm, 79.8 ppm and 62.6 ppm, which correspond to C-α, C-β and C-γ, respectively, in acetylated LIG-Gly.

Carbon atoms β, γ in β-O-4 structures in acetylated LIG-Gly, DMF gave signals at δ 79.8, 45 and 62.6 ppm and no signal was observed for C-α. The signal in acetylated LIG-DMF indicates only the cleavage of C-β in β-O-4 structure at δ 79.8 ppm.[5,23]

The presence of *p*-coumarate at the γ position of the lignin side chain could also contribute to this signal at δ 63 ppm[23] (Figure 9) in acetylated LIG-Gly and LIG-Gly, DMF. A very strong signal at δ 56 ppm is present in all spectra and it is attributed to the methoxy groups in syringyl and guiacyl units in all three types of lignins.[1,5,9,23]

In addition, other common C–C bonds, such as β-β, β-5, β-1 and 5-5′ type structures are also important substructures in lignin. The presence of β-β substructures in LIG-Gly can be seen from the C-γ signals at δ 69.4, 70.5 and 71.3 ppm, although C-α and C-β signals are overlapped with other signals. Also side chain carbons of C-γ and C-β in β-5 substructures can be observed at δ 62.6 ppm. Additionally, there is a β–5 structure in LIG-Gly, DMF, the signal at δ 124.7 ppm could also relate to 5-5′ substructures and that at δ 51 ppm was assigned to C-β in β–1 structures,[5,9,25] and were absent in acetylated LIG-DMF.

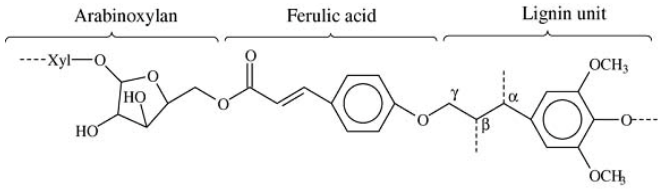


Figure 5: Lignin/phenolics–carbohydrate complex in wheat straw

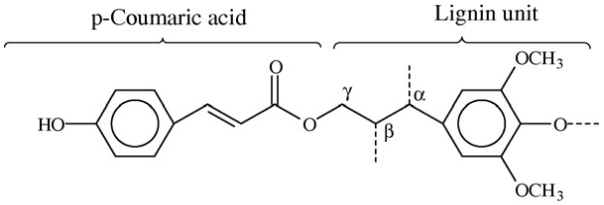


Figure 6: Ester bond between *p*-coumaric acid and lignin

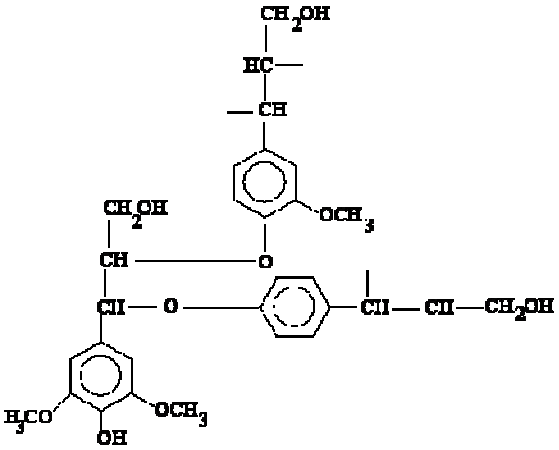


Figure 7: Simple representation of α-aryl and β-aryl ether linkages

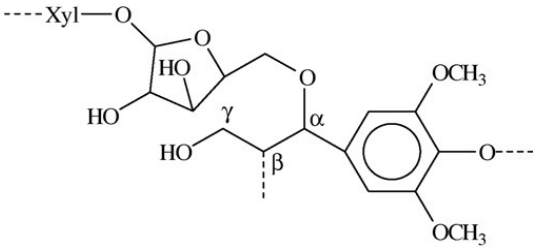


Figure 8: Direct attachment of arabinoxylan to lignin (benzyl ether linkage)

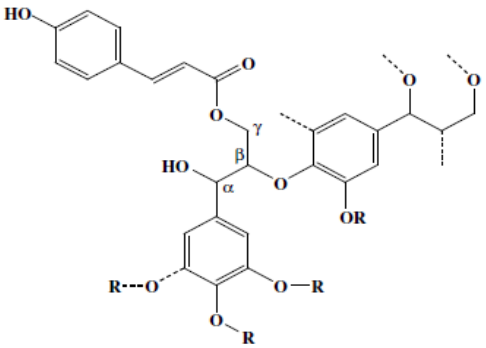


Figure 9: Representation of a coumarylated lignin fragment at γ position

The signals representing the γ-methyl, α and β-methylene groups in *n*-propyl side chains occurred at 10-14.5 and 21-23 ppm, respectively.[5] The known covalent linkages between carbohydrates and lignin, which are resistant to the isolation and purification steps, may explain the small proportions of polysaccharide suggested by the signal at 20 ppm (acetyl groups in hemicellulose).[30,32] In addition, CH_3 in acetyl group gave a strong signal at 22.0 ppm,[5,23] and it was observed in all three lignins.

CONCLUSION

The evaluation of FT-IR and ^{1}H NMR spectra showed no changes in the main structure of the lignins obtained by three methods of organosolv pulping. Based on ^{13}C NMR spectra, in acetylated LIG-Gly, C-α, C-β and C-γ in β-O-4 and C–C bonds, such as β-β and β-5, were observed. The signals C-β and C-γ in β-O-4 and β-1, β-5 and 5-5 type structures were detected for acetylated LIG-Gly, DMF. ^{13}C NMR signals related to ester linkage of *p*-coumaric acids, ferulic acids and ether linkage of ferulic acid and lignin were observed, while in acetylated LIG-DMF no fractures occurred.

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