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Physicochemical Characterization of Lignin Isolated with High Yield and Purity from Wheat Straw

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*Lignin preparation with high yield (85.71%) and purity (96.97%) from wheat straw was obtained by ball milling treatment and direct acidic dioxane extraction. This procedure gives a material that is representative of the whole lignin of wheat straw. The lignin fraction is distinguished by high β -O-4' structures and small amounts of condensed units (β -5', 5-5', and β -1') enriched in guaiacyl units. *p*-Coumaric acid is linked to lignin via γ -ester bond and a few ether bonds such as β -O-4', whereas ferulic*

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acid is linked to lignin by ether bonds, and its dehydrodimers (5-5'/8-O-4') are also incorporated into lignin polymer.

Keywords: Lignin; UV; Thermal analysis; NMR; Molecular weight; Wheat straw

Lignin is a phenolic polymer built up by oxidative coupling of three major C₆–C₃ (phenylpropanoid) units, namely, *trans-p*-coumaryl alcohol (H), guaiacyl (coniferyl) alcohol (G), and syringyl (sinapyl) alcohol (S), which form a randomized structure in a three-dimensional network inside the cell wall. The most frequent linkages in lignin are β-O-4', β-5', β-β', 5-5' and 4-O-5'. The physicochemical properties of straw lignins are known to be different from those of softwoods or hardwood, with straw lignins possessing characteristic alkali solubility^[1]. It has been shown that about 60–70% of the lignin from wheat straw is extractable with dilute alkali using temperatures lower than 100°C^[2]. The high solubility in alkali is recognized to arise from the considerable proportion of free hydroxyl groups present, G units, and smaller amounts of hydroxycinnamic acids, *p*-coumaric acid and ferulic acid. *p*-Coumaric acid is known to be extensively esterified or etherified with lignin, particularly at γ-position, comprising of 77% of total esters present in milled straw lignin. Ferulic acid is known to be etherified with lignin and esterified with arabinxylans at O-5 of arabinofuranyl residues^[3]. However, the actual chemistry of their attachment to lignin is not well understood^[1,3].

The content and structure of lignin differs among the species, among the mutants of a species, among the internodes of a culm, and among isolation methods^[4]. Wood contains 10–30% lignin, with high concentrations of lignin in the middle lamella and in the secondary wall. However, wheat straw comprises 14–17% lignin depending on morphological origin, with a low concentration of 31% lignin in the middle lamella of cortical fibers and 9% in the S2 region of the secondary wall^[5]. The composition of wheat straw is given in detail in Table I, according to the results obtained by Ernst et al.,^[6] Billa and Monties^[7], and Zhang et al.^[8] As can be seen, the content of lignin is much different among internodes, nodes and leaves in wheat straw. Lignin fractions from leaves, having a developed network of vessels that are enriched in G monomer units, are more condensed than those from internodes. The syringyl/guaiacyl (S/G) ratio is higher in the case of internode lignins^[9]. In general, the structural condensation of the lignin macromolecule is considered to decrease as the S/G ratio increases^[2].

TABLE I Composition within wheat straw

Fractions	Mass (%)	Klason lignin (%)	Total lignin (%)	Holocelluloses (%)
Internodes	68.5	18.9 ± 0.2	ND ^b	71.24
Nodes	4.2	14.8 ± 0.2	23.22	ND ^b
Leaves	20.3/5.5 ^a	13.5 ± 0.2	17.48	56.95

^a20.3/5.5 represent mass percent of sheaths and blades, respectively.

^bNot detectable.

Structurally, milled wood lignin is generally considered to be representative of native lignin; however, it typically consists of only 25–50% of theoretical lignin yield^[10], originating mainly from the secondary cell wall and middle lamella. Additionally, there is significant difference in the chemistry of lignin in the middle lamella and the secondary cell wall^[11]. The middle lamella of the fibers contains G and S units, whereas the secondary walls contain mainly S units in hardwood. The middle lamella lignin had a lower phenolic content and a lower non-condensed β -aryl ether structure than the secondary cell walls. Because of the heterogeneous nature of wheat straw lignin, milled lignin and alkali lignin could not represent the structure of whole lignin. Enzymatic hydrolysis has been used for lignin isolation since 1981. This method gives high lignin yields, but the isolated fraction also contains significant amounts of carbohydrates and some protein residues from enzymes^[12], likely derived from the secondary wall.

A modified method was proposed for the direct isolation of lignin with high yield and purity from wheat straw in this study, using ball milling treatment and acidic dioxane-water extraction. Toluene was added to ground wheat straw prior to ball milling treatment, which is found to improve the yield and purity of lignin. Utilization of acidic dioxane to isolate lignin was primarily targeted to reduce the level of contamination, particularly carbohydrates.

EXPERIMENTAL

Materials

Wheat straw (Variety Riband) was obtained from the Silsoe Research Institute (Silsoe, Bedfordshire). All weights and calculations were made on oven-dried samples (60°C, 16 h). The composition (w/w) of wheat straw used was cellulose 40.2%, hemicelluloses 38.8%, lignin 17.0%, wax 1.2%, ash 2.3%, and pectin 0.5% on a dry weight basis. The deviations of these

contents from their respective means were all less than 6%. All chemicals used were of analytical or reagent grade.

Extraction of Lignin Preparation

Dewaxed wheat straw (10 g) was ground, after adding 5 mL of toluene, in a one-gallon porcelain jar under a nitrogen atmosphere using porcelain balls. The jar was then placed on a rotary mill for a period of seven days. The ball-milled substrate was directly suspended in 200 mL dioxane-water (80:20 v/v) solution with a concentration of 0.05 mol/L of hydrochloric acid and refluxed at 85°C under nitrogen for 4 h. The resulting mixture solution was filtered and collected. The solid residue was washed with fresh dioxane until the filtrate was clear.

All the filtered solutions were then rotary-evaporated at 30°C to about 10 mL. Then 20 mL distilled water was added, followed by evaporation under vacuum that allowed complete elimination of dioxane with a pH solution above 1. The solubilized lignin preparation was obtained by precipitation at pH 1.5–2.0 after isolation of the degraded hemicelluloses in three volumes of 95% ethanol. The lignin preparation was then purified by washing with acidified water (pH 2.0) and freeze-dried.

Characterization of Lignin Preparation

To determine the neutral sugar composition, the lignin preparation was hydrolyzed with 2 M trifluoroacetic acid at 120°C for 2 h. The hydrolysates from lignin preparation were reduced, acetylated, and analyzed as their alditol acetates by gas chromatography (GC) according to the method of Blakeney et al.^[13]. The chemical compositions of phenolic acids and aldehydes, liberated from alkaline nitrobenzene oxidation of the lignin preparation at 185°C for 2.5 h, were determined on a Hichrom H50DS high-performance liquid chromatography (HPLC) column with dimensions 250 × 4.6 mm (Phenomenex Co., U.K.). The individual compounds were detected at 280 nm by computer comparison of retention times and peak areas with the authentic phenolics. Measurements of the molecular weight of the lignin were achieved according to the method described by Chen and Li^[14].

UV spectra were recorded on Hewlett-Packard 8452 A diode array spectrophotometer. Each lignin sample (5 mg) was dissolved in 95% (v/v) dioxane-water (10 mL). A 1 mL aliquot was diluted to 4 mL with 50% (v/v) dioxane-water, and the absorbances between 260 and 350 nm were measured. Fourier-transform infrared (FT-IR) spectra were obtained on an FT-IR spectrophotometer (Nicolet 510) using a KBr disc containing 1% finely ground samples. Solution-state 1-D and 2-D-NMR (nuclear magnetic resonance) spectra of the lignin preparation were recorded on a

Bruker Avance 500 MHz spectrometer on a 200 mg sample dissolved in DMSO-d₆ (1.0 mL). HMQC (heteronuclear multiple quantum coherence) analysis was performed by applying a 90° pulse width, a 0.2 s acquisition time, a 1.0 s pulse delay, and $^1J_{C-H}$ of 150 Hz. The DEPT (distortionless enhancement by polarization transfer) subspectrum was taken with a $\theta = 135^\circ$ and a coupling constant $^1J_{C-H}$ of 150 Hz. In particular, 30,000 scans were used for the acquisition of ^{13}C -NMR spectra.

Thermal stability of the lignin preparation was determined using thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) on a simultaneous thermal analyzer (Netzsch STA-409). The sample weighed between 10 and 15 mg. The scans were run from room temperature to 600°C at a rate of 10°C/min under a dry air atmosphere.

Molecular weight distribution of the lignin preparation was obtained from gel permeation chromatography (GPC), using 0.1 M NaNO₃ with pH 12 solution as mobile phase. Approximately 100 µL of the sample solution having a concentration of approximately 3 mg/mL were injected into the column in sequence. The flow rate was controlled at 0.6 mL/min. The molecular weight was based on polystyrene standards.

RESULTS AND DISCUSSION

Yield and Chemical Composition of Lignin Preparation

The treatment of ball-milled wheat straw with acidic dioxane-water solution for 4 h at 85°C solubilized 14.57% lignin (% dry matter), corresponding to 85.71% of the original lignin fractions. This indicated that acidic dioxane treatment at 85°C led to a substantial dissolution of lignin from all cell wall parts, and not only the secondary cell wall, by breaking linkages between lignin and hemicelluloses. More importantly, the yield of pure lignin preparation, 83.13%, was the highest in comparison with both milled straw lignin and enzymatic lignin^[15].

The data on neutral sugar composition in lignin preparation are given in Table II. Obviously, xylose and arabinose were the major sugar constituents in lignin preparation. This monosaccharide analysis revealed that lignin is linked to arabinoxylans. As shown in Table II, the high ratio of arabinose to xylose in lignin preparation reveals that arabinose plays an important role regarding cross-links between hemicelluloses and lignin. It is interesting to note that the lignin preparation contained rather low amounts of chemically linked polysaccharides, as can be seen by 3.03% neutral sugar content, indicating that the mild acidic dioxane-water treatment significantly cleaves the linkages between lignin and polysaccharides in the cell walls of wheat straw, such as ester bonds between ferulic acids and hemicelluloses, ether linkages between ferulic

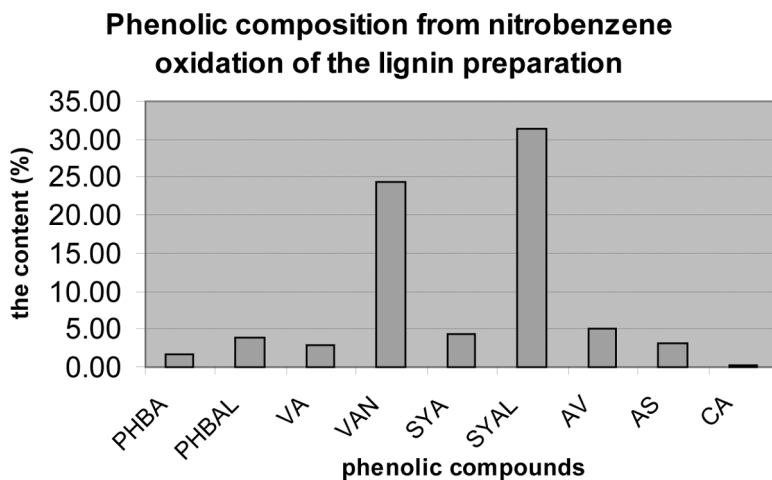
TABLE II Content of neutral sugars (% dry lignin, w/w) in isolated lignin

Components	Content (%)
Rhamnose	0.21
Arabinose	1.39
Xylose	1.18
Mannose	ND ^a
Galactose	0.03
Glucose	0.22
Total	3.03
Ara/Xyl ratio	1.18

^aNot detectable.

acids and lignins, and α -aryl ether linkages between lignins and hemicelluloses.

Alkaline nitrobenzene oxidation has been widely used for assaying and identifying the structure of lignins and the associated lignins in polysaccharides. The high yield (77%) of non-condensed phenolic compounds suggested that wheat straw lignin isolated with acidic dioxane treatment had a relatively low degree of condensation. As shown in Figure 1, the predominant oxidation products from lignin preparation were identified

**FIGURE 1** Yields (% sample, w/w) of phenolic acids and aldehydes from alkaline nitrobenzene oxidation of the isolated lignin.

to be vanillin (VAN, 24.50%) and syringaldehyde (SYAL, 31.50%), resulting from the oxidation of guaiacyl (G) and syringyl (S) units involved in the non-condensed structure of lignin, respectively. The presence of a small amount of *p*-hydroxybenzaldehyde (PHBAL, 3.90%) and *p*-hydroxybenzoic acid (PHBA, 1.80%) was considered most probably to be indicative of non-condensed *p*-hydroxyphenyl (H) units, indicating the incorporation of *p*-hydroxyphenyl alcohol in the lignin preparation. The molar ratio of G/S/H (4.6/4.7/1.0) indicated that a relatively higher amount of the non-condensed syringyl units appeared in the lignin preparation, and the mild acidic dioxane treatment was more effective in the release of syringyl units, particularly β -O-4' syringyl ethers.

During the alkaline nitrobenzene oxidation process, a large proportion of the ferulic acid is quantitatively oxidized to vanillin, and most of the *p*-coumaric acids are quantitatively oxidized to *p*-hydroxybenzaldehyde. However, the remaining occurrence of minor quantities of esterified or etherified *p*-coumaric (0.06%) and ferulic acid (0.08%) in the lignin preparation suggests that these two hydroxycinnamic acids are strongly linked to lignins. This observation also suggested that the acidic dioxane treatment resulted in a partial cleavage of these esterified or etherified linkages. Previously, Crestini and Argyropoulos^[1] showed through quantitative ³¹P NMR that acidic dioxane lignin contains about 10% of ester units expressed on the basis of 100 phenylpropane units. About 77% of the carboxyl fraction of ester bonds present in milled straw lignin was found to be composed of *p*-coumaric acid, while the rest comprised other aromatics acids bound to lignin via intra- and/or intermolecular ester bonds. Acidic dioxane lignin shows a somewhat lower amount of ester bonds compared to the milled straw lignin, and the amount of *p*-coumaric acid in dioxane lignin is about one-half of the amount detected in the milled straw lignin. In general, milled straw lignin contained more *p*-coumaric acid than ferulic acid^[16], whereas the reverse was true for acidic dioxane lignin since the esterified *p*-coumarates are more easily hydrolyzed than ether-linked ferulic acid in the acidic condition, and *p*-coumaric and ferulic acids were shown by NMR to be present in esterified and etherified forms, respectively, in the lignin preparation. In addition, cinnamic acid (CA) was found at 0.15% among the nitrobenzene oxidation products of the lignin preparation.

UV Absorption Spectra

Figure 2 shows the UV absorption spectrum of a lignin preparation. The spectrum exhibits two absorption maxima around 280 nm and 318 nm. The UV absorption of a specific lignin sample depends on both the concentrations of various structural units of lignin and the extinction coefficient of each structural unit. In the UV spectra of lignins in gramineous

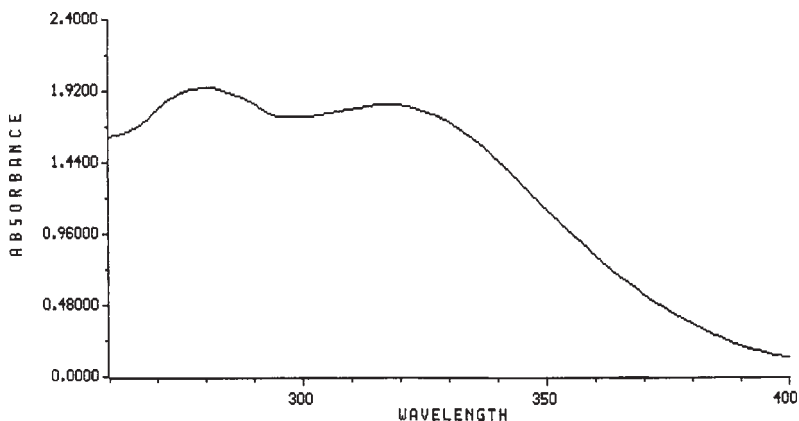


FIGURE 2 UV spectrum of lignin preparation dissolved in dioxane-water system with a concentration of 0.125 g/L.

monocotyledons, the absorption in the 280 nm region is mainly assigned to polyignol, a dehydrogenated copolymer of sinapyl alcohol, coniferyl alcohol, and a small amount of *p*-coumaryl alcohol, and the absorption at 310–320 nm is mainly attributed to the esters of *p*-coumaric and ferulic acids^[4]. All of these structural moieties give different absorption maxima and extinction coefficients. In the UV absorption spectrum, the absorption at 318 nm indicated that more ferulic acid is present in the lignin preparation than *p*-coumaric acid, because the absorption would be shifted to lower than 318 nm if the content of *p*-coumaric acid esters is higher than that of ferulic acid esters^[4]. The values of A_{280}/A_{260} and A_{320}/A_{280} were 1.20 and 0.94, respectively. In fact, the deposition of any units, such as S and H, other than G units during the lignification of cell walls will lead to a decrease of A_{280}/A_{260} to a level lower than 1.22^[17]. He and Terashima^[4] also found that both A_{280}/A_{260} and A_{320}/A_{280} are almost directly proportional to the increase of the content of hydroxycinnamic acid ester. In addition, the absorption maximum at 280 nm was shifted to longer wavelength with the increase in the hydroxycinnamic acid ester content, further implying that the content of hydroxycinnamic acid ester was limited in wheat straw.

Thermal Analysis

The thermal properties of lignin preparation were investigated by TGA and DSC. As can be seen from Figure 3, the lignin fraction started to decompose at about 180°C. The decomposition process of the lignin fraction covered a wide temperature range, between 180° and 600°C. At

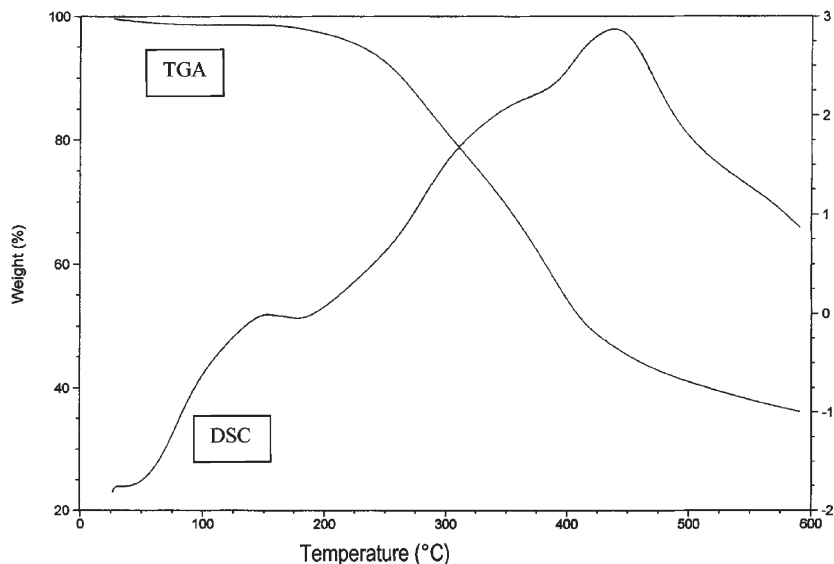


FIGURE 3 Thermogram of lignin preparation.

50% weight loss, the decomposition temperature of lignin was observed at 417°C. At 432°C, 63.5% of the lignin was decomposed, rendering charcoal as the residue product. The DSC curve showed one maximal exothermic reaction of the lignin occurring at 438°C. Lignin, as an amorphous polymer, did not show first-order transitions such as crystallization point (T_c) and melting point (T_m) but showed the glass transition point (T_g) as a second-order transition. A glassy state of lignin is in non equilibrium, and molecular rearrangement takes place depending on the time and temperature of the heat treatment. The glass transition temperature of the lignin preparation was observed by DSC and TGA to take place at 160°C, which falls between the reported values (147–167°C) for milled wood lignin (MWL) and dioxane lignin^[18]. It is thought that aryl ether linkages make lignin move easily, thus the higher occurrence of β -O-4' linkage leads to a lower T_g , and, in contrast, increased condensation structures such as carbon-carbon linkages may give rise to a higher T_g . Thus, the isolated lignin compares well with milled wood lignin.

FT-IR Spectrum

The FT-IR spectrum of a lignin sample provides an overall view of its chemical structure. As shown in Figure 4, the major peaks in the spectra

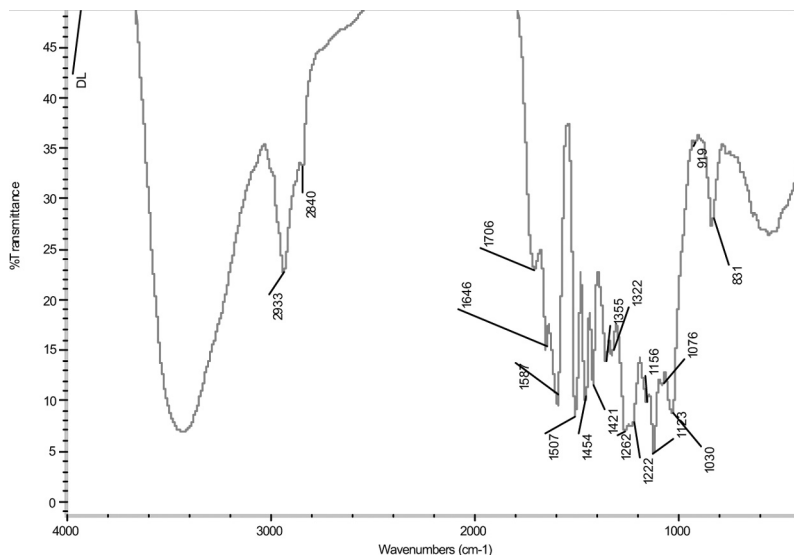


FIGURE 4 FT-IR spectrum of lignin preparation isolated with 80% dioxane in 0.05 M HCl solution at 85°C for 4 h from ball-milled wheat straw.

are the broad band at 3430 cm^{-1} , attributed to hydroxyl groups in phenolic and aliphatic structures, and the band at 2933 cm^{-1} , predominantly arising from C–H stretching in aromatic methoxy group and methylene groups. The shoulders at 1706 and 1646 cm^{-1} originate from conjugated carbonyl stretching, possibly indicating occurrence of hydroxycinnamic acids and are of particular interest since ball milling treatment should result in a slight increase in this band^[10]. Aromatic skeletal vibrations give three strong peaks at 1587 , 1507 , and 1421 cm^{-1} . Further bands are located at 1454 (asymmetric C–H deformations), 1355 (symmetric C–H bending), 1322 (syringyl ring breathing with C–O stretching), 1262 (guaiacyl ring breathing with C=O stretching), 1222 (aromatic ring breathing with C–O and C=O stretching), 1123 (aromatic C–H in-plane deformation, syringyl type), 1076 (C–O deformation, secondary alcohol and aliphatic ethers), 1030 (aromatic C–H in-plane deformation plus C–O in primary alcohols, guaiacyl type), 919 (C–H out of plane in aromatic rings), and the band at 831 cm^{-1} (aromatic C–H out of plane deformation). The weak signal at 1156 cm^{-1} shows the presence of *p*-coumaric ester group, typical for GSH lignins^[20,21]. In addition, it was found that the content of condensed guaiacyl units is relatively high in the lignin preparation, because the signals at 1587 and 1222 cm^{-1} are strong^[21]. In short, the FT-IR spectrum of the lignin preparation shows

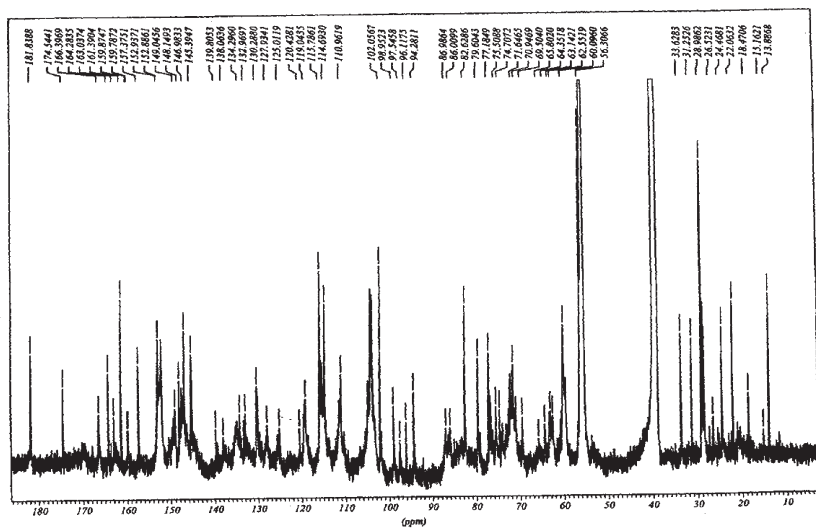
that it is of the GSH type with a low content of hydroxycinnamic acids. Analysis of the near infrared spectrum of the lignin preparation also confirmed the relative purity of the preparation as shown by two strong bands at 4407 (lignin polymer absorbance) and 5964 cm^{-1} (CH in aromatic skeletal)^[22].

¹³C and DEPT ($\theta = 135^\circ$) Spectra of the Lignin Fraction

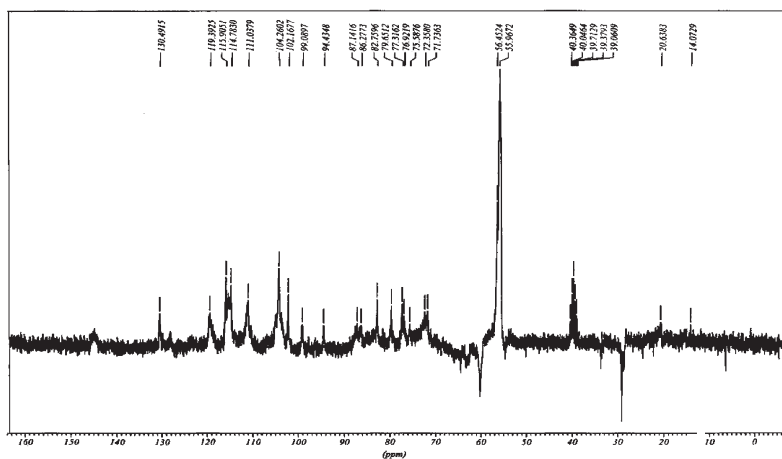
To further obtain more precise knowledge of composition and structural features, we isolated the lignin preparation from ball-milled wheat straw using acidic dioxane at 85°C for 4 h and investigated the preparation by both ¹³C and DEPT ($\theta = 135^\circ$) NMR experiments (Figure 5). Most of the observed signals have been previously assigned in wood and straw lignin spectra^[23,24].

As shown in Table III, the most striking characteristic of the ¹³C-NMR spectrum is the presence of *p*-coumarate ester (C- γ , 166.4 ppm; C-4, 159.8 ppm; C-1, 125.0 ppm; C- β , 115.3 ppm) and etherified ferulates (C- γ , 163.0 ppm; C-6, 122.3 ppm)^[15]. Ferulic acid also gives a signal at 174.5 ppm, indicating that the ester linkages between lignin and arabinxylans via ferulate bridge was significantly cleaved during acidic dioxane treatment. More importantly, 8-O-4' diferulates and *p*-coumarate ester in β -O-4' were identified by two signals, at 164.3 ppm and 161.4 ppm, respectively, assigned to C- γ and C'-4^[24,25]. It seems clear that *p*-coumaric acid is linked to lignin via ester bonds and a few ether bonds, whereas ferulic acid is linked to lignin by ether bonds, and its dehydromers are also incorporated into the lignin polymer. Characteristic aromatic carbon signals of etherified and non-etherified syringyl, guaiacyl, and *p*-hydroxylphenyl residues were detected in ¹³C and DEPT NMR spectra, as shown in Table III. It can be seen that more guaiacyl units do join in the construction of the condensed structures such as β -5', 5-5', and β -1' during lignification of wheat straw. In general, β -5', 5-5' and 4-O-5' are frequent linkage types in guaiacyl lignin. In syringyl lignin, however, intermonomeric couplings at C₅ in the aromatic ring cannot occur, due to the methoxy group present in this position^[26]. Crestini and Argyropoulos^[1] found that C₅substituted phenolic units are also selectively esterified. In addition, the occurrence of acetyl groups shown by two signals at 170 and 22.0 ppm implied that some of monolignols were acetylated during lignification of straw, which have been studied by Lu and Ralph^[27].

The ¹³C-NMR spectrum of the lignin preparation gives three resonances at 86.0, 72.2, and 60.1 ppm (very strong), assigned to C- β in β -O-4', C- α in β -O-4, and C- γ in β -O-4', respectively. These signals showed that the treatment with acidic dioxane solution under the condition given did not significantly attack the β -aryl ether structure, since in native



(a)



(b)

FIGURE 5 ^{13}C -NMR normal spectrum (a) and DEPT ($\theta = 135^\circ$) spectrum (b) of the lignin preparation (in DMSO-d_6) isolated with 80% dioxane in 0.05 M HCl solution at 85°C for 4 h from ball-milled wheat straw.

TABLE III Carbon chemical shifts (δ , ppm) of ^{13}C and DEPT NMR spectra in lignin isolated with acidic dioxane-water

δC ppm (Intensity)	Assignments
181.8 (m)	Carboxylic groups
174.5 (w)	C- γ in ferulic acids
166.4 (m)	C- in <i>p</i> -coumarate ester, in γ -ester
164.3 (w)	C- γ in 8-O-4' diferulate
163.0	C- γ in etherified ferulic acid
170.0 (w)	Acetyl group
161.4 (m)	C-4 in <i>p</i> -coumarate ester, in β -O-4'
159.8 (w)	C-4 in <i>p</i> -coumarate ester
157.4 (m)	C-4 in <i>p</i> -hydroxyphenyl units
152.9 (s)	C-3 in guaiacyl units with α -ether
152.1 (s)	C-3/C-5 in etherified syringyl units
149.1 (m)	C-3 in etherified guaiacyl unit
148.2 (s)	C-3 in non-etherified guaiacyl unit
147.2 (s)	C-3/C-5 in non-etherified syringyl unit
147.0 (s)	C-4 in etherified guaiacyl unit, in β -5'
145.4 (s)	C-4 in non-etherified guaiacyl units
139.8 (w)	C-4 in guaiacyl with α -aryl ether
138.1 (w)	C-4 in etherified syringyl unit
134.5 (s)	C-1 in etherified guaiacyl unit
134.3 (w)	C-1 in etherified syringyl unit
132.97 (w)	C-1 in non-etherified syringyl unit
130.3 (m)	CH-2/CH-6 in <i>p</i> -coumarate ester
127.9 (w)	CH-2/CH-6 in <i>p</i> -hydroxyphenyl units
125.0 (w)	C-1 in <i>p</i> -coumarate ester
120.4 (w)	C-6 in guaiacyl units, in 5-5' type
119.1 (s)	CH-6 in guaiacyl units
56.0 (s)	OCH ₃
34–29 (m)	CH ₂ - α and β , in dihydroconiferyl alcohol
22.0 (m)	Acetyl-(CH ₃)
18.5 (s)	Hexamethylbenzene (CH ₃) ₆ C ₆
115.8 (s)	CH-3/CH-5 in <i>p</i> -hydroxyphenyl units
115.3 (m)	C- β in <i>p</i> -coumarate ester
114.7 (s)	CH-5 in guaiacyl unit, in β -1' units
111.0 (m)	CH-2 in guaiacyl unit
102.1 (s)	CH-1 in xylans
104.2 (s)	CH-2/CH-6 in syringyl units
99.0 (w)	CH-1 in MeGlcA

(Continued)

TABLE III (Continued)

δC ppm (Intensity)	Assignments
97.5 (w)	C-1 in reducing xlyose, β -anomer
96.1 (w)	C-, unknown
94.3 (m)	CH-1, in xylose, α -anomer
87.0 (m)	CH- β , in syringyl β -O-4' (erythro)
86.0 (m)	CH- α , in syringyl units
82.6 (s)	CH- β , in β -O-4 with α -carbonyl groups
79.6 (m)	CH-3 in arabinofuranose
77.2 (m)	CH-4 in xylans with MeGlcA
77.0 (w)	CH-4 in xylose internal unit
75.5 (w)	CH- α , in β -1'
74.7 (w)	C- α , in β -O-4'
72.2 (s)	CH- α , in β -O-4' (erythro) guaiacyl
71.6 (s)	CH- γ , in β - β' units, and CH- α in β -O-4 (threo) guaiacyl
70.9 (w)	CH- γ in <i>p</i> -hydroxyphenyl units
69.5 (w)	CH-4 in xylose nonreducing end unit
65.8 (w)	CH ₂ -5 in esterified arabinose
64.4 (w)	CH ₂ - γ , in β -1
63.2 (w)	CH ₂ - γ , in β -5, C5-H ₂ in xylans
62.6 (w)	CH ₂ - γ , in β -O-4 with C α =O or β -1'
60.1 (s)	CH ₂ - γ , in β -O-4
54.0 (w)	CH- β , in β - β' unit
53.0 (w)	CH- β , in β -5' unit
15.2/13.9	Long chain CH ₂ and CH

Intensity abbreviations: s = strong, m = mean, w = weak.

wood/straw lignin, the β -O-4' linkage is the predominant inter-unit linkage, with smaller amounts of carbon-carbon and carbon-oxygen linkages being present. However, the content of β -aryl syringyl ethers decreased, possibly due to its more facile cleavage than β -aryl guaiacyl ethers during treatment^[28]. Interestingly, by comparison of the intensity of signals, the proportion of erythro- β -O-4' is higher than that of threo- β -O-4', thus partly giving rise to an increase of alkali solubility for straw lignins with a relatively low molecular weight average^[29,2]. β -O-4' linkages with α -carbonyl groups were detected in the lignin preparation by the signal at 62.6 ppm assigned to CH₂- γ in those structures, which is an important feature in MWL and dioxane lignin.

HMQC and ^1H Spectra of the Lignin Fraction

An examination of the proton and HMQC NMR spectra of the lignin fraction (Figure 6) further confirms the above results in detail. Analysis of lignin by HMQC has shown (Table IV) the major structures of native lignin, such as guaiacylglycerol- β -guaiacyl ether (β -O-4'), phenylcoumaran (β -5'), and pinosresinol (β - β') moieties. The so-called diarylmethane moieties of 5-CH₂-5' type, an important condensed structure usually formed during alkaline pulping, were thought to be indicated by signals at $\delta\text{C}/\delta\text{H}$ 29.5/1.2 ppm instead of 3.8 ppm^[30]. However, it was also detected in the lignin preparation. We suggest that the signal is not diagnostic of diarylmethane moieties of 5-CH₂-5' type. The signal at $\delta\text{C}/\delta\text{H}$ 34.0/2.2 ppm possibly originates from dihydroconiferyl alcohol^[31] or β - β' moieties. α -Esters were not detected by the absence of signals at $\delta\text{C}/\delta\text{H}$ 73–76/5.9–6.2 ppm in HMQC NMR spectrum of the lignin preparation. However, γ -esters were detected by the signal at $\delta\text{C}/\delta\text{H}$ 64.6/4.35 ppm (data not shown). In general, β -esters are not present in straw lignins, and acylation normally occurs at γ -positions^[1]. The absence of cross peaks in the region at δH 5.4– δC 81 ppm, indicating α -aryl ether bonds, confirmed that the α -O-4 structure occurs at lower levels than the detection limits of the HMQC experiment after mild acidic treatment, since it is easily hydrolyzed during acidic treatment.

Signals of lignin-carbohydrate linkages of the phenyl glycoside type at $\delta\text{C}/\delta\text{H}$ 102.5/5.0 ppm were detected in the lignin preparation, revealing that glycoside bonds are present between lignin and carbohydrates in wheat straw. The weak signals at $\delta\text{C}/\delta\text{H}$ 69.8/3.3 ppm (data not shown) have been assigned to CH of carbohydrates linked to the α -position of lignin via a primary hydroxyl group^[30]. Arabinoxylans were identified by several signals as shown in Figures 6 and 7, further suggesting that arabinoxylans form cross-links with lignins through ferulates via ether bond, glucuronic acid via ester bond, and xylose/arabinose via glycosidic and ether bonds. The HMQC NMR technique, providing high dispersion of carbohydrate signals, increases the likelihood of identification of other possible linkage sites of carbohydrates bonded to lignin. However, this analysis requires precise information on the chemical shifts of the corresponding moieties and the use of equipment with higher sensitivity^[30].

In ^1H NMR spectrum, a broad peak with various signals centered at 3.8 ppm was assigned to the resonance of the methoxy and side-chains protons in various structures, such as β -5', 5-5', and β -1' forms, and aliphatic hydroxyl groups as well as carbohydrates, and 8.8 H is observed from the integrated area. The signals from 6.6 to 7.6 ppm are attributed to the resonance of aromatic protons of the lignin, and 2.2 H is observed. This indicated that the lignin preparation has a relatively low degree of condensation, which is in agreement with the result of alkaline

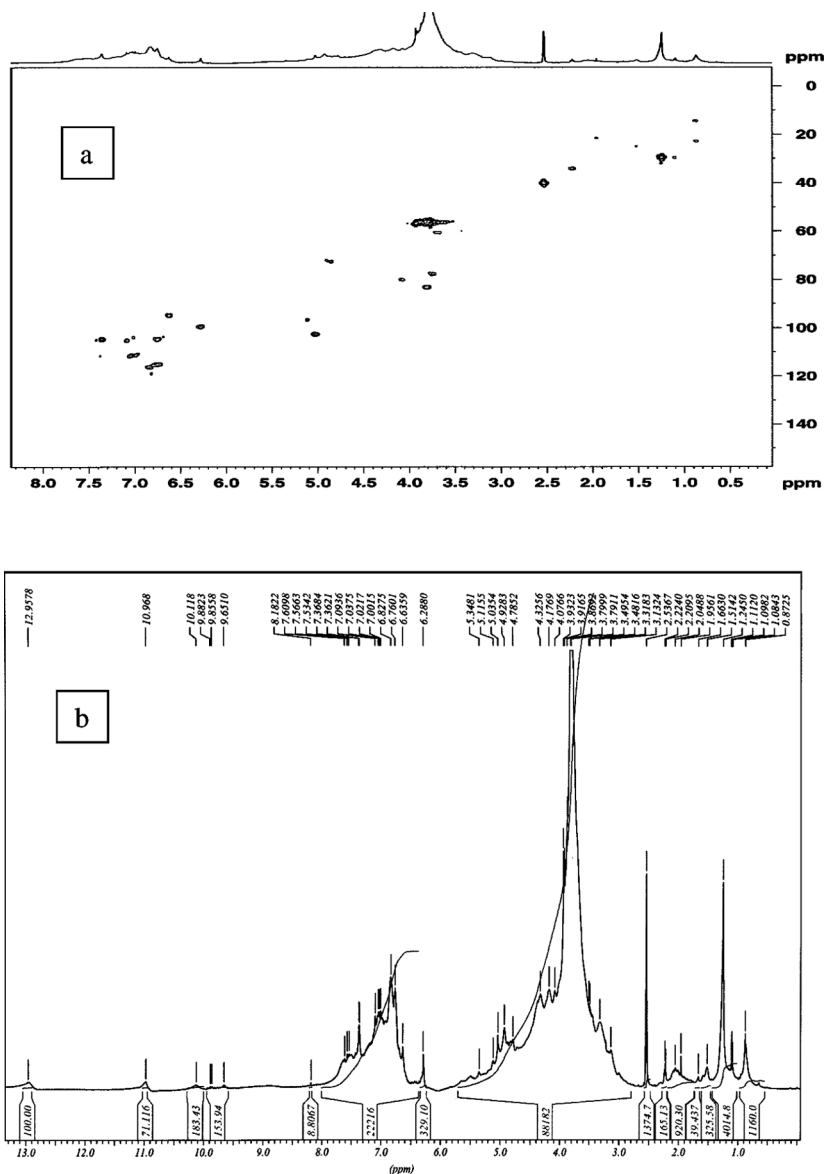


FIGURE 6 NMR HMQC spectrum (a) and ^1H spectrum (b) of the lignin preparation (in DMSO-d_6) isolated with 80% dioxane in 0.05 M HCl solution at 85°C for 4 h from ball-milled wheat straw.

TABLE IV Chemical shifts ($\delta\text{C}/\delta\text{H}$, ppm) of lignin preparation in HMQC NMR spectrum

$\delta\text{C}/\delta\text{H}$ ppm	Assignments
119.1/6.8	CH-6 in guaiacyl units
116.0/6.75	CH-3/CH-5 in <i>p</i> -coumarate ester
111.2/7.38	CH-2 in 5-5' diferulates
111.0/6.98	CH-2 in guaiacyl unit, in β -5'
111.3/7.02	CH-2 in guaiacyl unit
105.2/7.05	CH-2/CH-6 in etherified S units
104.7/7.32	CH-2/CH-6 in syringyl units with α -carbonyl groups
104.5/6.7	CH-2/CH-6 in syringyl units, β - β'
104.0/6.99	CH-2/C-6 in syringyl units
99.3/6.24	CH- α , possibly in β -1'
94.6/6.6	CH- α , unknown
83.3/5.13	CH- β , in β -O-4' with α -carbonyl groups
85.24/4.67	CH- β , in guaiacyl units, β - β'
22.2 /2.0/n.d.	Acetyl group
86.71/4.37	CH- β , in β -O-4'
83.04/3.78	CH-4 in arabinfuranose
79.76/4.05	CH-3 in arabinfuranose
77.53/3.70	CH-4 in xylans
72.5/4.81	CH- α , in β -O-4' (erythro)
60.7/3.64/3.4	CH ₂ - γ , in β -O-4'
55–57 /3.7–3.9	OCH ₃
102.5/5.0	CH-1 in xylans
34.0/2.0	CH in dihydroconiferyl alcohol
29.5/1.2/1.0	CH ₂ in aliphatic lignin moieties
22.7/0.83	CH in aliphatic lignin moieties
64.7/4.10/4.16	CH ₂ - γ , in β - β'
49.2/3.17	CH- β , in β -1'
14.3/0.84	Long chain CH

nitrobenzene oxidation. It has been observed that the lignin preparation arises from all cell wall parts, not only from middle lamella which, is considered to have a more condensed lignin structure^[31]. Signals of β -groups in β -1' (2.7–2.9 ppm), β -5', and β - β' dimers are located in the range of 3.5 to 2.3 ppm^[23], in which the two signals at 2.5 and 3.3 ppm arise from DMSO-*d*₆ (dimethyl sulfoxide) and HDO, respectively. The signals from 7.64 to 7.36 ppm can be assigned to the aromatic protons in positions 2 and 6, in structures containing a C _{α} =O group, and to

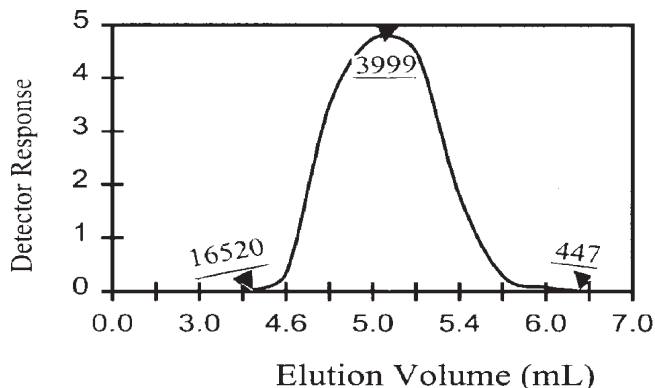


FIGURE 7 GPC chromatogram of lignin preparation; the numbers indicated on the figure are corresponding molecular weights calculated from polystyrene standards.

aromatic protons in positions 2 and 6 units conjugated with a double bond, to the proton in $\text{HC}_\alpha=\text{C}_\beta$ structure, confirming the presence of *p*-coumarate-type structure and hydroxycinnamic acid in lignin. The signal at 8.07 arises from phenolic hydroxyl groups and implied that the cleavage of benzyl-aryl-ether, particularly α -O-4', takes place during both acidic dioxane treatment and ball milling process. In addition, the contents of β -5' (about 2.01%) and 5-5' types are low, and only a trace of β -1' and β - β' is observed.

The contents of various aromatic groups containing aldehyde, acids, and acetoxy groups were also possibly estimated on the basis of total phenylpropanes by ^1H NMR spectrum, as shown in Table V. The lignin preparation contains 9.76% ester groups, which is close to the result obtained by ^{31}P NMR spectrum^[1]. Quantification of β -O-4', β -1', and 5-5' types and aliphatic hydroxyl groups is difficult in the ^1H NMR spectrum, due to the overlapping, broad signals. However, the number of free aliphatic and phenolic hydroxyl groups could be indicated from the corresponding aliphatic acetate (3.35%) and phenolic acetate (0.95%) signals^[21]. In fact, Gramineaene lignins are characterized by high contents of free phenolics in the β -O-4' structures, implicating guaiacyl units. It has been found that 40% of the guaiacyl units and only 5% of the syringyl units engaged in β -O-4' structures bear free hydroxyls^[1,28]. Therefore, the guaiacyl lignin structures engaged in β -O-4' structures were more easily degraded compared to syringyl units. Although more guaiacyl units join in the construction of condensed structures, their content is relatively low in wheat straw lignin.

TABLE V Contents of various aromatic groups containing aldehyde, acid, and acetoxy groups on the basis of total phenylpropanes calculated from ^1H NMR spectrum

Chemical shifts (ppm)	Assignments	Contents (%)
10.97–12.95	Carboxylic acids	1.84
9.6–10.1	Aldehydes and phenolic hydroxyl groups conjugated with carbonyl groups	3.62
6.29	Vinylic side chain	3.55
2.2–2.4	Aromatic acetates	0.95
1.7–2.0	Aliphatic acetates	3.35
1.0–1.2	Aliphatic $-\text{CH}_2-\text{CH}_2-$	10.85
0.87	Aliphatic $-\text{CH}-\text{CH}-$	6.30
Total esters		9.76

Molecular Weight Distribution

The lignin preparation isolated with 80% dioxane in 0.05 M hydrochloric acid at 85°C from ball-milled wheat straw had a relatively higher value of the weight-average molecular weight ($M_w = 3,900 \text{ g mol}^{-1}$) and number-average molecular weight ($M_n = 2,240 \text{ g mol}^{-1}$). This observation revealed that mild acidic dioxane treatment significantly breaks the linkages between lignin and hemicelluloses, and the isolated lignin is associated with only a small amount of hemicelluloses. A slight degradation of the macromolecular structure of lignin, such as cleavage of the aryl ether bonds between the lignin precursor, occurs only during acidic dioxane treatment, since, in all cases, acidic media were found to depolymerize the lignin macromolecule.

The GPC molecular weight distribution of the lignin preparation is shown in Figure 7. The elution maximum corresponds to polystyrene molecular weight of 4000 g mol^{-1} . The elution profile of the lignin showed a wide polydispersity, ranging from dimers up to a molecular weight over $16,000 \text{ g mol}^{-1}$, according to polystyrene references.

CONCLUSIONS

The ball milling treatment combined with a direct acidic dioxane extraction led to the release of 83.13% pure lignin from wheat straw. The lignin fraction had a relatively high value of weight-average molecular weight (M_w , $3,900 \text{ g mol}^{-1}$) and number-average molecular weight

(M_n , 2,240 g mol⁻¹) and a low degree of condensation. The value of T_g (160°C) lies between the values (147°–167°C) for milled wood lignin and dioxane lignin, further confirming that the lignin preparation is substantially native, with no significant change such as condensation reactions occurring during the extraction process. NMR analysis revealed that lignin is distinguished by high β -O-4' structures and by low amounts of condensed units (β -5', 5-5', and β -1'). The content of condensed guaiacyl units is relatively high in the lignin preparation. *p*-Coumaric acid is linked to lignin via γ -ester bond and a few ether bonds such as β -O-4', whereas ferulic acid is linked to lignin by ether bonds, and its dehydromers (5-5'/8-O-4') are also incorporated into lignin polymer.

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