Fractional and Structural Characterization of Ball-Milled and Enzyme Lignins from Wheat Straw

RUNCANG SUN,[1] B. XIAO,[2] J. M. LAWTHER[1]

[1] The BioComposites Centre, University of Wales, Bangor, LL57 2UW, United Kingdom

[2] Northwestern Forest College, Yangling, Shaanxi, People's Republic of China

Received 29 July 1997; accepted 29 October 1997

ABSTRACT: Ball-milled and enzyme lignins were extracted with 90 and 50% dioxane–water from 6 days ball-milled wheat straw, and subsequently cellulase-treated straw residues, respectively. The crude lignin preparations were purified using a two-step precipitation method instead of the traditional ether precipitation procedure, and fractionated into pure milled lignin (PML), pure enzyme lignin (PEL), hemicellulose-rich milled lignin (HRML), lignin-rich enzyme lignin (LREL), and solubilized lignin during enzyme treatment (SLET) fractions. The five lignin fraction were studied using spectroscopic and degradative tecyhniques. The PML and PEL fractions showed very low content of associated polysaccharides (2.36–2.86%). The PML is mainly composed of β-*O*-4 ether bonds in the lignin structural units. The less common β-5 and β-β carbon–carbon linkages are also present. The results obtained also indicated that the lignins in wheat straw cell walls appeared to be very closely associated to *p*-coumaric and ferulic acid, and glucuronic acid or 4-*O*-methylglucuronic acid. © 1998 John Wiley & Sons, Inc. J Appl Polym Sci 68: 1633–1641, 1998

Key words: ball-milled lignins; enzyme lignins; wheat straw

INTRODUCTION

Lignin, accounting for about 14% of the dry matter of wheat straw, seems to be of great importance in the topochemical effects of paper pulp manufacture and the generation of mechanical stress during the growth of plants. Furthermore, the qualitative characterizations of lignin, such as chemical composition and its structure, play a very important role in determining the properties of the cellulosic fibres.[1] Generally, there is very little information on the properties of Gramineae lignins compared to Gymnosperm and Dicotyledon lignins; this is particularly true for wheat straw lignin.

The straw lignin are known to be different from those softwoods or hardwoods. Straw lignins are composed of guaiacyl, syringyl, and *p*-hydroxyphenyl monomeric units, while wood lignins are composed of only guaiacyl units (softwood), guaiacyl and syringyl units (hardwood), or guaiacyl and *p*-hydroxyphenyl units (compression wood). Moreover, straw and grass cell walls are typified by the presence of ferulic and *p*-coumaric acids, linked to polysaccharides and/or lignins.[2] Ferulic acid is known to be esterified with hemicelluloses and etherified with lignin, whereas *p*-coumaric acid is known to be extensively esterified with lignin.[3]

Due to the above complex nature of straw and grass lignins, the study of their structure has been found to be more difficult. Meanwhile, the limitations of present isolation and purification methodologies for characterizing straw and grass lignins represents an important drawback in the study

Correspondence to: R. Sun.

Contract grant sponsor: LINK Collaborative Program in Crops for Industrial Use; contract grant number: CSA 2054.

Journal of Applied Polymer Science, Vol. 68, 1633–1641 (1998)

 CCC 0021-8995/98/101633-09

of lignocellulosic materials. Björkman's procedure of vibratory milling and solvent extraction is the preferred method for isolating relatively pure lignin from wood samples[4]; however, when it is applied to other plant material having a lower lignin content such as straw and grass, the results have not been as successful. A number of isolation and purification procedures has been previously attempted to reduce of polysaccharide content in isolated ball-milling and enzyme lignin preparations, but no one of these methods allowed obtaining the lignin fractions relatively free of polysaccharides.[5]

The aim of this study was to determine whether the Björkman method is suitable for isolation of the lignin fractions with relatively free polysaccharides from wheat straw. The dioxane-soluble lignins released by ball milling and subsequent cellulase treatment were isolated by a two-step precipitation method instead of the traditional ether precipitation procedure, and their physical and chemical properties studied.

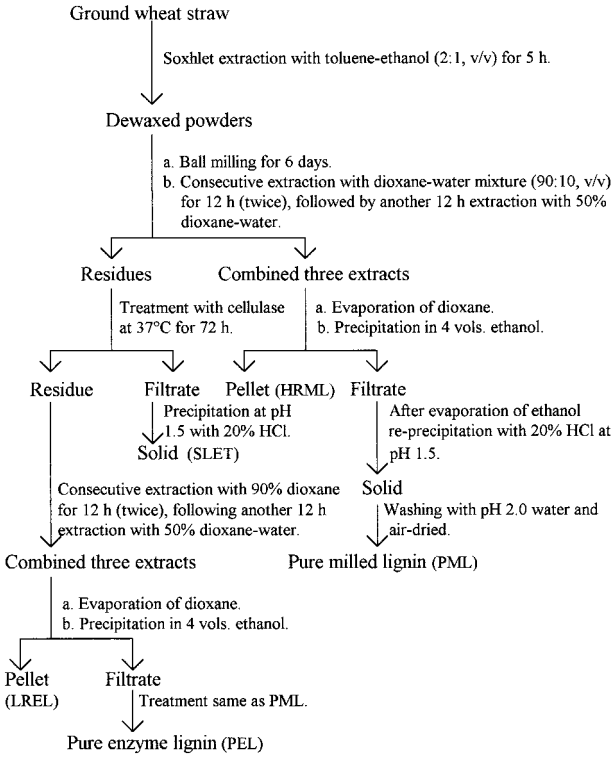

Figure 1 Scheme for isolation of ball-milled and enzyme lignin preparation from wheat straw.

EXPERIMENTAL

Fractionation and Isolation of Ball-Milled Lignin and Enzyme Lignins

The wheat straw was obtained from Compak Co. (Gainsborough, UK). The air-dried wheat straw was ground using Chritie Laboratory mill to pass a 60 mesh size screen. The ground straw was then extracted with toluene–ethanol (2 : 1, v/v) for 5 h in a Soxhlet apparatus. After dried in an oven for 16 h at 50°C, the extractive free powders were subjected to ball milling for 6 days in a rotary porcelain ball mill at 80 rpm, using mixture of 10 and 20 mm porcelain balls (1 : 1, w/w) (balls/straw weight ratio of 36). The ball-milled samples were then extracted twice with a dioxane–water mixture (90 : 10, v/v) for 12 h, followed by another 12-h extraction with a dioxane–water mixture (50 : 50, v/v). The extractions were performed using 10 g of a ball-milled sample to 250 mL of solvent at room temperature in darkness and under N_2. The three extractions were combined into one composite sample, and the solvents were removed by a rotary vacuum evaporator at 40°C. The HRML was obtained by precipitation in 4 vol ethanol. The PML was obtained by reprecipitation at pH 1.5 with 20% HCl from the supernatant solution.

After extraction of the HRML and PML, the dioxane–water-extracted residues were washed with water and treated with cellulase (1,4-[1,3; 1,4]-β-D-Glucan 4-glucano-hydrolase; EC 3.2.1.4, from *Aspergillus niger,* Sigma, St. Louis, MO) (4 g per 10 g of extractive free powder in 250 mL 0.2*M* HAc-NaAc buffer pH 4.7) at 37°C for 72 h. After filtration on a nylon cloth, the insoluble residues were washed with water, and LREL and PEL fractions were generated by successive extractions with 90 and 50% dioxane–water as before. The SLET fraction was obtained from the cellulase hydrolysates by precipitation at pH 1.5 with 20% HCl (Fig. 1).

Physicochemical Characterization of Lignin Fractions

UV spectra were recorded on a Hewlett-Packard 8452A Diode Array spectrophotometer. A lignin sample (5 mg) was dissolved in 95% (v/v) dioxane–water (10 mL). A 1-mL aliquot was diluted to 10 mL with 50% (v/v) dioxane–water, and the absorbances between 200 and 350 nm were measured.

The molecular-average weights of lignin fractions were determined by gel permeation chroma-

Table I The Yields (% Acidic Chlorite Lignin) of Milled Wheat Straw and Enzyme Lignin Fractions

Lignin Fractions	Yield (%)
Hemicellulose rich milled lignin (HRML)	33.12
Pure milled lignin (PML)	10.85
Solubilized lignin during enzyme treatment (SLET)	3.48
Lignin rich enzyme lignin (LREL)	19.79
Pure enzyme lignin (PEL)	7.80

tography on a PLgel 5 μ Mixed-D column. The samples were dissolved with tetrahydrofuran with a concentration of 0.2%, and a 200-μL sample in solution was injected. The columns were operated at 40°C and eluted with tetrahydrofuran at a flow rate of 1 mL min^{-1}. The column was calibrated using polystyrene standards.

FTIR spectra were obtained on an FTIR spectrophotometer (Nicolet, 750) using a KBr disc containing 1% finely ground samples. The solution state of ^{13}C-NMR spectrum was acquired with a Brucker 250 AC spectrometer operating in the FT mode at 62.4 MHz under total proton decoupled conditions. It was recorded at 25°C from a 250-mg sample dissolved in 1.0 mL DMSO-d_6 after 30,.000 scans. A 40° pulse-flipping angle, a 3.0 μs pulse width, and 0.85-s acquisition time were used.

Total and ester-linked hydroxycinnamic acids were released with 4 *N* NaOH at 170°C for 2 h and with 1 *N* NaOH under nitrogen atmosphere at 25°C for 16 h, respectively. Ether-linked hydroxycinnamic acids were calculated as the difference between total and ester-linked hydroxycinnamic acids.[6]

Neutral sugar composition of the lignin fractions was determined as alditol acetates.[7] Methods of uronic acid analyses, alkaline nitrobenzene oxidation of lignin, and determination of phenolic acids and aldehydes with HPLC were described in previous articles.[8,9] All nitrobenzene oxidation results represent the mean of at least triplicate, and each oxidation mixture was chromatographed twice. Other experiments were performed in duplicate.

RESULTS AND DISCUSSION

Lignin Yield

The yields of lignins resulting from the various fractionation procedures were expressed as a percentage of the total lignin determined by sodium chlorite oxidation in acidic conditions (14.1% by weight).

Table I shows the fractional yields of ball-milled and enzyme lignins. As expected, 6-day ball milling and 3-day cellulase treatment yielded about 75% of total lignin from wheat straw. The yields of PML (10.85%) and PEL (7.80%) are lower than the corresponding yields of HRML (33.12%) and LREL (19.79%) fractions, respectively, indicating that the majority of lignins are still linked to polysaccharides after 6-day ball milling and 3-day cellulase treatment, compared to the ball-milling lignin (PML+HRML). The lower yield of enzyme lignin (PEL + LREL) is probably due to a fraction of lignin being extracted during the ball-milling process.

UV Spectra

The PML and PEL fractions showed similar UV spectra having three absorption maxima at 320, 278, and 216 nm (Fig. 2). The first absorption maximum is attributable to bound hydroxycinnamic acid, such as *p*-coumaric and ferulic acids,[6] and the second one originates from a free and etherified hydroxyl group in a hydroxylated benzene nucleus.[10] As can be seen from the diagram, the PEL fraction gave the maximum absorption, indicating the purest preparation. A much lower absorption coefficient in LREL, SLET, and HRML fractions was undoubtedly due to more nonlignin material such as associated hemicelluloses.

Content of Associated Polysaccharides

The contents of neutral sugars and uronic acids in the isolated lignin fractions are given in Table

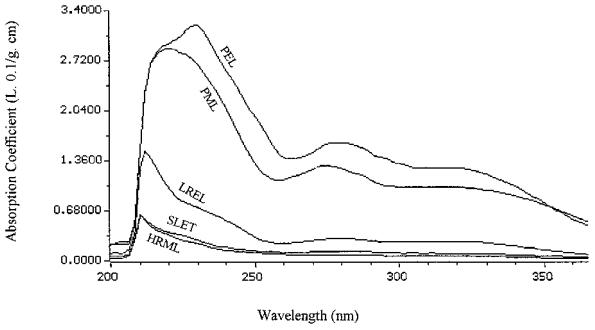

Figure 2 UV spectra of pure milled lignin (PML), pure enzyme lignin (PEL), lignin-rich enzyme lignin (LREL), hemicellulose-rich milled lignin (HRML), and solubilized lignin during enzyme treatment (SLET).

Table II The Content (%, w/w) of Neutral Sugars and Uronic Acids in Milled Wheat Straw and Enzyme Lignin Fractions Obtained from 6 Days Ball-Milled Wheat Straw and 3 Days Cellulase-Treated Residues, Respectively

Lignin Fractions	Neutral Sugars							Uronic Acids	Total
	Rha	Fuc	Ara	Xyl	Man	Glc	Gal		
HRML	0.69	Trace	4.20	10.69	0.50	5.24	3.11	7.33	32.16
PML	Trace	ND	0.20	0.90	ND	0.60	0.13	1.03	2.86
SLET	0.40	0.20	1.92	8.16	2.20	33.6	24.78	1.62	72.64
LREL	0.30	ND	2.16	7.53	0.33	3.93	1.11	5.50	20.86
PEL	ND	ND	0.20	0.58	ND	0.36	0.10	1.12	2.36

ND, not detected.

II. The PML and PEL contained a rather low level of associated polysaccharides—2.86 and 2.36%, respectively. The data observed indicated that both a ball milling and cellulase treatment can peel of the lignin from most of its neighboring polysaccharide moiety. This finding further confirms that the chemical bonds between lignin and hemicelluloses in PML and PEL fractions are mostly cleaved during the ball-milling and cellulase-treatment processes. However, as can be seen in Table II, HRML, SLET, and LREL fractions showed a much higher content of associated polysaccharides, 20.86–72.64%, suggesting that the native linkages between lignin and hemicelluloses in these fractions are only partly cleaved under the ball-milling and cellulase-treatment conditions. It was found that xylose is a major component with uronic acids, arabinose, glucose, and galactose as the secondary monosaccharides in HRML and LREL fractions. A high proportion of glucose in the SLET fraction was undoubtedly due to the degradation of cellulose by cellulase.

It is surprising that these relatively pure-milled and pure-enzyme lignins have not been isolated previously from grass and straw. Various isolation and purification procedures have been previously attempted to reduce polysaccharide content in ball-milling and enzyme lignin preparations, but none of these methods have enabled the isolation of lignin fractions, with relatively free polysaccharides. Scalbert and co-workers[5] purified ball-milled wheat straw lignin and enzyme lignin fractions by successive precipitations in 2% sodium sulfate aqueous solution and diethyl ether. The ball-milled lignin and enzyme lignin contained 7.1 and 16.8% polysaccharides. Ben-Ghedalia and Yosef [11] fractionated ball-milled lignin and enzyme lignin into eight fractions by ball milling for 7, 14, 21, and 28 days, respectively, and purified the lignin preparations by dissolution in 90% acetic acid and then precipitation in acidified water. The lignin preparations still contained 14.2–16.4% polysaccharide sugars. The crude lignin fractions, obtained by ball milling and subsequent cellulase treatment from orchardgrass were purified by dissolution in 90% (v/v) acetic acid and then precipitation into water. The precipitated lignin was further purified by dissolution in 90% acetic acid followed by precipitation into diethyl ether. The successfully purified lignin fractions, however, still contained 19.1–21.0% polysaccharides.[12] This high content of polysaccharides in ball-milled and enzyme lignin fractions was thought to be due to the specific structural patterns of association between lignin and polysaccharides in straw and grass cell walls.[5] However, it is easy to obtain the lignin fractions, with relatively free polysaccharides, by using this two-step precipitation method in the isolation of ball-milled and enzyme lignin fractions from straw and grass. This rapid and convenient method may be used for most studies on lignin from wheat straw and other plants, such as grass.

Interestingly, compared to the neutral sugar content, the relatively high content of uronic acids in PML and PEL fractions was probably due to the ester bonds between lignin and glucuronic acid or 4-*O*-methylglucuronic acid residue of hemicelluloses in wheat straw cell walls. Occurrence of the ester bond between lignin and glucuronic acid or 4-*O*-methylglucuronic acid was confirmed by two small signals at 170.2 and 171.8 ppm in ^{13}C-NMR spectrum (Fig. 6).[10]

Composition of Phenolic Acids and Aldehydes

The minor differences between the PML and PEL fractions were detected by alkaline nitrobenzene

Table III The Yields (% Sample, w/w) of Phenolic Acids and Aldehydes from Alkaline Nitrobenzene Oxidation of Wheat Straw Lignin Fractions

Phenolic Acids and Aldehydes	Extraction Fractions			
	HRML	PML	LREL	PEL
Gallic acid	0.66	1.58	1.7	3.08
Protocatechuic acid	ND	0.11	ND	0.055
p-Hydroxybenzoic acid	0.086	0.36	0.14	0.22
p-Hydroxybenzaldehyde	0.35	0.82	0.74	1.10
Vanillic acid	0.13	0.26	0.28	0.45
Syringic acid	0.14	0.91	0.52	0.86
Vanillin	1.43	7.06	3.67	8.11
Syringaldehyde	1.21	6.73	3.94	8.40
p-Coumaric acid	0.033	0.19	0.083	0.041
Acetonvanillone	ND	0.13	ND	0.2
Ferulic acid	ND	1.32	ND	0.12
Total	4.04	19.47	11.07	22.64

ND, not detected.

oxidation. The PML fraction yielded slightly higher vanillin than syringaldehyde, while the reverse yield values appeared in PEL fraction (Table III). This observation was supported by the FTIR spectroscopic results (Fig. 4). The slightly high vanillin content in PML fraction evidenced that the G-lignin is readily extracted during the ball-milling process. By contrast, in PEL fraction the S-lignin is preferential removal under the cellulase treatment conditions. The predominant degradation products, vanillin and syringaldehyde, resulted from the degradation of noncondensed guaiacyl and syringyl units, respectively. Occurrence of a low amount of *p*-hydroxybenzaldehyde is generally considered indicative of *p*-hydroxyphenyl units within the lignin "core." A slightly higher yield of nitrobenzene oxidation of PEL indicated less condensed PEL than PML. These results agreed with Billa and Monties' findings[1] and our previous studies.[13] These authors mentioned that ball-milled lignin and enzyme lignin obtained from wheat straw were slightly enriched in G and S units, respectively. The lower yield of oxidation products of HRML and LREL was undoubtedly due to the higher associated polysaccharides.

Straw and grass cell walls are typified by hydroxycinnamic acids, such as *p*-coumaric and ferulic acids. Ferulic acid is known to be esterified with hemicelluloses and etherified with lignin. Further studies confirmed that all of the esterified ferulic acid was also ether linked at the β-position of coniferyl alcohol.[2] *p*-Coumaric acid, on the other hand, is known to be extensively esterified at the γ-position on the side chain of lignin monomers.[12] In this study, the presence of ferulic acid and *p*-coumaric acid associated to cell walls through ester and or ether bonds was determined by alkali hydrolysis at a high temperature or at an ambient temperature, respectively. The results obtained showed that over 60% of ferulic acids were etherified to lignin, while about 77% of *p*-coumaric acids were esterified to lignin in PML and PEL fractions (Table IV). The data in Table IV also indicated that the PML and PEL contained 2.68 and 3.13% ferulic acids, respectively, whereas *p*-coumaric acid yields were only 0.71 and 1.20%, respectively. The significant low content of *p*-coumaric acid and ferulic acid in HRML suggested that most of the hydroxycinnamic acids were linked to lignin in wheat straw cell walls. On the basis of two-dimensional ^{31}P-NMR experiments, Crestini et al.[3] showed that 77% of the carboxyl fraction of the ester bonds present in ball-milled wheat straw lignin is composed of *p*-coumaric acid, while the rest are other aromatic acids bound to lignin *via* intra- and/or intermolecular ester bonds. On the other hand, the hydroxyl fraction of the ester bonds was found to be almost exclusively (86%) aliphatic. This finding confirmed once again that esterification occurs mainly with the lignin side chains, as previously stated by Kondo and co-workers.[12] Shimada et al.[14] reported that bamboo lignin contained both α- and γ-esters of *p*-coumaric acid, while maize lignin has been reported to contain

Table IV The Content of Hydroxycinnamic Acids (%, w/w) in Milled Wheat Straw and Enzyme Lignin Fractions Obtained from 6 Days Ball-Milled Wheat Straw and 3 Days Cellulase-Treated Residues, Respectively

Lignin Fractions	*p*-Coumaric Acid			Ferulic Acid		
	Total	Ester-Linked	Ether-Linked	Total	Ester-Linked	Ether-Linked
HRML	0.18	0.081	0.10	0.72	0.26	0.46
PML	0.71	0.55	0.16	2.68	0.96	1.72
LREL	0.69	0.46	0.23	1.46	0.58	0.88
PEL	1.20	0.92	0.28	3.13	1.23	1.900

p-coumarate esters, selectively esterifing the γ-position of the side chains.[15] Occurrence of esterified *p*-coumaric acid at the γ-position and etherified ferulic acid at the β-position of the lignin side chain in the PML fraction was confirmed by ^{13}C-NMR studies (Fig. 6), indicating that wheat straw lignin contained γ-esters of *p*-coumaric acid and β-ethers of ferulic acid.

Molecular Weight Distribution

The weight-average (M_w) and number-average (M_n) molecular weights and polydispersity (M_w/M_n) of each fraction are given in Table V. As can be seen, PML and PEL fractions have much lower molecular weights than those of LREL, SLET, and HRML fractions. The reason for these differences was probably due to the higher content of bound polysaccharides in LREL, SLET, and HRML fractions. The present data also illustrated that PML had a relatively higher molecular weight (2090) compared to the corresponding PEL fraction (1880), suggesting that the PML fraction consists mostly of large molecules, and ball milling for 6 days did not cause a significant subdivision of the lignin molecules.

Table V The Weight-Average (M_w), Number-Average (M_n) Molecular Weights, and The Polydispersity (M_w/M_n) of Isolated Lignin Fractions Obtained from Wheat Straw

Lignin Fractions	$\bar{M}_w$	$\bar{M}_n$	$\bar{M}_w/\bar{M}_n$
HRML	16430	9040	1.82
PML	2090	1550	1.35
SLET	7750	5600	1.39
LREL	7860	4520	1.74
PEL	1880	1480	1.26

The GPC molecular weight distribution of PML is shown in Figure 3. The elution maximum corresponds to polystyrene molecular weight of 2730. The elution profile of the lignin showed a wide polymolecularity, ranging from dimers up to polystyrene of molecular weight over 20,000. The second peak corresponded to the low molecular components.

FTIR Spectra

The FTIR spectra of PML and PEL showed minor changes in the peak intensities (Fig. 4), which confirmed that the "core" of the lignin structure does not change dramatically during the 6-day ball-milling and 3-day cellulase-treatment processes. The intensive bands of the carbonyl groups appear in the range between 1660 and 1725 cm^{-1}. The exact position of the band is dependent on whether the C=O groups are in conjugation with the aromatic ring (position below 1700 cm^{-1}, such as 1660 cm^{-1}) or not (position above 1700 cm^{-1},

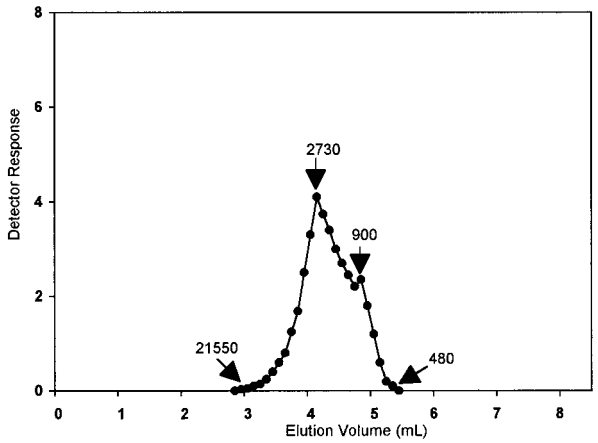

Figure 3 GPC molecular weight distribution of pure milled lignin (PML) obtained from wheat straw.

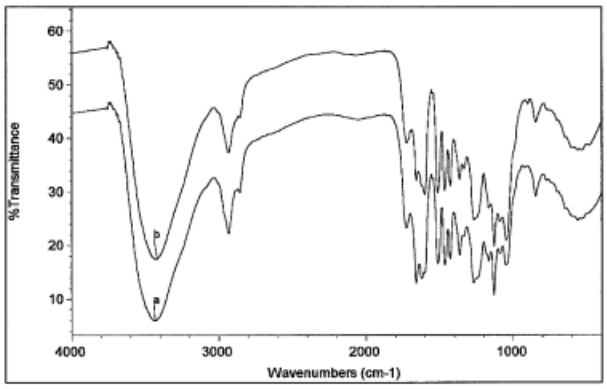

Figure 4 FTIR spectra of (a) pure milled lignin fraction (PML) and (b) pure enzyme lignin fraction (PEL) isolated from wheat straw.

e.g., 1720 cm^{-1}). Aromatic skeleton vibrations in two lignin fractions are assigned at 1595, 1510, and 1420 cm^{-1}.[16] Absorption at 1462 cm^{-1} indicates the C—H deformations and aromatic ring vibrations. The 1330, 1265, and 1235 cm^{-1} bands have been assigned to ring breathing with C—O stretch. The 1330 cm^{-1} band has been associated with sinapyl units, and 1265 and 1235 cm^{-1} bands with coniferyl units. Two small bands at 1364 and 1164 cm^{-1} correspond to aliphatic C—H stretch in CH_3 and C=O ester groups (conj.), respectively. The bands at 1129 and 1050 cm^{-1} indicate the aromatic CH in-plane deformation. Aromatic C—H out-of-plane bending appears at 843 cm^{-1}.

Figure 5 shows the FTIR spectra of SLET, HRML, and LREL. As can be seen from the diagram, the absorption intensities for lignin increased significantly from SLET through HRML to LREL. In contrast, the bands corresponding to polysaccharides decreased from SLET through HRML to LREL. Acetyl and uronic ester groups of hemicellulose residue absorb at 1740 cm^{-1} in the three fractions, and the absorption intensities decreased in the following order: HRML, LREL, and SLET. This phenomenon observed indicated that acetyl and uronic ester groups are enriched in polysaccharide–lignin complex fractions, such as HRML and LREL. The prominent bands in HRML spectrum corresponding to hemicelluloses appeared at 1650, 1040, and 900 cm^{-1}.

^{13}C-NMR Spectrum

The PML was also studied by ^{13}C-NMR spectroscopy (Fig. 6). Most of the observed signals have been previously assigned in straw and wood lignin spectra.[5,12,17–23] 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. This is due to the relatively free amount of bound polysaccharides in the PML fraction isolated by this two-step precipitation method. The spectrum does show signals at 62.9 (C-5, xyl internal unit), 65.3 (C-5, xyl nonreducing end unit), and 69.7 ppm (C-4, xyl nonreducing end unit) for polysaccharides; however, the peak intensities are rather weak. On the other hand, due to a large amount of polysaccharides associated in the ball-milled lignin fraction purified by ether precipitation from wheat straw in previous studies by Scalbert et al.,[5] all of the lignin spectra reported earlier showed rather large resonances for polysaccharides between 57 and 103 ppm. This made the assignments more difficult and overlap.

The region from 104.4 to 160.0 is assigned to be aromatic moiety of the lignin. The syringyl residues were indicated by signals at 153.0 and 152.3 (C-3/C-5, S), 138.0 (C-4, S etherified), 135.0 (C-1, S etherified), 133.0 (C-1, S nonetherified), 106.7 (C-2/C-6, S with α-CO), and 104.0 ppm (C-2/C-6, S), and guaiacyl residues gave signals at 149.4 (C-4, G etherified), 148.3–147.1 (C-3, G), 145.5 (C-4, G nonetherified), 135.0 (C-1, G etherified), 133.0 (C-1, G nonetherified), 120.5 and 119.2 (C-6, G), and 111.1 ppm (C-2, G), respectively. The *p*-hydroxyphenyl residues appeared signals at 128.8 and 128.0 ppm (C-2/C-6, H). These signals confirmed that pure milled lignin fraction could be justified as GSH-lignin. The signals at 166.2 (C-γ, PC ester), 129.7 (C-2/C-6, PC

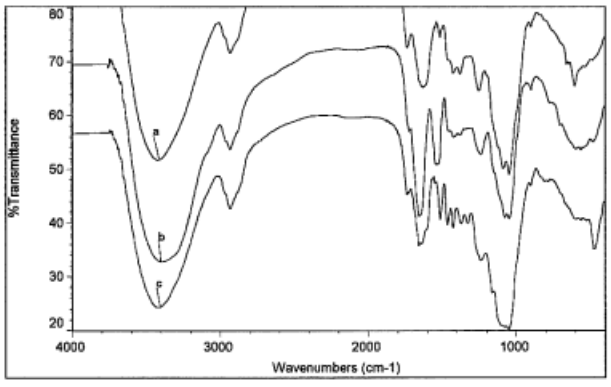

Figure 5 FTIR spectra of (a) hemicellulose-rich milled lignin fraction (HRML), (b) solubilized lignin during cellulase treatment (SLET), and (c) lignin-rich enzyme lignin fraction (LREL) isolated from wheat straw.

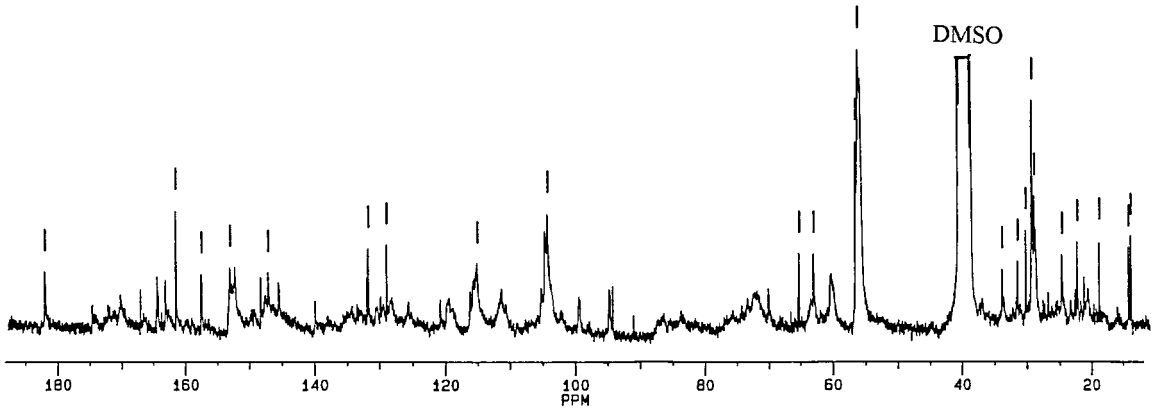

Figure 6 ^{13}C-NMR spectrum of pure milled lignin (PML, in DMSO-d6).

ester), 125.4 (C-1, PC ester), and 115.3 ppm (C-3/C-5, PC ester) indicated the esterified *p*-coumaric acid. Etherified ferulic acid was observed with signals at 167.1 (C-γ, FE ether) and 116.5 ppm (C-β, FE ether). Therefore, it seems very likely that the *p*-coumaric acids are linked by ester bonds at the γ-position of lignin side chains, while the ferulic acids are linked by ether bonds at the β- and γ-position of lignin side chains.

A very strong signal at 56.0 ppm corresponds to OCH_3 in syringyl and guaiacyl units. The intensive signals assignated to γ-methyl, α- and β-methylene groups in *n*-propyl side chains appeared in the spectrum between 13.6 and 33.8 ppm. The carbonyl resonances from uronic acids and esters may contribute to signals at 170.2 and 171.8 ppm, which indicates C-6 in methyl uronates.[10,17,20]

The ^{13}C-NMR spectrum over the range 160–100 ppm is more informative, yielding information on both the distribution of linkages and substitutions.[24] It is evident from the ^{13}C-NMR spectrum that the PML linkages, β-*O*-4 ether bonds (Fig. 7) (C-α in β-*O*-4, 72.4 ppm; C-β in β-*O*-4, 86.0–84.0 ppm; C-γ in β-*O*-4, 60.2 ppm), and less common β-5 (C-α in β-5, 87.0 ppm; C-γ in β-5, 65.3 ppm) and β-β (C-2/C-6 in β-β, 104.4 ppm; C-γ in β-β, 71.7 ppm) carbon–carbon linkages (Fig. 8) are present between the lignin structural units. This finding was in agreement with Crestini and co-workers' studies[3] on structural analysis of wheat straw lignin by quantitative ^{31}p- and 2D-NMR spectroscopy. The authors demonstrated that a β-*O*-4 aryl ether structure was evident, but not any evidence for the presence of α-*O*-4 substructures in milled wheat straw lignin.

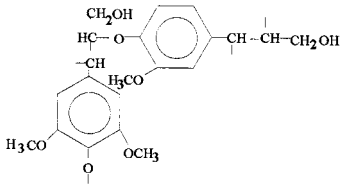

Figure 7 A simple representative β-aryl ether linkage.

SUMMARY

The current results showed that the Björkman method is suitable for the extraction of relatively pure lignins from both wood and straw when this two-step precipitation method is used instead of the traditional ether precipitation procedure in

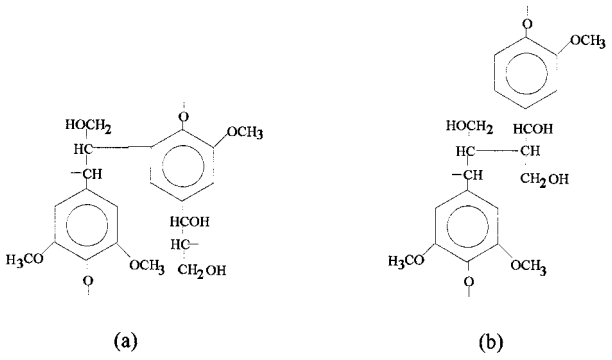

Figure 8 Simple representative carbon–cardon linkages: (a) β-5; (b) β-β.

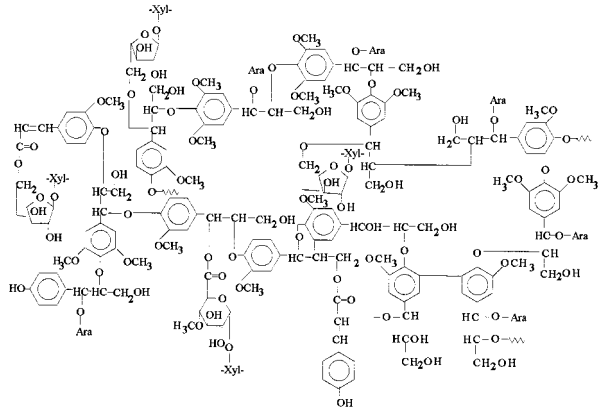

Figure 9 A structural model of wheat straw lignin.

the purification process. The isolated PML and PEL fractions contained much lower amounts of associated polysaccharides (2.36–2.86%). The PML fraction contained a slightly higher proportion of noncondensed guaiacyl units, while the PEL fraction showed a slightly higher proportion of noncondensed syringyl units. Fewer *p*-hydroxyphenyl units were found in all the lignin fractions. The isolated lignins are more condensed than hardwood lignins. Meanwhile, the lignin in wheat straw cell walls appeared to be very closely associated to hydroxycinnamic acid and glucuronic acid or 4-*O*-methylglucuronic acid. It was found that glucuronic acid or 4-*O*-methylglucuronic acid and over 77% *p*-coumaric acid is esterified to lignin, whereas about 60–64% ferulic acid is linked to lignin by ether bonds.

Based on the current results and our previous studies on wheat straw lignin,[25] a structural model of wheat straw lignin is proposed in Figure 9. However, it must be noted that the structural model in Figure 9 represents the linkages between hydroxycinnamic acids and polysaccharides and/or lignin, between lignin and polysaccharides, or among the lignin structural units, and that it has no quantitative meaning.

The authors are grateful for the financial support of this research from LINK Collaborative Program in Crops for Industrial Use under Agreement CSA 2054, and to Dr. James Bolton, Director of The BioComposites Centre, for the award of a research fellowship to Dr. R.-C. Sun.

REFERENCES

1. B. Billa and B. Monties, *Res. Chem. Intermed.,* **21,** 303 (1995).
2. G. Jacquet, B. Pollet, C. Lapierre, F. Mhamdi, and C. Rolando, *J. Agric. Food Chem.,* **43,** 2746 (1995).
3. C. Crestini and D. S. Argyropoulos, *J. Agric. Food Chem.,* **45,** 1212 (1997).
4. G. Wegener and D. Fengel, *Tappi,* **62,** 97 (1979).
5. A. Scalbert, B. Monties, E. Guittet, and J. Y. Lallemand, *Holzforschung,* **40,** 119 (1986).
6. T. Kondo, T. Ohshita, and T. Kyuma, *Anim. Feed Sci. Technol.,* **39,** 253 (1992).
7. A. B. Blakeney, P. J. Harris, R. J. Henry, and B. A. Stone, *Carbohydr.Res.,* **113,** 291 (1983).
8. R.-C. Sun, J. M. Lawther, and W. B. Banks, *Indust. Crops Prod.,* **4,** 127 (1995).
9. J. M. Lawther, R.-C. Sun, and W. B. Banks, *J. Agric. Food Chem.,* **43,** 667 (1995).
10. R.-C. Sun, J. M. Lawther, and W. B. Banks, *J. Agric. Food Chem.,* **62,** 1473 (1996).
11. D. Ben-Ghedalia and E. Yosef, *J. Agric. Food Chem.,* **42,** 649 (1994).
12. T. Kondo, T. Watanabe, T. Ohshita, and T. Kyuma, *J. Sci. Food Agric.,* **68,** 383 (1995).
13. R.-C. Sun, J. M. Lawther, and W. B. Banks, *Indust. Crops Prod.,* **6,** 1 (1997).
14. M. Shimada, T. Fukuzuka, and T. Higuchi, *Tappi,* **54,** 72 (1971).
15. J. Ralph, R. D. Hatfied, S. Quideau, R. F. Helm, J. H. Grabber, and H. J. G. Jung, *J. Am. Chem. Soc.,* **116,** 9448 (1994).
16. J. G. Buta, F. Zadrazil, and G. C. Galletti, *J. Agric. Food Chem.,* **37,** 1382 (1989).
17. D. S. Himmelsbach and F. E. Barton, II, *J. Agric. Food Chem.,* **28,** 1203 (1980).
18. H. H. Nimz, D. Robert, and O. Faix, *Holzforschung,* **35,** 16 (1981).
19. X.-Q. Pan, D. Lachenal, V. Neirinck, and D. Robert, *J. Wood Chem. Technol.,* **14,** 483 (1994).
20. T. Imamura, T. Watanable, M. Kuwahara, and T. Koshijima, *Phytochemistry,* **37,** 1165 (1994).
21. C. P. Neto, D. Evtuguin, and A. Robert, *J. Wood Chem. Technol.,* **14,** 383 (1994).
22. P. M. Froass, A. J. Ragauskas, and J. E. Jiang, *J. Wood Chem. Technol.,* **16,** 347 (1996).
23. M. C. Terrón, M. L. Fidalgo, G. Almendros, and A. E. Gonzalez, *Rapid Commun. Mass Spectrosc.,* **10,** 413 (1996).
24. D. Stewart, R. Brennan, and G. J. Provan, *Phytochemistry,* **37,** 1703 (1994).
25. R.-C. Sun, P. Rowlands, and J. M. Lawther, in *Recent Developments in Agriculture and Food Chemistry,* to appear.