FRACTIONAL AND STRUCTURAL CHARACTERIZATION OF BALL MILLED AND ENZYME LIGNINS FROM OIL PALM EMPTY FRUIT BUNCH FIBER

Run-Cang Sun
Senior Research Scientist

Laurence Mott
Pulp and Paper Group Research Manager

and

James Bolton
Director
The BioComposites Centre

University of Wales
Bangor LL57 2UW
United Kingdom

(Received October 1997)

ABSTRACT

The pure milled lignin (PML), hemicellulose rich milled lignin (HRML), pure enzyme lignin (PEL), lignin rich enzyme lignin (LREL), and solubilized lignin during enzyme treatment (SLET) fractions were obtained by 90% and 50% dioxane-water extractions from 6 days' ball milled oil palm empty fruit bunch fiber and the subsequently 3 days' cellulase-treated fiber residues, respectively. The purification was performed with a two-step precipitation method instead of traditional ether precipitation. The five lignin fractions were compared using spectroscopic and degradative techniques. The pure milled lignin and pure enzyme lignin fractions showed a very low content of associated polysaccharides (2.84–2.98%), and contained a large proportion of noncondensed syringyl units with some amounts of noncondensed guaiacyl units and fewer p-hydroxyphenyl units. They are composed mainly of β-O-4 ether bonds together with small amounts of β-5 carbon-carbon linkages. The lignin fractions also appeared to be very closely associated with hydroxycinnamic acid and glucuronic acid or 4-O-methylglucuronic acid. Glucuronic acid or 4-O methylglucuronic acid and over 83% of p-coumaric acid were identified to be esterified to lignin, and over 53% of ferulic acid was found to be linked to lignin by ether bonds.

Keywords: Oil palm empty fruit bunch fiber, lignin, polysaccharides, phenolic acids and aldehydes, molecular weight, FT-IR, ^{13}C-NMR spectroscopy.

INTRODUCTION

The fast-growing renewable biomass, such as straw of wheat, barley, rice, maize, oats, rye and cotton, sugar-beet pulps, bagasse, and bamboos, represents an enormous underutilized energy resource of great feed potential for ruminants and also a great potential as raw material for paper, chemicals, and other technical products.

Oil palm, being a species native to West Africa, has been introduced to various parts of the tropics to obtain palm oil from its fruits. The production of palm oil has tremendously increased since the 1970s, especially in South East Asian countries, such as Malaysia and Indonesia (Tomimura 1992). As a result, high amounts of palm tree by-products, that is, empty fruit bunches, fronds and trunks, are easily available. The annual production is 20.5 tons per hectare of oil palm biomass (dry weight), out of which only the palm oil and kernel are fully utilized, the remaining 67% being either discarded or underutilized (Kam-

Wood and Fiber Science, 30(3), 1998, pp. 301–311

ishima et al. 1988). Empty fruit bunch is a by-product in oil palm industry after the removal of the oil seeds for oil extraction, and represents a very abundant, inexpensive, and renewable resource. To date no commercial utilization of this empty fruit bunch has been reported (Sreekala et al. 1996). Therefore, the utilization of empty fruit bunches as lignocellulosic material has economical and ecological importance.

Oil palm empty fruit bunch fiber (OPEFBF) shows excellent mechanical properties and becomes a cost-effective replacement for synthetic fibers. The processing of this fiber is comparatively easier than that of other natural fibers (Sreekala et al. 1996). The overall aim of the present research into OPEFBF in our laboratories is to investigate the feasibility of using OPEFBF as a potential material for the production of chemical pulps and paper products. To achieve this aim, a thorough study and characterization of the starting material is necessary. In addition, lignin constitutes one of the major drawbacks for utilization of OPEFBF as raw material for pulp and paper production. Wood and straw lignins have been investigated in some detail (Björkman 1956; Himmelsbach and Barton 1980; Nimz et al. 1981; Froass et al. 1996; Sun et al. 1997a), but there is no information on the OPEFBF lignin. The specific objectives of this study were to isolate and characterize the ball milled and enzyme lignin fractions from OPEFBF.

MATERIALS AND METHODS

Fractionation and isolation of ball milled and enzyme lignins

Oil palm empty fruit bunch fiber was supplied by the Forest Research Institute of Malaysia, and it was processed by retting technique. The air-dried OPEFBF was ground using a Chritie Laboratory mill to pass a 60-mesh size screen. The ground fiber was then extracted with toluene-ethanol (2:1, v/v) for 5 h in a Soxhlet apparatus. After being dried in an oven for 16 h at 50°C, the extracted powders were subjected to ball milling for 6 days in a rotary porcelain ball mill at 80 rpm in dry state, respectively, using a mixture of 10-mm and 20-mm (1:1, w/w) porcelain balls at a balls/residue 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 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 extraction per sample, and the solvents were removed by a rotary vacuum evaporator at 40°C. The HRML was obtained by precipitation in 4 vols ethanol. The PML was obtained by re-precipitation 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, USA) (4 g per 10 g of the residue 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 the fractions were generated by successive extractions with 90% and 50% dioxane and separation as before. The SLET fraction was obtained by direct precipitation at pH 1.5 with 20% HCl from the cellulase-treated hydrolysates (Fig. 1).

Physico-chemical characterization of lignin fractions

UV spectra were recorded on a Hewlett-Packard 8452A Diode Array spectrophotometer. 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 240 and 350 nm were measured.

The molecular-average weight of lignin fractions was determined by gel permeation chromatography on a PLgel 5μ Mixed-D column. The samples were dissolved with tetra-

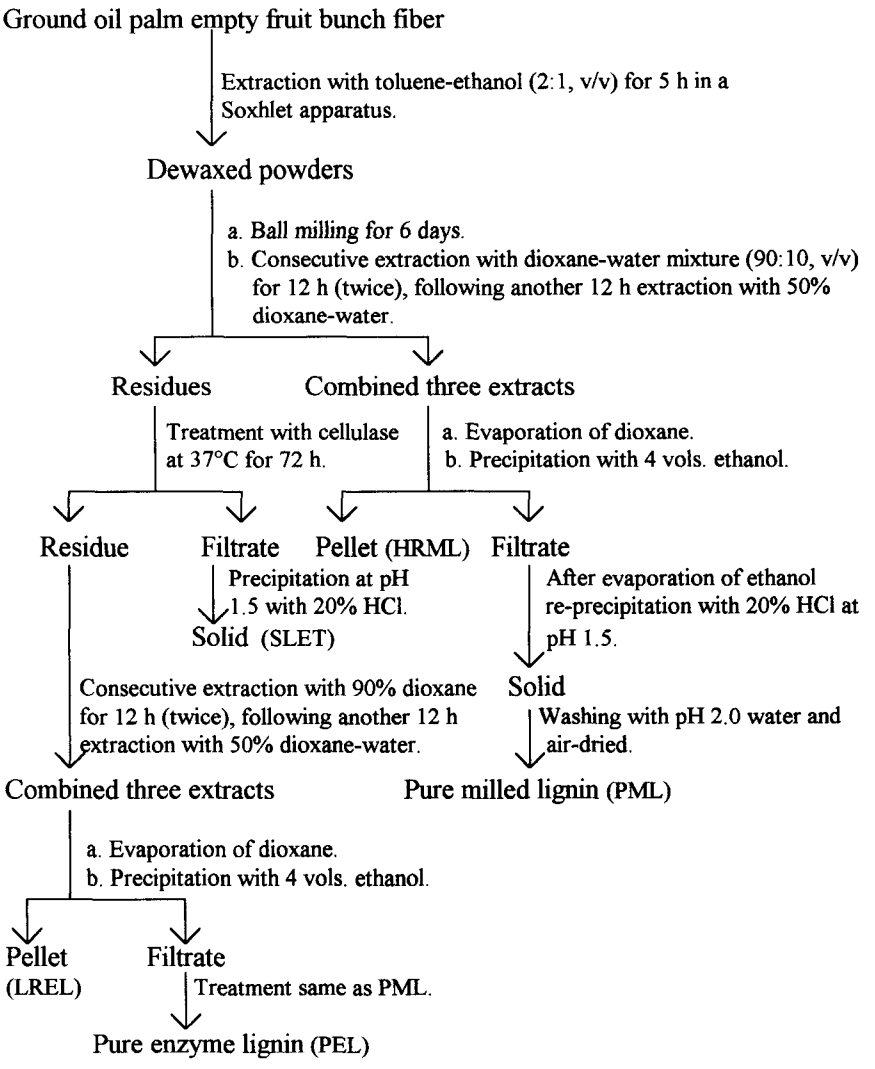

FIG. 1. Scheme for isolation of lignin fractions from oil palm empty fruit bunch fiber.

hydrofuran 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 (peak average molecular weights 1,320, 3,250, 9,200, 28,500, and 66,000).

FT-IR spectra were obtained on an FT-IR spectrophotometer (Nicolet, 750) using a KBr disc containing 1% finely ground samples. The solution-state of ^{13}C-NMR spectrum was obtained on 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 a 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.

TABLE 1. *The yields (% acidic chlorite lignin, w/w) of milled and enzyme lignins obtained from oil palm empty fruit bunch fiber.*

Lignin fractions	Yield (%)
Hemicellulose rich milled lignin (HRML)	23.31
Pure milled lignin (PML)	11.97
Solubized lignin during enzyme treatment (SLET)	4.44
Lignin rich enzyme lignin (LREL)	18.03
Pure enzyme lignin (PEL)	12.04

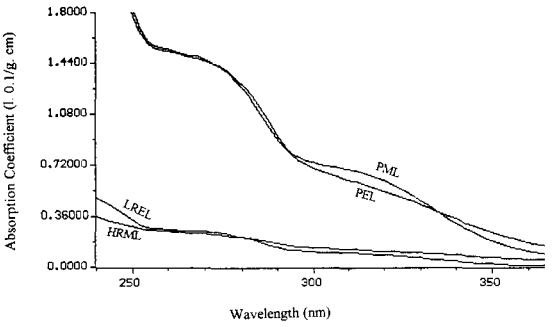

FIG. 2. UV spectra of pure milled lignin (PML), pure enzyme lignin (PEL), lignin rich enzyme lignin (LREL), and hemicellulose rich milled lignin (HRML).

Ether-linked hydroxycinnamic acids were calculated as the difference between total and ester-linked hydroxycinnamic acids (Kondo et al. 1992).

Neutral sugar composition of the lignin fractions was determined as alditol acetates (Blakeney et al. 1983). Methods of uronic acid analyses, alkaline nitrobenzene oxidation of lignin, and determination of phenolic acids and aldehydes with high performance liquid chromatography have been described in previous papers (Sun et al. 1995; Lawther et al. 1995). 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

Yield of lignin fractions

The yield of the lignins resulting from the various fractionation procedures was expressed as a percentage of the total lignin determined by acidic chlorite oxidation (14.2%, w/w, Lawther et al. 1995).

The fractional yields of ball milled and enzyme lignins are shown in Table 1. Six days' ball milling and three days' cellulase treatment yielded about 70% crude lignin fractions. The yields of PML (11.97%) and PEL (12.04%) are lower than the corresponding yields of HRML (23.31%) and LREL (18.03%) fractions, respectively, indicating that the majority of lignin is still linked to polysaccharides after 6 days' ball milling or 3 days' cellulase treatment. A slightly lower yield of enzyme lignin (PEL+LREL) than that of ball milling lignin (PML+HRML) is probable because some amount of lignins has been extracted during the ball milling process.

UV spectra

The UV spectra of PML, PEL, LREL, and HRML are shown in Fig. 2. The PML and PEL fractions exhibited the basic UV spectrum typical of lignins with a maximum at 225, 275, and 315 nm. The second absorption maximum was originated from non-conjugated phenolic groups (aromatic ring) in lignin (Scalbert et al. 1986). The third one was undoubtedly associated with the ferulic or p-coumaric acid. This phenomenon was also found in other samples of grass lignins, such as wheat straw lignin (Sun et al. 1997a). Much lower absorption coefficients of LREL and HRML fractions were undoubtedly due to the associated non-lignin materials such as polysaccharides.

Composition of bound polysaccharides

Because of lignin's close association with other polymers, the isolation of representative lignin preparations from grass or straw becomes more difficult, which is an important handicap in analytical research on lignin's structure and transformations. Björkman's procedure (1956) of vibratory milling and solvent extraction is the preferred method for isolating relatively unchanged lignin. This procedure

TABLE 2. *The content (% sample, w/w) of neutral sugars and uronic acids in milled and enzyme lignin fractions obtained from 6 days' ball milled oil palm empty fruit bunch fiber and 3 days cellulase treated the residues, respectively.*

Extraction fractions	Neutral sugars							Uronic acids	Total
	Rha	Dib	Ara	Xyl	Man	Glc	Gal		
HRML	1.68	0.50	7.89	15.52	1.30	2.15	4.83	10.19	44.06
PML	ND[a]	ND	0.12	1.02	ND	0.48	0.14	1.08	2.84
SLET	0.14	ND	0.66	7.26	2.16	32.43	20.61	1.28	64.54
LREL	0.14	ND	0.55	5.46	0.23	1.76	0.69	8.11	16.94
PEL	ND	ND	0.12	1.08	ND	0.47	0.15	1.16	2.98

[a] ND = not detected.

has been successful in the isolation of relatively pure milled lignins from wood samples; however, when applied to other plant material having a low lignin content, such as grass and straw, the results have not been as successful (Himmelsbach and Barton 1980). Scalbert and co-workers (1986) 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, however, contained 7.1% and 16.8% polysaccharides, respectively. With the study about the effect of isolation procedure on molecular weight distribution of wheat straw lignins, Ben-Ghedalia and Yosef (1994) fractionated ball milled lignin and enzyme lignin into 8 fractions by ball milling for 7, 14, 21, and 28 days, respectively, and purified the lignin fractions by dissolution in 90% acetic acid and precipitation in acidic water. The lignin preparations still contained 14.2–16.4% polysaccharide sugars. The crude lignin fractions, obtained by ball milling and by 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 still contained 19.1–21.0% polysaccharides (Kondo et al. 1995). This high content of polysaccharides in ball milled and enzyme lignin preparations was thought due to the specific structural patterns of association between lignin and polysaccharides in straw and grass cell walls (Scalbert et al. 1986). However, by using this two-step precipitation method in the fractionation and purification of Björkman lignin (1956) from grass, straw, and OPEFEF, it is easy to obtain the lignin fractions relatively free of polysaccharides. As shown in Table 2, the PML and PEL contained 2.84 and 2.98% associated polysaccharides, respectively, indicating that a ball milling can peel off the lignin from most of its neighboring polysaccharide moiety, that is, the bonds anchoring lignin to hemicelluloses in PML and PEL are mostly cleaved under the ball milling conditions. This is in line with our recent findings of wheat straw alkaline lignin, ball milling, and enzyme lignins (Sun et al. 1996; Sun et al. 1997a). On the other hand, the other three lignin fractions, HRML, SLET, and LREL showed a much higher content of associated polysaccharides, 16.94–64.54%, suggesting that the native linkages between lignin and hemicelluloses in the three fractions are only partly cleaved during the ball milling and cellulase treatment processes. Xylose was found to be the major component with arabinose, uronic acid, glucose, and galactose as the secondary monosaccharides in HRML and LREL fractions. A high proportion of glucose in the SLET fraction was due to the degradation of cellulose by cellulase. The current results showed that HRML and LREL fractions were more closely associated with polysaccharides, and PML and PEL fractions were less closely associated to polysaccharides, which may also reflect the situation occurring with the various areas of the cell wall.

It is of interest to note that 1.08–1.16% uronic acids exist in the purified PML and

TABLE 3. *The yield (% sample, w/w) of phenolic acids and aldehydes from alkaline nitrobenzene oxidation of the lignin fractions obtained from oil palm empty fruit bunch fiber.*

Phenolic acids and aldehydes	Extraction fractions				
	HRML	PML	SLET	LREL	PEL
p-Hydroxybenzoic acid	0.037	0.70	ND	0.073	0.33
p-Hydroxybenzaldehyde	0.12	0.53	0.32	0.24	0.29
Vanillic acid	0.11	0.60	0.21	0.21	0.28
Syringic acid	0.23	1.71	0.030	0.90	1.32
Vanillin	1.37	11.78	0.18	3.04	5.90
Syringaldehyde	3.16	22.04	0.36	10.54	20.83
p-Coumaric acid	0.11	0.24	ND[a]	0.18	0.28
Ferulic acid	Trace	1.01	ND	0.34	0.54
Total	5.14	38.61	1.10	15.53	29.77

[a] ND = not detected.

PEL fractions. This relatively high content of uronic acids was probably due to the ester bonds between lignin and glucuronic acid residue of hemicelluloses in OPEFBF cell walls, which was confirmed by two signals at 169.7 and 171.9 ppm in ^{13}C-NMR spectrum (Fig. 6).

Components of phenolic monomers

The simple phenolic monomers obtained from alkaline nitrobenzene oxidation of the five lignin fractions are given in Table 3. These phenolic acids and aldehydes resulted from the degradation of noncondensed lignin units. The major degradation product, syringaldehyde, resulted from the degradation of syringyl noncondensed units. The presence of the second major degradation product of vanillin was due to the degradation of guaiacyl noncondensed units. The syringaldehyde/vanillin molar ratios of five lignin fractions increased from 1.56 in PML to 2.95 in PEL fraction. Occurrence of fewer p-hydroxybenzaldehydes were generally considerated indicative of p-hydroxyphenyl units with the lignin 'core'. A similar result was reached by Gallacher et al. (1994), who proposed that the lignin in oil palm trunk cell walls appeared to contain a high proportion of aryl ether-linked syringyl units, but no ferulic or p-coumaric acid. Tomimura (1992) demonstrated that p-hydroxybenzaldehyde was not observed in the nitrobenzene oxidation products of oil palm trunk lignins. Obviously, small amounts of p-hydroxybenzaldehyde, ferulic acid (FE), and p-coumaric acid (PC) were found in the nitrobenzene oxidation products from our studies.

As can be seen from Table 3, the relatively high yields of alkaline nitrobenzene oxidation of PML and PEL confirmed the lower degree of condensation of PML and PEL, compared to the corresponding yields of wheat straw lignins (Sun et al. 1997a). Between the two pure lignin fractions, PML gave a higher yield in monomeric units, suggesting a relatively less condensed PML fraction. On the other hand, the much lower yield of oxidation products of LREL, HRML, and SLET was undoubtedly due to the higher content of associated polysaccharides, which corresponded with the sugar analyses.

The content of p-coumaric and ferulic acids in the isolated five lignin fractions is shown in Table 4. As expected, the PML and PEL contained more hydroxycinnamic acids than the corresponding HRML and LREL, respectively, indicating that the p-coumaric acid and fer-

TABLE 4. *The content of hydroxycinnamic acids (% sample, w/w) in milled and enzyme lignin fractions obtained from 6 days' ball milled oil palm empty fruit bunch fiber and 3 days cellulase treated residues, respectively.*

Lignin fractions	p-Coumaric acid					Ferulic acid				
	Total		Ester-linked		Ether-linked	Total		Ester-linked		Ether-linked
	cis	*trans*	*cis*	*trans*	*cis* + *trans*	*cis*	*trans*	*cis*	*trans*	*cis* + *trans*
HRML	0.003	0.038	ND[a]	0.039	0.002	0.15	0.084	0.15	ND	0.084
PML	0.02	0.18	ND	0.18	0.02	0.64	0.34	0.41	0.051	0.52
LREL	0.013	0.089	ND	0.082	0.02	0.37	0.14	0.18	0.030	0.30
PEL	0.017	0.083	ND	0.083	0.017	0.54	0.20	0.27	0.034	0.44

[a] ND = not detected.

ulic acid are mainly linked with lignin in OPEFBF cell walls. About 90% and 83% p-coumaric acid was esterified to lignin, whereas only 47% and 41% ferulic acid was esterified to lignin in PML and PEL fractions, respectively. The foregoing data indicated that a considerable proportion of p-coumaric acid was linked to lignin by esterified bonds, while over half of ferulic acid was linked by ether bond to lignin. These results agreed with our previous studies on wheat straw lignins (Sun et al. 1997a). The authors showed that about 90% p-coumaric acid in wheat straw cell walls is present in the ester-linked form to lignin, while 50–70% ferulic acid is ether-linked to lignin. Based on the study of cinnamic acid bridges between cell-wall polymers in wheat and phalaris internodes, Lam and co-workers (1992) reported that all of the etherified ferulic acid in the dioxane-water-soluble fractions of walls of wheat and phalaris internodes is also ester-linked, whereas p-coumaric acid is not involved in ester-ether bridges. The above findings suggested that ferulic acid probably formed an ester-ether bridge between lignin and polysaccharides in OPEFBF cell walls.

Further studies found that the substituted cinnamic acids were in both *cis* and *trans* forms. The amounts of *trans* isomer of p-coumaric acid were greater than those for the corresponding *cis* isomer, whereas the reverse was true for ferulic acid (Table 4). The results obtained did not agree with Newby and coworkers' study (1980) on free and bound phenolic acids of lucerne. The authors demonstrated that the *trans* forms of hydroxycinnamic acids in every case predominated, and if light had been excluded during the extraction and analyses, only the *trans* forms would have been present. These relatively higher amounts of *cis* isomers of p-coumaric and ferulic acids were probably due to occurring photoisomerization during the sample analyses in our studies.

Molecular weight distribution

The weight-average (M_w) and number-average (M_n) molecular weights and polydispersity (M_w/M_n) of each lignin fraction are given in Table 5. As expected, due to the low amount of associated polysaccharides, the isolated PML and PEL gave much lower molecular-average weights (1,990–2,720) compared to other two polysaccharide rich fractions. With the increase of associated polysaccharide content from 16.94% in LREL to 44.06% in HRML, the weight-average molecular weights increased significantly from 7,300 to over 18,000. These results were in good agreement with our previous findings from wheat straw lignins (Sun et al. 1997a). The present data also illustrated that PML had a relatively higher molecular weight (2,720) than the corresponding PEL fraction (1,990), suggesting that the PML fraction was composed mostly of large molecules, and ball milling for 6 days did not cause a significant subdivision of the lignin molecules.

TABLE 5. *The weight-average (M_w), number-average (M_n) molecular weights, and the polydispersity (M_w/M_n) of lignin fractions obtained from oil palm empty fruit bunch fiber.*

Extraction fractions	M_w	M_n	M_w/M_n
HRML	18,060	8,870	2.04
PML	2,720	1,910	1.43
SLET	7,700	5,700	1.35
LREL	7,300	4,020	1.81
PEL	1,990	1,520	1.31

The gel permeation chromatogram of PML is shown in Fig. 3. The elution maximum corresponded to polystyrene molecular weight of 3,200. The elution profile showed a wide polymolecularity, ranging from trimers up to polystyrene of molecular weight over 15,680. The second peak corresponded to the lower molecular components.

FT-IR spectra

The FT-IR spectra of PML and PEL fractions (Fig. 4) show several absorption bands that can be assigned empirically to structural groups, based on a multitude of results obtained both from model compounds and lignins (Jung and Himmelsbach 1989; Buta et al.

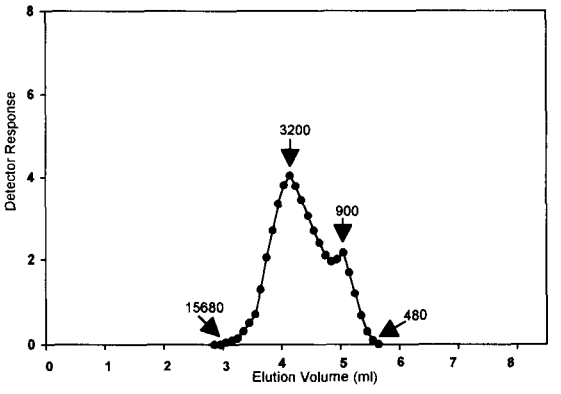

FIG. 3. GPC molecular weight distribution of pure milled lignin (PML) obtained from oil palm empty fruit bunch fiber.

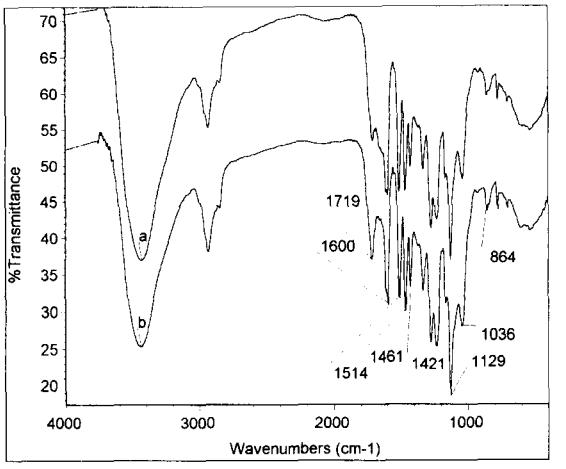

FIG. 4. FT-IR spectra of pure milled lignin fraction (PML, a) and pure enzyme lignin fraction (PEL, b) isolated from oil palm empty fruit bunch fiber.

1989; Almendros et al. 1992). The presence of a peak at 1,719 cm^{-1} is assignable to carbonyl groups (unconjugation with aromatic ring), and the absorption of this band is greater for PEL than for PML. Aromatic skeleton vibrations in lignin are assigned at 1,600, 1,514, and 1,421 cm^{-1}; and the C-H deformations and aromatic ring vibrations appear at 1,461 cm^{-1} (Sun et al. 1997b).

The different relations between the intensities of the bands at 1,514 and 1,600 cm^{-1} are used for a differentiation of softwood and hardwood lignins. In unconjugated syringyl model compounds, hardwood, and straw lignins, the intensity of these two bands is nearly the same or the second one is stronger than the first; while in unconjugated guaiacyl compounds and softwood lignins, the intensity of the 1,514 cm^{-1} band is considerably higher. As shown in Fig. 4, the band at 1,600 cm^{-1} was slightly more intensive than that at 1,514 cm^{-1} in the PML spectrum, and more intensive than at 1,514 cm^{-1} in the PEL spectrum, indicating that the isolated PML and PEL fractions have a higher syringyl unit content than that of guaiacyl unit. This was confirmed by nitrobenzene oxidation (Table 3). The current results suggested that oil palm empty fruit bunch fiber lignins can be classified as "hardwood type lignins."

Figure 5 shows the FT-IR spectra of HRML, SLET, and LREL. As can be seen, the absorption intensities for lignin decreased significantly from LREL to HRML, and to SLET, which corresponded with the yields of nitrobenzene oxidation. The prominent bands in HRML spectrum corresponding to hemicelluloses appeared at 1,660, 1,607, 1,043, and 904 or 897 cm^{-1}.

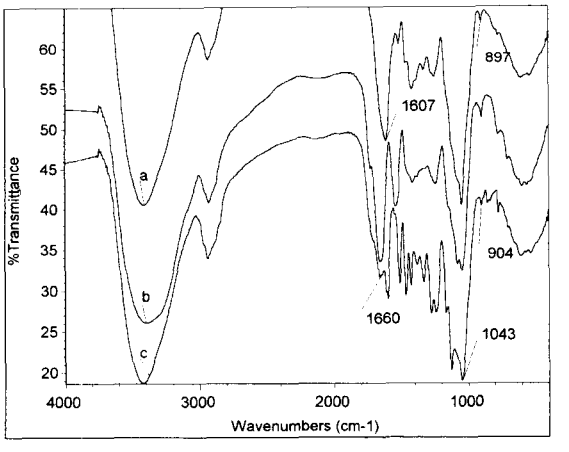

FIG. 5. FT-IR spectra of hemicellulose rich milled lignin fraction (HRML, a), solublized lignin during cellulase treatment (SLET, b), and lignin rich enzyme lignin fraction (LREL, c) isolated from oil palm empty fruit bunch fiber.

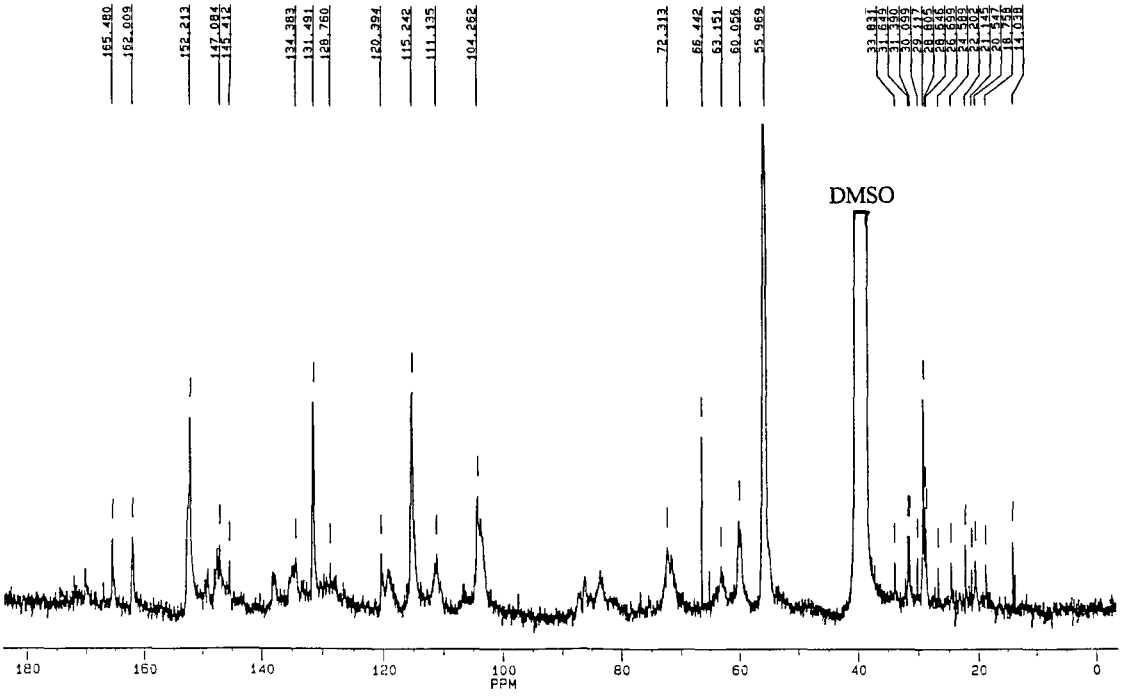

FIG. 6. ^{13}C-NMR spectrum of pure milled lignin (PML, in DMSO-d_6).

^{13}C-NMR spectrum

The PML fraction was also studied by ^{13}C-NMR spectroscopy and the spectrum is shown in Fig. 6. Most of the assignments could be made according to Nimz et al. (1981), Lapierre et al. (1984), Scalbert et al. (1986), Jung and Himmelsbach (1989), Pan et al. (1994), Imamura et al. (1994), Kondo et al. (1995), Terrón et al. (1996), and Froass et al. (1996). As shown in Fig. 6, the most striking characteristic of the ^{13}C-NMR spectrum is the near disappearance of typical polysaccharide signals between 57 and 103 ppm. This phenomenon was also observed in our previous studies on wheat straw alkaline lignin fractions isolated by a two-step precipitation method (Sun et al. 1996). Due to the low content of associated polysaccharides (2.84%) in the PML fraction, the spectrum showed only one signal at 63.2 ppm (C-5, xyl internal unit) for polysaccharides.

In the aromatic region (104.3 to 165.5 ppm) of the spectrum, the syringyl (S), guaiacyl (G), and p-hydroxyphenyl (H) residues were indicated by signals at 152.2 (C-3/C-5, S), 147.5 (C-3/C-5, S nonetherified), 138.0 (C-4, S etherified), 134.4 (C-1, S etherified), 106.5 (C-2/C-6, S with α-C=O), and 104.3 ppm (C-2/C-6, S); 149.5 (C-4, G etherified), 147.5 (C-3, G), 147.1 (C-3, G), 145.4 (C-4, G nonetherified), 134.4 (C-1, G etherified), 119.4 (C-6, G), 115.2 (C-5, G), 111.1 ppm (C-2, G); 162.0 (C-4, H), 131.5 (C-2/C-6, H), 115.2 (C-3/C-5, H), and 120.4 ppm (C-1, H), respectively. These signals confirmed that the PML fraction could be justified as GSH-lignin. Etherified and esterified ferulic acid was observed with a small signal at 166.8 (C-γ, FE ether/ester). There are very few coumaric and ferulic acid/ester structures indicated in the spectrum as both structures should have a strong α-C signal (protonated) in the range of 143.7–145 ppm (in DMSO). The only signals in this region are relatively weak compared to the very strong signals due to p-OH benzoate structures.

The strong resonance at 56.0 ppm corresponds to OCH_3 in syringyl and guaiacyl units. The intensive signals assigned to γ-methyl, α and β-methylene groups in n-propyl side chains appeared in the spectrum between 14.0 and 33.8 ppm (14.0–18.7 ppm for $-CH_3$, 22.2–33.8 ppm for $-CH_2-$). The integration values corresponding to the carbonyl region (two small signals at 169.7 and 171.9 ppm for carbons in aliphatic acids or esters) was in agreement with the above-indicated ester bonds between lignin and glucuronic acid or 4-O-methylglucuronic acid residue of hemicelluloses in OPEFBF cell walls (Himmelsbach and Barton 1980; Imamura et al. 1994).

The C-γ in β-O-4, C-α in β-O-4, and C-β in β-O-4 ether bond signals appeared at 60.1, 72.3, and 84.6 and 86.4 ppm, respectively. A low-intensity signal for C-α in β-5 carbon-carbon bond can be seen at 87.1 ppm. These signals indicated that oil palm empty fruit bunch fiber lignin is composed mainly of β-O-4 ether bonds together with small amounts of β-5 carbon-carbon linkages. These findings were in accordance with the Tanahashi and Higuchi's studies (1990) on the effect of the hydrophobic regions of hemicelluloses on dehydrogenative polymerization of sinapyl alcohol. The authors demonstrated that syringyl lignin is composed mainly of β-O-4 ether linkages, and the hemicelluloses are easily connected to the α-position of lignin.

Conclusions

Based on the above studies, it can be concluded that the Björkman method is suitable for extraction of relatively pure lignins from both wood and herbaceous plants, such as grass, straw, and oil palm when the two-step precipitation method is used instead of the traditional ether precipitation procedure in the purification process. The PML fraction showed a high amount of p-OH benzoate structures and somewhat lower amounts of hydroxycinnamic acids, such as p-coumaric acid and ferulic acid. The PEL fraction, on the other hand, included less p-coumaric and ferulic acids. Both PML and PEL fractions contained a large proportion of syringyl units with some amounts of guaiacyl units and fewer hydroxyphenyl units. The PEL preparation appeared to have more syringyl units than the PML fraction. They probably have the same degree of condensation of typical hardwood or softwood lignins, but less condensed than wheat straw lignin.

Acknowledgment

The authors are grateful for the financial support of this research from the Forestry Research Programme (Optimizing Pulping of Oil Palm Materials) of the UK Department for International Development (DFID) for the benefit of developing countries under Agreement R6353.

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