Isolation and Fractional Characterization of Ball-Milled and Enzyme Lignins from Oil Palm Trunk

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Ball-milled and enzyme lignins were isolated from the trunks of oil palms and subsequently studied. The lignins were fractionated into pure milled lignin (PML), pure enzyme lignin (PEL), lignin rich enzyme lignin (LREL), hemicellulose rich milled lignin (HRML), and solubilized lignin obtained during enzyme treatment (SLET) fractions by a two-step precipitation method. The chemical and structural composition of the five lignin preparations was determined by using UV, GPC, FT-IR, ^{13}C NMR spectroscopy and nitrobenzene oxidation. Pure milled lignin and pure enzyme lignin fractions were found to contain rather low amounts of neutral sugars, 2.2−3.1%. The lignin fractions contained a high proportion of noncondensed syringyl units with small amounts of noncondensed guaiacyl and *p*-hydroxyphenyl units. Trace amounts of *p*-coumaric and ferulic acids were also found to be associated with lignin in the oil palm trunk cell walls.

Keywords: *Polysaccharides; phenolic acids; aldehydes; alkaline nitrobenzene oxidation; molecular weight; FT-IR; ^{13}C NMR spectroscopy*

INTRODUCTION

Oil palms are monocotyledons with trunks that are anatomically different from wood, consisting of vascular bundles and parenchyma tissues (Tomimura, 1992). They are assumed to have a low content of pectin and xyloglucan and a high content of xylan in their primary (nonlignified) cell walls (McNeil et al., 1984), although the experiments on which these generalizations were based were restricted to the Gramineae. The Gramineaes have secondary walls containing a different type of lignin, attached to the arabinoxylan hemicelluloses, and contain abundant esterified ferulic and *p*-coumaric acids (Gallacher et al., 1994). It has been shown that the palms (Arecaceae) are one of the few monocotyledon families whose cell walls have a low pectin content, approaching that of the Gramineae (Jarvis, 1994). They are taxonomically close to the grasses (Dahlgren and Clifford, 1982).

Lignin's large molecular size and close association with other cell-wall polymers makes isolation of representative lignin preparations difficult, which is a significant handicap in analytical research on its structure (Terron et al., 1996). There are a series of standard preparations that have traditionally been used in studies on lignin. One of the most suitable is the Björkman procedure (1956) which involves extensive grinding and extraction with dioxane and is less destructive than other lignin isolation procedures (Terron et al., 1996). This procedure has been successful in the isolation of relatively pure milled wood lignins; however, when applied to other plant materials having a low lignin content, the results have not been as successful (Himmelsbach and Barton, 1980). Although lignins from wood and straw materials have been extensively studied and several structural models have been proposed (Himmelsbach and Barton, 1980; Nimz et al., 1981; Scalbert et al., 1986; Pan et al., 1994; Imamura et al., 1994; Neto et al., 1994; Kondo et al., 1995; Sun et al., 1997), the structure of oil palm trunk lignins has only recently received attention (Gallacher et al., 1984). This paper describes the application of a two-step precipitation method for the isolation and fractionation of unmodified ball milled lignin and enzyme lignin from the trunks of oil palms. The chemical and physicochemical characteristics of the lignin preparations are reported.

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MATERIALS AND METHODS

Fractionation and Isolation of Ball-Milled Lignin and Enzyme Lignin. Oil palm trunks were obtained from Forest Research Institute of Malaysia. The trunks were cut into lumber slabs, air-dried, and separated into vascular and parenchyma fractions (Gallacher et al., 1994). The air-dried vascular bundle fraction was ground to pass through a 60 mesh screen. The ground fiber was extracted with toluene−ethanol (2:1, v/v) for 5 h in a Soxhlet apparatus (Figure 1). After being dried in an oven for 16 h at 50 °C, the extractive free residues were subjected to ball-milling for 6 days in a rotary porcelain ball-mill at 80 rev min^{-1}, using a mixture of 10 mm and 20 mm porcelain balls (1:1, w/w) (balls/residue weight ratio of 36). The ball-milled samples were then extracted twice with dioxane−water (90:10, v/v) for 12 h, followed by another 12 h extraction with dioxane−water (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 under N_2. After filtration through a 20 μm nylon cloth, 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 with 4 vols 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 residue in 250 mL of 0.2 M HAc−NaAc buffer pH 4.7) at 37 °C for 72 h (Figure 1). After filtration through a 20 μm nylon cloth, the insoluble residues were

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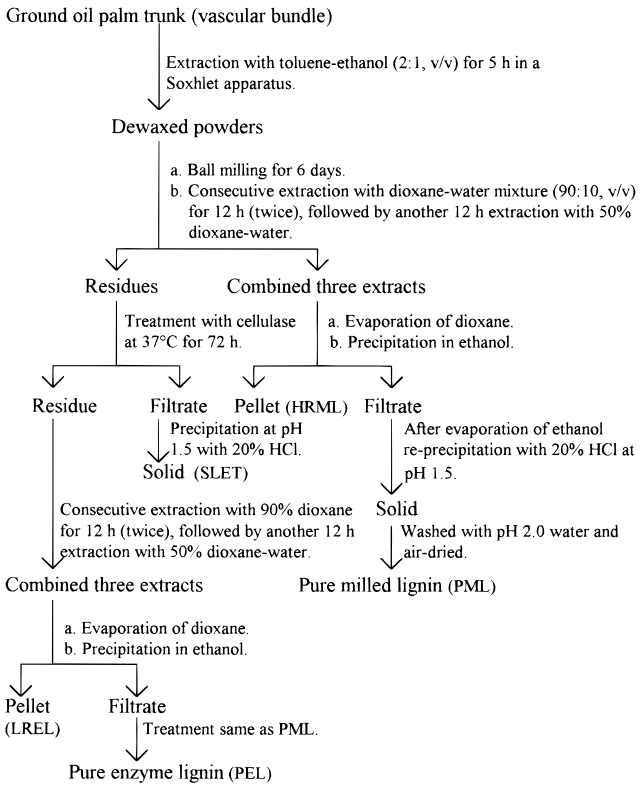

Figure 1. Scheme for isolation of lignin fractions from oil palm trunks.

washed with water, and LREL and PEL fractions were generated by successive extractions with 90% and 50% dioxane−water mixtures as before. The SLET fraction was obtained from the cellulose hydrolysate by precipitation at pH 1.5 with 20% HCl.

Physicochemical Characterization of Lignin Fractions. UV spectra were recorded on a Hewlett-Packard 8452A Diode Array spectrophotometer. Lignin samples (5 mg) were dissolved in 10 mL of 95% (v/v) dioxane−water. 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 average molecular weights of the lignin fractions were determined by gel permeation chromatography on a PLgel 5 μm Mixed-D column (Polymer Laboratories Inc., Amherst, MA). The samples (200 μL) were injected following dissolution in tetrahydrofuran at a concentration of 0.2%. 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.

FT-IR spectra were obtained on an FT-IR spectrophotometer (Nicolet, 750, Nicolet Instruments Limited, England) using KBr disks containing 1% of the samples, which were ground using Mikro-Dismembrator U (Mersungen AG, Germany) before making disks. The solution-state ^{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. The spectrum was acquired at 25 °C from a 250 mg sample dissolved in 1.0 mL of 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 (Sun et al., 1996).

Total and ester-linked hydroxycinnamic acids were extracted with 4 N NaOH at 170 °C for 2 h and with a 1 M NaOH extraction under a N_2 atmosphere at 25 °C for 16 h, respectively. Ether-linked hydroxycinnamic acids were calculated as being 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 for the quantification of uronic acids, alkaline nitrobenzene oxidation of lignin, and determination of phenolic acids and

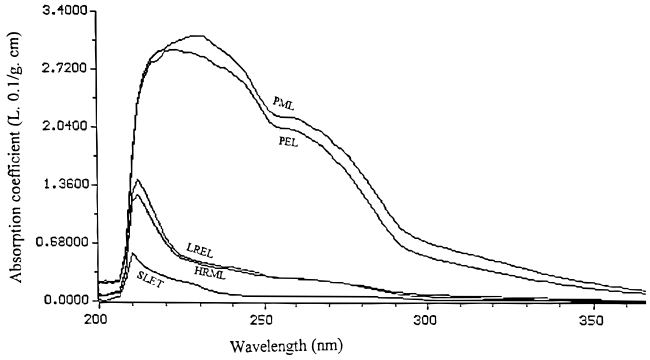

Figure 2. UV spectra of pure milled lignin (PML), pure enzyme lignin (PEL), lignin rich enzyme lignin (LRET), hemicellulose rich milled lignin (HRML), and solublized lignin obtained during enzyme treatment (SLET).

Table 1. Yields (Percent of Acidic Chlorite Lignin) of Milled and Enzyme Lignins Isolated from the Trunks of Oil Palms

lignin fractions	yield (%)
hemicellulose rich milled lignin (HRML)	23.76
pure milled lignin (PML)	10.42
solubilized lignin during cellulase treatment (SLET)	3.64
lignin rich enzyme lignin (LREL)	12.18
pure enzyme lignin (PEL)	8.42

aldehydes with high performance liquid chromatography have been reported previously (Sun et al., 1995; Lawther et al., 1995). All nitrobenzene oxidation results represent the mean of at least three replicate analyses. Other experiments were performed in duplicate.

RESULTS AND DISCUSSION

Lignin Yield. The yield of lignin resulting from the various fractionation procedures was expressed as a percentage of the total lignin determined by acidic sodium chlorite extraction (16.5%, w/w; Lawther et al., 1995).

Table 1 shows that the yields of PML and PEL are lower than the corresponding yields of HRML and LREL, respectively. This result indicated that the majority of lignin was still linked to polysaccharides after 6 days ball-milling. The reason for the lower yields of LREL and PEL than those of HRML and PML is probably due to a fraction of lignin being extracted during the ball-milling process.

UV Absorption. The UV spectra of the five lignin fractions (PML, PEL, LREL, HRML, and SLET) are shown in Figure 2. All the fractions exhibited the basic UV spectrum typical of lignins with a maximum at 210−230 nm. The lignin fractions of PML and PEL also exhibited a second maximum near 260 nm, originating from nonconjugated phenolic groups in the lignin (Scalbert et al., 1986). Much lower absorption coefficients of LREL, HRML, and SLET preparations was probably due to the association with nonlignin materials, such as polysaccharides.

Composition of Bound Polysaccharides. The two pure lignin fractions (PML and PEL) obtained by a two-step precipitation method contained rather low levels of associated polysaccharides (3.46−4.61%, Table 2), indicating that ball-milling can peel off the lignin from most of its neighboring polysaccharide moiety. Results therefore suggested that the covalent linkages between lignin and hemicelluloses in oil palm trunk cell walls tend to be cleaved during the ball-milling processes.

Table 2. Content (Percent Sample, w/w) of Neutral Sugars and Uronic Acids in Milled and Enzyme Lignin Fractions Isolated from 6-Day Ball-Milled Oil Palm Trunks and 3-Day Cellulase-Treated Residues

lignin fractions	neutral sugars							uronic acids	total
	Rha	Dib	Ara	Xyl	Man	Glc	Gal		
HRML	0.46	0.22	1.21	14.80	2.45	2.31	1.74	7.00	30.19
PML	ND[a]	ND	0.10	1.40	ND	0.56	0.14	1.26	3.46
SLET	0.12	ND	0.38	7.76	0.78	40.52	22.04	2.30	73.90
LREL	0.18	ND	0.48	9.70	0.62	4.51	0.87	8.45	24.81
PEL	ND	ND	0.10	1.82	ND	0.91	0.30	1.48	4.61

[a] ND = not detected.

These results are consistent with previous studies on alkaline wheat straw lignin (Sun et al., 1996, 1997). The other three lignin fractions (HRML, LREL, and SLET), however, had a much higher content of polysaccharides (24.81−73.90%). Xylose was found to be the major component with arabinose, glucose, galactose, and uronic acid as the secondary monosaccharides in HRML and LREL fractions (Table 2). The high proportion of glucose in SLET preparation was due to the degradation of cellulose by cellulase.

For lignin characterization purposes, the ideal procedure would be to isolate the entire lignin fraction free of polysaccharides. Scalbert et al. (1986) purified ball-milled wheat straw lignin and enzyme lignin fractions by successive precipitations in an aqueous 2% sodium sulfate solution and diethyl ether. The ball-milled lignin and enzyme lignin, however, contained 7.1% and 16.8% polysaccharides, which was about twice and four times higher than those of the PML and PEL fractions obtained by a two-step precipitation method proposed in this study. Similarly, Ben-Ghedalia and Yosef (1994) found that after ball-milling with additional cellulase treatment for 7 to 28 days, the fractions still contained 14.2−16.4% polysaccharide sugars. Although the crude lignin fractions were purified by dissolution in 90% (v/v) acetic acid, regenerated, and then purified again with 90% acetic acid followed by diethyl ether, the fractions still contained 19.1−21.0% polysaccharides (Kondo et al., 1995). The high content of polysaccharides in ball-milled and enzyme lignin preparations was thought to be due to the specific structural patterns of association between lignin and polysaccharides in straw and grass cell walls (Scalbert et al., 1986). However, it is easy to obtain the lignin fractions, which are relatively free of polysaccharides, by using this two-step precipitation method.

The relatively high concentration of uronic acids in PML and PEL fractions was probably due to the abundance of ester bonds between lignin and glucuronic acids of hemicelluloses in oil palm trunk cell walls. Occurrence of the ester bond between lignin and glucuronic acid (in PML fraction) was confirmed by two signals at 60.3 and 170.3 ppm in the ^{13}C NMR spectrum (Figure 6).

Components of Phenolic Monomers. The yield of alkaline nitrobenzene oxidation products and the components of phenolic acids and aldehydes are given in Table 3. The predominant oxidation product was found to be syringaldehyde, which comprised 66.3−70.8% of the total nitrobenzene oxidation products in the main four lignin fractions. Vanillin appeared as the second major degradation product. This suggested that oil palm trunk lignin was different from wheat straw lignin, which contains roughly equal amounts of non-condensed guaiacyl and syringyl units with relatively fewer *p*-hydroxyphenyl units. These results partly agreed with the studies by Gallacher et al. (1994) and Tominura (1992). By using solid-state ^{13}C NMR study of palm trunk cell walls, Gallacher et al. (1994) found that the lignin appeared to contain a high proportion of aryl ether-linked syringyl units, but no ferulic or *p*-coumaric acid. However, Tominura (1992) showed that milled wood lignins from oil palm trunk vascular bundles and parenchyma contained vanillin, *p*-hydroxybenzoic acid, syringaldehyde, and small amounts of phenolic acids such as vanillic acid and syringic acid, but *p*-hydroxybenzaldehyde was not observed in the oxidation products. In our studies, small amounts of *p*-hydroxybenzaldehyde, ferulic acid, and *p*-coumaric acid were found in the degradation products. Syringaldehyde and vanillin resulted from the degradation of syringyl and guaiacyl noncondensed units, respectively. The presence of small quantities of *p*-hydroxybenzaldehyde is generally considered to be indicative of *p*-

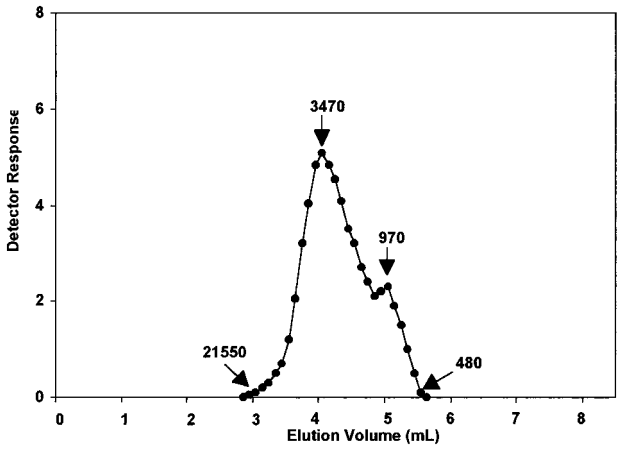

Figure 3. GPC molecular weight distribution of pure milled lignin (PML) obtained from oil palm trunks.

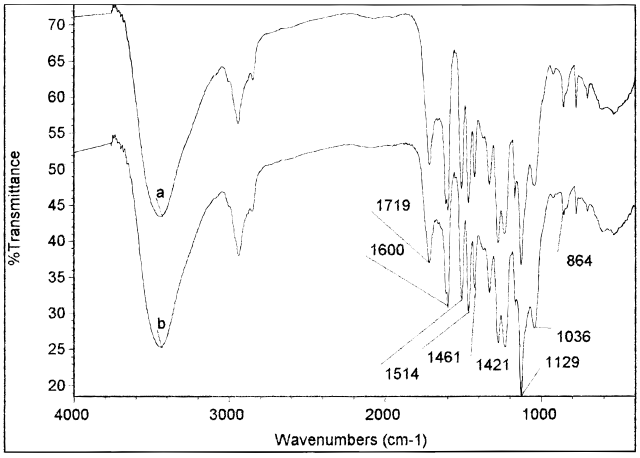

Figure 4. FT-IR spectra of pure milled lignin fraction (PML, a) and pure enzyme lignin fraction (PEL, b) isolated from oil palm trunks.

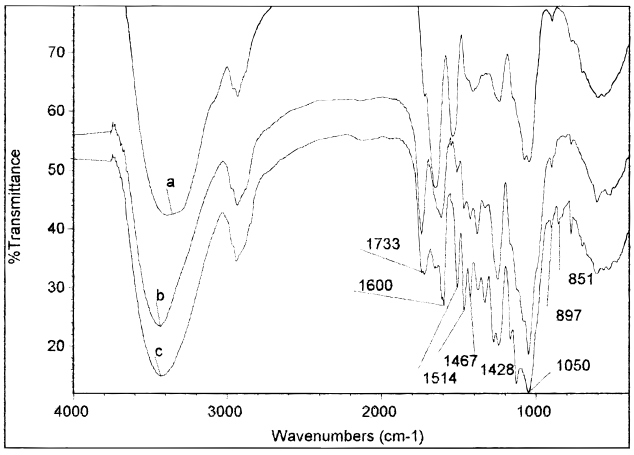

Figure 5. FT-IR spectra of solublized lignin obtained during enzyme treatment (SLET, a), hemicellulose rich milled lignin fraction (HRML, b), and lignin rich enzyme lignin fraction (LREL, c) isolated from oil palm trunks.

Table 3. Yield (Percent Sample, w/w) of Phenolic Acids and Aldehydes from Alkaline Nitrobenzene Oxidation of Oil Palm Trunk Lignin Fractions

phenolic acids and aldehydes	lignin fractions				
	HRML	PML	SLET	LREL	PEL
p-hydroxybenzoic acid	0.13	1.17	0.48	1.17	1.87
p-hydroxybenzaldehyde	0.11	0.35	0.33	0.15	0.20
vanillic acid	0.11	0.50	0.11	0.21	0.25
syringic acid	0.24	2.31	0.03	1.11	1.65
vanillin	1.18	8.48	0.12	2.86	5.36
syringaldehyde	4.27	27.05	0.30	13.78	24.30
p-coumaric acid	0.11	0.20	0.085	0.14	0.14
ferulic acid	0.034	0.74	ND	0.34	0.54
total	6.18	40.80	1.46	19.75	34.31

hydroxyphenyl units within the lignin "core" (Scalbert et al., 1986).

As can be seen from Table 3, the lower yields of oxidation products of HRML and SLET was due to the higher content of associated polysaccharides. It is also noteworthy to compare the relatively high yield of oxidation products found in the case of PML, PEL, and LREL fractions to the corresponding low yield of wheat straw lignin. A higher condensation degree was observed to appear in wheat straw lignins (Sun et al., 1996), while a lesser degree of condensation was found in oil palm trunk lignins, which compare with the condensation degree of hardwood or softwood lignins.

Table 4. Content of Hydroxycinnamic Acids (Percent Sample, w/w) in Milled and Enzyme Lignin Fractions Isolated from 6-Day Ball-Milled Oil Palm Trunks and 3-Day Cellulase-Treated Residues

lignin fractions	*p*-coumaric acid			ferulic acid		
	total	ester-linked	ether-linked	total	ester-linked	ether-linked
HRML	0.0066	0.0058	0.0008	0.065	0.020	0.045
PML	0.11	0.10	0.01	0.49	0.23	0.26
LREL	0.058	0.036	0.022	0.28	0.085	0.19
PEL	0.058	0.054	0.004	0.34	0.17	0.17

The presence of ferulic acid and *p*-coumaric acid associated with the cell walls (via ester and or ether bonds) was determined by alkali hydrolysis at high or low temperature, respectively. The results obtained showed that 50−70% of ferulic acids were etherified to lignin, while 62−93% of *p*-coumaric acids were esterified to lignin in oil palm trunk cell walls (Table 4). The data in Table 4 indicated that the PML and PEL contained 0.49 and 0.34% ferulic acids, respectively, whereas the content of *p*-coumaric acid was found to be only 0.11% and 0.058%, respectively; a level 4−6 times lower than the ferulic acid contents. The significantly lower content of *p*-coumaric acid and ferulic acid in the HRML fraction demonstrated that most of the hydroxycinnamic acids were linked to lignin in oil palm trunk cell walls. It is clear from the current study that the majority of ferulic acids were linked by ether bonds to lignin, while the majority of *p*-coumaric acids were linked by ester bonds to lignin in oil palm cell walls. These results were in agreement with previous studies on wheat straw lignins (Sun et al., 1997), in which 50−70% of ferulic acids were etherified to lignin and 70% of *p*-coumaric

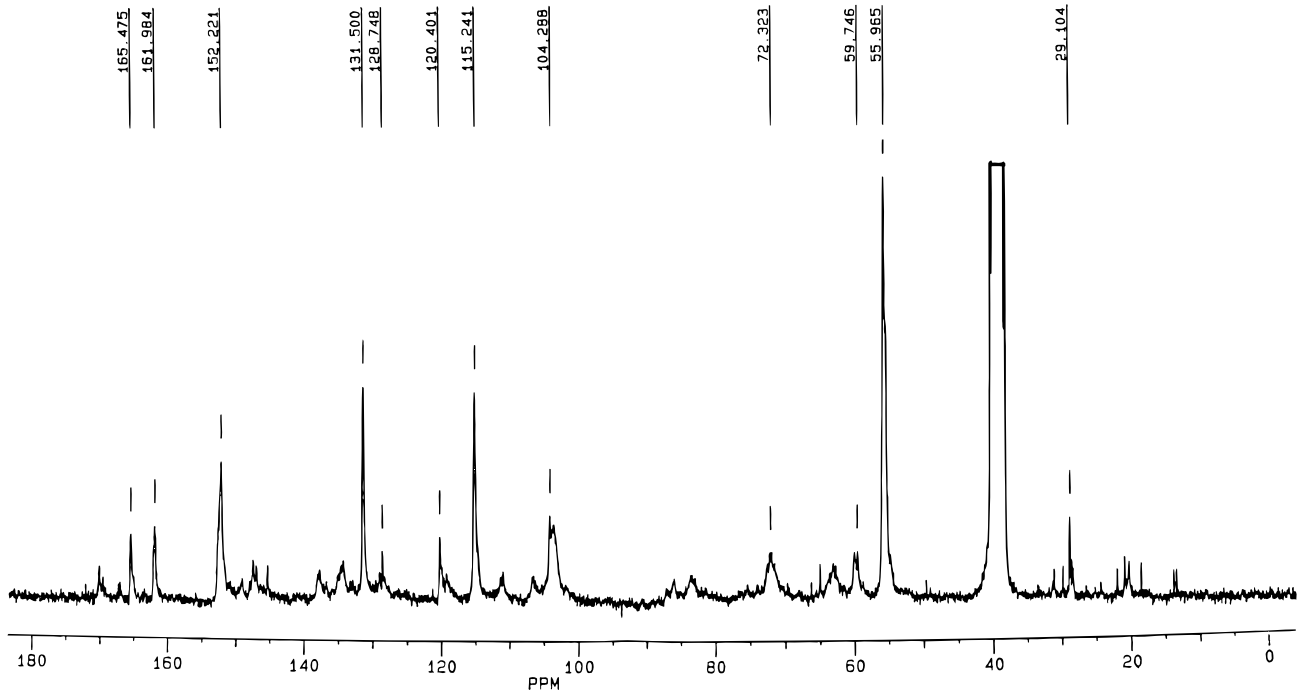

Figure 6. ^{13}C NMR spectrum of pure milled lignin (PML, in DMSO-d_6).

Table 5. Average-Weight ($\bar{M}_w$) and Average-Number ($\bar{M}_n$) Molecular Weights and Polydispersity ($\bar{M}_w/\bar{M}_n$) of Isolated Lignin Fractions Obtained from Oil Palm Trunks

lignin fractions	$\bar{M}_w$	$\bar{M}_n$	$\bar{M}_w/\bar{M}_n$
HRML	22980	9130	2.52
PML	3140	2100	1.49
SLET	12480	8260	1.51
LREL	4690	2460	1.91
PEL	1930	1390	1.39

acids were esterified to lignin in wheat straw cell walls. The results obtained in this study, however, were not consistent with the work done by Tomimura (1992) and Gallacher et al. (1994) on oil palm trunk cell walls. The authors mentioned that the oil palm trunk cell walls contained little or no ferulic and *p*-coumaric acids. The conflicting results were probably due to nature of samples and isolation conditions.

Molecular Weight Distribution. The average-weight ($\bar{M}_w$) and average-number ($\bar{M}_n$) molecular weights and polydispersity ($\bar{M}_w/\bar{M}_n$) of the lignin fractions are given in Table 5. As can be seen, PML, PEL, and LREL fractions had much lower molecular weights than those of HRML and SLET fractions. The reason for these differences is probably due to the higher content of bound polysaccharides in the HRML and SLET fractions. The present data also illustrated that the PML fraction had a relatively higher molecular weight (3140) than the corresponding PEL fraction (1930), suggesting that the PML fraction was made mostly of large molecules, and ball-milling for 6 days did not cause a significant subdivision of the lignin molecules.

The molecular weight distribution of PML is shown in Figure 3. The elution maximum corresponded to a polystyrene molecular weight of 3470. 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.

FT-IR Spectra. The FT-IR spectra of the PML and PEL fractions are shown in Figure 4. The spectral profiles and the relative intensities of the bands were similar in two spectra, which confirmed that the "core" of lignin structure does not change significantly during the ball-milling and cellulase treatment. The most striking characteristic of the FT-IR spectra of PML and PEL was the absence of polysaccharide bands. The presence of a peak at 1719 cm^{-1} is attributed to free carboxyl groups (C═O stretching nonconjugated to the aromatic ring). Aromatic skeleton vibrations in lignin are assigned at 1600, 1514, and 1421 cm^{-1} (Buta et al., 1989). Absorbances for these bands appeared to have similar intensities for the PML and PEL lignin fractions, indicating the same degree of aromaticity. The absorbance band at 1461 cm^{-1} corresponded with C−H deformations and aromatic ring vibrations. The intensity of the bands at 1514 and 1600 cm^{-1} can be used to differentiate softwood and hardwood lignins (Sun et al., 1997). In unconjugated syringyl model compounds, for example in hardwood, and straw lignins, the intensity of the these two bands are nearly the same, or the second one is stronger than the first (e.g. wheat straw lignin), while in unconjuated guaiacyl compounds and softwood lignins the intensity of the 1514 cm^{-1} band is considerably higher. As expected in Figure 4, the band at 1600 cm^{-1} was more intense than that at 1514 cm^{-1}, indicating more syringyl units in oil palm trunk lignins, which was in accordance with the results obtained by nitrobenzene oxidation. The oil palm trunk lignins, therefore, can be classified as 'hardwood type lignins'. The bands at 1129 and 1036 cm^{-1} corresponded with aromatic CH in-plane deformations. Aromatic C−H out of plane bending appears at 864 cm^{-1}.

Figure 5 shows the FT-IR spectra of SLET, HRML, and LREL. As can be seen, 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 1733 cm^{-1} in HRML and LREL fractions. The prominent bands in HRML spectrum corresponding to hemicelluloses appeared at 1050 and 897 cm^{-1}.

^{13}C NMR Spectrum. The PML fraction was also studied by ^{13}C NMR spectroscopy (Figure 6). Most of the observed signals have been previously assigned in straw and wood lignin spectra (Himmelsbach and Barton, 1980; Nimz et al., 1981; Scalbert et al., 1986; Pan et al., 1994; Imamura et al., 1994; Neto et al., 1994; Kondo et al., 1995). As expected, the most striking characteristic of the ^{13}C NMR spectrum is the absence 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 a two-step precipitation method. On the other hand, due to a large amount of polysaccharides associated in the ball-milled lignin fraction isolated by liquid−liquid extraction from wheat straw in previous studies by Scalbert et al. (1986), all of the lignin spectra reported earlier showed rather large resonances for polysaccharides between 57 and 103 ppm.

The region from 104.4 to 160.0 ppm is amenable to assignments as the aromatic part of the lignin. The syringyl (S) residues were indicated by signals at 152.3 (C-3/C-5, S), 138.0 (C-4, S etherified), 134.5 (C-1, S etherified), 131.5 (C-1, S nonetherified), 106.7 (C-2/C-6, S with α-CO), and 104.4 ppm (C-2/ C-6, S). Guaiacyl (G) residues gave signals at 149.3 (C-4, G etherified), 147.6 (C-3, G), 145.5 (C-4, G nonetherified), 134. 5 (C-1, G etherified), 131.5 (C-1, G nonetherified), 120.4 (C-6, G), and 111.1 ppm (C-2, G), respectively. The *p*-hydroxyphenyl (H) residues appeared as a signal at 128.7 ppm (C-2/C-6, H). These signals confirmed that a pure milled lignin fraction could be justified as GSH-lignin. The signals at 165.5 (C-γ, PC ester) and 115.2 ppm (C-3/C-5, PC ester) represented the esterified *p*-coumaric acid. Etherified ferulic acid was observed with a small signal at 166.6 (C-γ, FE ether). Therefore it appeared that the *p*-coumaric acids were linked to lignin by ester bonds, while the majority of the ferulic acids are linked by ether bonds.

The signals represented the γ-methyl, α and β-methylene groups in *n*-propyl side chains appeared in the spectrum between 13.7 and 31.5 ppm. A very strong signal at 56.0 ppm corresponded to the OCH_3 in syringyl and guaiacyl units. The carbonyl resonances from uronic acids and esters may contribute to signals at 170.3 and 60.3 ppm. A signal at 170.3 ppm indicates C-6 in methyl uronates (Himmelsbach and Barton, 1980), and the signal at 60.3 ppm partly originates from the 4-*O*-methoxyl group of the glucuronic acid residue in the xylan (Imamura et al., 1994). The polysaccharides associated with lignin are identified with two small

signals at 65.2 (C-5, xyl nonreducing unit) and 63.4 ppm (C-5, xyl internal unit), in agreement with the results obtained by sugar analyses.

The ^{13}C NMR spectra also indicated that β-*O*-4 linkages (C-α in β-*O*-4, 72.3 ppm; C-β in β-*O*-4, 86.2 ppm; C-γ in β-*O*-4, 60.3 ppm) were the major linkages between lignin structural units. The less common 5−5′ (132.8 ppm) and β-5 (87.1 ppm) carbon−carbon linkages were also present. These signals indicated that the trunk lignins of oil palms are composed mainly of β-*O*-4 ether bonds together with small amounts of 5−5′ and β-5 carbon−carbon linkages. These findings were in agreement with previous work (Tanahashi and Higuchi, 1990) on the effect of the hydrophobic regions of hemicelluloses on dehydrogenative polymerization of sinapyl alcohol. The authors showed that syringyl lignin is mainly composed of β-*O*-4 ether linkages and the hemicelluloses are easily connected to the α-position of lignin.

It is clear from the above results that the ball-milled and enzyme lignins can successfully be fractionated into PML, PEL, LREL, and HRML fractions by using a two-step precipitation method. The isolated pure milled and pure enzyme lignins contained very small amounts of associated polysaccharides. All the lignin preparations contained a high proportion of noncondensed syringyl units with small amounts of noncondensed guaiacyl and fewer *p*-hydroxyphenyl units. They seem less condensed than straw lignins, but corresponded to the condensation degree of hardwood lignins. Meanwhile, the lignin in oil palm trunk cell walls appeared to be very closely associated to glucuronic acid or 4-*O*-methylglucuronic acid by ester bonds. Small amounts of *p*-coumaric acid and ferulic acid were also found to be linked to lignin. Significant amounts of *p*-coumaric acids are esterified to lignin, whereas the majority of ferulic acids are linked by their phenolic groups via ether bonds to lignin. The lignin fraction of PML is mainly composed of β-*O*-4 ether bonds. The less common 5−5′ and β-5 carbon−carbon linkages are also present between the lignin structural units.

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