Physicochemical and Structural Characterization of Alkali Soluble Lignins from Oil Palm Trunk and Empty Fruit-Bunch Fibers

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Six alkali soluble lignin fractions were extracted from the cell wall materials of oil palm trunk and empty fruit-bunch (EFB) fibers with 5% NaOH, 10% NaOH, and 24% KOH/2% H_3BO_3. All of the lignin fractions contained rather low amounts of associated neutral sugars (0.8−1.2%) and uronic acids (1.1−2.0%). The lignin fractions isolated with 5% NaOH from the lignified palm trunk and EFB fibers gave a relatively higher degree of polymerization as shown by weight-average molecular weights ranging between 2620 and 2840, whereas the lignin fractions isolated with 10% NaOH and 24% KOH/2% H_3BO_3 from the partially delignified palm trunk and EFB fibers showed a relatively lower degree of polymerization, as shown by weight-average molecular weights ranging between 1750 and 1980. The results obtained by alkaline nitrobenzene oxidation showed that all of the lignin preparations contained a high proportion of noncondensed syringyl units with small amounts of noncondensed guaiacyl and fewer *p*-hydroxyphenyl units. The lignin fraction extracted with 5% NaOH from the lignified EFB fiber was mainly composed of *β*-*O*-4 ether-linked units. Small amounts of 5-5′, *β*-5, and *β*-*β*′ carbon−carbon linkages were also found to be present between the lignin structural units. Further studies showed that uronic, *p*-hydroxybenzoic, and ferulic acids in the cell walls of palm fibers were esterified to lignin.

Keywords: *Oil palm trunk fiber; empty fruit-bunch fiber; alkaline lignin; alkaline nitrobenzene oxidation; molecular weight; FT-IR; ^{13}C NMR spectroscopy*

INTRODUCTION

Oil palm is an economically important perennial crop. It originated in the tropical forests of West Africa and has been introduced to various parts of the tropics, mainly in southeastern Asian countries such as Malaysia, Indonesia, Thailand, and India. Large-scale cultivation has evolved in Latin America (Sreekala et al., 1997). The total plantation area in the world is expected to be 5 million ha by the year 2000. Because it is difficult to harvest fruits from a tree grown too tall, oil palm trees are usually replanted about every 25 years, although they can still produce fruit with high yields beyond that age (Tomimura, 1992). Oil palm trunk fiber and empty fruit-bunch (EFB) fiber are two important types of fibrous materials left during the periodic replanting and pruning, and in the palm oil mill. Studies on the utilization of the trunk and EFB fibers of the oil palm have shown the potential of this lignocellulosic raw material for a variety of applications including animal feed and papermaking (Mansor and Ahmad, 1991).

It was found that the major obstacle for the utilization of oil palm trunk fiber and EFB fiber as ruminant feed is due to the presence of lignin in the cell walls and its association with other cell wall polysaccharides (Mansor and Ahmad, 1991). Alkaline treatment of lignocellulosic substances such as straw, grass, and palm disrupts the cell wall by partially dissolving hemicelluloses, lignin, and silica, by hydrolyzing uronic, acetic, and hydroxycinnamic acid esters, and by swelling and decreasing the crystallinity of cellulose (Jackson, 1977). This increase of the degradability of the cell walls is also due to the cleavage of the bonds between lignin and hemicelluloses or between lignin and hydroxycinnamic acids. In general, aqueous alkaline treatments of grass cell walls result in the increase of the digestibility from ∼40−50% to 70% of the dry matter (Beckman, 1921). The objective of this study was to isolate and characterize the alkali soluble lignins from the lignified and partially delignified oil palm trunk and EFB fibers.

MATERIALS AND METHODS

Lignin Isolation. Oil palm trunk and EFB fibers were obtained from the Forest Research Institute of Malaysia. The fibers were first cleaned and dried. The air-dried fibers were then ground to pass through a 0.7 mm screen and further dried in an oven at 60 °C for 16 h. The dried samples were extracted with toluene/ethanol (2:1, v/v) for 6 h in a Soxhlet apparatus. After being dried in an oven for 16 h at 60 °C, the residues were subjected to extraction with 95% ethanol for 4 h in a Soxhlet apparatud and then with water at 100 °C for 2 h. Alkali soluble lignins were first extracted with 5% NaOH at 100 °C for 2 h. After treatment, the mixtures were screened by using a 20 μm nylon cloth to remove the 5% NaOH insoluble materials. The filtrates were neutralized to pH 5.5 with 20% HCl, concentrated using a rotary evaporator under reduced pressure at 40 °C, and then mixed with 3 volumes of ethanol. The precipitated hemicelluloses were filtered, washed with 70% ethanol, and air-dried. The 5% NaOH soluble lignins were obtained by reprecipitation at pH 1.5, adjusted with 20% aqueous HCl, from the supernatant solution. Note that treatments of the palm trunk and EFB fibers with 5% NaOH at 100 °C for 2 h were for fractions 1 and 4, respectively. Partial delignification was performed using sodium chlorite in acidic solution (pH 4.2−4.7, adjusted by 10% acetic acid) at 70 °C for 2 h. The residual hemicelluloses and lignins were further extracted using 10% NaOH for 16 h at 20 °C and 24% KOH/2% H_3BO_3 at 20 °C for 2 h from the above partially delignified residues, respectively. The soluble hemicelluloses and lignin were, respectively, recovered by using the two-step precipitation method mentioned above. The lignins solubilized during the 10% NaOH and 24% KOH/2% H_3BO_3 treatments from the

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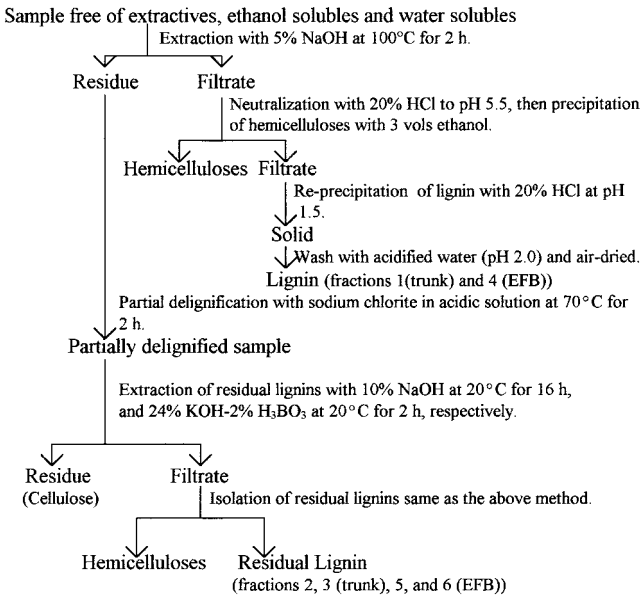

Figure 1. Scheme for isolation of lignin and hemicelluloses from oil palm trunk and EFB fibers.

palm trunk fiber were labeled fractions 2 and 3, and those from the palm EFB fiber were considered to be fractions 5 and 6, respectively (Figure 1).

Physicochemical Characterization of Lignin Fractions. 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 aldehydes with high-performance liquid chromatography have been reported previously (Lawther et al., 1995; Sun et al., 1995, 1997).

Total and ester-linked phenolics were extracted with 4 M NaOH at 170 °C for 2 h and with 1 M NaOH under a nitrogen atmosphere at 25 °C for 14 h, respectively. Ether-linked phenolics were calculated as the difference between total and ester-linked phenolics (Kondo et al., 1995).

UV spectra were recorded on a Hewlett-Packard 8452A diode array spectrophotometer. Lignin samples (5 mg) were dissolved in 10 mL of 90% (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 5μ Mixed-D column (Polymer Laboratories Inc.). The samples (200 μL) were injected following dissolution in tetrahydrofuran at a concentration of 0.2%. The column was 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) using KBr disks containing 1% of finely ground samples. The solution-state ^{13}C NMR spectrum was acquired with a Bruker AC 250 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 30000 scans. A 60° pulse flipping angle, a 3.9 μs pulse width, and 0.85 s acquisition time were used (Sun et al., 1996).

RESULTS AND DISCUSSION

Yield of Lignin. The yield of 5% NaOH, 10% NaOH, and 24% KOH/2% H_3BO_3 soluble lignins from oil palm trunk and EFB is given in Table 1. As expected, treatment of the palm trunk fiber and EFB fiber, which are free of extractives, ethanol solubles, and hot water solubles, with 5% NaOH resulted in release of 38.8 and 40.2% of the total lignin, respectively. The reason for

Table 1. Yield (Percent Dry Sample, w/w) of Lignin Obtained by Alkali Extraction of Oil Palm Trunk and EFB Fibers

	5% NaOH soluble[a]		10% NaOH soluble[b]		24% KOH soluble[c]		
sample	hemicelluloses	lignin[d]	hemicelluloses	lignin[d]	hemicelluloses	lignin[d]	lignin content[e]
trunk	17.3	6.6[1]	11.9	0.7[2]	13.5	0.3[3]	17.1
EFB	15.2	6.8[4]	12.7	2.0[5]	14.9	0.9[6]	16.9

[a] Extraction with 5% NaOH at 100 °C for 2 h from the fibers free of extractives, ethanol solubles, and water solubles. [b] Extraction with 10% NaOH at 20 °C for 16 h from 5% NaOH extracted and partially delignified sample. [c] Extraction with 24% KOH/2% H_3BO_3 at 20 °C for 2 h from 5% NaOH extracted and partially delignified sample. [d] Superscript 1−6 represent the lignin fractions 1, 2, 3, 4, 5, and 6, respectively. [e] Including 5% NaOH soluble lignin, acidic chlorite lignin, 10% NaOH soluble lignin, and 24% KOH/2% H_3BO_3 soluble lignin.

this high solubility of alkali lignin was probably due to the high amounts of ester-linked phenolic acids, such as *p*-hydroxybenzoic acid and ferulic acid, in the cell walls of oil palm trunk fiber and EFB fiber, which may play an important role in the release of lignin during the alkaline treatment process (Terrón et al., 1996). After partial delignification with sodium chlorite in acidic solution at 70 °C for 2 h, the residual lignin was extracted with 10% NaOH and 24% KOH/2% H_3BO_3 from the palm trunk fiber and EFB fiber, respectively. The yields of solubilized residual lignin during the 10% NaOH and 24% KOH/2% H_3BO_3 extraction processes from oil palm trunk fiber were rather low (0.7 and 0.3%). This observation implied that nearly all of the residual lignin was degraded or oxidized under the delignification condition given for the palm trunk fiber. On the other hand, the relatively higher yields (2.0 and 0.9%) of residual lignin released during the 10% NaOH and 24% KOH/2% H_3BO_3 extraction processes from the EFB fiber were presumed to be due to the stronger associations between lignin and hemicelluloses in the cell walls of EFB fiber, which prevented the lignin degradation or oxidation by sodium chlorite in acidic solution. The total lignin yields of extract-free and ethanol- and water-extracted oil palm trunk and EFB fibers were found to be 17.1 and 16.9%, respectively, which included 5% NaOH soluble lignin, chlorite lignin, 10% NaOH soluble lignin, and 24% KOH/2% H_3BO_3 soluble lignin.

UV Spectra. Ultraviolet and infrared spectroscopies are useful for the identification, determination, and characterization of analytical and technical lignins and lignin derivatives (Goldschmid, 1971; Fengel and Wegener, 1983). In the qualitative and quantitative UV spectroscopic determination of lignin the typical extinction maximum at ∼280 nm is mostly used (Ahlgren and Goring, 1971). However, the spectrophotometric determination at 280 nm involves the problems of uncontrolled influence of polysaccharides and their degradation compounds. At the short wavelength of ∼200 nm, however, the influence of those compounds is negligible (Wegener et al., 1983). In this study, the purity of the lignins obtained was determined by the UV spectra between 200 and 350 nm (Figure 2). As shown in the diagram, the four lignin fractions exhibit the basic UV spectra typical of lignins, which have an absorption maximum at λ 210−220 nm. The lignin fractions, extracted with 5% NaOH from the palm trunk fiber and EFB fiber, also exhibit a second maximum around 270 nm, originating from noncondensed phenolic groups (aromatic ring) in lignin (Scalbert et al., 1986). The

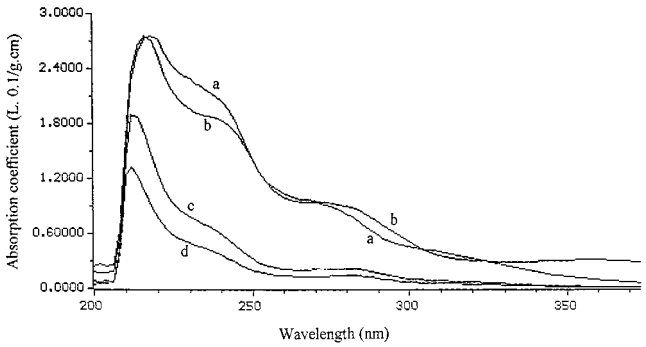

Figure 2. UV spectra of lignin fractions extracted with 5% NaOH (a) and 10% NaOH (d) from oil palm EFB fiber and with 5% NaOH (b) and 24% KOH (c) from oil palm trunk fiber.

relatively lower absorption of the lignin fractions (spectra c and d), isolated by 10% NaOH from the EFB fiber and 24% KOH/2% H_3BO_3 from the palm trunk fiber, is presumed to be due to the slightly higher amounts of coprecipitated nonlignin materials such as ash and salts.

Composition of Associated Polysaccharides. The composition of the associated polysaccharides in the isolated six lignin fractions was determined by their neutral sugar and uronic acid contents, and the analytical results are given in Table 2. All six lignin fractions showed rather low levels of associated neutral sugars (0.8−1.2%) and minor amounts of uronic acids (1.1−2.0%). This observation indicated that the chemical bonds between lignin and polysaccharides in the isolated lignin preparations are mostly cleaved during the alkali treatment processes. These results are consistent with previous studies on alkaline wheat straw lignins, isolated according to a two-step precipitation method (Sun et al., 1996). Xylose, glucose, and arabinose were found to be the three main sugar components. A relatively higher concentration of uronic acids in the lignin preparations, particularly in the 5% NaOH soluble lignin fractions, was probably due to the abundance of ester bonds between lignin and glucuronic acids of hemicelluloses in the cell walls of palm trunk and EFB fibers, and this was confirmed by a signal at 174.7 ppm (data not shown) in the ^{13}C NMR spectrum. This signal at 174.7 ppm originates from the C-6 in 4-*O*-methoxyl group of glucuronic acid residue in the xylan (Himmelsbach and Barton, 1980).

Component of Phenolic Acids and Aldehydes. The standard procedures for analyzing lignins by chemical degradative methods result in the formation of well-defined low molecular weight products. The amounts and relative distribution of such degradation products can then be used to derive information about the composition of the original polymer (Billa et al., 1996). Among these procedures, alkaline nitrobenzene oxidation represents a reference method, which is still one of the most frequently used method for the characterization of the structure of lignins (Iiyama and Lam, 1990). Table 3 gives the results concerning the yield and component of phenolic acids and aldehydes from the alkaline nitrobenzene oxidation of the six alkali soluble lignins. As can be observed, the predominant component of the phenolic monomers was found to be syringaldehyde, which represents 52.7−69.1% of the total phenolic monomers, resulting from the degradation of noncondensed syringyl (S) units. The second major degradation product, vanillin, results from the degradation of noncondensed guaiacyl (G) units. The occurrence of low amounts *p*-hydroxybenzaldehyde is generally considered to be indicative of *p*-hydroxyphenyl (H) units with the lignin "core". This observation is consistent with the results obtained by Jarvis (1994) from the solid-state NMR study of leaf cell walls of oil palm. He stated that linear chains of syringyl units comprised a significant part of the lignin in the palm trunk. Five phenolic acids such as *p*-hydroxybenzoic acid, vanillic acid, syringic acid, *p*-coumaric acid, and ferulic acid were also identified as minor quantities in all of the nitrobenzene oxidation products. Similar results have been reported by Gallacher et al. (1994). The authors indicated that the lignin in the palm trunk cell walls appeared to contain a high proportion of aryl ether-linked syringyl unit, but no ferulic or *p*-coumaric acid. With the studies on chemical characteristics and utilization of oil palm trunks, Tomimura (1992) reported that both ball-milled lignins from vascular bundles and parenchyma produced vanillin, *p*-hydroxybenzoic acid, syringaldehyde, and a small amount of phenolic acids such as vanillic acid and syringic acid, but *p*-hydroxybenzaldehyde was not observed in the degradation products. He, therefore, concluded that neither milled lignin contains a *p*-coumaric acid ester structure. Obviously, occurrence of small amounts of *p*-hydroxybenaldehyde, ferulic acid, and *p*-coumaric acid was found in the nitrobenzene oxidation products from our studies. The lower total yield of oxidation products found in the lignin fractions, isolated with 10% NaOH and 24% KOH/2% H_3BO_3 from the delignified palm trunk fiber and EFB fiber, may be explained by a higher content of non-lignin materials or a higher condensation degree of these four lignin fractions. The continuing occurrences of small amounts of *p*-coumaric acids in all of the nitrobenzene oxidation mixtures of the six lignin fractions and minor amounts of ferulic acids in the mixtures of lignin fractions 1 and 4 were presumed to be due to the conditions of nitrobenzene oxidation at the relatively low temperature (170 °C) and the relatively short oxidation period (3 h) used in this study because the recovery yields of ferulic acids and *p*-coumaric acids, obtained from the nitrobenzene oxidation, decreased with increase in temperature and reaction time. Ferulic acid was not detected among the oxidation products after 4 h at 170 °C or after 2 h at 180 °C, and the molar content in ferulic acids corresponded to an equivalent molar increase in vanillin (Billa et al., 1996). These results implied that some amounts of ferulic acids were quantitatively oxidized to vanillins by nitrobenzene under the reaction conditions used in our studies (170 °C, 3 h). Similarly, part of the *p*-coumaric acids appeared to be oxidized to *p*-hydroxybenzaldehydes or *p*-hydroxybenzoic acid under the conditions of alkaline nitrobenzene oxidation.

To avoid oxidation and/or degradation of hydroxycinnamic acids, the presence of *p*-coumaric and ferulic acids associated with cell walls through ester bonds, was determined by alkali hydrolysis at ambient temperature, and those associated through ether bonds at high temperature. The results showed that the palm trunk fiber contained minimal amounts of phenolic acids such as *p*-hydroxybenzoic acid (0.043%), vanillic acid (0.027%), syringic acid (0.14%), *p*-coumaric acid (0.012%), and ferulic acid (0.045%), in which 58% *p*-hydroxybenzoic acid, 66% *p*-coumaric acid, and 60% ferulic acid were esterified to lignin and/or polysaccharides, whereas 86% vanillic acid and 92% syringic acid were found to be etherified (Table 4). The considerable amounts of etheri-

Table 2. Content (Percent Dry Weight of Lignin, w/w) of Neutral Sugars and Uronic Acids in the Isolated Lignin Fractions

sample	lignin fraction	neutral sugars[a] Rha	Ara	Xyl	Man	Glc	Gal	uronic acids	total
trunk	1, 5% NaOH soluble lignin	T	0.41	0.10	N	0.18	0.12	2.00	2.81
	2, 10% NaOH soluble lignin	N	0.22	0.45	T	0.38	N	1.40	2.45
	3, 24% KOH soluble lignin	N	0.18	0.53	N	0.36	N	1.42	2.49
EFB	4, 5% NaOH soluble lignin	N	0.10	0.20	N	0.19	N	1.80	2.29
	5, 10% NaOH soluble lignin	N	T	0.65	N	0.45	N	1.10	2.20
	6, 24% KOH soluble lignin	N	0.10	0.68	N	0.40	N	1.20	2.38

[a] T, trace; N, not detectable.

Table 3. Content (Percent Lignin Sample, w/w) of Phenolic Acids and Aldehydes from Nitrobenzene Oxidation of Lignin Fractions

phenolic acids and aldehydes	lignin fractions[a] 1	2	3	4	5	6
p-OH-benzoic acid	0.24	0.13	0.15	0.48	0.13	0.15
p-OH-benzaldehyde	0.27	0.24	0.27	0.69	0.42	0.99
vanillic acid	0.38	0.72	0.45	1.02	1.35	1.16
syringic acid	2.27	1.05	1.13	2.23	1.58	2.24
vanillin	7.08	4.77	4.59	9.11	4.80	5.34
syringaldehyde	23.47	11.54	12.42	22.43	9.38	11.66
p-coumaric acid	0.21	0.18	0.17	0.18	0.15	0.17
ferulic acid	0.048	T	T	0.15	T	T
total	33.97	18.64	19.18	36.30	17.81	21.71

[a] Corresponding to the lignin fractions in Table 1; T, trace.

Table 4. Content (Percent Extract-Free Sample, w/w) of Phenolic Acids and Aldehydes Obtained from Alkaline Hydrolysis of Extract-Free Oil Palm Trunk and EFB Fibers

phenolic acids and aldehydes	trunk fiber total[a]	esterified[b]	etherified[c]	EFB fiber total[a]	esterified[b]	etherified[c]
p-OH-benzoic acid	0.043	0.025	0.018	0.039	0.024	0.015
p-OH-benzaldehyde	0.0073	0.0023	0.0050	0.012	0.0036	0.0084
vanillic acid	0.027	0.0044	0.023	0.039	0.0088	0.030
syringic acid	0.14	0.012	0.13	0.10	0.0087	0.091
vanillin	0.20	0.021	0.18	0.24	0.033	0.21
syringaldehyde	0.57	0.040	0.53	0.54	0.029	0.51
acetovanillone	0.081	0.0034	0.078	0.10	0.0012	0.099
acetosyringone	0.36	0.0074	0.35	0.30	0.0026	0.297
p-coumaric acid	0.012	0.0079	0.0041	0.020	0.014	0.0060
ferulic acid	0.045	0.027	0.018	0.069	0.043	0.026
total	1.49	0.15	1.34	1.46	0.17	1.29

[a] Represents the phenolics liberated during the alkali hydrolysis with 4 M NaOH at 170 °C for 2 h. [b] Represents the phenolics liberated during the alkali hydrolysis with 1 M NaOH at 25 °C for 14 h. [c] Calculated as being the difference between total and ester-linked phenolics.

fied benzaldehydes, such as syringaldehyde (0.53%) and vanillin (0.18%), and phenones, such as acetovanillone (0.078%) and acetosyringone (0.35%), were also composed. Similar results were observed for the palm EFB fiber, which contained 0.039% *p*-hydroxybenzoic acid (62% esterified, 38% etherified), 0.020% *p*-coumaric acid (70% esterified, 30% etherified), and 0.069% ferulic acid (62% esterified, 38% etherified). The significant amounts of vanillin (0.24%), syringaldehyde (0.54%), acetovanillone (0.10%), acetosyringone (0.30%), and syringic acid (0.10%) were found to be etherified to lignin and/or polysaccharides in the cells walls of the palm.

All nitrobenzene oxidation results represent the mean of at least triplicate analyses, and each oxidation mixture was chromatographed twice. However, it should be kept in mind that the contents of *p*-hydroxybenzoic acid (0.13−0.48%) in the six isolated alkali lignin fractions were much lower than that of the lignins obtained from oil palm frond by Suzuki et al. (1998). The authors reported that the content of *p*-hydroxybenzoic acid determined by alkaline nitrobenzene oxidation in the lignins isolated from steam-explosion pulps of oil palm frond was 1.72−2.30%, which exceeded those of the data determined in our experiments by >5−13-fold. This situation may reflect the influence of factors such as material, soil, climate, and method of analysis on the cell wall composition of the palm. This observation, however, needs further examination.

Table 5. Weight-Average ($\bar{M}_w$) and Number-Average ($\bar{M}_n$) Molecular Weights and Polydispersity ($\bar{M}_w/\bar{M}_n$) of the Lignin Fractions Extracted from Oil Palm Trunk and EFB Fibers

	lignin fractions[a] 1	2	3	4	5	6
$\bar{M}_w$	2840	1980	1950	2620	1750	1750
$\bar{M}_n$	1560	880	900	1650	730	850
$\bar{M}_w/\bar{M}_n$	1.82	2.25	2.16	1.59	2.40	2.06

[a] Corresponding to the lignin fractions in Table 1.

Molecular Weight Distribution. Table 5 shows the weight-average ($\bar{M}_w$) and number-average ($\bar{M}_n$) molecular weights, and polydispersity ($\bar{M}_w/\bar{M}_n$) of the six lignin fractions, which were computed from their chromatograms. The data in Table 5 indicate that treatment of the lignified palm trunk and EFB fibers with 5% NaOH solubilized a relatively large molecular size of lignin (2620−2840, fractions 1 and 4), whereas the extractions of the partially delignified fibers with 10% NaOH and 24% KOH/2% H_3BO_3 resulted in the release of a relatively small molecular size of lignin (1750−1980, fractions 2, 3, 5, and 6), indicating that extraction with a high concentration of alkali such as 10% NaOH and 24% KOH/2% H_3BO_3 has a greater effect on the degradation of lignin, such as cleavage of the ether bonds between the lignin precursors than does the extraction with low concentration of alkali (5% NaOH).

The molecular weight distribution of the lignin fraction, isolated by 5% NaOH from oil palm EFB fiber, is illustrated in Figure 3. The elution maximum corresponded to polystyrene molecular weight of 3240. The elution profile showed a wide polymolecularity, ranging from oligomers to polystyrene of molecular weights >26000.

FT-IR Spectra. The FT-IR spectra of the three lignin fractions, isolated with 5% NaOH from the lignified palm trunk fiber (spectrum a) and EFB fiber (spectrum b) and with 24% KOH/2% H_3BO_3 from the delignified palm trunk fiber (spectrum c), are shown in Figure 4. As can be seen in the diagram, the spectral profiles and the relative intensities of the bands in the two spectra a and b were rather similar, showing characteristic

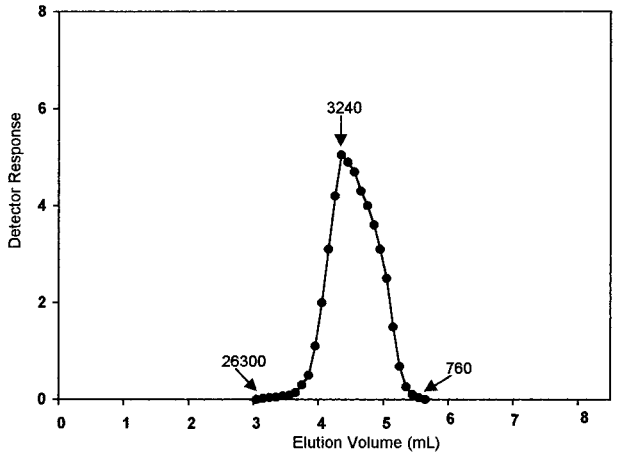

Figure 3. GPC molecular weight distribution of lignin fraction isolated by 5% NaOH from oil palm EFB fiber.

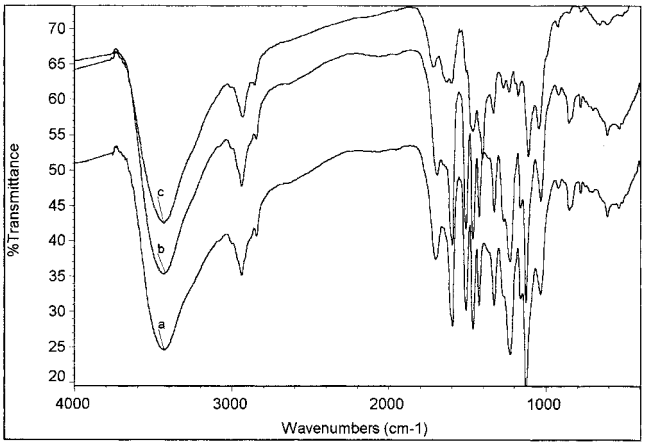

Figure 4. FT-IR spectra of 5% NaOH soluble lignin fractions obtained from lignified oil palm trunk fiber (a) and EFB fiber (b) and of 24% KOH/2% H_3BO_3 extracted residual lignin (c) obtained from delignified oil palm trunk fiber.

peaks at 1705, 1600, 1507, 1460, 1420, 1328, 1225, 1168, 1129, 1029, and 850 cm^{-1}, which can be assigned to the different units of lignin and *p*-hydroxycinnamic acids. This suggested that the two lignin fractions, extracted with 5% NaOH from the palm trunk and EFB fibers, have a similar lignin structure. Furthermore, the intense bands for polysaccharides were not detected, indicating a low amount of compounds containing these groups in the two lignin preparations. The presence of a peak at 1705 cm^{-1} is assignable to carbonyl groups (unconjugation with aromatic ring), and the absorption of this band is greater for spectrum a than for the spectra b and c. The aromatic skeleton vibrations in the two lignin fractions are assigned at 1600, 1507, and 1420 cm^{-1}. Absorption at 1460 cm^{-1} indicates the C−H deformations and aromatic ring vibrations. The syringyl ring breathing with CO stretching appears at 1328 and 1225 cm^{-1}. The bands at 1129 and 1029 cm^{-1} have been assigned to be the aromatic CH in-plane deformations in syringyl and guaiacyl units, respectively. Aromatic C−H out-of-plane bending appears at 850 cm^{-1} (Sun et al., 1996). The lignin-related absorbances at 1719, 1602, 1507, 1461, 1410, 1330, 1270, 1235, 1172, 1110, 1043, and 850 cm^{-1} in the lignin fraction (spectrum c), isolated with 24% KOH/2% H_3BO_3 from the partially delignified palm trunk fiber, are relatively weak, probably due to the more coprecipitated salt or ash.

^{13}C NMR Spectrum. The lignin fraction, isolated by 5% NaOH from the palm EFB fiber, was also studied by ^{13}C NMR spectroscopy, and the spectrum is shown in Figure 5. Most of the assignments could be made according to the results of 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), and Terrón et al. (1996). As shown in Figure 5, the most striking characteristic of the ^{13}C NMR spectrum is the near absence 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 according to a two-step precipitation method (Sun et al., 1996). Due to the rather low content of associated polysaccharides (2.3%) in the lignin fraction, the spectrum showed only

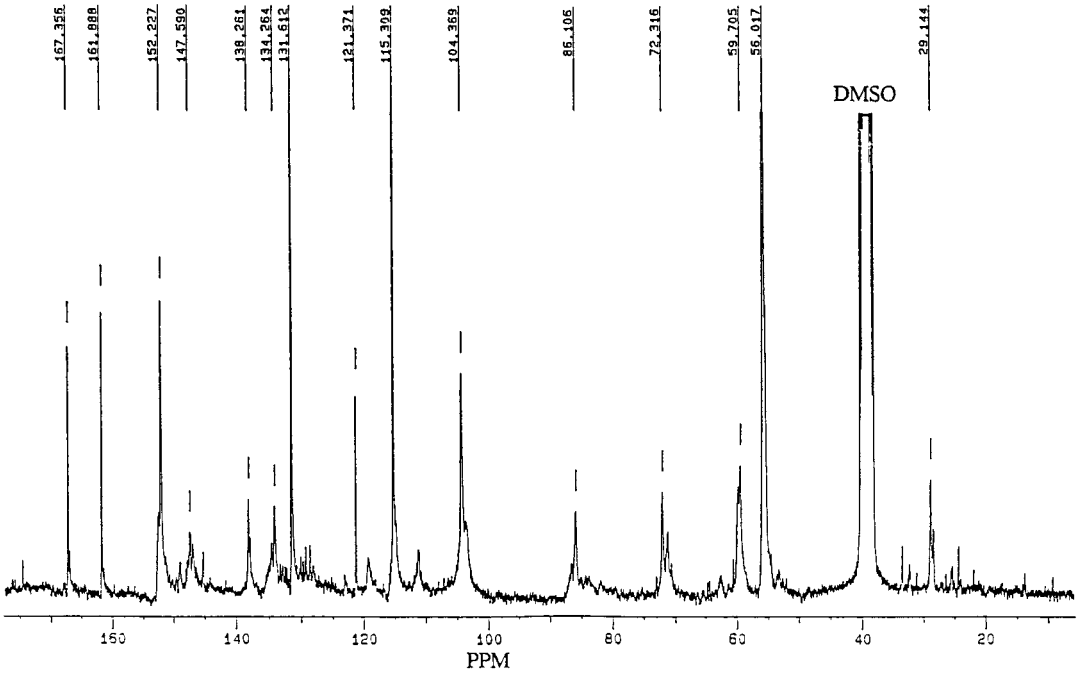

Figure 5. ^{13}C NMR spectrum of lignin fraction isolated by 5% NaOH from oil palm EFB fiber.

two small signals at 63.1 ppm (C-5, Xyl internal unit) and 174.7 ppm (C-6 in methyl uronates) for polysaccharides (Himmelsbach and Barton, 1980).

In the aromatic region (104.4 to 167.4 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), 138.3 (C-4, S etherified), 134.7 and 134.3 (C-1, S etherified), 133.4 (C-1, S nonetherified), and 104.4 ppm (C-2/ C-6, S); 149.4 (C-3, G etherified), 147.6 and 147.1 (C-3, G), 145.5 (C-4, G nonetherified), 134.7 and 134.3 (C-1, G etherified), 133.4 (C-1, G nonetherified), 119.3 (C-6, G), 114.9 (C-5, G), and 111.2 ppm (C-2, G); and 128.0 ppm (C-2/C-6, H), respectively. These signals confirmed that the lignin fraction could be justified as SGH-lignin, which corresponded with the lignins obtained from the grasses and cereal straws (Sun et al., 1996). The strong signals at 167.4, 161.9, 131.7, 121.4, and 115.3 ppm were assigned to CO, C-4, C-2/ C-6, C-1, and C-3/C-5 in esterified *p*-hydroxybenzoic acid, respectively. Esterified ferulic acid was detected with a small signal at 122.8 ppm (C-6, FE ester). This observation indicated that the lignin from oil palm EFB fiber contained a significant amount of esterified *p*-hydroxybenzoic acid and a small amount of esterified ferulic acid. This conclusion was in good agreement with the results obtained from oil palm frond by ^{13}C NMR (Suzuki et al., 1998), but not consistent with the results obtained by alkaline nitrobenzene oxidation, which yielded a minimal quantity of *p*-hydroxybenzoic acid in the lignin preparations obtained from the palm EFB fiber in our studies and a small amount in the lignin fractions obtained from the palm frond in the studies of Suzuki et al. (1998). The appearance of these strong signals for esterified *p*-hydroxybenzoic acid in the spectrum of ^{13}C NMR was presumed to be due to the signal overlaps with other phenolics. The reason for these different results obtained independently by alkaline nitrobenzene oxidation and ^{13}C NMR is currently under investigation.

The strong resonance at 56.0 ppm corresponds to OCH_3 in syringyl and guaiacyl units. The small signals assigned to γ-methyl and α- and β-methylene groups in *n*-propyl side chains appeared in the spectrum between 14.1 and 33.8 ppm. 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 59.7, 72.3, and 86.1 ppm, respectively. The low-intensity signals for C-β in β-β and C-α in β-5 carbon−carbon bonds can be seen at 53.5 and 87.0 ppm, respectively. The less common 5-5′ carbon−carbon linkage was detected at 132.8 ppm (C-5/C-5′ in 5-5′ units). These signals indicated that oil palm EFB fiber lignin is mainly composed of β-*O*-4 ether bonds together with small amounts of β-β, β-5, and 5-5′ carbon−carbon linkages.

Taken together, the above results show that all six alkali soluble lignin fractions are relatively free of associated polysaccharides and are composed of large amounts of syringyl units with small amounts of guaiacyl and fewer *p*-hydroxyphenyl units. It was found that the lignin fractions isolated with 10% NaOH and 24% KOH/2% H_3BO_3 from the partially delignified palm trunk and EFB fibers have a higher degree of condensation and a relatively smaller molecular size than the lignin fractions isolated with 5% NaOH from the lignified palm and EFB fibers. It was also found that uronic, *p*-hydroxybenzoic, and ferulic acids were esterified to lignin in the palm fiber cell walls. The lignin fraction, isolated with 5% NaOH from the palm EFB fiber, is mainly composed of β-*O*-4 ether bonds, together with small amounts of β-β, β-5, and 5-5′ carbon−carbon linkages between the lignin structural units.

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