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## Fractionation and characterization of oil palm (*Elaeis guineensis*) as treated by supercritical water

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**Abstract** In order to investigate the potential for efficient utilization of oil palm (*Elaeis guineensis*), supercritical water treatment (at 380°C and 100 MPa for 8 s) was applied to fractionate extractives-free samples into water-soluble portion and water-insoluble portion. The water-insoluble portion was further fractionated into methanol-soluble portion and methanol-insoluble residue. Samples were prepared from various parts of oil palm, i.e., trunks, fronds, mesocarp fibers, shells, empty fruit bunches, and kernel cake. These fractionated products were then characterized analytically. The water-soluble and methanol-soluble portions were determined to be mainly composed of carbohydrate-derived products and lignin-derived products, respectively. The methanol-insoluble residue was mainly composed of lignin (more than 84 wt%) and the phenolic hydroxyl contents determined by the aminolysis method was higher than for untreated oil palm samples. In addition, an alkaline nitrobenzene oxidation analysis indicated that the methanol-insoluble residue contained fewer oxidation products than untreated samples did. These findings imply that the water-soluble portion could be utilized for organic acid production, whereas the methanol-soluble portion and the insoluble residue could be used for the production of phenolic chemicals.

**Key words** Oil palm · Supercritical water treatment · Carbohydrate-derived products · Lignin-derived products · Lignin · Phenolic hydroxyl content · Alkaline nitrobenzene oxidation

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### Introduction

The increasing global demand of plant oils for cooking, food additives, cosmetics, industrial lubricants, and biodiesel has resulted in a rapid increase in the production of palm oil. In the production process of palm oil in Malaysia, Indonesia, and Thailand, a large amount of oil palm waste is generated, such as empty fruit bunch (EFB), mesocarp fiber, shell, and kernel cake. Trunk and frond of oil palm are also generated as unused biomass at the plantation site.

Although some of these wastes are utilized as mulching material, organic fertilizer, or boiler fuel,<sup>1</sup> much waste is not efficiently used. To help promote efficient utilization of the whole oil palm, our research group elucidated the chemical composition of each part of the plant. Consequently, it was found that all parts contain cellulose, hemicellulose, and lignin, although the monosaccharide composition of hemicellulose was different among the different parts. However, lignin from all parts except for kernel cake was similar to that of hardwood lignin.<sup>2</sup>

Supercritical water treatment (>374°C, >22.1 MPa) has been applied to cellulose<sup>3</sup> and wood<sup>4,5</sup> for its chemical conversion. It has been reported that, depending on the reaction conditions, various compounds such as saccharides, dehydrated and fragmented compounds, and organic acids could be obtained. Lignin in wood was also found to be converted to various useful phenolic compounds by supercritical water treatment.

In this study, therefore, we applied supercritical water treatment to various parts of the oil palm in an attempt to promote their efficient utilization and to establish the potential of oil palm as a biomass resource. The parts studied were trunk, frond, mesocarp fiber (hereinafter will be referred to as mesocarp), shell, EFB, and kernel cake.

## Materials and methods

### Sample preparation

Various parts of the oil palm, i.e., trunk, frond, mesocarp, shell, EFB, and kernel cake, were obtained from Malaysia, and these samples were first cleaned and air dried. The air-dried samples were then pulverized using a Wiley mill (Yoshida Seisakusho, Japan) and a Fritsch mill (Fritsch, Germany) to pass through 70-mesh sieves. The samples were then extracted with ethanol/benzene (1 : 2, v/v) for 8 h in a Soxhlet apparatus. Subsequently the samples were oven dried at 105°C for 10 h prior to use in the experiments.

### Supercritical water treatment

The supercritical water biomass conversion system used in this study was associated with a batch-type reaction vessel made of Inconel-625 with a volume of 5 ml.<sup>4</sup> Figure 1 shows the flowchart of supercritical water ( $T_c = 374^\circ\text{C}$ ,  $P_c = 22.1$  MPa) treatment and the fractionation of supercritically treated products. The extractives-free oven-dried sample (150 mg) was placed in the reaction vessel with 4.9 ml distilled water of high-performance liquid chromatography (HPLC) grade (Nacalai Tesque, Kyoto, Japan). The reaction vessel was then quickly heated by immersing it in a molten tin bath preheated to 500°C, which resulted in an average temperature inside the reaction vessel of 380°C during the 8 s treatment. After that, the reaction vessel was immersed in a water bath to quench the reaction. During this treatment, the temperature and pressure in the reaction vessel were monitored by a thermocouple and a pressure gauge.

Subsequently, the supercritical water-soluble portion and supercritical water-insoluble residue could be separated by

filtration. After 12 h refrigeration, the obtained supercritical water-soluble portion was again filtered to finally separate the water-soluble portion and the water-insoluble residue. The water-insoluble residue was then washed with 10 ml HPLC-grade methanol (Nacalai Tesque) for 10 min by sonication. The methanol-insoluble residue was then separated by filtration from the methanol-soluble portion as filtrates. The yields of these fractions were then determined quantitatively after complete evaporation of methanol from the methanol-insoluble residue. The yield of the water-soluble portion was then estimated by subtracting the yields of the methanol-soluble portion and methanol-insoluble residue from the initial weight of the oven-dried sample (150 mg).

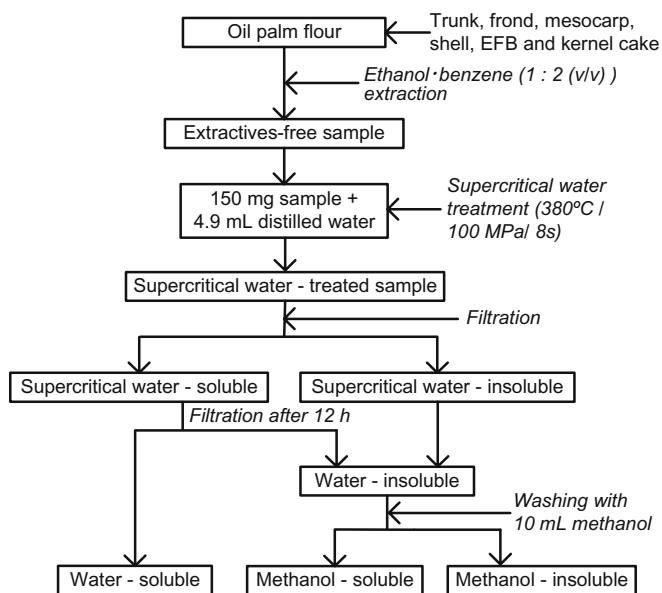
### Characterization of the fractionated portions

Characterization of the water-soluble portion was conducted using HPLC, ion chromatography (IC), capillary electrophoresis (CE), and ultraviolet-visible (UV-Vis) spectrophotometry. HPLC analysis was carried out to quantify the presence of monosaccharides and decomposed products of saccharides using a Shimadzu LC-10A under the following conditions: column, Shodex KS801 and KS802; flow rate, 1 ml/min; eluent, HPLC-grade distilled water; column temperature, 80°C. IC analysis was carried out to quantify the presence of oligosaccharides and monosaccharides using a Dionex ICS-3000 under the following conditions: column, CarboPac PA1; column temperature, 35°C; eluent, gradient-programmed mixture of HPLC-grade distilled water with 0.2 M sodium hydroxide and 1.0 M sodium acetate; flow rate, 1.0 ml/min.

CE analysis was carried out to quantify the presence of low-molecular-weight organic acids with an Agilent G1600A under the following conditions: column, fused-silica capillary (104 cm  $\times$  75  $\mu\text{m}\phi$ ); buffer, cation pH 5.6 (Agilent); voltage, -30 kV; temperature, 15°C; detector, UV<sub>270nm</sub>. Characterization of the lignin-derived products was conducted with a Shimadzu UV-2400 UV-Vis spectrophotometer at a wavelength of 205 nm.

Characterization of the methanol-soluble portion was conducted using Hitachi G7000M-M9000 gas chromatography/mass spectrometry (GC-MS) for qualitative analysis of low-molecular-weight products under the following conditions: column, Varian CP-Sil 8CB (30 m  $\times$  0.25 mm $\phi$ ); injector temperature, 250°C; column temperature: 30°C (1 min), 30°C  $\rightarrow$  250°C (5°C/min increment), 250°C (10 min); carrier gas, helium; flow rate, 1.5 ml/min; emission current, 20  $\mu\text{A}$ .

For characterization of the methanol-insoluble residue, the determination of Klason lignin and acid-soluble lignin was conducted according to Dence<sup>6</sup> and Whiting et al.<sup>7</sup> Ultraviolet (UV) spectra were recorded on a Shimadzu UV-2400 UV-Vis spectrophotometer. The absorptivity value used in the acid-soluble lignin determination was 110 l g<sup>-1</sup> cm<sup>-1</sup> for all samples at a wavelength of 205 nm. The lignin content of the samples was then determined as the sum of Klason lignin and acid-soluble lignin. These results were compared with the lignin content of untreated oil palm samples.



**Fig. 1.** Fractionation process of oil palm treated in supercritical water. EFB, empty fruit bunch

The phenolic hydroxyl content was determined by the aminolysis method according to the procedure described by Lai<sup>8</sup> to determine the number of phenolic hydroxyl groups present in 100 phenylpropane (C<sub>9</sub>) units of lignin (PhOH/100 C<sub>9</sub>); gas chromatography (Shimadzu GC14B) with a flame ionization detector (FID) was used to determine the phenolic hydroxyl content under the following conditions: column, Shimadzu stainless steel packed with 10 wt% polyethylene glycol 20 M on 60/80 mesh Shincarbon A (2 m × 3 mm $\phi$ ); column temperature, 180°C (isothermal); injection port temperature, 230°C; detector temperature, 250°C; carrier gas, helium. The average weight of the C<sub>9</sub> unit of lignin in oil palm samples was estimated by the weight of syringyl-type and guaiacyl-type C<sub>9</sub> units, considering the molar ratio of syringyl to guaiacyl moieties (S/V ratio).

The alkaline nitrobenzene oxidation treatment method employed in this study was that according to Katahira and Nakatsubo,<sup>9</sup> with veratraldehyde (Nacalai Tesque) as the internal standard. In order to quantify the nitrobenzene oxidation products, vanillin acetate, syringaldehyde acetate, and *p*-hydroxybenzaldehyde acetate were prepared with the respective reagent grade chemicals (Nacalai Tesque). Quantification of nitrobenzene oxidation products was carried out by gas chromatography (Shimadzu GC14B) with FID under the following conditions: column, Shimadzu CBP-5 column (25 m × 0.25 mm $\phi$ ); column temperature: 100°C (1 min), 100°C → 270°C (5°C/min increment), 270°C (10 min); injection port temperature, 250°C; detector temperature, 250°C; carrier gas, helium; flow rate, 1.5 ml/min.

## Results and discussion

As shown in Table 1, supercritical water treatment (at 380°C and 100 MPa for 8 s) was applied to various parts of the oil palm and the obtained yields for the fractionated water-soluble portion and the water-insoluble residue were investigated. The water-soluble portion was found to be 67–84 wt%, similar to those in Japanese beech as reported elsewhere.<sup>10</sup> However, the yields of the methanol-soluble portion, separated from the water-insoluble portion, were mostly lower than those of the methanol-insoluble residue. This is exactly the opposite of the situation for wood sample

such as the above-mentioned Japanese beech. The yields of the methanol-insoluble residue and methanol-soluble portion depend on the type of lignin linkages. If more condensed-type linkages are present, a larger amount of residue will remain. If fewer condensed-type linkages are present, with more ether linkages in the lignin, then a larger amount of the methanol-soluble portion will remain.

The methanol-soluble portion was originally soluble in supercritical water, because both hydrophilic and hydrophobic substances can be dissolved in supercritical water. However, as the supercritical water returns to the initial ambient condition, the hydrophobic substances become insoluble and condense as an oily substance. Therefore, washing the residue with methanol dissolves such oily substances in the methanol. The methanol-soluble portion was studied by GC-MS and will later be shown to be mainly composed of lignin-derived products, which explains its hydrophobic nature. The methanol-insoluble residue was studied using Klason lignin determination, and it was found that the majority was lignin (84%–99%). In addition, due to the low concentration of nitrobenzene oxidation products, the majority of the residue is condensed-type lignin (83%–96%).

### Characterization of water-soluble portion

In our previous work,<sup>3</sup> a similar study was carried out on microcrystalline cellulose using a rising time of 13 s in a molten tin bath at 550°C followed by a 380°C/100 MPa treatment in the reaction vessel for 2–8 s. The treatment conditions used in the present study were a little different, with a longer rising time in the molten tin bath (500°C), approximately 22 s, followed by 380°C/100 MPa treatment in the reaction vessel for 8 s. Thus, no oligosaccharides or monosaccharides were detected, only the decomposed products of saccharides such as dihydroxyacetone (DA), levoglucosan (LG), furfural (FR), and organic acids were detected (as shown in Table 2) along with lignin-derived products. The yield of unknown products, which was determined by subtracting the identified products from the total, is also shown. In the microcrystalline cellulose study,<sup>3</sup> only fructose (monosaccharide) was detected, but no oligosaccharides, for a 8 s treatment time. Indeed, the reason that these saccharides were not detected in the current study was

**Table 1.** Yields of fractionated water-soluble portion and water-insoluble residue for various parts of the oil palm treated by supercritical water at 380°C and 100 MPa for 8 s

Sample source	Yield (wt%)		
	Water soluble	Water insoluble	
		Methanol soluble	Methanol insoluble
Trunk	82.1	7.5	10.4
Frond	83.5	8.3	8.2
Mesocarp	67.1	5.0	27.9
Shell	70.3	11.3	18.4
EFB	75.6	7.6	16.8
Kernel cake	81.0	2.2	16.8

EFB, empty fruit bunch

**Table 2.** Yields of products in the fractionated water-soluble portion

Sample source	Proportion based upon untreated sample (wt%)									
	DA	LG	MG	FR	AA	GA	LA	LP	Unknown	Total
Trunk	5.4	1.1	0.4	0.5	6.4	4.8	1.7	12.2	49.6	82.1
Fronde	7.2	1.1	0.3	0.2	2.7	2.5	1.9	7.7	59.9	83.5
Mesocarp	6.3	0.8	0.2	0.2	5.1	3.8	1.0	8.7	41.0	67.1
Shell	3.9	1.0	0.1	0.5	3.1	1.6	0.4	9.7	50.0	70.3
EFB	5.5	1.1	–	0.6	2.6	2.0	1.0	8.1	54.7	75.6
Kernel cake <sup>a</sup>	4.8	0.4	0.3	0.2	4.2	2.1	1.1	0	61.1	81.0

DA, dihydroxyacetone; LG, levoglucosan; MG, methylglyoxal; FR, furfural; AA, acetic acid; GA, glycolic acid; LA, lactic acid; LP, lignin-derived product

<sup>a</sup>Contains 6.8% polymeric substances but not lignin

the different temperature-rising time. When the temperature-rising time is longer, further decomposition of saccharides is believed to occur during this period. It might, in addition, be the result of taxonomical differences in raw materials.

When lignocellulose is treated with supercritical water, a decomposition pathway has been proposed<sup>3</sup> whereby, with a prolonged treatment time, organic acids are expected to be formed at a high yield. Previously, it was reported that for wood samples treated in supercritical water with a short treatment time (5 s), organic acids were produced as a result of carbohydrate decomposition. However, during more prolonged treatment (4 min), the propyl sidechain of the phenylpropane unit of lignin was also decomposed to organic acids with a total yield of up to 35%.<sup>5</sup> Therefore, it is clear that supercritical water treatment is efficient in decomposing the carbohydrate portion of oil palm and that the water-soluble portion could be utilized for the production of organic acids, preferably using a prolonged treatment time. These organic acids could then be converted into methane by anaerobic fermentation, as reported by Yoshida et al.<sup>5</sup>

#### Characterization of methanol-soluble portion

For the methanol-soluble portion, GC-MS analyses were performed for all the various parts of the oil palm. The total ion chromatograms of the methanol-soluble portion obtained by GC-MS analysis are shown in Fig. 2. Based on this GC-MS analysis, the molecular weight (MW) and the compounds identified from the data in Fig. 2 are shown in Table 3. Identification of the peaks was conducted by comparing the retention times and mass fragmentation patterns with those of authentic compounds. However, peaks 4, 5, and 6 were determined from the mass fragmentation pattern reported by Hosoya et al.,<sup>11</sup> whereas peaks 16, 17, 19, 23, 24, and 25 were determined from the data of Ralph and Hatfield.<sup>12</sup> It could then be elucidated that these identified phenolic compounds must be mainly derived from lignin as indicated by G (guaiacyl-type) and S (syringyl-type) lignins. These compounds become insoluble as oily products when the critical state of water returned to the initial ambient state. Therefore, they are hydrophobic in nature. However, they could be fractionated mainly as the methanol-soluble portion.

**Table 3.** Products in the methanol-soluble portion identified by gas chromatography/mass spectrometry analysis

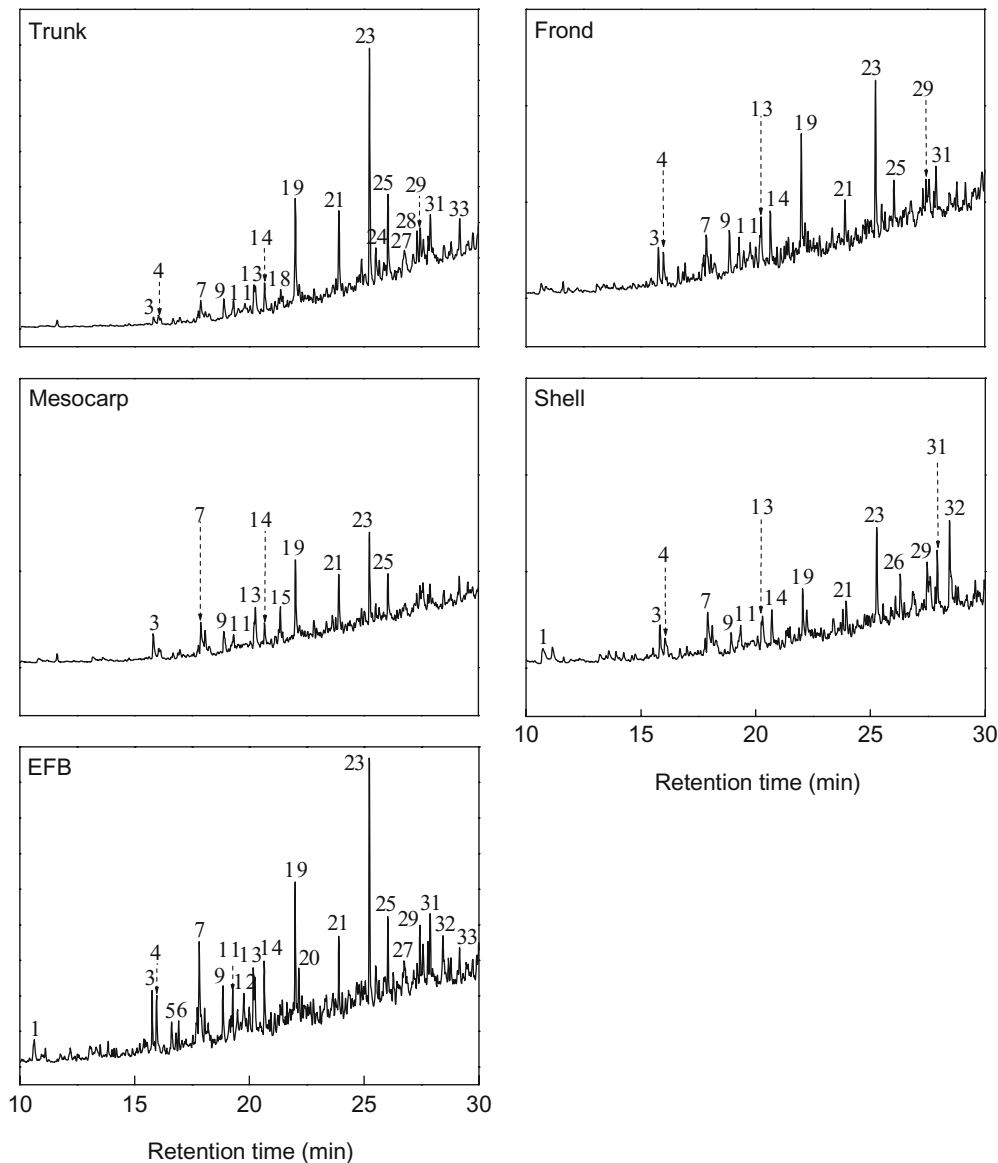
Peak	MW	Compound	Origin
1	124	Guaiacol	G
2	138	4-Methylguaiacol	G
3	152	4-Ethylguaiacol	G
4	134	2-Methyl-4-vinylphenol	G
5	148	2-Methyl-4-(1-propenyl) phenol	G
6	148	2-Methyl-4-(1-propenyl) phenol isomer	G
7	154	Syringol	S
8	166	4-Propylguaiacol	G
9		Unknown	
10	164	Eugenol	G
11		Unknown	
12		Unknown	
13	164	<i>cis</i> -Isoeugenol	G
14	164	<i>trans</i> -Isoeugenol	G
15		Unknown	
16	162	4-Propynylguaiacol	G
17	162	1-(4-Hydroxy-3-methoxyphenyl)allene	G
18	166	Acetoguaiacone	G
19	182	4-Ethylsyringol	S
20	180	Guaiacylacetone	G
21	196	4-Propylsyringol	S
23	194	<i>cis</i> -4-Propenylsyringol	S
24	192	4-Propenylsyringol	S
25	194	<i>trans</i> -4-Propenylsyringol	S
26	178	<i>trans</i> -Coniferylaldehyde	G
27	196	Acetosyringone	S
28		Unknown	
29	210	Syringylacetone	S
31		Unknown	
32	194	Ferulic acid	G
33		Unknown	

G, guaiacyl-type lignin; S, syringyl-type lignin

From a previous study,<sup>4</sup> it was found that in supercritical water, ether linkages are preferentially cleaved and the smaller fraction of lignin can be washed out with methanol. As a result, the residue becomes rich in condensed linkages. For various parts of the oil palm, it could be observed that the higher peaks detected were mainly from syringyl-type phenolic compounds. This is due to more syringyl-type lignin contained in oil palm, as reported previously.<sup>2</sup>

In Table 3, the corresponding peaks in Fig. 2 are indicated, and these are from syringyl-type as well as guaiacyl-type phenolic compounds similar in nature to those of hardwoods. Thus, the main components of the oil palm, such

**Fig. 2.** Total-ion chromatograms for the methanol-soluble portion from various parts of oil palm in gas chromatography/mass spectrometry analyses



as trunk, frond, and EFB, show similarity with hardwoods rather than softwoods. In addition, the methanol-soluble portion shows the potential for many phenolic compounds to be recovered from treatment by supercritical water.

#### Characterization of the methanol-insoluble residue

Table 4 shows the contents of Klason lignin and acid-soluble lignin in the methanol-insoluble residues from various parts of the oil palm: the lignin content as the sum of Klason lignin and acid-soluble lignin for these parts ranged between 84.1% and 99.2%. This suggests that the methanol-insoluble residue is mainly composed of lignin.

For untreated oil palm, it was found that the sum of Klason lignin and acid-soluble lignin ranged between 20.5% and 44.7%, with acid-soluble lignin being between 2.2% and 5.5%. However, acid-soluble lignin in the methanol-

insoluble residue was in the range 0.1%–1.6%, due perhaps to its removal during supercritical water treatment.

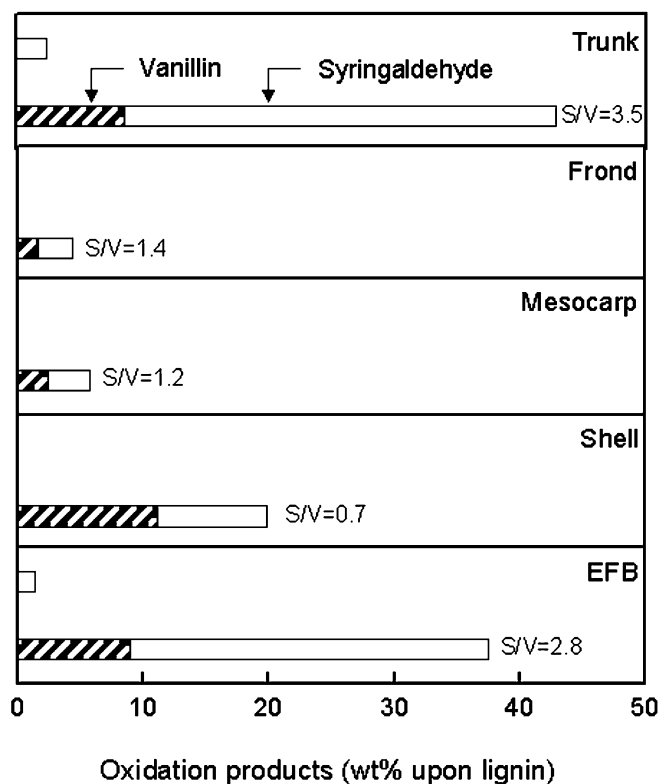
Table 4 also shows the number of phenolic hydroxyl groups (PhOH) present in 100 C<sub>9</sub> units of lignin for the methanol-insoluble residues. For comparison, results of the untreated oil palm samples are included. It is apparent that the methanol-insoluble residues have more phenolic hydroxyl groups than the untreated oil palm samples. Previously, it was demonstrated with lignin model compounds that condensed-type linkages, such as 5–5, were stable during supercritical water treatment, whereas noncondensed-type ether linkages, such as  $\beta$ -O-4, were easily cleaved by supercritical water hydrolysis.<sup>4</sup> After cleavage of noncondensed-type linkages, the proportion of phenolic hydroxyl groups increases. This explains the reason for the higher phenolic hydroxyl contents observed in methanol-insoluble residues compared with those of untreated samples and it suggests that many noncondensed-type

**Table 4.** Klason lignin and acid-soluble lignin contents and the number of phenolic hydroxyl groups present in 100 C<sub>9</sub> units of lignin (PhOH/100 C<sub>9</sub>) determined by the aminolysis method for the methanol-insoluble residues from various parts of the oil palm

Sample source	Methanol-insoluble residue (wt%)				PhOH/100 C <sub>9</sub>	Untreated oil palm (wt%)			
	KL	ASL	Lignin content <sup>a</sup>	Yield of lignin upon untreated oil palm		KL	ASL	Lignin content	PhOH/100 C <sub>9</sub>
Trunk	98.6	0.6	99.2	10.3	36.5	19.4	5.5	24.9	28.4
Frond	83.2	0.9	84.1	6.9	53.9	19.6	2.2	21.8	26.3
Mesocarp	85.3	0.7	86.0	24.0	81.3	39.3	5.4	44.7	21.8
Shell	84.6	0.1	84.7	15.6	22.1	37.1	2.2	39.3	13.1
EFB	86.1	1.6	87.7	14.7	72.0	16.0	4.5	20.5	12.8

KL, Klason lignin; ASL, acid-soluble lignin

<sup>a</sup>Lignin content = KL + ASL



**Fig. 3.** Yields of the alkaline nitrobenzene oxidation products of the methanol-insoluble residues for various parts of the oil palm (*upper bars*) and untreated oil palm samples (*lower bars*). *S/V*, molar ratio of syringyl to guaiacyl moieties

linkages were cleaved and that the residues are rich in condensed-type linkages.

It was observed that the increase in phenolic hydroxyl groups present in 100 C<sub>9</sub> units of lignin after supercritical water treatment was larger in oil palm mesocarp and EFB, compared with other parts of oil palm. This could be because the lignin in oil palm mesocarp and EFB is more susceptible to degradation under supercritical water treatment. Furthermore, for EFB, there is a strong possibility that it was originally rich in noncondensed-type linkages because the total yield of vanillin and syringaldehyde were nearly 40%, as shown in Fig. 3. However for mesocarp, such products are present in low amounts, at around 6%. This indicates that

mesocarp was not originally rich in noncondensed-type linkages.

As reported previously,<sup>2</sup> the molar ratio of syringyl to guaiacyl moieties (*S/V*), as measured by that of syringaldehyde and vanillin, was 3.5 in trunks, 1.4 in fronds, 1.2 in mesocarp, 0.7 in shells, and 2.8 in EFB. In contrast, the methanol-insoluble residues show a small presence or no presence of nitrobenzene oxidation products, as shown in Fig. 3. These results are to be expected because nitrobenzene oxidation products are mainly derived from the degradation of noncondensed-type lignin, and most of these linkages were already cleaved under supercritical water treatment, as mentioned above. The findings also suggest that methanol-insoluble residues are rich in condensed-type lignin. As a comparison,<sup>10</sup> the *S/V* ratio of Japanese beech is 2.3 for untreated samples, and yet none of the oxidation products were present in its methanol-insoluble residue.

## Conclusions

Various parts of the oil palm were fractionated and characterized after supercritical water treatment. The results presented in this study show that the water-soluble portion has the potential for conversion into organic acids, whereas the methanol-soluble portion lends itself to the recovery of many phenolic compounds. Overall, the characteristics of oil palm, as one of the monocotyledonous angiosperms, show some similarity to those of hardwoods, which are dicotyledonous angiosperms. Although further experiments are required to utilize various oil palm parts in industrial applications, this study has clarified that supercritical water technology is applicable to obtain various useful chemicals from oil palm. These findings show the great potential of oil palm not only in plant oil production but also as a source of lignocellulosics.

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