A COMPARATIVE EVALUATION OF PHENOLIC HYDROXYL CONTENT OF OIL PALM

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ABSTRACT

*Free phenolic hydroxyl content was determined in various parts of oil palm (*Elaeis guineensis*) such as trunk, frond, mesocarp, shell, empty fruit bunch and comparatively evaluated by aminolysis and periodate oxidation methods. This determination will elucidate the reactivity of lignin in oil palm and provide information for its future efficient utilisation. The results showed that aminolysis method consistently yielded higher values for phenolic hydroxyl content than the periodate oxidation method. More phenolic hydroxyl content was observed in the trunk, mesocarp and frond than in the shell and empty fruit bunch. As a comparison, phenolic hydroxyl content was also evaluated for hardwood, Japanese beech (*Fagus crenata*) and softwood, Japanese cedar (*Cryptomeria japonica*) for their sapwood portions. These samples have been selected because of their anatomical differences whereby, under botanical terms, oil palm is classified as one of monocotyledonous angiosperms, Japanese beech is classified as one of dicotyledonous angiosperms and Japanese cedar is classified as gymnosperms. Due to the abrupt increase in the formation of 1-acetylpyrrolidine in the aminolysis method, small discrepancy did exist between aminolysis and periodate oxidation methods, but the trend on the phenolic hydroxyl content was similar.*

Keywords: free phenolic hydroxyl content, oil palm, aminolysis method, periodate oxidation method, 1-acetylpyrrolidine.

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INTRODUCTION

Growing interest in the utilisation of lignin has prompted the development of simple and rapid methods for the determination of phenolic hydroxyl groups in lignin of biomass. This is because phenolic hydroxyl content provides pertinent information relating to the structure and reactivity of lignin (Lai, 1992; Goldschmid, 1954). For example, during hydrothermal treatment such as supercritical water treatment, new phenolic hydroxyl groups are formed due to the cleavage of, for example, β-O-4 linkages (Ehara *et al*., 2002). Similar trend also occurs during chemical pulp cooking and bleaching, whereby the newly formed phenolic structures increase the hydrophilicity of lignin and enhance the dissolution of degraded lignin (Liitiä and Tamminen, 2007).

In order to estimate the phenolic hydroxyl content of lignin, both physical and chemical methods or a combination of both have been used (Adler, 1977). Common physical methods include ionisation UV spectroscopy, potentiometric and conductometric titration methods (Lai, 1992). Meanwhile, chemical methods include the following: determination of the increase in methoxyl content from methylation, the increase in phenolic acetyl group content after acetylation, by a selective deacetylation in pyrrolidine (aminolysis), by oxidation of simple phenolic compound with sodium periodate

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(periodate oxidation) and NMR spectroscopic technique (Lai, 1992). Despite the abundance of methodology, each method has its own limitations. Therefore, selection of a suitable method is pertinent in determining the phenolic hydroxyl content.

Previously, Nasir *et al*. (2011) determined the phenolic hydroxyl group content in kraft and soda lignins, which were extracted from oil palm empty fruit bunch (EFB). They reported that the phenolic hydroxyl group content, which was determined via UV and ^{1}H NMR analyses, were 4.1076 mmol g^{-1} in kraft lignin and 2.5830 mmol g^{-1} in soda lignin. Meanwhile, Hazwan *et al*. (2013) determined phenolic hydroxyl content in kraft, soda and organosolv lignins, which were extracted from oil palm frond. They reported that the phenolic hydroxyl group content, which was determined via NMR analysis, were 0.49 per aromatic ring in kraft lignin, 0.39 per aromatic ring in soda lignin and 0.35 per aromatic ring in organosolv lignin.

In the present study, the phenolic hydroxyl content for the various parts of oil palm (*Elaeis guineensis*) such as trunk, frond, mesocarp fibre, shell and EFB was evaluated. For this purpose, two methods have been selected, which were aminolysis and periodate oxidation methods. These methods were selected based on their effectiveness to analyse not only isolated lignin but also lignocellulosics. In aminolysis method, for example, lignocellulosic sample was reduced with sodium borohydride prior to acetylation, whereas in periodate oxidation method, lignocellulosic sample could be used directly (Lai, 1992). By these two methods, the phenolic hydroxyl contents for various anatomical parts were evaluated, and compared with hardwood Japanese beech (*Fagus crenata*) and softwood Japanese cedar (*Cryptomeria japonica*). These samples were selected because of their anatomical differences whereby, under botanical terms, oil palm is classified as one of monocotyledonous angiosperms, Japanese beech as one of dicotyledonous angiosperms and Japanese cedar as gymnosperms.

MATERIALS AND METHODS

Sample Preparation

Various parts of oil palm such as trunk, frond, mesocarp, endocarp (shell) and EFB were obtained from Johor Bahru, Johor, Malaysia. Meanwhile, sapwood portions of Japanese beech and Japanese cedar were from Kyoto, Japan. These samples were first cleaned and air-dried. The air-dried samples were then pulverised using a Wiley mill (Yoshida Seisakusho, Japan) and a Fritsch mill (Fritsch, Germany) to pass through 70-mesh sieves. The pulverised samples were then extracted with ethanol/benzene (1:2, v/v) for 8 hr in a Soxhlet apparatus. Subsequently, these samples were oven-dried at 105ºC for 10 hr prior to use for experiments.

Lignin Content

Lignin content was determined as the sum of Klason lignin and acid-soluble lignin according to Dence (1992) and Sluiter *et al*. (2008). For Klason lignin determination, 72% H_2SO_4 aqueous solution was added to 1 g oven-dried sample. The mixture was allowed to stand in the water bath at 30±3°C for 1 hr with frequent stirring. The mixture was transferred to flask containing 400 ml distilled water and the total volume of the mixture was adjusted to 575 ml, equivalent to 3% H_2SO_4. It was then autoclaved at 121°C for 1 hr. The autoclaved sample was filtered, and the filtrate was separated for acid-soluble lignin determination. The residue was washed with hot water and the Klason lignin content was calculated from oven dried (105°C) weight.

Acid-soluble lignin was determined from the filtrate obtained above. It was placed in a quartz cell ('cuvette') and the absorbance at 205 nm was measured using 3% H_2SO_4 as a reference solution to obtain absorbance in the range of 0.2 to 0.7.

Aminolysis Method

The aminolysis method employed in this study was according to Lai (1992). Extractive-free sample (equivalent to 25 mg lignin) was placed in glass centrifuge tube. Four ml distilled water was then added together with 37.5 mg sodium borohydride. The mixture was subsequently centrifuged at 1300 rpm for 60 min. This procedure was repeated twice with sodium borohydride addition and centrifugation. The solution was then stirred for two days. After that, the solution was filtered and washed with distilled water, acetone and pyridine. It was acetylated with pyridine and acetic anhydride [1:1 (v/v)] for three days, whereby it was then filtered and washed with diethyl ether and dried *in vacuo* at 50ºC before 1 ml dioxane containing 5 ml internal standard (1-methylnaphthalene) and 1 ml pyrrolidine was added. The mixture was homogenised and stirred for 30 min before gas chromatography (GC) analysis. Five to six repeated analyses were conducted after homogenisation at an interval of 30-60 min.

GC (Shimadzu GC14B) with flame ionisation detector (FID) was used for the phenolic hydroxyl (PhOH) content over 100 phenylpropane (C_9) units of lignin (PhOH/100 C_9) under the following conditions: column, Shimadzu stainless steel packed with 10 wt.% polyethyleneglycol 20 M on 60/80 mesh Shincarbon A (2 m x 3 mm ø); column temperature, 180ºC (isothermal); injection port temperature, 230ºC; detector temperature, 250ºC; carrier gas, helium. The average weight of C_9 unit of lignin in oil palm samples was estimated by the

weight of syringyl-type and guaiacyl-type C_9 units, considering the molar ratio of syringyl to guaiacyl moieties, whereby it was 216, 208, 209, 204 and 213 for oil palm trunk, mesocarp, frond, shell and EFB, respectively. For wood samples, it was adapted from Lai (1992), whereby it was 209 for hardwood and 183 for softwood.

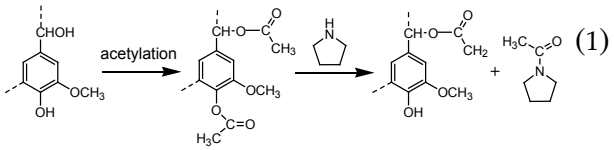
Periodate Oxidation Method

The periodate oxidation method employed in this study was according to Lai and Guo (1991). Extractive-free sample (400 mg) was placed in a glass centrifuge tube. Sodium periodate (800 mg), 6 ml cold distilled water and 1 ml cold distilled water containing 3 mg acetonitrile (internal standard) were added. The suspension was then homogenised and kept in the dark at 4°C refrigerated with occasional stirring. After two days, the mixture was homogenised and centrifuged to obtain clear solution prior to HPLC analysis.

HPLC analysis was carried out using Shimadzu LC-10A under the following conditions: column, Shodex KS801; flow-rate, 1 ml min^{-1}; eluent, HPLC grade distilled water; column temperature, 80°C.

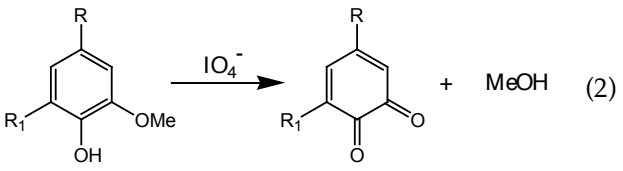
RESULTS AND DISCUSSION

The aminolysis method is a direct procedure based on the finding that the rate of deacetylation of aromatic acetates in pyrrolidine under mild conditions is considerably higher than that of aliphatic acetates. Thus, the phenolic acetyl group of acetylated lignin can be selectively deacetylated in pyrrolidine which, as indicated by the formation of 1-acetylpyrrolidine [Equation (1)], (Lai, 1992; Brunow *et al*.,1999).



(1)

The periodate oxidation method is, on the other hand, based on quantification of the methanol formed from methoxyl groups ortho to a phenolic hydroxyl group. In the process, nearly one mole of methanol per mole of phenolic hydroxyl group is released [Equation (2)], (Lai, 1992; Brunow *et al*., 1999).



(2)

where R = lignin sidechain; R_1 = H, OCH_3 or lignin unit.

The periodate oxidation method is based on methanol formation alone and cannot be applied to methoxyl-free phenolic units which are present in a large quantity in non-wood lignins (Lai *et al*., 1990). For oil palm, it was shown that it did not contain *p*-hydroxyphenylpropane moiety from our previous study (Shibata *et al*., 2008). However, other researchers have reported small amount of *p*-hydroxyphenylpropane moiety (<0.7 wt.%) and *p*-hydroxybenzoic acid (<0.6 wt.%) from isolated lignin fractions (Sun *et al*., 1999). Their percentages from the total lignocellulosics fraction were expected to be much lower. Therefore, the periodate oxidation method will be still valid for the determination of phenolic hydroxyl content in oil palm.

The lignin content and phenolic hydroxyl content obtained from both aminolysis and periodate oxidation methods are shown in *Table 1*. As indicated in *Table 1*, aminolysis method consistently yielded higher values for phenolic hydroxyl content than the periodate oxidation method, but the trend on its content was similar.

For aminolysis method, the phenolic hydroxyl content was evaluated based on the formation of 1-acetylpyrrolidine. It was observed that the increase in formation of 1-acetylpyrrolidine was not gradual as reported in the literature (Lai, 1992), at every 30-60 min interval. Periodate oxidation method, which was evaluated based on the formation of methanol, on the other hand, had already reached the maximum of methanol formation after two days prior to analysis. In addition, the reducing end groups of carbohydrate may behave like phenolic hydroxyl groups in the aminolysis method (Lai, 1992).

To elucidate these phenomena further, the phenolic hydroxyl contents for hardwood Japanese beech and softwood Japanese cedar were

TABLE 1. PHENOLIC HYDROXYL CONTENT (PhOH/100C_9) DETERMINED BY AMINOLYSIS AND PERIODATE OXIDATION METHODS IN VARIOUS PARTS OF OIL PALM AND SAPWOOD PORTIONS OF JAPANESE BEECH AND JAPANESE CEDAR

Sample	Lignin content	Aminolysis method	Periodate oxidation method
Oil palm			
Trunk	29.6	28.4	21.3
Mesocarp	36.0	21.8	16.4
Frond	23.7	26.3	19.8
Shell	54.0	13.1	10.8
EFB	24.7	12.8	8.8
Japanese beech	21.8	9.2	14.1*
Japanese cedar	28.0	20.1	16.7*

Note: *Adapted from Ehara *et al.* (2002). EFB - empty fruit bunch.

determined by aminolysis method, as shown in *Table 1*. It was observed that Japanese beech showed 9.2 PhOH/100C_9 and Japanese cedar showed 20.1 PhOH/100C_9 with aminolysis method. Japanese beech data was indeed higher if compared to American beech (8.1 PhOH/100C_9) and white birch (7.4 PhOH/100C_9) data obtained by periodate oxidation method (Sun *et al*., 1999), but lower if compared to quaking aspen (10.2 PhOH/100C_9), loblolly pine (11.7 PhOH/100C_9) (Lai *et al*., 1990; Sun *et al*., 1999), as shown in *Table* 2. Moreover, Japanese cedar data was much higher if compared

TABLE 2. PHENOLIC HYDROXYL CONTENT (PhOH/100C_9) FOR VARIOUS BIOMASS SAMPLES

Sample	Phenolic hydroxyl content	Method	Reference
Softwoods			
Norway spruce MWL[a]	19.5	Periodate oxidation	Chang *et al.* (1975)
Norway spruce MWL	20.2	UV spectroscopy	Chang *et al.* (1975)
Norway spruce MWL	18.0	Potentiometric titration	Lindner and Wegener, (1988)
Norway spruce MWL	24.0	NMR spectroscopic	Li and Lundquist (1994)
Norway spruce CELb	19.9	Periodate oxidation	Chang *et al*. (1975)
Norway spruce CEL	19.0	UV Spectroscopy	Chang *et al.* (1975)
Norway spruce wood	11.9	Aminolysis	Lai *et al.* (1990)
Norway spruce wood	12.9	Periodate oxidation	Lai *et al.* (1990)
Norway spruce Organocellc lignin	34.0	Potentiometric titration	Lindner and Wegener (1975)
Black spruce MWL	20.5	Periodate oxidation	Yang and Goring (1980)
Black spruce wood	10.0	UV spectroscopy	Yang and Goring (1980)
Loblolly pine wood	11.7	Periodate oxidation	Lai and Guo (1991)
Red pine wood	12.1	Periodate oxidation	Lai and Guo (1991)
Balsam fir wood	13.0	Periodate oxidation	Lai and Guo (1991)
Indulin AT[d]	46.0	UV spectroscopy	Tiainen *et al.* (1999)
Indulin AT	64.0	NMR spectroscopic	Tiainen *et al.* (1999)
Indulin AT	63.0	Aminolysis	Månsson (1983)
Pine kraft lignin	65.0	Aminolysis	Månsson (1983)
Pine kraft pulp (unbleached)	31.0	Periodate oxidation	Francis *et al.* (1991)
Pine kraft pulp (oxygen delignified)	22.0	Periodate oxidation	Francis *et al.* (1991)
Hardwoods			
Sweetgum MWL	14.4	Periodate oxidation	Chang *et al*. (1975)
Sweetgum CEL	13.1	Periodate oxidation	Chang *et al.* (1975)
Sweetgum wood	8.7	Periodate oxidation	Lai and Guo (1991)
Quaking aspen wood	9.2	Aminolysis	Lai *et al.* (1990)
Quaking aspen wood	10.2	Periodate oxidation	Lai *et al.* (1990)
Red oak wood	10.9	Periodate oxidation	Lai and Guo (1991)
Eastern cottonwood wood	9.4	Periodate oxidation	Lai and Guo (1991)
American beech wood	8.1	Periodate oxidation	Lai and Guo (1991)
White birch wood	7.4	Periodate oxidation	Lai and Guo (1991)
White birch MWL	18.0	NMR spectroscopic	Li and Lundquist (1994)
Monocotyledons			
Coconut husk lignin	12-21[e]	UV spectroscopy	Torres *et al.* (1992)

Note: [a]MWL- milled wood lignin; [b]CEL - cellulolytic enzyme lignin; [c]Organocell - two-stage sulphur-free chemical pulping process with methanol/water; [d]Indulin AT - industrial pine kraft (alkali) lignin; [e]Phenolic hydroxyl content differs based on the solvent used for lignin extraction.

to Norway spruce (12.9 PhOH/100C_9) data obtained by periodate oxidation method (Lai *et al*., 1990), and black spruce (10.0 PhOH/100C_9) data obtained by UV spectroscopy method (Lai and Guo, 1991). However, discrepancies between different analytical methods did exist, for example in the case of Norway spruce milled wood lignin (MWL) in the range of 18-24 PhOH/100C_9 (Yang and Goring, 1980; Lindner and Wegener, 1988; Chang *et al*., 1975). Therefore, these results show that discrepancy between aminolysis and periodate oxidation methods are possible to occur.

Furthermore, periodate oxidation method was reported to have certain limitations. The methanol formed could be partly converted to methyl acetate (Lai, 1992; Lai and Guo, 1991), thus possibly reducing the final methanol content and eventually reducing the phenolic hydroxyl content. Survey data collected from various researches (Lai *et al*., 1990; Lindner and Wegener, 1988; Li and Lundquist, 1994; Tiainen *et al*., 1999), also show slight variations in phenolic hydroxyl content from different methods, as shown in *Table 2*. Although in these studies, periodate oxidation method yielded higher value than aminolysis method, which was partly attributed to the larger number of steps involved in aminolysis method (Lai, 1992). Furthermore, different methods of sample preparation also yielded different values for phenolic hydroxyl content (Sun *et al*., 1999; Lindner and Wegener, 1988; Tiainen *et al*., 1999; Månsson, 1983; Torres *et al*., 1992). For example, sweetgum MWL yielded 14.4 PhOH/100C_9, cellulolytic enzyme lignin yielded 13.1 PhOH/100C_9 (Lindner and Wegener, 1988), whereas the wood portion yielded only 8.7 PhOH/100C_9v (Lai and Guo, 1991). This was caused by liberation of phenolic hydroxyl groups from lignin isolation procedure (Yang and Goring, 1980).

Although the above mentioned discrepancies exist, comparative results shown in *Table 1* clearly indicate a trend on phenolic hydroxyl contents for various parts of oil palm; more phenolic hydroxyl content in trunk, mesocarp and frond, compared with shell and EFB.

CONCLUSION

By using aminolysis method, higher amount of phenolic hydroxyl content was consistently determined for various parts of oil palm than periodate oxidation method. Overall, it shows there is a small discrepancy between these two methods. However, the trend of phenolic hydroxyl contents is similar.

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