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Antioxidative Activity of Lignin from Oil Palm Empty Fruit Bunch

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ABSTRACT. Improvement of oil palm plantation results in increased palm oil production. Empty fruit bunches (EFB) are oil palm solid waste containing lignocelluloses. In this study, the use of lignin content of EFB as antioxidant was assessed. Delignification of EFB was done by using ethanol in an organosolv pulping method. Antioxidative activity of lignin was characterized by using 1,1-diphenyl-2-picrylhydrazyl (DPPH), measured as IC_{50} (inhibition rate of 50%). Results of infrared spectrum analysis of EFB organosolv lignin showed a specific absorption of lignin compound. Hydroxyphenolic content was found to be 0.4% and lignin molecular weight was 4005 g/mol. The IC_{50} was 50.2 ppm and IC_{50} of the butylated hydroxytoluene as a control was 1883 ppm. This fact shows that EFB pf organosolv lignin is a potential antioxidant.

Keywords: antioxidant, empty fruit bunch, lignin, organosolv pulping.

Introduction

In 2010, oil palm plantation covered the area of 7.8 million hectares with oil palm production of about 19.8 million tons [1]. The improvement of oil palm plantation areas results in increased palm oil production. Consequently, waste production from palm oil industry also increases. Empty fruit bunches (EFB) are oil palm solid waste containing lignocelluloses. Today, EFB are commonly used as mulch in oil palm plantation or burned as fertilizer. However, when EFB are used as mulch, the transportation cost per unit of nutrition is considerably high and they may cause beetle pest population burst that can kill the plants. In addition, burning activity is prohibited by the government as it may cause air pollution [2]. Lignin utilization is one of the ways of EFB development.

The importance of lignin utilization is increasing. Lignin is the third wood macromolecule component covalently bonded with cellulose and hemicelluloses. It can be used commercially as binding material, filler, surfactant, animal feed, dispersant, and antioxidant. Lignin is a natural phenolic polymer compound. The phenoxyphenolic group allows lignin to act as antioxidant [3]. Studies have been done

to assess the radical catching activities of lignin. Barclay *et al.* characterized the radical scavenging activities of some lignin as natural antioxidant by relating the monomer structures of lignin to DPPH free radicals [4]. Dizhbite *et al.* characterized the radical scavenging activities of lignin as natural antioxidant [5]. Pan *et al.* (2006)[6] assessed the antioxidative activity of ethanol organosolv lignin from poplar hybrid as radical scavenger [6]. In addition, Vinardell *et al.* tested antioxidative activity of lignin from various sources (bagasse, lignosulphonate, steam explosion, and curan 100) in inhibiting hemolysis in human blood cell and in irritation test on human skin and rabbit eyes [7].

In general, the antioxidative effect of lignin depends on scavenging action of phenolic lignin structure. In order to characterize the antioxidative activity of phenolic component, DPPH is used as a free radical. This method gives some advantages as it is simple, easy, fast, sensitive, and it only uses small samples (Apak *et al.* 2007)[8]. An antioxidant in a reduced form will react with a free radical to form an oxidized form which is stable and has different color from its reduced form. This change in color can be detected by a spectrophotometer in appropriate wavelengths. The violet color of DPPH free radicals can be observed with a UV-Vis spectrophotometer at a wavelength of 515 nm. The reduced intensity of the color violet in DPPH is in line with the ability of lignin in scavenging DPPH free radicals [8].

This study was aimed at assessing the antioxidative activity of lignin from EFB by using a DPPH method. The assessment was based on hydroxyphenolic lignin content that allows lignin to possess antioxidative activity. This would be beneficial as an effort to develop the utilization of solid waste of oil palm industry and its lignin.

2. 2. Materials and Methods

2.1. Materials and Equipment

Materials used in this study included EFB of PTP Nusantara VIII of Sasungka Estate, Jasinga, West Java Province and DPPH (Merck). A spectrophotometer UV-VIS Hitachi U-2800 and a FTIR spectrophotometer Bruker type tensor 37 were used for measurements.

2.2. Methods

Delignification: delignification was done based on Sun *et al.* [9]. 250 g of dry EFB fiber was cooked in a digester to obtain EFB black liquor. The cooking was done in two stages. In the first stage, cooking was initiated at room temperature and increased to maximum temperature (reaction time) and in the second stage, cooking was done at the maximum temperature. Delignification was done in a composition of cooking solution of 1:1 (95% technical grade of ethanol:water). The composition of cooking solution to EFB dry fiber was 10:1 (v/b). NaOH as the pulping catalyst was used at 10%

[illegible] condition was as follows: maximum temperature of 170 °C, [illegible] hours, and 1 hour at maximum temperature.

[illegible] tion consist of two parts, namely black liquor and soften fibers [illegible] washed with 95% technical acetone and water. The remaining [illegible] then added into the black liquor. The black liquor was further [illegible] lon cloth of 20 µm to separate the filtrate and the precipitate.

[illegible] grom were isolated by referring to a method developed by Kim *et al.* [illegible] uor (500 mL) was titrated with H_2SO_4 20% to precipitate lignin. [illegible] ated until the pH of 2 was observed. The precipitate was left to [illegible] before it was separated the black liquor by using a centrifuge at [illegible] minutes. Lignin precipitate was then dissolved in NaOH 1N before it [illegible] ther with H_2SO_4 20% (as in the first stage). The resulted precipitate [illegible] nsecutively with H_2SO_4 0.01 N and distilled water before it was [illegible] cuum filter. The washed precipitate was dried in an oven at 50 °C [illegible] gnin meal was obtained.

[illegible] of **Hydroxyphenolic Content** [11]. Lignin (0.1 g) was dissolved in a [illegible] and diluted as a stock solution. Lignin alkali solution with the [illegible] g/L was obtained by taking 7.5 mL of stock solution. This stock [illegible] diluted up to 50 mL with a buffer solution with a pH of 12. Neutral [illegible] similar concentration was obtained by taking 7.5 mL stock solution [illegible] with 7.5 mL H_2SO_4 0.1 N and diluted to 50 mL with a buffer solution [illegible] difference of absorbance of the two solutions was measured at the [illegible] 280-400 nm with a neutral solution as a blank. Hydroxyphenolic [illegible] determined based on the following equation.

$$\text{[illegible] content} = \text{Aa}_{\text{as}} \times \frac{17}{4100} \times 100\%$$

[illegible] absorptivity

[illegible] ecular weight

[illegible] olar absorptivity

[illegible] of **Lignin Molecular Weight** [12]. Determination of lignin molecular [illegible] through an equivalent weight calculation. Lignin (0.5 g) was put in an [illegible] yer flask and wetted with 5 mL ethanol. NaCl (1 g) and 100 mL distilled [illegible] were then added into the mixture. The solution was further titrated with

NaOH 0.1 N until a pH of 7.5 was observed. The equivalent weight was then determined based on the following equation.

$$EW = \frac{1000 \cdot \text{gram sample}}{\text{mL NaOH} \cdot \text{N NaOH}}$$

Characterization of Lignin by Using FTIR [13]. Approximately 1 mg lignin meal was mixed with 150 mg KBr. The mixture was formed into pellet and analyzed with FTIR.

Lignin Antioxidative Activity [6]. Lignin solution was made in the concentration of 25, 50, 100, 150, and 200 mg/L and dissolved in 90% dioxane. Lignin dissolution was done gradually. Lignin solution (1.12 mL) was mixed with 4.13 mL DPPH solution of 6×10^{-5} M at 25 °C for 16 minutes. Butylated hydroxytolene (BHT) was used a positive control. The rates of DPPH radical scavenging were measured at 0 and 16 minutes at a wavelength of 515 nm.

$$IP(\%) = \frac{A(0) - A(16)}{A(0)} \cdot 100\%$$

Inhibition percentage was plotted as a function of lignin concentration. From the graph, the concentration of lignin needed to reach 50% IP was determined and measured as IC_{50}.

3. Results and Discussion

Delignification and Lignin Isolate

Delignification was done through an organosolv process. Delignification or pulping is a lignin dissolution process to separate lignin from cellulose. This process is selected based on several factors including environment, cost, and cooking solution recycle. Organosolv process is considered to be safe for the environment as it does not contain sulfur. In addition, this process is easier to do and the recycle of cooking solution results in lignin as solid material and carbohydrate as sugar material making this process more economical. Ethanol is the organic solvent used in this organosolv process. This process is also known as an *alcell* process. No sulfur content is used as the basis for solution selection as this kind of solution can reduce all sulfuric wastes resulted from conventional pulping. In addition, *alcell* process can also be used at high temperature so that the degradation of lignin-carbohydrate can occur in a more thorough way [14].

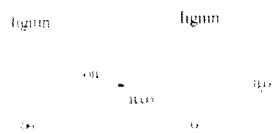
Lignin was isolated from black liquor through a precipitation by using H_2SO_4 20% until a pH of 2 was observed. The precipitate is lignin. In basic condition, it

[illegible]stance and in acidic condition, this phenolic form changes into [illegible] of acids also causes changes in lignin structural units, resulted in [illegible]out they are repolymerized to form lignin with higher molecular [illegible]tion also causes lignin to precipitate. Lignin precipitate was dissolved [illegible]ater to remove the organic compounds which are not dissolved [illegible] when pH was increased [10].

[illegible] of lignin in NaOH causes an increase in sodium ion content in the [illegible] can be minimized by washing the precipitate with dilute H_2SO_4. [illegible]emaining sulfuric ions can be reduced through washing with distilled [illegible] yielded in this study was 9.1%. The lignin content obtained in this [illegible]

[illegible]ontent of Phenolic Lignins

[illegible]yl group of phenolic lignins will undergo ionization in basic condition [illegible] ionization causes a bathochromic shift which can be used to [illegible]ally determine the number of phenolic hydroxyl groups in lignin. This [illegible]mp the absorption of lignin basic solution by using a neutral lignin [illegible]



[illegible] Ionization of lignin phenolic hydroxyl group in basic condition.

[illegible] of phenolic hydroxyl content determination by using a UV [illegible] revealed that lignin used in this study had a phenolic hydroxyl [illegible] In addition to benzylic and carbonyl groups, phenolic hydroxyl is [illegible]al group in lignin which determines lignin reactivity. This group can [illegible] between lignin and free radical allowing lignin to function as an [illegible]

[illegible] Molecular Weight

[illegible]lex polymer with a wide molecular weight distribution. Davin dan Lewis [illegible] of lignin is a bivalent chemical compound making its molecular weight [illegible]alent weight. The molecular weight of lignin in this study was 4005 [illegible] was in line with Sjöström [16] who stated that lignin molecular [illegible]ween 3000 and 5000 g/mol. The mean in molecular weight distribution [illegible] consistent, depend on pulping condition, lignin isolation process,

macromolecule degradation during isolation, and condensation effect especially in acidic condition. In addition, the inconsistence of lignin molecular weight might be caused by chemically randomized degradation of lignin in cell wall during isolation resulting in fragments with different sizes which were soluble but with rather consistent chemical compositions [17].

Lignin Functional Group Analysis

Infrared spectrum analysis of organosolv lignin done by comparing it with the Aldrich lignin model showed several typical absorption bands of lignin (Figure 2 and Table 1). The absorption bands at wavenumbers of 3409.74 cm^{-1} showed both by the EFB organosolv lignin and by Aldrich lignin indicates O-H stretches. The absorption band at wavenumbers of 2919.26 cm^{-1} in EFB organosolv lignin and 2933.34 cm^{-1} in Aldrich lignin showed C-H stretches from methyl groups. Two absorption bands at 1605.54 cm^{-1} and 1507.85 cm^{-1} in EFB organosolv lignin and 1596.20 cm^{-1} and 1508.29 cm^{-1} in Aldrich lignin showed the vibration characteristic of aromatic rings. The absorption bands at 1463.82 cm^{-1} in EFB organosolv lignin and 1464.39 cm^{-1} in Aldrich lignin showed asymmetrical C-H deformation. The most typical lignin absorption bands were found at 1510 cm^{-1} and 1600 cm^{-1} indicating the vibration of aromatic rings and between wavenumbers of 1470 cm^{-1} and 1460 cm^{-1} indicating C-H deformation [17].

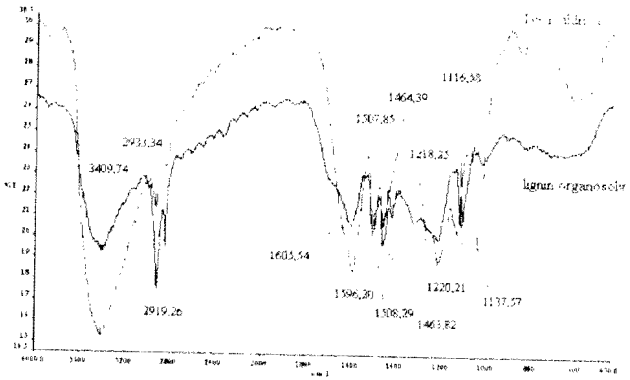


Figure 2 FTIR spectrums of Aldrich lignin and EFB organosolv lignin.

Absorption bands at wavenumbers of 1218.25 cm^{-1} in EFB organosolv lignin and 1220.21 cm^{-1} in Aldrich lignin showed guaiacyl ring vibration. Guaiacyl ring is a structure constructing lignin with coniferyl alcohol precursor. The absorption bands at 1116.58 cm^{-1} in EFB organosolv lignin and 1137.57 cm^{-1} in Aldrich lignin showed ether stretches (Table 1). Based on FTIR spectra of EFB organosolv lignin and Aldrich lignin, the lignin isolate obtained was comparable qualitatively. The absorption band

[illegible] to different lignin sources and isolation procedures applied.

[illegible] Activity

[illegible] activity was assessed by using DPPH method. This method [illegible] ability of antioxidant in inhibiting free radicals by donating its [illegible] BHT, a commercially chemical antioxidant was used as a positive [illegible]. The measurement of lignin antioxidative activity was conducted [illegible] 515.6 nm. The results showed that the IC_{50} value of organosolv [illegible] ppm and that of BHT was 1883 ppm. This indicated that lignin [illegible], was much higher than that of BHT. As a polyphenol, compared to [illegible] hidroxyphenolic content making lignin have higher antioxidative [illegible] atom in lignin is more acidic so that it is easier to release than [illegible] attributable to the fact that the electron expulsion group in lignin [illegible] longer than that in BHT (methyl) which reduces the acidity of lignin. [illegible] lignin from EFB has an IC_{50} value which is almost similar to that of [illegible] 651.6 ppm) as found in the study by Vinardell *et al.*[7].

[illegible] effect of lignin is caused by reactive free radical containing- [illegible] activities by phenolic structure of lignin [5]. Phenolic hydroxyl group [illegible] radical scavenging in lignin. After catching free radicals, phenolic [illegible] phenoxyl radicals, which are stable as they are stabilized by [illegible] The stability of phenoxyl radicals affects the antioxidative activity [illegible] structures with substitutes are able to stabilize phenoxyl radicals [illegible] activity [6].

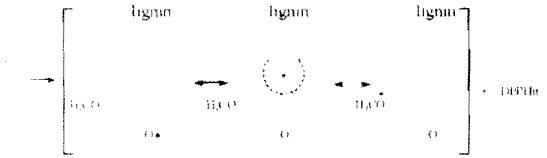


Figure 3 Reaction of lignin with DPPH free radicals [8].

[illegible] activity of lignin is affected by several factors, namely number of [illegible] hydroxyl groups, lignin molecular weight, and non-lignin components [illegible] lignin with low molecular weight has high antioxidative activity. Low [illegible] weight makes more reactive groups get exposed so that their reactivity [illegible] molecular weight is caused by depolmerization of lignin as a result of [illegible] breakage which creates new phenolic hydroxyl groups. This makes lignin [illegible] molecular weight have more phenolic hydroxyl groups than that with high [illegible] weight [6], as found that lignin isolated by organosolv in ethanol method [illegible] liquor with raw material of hybrid-poplar wood had an IC_{50} value of

8.20 ppm. This value indicated that lignin used in the study of Pan *et al.* [6] had higher antioxidative activity than EFB organosolv lignin isolate (with an IC_{50} value of 18.05 ppm) used in this study. This difference was caused by the fact that lignin used by Pan *et al.* [6] had higher molecular weight (2000-3000 g/mol) with phenolic hydroxyl content of about 2-3% than EFB organosolv lignin isolate (4005 g/mol) with phenolic hydroxyl content of only 0.4%.

Pouteau *et al.* [19] and Gregorova *et al.* [3] also stated that in its role lignin can stabilize polypropylene from thermal-oxidative degradation and inhibit hemolysis of human blood cell [7]. In their study, it was revealed that lignin inhibited hemolysis of human blood cell triggered by AAPH radicals with an IC_{50} value of 44.9 ppm. It was also known that lignin did not cause skin and eye irritation making it possible to use lignin in cosmetic formulation.

4. Conclusions

Lignin isolated from EFB showed higher antioxidative activity with an IC_{50} value of 50 ppm than that of BHT as a common antioxidant, with an IC_{50} value of 1880 ppm. Results of this study shows that EFB organosolv lignin has a potential as an antioxidant.

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