SEPARATION OF VANILLIN FROM SODA LIGNIN EXTRACTED FROM OIL PALM EMPTY FRUIT BUNCH

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Separation of vanillin from soda lignin extracted from black liquor of oil palm empty fruit bunch (EFB) using 20 % sulfuric acid is presented in this study. The degradation of soda lignin using alkaline nitrobenzene oxidation was carried out at 165 °C for 3 hours in a 2 M NaOH and nitrobenzene solution. The percentage of vanillin component was determined using high performance liquid chromatography (HPLC). The result showed that the percentage of vanillin in the soda lignin was 1.6 %. The crystallization process and thin layer chromatography (TLC) were used as methods to separate vanillin from other component. The isolated component was analyzed utilizing Fourier transform infrared spectrophotometry (FT-IR) and high performance liquid chromatography (HPLC). The results of the characterization studies proved that the isolated compound is vanillin.

INTRODUCTION

The *Elaesis guineensis* or commonly known as the oil palm was introduced to various parts of the tropics for its oil producing fruit. It is estimated that 2.28 million ha of land is being cultivated with oil palm trees in Malaysia [1]. Besides producing palm oil, the industry also generates massive amounts of lignocellulosic residues such as trunks, fronds and empty fruit bunches (EFB). Paper grade pulps from these fibres have since been established [2, 3, 4].

Lignin is an amorphous polyphenolic material arising from an enzyme-mediated dehydrogenates polymerization of tree major phenyl propanoid monomers, which are conyferyl, sinapyl and p-coumaryl alcohol [5]. Therefore, lignin structure was degraded to the monomer, which is has lower molecular weight by Nitrobenzene oxidation process before analyzed. Nitrobenzene oxidation is one of the standard procedures for analyzing lignin by chemical degradation technique in order to gain information about the composition of the original polymer [5]. Then, the components of lignin were identified using high performance liquid chromatography (HPLC).

Previous research [6] reported that vanillin found as the main product of fibrous lignin extraction. This is good opportunity since vanillin is widely used as an ingredient in food flavors, in pharmaceuticals and as a fragrance in perfumes and odor-masking products [7].

EXPERIMENTAL

Material

The empty fruit bunch (EFB) raw material in this study was supplied by Sabutek (M) Sdn. Bhd., Teluk Intan, Malaysia, a local company specializing in recycling of EFB.

Extraction of Lignin

The soda lignin was then precipitated from the concentrated black liquor by acidifying it to pH 2 using 20 % concentrated sulfuric acid (H_2SO_4). The precipitated lignin was then filtered and washed with water that was adjusted to pH 2 using H_2SO_4. Then, the soda lignin was dried in a vacuum oven at 55 °C for 24 hours [8].

Analysis of Lignin

Nitrobenzene oxidation was carried out by adding 50 mg dry soda lignin into a mixture of 2 M NaOH and 4 ml of nitrobenzene in a steel autoclave. Then, the autoclave was heated to 165 °C for 3 hours. After the autoclave was cooled to room temperature, the mixture was then transferred to a liquid-liquid extractor for continuous extraction with chloroform to remove any nitrobenzene reduction product and excess of nitrobenzene. The oxidation mixture was acidified by concentrated HCl to pH 3-4 and further extracted with chloroform. The solvent from the second chloroform solution was removed by using rotary evaporator at 40 °C. The mixture was then dissolved into dicloromethane and made it up to 10 ml. This mixture was used as a stock solution for high performance liquid chromatography (HPLC) analysis [5].

The nitrobenzene oxidation mixture was analyzed using HPLC. A 0.2 ml of stock solution was pipetted into 25 ml volumetric flask and made it up with acetonitril-water (1:2 v/v). The sample solution was injected into the HPLC system (Shimatzu) equipped with Hypersil bond C_{18} column to determine quantitatively vanillin component and another component were determine qualitatively. A mixture of acetonitril-water (1:8) containing of 1 % acetic acid was used as an eluent with a flow rate of 2 ml/min. The eluent was monitored with an UV (ultraviolet) detector at 280 nm [5].

Crystallization Process [9]

The nitrobenzene oxidation product was used in this process. The mixture was dissolve into acetone and made it up to 10 ml. The mixture was heated to 60 °C for 10 minutes using hot plate. The precipitate was filtered and washed using acetone. Then, the precipitate was analyzed using infra-red (IR) and high performance liquid chromatography (HPLC).

RESULT AND DISCUSSIONS

The components of lignin that has degraded using nitrobenzene oxidation is analyzed using HPLC. The components obtained from this method were labeled alphabetically as shown in Figure 1. From this analysis, 6 components were found in the fibrous lignin, which are vanillin, syringaldehyde, 4-hydroxybenzaldehyde, 4-hydroxybenzoic acid, vanillic acid and syringic acid.

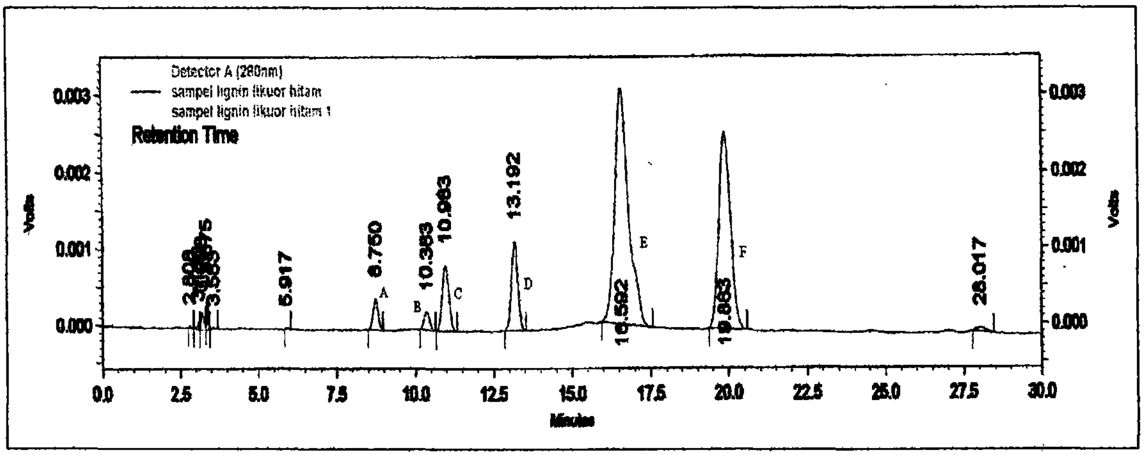


Figure 1: Lignin of black liquor chromatogram sample

The chromatogram peaks are able to be identified by differentiating the retention time between the standard component solution and the lignin sample component, Table 1 simplifies the identification of oxide peaks in lignin samples. The percentage of vanillin component in the lignin sample can be determined by injecting standard vanillin solution into different concentrations.

Table 1: Oxide peak identification in lignin sample

Peak	Retention time (R_t)	Oxide component
A	≈8.750	4-hydroxybenzoic
B	≈10.383	Vanillic acid
C	≈10.983	Syringic acid
D	≈13.192	4-hydroxybenzaldehyde
E	≈16.592	vanillin
F	≈19.883	syringaldehyde

The area of the standard vanillin solution against the standard vanillin solution concentration is plotted as presented in Figure 2. The area of vanillin component found in lignin is marked in the graph. In addition, the concentration of the sample in lignin was obtained.

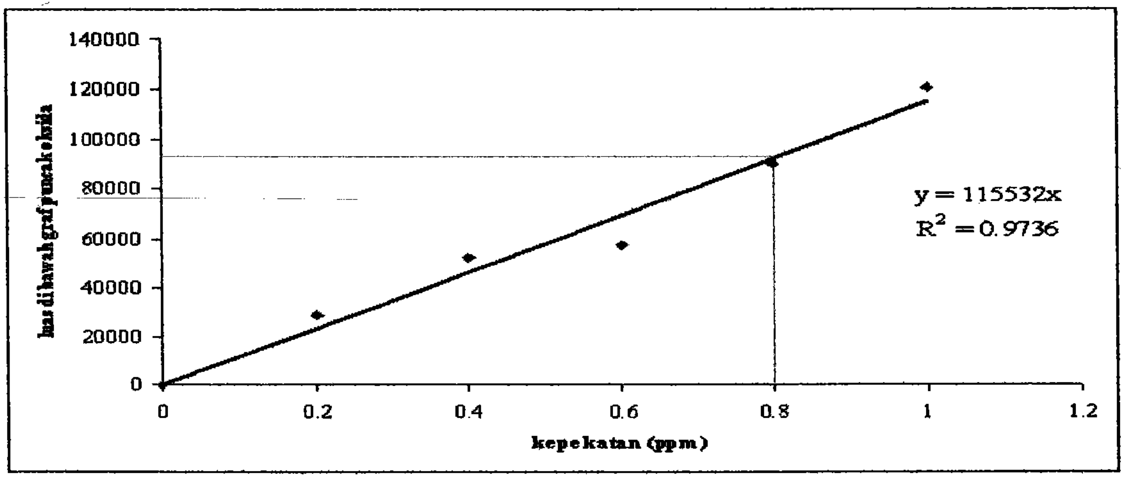


Figure 2 : Standard vanillin concentration graph against area of the standard vanillin graph

The linear equation for the graph is

$$y = 115532\,x,$$

From the equation, y representing the vanillin area, x representing the concentration, while 115532 representing the graph gradient. The HPLC chromatogram shows the area for vanillin as 92815, so, y = 92815.

$$\text{While, } x = \frac{92815}{115532}$$

$$= 0.8032 \text{ ppm}$$

Therefore, the concentration of vanillin in 50 mg lignin is 0.8032 ppm.

From the HPLC chromatogram, the area of vanillin in lignin sample is 92815. The concentration of vanillin in the lignin sample is 0.8034 ppm. The percentage of vanillin in lignin was calculated [10].

$$M_1 V_1 = M_2 V_2$$

$$0.8034 \text{ ppm } (25 \text{ mL}) = M_2 \,(0.25 \text{ mL})$$

$$M_2 = 80.34 \text{ ppm}$$

Thus, there are 80.34 μg vanillin in 1 mL of lignin sample. Therefore, 803.4 μg of vanillin can be found in 10 mL lignin sample that has been dilute from 50 mg lignin making the percentage of vanillin in 50 mg of lignin being

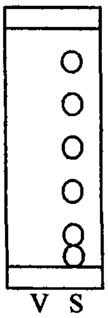
$$= \frac{803.4\ \mu\text{g vanillin}}{50 \times 10^{3}\ \mu\text{g lignin}} \times 100\ \%$$

$$= 1.6068\ \%\ \text{vanillin in 50 mg lignin}$$

Even though there is only a low percentage of vanillin in lignin, it is adequate as the research uses in lignin samples from black liquor industrial waste. Moreover, the research is aimed in producing new products from items which are commonly treated as a waste.

Thin layer chromatography (TLC) separation technique is used to identify and separate components in the solution sample by differentiating the sample spots and the standard solution spot [11]. Various solution ratios were used as the mobile phase because the solution composition may change based on the movement distance on the plate; means the mixture of solution provides better chromatogram. Amongst solvents used were chloroform, diethyl ether, methanol and hexane. The solvents were mixed in different ratios to obtain the best observation on the TLC plate.

As a result, we found that, chloroform and methanol mixture in 9:1 ratio v/v showed good separations on lignin sample. Figure 3 is showed the schematic picture of good vanillin separation redrawn for easier understanding. Six components were separated using chloroform and methanol mixture in ratio 9:1 v/v as the mobile phase. Moreover, the distance between the spots in the sample is clear. It can be concluded that, the vanillin component can separate from lignin using TLC technique is using chloroform and methanol mixture in ratio 9:1 v/v.



Chloroform : Methanol
9 : 1

Keyword : V is standard vanillin

S is lignin sample

Figure 3 : A good separation schematic redrawn for easier understanding

Vanillin components were separated from lignin using crystallization. The precipitate produced releases an odor alike to standard vanillin. Later, analysis has done to prove that the precipitate obtain in the crystallization process is vanillin. Infra red (IR) spectrometry analysis was used to determine the precipitate structure produced. Moreover, the peaks formed from the IR analysis are also easier to be traced and seen. Characterizing functioning groups for the precipitate sample was analyzed on the frequency of 4000-400 cm^{-1}.

According to the precipitate sample produced, there are a few obvious peaks. It clearly shows the characterization of some main functioning groups in vanillin structure. The typical IR spectrum of precipitate sample from crystallization process is shown in Figure 4. The strong and broad band at 3471.25 cm^{-1} is the characteristic of OH group or phenolic compound. Besides, the band at 3230.12 cm^{-1} is assigned to C-H stretching of the aromatic ring. Yet, the frequency does not show very obvious peak. A band at 1636.19 cm^{-1} is corresponding to carbonyl group (C=O). Stretching bands at 1399.83cm^{-1}

and 1338.6 cm^{-1} are characterize the C-H vibration on the methyl group. The vibration for C=C on the aromatic ring is band at 692.77 cm^{-1} [12].

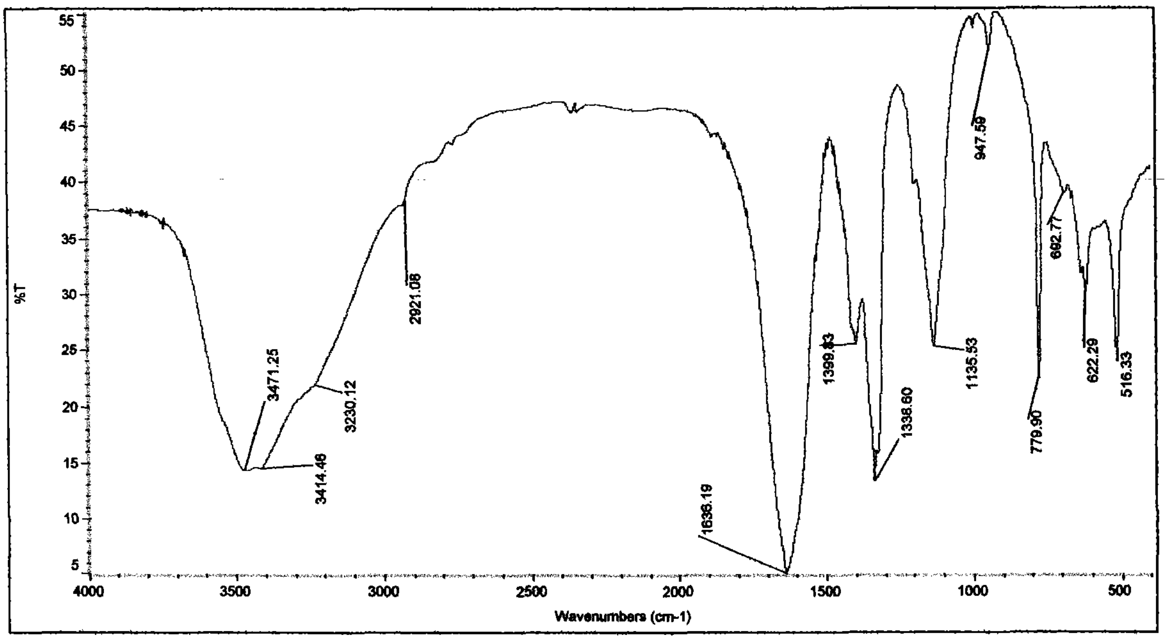
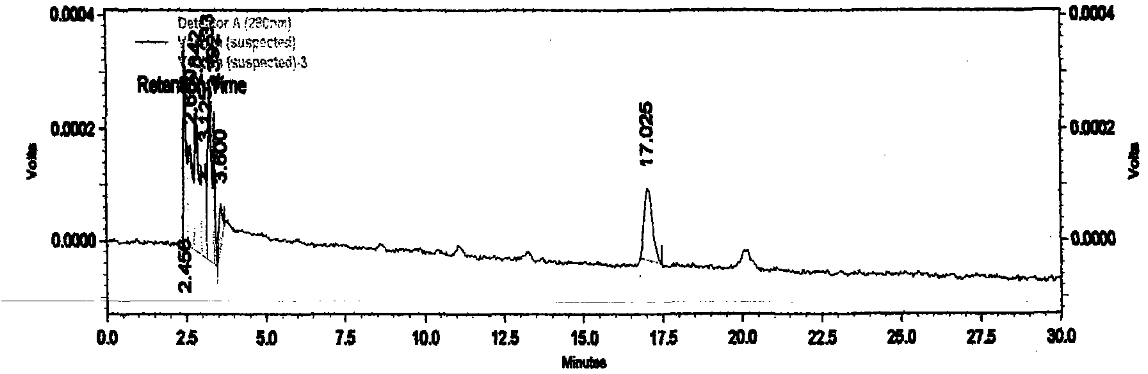
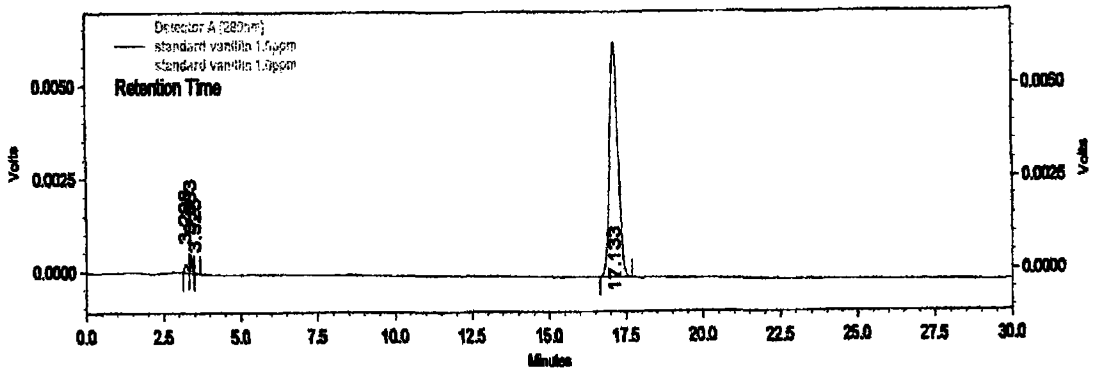


Figure 4 : IR spectrum precipitate sample from crystallization process

Thus the spectrum of precipitate product from crystallization can be identified as vanillin as there are some main functioning groups showed through the IR analysis. The precipitate was analyzed using high performance liquid chromatography (HPLC) to reaffirm the result characterized by IR analysis. The precipitate was collected and dissolved with acetone solution and water (1:2 v/v) as a solvent before the analysis is done. Finally, the sample with a concentration of 1.0 ppm was injected with a HPLC instrument. The chromatogram shows a peak at the retention time (R_t) which is almost the same as the retention time as the vanillin standard with the same concentration. Figure 5 presented the HPLC chromatogram of vanillin sample and vanillin standard.



Chromatogram HPLC of precipitate in crystallization process (1 ppm)



Chromatogram HPLC of vanillin standard (1 ppm)

Figure 5 : The chromatogram HPLC of precipitate in crystallization and vanillin standard (1ppm)

Therefore, the precipitate from crystallization process is identified as vanillin according to the HPLC and IR analysis. According to the HPLC analysis, vanillin contained impurities as there were several peaks that were not clear on the HPLC chromatogram.

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