

SEPARATION AND CHARACTERIZATION OF THE VANILLIN COMPOUND FROM SODA LIGNIN

**M. N. Mohamad Ibrahim¹, M. Y. Nor Nadiah¹, M. S. Norliyana¹
and S. Shuib²**

¹School of Chemical Sciences, Universiti Sains Malaysia
11800 Minden, Pulau Pinang, Malaysia
Fax: +604-6574854, E-Mail: mnm@usm.my

²School of Mechanical Engineering, USM Engineering Campus, Universiti Sains Malaysia, 14300 Nibong
Tebal, Pulau Pinang, Malaysia

ABSTRACT

The separation of vanillin from soda lignin that was extracted from black liquor of oil palm empty fruit bunches (EFB) using 20% sulfuric acid is presented in this paper. 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. One of the major products obtained from the breakdown of lignin is vanillin. The crystallization process was used as a method to separate vanillin from other compounds. Based on the solubility of vanillin in acetone, 15 mL of acetone was added to the residue of the oxidized products. The mixture was slowly heated to 40 °C-50 °C for 15 minutes until the vanillin crystalline precipitated. A yield of about 1.6 % vanillin was isolated from 50 mg sample of lignin. The isolated compound was analyzed utilizing high performance liquid chromatography, Fourier transform infrared spectrophotometry (FTIR) and nuclear magnetic resonance (¹H-NMR). The results of the characterization studies proved that the isolated compound was vanillin.

Keywords : Oil palm empty fruit bunches, black liquor, soda lignin, vanillin, crystallization process

INTRODUCTION

The *Elaeis 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 oil palm industry also generates massive amounts of lignocellulosic residues such as trunks, fronds and empty fruit bunches (EFB). EFB has been recognized as potentially being an alternative raw material for the paper and pulp industry [2, 3, 4]. The general objective of this project is to identify a more efficient way of managing the disposal of lignocellulosic residue and at the same time transforming the residue into useful products such as lignin.

The main source of lignin in Malaysia is from abroad where it was originally extracted from pine and acacia trees as a byproduct of the wood and pulp industry. However, this method is less effective due to foreign currency exchange lost in importing foreign product [5]. There is a need to search for alternative sources of lignin that can be derived from waste material and at the same time help to preserve natural treasures for future generations. One such source is the oil palm empty fruit bunch (EFB). This is because large amounts of EFB are collected every day at more than the 300 palm oil processing mills scattered throughout Malaysia.

Lignin is an amorphous polyphenolic material arising from an enzyme-mediated dehydrogenates polymerization of three major phenylpropanoid monomers, which are coniferyl, sinapyl and p-coumaryl alcohol [6]. The lignin structural elements are linked by carbon-carbon and ether bonds to form tri-dimensional network associated with the hemicelluloses polysaccharides inside the cell wall [7]. Lignin is usually insoluble in all solvents and can only be degraded by physical or chemical pulping process at high-temperature and high pressure. The delignification reactions involved the cleavage of non-phenolic β -O-4 linkage, phenolic α -O-4 linkage and releasing from the associated by the polysaccharide [8, 9].

Due to the high macromolecular complexity of lignin, lignin need to be degraded via the oxidation process to monomers, which have lower molecular weight before they are analyzed. Nitrobenzene oxidation was used as a method to break down the lignin into monomers before identifying them using high performance liquid chromatography (HPLC) analysis.

A previous study [10] reported that 8 components were found in degraded lignin. They are vanillin, syringaldehyde, 4-hydroxybenzaldehyde, syringic acid, 4-hydroxybenzoic acid, vanillic acid, p-coumaric acid and ferulic acid. Among these 8 compounds, vanillin was found to be the major component of soda lignin. Vanillin, a major component of vanilla, is widely used as an ingredient in food flavors, in pharmaceuticals and as a fragrance in perfumes and odor-masking products [11].

In this study the percentage of vanillin present in the soda lignin sample will be determined. Next, the vanillin compound will be separated from soda lignin via crystallization technique.

EXPERIMENTAL

Material

The oil palm empty fruit bunches (EFB) raw material in this study was supplied by Sabutek (M) Sdn. Bhd., Teluk Intan, Malaysia, a local company specializing in the recycling of EFB. The black liquor of EFB was supplied by the School of Technological Industry, Universiti Sains Malaysia (USM). This study was conducted from May 2005 until March 2006 at the School of Chemical Sciences, Universiti Sains Malaysia (USM).

Extraction of Lignin

The pH of the obtained black liquor was 12.45 and its density 1.02 g/mL. 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 adjusted to pH 2 using H_2SO_4 . Then, the soda lignin was dried in a vacuum oven at 55 °C for 24 hours prior to further analysis [12].

Nitrobenzene oxidation

Nitrobenzene oxidation was carried out by adding 50 mg of dry soda lignin into a mixture of 7 mL of 2 M NaOH and 4 mL of nitrobenzene in a 15 mL steel autoclave. Then, the autoclave was heated to 165 °C for 3 hours in a preheated thermostat oil bath. After the autoclave was cooled to room temperature, the mixture was then transferred to a liquid-liquid extractor for continuous extraction with chloroform (5×20 mL) in order to remove any nitrobenzene reduction product and excess nitrobenzene. The oxidation mixture was then acidified by concentrated HCl to pH 3-4 and further extracted with chloroform (5×15 mL). The solvent from the second chloroform solution was then removed using a rotary evaporator at 40 °C under reduced pressure in order to obtain the nitrobenzene oxidation mixture. The mixture was then dissolved into dichloromethane and made up to 10 mL. This mixture was then used as a stock solution for high performance liquid chromatography (HPLC) analysis [6].

The nitrobenzene oxidation mixture was analyzed using the HPLC method. 0.2 mL of the stock solution was pipetted into a 25 mL volumetric flask and acetonitril-water (1:2 v/v) was added to it. About 20 μ L of the sample solution was next injected into the HPLC system (Shimatzu) equipped with a Hypersil bond C_{18} column (particle size 5 μ m, 25 mm \times 4.6 mm i.d.) to quantitatively determine the vanillin component while another component was determined qualitatively. Acetonitril-water (1:8) containing 1 % acetic acid was used as an eluent with a flow rate of 2 mL/min. The eluent was then monitored with an UV (ultraviolet) detector at 280 nm [6].

Crystallization Process [13]

The nitrobenzene oxidation product was used in this process. The mixture was dissolved into acetone and made up to 10 mL. Next, the mixture was heated to 60 °C for 10 minutes using a hot plate. The precipitate was then filtered and washed using acetone. Then, the precipitate was analyzed using Fourier transform infra-red (FT-IR), nucleus magnetic resonance (1H -NMR) and high performance liquid chromatography (HPLC).

RESULTS AND DISCUSSION

Percentage of vanillin

The components of lignin obtained are listed in Table 1 and labeled alphabetically in Figure 1. The standard solution for each expected component to be present in lignin was injected into 3 different concentrations, 10 ppm, 100 ppm and 1000 ppm. This was done to ensure that the solution for each similar component at different concentrations had the same retention time. From the HPLC chromatogram, 6 components were detected in the soda lignin. They are vanillin, syringaldehyde, 4-hydroxybenzaldehyde, 4-hydroxybenzoic acid, vanillic acid and syringic acid. From the analysis we found that the concentration of vanillin in 50 mg lignin is 0.8032 ppm or equivalent to 1.6 %. Even

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

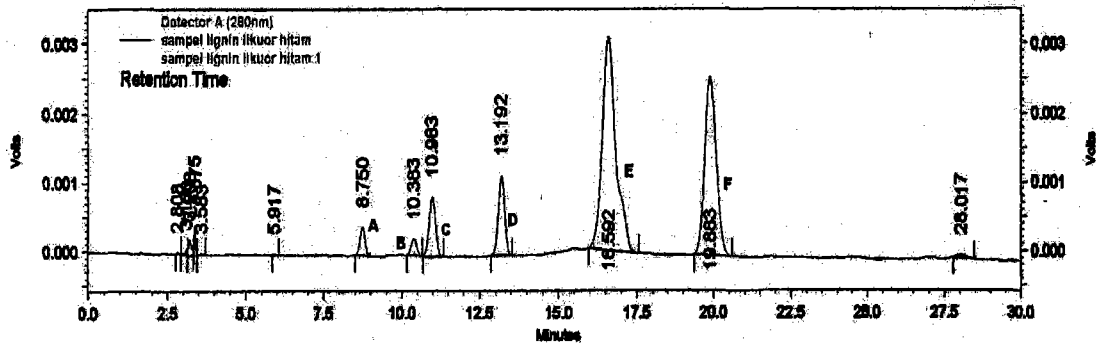


Figure 1: Chromatogram sample of lignin sample

Table 1: Oxide peak identification in the 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

Separation process via crystallization technique was used to separate vanillin from other components present in the lignin. The precipitate obtained from this process released an odor akin of the same kind to vanillin. Analysis was done in order to determine whether the precipitate obtained was vanillin. This involved the use of infra red (IR) spectrometry analysis in order to determine the functional groups present in the precipitate. This approach was selected as the precipitate easily formed pellets and was not destroyed when IR analysis was performed on it. The peaks formed from the IR analysis were also easier to be traced and observed. Characterization of the functional for the precipitate sample was performed on the 4000-400 cm^{-1} frequency range.

The IR spectrum clearly shows the characterization of several main functioning groups in the vanillin structure. A typical IR spectrum of precipitate sample from the crystallization process is shown in Figure 2. The strong and broad band at 3471.25 cm^{-1} is the characteristic of the OH group or the phenolic compound. A part from this, C-H

stretching of the aromatic ring was at the 3230.12 cm^{-1} band. Yet, the frequency does not show a very obvious peak. The band at 1636.19 cm^{-1} corresponds to that of the carbonyl group (C=O). Stretching bands at 1399.83 cm^{-1} and 1338.6 cm^{-1} are characteristic of C-H vibration on the methyl group. While the vibration for C=C on the aromatic ring is at 692.77 cm^{-1} band [14]. Figure 3 depicts the structure of vanillin (4-hydroxy-3-methoxybenzaldehyde). Thus, the spectrum of precipitate product from crystallization was identified as vanillin as the main functioning groups of the vanillin structure were revealed through the IR analysis.

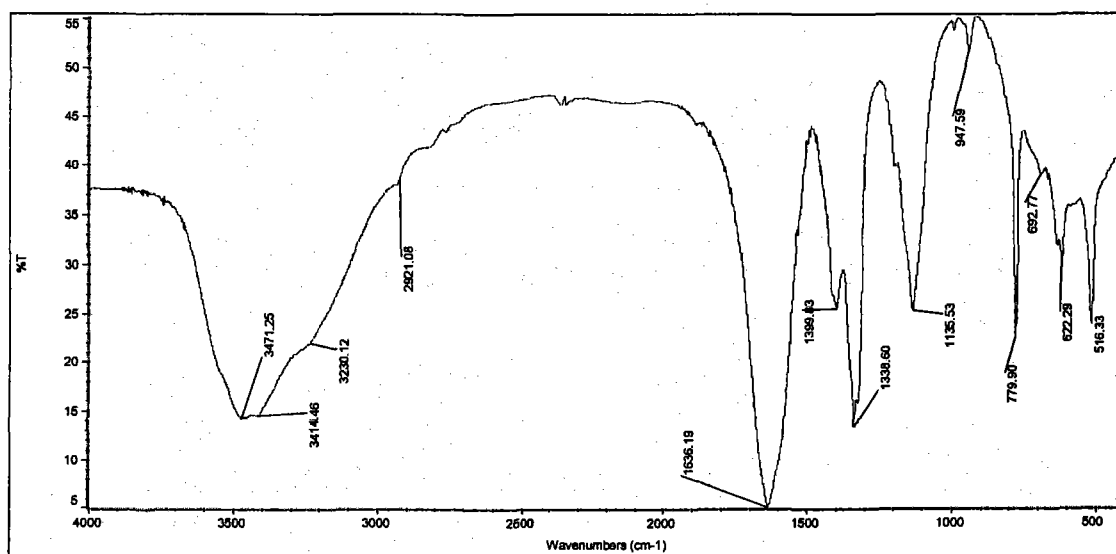


Figure 2 : IR spectrum of the precipitate sample (believe to be vanillin) from the crystallization process

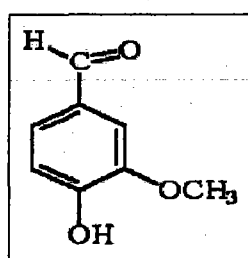


Figure 3 : Structure of vanillin compound

The precipitate was then analyzed using high performance liquid chromatography (HPLC) to reaffirm the results as characterized by the IR analysis. The precipitate was collected and dissolved in an acetone solution with water (1:2 v/v) acting as the solvent before the analysis was done. Finally, the 1.0 ppm concentrated sample was injected into

a HPLC machine. The chromatogram shows a peak at the retention time (R_t) which is almost the same as the retention time of standard vanillin with the same concentration. Figure 4 presents the HPLC chromatograms of the vanillin sample and the standard vanillin.

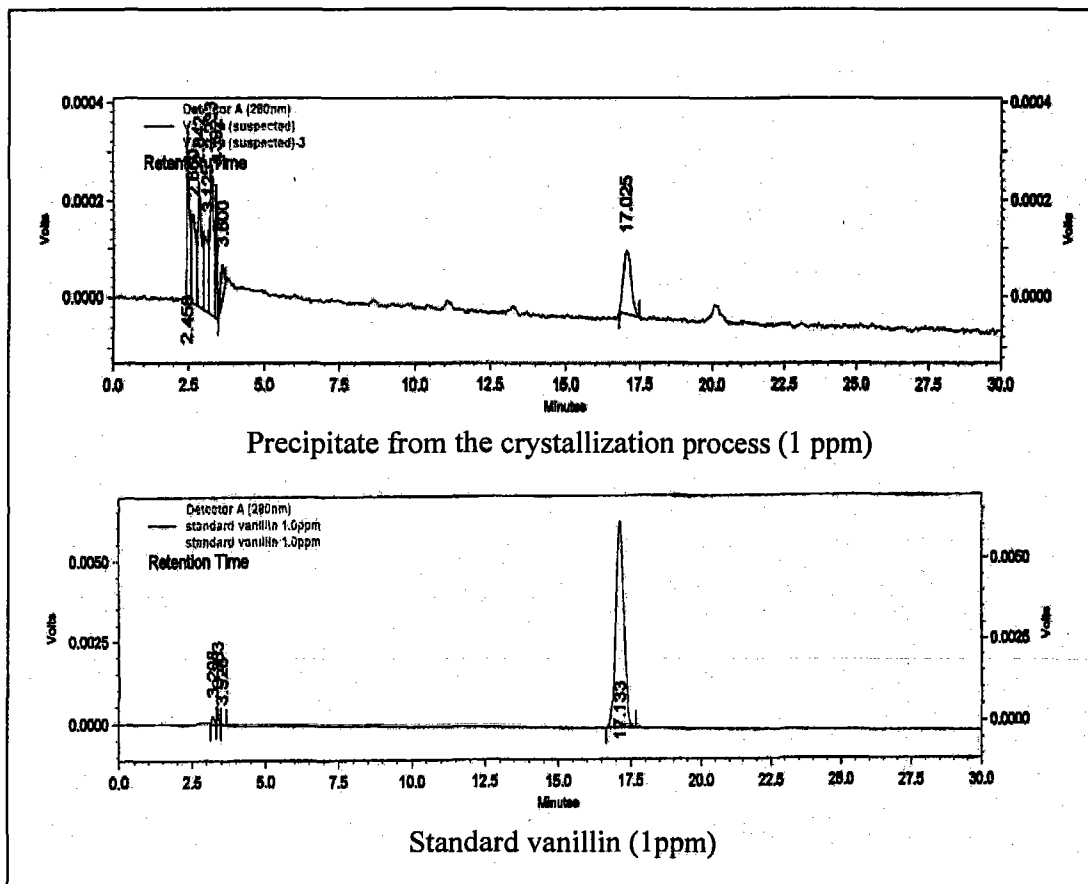


Figure 4 : The HPLC chromatograms of precipitate from the crystallization process and the standard vanillin (1ppm)

The ^1H -FT-NMR spectra were obtained from a Bruker Avance 300 operating in the FT mode at 400 MHz under total proton decoupled conditions. The spectra were recorded at 40 °C from 200 mg sample vanillin dissolved in 1 mL CDCl_3 after 3,000 scans. A 90 ° pulse flipping angle, a 26.6 μs pulse width and a 1.74s acquisition time were employed. There was no significant difference in the structure of vanillin precipitated from crystallization process and standard vanillin based on ^1H -NMR analysis (Figure 5). Incomplete dissolution of the sample may be because of the unexpected high signal/noise ratio. The peaks show that the chemical shifts for both of vanillins are very similar.

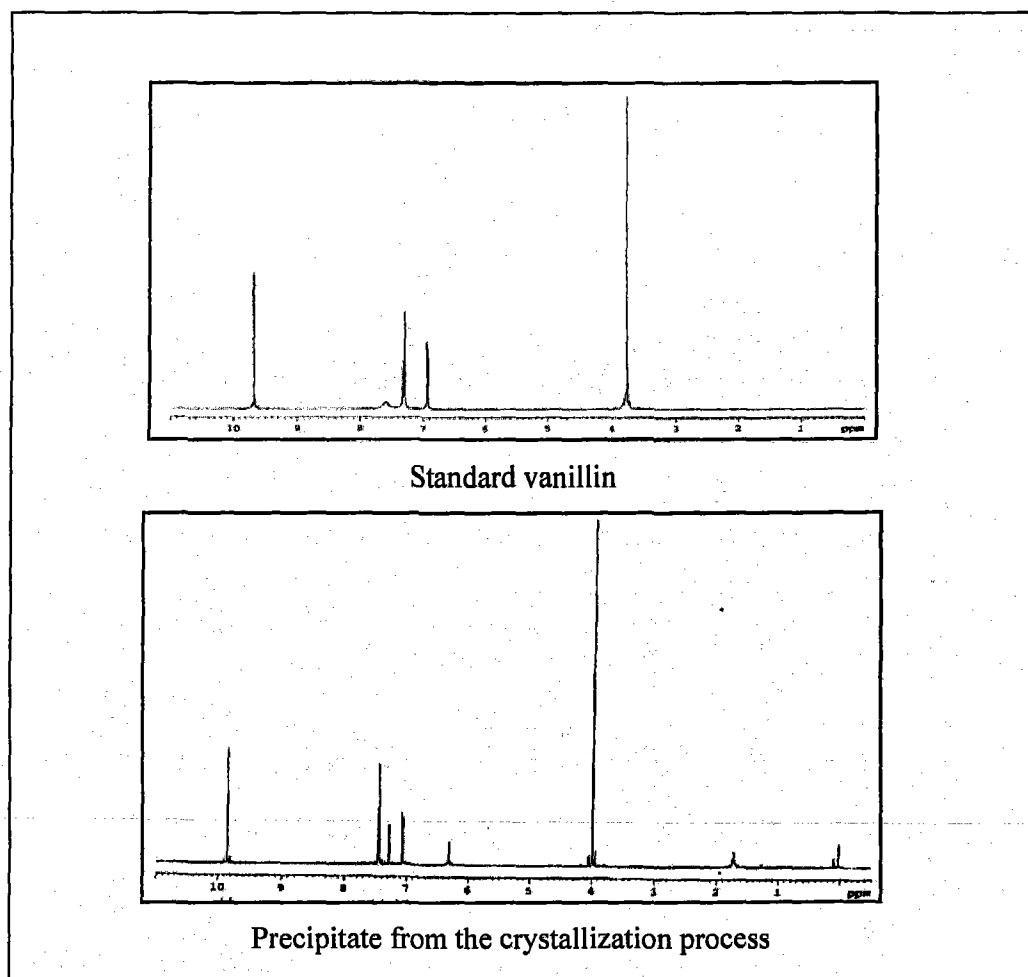


Figure 5 : ¹H-NMR spectrums for standard vanillin and sample vanillin percipitate from crystallization process

In conclusion, the HPLC, FT-IR and ¹H-FT-NMR analyses confirmed that the precipitate obtained via the crystallization process was vanillin.

ACKNOWLEDGEMENTS

The authors would like to express their appreciations to Universiti Sains Malaysia and the Malaysian Ministry of Science, Technology and Innovations for providing the financial support for this project through a research grant. The authors would also like to thank Sabutek (M) Sdn. Bhd. for supplying the EFB long fibers.

REFERENCES

1. Malaysian Palm Oil Board (MPOB), <http://www.porim.gov.my>, (accessed on 24/04/2005)
2. Akamatsu. S., Kobayashi. Y., Kamishima. H., Hassan. K.B., Mohamad Yusof. M.N, Husin. M. and Hassan., 1987. A., Industrial utilization of oil palm by-products: Termomechanical pulping of empty fruit bunches. *Cellulose Chemistry and Technology*, 21:191-197.
3. Khoo. K.C. and Lee T.W., 1991, Pulp and Paper from Oil Palm, *Appita Journal*, 44 (6):385-388.
4. Wan Rosli. W.D., Law. K.N. and Valade. J.J., 1998, Chemical Pulping of Oil Palm Empty Fruit Bunches, *Cellulose Chemistry and Technology*, 32 (1):133-143.
5. M.N. Mohamad Ibrahim., H. Azian. and M.R. Mohd Yusop, 2005, The Effect of Lignin Purification on the Performance of Iron Complex Drilling Mud Thinner, *Jurnal Teknologi C* (in press).
6. Stephen. Y.L. and Carlton, W.D., 1992, *Methods in Lignin Chemistry*, 1st Edition, Springer-Verlag Berlin Heidelberg, Germany.
7. Sun. R. C., Thomkinson. J., and James. B., 1999, Effect of precipitation pH on the physico-chemical properties of the lignin isolated from the black liquor of oil palm empty fruit bunches fibre pulping., *Polymer Degradation and Stability*, 68:195-200.
8. Gellerstedt. G. and Lindfors. E. L., 1983, Structural Changes in Lignin During Kraft Pulping, *Holzforchung*, 38 (3):151-158.
9. Groot. B. D., Dam. J. E. G. V., Zwan. R.P.V.D. and Riet. K. V., 2000, Simplified Kinetic Modeling of Alkaline Delignification of Hemp Woody Core, *Holzforchung*, 48:207-214.
10. M. N. Mohamad Ibrahim., M. Y. Nor Nadiah and H. Azian., 2006, Comparison Studies between Soda Lignin and Soda-Anthraquinone Lignin in Terms of Physico-Chemical Properties and Structural Features, *Journal of Applied Sciences*, 6 (2):292-296.
11. Greener Industry, http://www.uyseg.org/greener_industry/pages/vanillin/1vanillin_AP.htm, (accessed on 3/12/2005)
12. Mohamad Ibrahim. M.N., Chuah. S.B and Wan Rosli. W.D., 2004, Characterization of Lignin Precipitated from the Soda Black Liquor of Oil Palm Empty Fruit Bunch Fibers by Various Mineral Acids, *AJSTD*, 21(1):57-61.
13. Crystallization Theory, http://www.chem.ualberta.ca/.../theory/theory2_02L.html, (accessed on 28/02/2006).
14. Robert. M.S. and Francis. X.W., 1998, *Spectrometric Identification of Organic Compounds*, John Wiley and Son., USA.