Isolation and Characterization of Vanillin from Coconut Husk Lignin via Alkaline Nitrobenzene Oxidation

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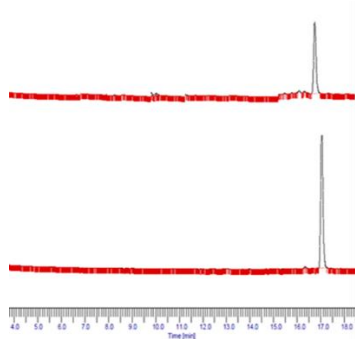
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Graphical abstract

Abstract

Isolation of vanillin from lignin extracted from coconut husk is presented in this study. Phosphoric acid (20%) is employed to precipitate the lignin from the black liquor produced by cooking the mixture of coconut husk and 1M NaOH in steam autoclave at 121ºC for one hour. Separation of vanillin from lignin was carried out via oxidation by adding 0.1 g of coconut husk lignin into a mixture of 2M NaOH (10 ml) and nitrobenzene (0.4 ml) in steel capillary bomb heated at 160ºC for 2.5 hours. Detection and quantification of vanillin presents in the lignin oxidized mixture was performed by liquid and gas chromatography. The recovery of vanillin from the mixture was done via crystallization process by dissolving the mixture in acetone and heats up to 60ºC with periodic stirring. Vanillin crystal appeared by rapidly cooled the heated mixture in the ice bath. The vanillin obtained was characterized by Infrared Spectroscopy (FTIR) for component verification. Component isolation by HPLC and GC-FID denoted the retention time of vanillin appeared around 18.852 minutes and 16.913 minutes respectively. A yield of 2.83 % vanillin was isolated from 0.1 g lignin sample and the result of FTIR characterization has suggested that the isolated compound is vanillin.

Keywords: Vanillin; lignin; coconut husk; nitrobenzene oxidation; crystallization

■1.0 INTRODUCTION

Coconut husk is a lignocellulosic biomass which is one of the abundance agricultural residues produced annually by most countries. This fibrous biomass majorly consists of short length fiber and the pithy tissue. It existed at the outer shell of coconut fruit (mesocarp) originated from the coconut palm tree, Cocos nucifera L. (Palmae) that mostly grown around the coastal areas of the tropical countries [1-2]. The world production of coconuts was approximately 55 billion per year, but only 15% of the husk fibers are being utilized and the rest are remained unconsumed and dumped in the nature which lead to the environment pollution [3]. Normally, coconut husk composed high lignin content which makes this fiber tougher and stiffer [4]. This natural fiber consists of 46% of lignin in average [5-7]. Due to this reason, coconut fibers are preferably to be used as the alternatives for synthetic fibers in composites materials. In addition, the presence of higher lignin content compared to the cellulose content in the coconut husk makes this fiber quite unfavorable due to the limited use of lignin. However, the potential of lignin as renewable natural resource for aromatic compound derivation [8], has made a sense for the utilization of coconut husk as one of the source for sufficient amount of lignin compound.

Lignin is one of the predominant compounds existed in the lignocellulosic materials. This compound is originally occurred as a macromolecule that presented as the second major constituent after cellulose [8]. It was developed from the copolymerization of three primary monomers which are sinaply alcohol, coniferyl alcohol and p-coumaryl alcohol [8-10]. Each of these monomers give rise to different aromatic nuclei in the lignin structure which are syringyl (S), guaiacyl (G) and p-hydroxyphenyl (H) respectively. Polymeric structure of lignin can be degraded and converted into other molecules via thermochemical treatments with or without the aid of chemicals additives, solvents and catalysts [10]. Degradation of lignin is a complicated process because this polymer has many constituents with different breakdown route, either competitive or consecutive reactions. This process also occurs over a broad temperature range because of the cleavage of various oxygen functional groups which takes place at different temperatures due to their different thermal stability [11]. The breakdown of the functional groups would give low molecular weight products. Furthermore, the cleavage of the aryl-ether linkages will lead to the creation of unstable and highly reactive

free radicals that might form products with increased stability through the rearrangement, electron abstraction or radical-radical interaction [11]. Normally, the degradation of lignin polymeric structure initially occurs at high temperature around 200-275ºC. During this temperature, aromatic compounds will be formed and most of them having phenolic groups [12]. Degradation of lignin via oxidation route is normally applied for conversion of lignin into aromatic aldehyde compound such as vanillin [13].

Vanillin is a primary compound of vanilla extracts. It is essential in lots of various applications in food flavors formulations, chocolate, confectionery, baking, beverage, dairy and fresh products, perfumes and fragrance, odors masking, formulation for home and personal care, toiletries, detergency, feed applications, pharmaceutical and agrochemicals intermediated [14-16]. Instead of natural production from vanilla orchid bean, vanillin can be chemically derived via controlled oxidation of lignin. Oxidant gives significant effect towards aldehyde production. Milder oxidants such as nitrobenzene, metal oxides and oxygen were consumed in order to preserve the lignin aromatic ring, since the reaction will occurred at the side chains. Hydrogen peroxide, permanganate, chlorine, chlorine dioxide or hypochlorite are less preferable oxidants for aldehyde production due to their strong oxidizing effect that may destroy the aromatic ring and lead to the production of corresponding phenolic acids [17-18]. However, nitrobenzene has been proven as an effective oxidant to produce reasonable amount of vanillin as this oxidant produced vanillin as the major compound [19]. In this study, a new source of vanillin is explored, which is from coconut husk waste. Due to the significant amount of coconut husk being generated every year, there is a need to utilize this waste effectively. Besides producing a useful value added product from coconut husk, this study could also partly help with solving the solid waste management problem.

■2.0 EXPERIMENTAL

2.1 Materials

Coconut husk was purchased from a local supplier which is MD Interactive's company, located at Tampoi Industrial Park, Johor Bahru. The sample was air dried for 24 hours before cut into small pieces and ground to pass a 1-mm size screen. The powdered-form husk then was extracted with toluene-ethanol (2:1 v/v) in a Soxhlet extractor for 6 hours. The dewaxed sample was further dried in an oven at 60 °C for 24 hours.

2.2 Lignin Isolation by Alkaline Hydrolysis and Acid Precipitation

The treated sample of coconut husk was soaked in 1 M aqueous NaOH solution which the ratio of sample to the extraction solution is 3:100 (g/ml). The mixture was stirred gently and poured into a 200 ml glass bottle equipped with a plastic cap, prior to the cooking process in a steam autoclave operated at 121°C for 1 to 1.5 hours. The cooked mixture then was allowed to cool to ambient temperature prior to the separation of the solid residues and the filtrate by filtration. The supernatant fluid which exists in the form of black liquor was stored overnight at 4°C in the refrigerator before subjected to an acidification process. Coconut husk lignin was recovered from black liquor (pH 12) by mineral acid precipitation. The black liquor was acidified by H_3PO_4 (20%) at pH 2. Around this pH, the lignin precipitates formed. The precipitates were separated via vacuum filtration using 0.2 µm pore filter and washed with distilled water of pH 2, which prepared using the same acid used in previous. Finally, the isolated lignin was subsequently oven dried at 55°C for 24 hours to 48 hours until the lignin was completely dried.

2.3 Vanillin Derivation via Nitrobenzene Oxidation

1.0 g of dried lignin separated from coconut husk was mixed with 10 ml of 2 M NaOH aqueous solution and 0.4 ml nitrobenzene in a 50 ml stainless steel capillary bomb equipped with screw cap fitted with a teflon gasket. The bomb contained the mixture was sealed tightly before heated at 160ºC for 2.5 hours in an oil bath containing silicone oil as the heating medium. The oxidative cleavage of lignin side chains was occurred during the cooking process. After oxidation completed, the cooked mixture was cooled in the ice water prior to liquid-liquid extraction by chloroform. The oxidized mixture was extracted with five times of 20 ml chloroform to remove any excess nitrobenzene or its reduction products. After extraction, the oxidized mixture was acidified by 20 % H3PO4 until achieved to pH3. The acidified oxidation mixture then was extracted again with five times of 15 ml chloroform and the remaining chloroform was removed through rotary evaporator at 40ºC. Finally, the concentrated nitrobenzene oxidation mixture containing vanillin, left in the flask was dissolved into dichloromethane and made up to 10 ml as a stock solution for further analysis by High Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC).

2.4 Separation and Identification of Vanillin in the Oxidized Mixture

2.4.1 High Performance Liquid Chromatography (HPLC)

Perkin Elmer High Performance Liquid Chromatography (autosampler) equipped with Hypersil C18 column type (particle size, 5 μm, 150 x 4.6 mm inside diameter) was being used to separate and detect the vanillin compound based on the standard vanillin as the reference. Column temperature was 35ºC and injection volume was 20 μl. A mixture of acetonitrile: water (1:8) containing 1% acetic acid was used as the mobile phase with a flowrate of 2 ml/min. Stock solution of the oxidized mixture was prepared by dissolving the mixture into dichloromethane and made up to 10 ml.

2.4.2 Gas Chromatography (GC)

Perkin Elmer Autosystem XL Gas Chromatography has been employed. The type of capillary column used is DB-Wax (30 m x 0.53 mm inside diameter x 0.5 µm film thickness). The split ratio is 1:100 at 250ºC and the sample injection is 3 µL. Initial temperature for oven is set to 40ºC at a rate of 10ºC /min and 5 minutes retention time, while the final temperature is 210ºC. The detector (FID) operates at 280ºC and helium is used as the carrier gas with a flow rate at 32 cm/s. The standard and sample solutions were prepared by using acetone as a solvent.

2.5 Purification of Vanillin by Crystallization

The oxidized mixture was dissolved in acetone and made up to 10 ml. The dissolved mixture then was heated under temperature range of 40 to 60ºC and slowly stirred for 10 to 15 minutes. Next, the heated mixture was soaked in the ice bath until some crystal-like precipitates formed. The brownish precipitates claimed as the vanillin was collected by filtration and washed with acetone.

2.6 Structural Verification by Fourier Transform Infrared Spectroscopy (FTIR)

The IR spectra of the vanillin sample recovered by crystallization were recorded by Perkin Elmer FTIR spectrometer. The sample was prepared by embedding 1% of finely ground vanillin sample in potassium bromide (KBr) pellets. The spectra were recorded within the absorption band in a range of 4000-400 cm^{-1}.

■3.0 RESULTS AND DISCUSSION

3.1 Detection of Vanillin in Oxidized Mixture by HPLC

The presence of vanillin compound in the oxidized mixture was verified by comparing the retention time of vanillin chromatogram with the reference standard. The chromatograms of standard vanillin and derived vanillin contained in the oxidized mixture as detected by HPLC are shown in Figure 1 (a) and (b) respectively. According to the chromatograms, the retention time of standard vanillin is 18.93 minutes while the retention time of derived vanillin is 18.85 minutes. The percentage of the similarity between the retention times is 99.57% which means vanillin is clearly separated and identified from the assorted compound of oxidized mixture. The presence of other compounds which derived along vanillin during the nitrobenzene oxidation stage also have been detected and separated by HPLC at different retention time. These compounds were possibly consisted of other phenolic or aromatic aldehyde such as syringaldehyde, 4-hydroxybenzaldehyde and their respected acid such as vanillic acid,syringic acid ,p-hydrobenzoic acid as well as hydrocinnamic acids (ferulic and p-coumaric acid) [12, 19-21].

3.2 Verification and Quantification of Vanillin in Oxidized Mixture by GC-FID

Identification of vanillin existence in the lignin oxidized mixture was verified by GC-FID analysis. Similar to HPLC, GC is used to separate the vanillin from the other derived compounds in the oxidized mixture based on the volatility of the component at different retention time. The chromatograms of standard vanillin and derived vanillin contained in the oxidized mixture as detected by GC-FID are shown in Figure 2 (a) and (b), respectively. Based on the chromatograms, the lignin derived-vanillin exhibited a prominent peak at 16.91 minutes while standard vanillin emerged at 17.16 minutes. The degree of similarity between the retention times is 98.56% which supported the HPLC result and verified the presence of vanillin compound in the mixture. Similar to HPLC, GC-FID analysis is also exhibits the existence of other phenolic compounds produced along with vanillin.

GC-FID also was employed in this study for component quantification by determining the concentration of vanillin in the oxidized mixture. The concentration of vanillin has been computed based on the standard vanillin calibration curve where a range of standard vanillin concentrations were plotted against the chromatographic peak areas. By identifying the concentration of vanillin in the oxidized mixture, the percentage of vanillin separated from the lignin also was determined. Approximately 2.83% of vanillin was successfully separated from coconut husk lignin in this process.

3.3 Structural Verification by FTIR Spectroscopy

Figure 3 (b) indicates that IR spectrum of the derived vanillin sample matches almost perfectly with the standard vanillin spectrum in Figure 3 (a). A broad band at 3211 cm^{-1} in the spectra of derived sample is appeared due to the characteristic of hydroxyl (OH) group in the phenolic structure. A noticeable shoulder band arose at 2854 cm^{-1} is attributed to the vibration of methoxy (OCH3) group. The existence of aldehyde group was indicated by the appearance of a sharp and intense band occurred at 1680 cm^{-1} which is due to the carbonyl (C=O¬) stretching. Other significant bands appeared at 1586, 1503 and 1425 cm^{-1} in the spectrum of derived sample is related to the characteristic of aromatic rings due to the aromatic skeleton vibrations. The presence of ether group is exhibited by the C-O stretching bands which appeared at 1019 and 1259 cm^{-1}. Meanwhile, the band occurred at 815 cm^{-1} denotes the C-H deformation and ring vibration in the vanillin structure [22-23]. The IR spectrum results have suggested that the derived sample is vanillin since it consists of all main functional groups that are present in the standard vanillin structure.

■4.0 CONCLUSION

Production of synthetic vanillin from lignin isolated from biomass source is regarded as an excellent alternative for vanillin production as it involves the transformation of agricultural waste into a high value-added product. The study was intended to produce vanillin originate from biomass sources, which abundantly generated as residues by most industrial sectors. The utilization of biomass sources was not only provide cheaper, safe and renewable sources for lignin separation, but also could reduce the environment polluting issues. From this study, 2.83% of vanillin was successfully separated from coconut husk lignin using the crystallization process based on the HPLC and GC analysis. The isolated compound was structurally verified by FTIR analysis.
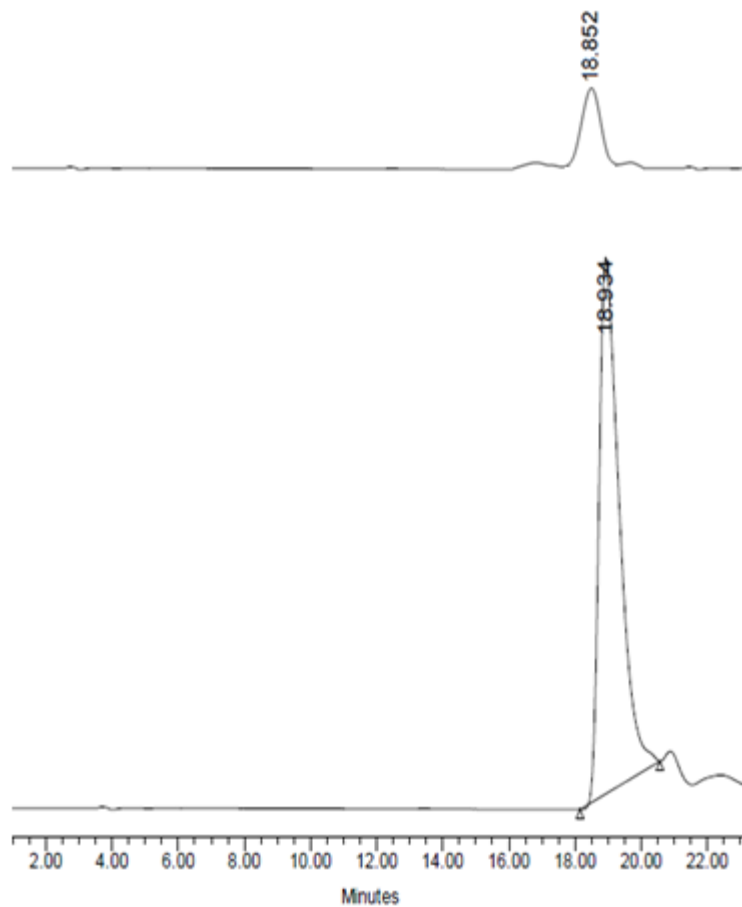
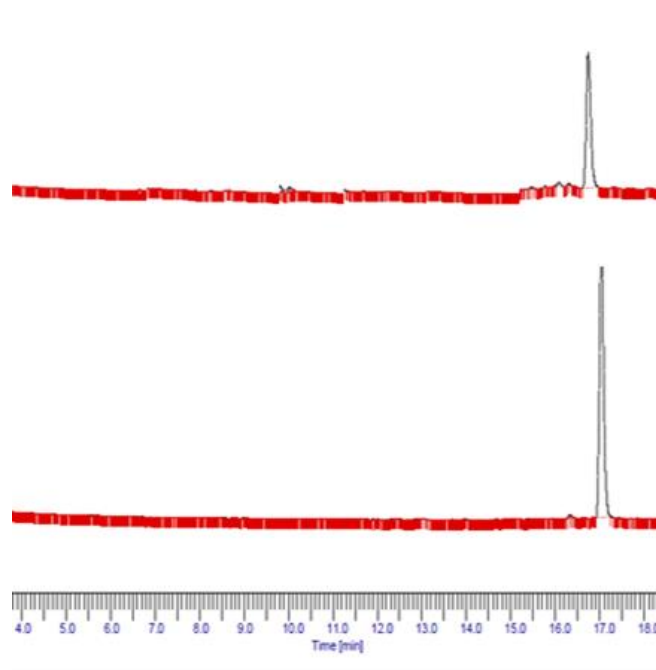**Figure 1** HPLC chromatogram of (a) standard vanillin and (b) derived vanillin contained in the oxidized mixture

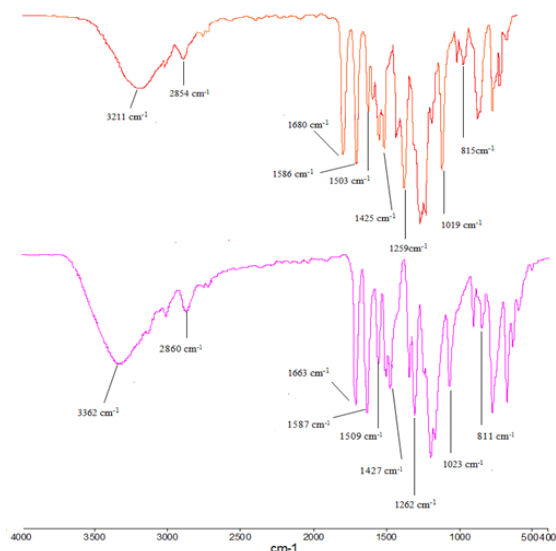**Figure 2** GC-FID chromatogram of (a) standard vanillin and (b) derived vanillin contained in the oxidized mixture

Figure 3 FTIR spectrum of (a) Standard vanillin (b) Derived vanillin

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