PRODUCTION OF SYNTHETIC VANILLIN FROM COCONUT HUSK LIGNIN VIA ALKALINE NITROBENZENE OXIDATION

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To myself, dearest mother, beloved late father and darling husband.

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ABSTRACT

Vanillin is one of the essential constituents that provide the flavor and aroma characteristics to the vanilla extract. Due to the increasing market demands, the production of synthetic vanillin has gradually developed as an alternative to the complex production of natural vanillin from vanilla orchid beans. Recently, the chemical production of synthetic vanillin from biomass lignin has gained significant attention as the more environmental friendly alternative for vanillin production. Accordingly, lignin isolated from three sources of lignocellulosic biomass which are oil palm empty fruit bunch (OPEFB) fiber, coconut husk and kenaf fiber were studied and compared prior to be used in the vanillin derivation process. Among the biomass studied, the highest lignin fractions were recovered from the coconut husk which exhibits the best characteristics of lignin in term of structure, purity and thermal behavior. Therefore, only the coconut husk lignin was used for further study. Coconut husk lignin was subjected to an alkaline oxidation at 160 ºC for 2.5 hours, using 0.40 mL nitrobenzene as the oxidant. Approximately 2.83 % of vanillin was successfully produced and its presence in the oxidized mixture was identified by using the high performance liquid chromatography (HPLC) and gas chromatography (GC) method. Meanwhile, the crystalline structure of purified vanillin was verified using the Fourier transform infrared (FTIR) spectroscopy and proton nuclear magnetic resonance (H^1NMR) method. In order to maximize the production yield of vanillin, several variables were considered for optimization using the response surface methodology (RSM) which includes oxidation temperature, oxidation time and volume of nitrobenzene. A maximum yield of 4.01 % vanillin was obtained from coconut husk lignin at 140 ºC for 2.8 hours using 0.44 mL nitrobenzene. The production yield of vanillin at these optimum conditions was compared with the oxidation of OPEFB and kenaf fibers lignin. The comparison shows the highest yield was obtained for the coconut husk lignin oxidation. As a conclusion, the yield of vanillin was improved at low oxidation temperature.

ABSTRAK

Vanilin adalah salah satu juzuk utama yang memberikan ciri-ciri perisa dan aroma kepada ekstrak vanila. Oleh kerana permintaan pasaran yang semakin meningkat, penghasilan vanilin sintetik telah berkembang secara beransur sebagai alternatif kepada penghasilan vanilin semulajadi yang kompleks daripada kekacang orkid vanila. Kini, penghasilan vanilin sintetik secara kimia daripada lignin biojisim telah mendapat perhatian yang signifikasi sebagai alternatif yang lebih mesra alam sekitar. Oleh itu, di dalam kajian ini, lignin telah diasingkan daripada tiga sumber biojisim lignoselulosa iaitu gentian tandan kosong buah kelapa sawit (OPEFB), sabut kelapa dan gentian kenaf untuk dibandingkan sebelum digunakan dalam proses penghasilan vanilin. Antara biojisim-biojisim tersebut, pecahan lignin tertinggi diperolehi daripada sabut kelapa yang mempamerkan ciri-ciri lignin yang terbaik dari segi struktur, ketulenan dan sifat terma. Oleh itu, hanya lignin sabut kelapa telah digunakan untuk kajian seterusnya. Lignin sabut kelapa kemudiannya digunakan dalam pengoksidaan beralkali pada suhu 160ºC selama 2.5 jam, dengan menggunakan 0.40 mL nitrobenzena sebagai agen pengoksida. Kira-kira 2.83 % vanilin telah berjaya dihasilkan dan kehadirannya di dalam campuran oksida telah dikenal pasti menggunakan kaedah kromatografi cecair berprestasi tinggi (HPLC) dan kromatografi gas (GC). Sementara itu, stuktur kristal vanilin yang ditulenkan telah disahkan menggunakan kaedah spektroskopi inframerah transformasi Fourier (FTIR) dan proton resonans magnetik nuklear (H^1NMR). Bagi memaksimumkan penghasilan vanillin, beberapa pemboleh ubah iaitu, suhu pengoksidaan, masa pengoksidaan dan isipadu nitrobenzena telah dipertimbangkan untuk proses pengoptimuman menggunakan kaedah tindakbalas permukaan (RSM). Hasil maksimum sebanyak 4.01 % vanillin telah diperolehi daripada lignin sabut kelapa pada suhu 140 ºC selama 2.8 jam menggunakan 0.44 mL nitrobenzena. Hasil vanillin menggunakan keadaan optimum ini dibandingkan dengan proses pengoksidaan lignin gantian kenaf dan OPEFB. Perbandingan tersebut menunjukkan hasil tertinggi vanillin diperolehi daripada proses pengoksidaan lignin sabut kelapa. Kesimpulannya, hasil vanillin telah diperbaiki pada keadaan suhu pengoksidaan yang rendah.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ANOVA	-	Analysis of Variance
$(CH_3)_2CO$	-	Acetone
C=O	-	Carbonyl
CCD	-	Central Composite Design
$CDCl_3$	-	Deuterated Chloroform
$CHCl_3$	-	Chloroform
CH_2Cl_2	-	Dichloromethane
CH_3CN	-	Acetonitrile
CH_3COOH	-	Acetic Acid
C_2H_6O	-	Ethanol
$C_4H_8O_2$	-	Dioxane
$C_6H_5NO_2$	-	Nitrobenzene
C_7H_8	-	Toluene
DF	-	Degree of Freedom
DMSO-d_6	-	Dimethylsulphoxide
DOE	-	Design of Experiment
FDA	-	Food and Drug Administration
FTIR	-	Fourier Transform Infrared Spectroscopy
g	-	Gram
G	-	Guaiacyl
GC	-	Gas Chromatography
GC-FID	-	Gas Chromatography-Flame Ionization Detector
H	-	*p*-Hydroxyphenyl
HCl	-	Hydrochloric Acid
HPLC	-	High Performance Liquid Chromatography

Hrs	-	Hours
H^1 NMR	-	Proton Nuclear Magnetic Resonance
H_2SO_4	-	Sulphuric Acid
H_3PO_4	-	Phosphoric Acid
KBr	-	Potassium Bromide
kg	-	Kilogram
LAP	-	Laboratory Analysis Procedure
M	-	Molar
MHz	-	Mega Hertz
mL	-	Milliliter
mm	-	Millimeter
MS	-	Mean Square
NaOH	-	Sodium Hydroxide
$NaClO_3$	-	Sodium Chlorite
NREL	-	National Renewable Energy Laboratory
OH	-	Hydroxyl
OCH_3	-	Methoxyl
OPEFB	-	Oil Palm Empty Fruit Bunch
ppm	-	Part per million
RSM	-	Response Surface Methodology
Rt	-	Retention Time
S	-	Syringyl
SS	-	Sum of Square
t	-	Time
T	-	Temperature
TG	-	Thermogravimetric
TGA	-	Thermogravimetric Analysis
V_{BN}	-	Volume of Nitrobenzene
UV-Vis	-	Ultraviolet Visible
WADA	-	World Anti Doping Agency
µL	-	Microliter
4-HBA	-	4-hydrobenzoic Acid

LIST OF SYMBOLS

R^2	-	Regression Coefficient
$	-	United States currency
%	-	Percentage
°C	-	Degree Celsius
α	-	Alpha
β	-	Beta
δ	-	Delta
γ	-	Gamma
μ	-	Micro
n	-	Integer Number
~	-	Approximately
=	-	Equals

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

This chapter presents the background of the conducted study and the general ideas regarding vanillin production. The focal purposes, problem statement, scope, significance of the study and the outline of the thesis are also stated in details.

1.1 Background of Study

Vanillin (4-hydroxy-3-methoxybenzaldehyde) is the most prominent aromatic compound existed in natural vanilla extract (Walton *et al*., 2003). This compound presents as the essential constituent that contributes to the flavor and aromatic characteristic to the vanilla. Therefore, vanillin is one of the principal key additives to food products, perfumery, beverage and intermediate chemicals in the pharmaceutical industries. Vanillin can be derived naturally or synthetically through different sources and methods. Natural vanillin is originated from vanilla orchid beans, while synthetic vanillin can be produced through various sources such as, coniferin, eugenol, plant ferulic acid, petroleum guaiacol and lignin via chemical or biotechnological routes (Mathias *et al*., 1995). Previously, the production of vanillin was mainly depends through the direct extraction from cured vanilla orchid beans. However, regarding to the complexity of the process and constantly increasing market demands, alternative in chemical and biotechnological routes were gradually developed. Nowadays, synthetic vanillin is widely used as the flavoring agent instead of natural vanilla extracts. Thus, the competition of markets is longstanding and turns more intense when the prices of natural vanilla rockets.

However, due to high investment and risky procedures in biotechnological methods, synthetic vanillin was preferable to be produced commercially by the chemical method. In general, vanillin was chemically prepared either from guaiacol (petroleum-derived compound) or from lignin (constituent of woody materials commonly obtained from a byproduct of the pulping industry or lignocellulosic biomass) (Wong *et al.*, 2010). Based on a recent statistic, only 15% of vanillin is synthesized via oxidation of lignin while the rest is majorly from petroleum guaiacol (Sinha *et al.*, 2008). However, the utilization of lignin is estimated to expand in the future due to the significant discovery of lignin potential to be used as the renewable source for vanillin. In addition, Esposito (1997) has reported that lignin-based vanilla flavoring is alleged to have richer flavor profile compared to the guaiacol-based flavoring due to the presence of acetovanillone in the lignin derived product.

Previously, most of lignin was obtained from the black liquor produced by paper and pulp industries. However, in recent studies, the extraction of lignin from lignocellulosic biomass, abundantly generated from agriculture and forestry residues, has getting attentions as the new alternative for a greener and safer resource for vanillin production. Extensive studies have been conducted previously in order to improve the production yield of vanillin. Different methods have been applied using different raw materials and chemicals to obtain the maximum vanillin yield. Thus, various chemical methods have been carried out in order to convert the lignin into vanillin, including oxidation process by oxygen, hydrogen peroxide, metal oxides and nitrobenzene. However, nitrobenzene oxidation method was preferably employed due to the ability to produce higher yield of vanillin compared to other methods (Stephen and Carlton, 1992 and Mohammad Ibrahim *et al.*, 2008).

Therefore, this study has been conducted to serve the purpose on improving and maximizing the yield of vanillin isolated from biomass sources. Besides of considering the raw material selection, the chemicals used as the oxidant and precipitating agent particularly for lignin oxidation and extraction processes were taken into account. Hence, high selective chemicals such as nitrobenzene oxidant for lignin oxidation process have been used to increase the yield of vanillin. Part of that, the crucial factors of lignin oxidation such as oxidation temperature, oxidation time and volume of oxidant that might affect the production yield of vanillin were also optimized and studied.

1.2 Problem Statement

The potential of vanillin to be consumed in various useful products makes vanillin as a molecule of interest and received a continuous market demand. However, the production of natural vanillin from the vanilla orchid pods is very limited due to the several hindrances such as limited cultivation area with suitable climate and involves a long and laborious process. Thus, various methods to synthesis a synthetic vanillin via biotechnological and chemical routes had been studied and introduced in order to find new alternatives for substitution of limited natural vanillin from vanilla orchid.

Nowadays, lignin isolated from woody or lignocellulosic material is among of propitious feedstock used to produce synthetic vanillin either by chemical or biotechnological methods. However, both of the methods come with their own pros and contras and till now the most optimal alternative to produce good quality and high quantity of vanillin is still being studied by the researchers. The production of vanillin via biotechnological route is considered clean and safer compared to chemical route because the processes involved were free from the consumption of hazardous chemical substances. Though, this method requires high investment and strict control of microorganism and enzymes. In contrast, vanillin can be produced cheaply by chemical routes, but the safety quality of the vanillin produced might be lower due to the use of hazardous chemicals.

Thus, in order to improve the quantity and reduced the harmful effect of synthetic vanillin produced by chemical method, an alternative involving the utilization of safer and renewable resource as the starting material and less hazardous chemical had been studied in this research. Meanwhile, to maximize the yield of vanillin via oxidation of lignin, selection of the right lignin source, chemicals for extraction and oxidation as well as the severity of the oxidation process are very important as these factors give the significant effects to the vanillin yield. Severity of oxidation is mainly referred to the oxidation conditions where the conversion of lignin into vanillin occurred. Thus, optimization study of the oxidation conditions had been conducted in this study in order to produce maximum vanillin yield.

In terms of feedstock, most of the developing countries such as Malaysia generate excessive amount of agricultural waste every year which remained unutilized and lead to an environmental issues. Hence, the utilization of these wastes as the renewable resources for lignin to produce synthetic vanillin is considered as the best step as it encourages a greener production through the consumption of ready available, abundant, cheap, safe and natural agriculture wastes to produce the value added products such as vanillin.

1.3 Objectives of Study

In an attempt to achieve a maximum yield of vanillin, the raw material selection, characteristic of lignin as an intermediate product, and the effects of oxidation conditions are significantly studied. Therefore, the research embarks on the following objectives:

(i) To screen and study the characteristics of lignin over different type of biomass in order to determine the suitable lignin for vanillin production.

(ii) To produce and verify the vanillin derived via alkaline nitrobenzene oxidation of lignin.

(iii) To optimize the lignin oxidation conditions via response surface methodology (RSM).

1.4 Scope of Study

In order to achieve the objectives, the scopes of the study are listed and described by the following stage;

a) Raw material screening

(i) Raw material preparation;

Oil palm empty fruit bunch (OPEFB) fiber, coconut husk and kenaf fiber were physically prepared prior to the pretreatment by Soxhlet extraction. The chemical compositions of the samples are determined and the contents of lignin are compared.

(ii) Lignin extraction;

Lignins were extracted from the treated OPEFB fiber, coconut husk and kenaf fiber via alkaline hydrolysis. Hydrochloric acid (HCl), sulphuric acid (H_2SO_4) and phosphoric acid (H_3PO_4) are screened for lignin precipitation process.

(iii) Lignin characterization;

The characteristics of all lignins were studied by Fourier transform infrared (FTIR) spectroscopy, proton nuclear magnetic resonance (H^1 NMR), ultraviolet-visible spectroscopy (UV-Vis) and thermogravimetric analyzer (TGA).

b) Vanillin production

(i) Derivation of vanillin via lignin oxidation;

The extracted lignin was oxidized by nitrobenzene in alkaline medium to derive vanillin.

(ii) Identification and quantification of vanillin in lignin oxidized mixture;

The vanillin compound in the oxidized mixture was identified and quantified by high performance liquid chromatography (HPLC) and gas chromatography -flame ionization detector (GC-FID).

(iii) Crystallization and verification of vanillin;

Vanillin was purified by crystallization process. The crystalline-formed vanillin was characterized by FTIR and H^1NMR for structural verification.

c) Optimization study

(i) Optimization of the operational conditions;

The oxidation conditions which are oxidation temperature (120-160 ºC), oxidation time (2-4 hours) and volume of nitrobenzene (0.2-0.6 mL) were optimized by RSM to obtain the maximum vanillin yield.

(ii) Comparative study;

The yield of vanillin was compared between coconut husk, OPEFB fiber and kenaf fiber lignin at the optimized oxidation conditions.

1.5 Significance of Study

Utilization of vanillin is gradually expanding over the years not only limited to flavor and fragrance industries but also as intermediate chemicals in pharmaceutical products manufacturing. Accordingly, the continuous production of this compound is being looked-for in order to satisfy the market demand for the vanillin based products. This study has proposed and provides another alternative for vanillin derivation. The proposed method also suggests a greener approach of production process through the consumption of cheap, abundant, safe and renewable biomass feedstock as well as less harmful chemical substance. Hence, this study is important to the environmental and economic concerns as it presents a continuous process that uses renewable sources is more desirable. In addition, the effect of operational conditions and optimization study had been examined. This research might lead towards an advancement of knowledge in future production of vanillin in order to improve the poor vanillin yield problem.

Basically, the implementation of this research involved a chemical engineering multidisciplinary which not only focused on alternative for chemical derivation of vanillin from different source but it also covered the engineering branch whereas the operations and optimization were taken into consideration. Accordingly the influences of operating parameters towards product yield were studied in order to boost up the production. For future view, the study regarding on the effect of operating parameter and the optimized operating conditions could be used as a point of references for advance engineering discipline such as reactor design and process modeling, particularly in vanillin-related industries.

1.6 Thesis Outline

There are 5 chapters in this thesis and each chapter describes the sequence of this research. Chapter 1 presents brief overview of vanillin production via natural, biological and chemical methods. This chapter also presents the problem statement, objectives, scopes and the significance of the study that have been conducted.

Chapter 2 elaborates the deeper knowledge on vanillin production methods either by natural, biological or chemical routes with their respective pros and contras. This chapter also explains a detail information concerning on vanillin, lignin as the intermediate product and biomass sources as the raw material used in this study. A part of that, the previous study about vanillin production done by researches were also reviewed.

Chapter 3 covers the specific method used in the study. This chapter described the detail experimental procedures involving in raw material screening and preparation, extraction of lignin, characterization of lignin, lignin oxidation to derive vanillin, analysis of vanillin and optimization study by RSM.

Chapter 4 presents all of the results obtained in the study which cover the results of raw material screening, chemical composition of the raw material, fractional yield of lignin extracted, the characteristic of lignin, detection and quantification of vanillin, structural verification of vanillin, optimization vanillin oxidation conditions and finally the comparative results of vanillin yield among different biomass employing the optimized conditions. The corresponding discussions and justifications for the results also have been made accordingly.

Chapter 5 concludes the overall findings achieved in this study. The recommendations for future research were also discussed in this chapter to improve the structure of the study as well as the findings.

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