

UNIVERSITI PUTRA MALAYSIA

ALKALINE HYDROLYSIS OF OIL PALM EMPTY FRUIT BUNCH FOR ENHANCED FERULIC ACID RELEASE

FAIROUZ JAHAAN BINTI MOHD AANIFAH

FBSB 2013 24



ALKALINE HYDROLYSIS OF OIL PALM EMPTY FRUIT BUNCH FOR ENHANCED FERULIC ACID RELEASE



FAIROUZ JAHAAN BINTI MOHD AANIFAH

MASTER OF SCIENCE UNIVERSITI PUTRA MALAYSIA

2013



ALKALINE HYDROLYSIS OF OIL PALM EMPTY FRUIT BUNCH FOR ENHANCED FERULIC ACID RELEASE



FAIROUZ JAHAAN BINTI MOHD AANIFAH

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

July 2013

COPYRIGHT

All material contained within the thesis, including without limitation text, logos, icons, photographs and all other artwork, is copyright material of Universiti Putra Malaysia unless otherwise stated. Use may be made of any material contained within the thesis for non-commercial purposes from the copyright holder. Commercial use of material may only be made with the express, prior, written permission of Universiti Putra Malaysia.

Copyright © Universiti Putra Malaysia





G

DEDICATED TO MY PRECIOUS PARENTS AND BROTHERS

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for degree of Master of Science

ALKALINE HYDROLYSIS OF OIL PALM EMPTY FRUIT BUNCH FOR ENHANCED FERULIC ACID RELEASE

By

FAIROUZ JAHAAN BINTI MOHD AANIFAH

July 2013

Chairman : Professor Suraini Abd-Aziz, PhD

Faculty

Biotechnology and Biomolecular Sciences

Malaysia is the second largest producer and biggest exporter of palm oil worldwide. Owing to this fact, the biomass produced is in abundance, namely; oil palm empty fruit bunches (OPEFB), mesocarp fibres, palm kernel shells, oil palm fronds and trunks. Among these, OPEFB is the hugely generated lignocellulosic waste. Accordingly, the National Biomass Strategy 2020 was developed to create wealth through biofuels and bio-based chemicals production from excess biomass. Ferulic acid (FA), a hydroxycinnamic acid, exists in various agricultural residues such as maize bran, corn cob, wheat straw and also OPEFB. FA serves as a raw material for production of pharmaceuticals, cosmetics and flavours. It is mainly utilized in flavour synthesis, especially vanilla flavour, due to its property as a precursor for vanillin, the key ingredient of vanilla aroma. FA from nature provides biovanillin through biotechnological route. In order to obtain FA from the OPEFB fibres for biovanillin production, this study was conducted to examine the methods and conditions of FA release from OPEFB fibres through alkaline hydrolysis. The selected treatment strategy (Treatment B1) that involved autoclaving OPEFB fibres

(120°C, 3 hours) followed by alkaline hydrolysis (90°C, 3 hours, agitated at 120 rpm in water bath shaker), showed significant yield of FA release from OPEFB fibres. Alkaline hydrolysis using 0.5 to 5.0% (w/v) of NaOH, KOH and K₂CO₃ gave both 2.0% (w/v) KOH and NaOH as the best alkalis concentration for better FA release from OPEFB fibres compared to other alkali concentrations. The addition of 98 µL sodium bisulfite (NaHSO₃) to KOH treatment yielded 4.23 mg/L higher FA compared to the hydrolysis without NaHSO₃ as it reduced repolymerization and oxidation of FA. It was also observed that the FA release was affected by the different reaction times at high and ambient temperature during alkaline hydrolysis. FA was observed to decrease due to longer hydrolysis time at high temperature and treatment at 37°C for 16 hours yielded only an average of 24 to 42 mg/L FA only. About 66.18 \pm 3.24 mg/L and 56.94 \pm 3.52 mg/L FA was released using 2.0% (w/v) NaOH and KOH, respectively through Treatment B1 (with the addition of NaHSO₃) from the esterified FA in the OPEFB fibres. Fourier transform infrared (FTIR) analysis showed evidence of decrease in aromatic groups, lignin and ester linkages stretching thus showed that FA, the lignin monomer has been released from OPEFB fibres. It was suggested that mild alkaline hydrolysis was sufficient in solubilising FA that is esterified in the OPEFB lignin and hemicellulose.

iii

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

HIDROLISIS BERALKALI TANDAN KOSONG BUAH KELAPA SAWIT BAGI MENINGKATKAN PENGEKSTRAKAN ASID FERULIK

Oleh

FAIROUZ JAHAAN BINTI MOHD AANIFAH

Julai 2013

Pengerusi

Fakulti

Profesor Suraini Abd-Aziz, PhD

: Bioteknologi dan Sains Biomolekul

Malaysia merupakan negara penghasil kedua terbesar dan pengeksport utama minyak kelapa sawit di dunia. Berdasarkan fakta ini, banyak biomas yang terhasil seperti tandan kosong buah kelapa sawit (OPEFB), sabut mesokap, tempurung isirong sawit, pelepah sawit dan batang sawit. OPEFB merupakan penyumbang utama kepada kuantiti sisa lignoselulosik yang terhasil. Oleh yang demikian, Strategi Biomas Kebangsaan 2020 dibentuk untuk membina kekayaan melalui penghasilan biotenaga dan biokimia daripada biomas. Asid ferulik (FA) adalah asid hidroksisinamik yang wujud dalam pelbagai sisa pertanian seperti dedak jagung, tongkol jagung, jerami gandum dan juga OPEFB. FA bertindak sebagai bahan mentah dalam pembuatan bahan farmaseutikal, kosmetik dan perisa. FA digunakan secara meluas dalam sintesis perisa, terutamanya perisa vanila kerana sifatnya sebagai prekursor kepada vanilin, sebatian penting yang memberi aroma vanila. FA yang diekstrak dari sumber semulajadi boleh menghasilkan biovanilin melalui kaedah bioteknologi. Kajian ini bertujuan menyelidik kaedah dan keadaan pengekstrakan FA daripada sabut OPEFB melalui hidrolisis beralkali untuk dijadikan bahan mentah bagi penghasilan biovanillin. Strategi rawatan terpilih (Rawatan B1) yang terdiri daripada mengautoklaf sabut OPEFB (120°C, 3 jam) dan diikuti dengan hidrolisis beralkali (90°C, 3 jam, dicampurkan pada 120 rpm dalam penggoncang rendaman air), menunjukkan penghasilan FA yang bagus daripada sabut OPEFB. Hidrolisis beralkali menggunakan NaOH, KOH dan K₂CO₃. dengan kepekatan 0.5 hingga 5.0% (berat/isipadu) menunjukkan bahawa 2.0% (berat/isipadu) KOH dan NaOH adalah kepekatan alkali yang dapat mengekstrak kuantiti FA yang lebih tinggi berbanding alkali dan kepekatan yang lain. Tambahan 98 µL natrium bisulfite (NaHSO₃) dalam rawatan menggunakan KOH, menyumbang kepada peningkatan FA sebanyak 4.23 mg/L berbanding hidrolisis tanpa NaHSO₃ kerana sebatian ini bertindak mengurangkan kadar polimerisasi semula dan oksidasi FA. Selain itu, pengekstrakan FA juga dipengaruhi oleh jangka masa rawatan pada suhu tinggi dan ambien. Kajian menunjukkan konsentrasi FA berkurangan disebabkan oleh masa hidrolisis yang lama pada suhu tinggi dan hidrolisis pada 37°C untuk 16 jam menghasilkan 24 hingga 42 mg/L FA sahaja. Melalui Rawatan B1, sebanyak 66.18 ± 3.24 mg/L dan 56.94 ± 3.52 mg/L FA berjaya diekstrak menggunakan 2.0% (berat/isipadu) NaOH dan KOH masingmasing (dengan penambahan NaHSO₃), daripada FA yang terester dalam OPEFB. Analisis infra merah transformasi Fourier (FTIR) menunjukkan pengurangan regangan pada kumpulan aromatik, lignin dan ikatan ester, sekaligus membuktikan pembebasan monomer lignin (FA) dari sabut OPEFB. Dengan itu, dicadangkan bahawa keadaan hidrolisis beralkali yang sederhana mencukupi untuk membebaskan FA yang teresterifikasi dalam lignin dan hemiselulose OPEFB.

ACKNOWLEDGEMENTS

First and foremost, glory and praise to The Almighty Allah s.w.t for His blessings. Alhamdulillah, I am able to complete this research work to gain my Master of Science (Environmental Biotechnology) degree.

My heartfelt gratitude to my main project supervisor, Professor Dr. Suraini Abd. Aziz to have belief in me and for this knowledgeable opportunity. I greatly appreciate her dedicated guidance, kind assistance during thesis and journal paper writing, endless support and care throughout this study. My sincere thanks to my co-supervisors, Dr. Phang Lai Yee and Dr. Helmi Wasoh for their guidance and support during experimental work.

I would like to extend my thanks to the Assistant Science Officer, Mr. Rosli Aslim in Central Teaching Laboratory, Faculty of Biotechnology and Biomolecular Sciences, UPM. My thanks also goes to the lab assistances, Mrs. Renuga, Mrs. Aluyah, Mrs. Norazlina and Mrs. Noraishah for their kind help.

I am thankful to my fellow lab mates; Mr. Rozaimi, Mrs. Zuraidah, Miss Ezyana, Miss Siren, Mr. Faizal, Mrs. Elmy Nahida and Mrs. Ida Amalina for their best assistance during experimental work and writing. Special thanks to Mr. Shankar Ramanathan from UTM. My warm gratitude to Professor Dr. Mohd Ali Hassan, leader of Environmental Biotechnology Research Group, UPM and all the group members for knowledge sharing and concern shown.

Last but not least, I feel blessed to be surrounded by supportive family members; my father: Mr. Mohd Aanifah, my mother: Mrs. Zainab Bibi and my brothers: Muhammed Rafeeqh and Muzzammil. Thanks for the endless love and care. I certify that a Thesis Examination Committee has met on 29th July 2013 to conduct the final examination of Fairouz Jahaan Bt. Mohd Aanifah on her thesis entitled "Alkaline Hydrolysis of Oil Palm Empty Fruit Bunch for Enhanced Ferulic Acid Release" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

Norazizah Shafee, PhD

Associate Professor Faculty of Biotechnology and Biomolecular Sciences Universiti Putra Malaysia (Chairman)

Syahida Ahmad, PhD

Senior Lecturer Faculty of Biotechnology and Biomolecular Sciences Universiti Putra Malaysia (Internal Examiner)

Salmiaton Ali, PhD

Associate Professor Faculty of Engineering Universiti Putra Malaysia (Internal Examiner)

Madihah Md Salleh, PhD

Associate Professor Universiti Teknologi Malaysia Malaysia (External Examiner)

NORITAH OMAR, PhD

Associate Professor and Deputy Dean School of Graduate Studies Universiti Putra Malaysia

Date: 19 September 2013

The thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Suraini Abd. Aziz, PhD

Professor Faculty of Biotechnology and Biomolecular Sciences Universiti Putra Malaysia (Chairman)

Phang Lai Yee, PhD

Senior Lecturer Faculty of Biotechnology and Biomolecular Sciences Universiti Putra Malaysia (Member)

Helmi Wasoh @ Mohamad Isa, PhD

Senior Lecturer Faculty of Biotechnology and Biomolecular Sciences Universiti Putra Malaysia (Member)

BUJANG BIN KIM HUAT, PhD

Professor and Dean School of Graduate Studies Universiti Putra Malaysia

Date:

DECLARATION

Declaration by graduate student

I hereby confirm that:

- this thesis is my original work
- quotations, illustrations and citations have been duly referenced
- this thesis has not been submitted previously or concurrently for any other degree at any other institutions
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia
- written permission must be obtained from supervisor and Deputy Vice-Chancellor (Research and Innovation) before thesis is published in book form
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity was upheld as according to Rule 59 in Rules 2003 (Revision 2012-2013). The thesis has undergone plagiarism detection software.

Signature:	Date:
Name and Matric No.:	

Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in Rule 41 in Rules 2003 (Revision 2012-2013) were adhered to.

Signature : Name of Chairman of Supervisory Committee:	
Signature : Name of Member of Supervisory Committee:	
Signature : Name of Member of Supervisory Committee:	

TABLE OF CONTENTS

Page

49

ABSTRACT	ii
ABSTRAK	iv
ACKNOWLEDGEMENTS	vi
APPROVAL	vii
DECLARATION	ix
LIST OF TABLES	xiv
LIST OF FIGURES	XV
LIST OF APPENDICES	xvi
LIST OF ABBREVIATIONS	xvii

CHAPTER

1	INTRODUCTION –	
	1.1 Background	1
	1.2 Research Problem	3
	1.3 Objective	5
	1.4 Scope of Work	5
2	LITERATURE REVIEW	
	2.1 Lignocellulosic Biomass	6
	2.2 Oil Palm Biomass	10
	2.3 Oil Palm Empty Fruit Bunch	14
	2.4 Lignin and Hemicellulose of Oil Palm Empty Fruit Bunch	16
	2.4.1 Lignin	16
	2.4.2 Hemicellulose	27
	2.5 Ferulic Acid	28
	2.5.1 Applications of Ferulic Acid	31
	2.6 Modes of Vanillin Production	33
	2.6.1 Chemical Pathway of Vanillin Production	33
	2.6.2 Biological Pathway of Biovanillin Production	34
	2.7 Ferulic Acid Release from Oil Palm Empty Fruit Bunch	36
	2.7.1 Physical Treatment	38
	2.7.2 Biological Treatment	40
	2.7.3 Chemical Treatment	41
	2.8 Concluding Remarks	44
	Ū.	
3	MATERIALS AND METHODS	
	3.1 Oil Palm Empty Fruit Bunch (OPEFB)	46
	3.2 Chemical and Reagents	46
	3.3 Phenolic Acids Standard Preparation	47
	3.4 Flow Chart of Experimental Design	48

Bunch Fibres for Ferulic Acid Release 3.6 Effects of Chemical and Physical Parameters on Ferulic Acid 50 Release

3.5 Physico-Chemical Treatments of Oil Palm Empty Fruit

	3.6.1 Effects of Different Types of Alkalis and Their	50
	Concentrations	50
	3.6.2 Effects of Different Sodium Bisulfite Concentrations,	50
	Reaction Times and Ambient Temperature on Ferulic	
	2.7 Drofiling of Dhanolia Agida Dalaasa	51
	3.7 Profiling of Phenolic Acids Release	51
	3.8 Analytical Methods	52 52
	5.8.1 Sample Preparations prior to High Performance Liquid	52
	2.8.2 Earrylia Acid Determination	52
	2.8.2 Characterisation of Untrasted and Trasted Oil Dalm	52
	5.6.5 Characterisation of Ontreated and Treated On Pann Empty Emit Runch Eibros	55
	2.8.4 Equation Transform Infrared Analysis of Oil Dalm	57
	5.8.4 Fourier Transform Infrared Analysis of Off Palm	57
	2.8.5 Statistical Analysia	57
	5.8.5 Statistical Analysis	57
4	RESULTS AND DISCUSSION	
_	4.1 Effects of Different Treatment Strategies on Oil Palm Empty	58
	Fruit Bunch Fibres for Enhanced Ferulic Acid Release	
	4.1.1 Treatment A	59
	4.1.2 Treatments B1 and B2	61
	4.1.3 Treatment C	63
	4.1.4 Overall Comparison of the Treatment Strategies	64
	4.2 Effects of Different Alkalis and Their Concentrations on	68
	Ferulic Acid Release	
	4.2.1 Sodium Hydroxide Hydrolysis	69
	4.2.2 Potassium Hydroxide Hydrolysis	71
	4.2.3 Potassium Carbonate Hydrolysis	73
	4.2.4 Overall Comparison of Effects of Different Alkalis and	75
	Their Concentrations on Ferulic Acid Release	
	4.3 Effects of Different Sodium Bisulfite Concentrations.	77
	Reaction Times and Ambient Temperature on Ferulic Acid	
	Release	
	4.3.1 Effects of Different Sodium Bisulfite Concentrations	77
	on Ferulic Acid Release	
	4.3.2 Effects of Reaction Times on Ferulic Acid Release	80
	4.3.3 Effects of Ambient Temperature on Ferulic Acid	82
	Release	
	4.4 Characterisation of Untreated; Treated Oil Palm Empty Fruit	85
	Bunch and Its Alkaline Hydrolysate	
	4.4.1 Fourier Transform Infrared Analysis	85
	4.4.2 Lignocellulosic Contents of Untreated and Treated Oil	88
	Palm Empty Fruit Bunch Fibres	
	4.4.3 Profiling of Phenolic Acids in Oil Palm Empty Fruit	93
	Bunch Alkaline Hydrolysate	
_		
5	CUNCLUSIONS AND RECOMMENDATIONS FOR	
	ГОТОКЕ КЕЗЕАКОП 5-1 Summary	00
	5.2 Conclusions	70 100
		100

5.3 Recommendations for Future Work	101
REFERENCES	103
APPENDICES	122
BIODATA OF STUDENT	137
LIST OF PUBLICATIONS	138

