

UNIVERSITI MALAYA

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Name of Degree: **MASTER OF SCIENCE**

Title of Project Paper/Research Report/Dissertation/Thesis ("this Work"):

ENZYMATIC ASSISTED AQUEOUS OIL EXTRACTION FROM *JATROPHA CURCAS*

Field of Study: **OLEOCHEMISTRY**

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ABSTRACT

Enzymatic assisted aqueous oil extraction (EAAOE) of *Jatropha curcas* was investigated in this research. The presence of Alcalase[®] 2.4 L and Celluclast[®] 1.5 L facilitates the recovery of the oil up to 58%. Parameters involved in EAAOE such as selection and concentration of enzymes, ratio of *Jatropha curcas* seeds: water (mass/volume), pH, temperature, duration of incubation time, effect of ultrasonication, and oil recovery from oil-water mixture were optimised. The highest oil recovery (58%, m/m) was obtained with 6 mL/1g water/oil, 6% (m/m) enzyme, temperature at 50°C, seed particle size less than 1 mm, pH 5.0 and pH 8.0 for Celluclast[®] and Alcalase[®] respectively, incubation for 4 hours (Alcalase[®]) and 8 hours (Celluclast). In order to enhance oil recovery, centrifugation was conducted at 4°C and 8000 rpm, coupled with hexane-aided separation and subsequent 2 hours freezing of the aqueous layer. The alkaline protease was found to be more efficient than cellulase. In addition, the step-wise addition of enzyme studies was carried out to investigate the sequential enzymatic mechanism on the oil extraction. A comparison between the one-step and step-wise addition of Alcalase[®] 2.4 L and Celluclast[®] 1.5 L at the same concentration found that the cellulosic materials were broken down first and followed by protein hydrolysis to facilitate oil releasing and extraction process. The present study had also isolated and elucidated toxic substances, phorbol esters which are responsible for the toxicity of *Jatropha curcas* to animals and humans, by using high performance liquid chromatography and liquid chromatography - mass spectrometry. The result demonstrated that the phorbol ester content of *Jatropha* oil extracted by the EAAOE method were 0.2% and 0.3% for enzyme Alcalase[®] and Celluclast[®], respectively. The oil extracted from EAAOE exhibited good physicochemical properties and is useful as a biodiesel feedstock in industrial applications.

The oil exhibited iodine values of 96.5 and 95.1, peroxide values of 1.83 meq/kg and 1.77 meq/kg, saponification values of 207.9 and 205.1, density values of 0.9130 g/cm³ and 0.9134 g/cm³, moisture content of 0.11% and free fatty acid (FFA) content (% as oleic acid) of 2.15 and 2.30 for enzyme Alcalase[®] and Celluclast[®], respectively. Oleic and linoleic acid were detected as the dominant unsaturated fatty acids which was about 77%, while stearic and palmitic were the highest saturated fatty acids content in *Jatropha* oil with 23%. Therefore, *Jatropha* oil can be categorised as oleic–linoleic oil. The favourable method for *Jatropha curcas* oil with high FFA transesterification was direct esterification of the FFA prior to transesterification reaction. In comparison with double-stage transesterification process, it was found that the former method managed to achieve higher total methyl ester (62.1% m/m), near 30% greater than the latter transesterification method. In addition, acid pre-treatment in esterification has shortened the total reaction duration from 6 hours 30 minutes to 4 hours 50 minutes. The analysis of *Jatropha* oil methyl ester by this method consist of FFA content of 0.26%, moisture content of 0.043%, density value of 0.8709 g/cm³ at 25°C and pour point of 3°C. Therefore, the findings showed that the quality analysis of *Jatropha* oil methyl ester was well within the biodiesel specifications, namely, American standard ASTM D6751 and European standard EN 14214.

ABSTRAK

Kaedah pengekstrakan minyak secara akueus dengan kehadiran enzim (EAAOE) telah dijalankan dalam penyelidikan ini. Kehadiran enzim Alcalase[®] 2.4 L dan Celluclast[®] 1.5 L telah meningkatkan pemulihan minyak sehingga 58%. Pembolehubah yang terlibat dalam EAAOE seperti pemilihan dan kepekatan enzim, nisbah biji kacang *Jatropha curcas*: air (jisim/isipadu), pH, suhu, tempoh masa pengeraman, kesan ultrasonikasi, dan kaedah pemulihan semula minyak daripada campuran minyak - air yang telah dioptimumkan. Hasil pemulihan minyak yang tertinggi (58% m/m) telah diperolehi, dengan 6 mL/1g air / minyak; 6% (m/m) enzim, suhu pada 50°C; benih saiz zarah kurang daripada 1 mm, pH 5.0 dan pH 8.0 untuk Celluclast[®] dan Alcalase[®]; dieram selama 4 jam (Alcalase[®]) dan 8 jam (Celluclast[®]). Dalam usaha untuk meningkatkan pemulihan minyak, proses pengemparan telah dijalankan pada 4°C dan 8000 rpm, ditambah pula dengan sedikit larutan heksana dan 2 jam pembekuan lapisan akueus. Hasil kajian mendapati protease alkali lebih cekap daripada sellulase. Di samping itu, penambahan enzim secara berperingkat telah dilakukan untuk mengkaji mekanisme enzim. Perbandingan antara kaedah penambahan enzim Alcalase[®] 2.4 L dan Celluclast[®] 1.5 L secara tunggal dan berperingkat pada kepekatan yang sama telah mendapati bahawa bahan sellulosa harus diuraikan terlebih dahulu diikuti oleh proses hidrolisis protein bagi memudahkan pengeluaran minyak dan proses pengekstrakan. Dalam kajian ini juga, bahan toksik, ester phorbol yang bertanggungjawab bagi ketoksikan dalam *Jatropha curcas* kepada haiwan dan manusia, telah diasingkan dan dinilai dengan menggunakan kromatografi cecair prestasi tinggi dan kromatografi cecair - spektrometri jisim. Hasil kajian menunjukkan bahawa kandungan ester phorbol minyak *Jatropha* diekstrak dengan kaedah EAAOE terdiri daripada, masing-masing 0.2% dan 0.3% bagi enzim Alcalase[®] dan Celluclast[®].

Minyak yang diekstrak daripada EAAOE mempamerkan ciri fisikokimia yang baik dan berguna sebagai bahan mentah biodiesel dalam aplikasi perindustrian. Keputusan menunjukkan nilai iodin adalah 96.5 dan 95.1, nilai peroksida adalah 1.83 meq/kg dan 1.77 meq/kg, nilai saponifikasi adalah 207.9 dan 205.1, nilai ketumpatan adalah 0.9130 g/cm³ dan 0.9134 g/cm³, kandungan lembapan adalah 0.11% dan kandungan asid lemak bebas (% asid oleik) adalah 2.15 dan 2.30 untuk enzim Alcalase[®] dan Celluclast[®]. Asid oleik dan linoleik telah dikesan sebagai asid lemak tak tepu yang dominan kira-kira 77.0% dan 77.8% untuk enzim Alcalase[®] dan Celluclast[®] manakala stearik dan palmitic merupakan kandungan asid lemak tepu yang tertinggi dalam minyak *Jatropha* dengan 23.0% dan 22.2% bagi enzim Alcalase[®] dan Celluclast[®] dalam EAAOE. Oleh itu, minyak *Jatropha* boleh dikategorikan sebagai minyak oleik-linoleik. Kaedah pengesteran terus sebagai rawatan sebelum tindak balas transesterifikasi telah dipilih untuk dijalankan bagi minyak yang mengandungi asid lemak bebas yang tinggi (FFA). Dalam perbandingan proses pra-rawatan asid dengan proses transesterifikasi secara dua peringkat telah mendapati bahawa kaedah pertama berjaya mencapai jumlah ester metil yang lebih tinggi (62.1% m/m), 30% lebih tinggi daripada kaedah transesterifikasi kedua. Di samping itu, kaedah pra-rawatan asid dalam proses pengesteran telah memendekkan tempoh masa tindak balas daripada 6 jam 30 minit kepada 4 jam 50 minit. Analisis ester metil daripada minyak *Jatropha* melalui kaedah ini mendapati kandungan asid lemak bebas (FFA) adalah sebanyak 0.26%, kandungan kelembapan adalah 0.043%, nilai ketumpatan adalah 0.8709 g/cm³ pada 25°C dan takat tuangan adalah 3°C. Oleh itu, hasil analisis menunjukkan bahawa kualiti ester metil daripada minyak *Jatropha* adalah baik dan menepati spesifikasi yang dibenarkan oleh piawaian Amerika ASTM D6751 dan piawaian Eropah EN 14214.

ACKNOWLEDGEMENTS

Alhamdulillah, all praises to Allah for the strengths and His blessing in completing this thesis. First and foremost, I want to express my heartfelt gratitude especially to my supervisor, Dr Cheng Sit Foon, for her constant supervision, constructive criticisms, patience and support in academic and in real life. I am indebted to her guidance and advices that inspired me to see things positively. I would like to thank my co-supervisor, Prof Chuah Cheng Hock for his continuous support, guidance, suggestion and advice. I am very much grateful for the knowledge he has imparted to me for the improvement of this work. Without their supervision, this course would not been successfully completed. My sincere and special appreciation to Y. Bhg. Tan Sri Augustine Ong Soon Hock, the founder of Unit of Research on Lipids (URL), University of Malaya for his guidance, knowledge and support.

I would like to take this opportunity to convey my gratitude to University of Malaya for the facilities and grant offered. I would also like to acknowledge Universiti Tun Hussein Onn Malaysia and Ministry of Higher Education Malaysia with gratitude for granting me the scholarship to pursue my M.Sc. studies.

I would like to thank the staff of Department of Chemistry and Department of Biochemistry, University of Malaya who had rendered their kind assistance in one way or another. To my fellow colleagues and friends in Unit of Research on Lipids (URL); Gouk Shiou Wah, Ang Khoon Poh, Ang Chun Hui, Wong Siew Pui, Alinda Samsuri, Mohd Nor Latif and Jason Tan, my sincere thanks for all their encouragements and helpful discussion. Their support and help always give me motivation to finish the study.

Last but not least, my special gratitude and appreciation to my dad, Wazir bin Haron, my mum, Salabiah bt Serbaini, my siblings, Shairah, Anwar Faiz and Akmal Farid, my best friends, Iman, Fatimah and Noorbaiti for their patience, tolerance and support. When I felt down, their love will always give me strength and extra push in times of difficulties.

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LIST OF ABBREVIATIONS

ASTM	American Society of Testing and Materials
A. R.	analytical reagent
Bhd.	Berhad
BSTFA	N,N-Bis (trimethylsilyl)trifluoroacetamide
CPO	crude palm oil
°C	degree Celcius
CDCl ₃	deuteriochloroform
D	diameter
DAG	diacylglycerol
DV	density value
AU-A/g	enzyme activity of alkaline
EGU/g	enzyme activity of cellulase
EAAOE	enzymatic assisted aqueous oil extraction
EN	European Standard
F	correction factor
FAC	fatty acid composition
FFA	free fatty acids
FAME	fatty acid methyl esters
FID	flame ionisation detector
g	gram
GC	gas chromatography
GC-FID	gas chromatography-flame ionisation detector
GC-MS	gas chromatography-mass spectroscopy
HCl	hydrochloric acid

HU	hemagglutinating units
HIV	human immunodeficiency virus
HPLC	high performance liquid chromatography
ID	internal diameter
IS	internal standard
IV	iodine value
JO	<i>Jatropha</i> oil
JOME	<i>Jatropha</i> oil methyl ester
kg	kilogram
K ₂ Cr ₂ O ₇	potassium dichromate
KHP	potassium hydrogen phthalate
KOH	potassium hydroxide
L	litre
LCMS	liquid chromatography mass spectrometry
ME	methyl esters
mL	millilitre
mg	milligram
min	minutes
MAG	monoacylglycerol
NaOH	sodium hydroxide
NaCl	sodium chloride
Na ₂ SO ₄	sodium sulphate
Na ₂ S ₂ O	sodium thiosulphate
H ₂ SO ₄	sulphuric acid
PDBu	4β-phorbol-12,13-dibutyrate
PMA	phorbol 12-myristate 13-acetate

PORIM	Palm Oil Research Institute Malaysia
PV	peroxide value
RBD	refined bleached and deodorised
R _f	retention factor
SG	specific gravity
SV	saponification value
TPA	4β-12-O-tetradecanoylphorbol-13-acetate
TLC	thin layer chromatography
TAG	triacylglycerols
VOCs	volatile organic compounds

LIST OF APPENDICES

Appendix

- A Product data sheet of Alcalase[®] 2.4 L from Novozymes
- B Product data sheet of Celluclast[®] 1.5 L from Novozymes