

ESTERIFICATION OF PALM OIL IN CROSSLINKED
SOLVENT BY IMMORTALIZED *Aspergillus niger* LI-PASE

NIP. LOCAL PRINT. MACHINERY

FAVOURABLE FOR TECHNOLOGY

COLLEGE OF ENGINEERING, DABLA TECNICO, MILANSHAH

2005

Perustakooan

Kolej Universiti Sains Dan Teknologi Malaysia (KUSTEM)

1100036824

LP 3 | EST | 2005



1100036824

Transesterification of palm olein in organic solvent by an immobilized (*Aspergillus niger*) lipase / Nur Asiah Hashim.



PERPUSTAKAAN

PERPUSTAKAAN
KOLEJ UNIVERSITI SAINS & TEKNOLOGI MALAYSIA
21030 KUALA TERENGGANU

1100036824

Lihat sebelah

HAK MILIK
PERPUSTAKAAN KUSTEM

**TRANSESTERIFICATION OF PALM OLEIN IN ORGANIC SOLVENTS BY AN
IMMOBILIZED *Aspergillus niger* LIPASE**

By

Nur Asiah binti Hashim

Research Report submitted in partial fulfillment of
the requirements for the degree of
Bachelor of Science (Biological Sciences)

Department of Biological Sciences
Faculty of Science and Technology
KOLEJ UNIVERSITI SAINS DAN TEKNOLOGI MALAYSIA
2005

This project should be cited as:

Nur Asiah, H. 2005. Transesterification of palm olein in organic solvents by an immobilized *Aspergillus niger* lipase. Undergraduate thesis, Bachelor of Science in Biological Sciences, Faculty of Science and Technology, Kolej Universiti Sains dan Teknologi Malaysia, Terengganu. 56p.

No part of this project report may be produced by any mechanical, photographic, or electronic process, or in the form of phonographic recording, nor may be it be stored in a retrieval system, transmitted, or otherwise copied for public or private use, without written permission from the author and the supervisor (s) of the project.



**JABATAN SAINS BIOLOGI
FAKULTI SAINS DAN TEKNOLOGI
KOLEJ UNIVERSITI SAINS DAN TEKNOLOGI MALAYSIA**

**PENGAKUAN DAN PENGESAHAN LAPORAN
PROJEK PENYELIDIKAN I DAN II**

Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk: TRANSESTERIFICATION OF PALM OLEIN IN ORGANIC SOLVENTS BY AN IMMOBILIZED *Aspergillus niger* LIPASE oleh NUR ASIAH BT. HASHIM, No. Matrik UK 6600 telah diperiksa dan semua pembetulan yang disarankan telah dilakukan. Laporan ini dikemukakan kepada Jabatan Sains Biologi sebagai memenuhi sebahagian daripada keperluan memperolehi IJAZAH SARJANA MUDA SAINS—SAINS BIOLOGI Fakulti Sains dan Teknologi, Kolej Universiti Sains dan Teknologi Malaysia.

Disahkan oleh:

Penyelia Utama

Nama: **HAZLINA AHAMAD ZAKERI**
Pensyarah
Cop Rasmi: **Jabatan Sains Biologi**
Fakulti Sains dan Teknologi
Kolej Universiti Sains dan Teknologi Malaysia (KUSTEM)
Mengabang Telipot
21030 Kuala Terengganu, Terengganu Darul Iman.

Tarikh: **7/4/2005**

Penyelia Kedua (jika ada)

Nama:

Cop Rasmi

Tarikh:

Ketua Jabatan Sains Biologi

Nama: **PROF. MADYA DR. NAKISAH BT. MAT AMIN**
Ketua
Cop Rasmi: **Jabatan Sains Biologi**
Fakulti Sains dan Teknologi
Kolej Universiti Sains dan Teknologi Malaysia
(KUSTEM)
21030 Kuala Terengganu.

Tarikh: **7/4/2005**

ACKNOWLEDGEMENT

First and foremost, I would like to thank Allah The Almighty for with His blessings, I acquired the spiritual strength to complete this thesis. I would also convey my sincere appreciation and gratitude to my supervisor, Cik Hazlina binti Ahamad Zakeri from the Department of Biological Sciences, Faculty of Science and Technology for her advice, comments and guidance throughout my final year project. Her views and advices play important role in the success completion of the thesis.

Secondly, I would like to dedicate my thanks to both the Biochemical Lab officers namely Cik Nor Azlina and Cik Ku Naiza for their assistance in coping with the technical problems of using reverse-phase high performance liquid chromatography (HPLC). Their kind cooperation and patient are highly appreciated.

Thank you to my fellow labmates, Alia, Siti, Ika, Ain, Syida and Adun for helping me in many ways and encouraging me throughout my project.

Finally, I would like to express my whole hearted love and gratitude to my family especially my parents, for their moral, financial and mental support to make this thesis a success. Thank you very much.

TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENT	ii
LIST OF TABLES	v
LIST OF FIGURES	vi
LIST OF ABBREVIATIONS	vii
LIST OF APPENDICES	viii
ABSTRACT	ix
ABSTRAK	x
CHAPTER 1 INTRODUCTION	1
CHAPTER 2 LITERATURE REVIEW	
2.1 Palm olein	4
2.1.1 Introduction to palm olein	4
2.1.2 Compositions of palm olein	8
2.1.3 Applications of palm olein	9
2.2 Lipases	10
2.2.1 Introduction to lipases	10

2.2.2	Lipase specificity	12
2.2.3	Immobilization of lipases	13
2.2.4	Organic solvents	15
2.2.5	Application of lipases in organic solvents	19
2.3	Transesterification reaction	21
2.3.1	Definition	21
2.3.2	Lipase-catalyzed transesterification	23
CHAPTER 3 METHODOLOGY		
3.1	Materials	25
3.2	Methods	25
3.2.1	Transesterification reaction	25
3.2.2	Effect of different organic solvents	26
3.2.3	Removal of free fatty acids (FFA)	26
3.2.4	Determination of peak composition by Reversed-Phase High Performance Liquid Chromatography (RP-HPLC)	27
CHAPTER 4 RESULTS		29
CHAPTER 5 DISCUSSION		38
CHAPTER 6 CONCLUSION		40
REFERENCES		41
APPENDICES		48
CURRICULUM VITAE		56

LIST OF TABLES

Table	Page
2.1 Fatty acids composition of palm olein	9
2.2 Triglycerides composition of palm olein	9
2.3 Log <i>P</i> values of organic solvents	16
2.4 Industrial applications of microbial lipases	19
4.1 Percentage and concentration of peak observed HPLC profiles of non-transesterified and transesterified palm olein in various organic solvents.	35
A.1 The percentage of free fatty acid (%FFA) removed for non-transesterified palm olein	50
A.2 The percentage of free fatty acid (%FFA) removed for transesterified palm olein	50

LIST OF FIGURES

Figure		Page
2.1	Palm dry fractionation route	7
2.2	The dependence of the activity of immobilise enzymes in biphasic system, on the log P of the organic phase	18
4.1	HPLC profile of non-transesterified palm olein (a) and palm olein transesterified in dimethylsulphoxide (b)	30
4.2	HPLC profile of non-transesterified palm olein (a) and palm olein transesterified in tetrahydrofuran (b)	31
4.3	HPLC profile of non-transesterified palm olein (a) and palm olein transesterified in dietylether (b)	32
4.4	HPLC profile of non-transesterified palm olein (a) and palm olein transesterified in heptane (b)	33
4.5	HPLC profile of non-transesterified palm olein (a) and palm olein transesterified in isooctane (b)	34
B.1	The sample of palm olein that has been used in this study	51
C.1	The orbital shaker was used to carry out transesterification reaction at 60°C and 200 rpm or 6 hours	52
D.1	The HPLC used to determine the peak for each sample	53
E.1	Separatory funnel showed the upper yellow layer of organic phase (glyceride) and lower pink layer of aqueous phase (FFA).	54
F.1	Example of samples that has been injected into the HPLC	55

LIST OF ABBREVIATIONS

MUFA	Monounsaturated fatty acid
PUFA	Polyunsaturated fatty acid
RP-HPLC	Reversed-Phase High Performance Liquid Chromatography
REFPO	Refined palm olein
RPO	Red palm olein
POP	Palmitic-Oleic-Palmitic
IV	Iodine value
CBE	Cocoa butter equivalent
CBI	Cocoa butter improver
PMF	Palm mid fraction
C14:0	Myristic acid
C16:0	Palmitic acid
C18:0	Stearic acid
C18:1	Oleic acid
C18:2	Linoleic acid
PPP	Tripalmitin
SOS	Disteroolein

LIST OF APPENDICES

Appendix		Page
A	The percentage of free fatty acid (FFA) removed	49
B	Figure of palm olein	51
C	Figure of orbital shaker	52
D	Figure of High Performance Liquid Chromatography (HPLC)	53
E	Figure of separatory funnel filled with sample	54
F	Figure of sample of glyceride	55

ABSTRACT

The effect of different organic solvents as reaction media for transesterification of palm olein was studied. The organic solvents studied used were: dimethylsulphoxide ($\log P$ -1.3), tetrahydrofuran ($\log P$ 0.49), diethylether ($\log P$ 0.85), heptane ($\log P$ 4.0) and isoctane ($\log P$ 4.52). Transesterification reaction was carried out at 60°C and 200 rpm for 6 hours using an immobilized lipase from *Aspergillus niger* as catalyst. The catalytic performance of the lipase was appraised by determining the changes in peak composition and concentrations by Reversed-Phase High Performance Liquid Chromatography (RP-HPLC) and the calculated degree of hydrolysis (DoH) as well as degree of transesterification (DoT). Transesterification resulted in an increase in Peak 3 for all the solvents studied except for diethylether. Peak 8 was observed to increase in at least three of the solvents (i.e. tetrahydrofuran, heptane and isoctane) whilst Peak 1 was observed to increase in at least two of the solvents (i.e. tetrahydrofuran and diethylether) studied. Peak 2 and Peak 6 was increased when palm olein was transesterified in dimethylsulphoxide and heptane, respectively. A new peak, Peak 4 was only observed in diethylether. DoH was the highest when isoctane was used as medium with 0.4%. This was followed by palm olein transesterified in heptane (0.11%), diethylether (0.09%), tetrahydrofuran (0.03%) and dimethylsulphoxide (0.03%). Isooctane also gave the highest DoT with 5.47%, followed by tetrahydrofuran (2.97%), diethyleter (2.25%), dimethylsulphoxide (0.84%) and heptane (0.05%). The results obtained show that the lipase was active in all range of organic solvents with isoctane being the best medium to be used in this study.

TRANSESTERIFIKASI KE ATAS MINYAK OLEIN KELAPA SAWIT DALAM PELARUT ORGANIK MENGGUNAKAN *Aspergillus niger* LIPASE TERSEKAT-GERAK.

ABSTRAK

Kesan ke atas pelbagai pelarut organik sebagai media tindak balas untuk transesterifikasi ke atas minyak olein kelapa sawit telah dijalankan. Pelarut organik yang digunakan adalah dimetilsulfida ($\log P = 1.3$), tetrahidrofuran ($\log P = 0.49$), dietileter ($\log P = 0.85$), heptana ($\log P = 4.0$) dan isooktana ($\log P = 4.52$). Tindak balas diteruskan pada 60°C dan 200 rpm selama 6 jam menggunakan lipase dari *Aspergillus niger* sebagai katalisis. Persembahan katalitik dari lipase ditentukan dengan melihat perubahan pada komposisi dan kepekatan puncak yang didapati dari RP-HPLC dan juga pengiraan darjah hidrolisis serta transesterifikasi. Hasil transesterifikasi menunjukkan peningkatan pada puncak ke 3 untuk semua pelarut kecuali dietileter. Puncak ke 8 didapati menunjukkan peningkatan pada sekurang-kurangnya tiga pelarut (tetrahidrofuran, heptana dan isooktana) manakala Puncak 1 didapati meningkat pada sekurang-kurangnya dua pelarut (tetrahidrofuran dan dietileter). Puncak 2 dan 6 menunjukkan peningkatan apabila transesterifikasi dalam dimetilsulfida dan heptana. Puncak yang baru, iaitu Puncak 4 diperhatikan dalam dietileter. Darjah hidrolisis yang tertinggi didapati apabila isooktana digunakan sebagai media dengan nilai (0.4%). Ini diikuti oleh minyak olein kelapa sawit yang ditransesterifikasi dalam heptana (0.11%), dietileter (0.09%), tetrahidrofuran (0.03%) dan dimetilsufida (0.03%). Isooktana juga memberi nilai darjah transesterifikasi tertinggi dengan nilai 5.47%, diikuti oleh tetrahidrofuran (2.97%), dietileter (2.25%), dimetilsulfida (0.84%) dan heptana (0.05%). Keputusan ini menunjukkan bahawa lipase aktif dalam semua julat pelarut organik dan isooktana adalah media terbaik untuk kajian ini.