

ENZYMATIC SYNTHESIS OF ALKYL ESTERS BY IMMOBILIZED
REIZOMUCOR MITSUBII WITH
PALM OLEIN AS SUBSTRATE

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PALM OLEIN AS SUBSTRATE

By

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**JABATAN SAINS BIOLOGI
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**PENGAKUAN DAN PENGESAHAN LAPORAN
PROJEK PENYELIDIKAN I DAN II**

Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk: ENZYMATIC SYNTHESIS OF ALKYL ESTERS BY IMMOBILIZED *RHIZOMUCOR MIEHEI* LIPASE WITH PALM OLEIN AS SUBSTRATE oleh Farizul Bin Abdul Rahman, no. Matrik: UK 9116 telah diperiksa dan semua pembedahan 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.

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LIST OF ABBREVIATIONS

MPOB	-	Malaysia Palm Oil Board
PORIM	-	Palm Oil Research Institute of Malaysia
RBD	-	Refined, Bleached and Deodorized
U	-	Unit
M	-	Molar
g	-	Gram
PPP	-	Tripalmitin
SOS	-	Oleodistearin
OSA	-	Stearodiolein
POP	-	Oleodipalmitin
OPO	-	Palmitodiolein
OOO	-	Tridiolein
OLO	-	Linoleodiolein
LOL	-	Oleodilinolein

ABSTRACT

Alkyl esters of fatty acids, better known as biodiesel, are nontoxic, biodegradable, and an excellent replacement for petroleum diesel. The conventional biodiesel technology for the production of fatty acid alkyl esters from vegetable oils and animal fats, involves the use of an inorganic base or acid catalyst at or near the boiling temperatures of the triglyceride/alcohol mixture. Enzymatic conversion of triglycerides has been suggested as an alternative. In this study, the synthesis of alkyl esters by immobilized *Rhizomucor miehei* lipase using RBD palm olein as substrate was investigated. The production of alkyl esters involved a two-steps process: hydrolysis of palm olein to release fatty acids from the triglycerides backbones, followed by alcoholysis of the free fatty acids to produce esters. Several reaction parameters were studied to obtain the optimum conditions for lipase activity: incubation time, temperature, amount of palm olein as substrate and amount of enzyme for hydrolysis; type of alcohol, substrate ratio and type of solvent for alcoholysis/transesterification. It was found that optimum fatty acids were released after four hours of incubation, at 45°C, with 12 g palm olein as substrate and 0.6 g of lipase. As for transesterification, the amount of alkyl esters formed was optimum with propanol as reactant, at palm olein:propanol ratio of 1:2 (w/v) and in diethylether as solvent. These results indicate that *R. miehei* lipase is capable of catalyzing the alkyl ester production from palm olein. Further investigation involving several other parameters is recommended to ensure highest yield of alkyl esters for use as biodiesel.

SINTESIS BERENZIM ALKIL ESTER OLEH LIPASE *Rhizomucor miehei* TERSEKAT-GERAK MENGGUNAKAN PALM OLEIN SEBAGAI SUBSTRAT

ABSTRAK

Alkil ester daripada asid lemak juga dikenali sebagai biodisel, adalah tidak bertoksik, boleh diurai secara biologi, dan merupakan bahan ganti yang baik untuk bahan bakar disel. Penghasilan biodisel daripada minyak tumbuhan dan lemak haiwan secara teknologi konvensional melibatkan penggunaan bahan asas bukan organik/pemangkin berasid pada/takat hampir mendidih trigliserida/campuran alkohol. Penukaran trigliserida secara berenzim telah menjadi satu alternatif. Dalam kajian ini, sintesis alkil ester oleh enzim lipase *Rhizomucor miehei* tersekat gerak menggunakan RBD olein kelapa sawit sebagai substrat telah diuji. Penghasilan alkil ester melibatkan dua langkah: hidrolisis olein kelapa sawit untuk membebaskan asid lemak daripada ikatan trigliserida, diikuti oleh tindak balas alkoholisis keatas asid lemak bebas untuk menghasilkan ester. Beberapa parameter tindak balas dikaji untuk menentukan keadaan optimum untuk aktiviti lipase: masa pegeraman, suhu, kuantiti olein kelapa sawit sebagai substrat dan kuantiti enzim untuk hidrolisis; jenis alkohol, nisbah substrat dan jenis pelarut untuk alkoholisis/transesterifikasi. Keadaan optimum untuk penghasilan asid lemak bebas berlaku selepas 4 jam pegeraman, pada suhu 45°C, menggunakan 12 g olein dan 0.6 g lipase. Kuantiti alkil ester yang terhasil dalam transesterifikasi adalah optimum dengan propanol sebagai substrat, pada nisbah olein:propanol 1:2 (w/v) dan didalam pelarut dietileter. Didapati lipase *Rhizomucor miehei* berupaya untuk memangkinkan penghasilan alkil ester daripada olein minyak sawit. Kajian seterusnya

melibatkan beberapa parameter lain dicadangkan untuk memastikan penghasilan tertinggi alkil ester, untuk digunakan sebagai biodiesel.