



UNIVERSITI PUTRA MALAYSIA

***PRODUCTION OF DIACYLGLYCEROL FROM PALM OLEIN USING
ENZYMATIC GLYCEROLYSIS, AND ITS RECOVERY***

YEOH CHIOU MOI

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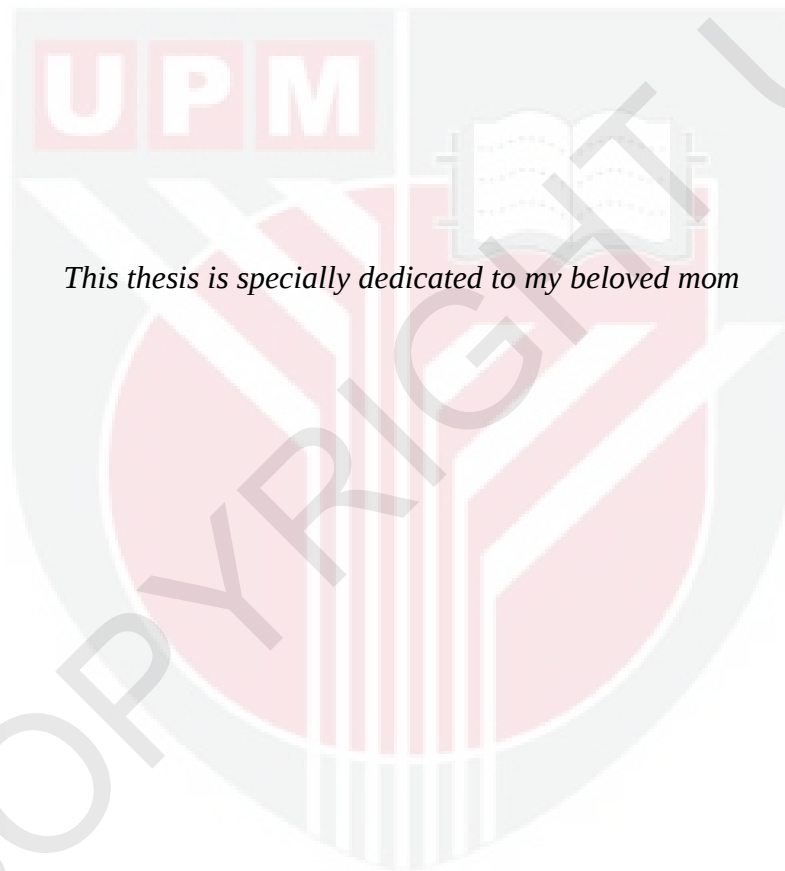
**PRODUCTION OF DIACYLGLYCEROL FROM PALM OLEIN USING
ENZYMATIC GLYCEROLYSIS, AND ITS RECOVERY**

By

YEOH CHIOU MOI

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirements for the Degree of Doctor of Philosophy**

October 2010



This thesis is specially dedicated to my beloved mom

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in
fulfilment of the requirement for the degree of Doctor of Philosophy

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October 2010

Chairman : Associate Professor Thomas Choong Shean Yaw, PhD

Faculty : Engineering

Diacylglycerol (DAG) is one of the minor components that is available in edible oils and fats. DAG has novel nutritional function in suppressing the serum triacylglycerol (TAG). Enzymatic reaction is preferred over chemical reaction as it usually requires mild operating condition. Among the available enzymatic reaction, glycerolysis is selected as the reaction process since abundant supply of glycerol (GLY) is expected from the biodiesel industry. The common edible oil that is available in Malaysian market, palm olein, is selected as the raw material for the reaction. The main objective of this study was to produce in a cost effective way of edible oil of at least 80 wt-% of DAG purity and less than 0.1 wt-% of free fatty acid (FFA).

The research began with the screening a few commercial enzymes. After consideration of the enzyme cost, Lipozyme TL IMTM was selected as the catalyst for the glycerolysis reaction. The operating parameters affecting the reaction were studied in order to optimize the DAG yield. Introduction of silica gel as additional solid support for the GLY significantly enhanced the reaction. However, the tocopherols and tocotrienols (tocols) content in the oil were reduced. The optimum temperature for the glycerolysis reaction was 55 °C.

As the addition of silica gel reduced the tocols content, the glycerolysis reaction was conducted without the presence of the silica gel. In this approach, the glycerol-enzyme (G/E) mass ratio was greatly affecting the reaction yield. The optimum G/E mass ratio was found to be ranged between 0.8 and 1.5. The mass ratio below 0.8 and above 1.5 was not recommended for the reaction as the reaction is either slow or inhibited. It was found that increasing of the enzyme load could shorten the reaction time.

Kinetic study of the glycerolysis reaction was developed based on the Michaelis-Menten mechanism. Three models were studied which were simple ternary model, simple ping-pong bi bi model, and complex ping-pong bi bi model. Among these models, the complex ping-pong bi bi model was the most appropriate model to describe the glycerolysis reaction.

DAG purity in the product was increased by purification using short-path distillation (SPD). Besides increasing the DAG purity, the FFA content in the product was also need reduced below 0.1 wt-%. A single-stage purification process was not able to increase the DAG purity to above 80 wt-%. Double-stage purification process was recommended as it could increase the DAG purity to above 80 wt-%. It was found that the removal of TAG at the first stage and the removal of the FFA at the second stage was able to achieve 89.9 wt-% of DAG purity without exceeding the limit of 0.1 wt-% of FFA.

A detailed DAG profile was identified and other product characterization such as fatty acid composition (FAC), slip melting point (SMP), and solid fat content (SFC) profile were also carried out. It was found that high DAG product had lower iodine value (IV) and higher SMP than raw material palm olein. The tocols content in the high DAG product was 1134 ppm.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**PENGHASILAN DIASILGLISEROL DARIPADA OLEIN MINYAK
SAWIT MELALUI GLISEROLISIS BERENZIM, DAN PEMULIHANNYA**

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Diasilgliserol (DAG) ialah satu daripada komponen-komponen kecil yang terdapat dalam minyak dan lemak makan. Diasilgliserol mempunyai fungsi pemakanan novel dalam menekan serum triasilgliserol. Reaksi enzim lebih disukai daripada tindak balas kimia kerana ianya memerlukan keadaan kendalian yang serderhana. Antara tindak balas berenzim yang ada, gliserolisis telah dipilih sebagai proses tindak balas kerana terdapat bekalan berlebihan gliserol (GLY) yang dijangka dari industri biodiesel. Olein minyak sawit adalah minyak makan yang paling biasa di pasaran Malaysia dan dipilih sebagai bahan mentah untuk tindak balas. Objektif utama kajian ini adalah untuk menghasilkan sekurang-kurangnya 80 bt-% diasilgliserol dan lemak bebas (FFA) kurang daripada 0.1 bt-% dengan cara yang paling menjimatkan.

Kajian bermula dengan pemeriksaan beberapa enzim-enzim komersial. Selepas pertimbangan kos enzim, Lipozyme TL IMTM telah dipilih sebagai pemangkin untuk tindak balas gliserolisis. Parameter operasi yang mempengaruhi tindak balas akan dikaji untuk mengoptimumkan kuantiti diasilgliserol. Pengenalan silika gel sebagai bahan sokongan tambahan untuk gliserol nyata sekali meningkat kadar tindak balas. Bagaimanapun, kandungan tocopherol and tocotrienol (tocols) dalam minyak telah berkurang. Suhu optimum untuk gliserolisis adalah 55 °C.

Oleh kerana pengenalan silika gel telah mengurangkan kuantiti tocopherol and tocotrienol, tindak balas gliserolisis dijalankan tanpa kehadiran silika gel. Dalam pendekatan ini, nisbah jisim gliserol-enzim (G/E) sangat mempengaruhi hasil tindak balas. Dalam kajian optimum, didapati bahawa nisbah jisim gliserol-enzyme adalah dalam lingkungan 0.8 hingga 1.5. Nisbah jisim yang kurang daripada 0.8 dan melebihi 1.5 adalah tidak disarankan untuk tindak balas kerana tindak balas adalah lambat atau berhenti. Ia juga didapati bahawa peningkatan kuantiti enzim akan memendekkan masa tindak balas.

Kajian kinetik gliserolisis adalah berasaskan mekanisma Michaelis-Menten. Tiga model telah dikaji iaitu model ternary ringkas, model dwi ping-pong ringkas dan model dwi ping-pong yang kompleks. Antara model-model ini, model dwi ping-

pong yang kompleks ialah model yang paling sesuai untuk menggambarkan tindak balas gliserolisis.

Ketulenian diasilgliserol dalam produk boleh dinaikkan dengan proses penyulingan berjarak pendek (SPD). Selain daripada menaikkan ketulenian DAG, kandungan FFA juga perlu dikurangkan dibawah 0.1 wt-%. Process penyulingan satu peringkat tidak berupaya mempertingkatkan ketulenian DAG melebihi 80 wt-%. Proses penyulingan dua peringkat disarankan kerana ia boleh meningkatkan ketulenian DAG melebihi 80 wt-%. Ia juga didapati bahawa penyingkiran TAG di peringkat pertama dan penyingkiran FFA pada peringkat kedua mampu mencapai 89.9 wt-% ketulenian tanpa melebihi had 0.1 wt-% FFA.

Profil DAG yang terperinci telah dikenalpasti dan pencirian produk lain seperti komposisi asid lemak (FAC), gelincir takat lebur (SMP) dan kandungan lemak padu (SFC) profil turut dijalankan. Ia didapati bahawa produk berdiasilgliserol tinggi mempunyai nilai iodin yang lebih rendah dan SMP yang lebih tinggi daripada bahan mentah iaitu olein kelapa sawit. Kandungan tocols dalam produk berdiasilgliserol tinggi adalah 1134 ppm.

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I certify that a Thesis Examination Committee has met on 29 October 2010 to conduct the final examination of Yeoh Chiou Moi on her thesis entitled “Production of Diacylglycerol from Palm Olein by Enzymatic Glycerolysis, and its Recovery” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Putra Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree.

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declared that it has not been previously and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or other institutions.

YEOH CHIOU MOI

Date: 29 October 2010

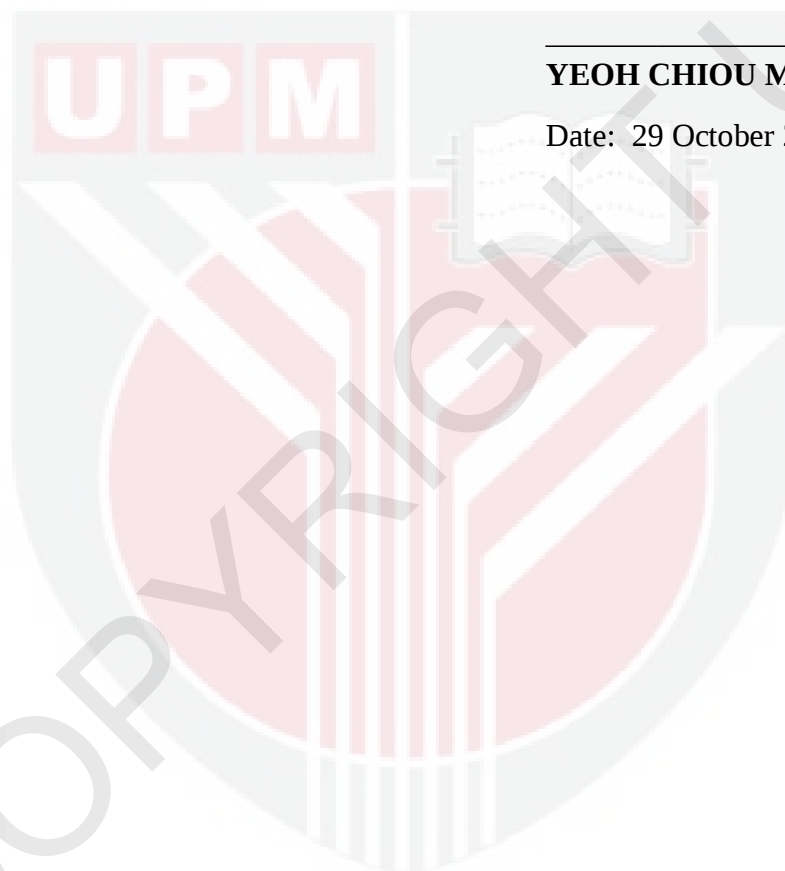


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