



**UNIVERSITI PUTRA MALAYSIA**

***ENZYMATIC SYNTHESIS OF DIACYLGLYCEROL FROM PALM KERNEL  
OIL AND SOY-CANOLA OIL BLEND, AND ITS ANTI-OBESITY EFFECTS  
IN C57BL/6N MICE***

**TANG TECK KIM**

**IB 2013 11**



**ENZYMATIC SYNTHESIS OF DIACYLGLYCEROL FROM PALM KERNEL  
OIL AND SOY-CANOLA OIL BLEND, AND ITS ANTI-OBESITY EFFECTS  
IN C57BL/6N MICE**

**By**

**TANG TECK KIM**

**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**

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

**ENZYMATIC SYNTHESIS OF DIACYLGLYCEROL FROM PALM KERNEL OIL AND SOY-CANOLA OIL BLEND, AND ITS ANTI-OBESITY EFFECTS IN C57BL/6N MICE**

By

**TANG TECK KIM**

**July 2013**

**Chairman : Lai Oi Ming, PHD**

**Faculty : Institute of Bioscience**

The anti-obesity effect of long chain unsaturated diacylglycerol (DG) oil have been well studied but not for short or medium chain saturated DG. Intake of different chain length and degree of saturation of fatty acid in the form of triacylglycerol has different impact on the body. Besides, fatty acid in the form of DG with different chain length and degree of saturation may have different health effect on the body. Therefore, in this study, two types of DG were produced from 1) palm kernel (PK) oil of medium chain saturated fatty acid and 2) soy-canola (SC) blend oil of long chain unsaturated fatty acid through enzymatic glycerolysis process and its health effect was investigated in C57BL/6N mice. The palm kernel diacylglycerol (PKDG) and soy-canola diacylglycerol (SCDG) were produced in 10L pilot-scale packed reactor. The present study involved the determination of the equilibrium time for the optimum reaction PKDG and SCDG while other parameters: glycerol to oil ratio (1.45:10 for PK and 1:10 for SC), temperature (65°C) and enzyme (Novozyme 435

lipase, 8% w/w) substrates ratio were kept constant. The result showed that optimum reaction times for DG production for PKDG and SCDG were 9 hours and 10 hours respectively and is utilized in pilot scale production. Ten batches of PKDG and SCDG were successfully produced giving yield of 40% DG. Consecutively, PKDG purification was carried out in 10L short path distiller. The optimum temperature to remove free fatty acid and monoacylglycerol from crude PKDG was 200°C while optimum temperature and maximum recovery of PKDG from crude PKDG was at 250°C with flow rate fixed at 1.008 l/h, roller wiper speed at 280 rpm. Purity for PKDG obtained was 88.77% and percentage of recovery was 74%. The purified PKDG and SCDG were incorporated into animal feed and their dietary effect in diet-induced obese C57BL/6N mice was investigated. Sixteen weeks feeding trial on C57BL/6N mice with high fat diet consisting of 30% PKDG and 30% SCDG were found to significantly reduce the fat accumulation in epididymal and retroperitoneal region as compared to high fat diet 30% palm kernel TG (PKTG) although there were no significant difference ( $P < 0.05$ ) in body weight. Serum glucose, cholesterol, leptin and insulin were significantly suppressed by PKDG and SCDG compared to PKTG. No significant differences ( $P < 0.05$ ) were found in the health benefit effects between PKDG and SCDG. Compared to TG, the structural differences in DG is the main factor contributing to its anti-obesity effect. Fatty acid composition doesn't seem to influence this ability.

In terms of gene expression PKDG induced the high expression of acyl-CoA synthase long chain (ACSL) and acyl-CoA synthase medium chain mRNA in small intestine while SCDG diet induced the high expression of ACSL in liver as well as small intestine suggesting that difference in fatty acid composition (FAC) of DG may potentially induce  $\beta$ -oxidation in different organs in mice. On the other hand, PKDG and SCDG-fed mice showed reduction expression of apolipoprotein B mRNA and have potential to reduce low density lipoprotein level in the body as compared to PKTG. Up-regulation of uncoupling protein-2 in liver and small intestine only can be observed in PKDG-fed mice.

Abstrak tesis yang dikemukakan kepada senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**SINTESIS BERENZIMATIK DIASILGLISEROL DARIPADA MINYAK ISIRONG SAWIT DAN GABUNGAN MINYAK SOYA-KANOLA, SERTA KESAN ANTI-KEGEMUKAN TERHADAP TIKUS C57BL/6N**

Oleh

**TANG TECK KIM**

**Julai 2013**

**Chairman : Lai Oi Ming, PHD**

**Faculty : Institute of Biosains**

Banyak kajian telah dijalankan untuk mengenai kesan anti-obesiti minyak diasilgliserol (DG) berantai panjang tidak tepu tetapi bukan untuk minyak DG rantai pendek atau sederhana tepu. Pengambilan minyak dalam bentuk triasilgliserol yang berbeza rantai dan tahap ketepuan asid lemak boleh memberi kesan yang berbeza kepada badan. Oleh itu, dalam kajian ini, dua jenis minyak DG telah dihasilkan daripada 1) minyak isirong sawit (PK) yang berantai sederhana and berasid lemak tepu serta 2) minyak gabungan minyak soya-kanola (SC) berrantai panjang and berasid lemak tak tepu melalui proses glycerolisis berenzim dan kesan terhadap kesihatan dikaji menggunakan tikus C57BL/6N. PKDG dan SCDG dihasilkan dalam bioreaktor terpadat 10L skala pilot dan penentuan masa keseimbangan bagi tindak balas PKDG dan tindak balas SCDG yang optimum telah dikaji. Parameter lain seperti nisbah gliserol: minyak 1.45:10 (bagi PK) dan 1:10 (bagi SC), suhu (65°C) dan nisbah enzim : substrat (Novozyme 435 lipase, 8% w/w) ditetapkan sepanjang

eksperimen. Keputusan menunjukkan bahawa masa tindak balas optimum untuk pengeluaran PKDG dan SCDG adalah 9 dan 10 jam masing-masing dan keputusan ini diaplikasikan dalam pengeluaran berskala pilot. PKDG dan SCDG sebanyak 10 kali telah berjaya dihasilkan dengan peratus hasilan DAG sebanyak 40%. Seterusnya, penulenan PKDG telah mengguna penyuling berjarat pendek (10L). Suhu optimum untuk mengeluarkan asid lemak bebas dan monoasilgliserol dari PKDG mentah adalah 200°C manakala suhu optimum dan pemulihan maksimum PKDG dari PKDG mentah adalah 250°C dengan kadar aliran yang ditetapkan pada 1.008 l/h, kelajuan roller pengelap pada 280 rpm. Ketulenan PKDG yang diperolehi adalah 88.77% dan peratus pemulihan adalah 74%.

PKDG yang ditulenan serta DAG SC masing-masing dicampurkan ke dalam makanan haiwan dan kesannya telah dikaji dalam C57BL/6N. Enam belas minggu selepas tikus C57BL/6N dengan diberikan diet kandungan lemak tinggi yang terdiri daripada 30% PKDG dan 30% SCDG didapati dapat pengumpulan lemak dengan ketara dibahagian epididimal dan retroperitoneal berbanding 30% PKTG walaupun tiada perbezaan yang signifikan ( $P < 0.05$ ) dalam berat badan diperhatikan. PKDG and SCDG didapati dapat mengurangkan paras serum glukosa, kolesterol, leptin dan insulin dengan ketara berbanding PKTG. Tiada perbezaan yang signifikan antara PKDG dan SCDG ( $P < 0.05$ ) dari segi kesan manfaat kesihatan yang diberikan. Berbanding dengan PKTG, perbezaan struktur di DG berbanding TG adalah faktor utama yang menyumbang kepada kesan anti-obesiti. Komposisi asid lemak tidak begitu ketara dalam mempengaruhi keupayaan ini.

Dari segi gen PKDG mendorong penhasilan tinggi acyl-CoA sintase rantai panjang (ACSL) dan acyl-CoA sintase rantai sederhana mRNA dalam usus kecil manakala diet SCDG mendorong penghasilan tinggi ACSL dalam hati serta usus, yang menunjukkan bahawa perbezaan dari segi komposisi asid lemak dari DG mempunyai potensi untuk menyebabkan  $\beta$ -pengoksidaan berlaku dalam organ yang berlainan dalam tikus. Sebaliknya, tikus yang diberi makanan PKDG dan SCDG menunjukkan penghasilan mRNA B apolipoprotein yang berkurangan dan mempunyai potensi untuk mengurangkan tahap lipoprotein ketumpatan rendah dalam badan berbanding dengan PKTG. Selain itu, peningkatan protein pemisahan-2 (uncoupling protein-2) dalam hati dan usus kecil hanya boleh diperhatikan dalam tikus yang diberi makanan PKDG.



## ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to my supervisor Prof. Dr. Lai Oi Ming for giving me this opportunity to carry out this project work. Your valuable guidance and assistance during this period enable the project to run successfully. My Alma Mater, University Putra Malaysia. Sime Darby Research Sdn Bhd for the financial support and cooperation on this project. Staff in Sime Darby for their kind assistance. My co-supervisor, Assoc. Prof. Dr. Noorjahan Banu Alitheen for providing the facility to carry out this project. I also thank my supervisory committee for their invaluable time to check on the thesis. My labmates: Beh Boon Kee, Phuah Eng Tong for their general opinion. Last but not least, I am forever indebted to my family for their understanding, endless patience and encouragement.

## APPROVAL SHEETS

I certify that a Thesis Examination Committee has met on \_\_\_\_\_ to conduct the final examination of TANG TECK KIM on his thesis entitled “ENZYMATIC SYNTHESIS OF DIACYLGLYCEROL FROM PALM KERNEL AND SOY-CANOLA OIL BLEND, AND ITS ANTI-OBESITY EFFECTS ON C57BL/6N MICE.” 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:

Name of Chairperson, PhD

Title (e.g. Professor/Associate Professor/Ir) – omit if not relevant

Name of Faculty

Universiti Putra Malaysia

(Chairman)

Name of Examiner 1, PhD

Title (e.g. Professor/Associate Professor/Ir) – omit if irrelevant.

Name of Faculty

Universiti Putra Malaysia

(Internal Examiner)

Name of Examiner 2, PhD

Title (e.g. Professor/Associate Professor/Ir) – omit if irrelevant

Name of Faculty

Universiti Putra Malaysia

(Internal Examiner)

Name of External Examiner, PhD

Title (e.g. Professor/Associate Professor/Ir) – omit if irrelevant

Name of Department and/or Faculty

Name of Organisation (University/Institute)

Country

(External Examiner)

---

**Zulkarnain Zainal, PhD**

Professor/Deputy Dean

School of Graduate Studies

Universiti Putra Malaysia

Date:

This 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:

**Lai Oi Ming, PHD**

Professor

Faculty of Biotechnology and Biomolecular Sciences

Universiti Putra Malaysia

(Chairman)

**Noorjahan Banu Alitheen, PhD**

Associate Professor

Faculty of Biotechnology and Biomolecular Sciences

Universiti Putra Malaysia

(Member)

**Lo Seong Koon, PhD**

Cheif Scientist II,

Sime Darby Research SDN BHD

(member)

---

**BUJANG BIN KIM HUAT, PhD**

Professor and Dean

School of Graduate Studies

Universiti Putra Malaysia

Date:

## DECLARATION

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



---

**TANG TECK KIM**

Date: 19 July 2013

## TABLE OF CONTENTS

<b>ABSTRACT</b>	ii
<b>ABSTRAK</b>	v
<b>ACKNOWLEDGEMENTS</b>	viii
<b>APPROVAL</b>	ix
<b>DECLARATION</b>	xi
<b>LIST OF TABLES</b>	xv
<b>LIST OF FIGURES</b>	xvi
<b>LIST OF APPENDICES</b>	xvii
<b>LIST OF ABBREVIATIONS</b>	xviii

### CHAPTER

<b>1</b>	<b>INTRODUCTION</b>	<b>1</b>
<b>2</b>	<b>LITERATURE REVIEW</b>	<b>5</b>
	2.1 Palm kernel oil	5
	2.1.1 Health impact of palm kernel oil	6
	2.1.2 PKO the applications	8
	2.2 Overweight and obesity	8
	2.2.1 Obesity - the cause, prevention and treatment	9
	2.2.2 Obesity and dietary fat	10
	2.2.3 Diabetes	11
	2.2.4 Impaired glucose tolerance or impaired fasting glycaemia	11
	2.3 Diacylglycerol (DG)	12
	2.3.1 Introduction	12
	2.3.2 Metabolism process of dietary DG	14
	2.3.3 Health benefits of DG	17
	2.3.4 Source of vegetable oil used for DG oil production	19
	2.3.5 Production of DG oil via enzyme lipase	20
	2.3.6 Safety aspects / regulations	24
	2.3.7 Application	25
	2.3.7.1 DG as frying oil	25
	2.3.7.2 DG as emulsion	26
	2.3.7.3 DG as bakery fat	27
	2.3.8 DG effect on blood lipid in animal model	28
	2.3.9 DG effect on blood lipid in human model	30

2.3.10	Body Weight Effect of DG in animal pre-clinical trial	32
2.3.11	Body Weight Effect of DG in Human Clinical Trial	35
<b>3</b>	<b>MATERIALS AND METHODS</b>	<b>39</b>
3.1	Materials	39
3.2	Methods	39
3.2.1	DG production	39
3.2.2	DG oils Purification	40
3.2.3	Free fatty acid (FFA) test	42
3.2.4	Peroxide value (PV) test	42
3.2.5	Acylglycerol and fatty acids composition Analysis	43
3.2.6	Determination of fatty acid composition (FAC) using gas chromatography	43
3.2.7	Feed formulation	44
3.2.8	Proximate analysis for animal feed	45
3.2.8.1	Crude Ash Determination	46
3.2.8.2	Crude protein determination using Kjeldahl method	46
3.2.8.3	Crude fat determination using soxhlet extraction method	47
3.2.8.4	Crude fiber determination using acid digestion method	48
3.3	Animals and diets	49
3.3.1	Body weight and food intake.	49
3.3.2	Total fecal lipids content.	49
3.3.3	Blood Serum analysis	49
3.3.4	Fat pad weights	50
3.4	Gene expression analysis	50
3.4.1	Primers design	50
3.4.2	RNA extraction	51
3.4.3	RNA Gel Electrophoresis	51
3.4.4	Complementary DNA (cDNA) synthesis	52
3.4.5	Annealing Temperature Optimization for primers	52
3.4.6	Standard curve construction	53
3.4.7	Gene Expression Analysis of ACSM, ACSL, ApoB and UCP-2.	53
3.5	Statistical analysis	54

<b>4</b>	<b>RESULTS AND DISCUSSION</b>	<b>55</b>
4.1	DG production.	55
4.2	Purification of DG	62
4.2.1	Removal of free fatty acids and Monoacylglycerol from first step distillation	62
4.2.2	Purification of Diacylglycerol from first cut residue	66
4.3	Proximate analysis	71
4.4	Feeding trial	72
4.5	Fasting serum analysis	75
4.6	Gene expression analysis	82
4.6.1	Total RNA extraction	82
4.6.2	Annealing temperature optimization	83
4.6.3	Evaluation using standard curve	85
4.6.4	Gene expression of ACSM, ACSL,Apo and UCP-2 in liver and small intestine of C57BL/6 mice	91
<b>5</b>	<b>SUMMARY, CONCLUSIONS AND RECOMMENDATION FOR FUTURE WORK</b>	<b>94</b>
5.1	Summary	94
5.2	Conclusion	95
5.3	Recommendations for future work	96
	<b>REFERENCES</b>	<b>97</b>
	<b>APPENDICES</b>	<b>109</b>
	<b>BIODATA OF STUDENT</b>	<b>117</b>
	<b>LIST OF PUBLICATIONS</b>	<b>118</b>