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

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By

TANG TECK KIM

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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^{o}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 successful 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^{o}C while optimum temperature and maximum recovery of PKDG from crude PKDG was at 250^{o}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 β-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 dikemukan 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 triasigliserol 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^{o}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^{o}C manakala suhu optimum dan pemulihan maksimum PKDG dari PKDG mentah adalah 250^{o}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 ditulenkan 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 megurangkan 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 serderhana mRNA dalam usus kecil manakala diet SCDG mendorong penghasilan tinggi ACSL dalam hati serta usus, yang menunjukkan bahawa perbezaan dari segi komposis asid lemak dari DG mempunyai potensi untuk menyebabkan β-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.

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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

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