UNIVERSITI PUTRA MALAYSIA

ENZYMATIC SYNTHESIS, ENCAPSULATION AND ANTI-OBESITY EFFECT OF PALM-BASED MEDIUM-AND LONG-CHAIN TRIACYLGLYCEROL FUNCTIONAL OIL

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By

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Medium-and long-chain triacylglycerol (MLCT) is a type of structured lipid that can act to suppress visceral fat accumulation. In the present work, MLCT was synthesized from palm sources and its anti-obesity effect was evaluated in mice prior to its application as powdered oil. This study was aimed to produce a palm-based healthy functional oil in liquid and powdered form that had the ability to reduce body fat accumulation. In the first part of this study, response surface methodology (RSM) with face centered composite design (FCCD) was employed to optimize the palm-based MLCT (P-MLCT) production. The optimized conditions obtained: substrate ratio palm kernel oil: palm oil (PKO:PO) of 90:10 (w/w), reaction time of 7.26 h, reaction temperature of 50 ˚C and 5 % (w/w) Lipozyme TLIM lipase successfully yielded around 60 % MLCT acylglycerol.

Subsequently, the influence of P-MLCT on body fat accumulation was evaluated in Diet Induced Obesity (DIO) C57BL/6J mice for 2 and 4 months period, respectively. Blood serum was collected and fat pad (perirenal, mesenteric, epididymal, and retroperitoneal) were dissected at the end of feeding trials. The 2 months feeding trial revealed that mice fed with P-MLCT attained the lowest total fat pad accumulation. Visceral fat accumulation of P-MLCT fed mice was 0.921 g (7 % P-MLCT) and 3.15 g (30 % P-MLCT) compared to non-enzymatically modified palm kernel: palm oil blend (PKO-PO Blend) having 1.07 g (7 % PKO-PO Blend) and 3.29 g (30 % PKO-PO Blend), and commercial MLCT (C-MLCT) with 1.03 g (7 % C-MLCT) and 3.18 g (30 % C-MLCT). Four months trial showed a more significant reduction ($P<0.05$) where consumption of 7 % P-MLCT managed to reduce ~30 % of body weight gain and ~37 % of body fat mass, compared to PKO-PO Blend and C-MLCT. Both 2 and 4 months trial resulted in similar trend on the blood serum parameters. P-MLCT was found to be efficient in controlling blood glucose and total triglyceride level, as compared to C-MLCT and PKO-PO Blend. Nevertheless, PKO-PO Blend and P-MLCT lead to a significantly higher ($P<0.05$) total cholesterol level compared to C-

MLCT. Both PKO-PO Blend and P-MLCT when fed for 2 months period resulted in mice having 1.14x (low fat diet) and 1.30x (high fat diet) higher cholesterol level. Similarly, 4 months feeding trial also led to a 1.25x (low fat diet) and 1.40x (high fat diet) increase in cholesterol level.

Lastly, P-MLCT powdered oil was developed *via* microencapsulation process with Maillard Reaction Products (MRP) as the encapsulating agent. MRP was prepared from heat aqueous solution containing mixture of sodium caseinate (SC), soy protein (SP) and maltodextrin (M) prior emulsification with P-MLCT oil and subsequently spray dried. The effect of MRP prepared at various conditions (temperature, incubation time, ratio of SC:SP:M and carbohydrate+protein:water content) on the degree of glycation and the physical properties of emulsion as well as spray dried powder was investigated. It was found that increase in the heat treatment (20 ˚C, 40 ˚C, 60 ˚C, 80 ˚C, 100 ˚C) and incubation time (2 h, 6 h, 8 h) of Maillard reaction led to the elevation of glycation. This subsequently caused a reduction in emulsion droplet size as well as lowering of moisture and surface oil content of the spray dried P-MLCT powder. The emulsion droplet size was reduced from 3.86 um to 0.29 um with the increase in temperature from 20 ˚C to 100 ˚C and 0.51 um to 0.26 um when incubation time was prolonged from 2 h to 8 h. Additionally, moisture content of spray dried powder was 20 % and 12 % lesser when the reaction temperature elevate from 20 ˚C to 100 ˚C and reaction time was extended from 2 h to 8 h. Also, MRP resulted in powder with smoother surface.

In summary, P-MLCT oil having ~60 % MLCT acylglycerol can act as healthy functional oil/powder in managing obesity. Both the liquid and powdered P-MLCT oil can be incorporated with ease into various food products to enhance their functionality.

Abstrak tesis yang dikemukan kepada senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

ENZIM SINTESIS, PEMERANGKAPAN DAN KESAN ANTI-OBESITI MINYAK BERFUNGSI TRIASIGLISEROL BERANTAI SEDERHANA DAN PANJANG BERASASKAN KELAPA SAWIT

Oleh

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Triasigliserol berantai sederhana dan panjang (MLCT) adalah antara minyak berstruktur yang dapat mengurangkan pengumpulan lemak di badan. Kajian ini melibatkan penghasilan minyak MLCT dari sumber kelapa sawit serta kajian praklinikal untuk melihat kesan anti-obesiti minyak MLCT ini terhadap tikus sebelum mengubahnya menjadi minyak serbuk. Tujuan kajian ini dijalankan adalah untuk menghasilkan minyak berfungsi berasaskan kelapa sawit yang sihat dalam bentuk cecair ataupun serbuk yang mempunyai keupayaan untuk mengurangkan pengumpulan lemak badan. Dalam bahagian pertama kajian ini, kaedah greak balas permukaan (RSM) berasaskan reka bentuk komposit berpusat muka (FCCD) digunakan untuk mengoptimumkan kandungan MLCT yang berasaskan kelapa sawit (P-MLCT). Didapati keadaan optimanya iaitu: nisbah substrak minyak sawit: minyak isirong sawit (PKO: PO) 90:10 (w/w), masa 7.26 jam, suhu 50 °C dan kandungan Lipozyme TLIM lipase 5 % (w/w) berjaya menghasilkan kira-kira 60 % P-MLCT.

Seterusnya, pengaruh P-MLCT terhadap pengumpulan lemak badan disiasat pada tikus jenis Makanan Induksi Obesiti C57BL/6J bagi tempoh 2 dan 4 bulan. Serum darah dianalisis manakala berat lemak viseral (mesenterik, perirenal, retroperitoneal, epididymitis) dibedah dan diukur pada akhir eksperimen. Hasil kajian praklinikal selama 2 bulan menunjukkan tikus diberi makanan P-MLCT memberikan ukuran yang paling rendah dari segi jumlah pengumpulan lemak badan. Lemak viseral bagi tikus diberi makanan P-MLCT adalah sebanyak 0.921 g (7% P-MLCT) dan 3.15 g (30 % P-MLCT) berbanding dengan campuran minyak isirung sawit: minyak sawit (campuran PKO-PO) iaitu 1.07 g (7 % campuran PKO-PO) dan 3.29 g (30 % campuran PKO-PO) serta minyak komersial MLCT (C-MLCT) iaitu 1.03 g (7 % C-MLCT) dan 3.18 g (30 % C-MLCT). Kajian praklinikal selama empat bulan menunjukkan pengurangan lemak badan yang lebih ketara ($P<0.05$). Pengambilan 7 % P-MLCT dapat mengurangkan ~30 % berat badan badan dan ~37 % berat lemak badan berbanding dengan campuran PKO-PO dan C-MLCT. Pengaruh terhadap serum darah menunjukkan implikasi yang sama

bagi kedua-dua ujian praklinikal. P-MLCT didapati cekap dalam mengawal tahap gula dan tahap trigliserida dalam darah. Walau bagaimanapun, campuran PKO-PO dan P-MLCT menyebabkan peningkatan paras kolesterol yang ketara ($P<0.05$) berbanding dengan C-MLCT. Tikus yang diberi makanan mengandungi campuran PKO-PO dan P-MLCT selama 2 bulan menujukkan kenaikan tahap kolesterol sebanyak 1.14x (diet rendah lemak) dan 1.30x (diet tinggi lemak). Selarasnya, ujian praklinikal 4 bulan juga menunjukkan peningkatan tahap kolesterol sebanyak 1.25x (diet rendah lemak) dan 1.40x (diet tinggi lemak).

Bahagian terakhir kajian ini melibatkan penghasilan serbuk minyak P-MLCT melalui teknik microenkapsulasi dengan menggunakan Produk Reaksi Maillard (MRP) sebagai bahan pengkapsul. MRP disediakan dengan memanaskan larutan yang terdiri daripada campuran natrium kaseinat (SC), protein soya (SP) dan maltodekstrin (M) sebelum diemulsikan dengan P-MLCT dan seterusnya dikeringkan dengan pengering semburan. Kajian ini meneliti kesan suhu, masa inkubasi, nisbah SC: SP: M serta nisbah karbohidrat+protein: air atas respons glikasi, sifat-sifat fizikal emulsi dan serbuk partikel kering. Seperti yang dijangka, peningkatan rawatan suhu dan masa inkubasi meningkatkan ($P<0.05$) tahap glikasi. Peningkatan glikasi didapati berhubung kait dengan pengurangan saiz titisan emulsi serta merendahkan kelembapan dan kandungan minyak atas permukaan serbuk. Saiz titisan emulsi dapat dikurangkan dari 3.86 um kepada 0.29 um dengan peningkatan suhu dari 20 ˚C kepada 100 ˚C dan 0.51 um kepada 0.26 um apabila masa tindak balas dipanjangkan dari 2 h kepada 8 h. Selain itu, peningkatan suhu tindak balas dari 20 ˚C kepada 100 ˚C dan masa tindak balas dari 2 h kepada 8 h masing-masing juga dapat menrendahkan kandungan kelembapan serbuk MLCT sebanyak 20 % and 12 %. Glikasi juga mangakibatkan permukaan yang licin pada serbuk partikel kering.

Sebagai kesimpulannya, minyak P-MLCT yang mengandungi 60 % MLCT acylglycerol boleh bertindak sebagai minyak/serbuk fungsi yang sihat terutamanya dari segi keupayaannya dalam menguruskan obesiti. Minyak P-MLCT dalam bentuk cecair ataupun serbuk boleh dimasukkan ke dalam pelbagai produk makanan.

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I certify that a Thesis Examination Committee has met on 28 April 2016 to conduct the final examination of Lee Yee Ying on her thesis entitled "Enzymatic Synthesis, Encapsulation and Anti-Obesity Effect Palm-based Medium–And Long-Chain Triacylglycerol Functional Oil" 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 Committe recommends that the student be awarded the Doctor of Philosophy.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

C-MLCT	Commercial MLCT
DIO	Diet induced obesity
DSC	Differential scanning calorimeter
FAC	Fatty acid composition
FAME	Fatty acid methyl ester
FFA	Free fatty acid
HDL	High density lipoprotein
HOMA-IR	Homeostatic model assessment insulin resistance
HPLC	High performance liquid chromatography
LCFA	Long chain fatty acid
LCT	Long chain triacylglycerol
LDL	Low density lipoprotein
MAG	Monoacylglycerol
MCFA	Medium chain fatty acid
MCT	Medium chain triglyceride
MLCT	Medium-and long-chain triacylglycerol
MRP	Maillard reaction products
MUFA	Monounsaturated fatty acid
P-MLCT	Palm-based medium-and long-chain triacylglycerol
PKO	Palm kernel oil
PKO-PO blend	Physical blend palm kernel and palm oil
PO	Palm oil
RBD	Refined bleached deodorized
RSM	Response surface methodology
SALATRIM	Short and long chain triacylglycerol molecule
SC	Sodium caseinate
SEM	Scanning electron microscope
SP	Soy protein
TAG	Triacylglycerol

CHAPTER 1

INTRODUCTION

Fats and oils play a vital role in maintaining the physiological functions in our body besides contributing to the texture, flavor, and aroma of a wide variety of foods. Often, the high fat foods are the one that is most palatable and often associated with incidence of obesity that are linked to several metabolic syndromes such as cardiovascular disease, stroke, type 2 diabetes, and certain types of cancer (Panel, 1998). With the advancement in lipid technology, our conventional fats and oils can be structurally modified with the aid of enzyme or chemical catalyst to produce lipid with enhanced functionality that is more healthful than the conventional oil. Structured lipid can be made to be low or zero calories and more nutritious in terms of its ability to deliver specific essential fatty acid to our body, catering for the growing consumers' interest for healthier food choice (Lee and Akoh, 1998). Resetta™ (Nisshin Oillio Group Limited, Japan) or the so called Medium-and Long-Chain Triacylglycerol (MLCT) is a type of structured lipid that has the ability to restrain body fat accumulation and reduce blood triglyceride level (Kasai et al., 2003; Matsuo and Takeuchi, 2004; Matulka et al., 2006; Zhang., 2010). It was claimed to have FOSHU (Food for Specific Health Uses) status in Japan in 2002 and gained its GRAS (Generally Recognized as Safe) status in 2006 by the U.S. Food and Drugs Administration. MLCT is made up of medium chain fatty acid (MCFA) and long chain fatty acid (LCFA) attached to the individual glycerol backbone. MCFA with C6-C12 carbon chain length is included in MLCT as it has rapid metabolism compared to LCFA due to its small molecular size and greater solubility. MCFA can be transported directly to the liver through portal vein to undergo beta-oxidation process producing instantaneous energy (Aoyama et al., 2007; Papamandjaris et al., 1998). In contrast, LCFA needs to be cycled back into the intestinal lymphatic ducts and transported as chylomicron to the thoracic ducts into the systematic circulation to be deposited in the body as fat. Still, LCFA is incorporated in MLCT molecule as its presence are crucial in providing the essential fatty acids to body and to increase the smoke point of MLCT.

Ever since its introduction 50 years ago, microencapsulation process that encloses small particle, liquid or gas within a thin film of coating has widely been used in food industries to entrap and protect the flavor and bioactive materials (Luckham, 1994). Among the several methods, spray drying which has the ability to convert liquid (solution, emulsion, slurries, paste and even melts) into free flowing powder is the most common, well established and economical technique used for microencapsulation process. For microencapsulation, selection of wall material is of utmost important. For hydrophobic core material, protein is commonly selected as the wall material due to its amphiphilic and emulsifying property. Lately, study found that products (MRP) formed during Maillard reaction/ non enzymatic browning (reaction between the reducing end of a sugar with the free amino group of a protein under heat) also acquired emulsifying characteristic which is even more superior than the native protein alone giving a more stable and smaller emulsion droplet size (Augustin et al., 2006; Kato et al., 1992; Li and Tang, 2013). Several studies also found MRP to have anti-oxidative and anti-microbial effect (Augustin et al., 2006; Kato et al., 1992; Kato et al., 1993). As a result, MRP was widely used in pharmaceuticals, cosmetics and food applications for the past

few years (O'Brien et al., 1998). Not until recently that considerable studies utilized MRP for encapsulation purposes (Augustin et al., 2006; Li and Tang, 2013a).

The anti-obesity and health effects of MLCT produced from MCT and rapeseed oil had been well studied (Kasai et al., 2003; Matsuo et al., 2001; Matsuo and Takeuchi, 2004a; Shinohara et al., 2005; Shinohara et al., 2006). Additionally, work had also been conducted to examine the ability of MLCT to act as frying oil, margarine and shortening (Arifin et al. 2011; Koh et al., 2009). Nonetheless, no work had been done so far to produce MLCT from a palm-based source through enzymatic intertesterification reaction as well as to investigate the application of powdered MLCT. Thus, the aims of the present study was to produce and optimize a palm-based type MLCT through enzymatic interesterification reaction between palm kernel oil (PKO) and palm oil (PO). PKO will provide the MCFA and PO will contribute to the LCFA in MLCT molecule. Face centered composite design was chosen for the optimization as the region of interest falls within the region of operability. The optimized conditions for parameters: substrate ratio PKO:PO (w/w), reaction temperature, reaction time, enzyme load was utilized for further up-scale in pilot plant production *via* 10 L stirred tank reactor. This was followed by a preclinical investigation to examine the effect of palm-based type MLCT on body fat suppression and blood serum parameters as compared to commercial MLCT and physical mixture of PKO:PO blend on DIO C57BL/6J mice for short term 8 weeks and mid term 16 weeks duration, respectively. Since the genetic, biological and characteristic of mice resemble that of human, it was selected as the subject for the preclinical investigation. Additionally, mice is inexpensive, can provide larger sample size and is easier to handle. Subsequently, the palm-based type MLCT oil was transformed into powdered functional oil through microencapsulation *via* spray drying process, utilizing the MRP as the cell wall material.

The specific objectives of this study were:

i. To optimize TLIM lipase catalyzed enzymatic interesterification reaction conditions for P-MLCT production.

ii. To investigate the anti-obesity effect of P-MLCT for a short term 8 weeks period on DIO C57BL/6J mice.

iii. To examine the anti-obesity effect of P-MLCT for a mid term 16 weeks period on DIO C57BL/6J mice.

iv. To produce P-MLCT powdered functional oil using microencapsulation technique with MRP as the encapsulating agent.

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