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ENZYMATIC INTERESTERIFICATION, FRACTIONAL CRYSTALLISATION AND ITS PHYSICOCHEMICAL PROPERTIES OF PALM-BASED COCOA BUTTER EQUIVALENT WITH HIGH PALMITOYL-OLEOYL-STEREOYL LEVEL

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science

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Cocoa butter is important in the confectionary industry because of its organoleptic attributes. This fat provides cool melting in the mouth sensation that gives a satisfying feeling to whoever eats chocolate. Cocoa butter was made from cocoa beans which undergo processes such as, drying, roasting, refining and fractionation. The end products, namely cocoa butter, cocoa liquor and cocoa mass are used mainly for chocolate industry.

However, the supply and price of cocoa butter seems to deteriorate over the years, alluring researchers to study and produce alternatives to cocoa butter. Even though exotic fats are available as Cocoa Butter Equivalents, they also have instability issues as cocoa butter. Therefore, palm oil, rich in palmitic acids is explored to be one of the alternatives.

In this study, crude palm-based cocoa butter equivalent (CBE) was developed via enzymatic interesterification between palm-based fat and fully hydrogenated fat. Fully hydrogenated fat with low Iodine Value used as the stearic donor to obtain high levels of POS (1-palmitoyl-2-oleoyl-3-stearoyl-*sn*-glycerol) triacylglycerols. Response Surface Methodology was used to obtain the optimum percentage of POS response of the interesterified product. The major parameters that influence the enzymatic interesterification reactions such as substrate ratio Palm Oil: Fully Hydrogenated Palm Oil (50:50-100:0 w/w), temperature (65-75°C), incubation time (30-600 minutes) and enzyme load (2-12 % w/w) were used for optimization. Based on the highest yield of POS produced using RSM, the optimal enzymatic parameters were: ratio of palm oil/fully hydrogenated palm oil is 1:1, 9.5% of enzyme load, 172.5 minute of retention time at 63.75°C. To purify the fat, the enzymatic interesterified product was subjected

to fractionation process at various cooling temperatures (32-45°C) and reaction time (12-36 hour).

At 34°C, POS achieved at the highest level which was approximately 31% after 12 hour of cooling process. The study of physiochemical properties of the CBE fat were done for characterization identification. The properties were solid fat content, slip melting point and iodine value. The Iodine Value (IV) and Slip Melting Point (SMP) are 44.30 and 29°C, respectively.

Physicochemical characteristics of Cocoa butter Equivalent fractionated at 34°C (CBE34) fat proposed that it could be utilized in confectionery industry as CBE. Though the CBE produced was slightly softer compared to Cocoa Butter (CB), it fulfills the growing trend of softer chocolates that quickly dissolves in the mouth. The characteristics of the CBE produced, having high yield of POS contributed to the compatibility with CB. The thermo profile indicated that CBE34 fat melted below body temperature (37°C), which contributes to the fast melting in the mouth, leaving no waxy taste. According to European Union regulations, only 5% of CBE can be used in chocolate formulations, therefore, CBE34 will be easily blended with CB. The crystallization profile of CBE34 is (β′+β), which is similar to cocoa butter. Hence, from this study, CBE 34 is recommended for utilization in the confectionery industry as CBE.

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

INTERESTERIFIKASI ENZIM, PENGHABLURAN SECARA FRAKSI DAN CIRI-CIRI FISIOKIMIA PENGGANTIAN LEMAK KOKO BERASASKAN KELAPA SAWIT DENGAN KADAR PALMITOYL-OLEOYL-STEREOYL YANG TINGGI

Oleh

SITI MASLINA BINTI MOHAMAD ALWI

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Lemak koko penting dalam industri konfeksi kerana sifat-sifat organoleptiknya. Lemak ini memberikan sensasi pencairan dalam mulut yang memberi kenikmatan kepada sesiapa yang memakan coklat. Lemak koko dibuat daripada biji koko yang menjalani proses seperti pengeringan, pemanggang, proses penapisan dan fraksionasi. Produk akhir, iaitu lemak koko, koko likor dan serbuk koko digunakan terutamanya untuk industri coklat.

Bagaimanapun bekalan dan harga lemak koko didapati merosot dari tahun ke setahun, menggalakkan penyelidik untuk mengkaji dan menghasilkan alternatif kepada lemak koko. Walaupun lemak eksotik boleh didapati sebagai pengganti lemak koko, mereka juga mempunyai masalah ketidakstabilan harga dan bekalan seperti lemak koko. Oleh itu, minyak kelapa sawit, yang kaya dengan asid palmitik dieksplorasi dan menjadi salah satu alternatif.

Suatu lemak ekuivalen kepada lemak koko yang berasaskan sawit mentah (CBE) telah dibangunkan melalui penjanaan reaksi enzimatik antara lemak berasaskan sawit dan lemak hidrogenasi sepenuhnya. Lemak hidrogenasi sepenuhnya dengan nilai iodin yang rendah akan digunakan sebagai penderma asid stearik untuk mendapatkan triasilgliserol POS (1-palmitoyl-2-oleoyl-3-stearoyl-sn-gliserol) pada tahap tinggi yang tinggi. Kaedah 'Response Surface' digunakan untuk mendapatkan tindakbalas optimum produk terinteristifikasi iaitu peratusan POS. Parameter utama yang mempengaruhi tindak balas penjanaan enzimatik seperti nisbah substrat kelapa sawit: minyak sawit terhidrogenasi sepenuhnya (50: 50-100: 0 w / w), suhu (65-75°C), masa

inkubasi (30-600 minit) dan peratusan enzim (2-12% w / w) yang digunakan untuk proses pengoptimuman. Berdasarkan peratusan POS yang dihasilkan berlandaskan RSM, parameter tindakbalas enzim yang optimum adalah: nisbah minyak kelapa sawit/minyak sawit hidrogenasi sepenuhnya adalah 1:1, 9.5% beban enzim, 172.50 minit masa inkubasi pada suhu 63.75°C. Untuk menulenkan lemak yang telah diinteresterifikasi, produk melalui proses fraksionasi pada pelbagai suhu penyejukan (32-45°C) dan masa tindak balas (12-36 jam). Pada 34°C, POS dapat dicapai pada tahap tertinggi iaitu kira-kira 31% selepas proses penyejukan 12 jam. Kajian terhadap sifat-sifat fisiokimia lemak lemak gantian koko dilakukan untuk mengenal pasti sifat-sifat lemak tersebut. Ciri-ciri yang digunakan untuk menegenalpasti sifat-sifat tersebut adalah kandungan lemak (SFC), titik lebur (SMP) dan nilai iodin (IV). IV dan Takat lebur (SMP) masing-masing adalah 44.30 dan 29.05°C.

Ciri-ciri fizikokimia Lemak koko setara yang difraksi pada suhu 34°C (CBE34) mencadangkan bahawa ia boleh digunakan dalam industri coklat sebagai lemak gantian koko. Walaupun pengeluaran lemak gantian koko lebih lembut sedikit berbanding lemak koko, ia memenuhi trend terkini yang lebih cenderung kepada coklat yang lebih lembut yang cepat cair dalam mulut. Ciri-ciri lemak gantian koko yang dihasilkan, sesuai dengan lemak koko kerana POS yang dihasilkan adalah tinggi. Profil thermo menunjukkan bahawa lemak CBE34 cair di bawah suhu badan (37°C), yang menyumbang kepada pencairan yang cepat di dalam mulut, tidak meninggalkan rasa melekit. Menurut peraturan EU, hanya 5% lemak gantian koko boleh digunakan dalam formulasi coklat, oleh itu, membuatkan CBE34 mudah dicampur dengan lemak koko. Profil kristalisasi CBE34 adalah (β′ + β), sama dengan lemak koko. Berpandukan ciri-ciri kondisi penghasilan CBE34, lemak gantian koko ini disyorkan untuk digunakan dalam industri coklat.

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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 Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

CB	Cocoa butter
CBA	Cocoa butter alternative
CBE	Cocoa butter equivalent
CBR	Cocoa butter replacer
CBS	Cocoa butter substitute
CNO	Coconut oil
CPO	Crude palm oil
DSC	Differential scanning calorimeter
EIE	Enzymatic interesterification
FHPO	Fully hydrogenated palm oil
HPLC	High performance liquid chromatography
IV	Iodine value
LLL	Middle chain triacyglyceride
LLM	1,2-lauric-3-myristic
LMM	1-lauric-2,3-myristic
MCT	Middle chain triglyceride
PMF	Palm mid-fraction
PEE	1-palmitoyl- 2,3 dielaidoyl-rac-glycerol
POP	1,3-dipalmitoyl-2oleoyl-glycerol
POS	1-palmitoyl-2-oleoyl-3-stearoyl-rac-glycerol
PKO	Palm kernel oil
RSM	Response surface method
SEE	1-Stearoyl-2,3-dielaidoyl-rac-glycerol
SFC	Solid fat content
SMP	Slip melting point

SOS	1,3-distearoyl-2-oleoyl-glycerol
TAG	Triacyglycerol/Triacylglyceride
TLIM	*Thermomyces lanuginosus* immobilized
XRD	X-ray Diffraction

CHAPTER 1

INTRODUCTION

The physical properties and significant organoleptic attributes of cocoa butter make it an essential fat in the confectionary industry. The triacylglycerols that exist in cocoa butter are very unique, making it a luxury product. It is composed of three main triacylglycerols which are 1, 3-dipalmitoyl-2oleoyl-glycerol (POP, 15-16%), 1-palmitoyl-2-oleoyl-3-stearoyl-rac-glycerol (POS, 35-38%), and 1, 3-distearoyl-2-oleoyl-glycerol (SOS, 23-26%) (Oracz *et al*., 2015). The crystallization and melting attributes that is crucial in providing sharp melting at body temperature is primarily provided by these triacylglycerols which are a very important in chocolate confectionery (Talbot, 2009; Lipp and Anklam, 1998).

Over the years, researchers have been trying to replace cocoa butter with exotic fats such as illipe, sal and shea which have unstable issues in terms of supply, price and quality (Gunstone, 2011). Because of its similar physical and chemical properties, these cocoa butter equivalents are popular in the chocolate industry. Cocoa butter equivalent are also mixable with cocoa butter without changing the properties. The 1-palmitoyl-2-oleoyl-3-stearoyl-*s n*-glycerol (POS) contributes the highest level of triacylglycerol in cocoa butter.

High value added products, especially the specialty lipids can be developed from cheap commercial oils by enzymatic lipid modification reactions (Forssell *et al*., 1992). Palm oil is one of the most popular oil used to produce cocoa butter-like fats because it is cheap, convenient and has constant supply. Palm oil which has two fractions; solid and liquid, has the potential to be used as a cocoa butter equivalent (CBE) or substitute (CBS). At present, Malaysia holds 39% of world palm oil production and 44% of world exports. If other oils & fats produced in the country are included in the picture, Malaysia accounts for 12% and 27% of the world's total production and exports of oils and fats respectively (MPOC, 2017). Palm oil and its fractions have long been used as edible oils and fats for a wide range of applications. However, past negative perceptions and campaigns struck by non-government organizations (NGOs) of palm oil had painted an unhealthy picture of palm oil, thus creating a great challenge for palm oil to penetrate the functional oils market. Having said that, this scenario can be advantageous for researchers to study more on creating better impact of palm oil usage towards healthier aspects in environment and human well-being. Thus, in order to stay competitive in the edible oils and fats market, it is a necessity for the development of functional oils from palm oil.

Extensive research has been done to produce these specialty fats and many methods have been attempted using all sorts of techniques such as enzymatic and chemical

reactions. Further purification, another crucial step, needs to be done using fractionation, supercritical fluid extraction, short path distillation and many more to obtain CBE.

Many studies use palm oil and its derivatives alone or mix them with other oils and fats to produce CBE. For instance, Pinyaphong and Phutrakul (2009), produced CBE using palm oil and methyl stearate, whereas, Chong *et al.*, (1992) generated CBE by enzymatically modifying palm olein and stearic acid. Palm mid-fraction and stearic acid also yielded high POS and SOS levels (Undurraga *et al*., 2003). Many works have concentrated on enzymatic modification and purifications such as done by Chiel Jedang of Korea, in 2010 using palm oil and fatty acid ethyl ester to create high SOS CBEs by EIEs as well as distillation processes. Palm oil and fully hydrogenated soybean oil was used to produce CBE with high POS and POP levels via EIE and solvent fractionation (Abigor *et al.*, 2003). Sonwai and Kaphueakngam, (2014), produced cocoa butter equivalent using palm-mid fraction and mango kernel fat contained high SOS percentage (approximately 27%) and same amount of POP level as cocoa butter (13%).

In this study, the production of palm-based CBE using enzymatic interesterification (EIE) of palm oil and fully hydrogenated palm oil was investigated. The catalyst, a commercially immobilized lipase, *Thermomyces lanuginosus* lipase (Lipozyme TLIM) was used. The effects of various reaction parameters on the yield of POS, POP and SOS were evaluated and optimized by response surface methodology (RSM). Further purification of CBEs was done by the fractionation process. Physicochemical characteristics and thermo profiles of the CBEs produced were also studied. In short, the objectives of this study were as follows:

i. To optimize various reaction parameters of a commercially immobilized lipase (TL IM Lipozyme) on the production yield of POS which are the ratio of palm oil to hydrogenated palm oil (0-100:100-0), enzyme load (2-12%), incubation time (30-600 minute) and reaction temperature (60-75°C).

ii. To determine the crystallization temperature and retention time of crystallization fractionation process parameters to yield the highest percentage of POS.

iii. To study the physicochemical properties such as solid fat content, slip melting point, iodine value and thermo gram profile of the high yield POS CBE produced.

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