PRODUCTION AND CHARACTERISATION OF COCOA BUTTER EQUIVALENT FROM FORMULATED HARD PALM OIL MIDFRACTION AND CANOLA OIL BLENDS

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AND CANOLA OIL BLENDS

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For My Beloved Family,

My father Azwir M. Zen and mother Rita Hendryyaty
My brothers Mohd. Riansyah Putra and Rofi Rendra Ramadhan
Big family of my grandparents (alm. Hj.Syamsudin Maisa and alm. M. Zen)

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ABSTRACT

There has been increased interest in cocoa butter equivalent (CBE) due to high price, uncertainty in supply and variability in quality of authentic cocoa butter (CB). This study investigated the blending of hard palm oil midfraction (PMF) with canola oil to produce CBEs which contain omega-3 and omega-6 fatty acids using immobilized lipase from *Rhizomucor miehei.* The experiments were designed using Response Surface Methodology (RSM) to optimize the percentage of triacylgycerols (TAGs) including palmitic-oleic-palmitic (POP), palmitic-oleic-stearic (POS), stearic-oleic-stearic (SOS) and diacylglycerol (DAG). The experiment was performed at hard PMF concentration of 50 to 90%, lipozyme load between 5% and 10% with reaction time of 2 to 14 hours. The best reaction conditions to attain these targets were 58.85% of hard PMF concentration, 2 hours of reaction time and 7.29% of lipozyme load (based on the weight of substrate). Under these conditions, the produced CBE contained 28.65±2.00% of POP, 19.52±0.96% of POS, 3.57±0.11% of SOS and 4.81±0.47% of DAG. The addition of canola oil improved the nutritional value of CBEs which was marked by the higher percentage of linoleic acid (omega-6, 7.98±0.92%) and linolenic acid (omega-3, 2.47±0.47%) in CBE than commercially available CBE (omega-6, 2.63±1.01%) and CB (omega-6, 2.68±0.34%). Enzymatic interesterification has not altered fatty acid content in the CBE, especially linoleic acid (omega-6) and linolenic acid (omega-3) which was characterised by no significant difference ($p > 0.05$) between the fatty acid profile of initial mixture and CBE. Slip melting point (SMP) value of CBE (46.25 °C) was significantly higher ($p < 0.05$) than the SMP value of CB (32-35 °C). In addition, the solid fat content (SFC) value of CBE was different from CB. It was due to the high amount of POP, free fatty acid (FFA) or saturated-saturated-saturated (StStSt) type of TAGs in CBE produced and also lower amount of TAGs which has oleic acid at sn-2 position. Moreover, the produced CBE behaved as pseudoplastic flow. Although there were differences of characteristics between the produced CBE with the authentic CB, it was possible to produce a high nutritional value of CBE with a physicochemical composition that was very close to that of CB by using enzymatic interesterification.

ABSTRAK

Pencarian kepada kesetaraan mentega koko (CBE) telah berkembang, kerana masalah harga premium, ketidaktentuan dalam bekalan dan kepelbagaian dalam kualiti mentega koko (CB). Kajian ini menyiasat pengadunan peringkat tengah minyak sawit keras dengan minyak *canola* untuk menghasilkan CBE yang mengandungi asid lemak omega-3 dan omega-6 dengan menggunakan lipase imobilisasi dari *Rhizomucor miehei*. Eksperimen direka bentuk menggunakan Kaedah Tindakbalas Permukaan (RSM) untuk mengoptimumkan peratusan triasilgliserol (TAG) termasuk palmitik-oleik-palmitik (POP), palmitik-oleik-stearik (POS), stearik-oleik-stearik (SOS) dan diasilgliserol (DAG). Keadaan proses dijalankan pada kepekatan peringkat tengah minyak sawit keras sebanyak 50 hingga 90%, beban *lipozyme* antara 5% dan 10% dengan masa tindak balas 2 hingga 14 jam. Syarat-syarat terbaik untuk mencapai sasaran ini ialah 58.85% kepekatan peringkat tengah minyak sawit keras, 2 jam masa tindak balas, dan 7.29% beban *lipozyme* (berdasarkan berat substrat). CBE yang dihasilkan mengandungi 28.65±2.00% POP, 19.52±0.96% POS, 3.57±0.11% SOS dan 4.81±0.47% DAG. Penambahan minyak *canola* meningkatkan nilai pemakanan CBE yang ditandai dengan peratusan asid linoleik (omega-6, 7.98±0.92%) dan asid linolenik (omega-3, 2.47±0.47%) yang lebih tinggi dalam CBE daripada CBE komersial (omega-6, 2.63±1.01%) dan CB (omega-6, 2.68±0.34%). Interesterifikasi enzim tidak mengubah kandungan asid lemak dalam CBE, terutamanya asid linoleik (omega-6) dan asid linolenik (omega-3) di mana campuran awal mempunyai ciri-ciri profil asid lemak yang tidak berbeza (p > 0.05) daripada CBE. Nilai slip takat lebur (SMP) CBE (46.25 °C) adalah jauh lebih tinggi (p < 0.05) dari nilai slip takat lebur CB (32 − 35 °C). Di samping itu, nilai kandungan lemak pepejal (SFC) daripada CBE adalah berbeza dari CB. Ia adalah disebabkan oleh jumlah POP, asid lemak bebas (FFA) atau jenis tepu-tepu-tepu TAG (StStSt) yang tinggi dalam CBE yang dihasilkan dan jumlah TAG yang mempunyai asid oleik di kedudukan sn-2 yang lebih rendah. Sebagai tambahan, CBE yang dihasilkan berkelakuan sebagai aliran pseudoplastik. Walaupun terdapat perbezaan ciri-ciri antara CBE yang dihasilkan dengan CB, ia adalah mungkin untuk menghasilkan CBE yang mempunyai nilai pemakanan yang tinggi dengan komposisi fizikokimia yang sangat dekat dengan CB menggunakan interesterifikasi enzim.

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LIST OF ABBREVIATIONS

A	-	Arachidic acid
AOCS	-	American Oil and Chemists' Society
CB	-	Cocoa butter
CBA(s)	-	Cocoa butter alternative(s)
CBE(s)	-	Cocoa butter equivalent(s)
CBR(s)	-	Cocoa butter replacer(s)
CCD	-	Central Composite Design
DAG(s)	-	Diacylglycerol(s)
DF	-	Degree of freedom
FAM	-	Fatty acid mixture
FFA(s)	-	Free fatty acid(s)
FHSO	-	Fully hydrogenated soybean oil
GC	-	Gas chromatography
HOSO	-	High oleic sunflower oil
ICCO	-	International Cocoa and Commodities Organisation
IM	-	Immobilized
L	-	Linoleic acid
LDL	-	Low density lipid
Ln	-	Linolenic acid
MAG(s)	-	Monoacylglycerols
O	-	Oleic acid
P	-	Palmitic acid
PMF	-	Palm oil midfraction
pNMR	-	Pulse nuclear magnetic resonance
PORIM	-	Palm Oil Research Institute of Malysia
RBD-PO	-	Refined, bleached, and deodorized palm oil
RM	-	*Rhizomucor miehei*

ROPO	-	Refined olive pomace oil
RSM	-	Response surface methodology
S	-	Stearic acid
SF(s)	-	Specialty fats
St	-	Saturated fatty acid
SMP	-	Slip melting point
SFC	-	Solid fat content
TAG(s)	-	Triacylglycerol(s)
TL	-	*Thermomyces lanuginosis*
U	-	Unsaturated fatty acid

LIST OF SYMBOLS

$^{\circ}$C	-	Degree celcius
$^{\circ}$K	-	Degree kelvin
%	-	Percentage
C_{16}	-	Palmitic acid
C_{18}	-	Stearic acid
$C_{18:1}$	-	Oleic acid
$C_{18:2}$	-	Linoleic acid
α	-	Alpha
β	-	Beta
γ	-	Shear rate
τ	-	Shear stress
H_2	-	Gas of hydrogen
rpm	-	Revolutions per minute
Y_i	-	Responses
βo	-	Constant
βi	-	Linear
βii	-	Quadratic
βij	-	Interactive coefficient
k	-	The number of independent variables
k	-	The consistency coefficient
$\sum$	-	Sigma
X	-	Independent parameter
Wt	-	Weight
V	-	Volume
v	-	Velocity
d	-	Diameter

m	-	Meter
cm	-	Centimeter
mm	-	Millimeter
μm	-	Micrometer
ml	-	Mililiter
n	-	Flow behavior index
Psig	-	Pounds per square inch gauge
R^2	-	Coefficient determination
p	-	Probability
Pa	-	Pascal
MPa	-	Megapascal
s	-	Second
mPas	-	Milipascal second

CHAPTER 1

INTRODUCTION

1.1   Background of the Research

Cocoa butter (CB) is a highly valued and important ingredient of chocolate and confectionery products. It gives desired physical properties to the chocolate product like gloss, snap, and melting properties (Lipp *et al.*, 2001). High and fluctuated price, uncertainty in supply and variability in quality have led the industry to find alternatives for CB (Wang *et al*., 2006; Zaidul *et al*., 2007; Jahurul *et al*., 2013).

Numerous fats and oils can be used for replacing the CB, collected under the name of cocoa butter alternatives (CBAs). One of CBAs groups is cocoa butter equivalents (CBEs). These non-lauric fats have similar physicochemical characteristics as CB and are therefore compatible with it. Compatibility in this context corresponds to the ability of the triacylglycerol (TAG) of two distinct fats to crystallize together without forming a eutectic (Lonchampt and Hartel, 2004). Therefore, CBEs may be combined with CB in any ratio for coating formulation of chocolate products.

The increasing of healthy lifestyle awareness leads consumers to consume products with high polyunsaturated fatty acids, low calories, enriched lipids and structured lipids. High oleic acid oils, such as canola oil, olive oil, sunflower oil, contain a low level of saturated fatty acids and an appreciable amount of

polyunsaturated fatty acid, which has the potential to be modified into other products (Rousseau and Marangoni, 2002). By using proper methods, modification of high oleic acid oil can be conducted properly without altering the nutrition of oil.

Canola oil, which contains a balanced ratio (2:1) of linoleic acid (omega-6) and linolenic acid (omega-3), provides more of unique healthy benefits than many other vegetable oils (Prsybylski *et al*., 2005). Moreover, the presence of oleic acid in canola oil makes it potential as a raw material for CBE production. This advantage is required to maintain sharp melting point characteristic on CBE. Therefore, canola oil was chosen as raw material in this study.

Modification of fats and oils through lipase-catalyzed interesterification has been studied since the 1890's (Undurraga *et al*., 2001). Recently, preparation of CBEs through 1,3-specific lipase-catalyzed interesterification has received much attention due to certain advantages of enzymes over chemical catalysts (Wang *et al*., 2006). One of the advantages of using 1,3-specific lipase is the ability of incorporating fatty acids, only at the 1- and 3- positions of the glycerol, without changing the fatty acid residues in the sn-2-position, while chemical catalysts will randomize all of the fatty acids in a TAG mixture (Wang *et al*., 2006). This advantage gives a product which is unobtainable by chemicals such as structured lipids in medical foods or enriched lipids with specific fatty acids to improve the nutritional properties of fats and oils (Willis and Marangoni, 2002). Therefore, the use of enzymatic interesterification was expected to maintain the nutritional content of CBE produced in this study.

Previous research has been using mixture of hydrogenated cottonseed oil and olive oil (1:1) to produce CBE (Chang *et al*., 1990). Slip melting point (SMP) of CBE produced from this study was 39˚C. It has TAG composition, 23% of palmitic-oleic–stearic (POS) and 28% of stearic-oleic-stearic (SOS). Furthermore, CBE from refined olive pomace oil (ROPO), palmitic acid, and stearic acid (1:2:6) was made by Ciftci *et al*. (2009). CBE produced has TAG composition 11% of palmitic-oleic-palmitic (POP), 21.8% of POS and 15.7% of SOS. It has SMP around 29.9˚C. Soekopitojo *et al*. (2009) used the mixture of PMF and fully hydrogenated soybean

oil (FHSO) at 1:2 ratio. The yield of CBEs obtained was 20.5% (based on the weight of the original substrate) with TAG composition 9.5% of POP, 26% of SOS, and 37.7% of POS. It has SMP around 30-34.5˚C.

All previous studies mentioned only concerned about the physicochemical of CBE produced, but neglected the fatty acid composition. In fact, the raw materials used in previous studies contained a lot of nutrition and might have given more nutritional value into CBE produced. Therefore, current study tried to modify fatty acid composition by adding canola oil to the CBE production, expecting CBE with higher nutritional value.

Lipase immobilization is necessary in order to improve the reusability and stability of lipase. Due to its reusability for multiple times, immobilized enzymes become economically attractive for interesterification (Goh *et al*., 1993). Therefore, this study used immobilized enzyme for producing CBE.

CBE production by enzymatic interesterification is affected by many factors such as raw material composition, lipozyme load, and reaction time. Thus, a statistical tool is needed to optimize the process condition. The optimization can be carried out effectively by response surface methodology (RSM). It helps in achieving optimum conditions with minimum number of experiments, but the results are statistically acceptable (Kadivar and Shekarchizadeh, 2012). Therefore, this study used RSM to determine the number of experiments and optimum condition of CBE production.

Previous research used RSM to produce CBE from high oleic sunflower oil (HOSO) (Naessens, 2012). RSM was used to maximize the percentage of saturate-unsaturated-saturated (StUSt) TAGs and minimize saturate-unsaturated-unsaturated (StUU) TAG from five factors, namely reaction time, temperature, water content, enzyme load, and substrate ratio. Optimum conditions given by this method were 7.98 of reaction times, 65ºC of reaction temperature, 1% of water content, 8.54 of enzyme load, and 7.99 of substrate ratio. These conditions generated 70.14% of SUS

and 26.76% of SUU. Kadivar *et al*., (2013) used RSM to maximize (StUSt) TAGs on CBE production from high oleic sunflower oil (HOSO). CBE containing 70.03% of StUSt was produced by combining five selected factors (1:7 of HOSO and fatty acid ratio with 8.5% enzyme and 1% water at 65∘C for 8 hours).

1.2 Problem Statement

Due to its unique composition and specific characteristic, CB becomes highly appreciated and is expensive compared with all other natural vegetable fats and oils. On the other hand, uncertainty in supply due to the low amount of CB in cocoa beans (yield of CB around 50-57% based on dry weight basis of cocoa beans), the limitation of cacao plant cultivation (the major cocoa beans growing countries in the world are Ivory Coast, Ghana, Indonesia, Cameroon, Nigeria, Brazil, Ecuador, Dominician Republic, and Malaysia), and the declined of the world total cocoa beans production from 3,752 million tonnes in 2007/2008 to 3,613 million tonnes in 2009/2010 contributed to the higher price of CB (Zaidul *et al*., 2007, Jahurul *et al*., 2013). Moreover, CB has some weaknesses when it is used in confectionery product, including complicacy for distributing in hot climates due to the low slip melting point, variability in quality by depending on source location, and the *blooming* problem which exists if whole product uses CB (Torbica *et al.*, 2006). Therefore, the alternative solutions, either full replacement or partial replacement of CB, are needed.

Cocoa butter equivalent (CBE) is modified to possess TAG composition similar to CB so it can be used as an alternative. It is usually prepared from lower value fats and oils, so it is able to reduce the selling price. Besides, CBE has several purposes, including: improving chocolate tolerance of milk fat, increasing the stability of chocolate at high temperature, and controlling chocolate blooming (Wainwright, 1999).

Many efforts have been made to produce CBE, including blending natural fats, fractionation, hydrogenation and interesterification, chemically or enzymatically. The simplest way to generate CBE is by blending exotic fats. But, exotic fats have a problem in supply because it is collected in the wild. This problem can make uncertainty in supply and triggering higher price of CBE (Kang and Kim, 2013). Moreover, the other method like hydrogenation is not recommended due to the compatibility issue of vegetable fat with the CB. After all, CBE is not normally containing trans fatty acids, unless it is modified or combined with another method to reduce trans fatty acid content in the final product (Talbot, 2007; Zarringhalami *et al.*, 2012). Furthermore, fractionation could be an effective method for obtaining CBE. This method is usually combined with others, such as blending or interesterification to produce CBE (Hashimoto *et al.*, 2001; Ali *et al.*, 1997; Kang and Kim, 2013)

Interesterification, either chemically or enzymatically, is a common method used for obtaining CBE and has been developed by many studies. Chemical interesterification is the popular method used by industry due to the lower costs of initial investment and ease of processing. Nevertheless, this method has less conformity for CBE production since the harsh condition process relieves the fine flavor of the product (Rousseau and Marangoni, 2002). On the other hand, enzymatic interesterification condition process is mild and easy to control. Since the main objective of this study was to produce high nutritional CBE, enzymatic interesterification became the chosen option. This method was expected to retain the linolenic acid (omega-3) and linoleic acid (omega-6) content on CBE properties by utilizing its benefits such as milder processing condition, unchanged the fatty acid composition and its regiospecifity.

1.3   Objective of the Study

i.   To produce a high nutritional value of CBE by blending hard palm oil midfraction (PMF) with canola oil by using enzymatic interesterification.

ii.   To optimize the CBE characteristic by using Response Surface Methodology (RSM).

1.4   Hypothesis

i.   The blending of hard palm oil midfraction (PMF) with canola oil, which contains high linolenic acid (omega-3) and linoleic acid (omega-6) will produce CBE containing a high nutritional value.

ii.   Enzymatic interesterification will not affect the fatty acid profile (including linolenic acid (omega-3) and linoleic acid (omega-6)) of CBE.

1.5   Research Scopes

The scopes of the study consist of:

i.   Physical and chemical characterizations of hard PMF and canola oil used in this study.

ii.   Preliminary study to investigate the significant effect of lipozyme load, reaction time, and stearic acid addition on TAG and SMP of CBEs.

iii.   Investigation of the chosen parameters such as hard PMF concentration, reaction time, and lipozyme load on CBE production to optimize the percentage of POP, POS, SOS and DAG, which were determined by using response surface methodology (RSM).

iv.   Characterization of CBE obtained, including fatty acid profile, triacylglycerol (TAG) composition, solid fat content (SFC), slip melting point (SMP), and rheological properties.

1.6   Significance of Study

By conducting this study, a method and formulation to obtain a partial replacement of CB in chocolate product could be determined. High nutritional cocoa butter equivalents (CBEs) were produced to improve the commercial benefit of the product. Futhermore, the influences of the process variables were studied and the model for optimization of CBEs production from enzymatic interesterification could be obtained.

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