SUPERCRITICAL CARBON DIOXIDE EXTRACTION OF MANGO SEED KERNEL FAT BLENDED WITH PALM OIL MID-FRACTION AND PALM STEARIN TO FORMULATE COCOA BUTTER REPLACERS

MD. JAHURUL HAQUE AKANDA

UNIVERSITI SAINS MALAYSIA

2013

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by

MD. JAHURUL HAQUE AKANDA

Thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

November 2013

ACKNOWLEDGEMENTS

Firstly, praise to God, Almighty Allah, who created and control the universe. This humble work could reach the present form through His blessing. My profound gratitude and appreciation are assigning to my supervisor Professor Ir. Mohd Omar Ab. Kadir and my field-supervisor Professor Md. Zaidul Islam Sarker for their insightful guidance's, encouragements and important supports throughout this work. I would like to express my thanks to Professor Nik Norulaini Nik Ab. Rahman for her valuable advices and comments throughout my Ph.D. program. I wish to acknowledge University Postgraduate Research Grant Scheme (PRGS, No. 1001/PTEKIND/845026) for the financial support and Universiti Sains Malaysia for the fellowship provided to me.

I would like to acknowledge Sime Darby Research Sdn Bhd for giving me chance to do some analysis of this thesis. Nur Azwani Ab. Karim, Scientist, Sime Darby Research Sdn Bhd is gratefully appreciated. I wish to express my warmest thanks to all staff of the School of Industrial Technology at Universiti Sains Malaysia for their kind help and cooperation. My special thanks to Mr. Mazlan Mohamed, Mr. Ravi Vinayagamuertty, Mr. Khairul, Mr. Shamsul Zoolkiffli, Ms. Noraida Bukhari, Mr. Azmaizan Yaakub for their accommodating the chemical analysis lab-work throughout this work. I also would like to thank my colleagues in the school of industrial Technology and specially Sahena, Shalima, Zainul, Sohrab, Moftah, Mark and other lab mate for the pleasant time and for sharing the lovely moments.

Finally, I would like to acknowledge my family: my parents Kalim Uddin and Mrs. Jhora Begum, my wife Maksuda, and son Mausoof for their love, support and kind encouragement.

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LIST OF ABBREVIATIONS AND SYMBOLS

ANOVA	Analysis of variance
AR	Analytical reagent
AV	Acid value
CB	Cocoa butter
CBE	Cocoa butter equivalent
CBEXs	Cocoa butter extenders
CBIs	Cocoa butter improvers
CBR	Cocoa butter replacer
CBS	Cocoa butter substitute
C14	Myristic acid
C15	Pentadecanoic acid
C16	Palmitic acid
C16:1	Palmitoleic acid
C17	Heptadecanoic acid
C18:0	Stearic acid
C18:1	Oleic acid
C18:2	Linoleic acid
C18:3	Linolenic acid
C20	Arachidic acid
C20:1	11-eicosenoic acid
C22	Behenic acid
C24	Lignoceric acid
^{o}C	Degree celsius

DOE	Design of experiment
DSC	Differential scanning calorimetry
FAME	Fatty acid methyl ester
FID	Flame ionization detector
GC	Gas chromatography
GC/MS	Gas chromatography/mass spectrometry
HPLC	High performance liquid chromatography
IV	Iodine value
MPa	Mega pascal
MSKF	Mango seed kernel fat
NMR	Nuclear magnetic resonance
PKO	Palm kernel oil
PLM	Plan polarized light microscopy
POMF	Palm oil mid-fraction
POP	1,3-dipalmitoyl-2-oleoyl-glycerol
PORIM	Palm oil research institute malaysia
POS	1-palmitoyl-3-stearoyl-2-oleoyl-glycerol
PS	Palm stearin
RSM	Response surface methodology
SC-CO_2	Supercritical carbon dioxide
SFC	Solid fat content
SMP	Slip/sharp melting point
SOS	1,3-distearoyl-2-oleoyl-glycerol
SV	Saponification value
TG	Triglyceride

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PENGEKSTRAKAN LEMAK ISIRONG BIJI MANGGA MENGGUNAKAN KARBON DIOKSIDA LAMPAU GENTING SEBATI DENGAN MINYAK SAWIT PECAHAN PERTENGAHAN DAN STEARIN PALMA UNTUK MEMBENTUK PENGGANTI MENTEGA KOKO

ABSTRAK

Mangga (*Mangifera tindan* L.) ialah sejenis buah tropika tahunan yang penting. Isirung biji mangga adalah hasil sampingan perindustrian yang mengandungi mentega koko hampir serupa dengan lemak. Pada masa ini tidak terdapat laporan atas pengekstrakan lemak isirung biji mangga menggunakan karbon dioksida lampau genting (BLG-CO_2). Tujuan kajian ini adalah untuk mencampurkan ekstrak lemak isirung biji mangga (LIBM) dengan fraksi pertengahan minyak sawit (FPMS) dan stearin sawit (SS) untuk menjanakan rumusan pengganti mentega koko (PMK). Ciri fiziko kimia, termal, kandungan lemak pepejal dan morfologi campuran LIBM: FPMS dan LIBM:SS dijalankan dengan menggunakan teknik kromatografi dan termal berbeza. Pengoptimuman parameter pengekstrakan SC-CO_2 lemak isirung biji mangga (LIBM) dari isirung biji mangga (IBM) dijalankan menggunakan kaedah permukaan gerak balas rekabentuk komposit berpusat. Pembolehubah yang dipertimbangkan dalam kajian itu ialah tekanan (20-50 MPa), suhu (40-80 °C), dan kadar aliran CO_2 (1-4 ml/min). Hasil lemak yang dioptimumkan telah diramalkan sebanyak 11.29% pada 44.2 MPa, suhu 72.2 ºC dan kadar aliran CO_2, 3.4 ml/min yang nilai hampir sama dengan hasil lemak pengekstrakan dengan Soxhlet. Campuran yang mengandungi 70 hingga 85% MSKF mempunyai sifat fiziko-kimia, profil asid lemak, nilai iodin (IV), takat lebur 'slip' (SMP), nilai saponifikasi (SPV) dan nilai asid (AV) yang hampir sama dengan

mentega koko komersial. Hasil kajian menunjukkan bahawa julat kandungan trigliserida (TG) utama bagi semua campuran adalah POP: 11-38.8%, SOS: 22.1-36.9%, dan POS: 15.4-16.2%,. Ciri daripada pencairan menunjukkan satu lengkung tunggal dengan hanya satu titik maksima dan satu bahu kecil untuk campuran no. 3 hingga 6 (LIBM: FPMS) dan 1 hingga 4 (LIBM: SS). Campuran sama mempunyai 70 hingga 95% LIBM yang menunjukkan corak penghabluran serupa dengan satu lengkung tunggal yang mempunyai satu puncak maksimum pada suhu-suhu 10.17, 10.58, 11.54 , dan 11.66 °C. Kandungan lemak pepejal (KLP) campuran no. 1 hingga 5 didapati hamper sama dengan mentega koko (MK) komersial pada suhu 10 hingga 20 °C. Pada 35 °C, KLP kesemua campuran mempamerkan cirri seperti MK. Mikroskop cahaya terkutub digunakan untuk memantau mikrostruktur rangkaian hablur semua campuran itu. Mikrostruktur hablur campuran no. 3 hingga 6 dan 1 hingga 4 ialah sferulit yang terdiri daripada jarum seperti hablur bersinar dan bercabang menuju ke luar daripada teras pusat, serupa dengan MK. Kajian mendedahkan bahawa PMK yang berciri mesra alam boleh disediakan dengan pengadunan LIBM dengan FPMS tempatan and SS dan boleh digunakan secara langsung dalam produk coklat.

SUPERCRITICAL CARBON DIOXIDE EXTRACTION OF MANGO SEED KERNEL FAT BLENDED WITH PALM OIL MID-FRACTION AND PALM STEARIN TO FORMULATE COCOA BUTTER REPLACERS

ABSTRACT

Mango (*Mangifera indica* L.) is an important annual tropical fruit. Mango seed kernel (MSK) which is industrial by-product contains considerable amount of cocoa butter analogy fats. It is remarkable that no reports on the extraction of the mango seed kernel fat (MSKF) using supercritical carbon dioxide **(**SC-CO_2) have been published. The aim of this work is to blend SC-CO_2 extracted MSKF with palm oil mid-fraction **(**POMF) and palm stearin (PS) to formulate new cocoa butter replacers (CBRs). The physico-chemical properties, thermal properties, solid fat content (SFC) and morphology for the blends of MSKF: POMF and MSKF: PS conducted using different chromatographic and thermal techniques. Optimization of the SC-CO_2 extraction parameters of MSKF from MSK were conducted using central composite design (CCD) of response surface methodology (RSM). The variables considered in the study are pressure (20-50 MPa), temperature (40-80 °C), and CO_2 flow rate (1-4 ml/min). The optimized fat yield was predicted to be 11.29% at 44.2 MPa, 72.2 ºC and CO_2 flow rate of 3.4 ml/min which was close to the fat yield (11.7%) of Soxhlet extraction. The blends containing 70 to 85% of MSKF had physico-chemical properties like fatty acid profiles, iodine value (IV), slip melting point (SMP), saponification value (SPV) and acid value (AV) close to that of commercial CB. Results showed that the major TG ranges in all blends were POP 11-38.8%, SOS 22.1-36.9%, and POS 15.4-16.2%, respectively. The melting

behaviour indicated a single curve with only one maximum and one small shoulder for blends no. 3 to 6 (MSKF: POMF) and 1 to 4 (MSKF: PS). The same blends having 70 to 95% of MSKF indicated similar crystallization pattern with a single curve having one maximum peak heights at 10.17, 10.58, 11.54, and 11.66 °C temperatures. The SFC of blends no. 1 to 5 was found to be close to that of commercial CB at temperatures 10 to 20 °C. At 35 °C the SFC of all blends were depicting that of CB. Polarized light microscopy was used to monitor microstructure of crystal network of the all blends. The microstructure of the crystals of blends no. 3 to 6 and 1 to 4 was spherulites consisting of needle like crystals radiating and branching outward from the central nuclei, similar to that of CB. The studies revealed that environmental friendly CBRs could be prepared by blending MSKF with local POMF and PS and could be used directly in chocolate products.

CHAPTER 1

INTRODUCTION

1.1 Research background

Mango (*Mangifera indica* L.) fruit belongs to the family of *Anacardiaceae.* It is the most important popular seasonal tropical fruit around the world and in some countries it is known as the “king of fruits”. As it is a seasonal fruit, about 20% of mango fruits are processed for the purpose of making puree, leather, pickles, canned slices, jam, jelly, and chutney (Loelillet, 1994). Moreover, mango juice is the predominant fruit juice especially in tropical and subtropical areas. It has been well acknowledged that mango fruit and its processed products have gained popularity in both the national and international market due to their nutritional quality, delicious, succulence, sweet taste, and delicate flavor. In the industry, only large edible portion of mangoes are used. Consequently, considerable amounts of mango seeds are discarded as agricultural wastes which are produced either by industrial processing or after consumption of the fruit, amounting 35 to 60% of the total fruit weight (Puravankara et al., 2000). Depending on the mango varieties, the seed represents from 10 to 25% of the weight of the total fruit and the kernel inside the seed represents from 45 to 85% of the seed and about 20% of the whole fruit (Hemavathy et al., 1988; Arogba, 1997; Solís-Fuentes and Durán-de-Bazúa, 2011).

Mango seed kernels (MSK) contain considerable amounts of cocoa butter analogy fats. On a dry basis, MSK contains about 7.1 to 15% crude fat (Ali et al., 1985; Hemavathy et al., 1988; Abdalla et al., 2007; Gunstone, 2011). It is the rich sources of palmitic ($C_{16:0}$), stearic ($C_{18:0}$) and oleic acids ($C_{18:1}$) containing about 3 to 18%, 24 to 57% and 34 to 56%. The other major fatty acids are linoleic, $C_{18:2}$ (up to 13%), linolenic, $C_{18:3}$ (0.6 to 1.0%), arachidic, $C_{20:0}$ (1.7 to 2.6%), and myristic, C_{14} (0.5 to 8%) acids. This group of fats received considerable attention for their particular physical and chemical properties which make them very suitable for the fabrication of confectionary. Therefore, the lipid composition of MSK has drawn immense research interest because of their potential application in the confectionery industry as a source of cocoa butter alternatives (Lakshminarayana et al., 1983; Ali et al., 1985; Hemavathy et al., 1988; Muchiri et al., 2012).

Currently, mango seed kernel fats (MSKF) are extracted and fractionated using solvents and their quality evaluated by many researchers in the literature (Gaydou and Bouchet, 1984; Ali et al., 1985; Abdalla et al., 2007). These organic solvent has adverse effects and it is non-selective, costly and requires long extraction time. Therefore, it is essential to develop improved method for MSKF extraction. Moreover, greater concern over the disposal of such toxic organic solvents and their effect on the human health as well as on the environment has led to a move towards cleaner extraction methods such as SC-CO_2 extraction (Staby and Mollerup, 1993; Hultin, 1994). Until now, no studies have been conducted for the extraction of MSKF using SC-CO_2.

Food industries are keen to find for alternative fats to cocoa butter (CB) from natural matrices that are denoted as cocoa butter replacers (CBRs), cocoa butter equivalents (CBEs), and cocoa butter substitutes (CBSs) fat. CB is a natural fat obtained from cocoa seeds (*Theobroma cacao*). It is commonly used as an essential major ingredient of chocolate and other confectionary products due to its specific physical and chemical properties. CB is solid at room temperature (below 25 ºC) and at body temperature (~37 ºC) it is liquid. CB mainly consists of C_{16}, $C_{18:0}$, $C_{18:1}$, and $C_{18:2}$ acids but low amount of C_{12} and C_{14} acids. CB can crystallize into several polymorphic forms, having α, γ, β', and β crystals, with melting points of 17, 23, 26, and 35–37 °C, respectively. In the chocolate production, only β crystal is used because it has a high melting point. This crystal structure confers chocolate products an excellent quality in terms of sheen, snap, and smooth texture. In addition, CB exhibits resistance to fat bloom, arising from changes in the fat in the chocolate during storage. This can be seen as undesirable white or streaky grey-white spots on the chocolate surface.

CB is highly appreciated and is expensive compared with all other vegetable fats and oils because of its specific characteristics. Another important reason is that cocoa beans contain low amount of CB (Zaidul et al., 2007a). Moreover, cocoa has only been cultivated in a few countries (Moreton, 1988; Hassan et al., 1995). Therefore, many researchers have produced cocoa butter alternative fats either by fractionation and blending or enzymatic interesterification of palm kernel oil (PKO) and palm oil (PO) (Bloomer et al., 1990; Hashimoto et al., 2001; Undurraga et al., 2001; Calliauw et al., 2005; Zaidul et al., 2007a), mango seed fat (Lakshminarayana et al., 1983; Ali et al., 1985; Jiménez- Bermúdez et al., 1995; Solís-Fuentes, 1998), kokum butter (Reddy and Prabhakar, 1994; Maheshwari and Reddy, 2005), sal fat (Reddy and Prabhakar, 1989; Gunstone, 2011), shea butter (Olajide et al., 2000), and illipé fat (Gunstone, 2011).

1.2 Problem statement

CB is the most important class of fats with demonstrated health benefits, and is used entirely for making chocolate and other confectionery products (Wollgast and Anklam, 2000; Kim et al., 2011). Moreover, it is a highly demanded fat among tropical plant fats, but its world production is decreasing by the day due to the cultivation difficulties, weather event, low productivity, and pest attacks (Bootello et al., 2012). As only a few countries cultivate cocoa, supply can be unstable. In recent years, cocoa price is increasing due to increasing consumption of chocolate products that contain higher level of CB (Afoakwa, 2010). Therefore, the food industries are in need for CB alternatives that might have similar quality of natural CB to meet the demand of premium grade and relatively cheaper CB overcoming the uncertainty in the supply of original CB due to its less existibility in the cocoa beans. Thus, interests of CB analogy fats from various natural sources are increasing.

Like CB, MSKF is a natural fat containing high saturated and monounsaturated fatty acids. These fatty acid constituents and other physico-chemical properties of MSKF make it a valuable fat that is comparable to that of commercial CB (Muchiri et al., 2012). In the industry, only large edible portion of mangoes are used or consumed. As a result considerable amounts of seeds are discarded as industrial waste or raised as by-products. These by-products particularly come from the tropical or subtropical fruit processing industries which have adverse effects on the environments, human health as well as creating both disposal and pollution problems. Due to increasing popularity and production of mangoes worldwide, disposal amounting 35 to 60% of the fruit weight represents a growing problem as the plant material is prone to microbial spoilage (Larrauri et al., 1996).

Moreover, drying cost, storage, and shipment of these by- products are economically limiting factors (Schieber et al., 2001).

Traditional solvent extraction is the current method for the extraction of MSKF (Lakshminarayana et al., 1983; Ali et al., 1985; Solis-Fuentes and Durán-de-Bazúa, 2004; Abdalla et al., 2007). In many countries, health and safety regulations are getting stricter in addressing environmental problems created by the use of organic solvents and these issues are forcing the industries to search for alternative processing methods. The solvents are unsafe to handle and unacceptable as it is harmful to human health and the environment, restricting its use in the food, cosmetic and pharmaceutical industries (Liu et al., 2009a). Moreover, there is an increasing concern of the health and safety hazards associated with the use of organic solvents often introduces contaminants into the MSKF that must be removed later. The major demerits of solvent extraction methods are (a) they require high temperature that affects nutritional quality of the products, (b) they are heat sensitive labile natural compounds, and (c) they contain toxic solvent left in the final products which have diverse adverse human health effects (Staby and Mollerup, 1993; Hultin, 1994).

1.3 Objectives of Study

The objectives of the study are:

1. To extract mango seed kernel fat from different varieties of mango seed kernel using conventional and SC-CO_2 methods for screening cocoa butter analogy fats.

2. To optimize the $SC\text{-}CO_2$ extraction parameters extracting CB analogy fat from MSK using response surface methodology (RSM).
3. To formulate CBRs from $SC\text{-}CO_2$ extracted MSKF blended with palm oil mid-fraction (POMF) and palm stearin (PS).
4. To characterize the physico-chemical, thermal properties and solid fat content for the blends of MSKF: POMF and MSKF: PS as CBRs using DSC and pNMR.

1.4 Research Contributions

This work does indeed offer some significant contributions to the current body of knowledge on research involving $SC\text{-}CO_2$ extraction and CBRs formulation. The physico-chemical properties of the $SC\text{-}CO_2$ extracted MSKF are ideal for use in CBRs formulation in food industry in particular chocolate industry. According to the Malaysian Palm Oil Council (MPOC), POMF and PS are obtained by fractionation of palm oil. PS is always traded at a discount compared to any other palm oil products; making it a cost effective ingredient for various applications. Blending of MSKF with POMF and PS can lower the costs comparative to commercial CB, while having the desired taste of CB. $SC\text{-}CO_2$ extraction and direct blending processes have the additional advantage that no chemical process is involved, consistent with the consumer trend toward natural and chemical free products.

The results of this study suggest that certain blends of MSKF and PS could be used to prepare CBRs without altering the physical and chemical properties of the product significantly. These blends could be used for the production of high temperature resistant hard butter for countries with hot climate. Moreover, blends of

MSKF and PS could solve the tempering difficulties faced by chocolate manufacturers in tropical countries like Malaysia or in countries with a moderate climate during the summer season. This blending method is a feasible alternative to expand the use of Malaysian POMF, and PS through the development of new CBRs.

1.5 Scope of Study

The following scopes of the study were performed in order to achieve the objectives:

- Examination of the effect of continuous and soaking techniques of SC-CO_2 extraction on mango seed kernel fat yield.
- Examination of the effect of SC-CO_2 extraction parameters on mango seed kernel fat yield.
- Comparison the fatty acids content in mango seed kernel fat obtained by SC-CO_2 and Soxhlet methods.
- Determination of the physico-chemical properties such as fatty acid constituents, iodine value, saponification vale, slip/sharp melting point, and acid value of SC-CO_2 extracted mango seed kernel fat.
- Comparison of the physico-chemical properties of SC-CO_2 extracted mango seed kernel fat with that of commercial CB.
- Preparation of cocoa butter replacers by blending of SC-CO_2 extracted MSKF with POMF and PS.
- Determination of the physico-chemical properties such as fatty acid and triglyceride compositions, iodine value, saponification value, acid value, slip/sharp melting point, solid fat content, and melting and crystallization profiles of MSKF: POMF and MSKF: PS blends.

- Examination of the effect of blending on physico-chemical properties in the formulated blends.
- Comparison the physico-chemical properties of all blend with that of commercial CB.

1.6 Limitation of Study

As discussed in earlier sections, SC-CO_2 could be advantageous for extracting of MSK fat and be blended again in desired ratios to formulate cocoa butter alternative fats according to the market demand. Supercritical CO_2 being a cheaper recyclable solvent and it will not be costly to produce the desired products and bear no threat to health and environment. The major hurdle to the application of SC-CO_2 in fats and oils industry is its instrumentation to an industrial scale. Another problem is the maintenance of the SC-CO_2 equipment which is somewhat costly as it goes frequent gas-leakage during high pressure operation. Attention should be paid while constructing the heavy duty equipment for use in the industry. It is also important to keep in mind during construction of the equipment to link a supercritical fluid chromatography to SC-CO_2 equipment to be used in special purpose to concentrate desired components. By this time SC-CO_2 has been established to industrial scale in essential oil extraction from different spices and in extraction of some pharmaceutical or phytochemicals from different plant parts. Once the industry adopts SC-CO_2 equipment in fats and oils extraction, the process can appear cost–effective or more profitable than the conventional methods.

1.7 Overview of Thesis

This thesis consists of eight chapters which begins with the introduction as the first chapter. The introduction describes in detail the background of the research. In this chapter, the problem statement, research objectives, research contributions, scope and limitation of the study have been identified and described. Chapter 2, covers the literature review regarding the application of SC-CO_2 in the extraction of fats and oils from various plant sources presented in detail to further understand the principles of this research. Blending of fats and oils to obtain CBRs is also reviewed as well as appraisal on MSKF, CB and cocoa butter alternative fats. In Chapter 3, screening of cocoa butter analogy fats from different varieties of MSK are presented and discussed. In Chapter 4 presents and discusses the optimization of the SC-CO_2 extraction parameters of CB analogy fat from MSK using RSM are presented and discussed. In Chapter 5, SC-CO_2 extracted MSKF blended with palm oil mid-fraction and palm stearin and investigated their physico-chemical properties. In Chapter 6 and 7, the thermal properties and SFC of MSKF: POMF and MSKF: PS blends are discussed. The conclusion of this research and some recommendations for the future work are addressed in Chapter 8.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

In this chapter, literature reviews regarding the application of SC-CO_2 in the extraction of fats and oils from various plant sources are presented. Blending of those fats and oils to obtain CBRs are also reviewed. Moreover, reviews on mango seed kernel fat (MSKF), cocoa butter and cocoa butter alternative fats are summarised.

2.2 Cocoa Butter

2.2.1 Background of Cocoa

The generic name of cocoa is *Theobroma* belonging to the family of Sterculiaceae, also called “Food of God”. It contains about 30 to 50 beans, covered with pulp. About 500 years ago, cocoa beans were originated from Latin America, and within a few years it spread to Europe. From there it was then distributed throughout the world (ICCO, 2000).

2.2.2 Cocoa Production

The major cocoa beans growing countries in the world are Ivory Coast, Ghana, Indonesia, Cameroon, Nigeria, Brazil, Ecuador, Dominician Republic, and Malaysia, contributing almost 90% of world production (ICCO, 2009/2010; FAO, 2012). The world total cocoa beans productions in season 2007/08 to 2009/10 are shown in Table 2.1. The Global cocoa beans production declined at 3.613 million tonnes in 2009/2010 season, while in 2007/2008 it was 3.752 million tonnes (Table 2.1). In season 2007/2008 to 2009/2010, the cocoa beans production declined by 3% in Africa, while it increased by 1.9% and 1.8% in the Americas and in Asia and Oceania to 14.4% and 17.5%, respectively. Africa is still the largest cocoa producing region, contributing 68.0% of the total world production followed by Asia and Oceania and the Americas in 2009/2010.

In Malaysia, cocoa beans production has also declined gradually. According to the Malaysian Cocoa Board, cocoa beans production in 2011 was 15000 tons, while in 2007 it was 35180 tons (MCB, 2011). The International Cocoa Organization (2009/2010) reported that weather has a great impact on the cocoa beans production. de Magalhães et al. (2011) reported that about 71% of the cocoa consumed around the world comes from the Africa’s, especially from Ivory coast, Ghana and Nigeria. In the last decade, the consumption of cocoa has increased while its production has been declining day by day (Jonfia-Essien et al., 2008).

Table 2.1: Production of cocoa beans in the world (thousands of tonnes)

	2007/2008		2008/2009		2009/2010	
Africa	2693	71.8%	2518	69.9%	2458	68.0%
Ivory Coast	1382		1222		1242	
Ghana	729		662		632	
Nigeria	230		250		240	
Cameroon	185		227		190	
Others	166		158		154	
America	469	12.5%	488	13.5%	522	14.4%
Brazil	171		157		161	
Ecuador	118		134		160	
Others	180		197		201	
Asia and Oceania	591	15.8%	599	16.6%	633	17.5%
Indonesia	485		490		535	
Papua New Guinea	52		59		50	
Others	55		50		48	
World Total	3752	100.0%	3605	100.0%	3613	100.0%

Source: ICCO Quarterly Bulletin of Cocoa Statistics, Vol. xxxvi, No. 4, Cocoa year 2009/2010.

2.2.3 Cocoa Butter Extraction

The cocoa seeds are referred to as cocoa beans consist about 85% cotyledon (nib) and 15% shell. The nibs contain about 55% fat. The nibs ground to a paste are called cocoa liquor or mass, some are directly used in chocolate. Cocoa beans are natural oil seeds like palm kernel, groundnut, sesame seed, or any other oil seeds. Processing of cocoa beans for getting the fats or oils is not same like other oilseed due to the unique physico-chemical properties of cocoa beans, especially the fats (Adeyeye et al., 2010). Presently, several methods are employed for the extraction of CB from mass or liquor, or from other sources including hydraulic press, mechanical press, screw presses, SC-CO_2, and solvent extractions (Asep et al., 2008; Nair, 2010). Recently, Nair (2010) reported that the hydraulic press and screw presses are not successful methods for the extraction of CB. The major demerits of hydraulic press, mechanical press, screw presses, and solvent extractions are a) they require high temperature that affects nutritional quality of the CB, b) they are heat sensitive labile natural compounds, and c) they contain toxic solvent left in the final products which have diverse adverse human health effects (Staby and Mollerup, 1993; Hultin, 1994).

Solvent extraction with hexane has been widely used to extract the CB as well as fats and oils from the oil-contained sources. However, there is an increasing concern of the health and safety hazards associated with the use of organic solvents, while expression by hydraulic method often introduces contaminants into the CB that must be removed later.

Meanwhile, several studies have been carried out for the extraction of CB from cocoa liquor and shell by SC-CO_2 at different temperatures and pressures (Li and Hartland, 1992; McHugh and Krukonis, 1994; Li and Hartland, 1996; Rossi, 1996; Asep et al., 2008). The studied pressure and temperature ranges from 15 to 40 MPa and 40 to 80 ºC. The yield of CB extracted with SC-CO_2 at 30-40 MPa and 50-80 ºC depend on the degree of disruption of lipid bearing cells reported by McHugh and Krukonis (1994), Rossi (1996). At higher pressure, the yield of CB was reported to be higher and temperature range being studied. Moreover, in their study, the triglycerides in terms of fatty acid compositions of extracted CB were within the required range and retained the aroma of the residue. Recently, Li and Hartland (1996), Rossi (1996), Asep et al. (2008), and Jinap et al. (2013) studied the effect of co-solvent such as ethanol on SC-CO_2 extraction of CB. Their results showed that addition of co-solvent improve the efficiency of the CB extraction. By adding co-solvent such as ethanol (20-25%, w/w), the solubility of CB increased and maximum yield was also reported by Li and Hartland (1992), Asep et al. (2008), and Jinap et al. (2013).

2.2.4 Composition of Natural Cocoa Butter

CB obtained from cocoa beans and on dry weight basis accounts for 50 to 57% and is responsible for the melting properties of chocolate (Steinberg et al., 2003). The major fatty acids of CB are C_{16} (25 to 33.7%), $C_{18:0}$ (33.7 to 40.2%), $C_{18:1}$ (26.3 to 35%) and $C_{18:2}$ (1.7 to 3%) which contribute about 98% of the total fatty acids (Bracco, 1994; Asep et al., 2008). The fatty acid compositions of CB differ depending on the country of origin. The key fatty acid compositions of CB from different countries are shown in Table 2.2. 1,3-dipalmitoyl-2-oleoylglycerol (POP), 1-palmitoyl-2-oleoyl-3-stearoyl-glycerol (POS) and 1,3-distearoyl-2-oleoyl-glycerol (SOS) are the three main triglycerides (TGs) account for 92 to 96% of total lipid composition of CB (Lehrian and Keeney, 1980; D'Alonzo et al., 1982; Davis and Dimick, 1989; Lipp and Anklam, 1998; Lipp et al., 2001; Asep et al., 2008). Among these three TGs, POS is the major leading TG component present in CB with range 42.5 to 46.4% yield followed by SOS (27.8 to 33.0%) and POP (18.9 to 22.6%) (Asep et al., 2008).

Table 2.2: The major fatty acid composition (%) of natural cocoa butter produced from various countries.

Country	Fatty acid (%)				References
	$C_{18:0}$	$C_{18:1}$	C_{16}	$C_{18:2}$	
Ivory Coast	36.9	32.9-33	25.8-26.6	2.6-2.8	Davis and Dimick (1989); Lipp and Anklam (1998)
Ghana	36.69-37.6	32.7-32.99	25.3-25.46	2.51-2.8	Spangenberg and Dionisi (2001); Lipp and Anklam (1998)
Indonesia	36.88-37.3	33.06-34.3	24.1-25.13	2.5-2.7	Spangenberg and Dionisi (2001); Lipp and Anklam (1998)
Brazil	33.3-33.8	34.5-36.5	25.1-27.9	3.5-3.6	Lehrian and Keeney (1980); Lipp and Anklam (1998)
Ecuador	34.62-36	34.6-34.91	25.2-25.6	2.6-3.04	Spangenberg and Dionisi (2001); Lipp and Anklam (1998)
Malaysia	36-37.4	33.5-34	24.9-26	2.6-3.0	Kheiri (1982); Lipp and Anklam (1998)

2.2.5 Physico-chemical Properties of Natural Cocoa Butter

The physico-chemical properties of cocoa beans and the characterization of cocoa matrix are greatly influenced by processing method (Hoskin and Dimick, 1984, 1994; Jinap, 1994; Amin et al., 1997, 1998, 2002; Puziah et al., 1998). The properties of CB fat are the properties of the mixture of TGs. CB is commercially available fat that contains significantly higher amount of saturated acids, leading to TGs of POS, SOS, and POP. Due to such types of TGs in CB, it confers the short melting behavior which is appreciated by consumers. CB has relatively low and sharp melting point which ranges from 27 to 35 ºC. At room temperature, CB is hard and brittle and its hardness depends on the solid fat content (SFC) and it might be 0% just above 37 ºC temperature. Furthermore, the nature of crystalline lattice also renders the hardness/consistency of the CB (Kheiri, 1982).

The other physico-chemical properties of CB such as IV and SPV are shown in Table 2.3. Iodine values have great impact on oil quality; it indicates the degree of unsaturation in the fats or oils. Typically, at room temperature fats are liquid because of the higher unsaturated fatty acid components, while it is solid at lower unsaturated fatty acid components. Chaiseri and Dimick (1989) reported that the higher IV contributes to the softness of CB. They also stated that higher IV content CB is softer than the lower IV content CB. The commercial CB from different countries shows different IV that ranges from 34.40 to 38.65.

The SPV indicates that the average chain length of fatty acid present in fat. If the SPV value of the fat is high, then the chain length of fatty acid will be shorter, and vice versa. The AV is defined as the weight in milligrams of potassium hydroxide necessary to neutralize the free fatty acid present in 1 g of fat and it is used to quantify the free fatty acids present in fats or oils. The cloud point (CP) is related to the unsaturation of oil, that is, the unsaturation of oil is higher, when its CP is low.

Table 2.3: Chemical characteristics of commercial CB from different countries.

Country	**Iodine value**	**Saponification value**
Bolivia	36.02	195.43
Brazil	37.46	195.07
Colombia	36.56	195.75
Ecuador	36.68	195.85
Peru	37.94	195.92
Costa Rica	36.64	195.27
Dominican Republic	36.72	194.92
Mexico	35.79	193.72
Panama	36.86	196.71
Ivory Coast	35.54	193.58
Nigeria	37.33	193.62
Malaysia	34.74	194.36

Source: Chaiseri and Dimick, 1989

2.2.6 Cocoa Butter Replacer (CBR)

The CBR or cocoa butter alternatives are defined as non-lauric fats that could replace CB either partially or completely in the chocolate or other food products (Kheiri, 1982). The fatty acid compositions of CBR are similar to that of commercial CB with more or less similar TGs structure. It should be cheaper than that of commercial CB. CBR can be divided into the following groups.

2.2.6.1 Cocoa Butter Equivalent (CBE)

CBEs are vegetable fats which have similar physical and chemical characteristics like CB. Therefore, CBEs can be mixed with CB in any amount without changing the behavior of the final product. The major fatty acids contained in CBEs are C_{16}, $C_{18:0}$, and $C_{18:1}$ acids, which are similar to that of CB. CBEs are divided into two subgroups, namely cocoa butter extenders (CBEXs) and cocoa butter improvers (CBIs) (Lipp and Anklam, 1998). CBEXs cannot be mixed with CB in every proportion, while CBIs are similar to CBEs, contain higher level of solid TGs, and because of this characteristic it is commonly used for improving soft CB.

2.2.6.2 Cocoa Butter Substitute (CBS)

CBSs are lauric and myristic plant fats (containing lauric and myristic acids) with some physical similarities to CB, but chemically they are completely different. Therefore, they are suitable for wholly replacement of CB.

2.2.7 Alternative Sources of Cocoa Butter

Many researches have carried out work for the production of CBRs, CBEs and CBSs from various natural sources. All of these are obtained from fats of natural plant, such as PKO, PO, MSKF, kokum butter, sal fat, shea butter, illipé butter, soya oil, rape seed oil, cotton oil, ground nut oil, and coconut oil. Replacing the CB either partially or wholly with other natural fats has been investigated due to the technological and economical advantages. These methods include chemical or enzymatic fractionation or SC-CO_2 extraction of fats and their blends from various sources.

2.2.7.1 Cocoa Butter Equivalent (CBE) from Mango Seed Kernel Fat (MSKF)

Up till now, lots of research have been conducted for the extraction and fractionation of various MSKF as their lipid compositions as well as unique physical and chemical properties are similar to that of CB (Narashima-Char et al., 1977; Lakshminarayana et al., 1983; Ali et al., 1985; Dhinigra and Kapoor, 1985; Ali and Dimick, 1994; Jiménez-Bermúdez et al., 1995; Solís-Fuentes, 1998). Recently, Kaphueakngam et al. (2009) produced CBE by blending mango seed almond fat (MAF) with POMF. Seven blends of MAF-POMF with different ratios (100/0, 90/10, 80/20, 70/30, 60/40, 50/50, 40/60, 30/70, 20/80, 10/90 and 0/100) have been studied using various techniques. Their results showed that C_{16}, $C_{18:0}$, and $C_{18:1}$ acids were predominant fatty acids in all blends, which were similar to commercial CB components. They reported that the major fatty acids, the melting behavior and slip melting point of the 80/40 (%wt) blend resemble to that of commercial CB.

In another study, Solís-Fuentes and Durán-de-Bazúa (2004) studied the thermal behaviour of MAF and its mixtures with CB. They reported that the fatty acid constituents and the physico-chemical properties of MAF are similar to that of commercial CB. They also showed that the MAF curves for solid–liquid phase change and the solid fat content of MAF profiles are great similar to that of CB. The properties of MAF, thermal conduct and the presence of α and β crystalline forms in MAF made it a suitable fat like CB. The iso-solid diagrams showed the compatibility between the mixture of MAF and CB, even better than that of mixtures of CB with milk fat, lauric fats, or hydrogenated cottonseed oil (Solís-Fuentes and Durán-de-Bazúa, 2004).

2.2.7.2 Cocoa Butter from Palm Oil and Palm Kernel Oil (PKO)

The level of fatty acids such as C_{12} and C_{14} are present in CB as trace or very low amount while the amount of C_{16}, $C_{18:0}$, and $C_{18:1}$ acids are high. On the other hand, PKO contains a high level of C_{12} constituent but low in $C_{18:0}$ and $C_{18:1}$ constituents compared with commercial CB. Although PKO contains high level of C_{12} and C_{14} acids, it is widely used as a suitable raw material in confectionery (Pantzaris and Ahmad, 2001). To produce CBRs blend components, Zaidul et al. (2006) successfully fractionated the PKO to reduce its C_{12} content and increase $C_{18:0}$ and $C_{18:1}$ constituents using SC-CO_2. About 28% of C_{12} yield was reduced in fraction 4, while 31% of $C_{18:1}$ constituent increased in yield which is closer to the fatty acid composition of CB. The authors extracted PKO into four fractions and observed that at higher temperature (80ºC) the total PKO yield increased with increasing pressures. At higher pressure of 48.3 MPa and temperature of 80ºC, the highest yield reported 99.6%. They also reported that the highest level of short chain fatty acid such as C_8, C_{10} and C_{12} constituents was found in fraction 1, while longer chain fatty acid such as $C_{16,}$ $C_{18:0}$ and $C_{18:1}$ constituents were seen in fraction 4. The lower melting point was reported in fraction 4 than in other studied fractions. The authors fractionated

PKO to produce triglycerides in terms of fatty acid constituents that could be used in CBRs blend.

In another study, Zaidul et al. (2007a) produced CBRs by blending of SC-CO_2 extracted PKO fractions with conventionally extracted PO and commercial $C_{18:0}$ and $C_{18:1}$ constituents at various ratios. They also fractionated PKO into four fractions using SC-CO_2 and are thus called f-PKO-1, f-PKO-2, f-PKO-3 and f-PKO-4. To obtain CBRs, the authors blended the f-PKO-3 and f-PKO-4 fraction as blending components (denoted as low lauric and high oleic acid constituents) with PO and commercial fatty acids such as $C_{18:0}$ and $C_{18:1}$ constituents at different ratios and labeled as blends 1 to 10.

The fatty acid compositions of different blends of f-PKO-3, f-PKO-4, PO, commercial $C_{18:0}$ and $C_{18:1}$ constituents are shown in Table 2.4. In both blends (f-PKO-3 and f-PKO-4) 1 to 10 low levels of C_8 and C_{10} were found while large amounts of the longer chain fatty acid such as $C_{16,}$ $C_{18:0}$, $C_{18:1}$ and $C_{18:2}$ were reported which are closer to that of commercial CB. The authors reported that increasing the amount of f-PKO (f-PKO-3 and f-PKO-4), more than 50% increased shorter chain fatty acid and decreased longer chain fatty acid constituents. Moreover, their results showed that the physico-chemical properties like SMP, SFC, IV, SPV and AV of blends 2 to 10 were closer to that of commercial CB.

Table 2.4: Fatty acid constituents for blends 1-10 (referred to f-PKO-3 and f-PKO-4 as a blend component) and commercial CB. (Zaidul et al., 2007a).

f-PKO-3									f-PKO-4								
Blend number	**Fatty acid constituents (%)**								**Blend number**	**Fatty acid constituents (%)**							
	C_8	**C_{10}**	**C_{12}**	**C_{14}**	**C_{16}**	**C_{18}**	**$C_{18:1}$**	**$C_{18:2}$**		**C_8**	**C_{10}**	**C_{12}**	**C_{14}**	**C_{16}**	**C_{18}**	**$C_{18:1}$**	**$C_{18:2}$**
1	0.1	0.1	1.8	1.3	28.7	25.2	34.6	8.1	1	0.0	0.0	1.4	1.1	28.8	25.2	35.2	8.2
2	0.1	1.1	3.6	1.9	27.0	19.9	37.8	8.5	2	0.0	0.0	2.8	1.7	27.8	20.5	38.4	8.7
3	0.1	1.1	5.1	2.6	27.5	19.9	35.4	8.2	3	0.0	0.0	4.2	2.3	28.1	20.4	36.5	8.4
4	0.2	0.2	7.2	2.7	27.1	19.6	34.9	8.0	4	0.1	0.1	5.6	2.9	26.7	20.4	35.8	8.3
5	0.3	0.3	9.0	4.0	24.6	19.3	34.6	7.8	5	0.1	0.1	7.1	3.4	25.2	20.3	35.5	8.2
6	0.3	0.3	10.8	4.7	23.2	19.1	33.9	7.6	6	0.1	0.1	8.5	4.0	23.8	20.2	35.2	8.0
7	0.4	0.4	12.6	5.4	21.4	18.8	33.5	7.4	7	0.1	0.1	9.8	4.5	22.7	19.9	34.9	7.9
8	0.4	0.4	14.3	6.1	20.1	18.5	32.9	7.2	8	0.1	0.1	11.3	5.1	20.9	19.8	34.8	7.8
9	0.5	0.5	16.1	6.7	18.4	18.2	32.5	7.0	9	0.1	0.1	12.1	5.6	20.2	19.7	34.4	7.7
10	0.5	0.5	17.9	7.4	16.7	17.9	32.2	6.8	10	0.1	0.1	14.0	6.2	18.2	19.5	34.2	7.6
CB[a]	0.0	0.0	Trace	0.7	25.2	35.5	35.2	3.2	CB[a]	0.0	0.0	Trace	0.7	25.2	35.5	35.2	3.2

[a]Kheiri (1982) and Pease (1985)

Many other researchers have produced CBS from the fractionation of PKO in the literature (Hashimoto et al., 2001; Calliauw et al., 2005). Calliauw et al. (2005) developed a two-stage dry static fractionation method for the fractionation of PKO. They also studied single-stage static fractionation method. When both methods were compared, the major advantage of the two-stage method was that it produces a higher yield of good quality palm kernel stearin (PKS) and reduces the additional hydrogenation that would be advantageous for the production of CB like fat. By using this method, they produced CBS from PKO without hydrogenation. They also reported that about 30% of PKS produced by the two-stage process needs to be hydrogenated for use as CBS. Ali (1996) produced CBEs by blending of sal fat with co-fractionated PO. Their results showed that the TG composition and solidification characteristics were similar to that of the Malaysian CB. They also reported that the co-fractionation method increases the compatibility between CBE triglyceride components.

Due to the absence of any waxy taste, *trans* fatty acids and low level of linoleic acid in POMF, Samsudin and Rahim (1996) used it in white chocolate formulation. Two types of POMF (POMF I, a commercial sample and POMF II**,** from a laboratory-scale acetone fractionation of POMF I) and Malaysian CB were used in white chocolate formulation. They investigated the effect of the tempering process and bloom resistance of the produced white chocolate products. They found that the tempering time to produce a well-tempered chocolate using POMF I was longer than that using POMF II**,** while the time to produce a well-tempered CB chocolate increased with increasing tempering temperature.

Since 1980s the modification of fats through interesterification reactions catalyzed by enzyme has been widely studied. It is a method which has several advantages (lower energy consumption, absence of isomerization by products, and better control of products) over the conventional methods of the chemical interesterification (Undurraga et al., 2001). Many researchers produced CBEs through enzymatic interesterification of POMF in the literature (Bloomer et al., 1990; Undurraga et al., 2001). To produce CBEs, Bloomer et al. (1990) studied the interesterification reaction of POMF using lipase enzyme as catalysts. They studied the purity of the product and the influence of the solvent concentration on the reaction rate at different temperatures. Their results showed that the solvent concentration and temperature have great effect on the reaction rate. The optimum temperature was 40 ºC and the solvent concentration was between 1 to 1.15 grams of solvent/gram of substrate reported in that study. They also showed that above this temperature the rate of interesterification reduced and the product purity decreased with increase of solvent.

In another study, Undurraga et al. (2001) produced CBEs through enzymatic interesterification of POMF with stearic acid in a solvent free system using Novo lipase Lipozyme™ as a catalyst. Their study performed in batch and in a continuous packed bed reactor. They investigated the effect of different parameters such as stearic acid-POMF ratio, enzyme-substrate ratio, and humidity of the enzyme preparation on productivity. Their results showed that the highest specific productivity obtained in shake flask was 0.0393 g/Batch Interesterification Unit (BIU) h at a stearic acid–POMF ratio of 1.6 and enzyme–substrate ratio of 23 BIU/g. On the other hand, the highest mass productivity observed was 1.54 g/g·h, using an enzymic load of 73 BIU/g in the continuous packed bed reactor. They reported that the thermograms of their products obtained by differential scanning calorimetry (DSC) were similar to that of CB, but exhibited several distinct peaks due to the presence of diglycerides and trisaturated TGs. They also reported that POMF could be used as a suitable raw material for interesterification with stearic acid using Lipozyme™, leading to the production of CBEs whose composition resembles closely to that of CB. Table 2.5 shows the composition of POMF, pure CB, and CBEs which were produced in their study. In comparison with all extraction methods, SC-CO_2 is feasible in terms of quality product and has the potential to produce a higher yield and good quality CBRs blends.

Table 2.5: TG compositions of POMF, CB and CBEs produced by interesterification reaction.

Sample	POP	POS	SOS	Other TGs	DGs
PMF	74.3	14.3	2.0	9.4	-
CB	23.4	42.8	27.5	3.6	2.7
CBE	23.4	38.5	20.2	8.2	9.7

Source: Undurraga et al., 2001

2.2.7.3 Cocoa Butter Replacers from Kokum Butter

Kokum butter (*Garcinia indica* Choisy *Syn Brindonia indica*) belongs to the family of Guttiferae. It is found in the western peninsular coastal regions and the states of Maharashtra, Goa, Karnataka and Kerala India and parts of Eastern India in the states of West Bengal, Assam, and North Eastern Hill regions and other parts of peninsular India. It is a small evergreen tree and is also known as mangosteen, goa butter tree, kokum butter tree (Baliga et al., 2011). The mature tree yields fruits annually and takes five months to complete its fruiting process and by March to May