FRACTIONATION OF BAMBANGAN KERNEL FAT BY TWO-STAGE FRACTIONATION AND THEIR CHARACTERISTICS

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ABSTRACT

Given the demand for new fats and oils and the limited research on bambangan kernel fat (BKF), this study aimed to fractionate BKF by two-stage acetone fractionation and to determine its physicochemical properties such as fatty acids by gas chromatography (GC), triglycerides (TAGs) by high-performance liquid chromatography (HPLC), iodine value (IV), acid value (AV), Free fatty acid (FFA), peroxide value (PV), slip melting point (SMP) and solid fat content (SFC) by pulse nuclear magnetic resonance (pNMR). The thermal (melting and crystallization) and polymorphic behaviors, as well as the morphological structures, were also determined using differential scanning calorimetry (DSC), X-Ray Diffraction (XRD), and polarized light microscopy (PLM). The major fatty acids of all stearin fractions were palmitic, stearic, and oleic acids, with the first stearin (Fraction-I) having a higher stearic acid (48.50%) but lower oleic acid (33.76%) content than the second stearin (Fraction-III). High SMP (36.3°C) and low IV, PV and AV (39.9 g iodine/100 g, 1.03 mEq/g and 0.93 mgKOH/g respectively) values were found in Fraction-III compared to Fraction-I. The SFC did not drop to 0% at body temperature, which was shifted to 0% above 40°C for both stearin fractions, which indicated it is heat-resistant. FI and FIII that composed of symmetrical SOS of 55.83% and 64.70% respectively are the valuable heat-resistance cocoa butter improver (CBI) fat produced from the fractionated BKF. The melting curve of the stearin fractions showed a similar single curve with cocoa butter (CB) but shifted towards the right. Both the stearin fractions melted and crystallized rapidly at high temperatures with one maximum peaks starting at 20.30−21.74°C and ending at 38.72−42.45°C (melting), and 17.05−18.46°C and ended at 5.63−8.20°C (crystallization). The melting curve for the stearin tended to be sharper and showed higher melting properties than pure BKF and CB. They exhibited β polymorphic form, which was similar to that of CB. Moreover, the stearin fractions showed spherulites microstructure consists of needle-like crystals radiating from central nuclei. This study shows that the BKF fractions (stearins) could be used as suitable raw materials for developing new fatty products in the food industry, especially the confectionery industries.

ABSTRAK

PEMECAHAN LEMAK KERNEL BAMBANGAN MELALUI PEMECAHAN DUA PERINGKAT SERTA CIRI-CIRINYA

Berikutan permintaan yang tinggi terhadap lemak dan minyak yang baru, serta kajian yang terhad bagi lemak kernel bambangan (BKF), kajian ini bertujuan untuk mendapatkan pecahan BKF melalui pemecahan aseton secara dua peringkat dan untuk menentukan sifat fizikokimia seperti asid lemak menggunakan kromatografi gas (GC), trigliserida (TAG) menggunakan kromatografi cecair berprestasi tinggi (HPLC), nilai iodin (IV), nilai asid (AV), Asid lemak bebas (FFA), nilai peroksida (PV), takat lebur (SMP) dan kandungan lemak pepejal (SFC) menggunakan nadi resonans magnet nuclear (pNMR). Ciri termal (sifat lebur dan penghabluran) dan bentuk polimorfik, serta struktur morfologi juga ditentukan dengan menggunakan pengimbasan kalori simetri (DSC), difraksi X-Ray (XRD), dan mikroskop cahaya polarisasi (PLM). Asid lemak utama bagi semua pecahan stearin adalah asid palmitik, stearik, dan oleik, dengan stearin pertama (Pecahan-I) mempunyai asid stearik yang lebih tinggi (48.50%) tetapi kandungan asid oleik (33.76%) lebih rendah daripada stearin kedua (Pecahan-III). Pecahan-III mempunyai nilai SMP (36.3° C) yang tinggi dan nilai IV , PV dan AV yang rendah (39.9 g iodin / 100 g1.03 mEq/g dan 0.93 mgKOH/g) berbanding Pecahan-I. Nilai SFC tidak jatuh kepada 0% pada suhu badan, dan beralih kepada 0% di atas 40° C bagi kedua-dua pecahan stearin, yang menunjukkan ia tahan panas. FI dan FIII yang terdiri daripada SOS simetri masing-masing sebanyak 55.83% dan 64.70% adalah lemak tahan panas yang bernilai yang memperbaiki lemak koko (CBI) yang dihasilkan dari pemecahan BKF yang meningkatkan ketahanan lemak koko. Keluk lebur dari pecahan stearin menunjukkan keluk tunggal yang sama dengan lemak koko (CB) tetapi beralih ke arah kanan. Kedua-dua pecahan stearin cair dan mengkristal dengan cepat pada suhu tinggi dengan satu puncak maksimum bermula pada 20.30-21.74 ° C dan berakhir pada 38.72-42.45 ° C (lebur), dan 17.05-18.46 ° C dan berakhir pada 5.63-8.20 ° C (kristalisasi). Keluk lebur untuk stearin cenderung lebih tajam dan menunjukkan sifat lebur yang lebih tinggi daripada BKF tulen dan lemak koko (CB). Kedua-dua pecahan juga mempamerkan bentuk polimorfik β, yang serupa dengan CB. Selain itu, pecahan stearin menunjukkan struktur mikro sferulit terdiri daripada Kristal berbentuk seperti jarum yang menuju ke luar pusat nukleus. Kajian ini menunjukkan bahawa pecahan BKF (stearin) boleh digunakan sebagai bahan mentah yang sesuai untuk membangunkan produk lemak baru dalam industri makanan, terutamanya industri konfeksioneri.

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LIST OF SYMBOLS

α	-	Alpha
λ	-	Lambda
θ	-	teta
μ	-	micro
β	-	Beta
ɣ	-	gamma

LIST OF ABBREVIATIONS

BKF	-	Bambangan kernel fat
CB	-	Cocoa butter
CBA	-	Cocoa butter alternative
CBE	-	Cocoa butter equivalents
CBR	-	Cocoa butter replacer
CBS	-	Cocoa butter substitutes
CBI	-	Cocoa butter improver
FO	-	Fats and Oils
FI	-	Fraction I
FII	-	Fraction II
FIII	-	Fraction III
FIV	-	Fraction IV
FV	-	Fraction V
FVI	-	Fraction VI
MSF	-	Mango seed fat
RSF	-	Rambutan seed fat
PO	-	Palm oil
PS	-	Palm stearin
PMF	-	Palm oil-mid fraction
PKO	-	Palm kernel oil
TAGs	-	triacylglycerides
OLL	-	1-oleoyl-2,3-dilauroyl-glycerol
PLL	-	1-palmitoyl-2,3- dilauroyl-glycerol
OLO	-	1-oleoyl-2-lauroyl-3-oeloyl-glycerol
POL	-	1-palmitoyl-2-oeloyl-3-lauroyl-glycerol
PLP	-	1-palmitoyl-2-lauroyl-3-palmitoyl-glycerol
OOO	-	1,2,3-trioleoyl-glycerol
POO	-	1-palmitoyl-2,3-oleoyl-glycerol
POP	-	1-palmitoyl-2-oleoyl-3-palmitoyl-glycerol
SOO	-	1-stearoyl-2-oleoyl-3-glycerol
POS	-	1-palmitoyl-2-oleoyl-3-stearoyl-glycerol

SOS	-	1,3-distearoyl-2-oleoyl-glycerol
SSS	-	1,2,3-tristearoyl-glycerol
SSP	-	1,2-distearoyl-3-palmitoyl-glycerol
SFC	-	Solid fat content
IV	-	Iodine value
PV	-	Peroxide value
AV	-	Acid value
FFA	-	Free fatty acid
SMP	-	Slip melting point
Hm-SMT	-	High-melting symmetrical triglycerides
HCO	-	Hydrogenated conola Oil
HPO	-	Hydrogenated palm Oil

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CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Cocoa butter (CB) is the common desired fat resources in chocolate and other confectionery products that are obtained from the beans of cocoa (Jahurul *et al.,* 2018a). It is solid at room temperature (25°C), and liquid at ~37°C and mainly consists of palmitic, stearic and oleic acids (Luma & Yang, 2016). However, CB is the most expensive material for chocolate formulations, and the surplus in demand caused the shortage in the production and supply of CB (Beckett, 2000; Gross, 2014). Furthermore, CB has a low melting point (33.8°C) that cause chocolate products to melt easily in the tropical and subtropical region (Jin *et al.,* 2017b). Thus, cocoa butter alternatives (CBAs) such as cocoa butter equivalent (CBE); cocoa butter improver (CBI) from natural fat resources are required to replace CB (partially/wholly) or as a blending component with other CBA for the production of chocolate in the confectionery industry.

Natural fats resources present different polymorphism (three major forms; β, β′, α) that gives different melting points behavior (Rao, 2003). These fat resources should have similar characteristics as CB so that it could be used as an alternative (CBA) in the confectionery industry. The main intention was to improve the properties of final products, reduce the cost, and coop with the shortage of CB supply since it is only cultivated in only a few countries (Hassan *et al.*, 1995). Thus, the food industry are keen to find an alternative from natural fat resources for the production of chocolate and other confectionery due to the specific properties and economic reasons.

Bambangan (*Mangifera pajang*) fruit is an underutilized fruit that belongs to the mango (*Anacardiaceæ*) family and distributed around the Southeast Borneo island, such as Sabah, and Sarawak, Malaysia (Azrina *et al.*, 2016; Jahurul *et al.*, 2018a). Although it is known as a forest fruit in Peninsular, Malaysia, bambangan has been recognized as an iconic fruit of East Malaysia, especially in Sabah. It has an ovoid shape (the weight is 0.5 to 1kg per fruit), brown skin colour with a thickness of 5 - 7 mm, and mango-like pulp (Lim, 2012). It has sweet-sour flesh, which constitutes 60-65% while the peel and kernel represent 15-20% of total weight, respectively (Abu Bakar *et al.,* 2009).

Bambangan fruit usually eaten fresh or used as a flavor adding into the dish, as a pickle and juice production, but the peel and kernel was discards as a waste by-product. Nowadays, bambangan has accepted considerable attention among the scientist in various area of expertise such as food, health and pharmaceutical industry due to its properties and nutritional value. It has economic potential as it has the ability to grow in the adverse climatic environment, rich sources of vitamins, macronutrients, and minerals (Bakar & Fry, 2013). New information on the nutritional value of bambangan fruit leads to the commercialization of the fruit, thus gives an economic opportunity to the people primarily in Sabah by planting a bambangan tree.

Malaysia Department of Agriculture (2015) reported that the cultivation production of bambangan fruit increased from 2013 to 2015 (from 115.3 to 121.6 metric tons). The tree can grow up around 30 meters tall and can bear up to hundreds of fruit during the season. The fruits are three times bigger in size compared to commercial mango fruit (Hassan *et al.,* 2011a). Besides, bambangan fruit was used in cooking and in the production of a functional food product (Bakar & Fry, 2013) as well as abundant sources of fiber (Ibrahim *et al.*, 2010). Meanwhile, the kernel that was considered as the waste by-product contains approximately 9.9% fat (BKF) on a dry basis (Jahurul *et al.*, 2018a,b).

Bambangan kernel fat (BKF) was previously reported as a new CB alternative source because of the similarity in its physicochemical and thermal composition with CB (Azrina *et al*., 2016; Haron & Said, 2004; Jahurul *et al.,* 2018a,2019a). Therefore, new fat resources such as BKF are extracted from the kernel of bambangan fruit and available as an agricultural waste product (Haron & Said, 2004; Azrina *et al.,* 2016; Jahurul *et al.,* 2018a; Jahurul *et al.*, 2019a). They reported comparable physicochemical properties such as iodine value, fatty acid, and triglycerides compositions with CB. The blending of SUS-TAGs-rich fats with other vegetable fats in cocoa butter or chocolate products increases their desirable physicochemical and thermal properties (Reddy & Prabhakar, 1994; Maheshwari & Reddy, 2005; Bootello *et al.,* 2012; Aydemir, 2019). Thus, it is necessary to fractionate BKF to obtain new fat fractions, which could be used in chocolate formulations with desirable characteristics.

Furthermore, the properties of BKF fractions produced from multi-stages fractionation are still unexplored area of research. Fractionation is a well-known reversible modification process applied widely today in the edible oil industry. The main purpose of this process is to modify the properties of crude fats through physical separation or crystallization by producing high-melting pure crystals with improved functionality. Moreover, the European Union (Directive 2000/36/EC) allowed fractionation in the edible industry as the only lipid modification technique that is used for CBE and CBR production due to safety concerns. Techniques such as fractional crystallization, fractional distillation, supercritical fluid extraction, liquid-liquid extraction, adsorption and membrane separation are used to separate different compounds based on their solubility, solidification, or volatility (Gibon, 2006; Timms, 2006; Harris 2017).

Currently, the fats and oils processing industry use fractionation to extend their applications as well as to replace, fully or partially, chemical modification processes (Kellens *et al.,* 2007). The controlled crystallization of fats in industrial applications gives to an end products such as chocolate as it can isolate the fats (with specific functions) from its natural resources. Therefore, pure BKF is fractionated and the current study aimed to determine the physicochemical properties, thermal properties and morphology behavior of BKF fractions produced by two-stage solvent fractionation to identify their potential applications. Solvent fractionation is referred to as fractional crystallization and is based on the different melting points as well as the solubility of fat fractions at a certain temperature (Mahisanunt *et al.,* 2017).

One stage fractionation is the common technique for preparation of fat fractions but fats obtained from multi-stage fractionation is characterized as a high-quality fat (Mondal *et al.*, 1999). Solvent fractionation of BKF and its applications is still an unexplored area of research. SUS-TAG-rich BKF fractions could be used as blending components with palm oils for the production of cocoa butter equivalents (Reddy & Prabhakar, 1994; Maheshwari & Reddy, 2005; Bootello *et al.,* 2012; Tran *et al.,* 2015). Moreover, SUU and UUU-TAGs BKF fractions would be an ideal source for producing cooking oils, frying fats, and hard structured lipids by further blending or chemical and enzymatic inter-esterification (Sue, 2009; Kim & Akoh, 2015; Jin *et al.,* 2017a).

Thus this technique are superior to modify the properties of pure BKF crude and increase its value. In this work, the properties (physicochemical, thermal, and morphology) and potential uses of bambangan kernel fractions produce from two-stage fractionation were analyzed to provide high-quality fat with diverse applications in food industries.

1.2 Problem Statement

Southeast Asia is diverse with commercial fruits and more than 200 species of edible fruit, which is beneficial to the health but remains underutilized (Abu Bakar *et al.,* 2009). *Mangifera pajang* (bambangan) is one of the underutilized fruit distributed in Borneo islands in which the pulp and juice powder is used for the production of functional food and beverages due to its nutritional values (Jahurul *et al.*, 2018c; Hassan *et al.,* 2011). However, bambangan still did not reach full utilization to widen its application into various industries, especially in confectionery products. Bambangan is usually eaten fresh or made into local cuisine, but the kernel that has nutraceutical constituents are discarded as waste (Azrina *et al.,* 2016). The kernel represents about 10-25% of bambangan fruits weight; thus, it is available in a large amount as a waste by-product (Jahurul *et al.,* 2018b).

The disposal of this waste by-product will lead to a loss in natural resources as the kernel might be available in large amounts after the disposal of the wastes from the production of bambangan juice. Besides that, BKF composition showed similar characteristics with commercial CB in the confectionery industry. Previously, Jahurul *et al.* (2018a) reported that BKF has identical attributes with CB, but it contained lower palmitic and higher stearic contents compared to CB. Thus the different technique are required to improve the properties of this fat to have desired characteristics in the confectionery industry or as CB alternatives (CBE/CBI).

Although CB ideally used in food and confectionery industries, especially chocolate, increasing in demand for chocolate caused the manufacturer and researcher to look for CB alternatives (Silva *et al.,* 2017). However, it is the most expensive ingredient for chocolate production, has a low melting point, and raising in demand for chocolate worldwide cause a shortage in CB supply (Jin *et al.,* 2017c). This chocolate may melt quickly in tropical or sub-tropical conditions. Therefore, similar fats and oils are needed to be used or substitute partially/wholly with CB in the manufacturing of confectionery product.

1.3 Significant of the Study

From this study, different characteristics of BKF fractions produced from the two-stage fractionation were determined for the utilization of fat in various industries especially confectionery industry. The potential usage of BKF fractions, uniquely as cocoa butter alternatives (CBE/CBI), identified based on their physicochemical and thermal properties. Other applications, such as in cooking and chocolate manufacturing were determined through the properties of the fractions. The modification method of solvent fractionation used in this study was to improve the properties of BKF so that it has desirable characteristics to be applied as multifunctional fats and oils in the industry.

According to Harris (2017), fractionation are used to produce fat with sharp, high melting with specific functionality. Therefore, BKF fractions produced from the two-stage fractionation have broad application, especially in confectionery products as cocoa butter alternatives. On the other hand, Jun *et al.,* (2018) mentioned that one stage fractionation is the conventional method used to prepare mango kernel fat while fats obtained from two or multi-stage fractionation usually characterized as high-quality fat. Similar to mango, the properties of the BKF were enhanced through