FRACTIONATION AND CHARACTERIZATION OF RAMBUTAN SEED FATS (*NEPHELIUM LAPPACEUM L.*) AND THEIR POTENTIAL APPLICATIONS AS COCOA BUTTER IMPROVER

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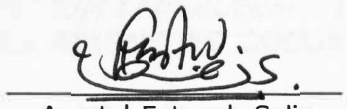

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ABSTRACT

Rambutan (*Nephelium lappaceum* L.) is a commercial tropical crop which is appreciated by consumers because of its pleasant aroma, refreshing flavor, and exotic appearance. Rambutan seeds are the main by-products in the canning industry and has attracted attention for their feasibility in industrial applications. In this study, rambutan seed fat (RSF) was fractionated by two-stage acetone fractionation and their physicochemical, thermal properties (melting and crystallization) and morphology of RSF fractions were determined in order to identify their potential applications. The results showed that the second solid fraction (F_2-S) exhibited the highest SMP (49.03 °C) and lowest IV (27.57 g iodine/g). The major fatty acids in all solid fractions were stearic (15.1-21.6%), oleic (25.0-35.5%), and arachidic (42.7-46.9%) acids. The high-melting symmetrical monounsaturated triacylglycerols (Hm-SMT) of F_2-S which is the sum of SOS (3.82%) and POS (1.19%) were also found to be higher than first solid fraction (F_1-S). The SFC of F_2-S at 20 °C (78.57%) and 35 °C (22.95%) were found to be higher than F_1-S, indicating a harder solid fraction. The melting onset (26.92-31.24 °C) and offset (49.57-52.84 °C) temperatures for both F_1-S and F_2-S increased from the crude RSF and shifted towards higher temperatures. F_2-S showed the densely packed microstructure compared to that of crude RSF and F_1-S with the diameter of 20–30 µm under microscopic observation. This study revealed that by performing fractionation of RSF, a cocoa butter improvers (CBI) could be prepared by blending them with other fats that has the potential to be utilized in food industry.

ABSTRAK

PEMERINGKATAN LEMAK BIJI BUAH RAMBUTAN (NEPHELIUM LAPPACEUM L.) DAN POTENSI KEGUNAANNYA DI DALAM INDUSTRI MAKANAN.

Rambutan (Nephelium lappaceum L.) adalah tanaman tropika komersial yang dikenali oleh pengguna kerana aromanya yang menyenangkan, rasa yang menyegarkan, dan rupa yang eksotik. Biji rambutan adalah produk sampingan utama dalam industri pengetinan dan kelebihannya untuk diaplikasikan dalam industri telah menarik perhatian. Melalui penyelidikan ini, lemak biji rambutan (RSF) dipecahkan melalui pemisahan aseton dua peringkat, dan ciri fizikokimia, sifat terma (peleburan dan penghabluran) dan morfologi pecahan RSF dikaji untuk mengenal pasti potensi kegunaannya. Dapatan kajian menunjukkan bahawa pecahan pepejal kedua (F_2-S) menunjukkan SMP tertinggi (49.03 °C) dan IV terendah (27.57 g iodine/g). Asid lemak utama dalam semua pecahan pepejal adalah stearik (15.1-21.6%), oleik (25.0-35.5%), dan asid arakid (42.7-46.9%). Triasilgliserol simetri mono-tak tepu bertakat lebur tinggi (Hm-SMT) F_2-S, di mana ianya jumlah daripada SOS (3.82%) dan POS (1.19%) didapati lebih tinggi daripada pecahan pepejal pertama (F_1-S). SFC F_2-S pada suhu 20 °C (78.57%) dan 35 °C (22.95%) didapati lebih tinggi daripada pecahan pepejal pertama (F_1-S), yang menunjukkan ianya pecahan pepejal yang lebih keras. Suhu awal (26.92-31.24 °C) dan akhir (49.57-52.84 °C) bagi kedua-dua F_1-S dan F_2-S meningkat dari RSF mentah dan beranjak ke suhu lebih tinggi. F_2-S menunjukkan mikro-struktur yang lebih padat berbanding RSF dan F_1-S dengan saiz 20-30 μm di bawah pemerhatian mikroskopik. Kajian ini mendedahkan bahawa dengan melakukan pemeringkatan RSF, penambah baik mentega koko (CBI) boleh dihasilkan dengan mencampurkan mereka dengan lemak lain yang mempunyai potensi untuk digunakan dalam industri makanan.

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LIST OF ABBREVIATIONS/SYMBOLS

%	-	Percentage
µL	-	microliter
°C	-	Celsius
>	-	More than
<	-	Less than
ABTS	-	2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)
ANOVA	-	Analysis of Variance
AOAC	-	Association of Official Analytical Chemists
AOCS	-	American Oil Chemists' Society
CB	-	Commercial cocoa butter
cm	-	centimeter
CNS	-	Central Nervous System
DPPH	-	2,2-diphenyl-1-picrylhydrazyl
DSC	-	Differential scanning calorimetry
FAO	-	Food and Agriculture Organization of the United Nations
g	-	Gram
GC-FID	-	Gas Chromatography-Flame Ionization Detector
h	-	Hour
HPLC	-	High Performance-Liquid Chromatography
HSD	-	Tukey Honest Significance
KOH	-	Potassium hydroxide
m	-	Meter
mg	-	Milligram
ml	-	Milliliter

Mono-UTAG	-	Mono-unsaturated TAG
NaOH	-	Sodium hydroxide
RSAC	-	Rambutan seed albumin concentrate
RSF	-	Rambutan seed fat
SC-CO2	-	Supercritical carbon dioxide
SFA	-	Saturated fatty acids
SFC	-	Solid fat content
TAG	-	Triglyceride
USFA	-	Unsaturated fatty acids
WHO	-	World Health Organization
RSAC	-	Rambutan seed albumin concentrate

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

In many food industries, Fats and oils are important ingredients in food that could providing an attractive flavour when consumed and giving a sensational moist in mouth. These characteristics have driven a vast potential application of fats and oils in food industries particularly when it used as an ingredient in food formulation such as bakery, confectionary, and chocolate products (Rios *et al.*, 2014). Fats and oils (i.e., cocoa, illipe, kokum, sal, shea, mango, rambutan, palm kernel, sunflower oils) are extracted from fruit seed and the fatty acids differ in terms of plant growth (Jahurul *et al.*, 2014a; Sonwai *et al.*, 2012; Kumar *et al.*, 2016; Solís-Fuentes *et al.*, 2010; Winayanuwattikun *et al.*, 2008). There is an attempt to find another fats and oils alternative with low cost and highly available such as mango and rambutan fruit seed and these natural sources of fats have been used in food formulation to develop a food product with desired properties. However, some of these physicochemical and thermal properties have limit their potential application in food product development (Jahurul *et al.*, 2019). Therefore, modification of fats and oils is needed in order to improve their properties, thus improving of food properties and increasing its consumption.

Pure fats and oils often do not have satisfactory physicochemical and thermal properties for the use in food products. Modification offers the possibility of changing the properties of oils and fats within wide ranges, thus making them suitable for many uses or for making oils and fats with desirable properties available in sufficient quantities. The techniques which are blending, hydrogenation, fractionation, and chemical and enzymatic interesterification used to produce fats for margarine,

confectionery, cosmetic, and chemical industries (Momeny *et al.*, 2013; Kang *et al.*, 2013; Jin *et al.*, 2016; Kadivar *et al.*, 2016; Lakum & Sonwai, 2018). Solvent fractionation is applied to produce structural lipids such as hard fats (high-melting-point triacylglycerols), polyunsaturated fatty acid-rich oil, creams, and margarines due to the high separation efficiency and yield of aimed fractions with sharper melting properties (Gibon, 2006; Kellens *et al.*, 2007; Mu *et al.*, 2016; Jin *et al.*, 2016). For example, palm oil is fractionated into palm stearin and liquid and is the most widely fractionated oil worldwide (Senanayake & Shahidi, 2005; Gibon, 2006). Meanwhile, these palm oil fractions have been used to prepare wide variety of food products, especially confectionery products (Jahurul *et al.*, 2014a; Lakum & Sonwai, 2018).

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; Baliga & Shitole, 1981). Solvent fractionation of RSF and its applications is still an unexplored area of research. Therefore, this technique is superior to alter the properties of RSF crude and increase its value. In this work, the properties (physicochemical, thermal, and morphology) and potential uses of rambutan seed fractions produce from two-stage fractionation were analyzed to provide high-quality fat with diverse applications in food industries.

In food industry, fats play crucial role in order to produce desirable physicochemical characteristics, smoothness, velvety/valuable texture, appearance, alluring aromas and satisfying dietary outcomes (Rios *et al.*, 2014). The utilization of such fats (i.e., cocoa spread, illipe margarine, kokum spread, sal margarine, shea spread, mango, rambutan, and palm oil) are important in chocolate products as they provide the perfect qualities of polish, desirable melting properties at body temperature and preferential consistency (Jahurul *et al.*, 2014a; Bootello *et al.*, 2012; Sonwai & Ponprachanuvut, 2012; Munchiri *et al.*, 2012; Kumar *et al.*, 2016; Okullo *et al.*, 2010; Solís-Fuentes *et al.*, 2010; Winayanuwattikun *et al.*, 2008).

Rambutan (*Nephelium lappaceum* L.) is one of the most important tropical commercial fruit widely cultivated in Southeast Asia, Australia, South America, and

African countries. It is closely related to the subtropical fruits such as lychee (*Litchi chinensis* Sonn.), longan (*Euphoria longan* Steud.), and pulasan (*Nephelium mutabile* Blume) in the same family Sapindaceae. Thailand, Malaysia, and Indonesia are the leading producer (80% of the total world production) and exporter of the rambutan fruit (Tindall, 1994; Ahmad & Chua, 2013). Recently, Mahisanunt *et al.* (2017) reported that Thailand produce 318,000 tons rambutan from 2014 to 2015. It is rich in sugars (glucose and sucrose), proteins, vitamins, minerals, antioxidants, and valued for its refreshing flavor, pleasant aroma, and exotic appearance (Ong *et al.*, 1998; Zhuang, *et al.*, 2017; Li *et al.*, 2018). Generally, rambutan is consumed as fresh. Rambutan juice, jam, jelly, chips, marmalade, spread, rambutan stuffed with a chunk of pineapple and canned in syrup are the main industrial products in Malaysia and Thailand (Morton, 1987, Chai *et al.*, 2019a). After direct consumption or industrial processing, the residues mainly seed and peels are discarded as by-products (Solís-Fuentes *et al.*, 2010). Recently, Mahisanunt *et al.* (2017) reported that the yearly average 1900 tons of rambutan seeds are discarded as by-products in Thailand. The huge quantities of this industrial by-products cause serious environmental problems and also results in economic losses if not utilized effectively (Chai *et al.*, 2018).

Although the rambutan seeds are a potential functional ingredient in food processing (Harahap *et al.*, 2012; Vuong *et al.*, 2016), most of these by-products are considered industrial waste becomes a source of environmental pollution (Chai *et al.*, 2018; Evaristus *et al.*, 2018). In developed countries, food industries produced around 39% food wastes and these wastes are being used as raw material for making various food products (Mirabella *et al.*, 2014). These by-products are not only growing problem but also are economically limiting factors for their disposal (costs to dry, store and shipping) (Schieber *et al.*, 2001; Jahurul *et al.*, 2015). Shalini and Gupta (2010) reported that annually $10 million spent for the disposal of only apple pomace in the USA. The proper use of rambutan seed could generate economic gains for industry, contributing as potential protein ingredient to reduce nutritional deficiencies, promoting health (as α-amylase inhibitory peptides), reducing disposal cost, and reducing the environmental problems. This review examines the nutritional and functional potential of the rambutan

seeds and the mainstream sectors of applications such as in the food, pharmaceutical, nutraceutical, and cosmetic industries through scientifically proven information.

Cocoa butter (CB), is a high-priced constituent and crucial ingredient in chocolate formulations, and responsible for the melting behavior and glossy texture of chocolate products (Jin *et al.*, 2016; Kadivar *et al.*, 2016). Despite being the most ideal constituent and ingredients in chocolate and other confectionery products, the low melting point of CB causes the products, especially chocolate to melt easily in subtropical and tropical areas (Shahidi, 2005). Furthermore, demand for chocolate products is keep increasing despite the high-cost and uncertainty in the supply of CB increasing the interests among manufacturers and researchers to find for alternatives such as cocoa butter equivalents (CBE) (Tchobo *et al.*, 2009).

Afoakwa *et al.* (2008) reported that the crystallization and component of cocoa butter play a crucial role in the final product's quality during chocolate manufacture. The melting point of the chocolate product is highly affected by the crystalline state and the ratio of solid fat content. Solís-Fuentes *et al.* (2010) mentioned that the last peak curve of the rambutan seed fat melting point (~45 °C) was found to be higher than of cocoa butter and this gives advantages in the manufacturing process in most tropical countries. Meanwhile, at low temperature, rambutan seed fat was found to be smoother than cocoa butter, yet more consistent and solid at higher temperatures. This is due to the disparity in the composition of cocoa butter and rambutan seed fat (Solís-Fuentes *et al.*, 2004).

1.2 Problem Statements

Rambutan seed is considered as a waste in rambutan canning manufactures with a noteworthy value as much as 94,500 tonnes/year from Thailand, Indonesia and Malaysia alone (Norlia et al., 2011). This massive value has become an issue that need to be solved. Hence, it has potential to be the new source of natural edible fat such to produce confectionary products that would be beneficial in food industry (Solís-Fuentes et al., 2010; Morton, 1987). However, RSF crude has wide crystallization and melting ranges and varies in triglyceride patterns which are unsuitable for use in specific food

products (Sirisompong *et al.*, 2011). Thus, a modification process should be applied to improve the crystallization and melting properties of RSF.

Cocoa butter (CB) is known as the ideal ingredient in most confectionary products especially chocolate, but chocolate can easily melt in tropical and subtropical areas due to the low melting point of CB (Shahidi, 2005). High price and rising cost, increasing demand and low supply due to only few countries have been cultivated and became cocoa butter supplier (Dewettinck & Depypere, 2011; Tchobo *et al.*, 2009). Thus, in the manufacture of chocolates produced from natural sources, it is important to consider a cheaper and more accessible alternative fats of cocoa butter to be used partially/wholly with CB in order to increase the quality of the product and reduce the cost of production, as well as to alter the production process and to create new business values (Khairy & Yang, 2016).

Fractionation is used to produce fat with sharp, high melting with specific functionality. Therefore, the two-stage fractionation produced RSF fractions produced from with wide applications, especially in confectionery products as alternatives to cocoa butter, specifically as cocoa butter improver (Harris, 2017). On the other hand, Jun *et al.*, (2018) stated that one stage fractionation is the common method used to prepare mango kernel fat. However, the author found that fats obtained from two or multi-stage fractionation usually characterized as high-quality fat. Similar to mango, the properties of the RSF have been enhanced through the solvent fractionation, which yields fats with high melting properties and specific industrial applications. Fractionation improves the quality of rambutan seed fat produced from the fractionation as well as increases its values. Therefore, the rambutan seeds could become new fat resources and could help in reducing the manufacturing cost for one product. Commercial cocoa butter would have a new alternative that has similar desirable characteristics, which is beneficial in various industries, especially confectionery products. Therefore, new cocoa butter alternatives (CBI/CBE) or blending component as a source of CB are expected to be produced from this study for the use as a functional food in the confectionery industry.

This study will discuss the by-product of rambutan, specifically the rambutan seeds for rambutan seed fat production. The aim of this study was therefore to use fats obtained from the rambutan seed fat fractionation to study their physicochemical properties, thermal properties, and morphology. This information will contribute to a good evaluation of the potential of rambutan seed fat and its fractions as alternatives to cocoa butter (CBAs) in the food industry.

1.3 Objectives

This study aims to:

1. To produce new fats from the two-stage fractionation of rambutan seed fats.

2. To evaluate and compare the physicochemical and thermal properties (fat yield, iodine value, slip melting point, free fatty acid, solid fat content, triglycerides, and fatty acids) of rambutan seed fat and its solid fractions using different chromatographic and thermal techniques

3. To study and compare the morphology of rambutan seed fat and its solid fractions using PLM.

1.4 Significances of Study

The improved physical characteristics (thermally) of rambutan seed fat may make it a suitable to be cocoa butter equivalent (CBE) or cocoa butter improver (CBI) in chocolate and other confectionary products. To the best of the authors' knowledge, the two-stage fractionation of rambutan seed fat have not been studied yet. Hence, this study is aimed to provide new information about the product of fractionation of rambutan seed fat. Rambutan seed fat has good potential as a promising raw material for the industrial application especially in food industry. The potential usage of RSF, uniquely as cocoa butter alternatives (CBA/CBI) in tropical countries, 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 RSF so that it has desirable characteristics to be applied as multifunctional fats and oils in the industry. The utilization of rambutan seeds to produce rambutan seed fat and its fractions could be one of the solutions for a better waste management and ensure sustainable production.