EFFECTS OF FERMENTATION AND ROASTING TREATMENT ON FAT OF RAMBUTAN (*Nephelium lappaceum*) SEED AND ITS POTENTIAL UTILIZATION AS CONFECTIONERY FAT

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Alhamdulillahirobbil'alamiin, all praise is due to Allah SWT, the Lord of the Worlds, Most Gracious, Most Merciful. Master of the Day of Judgment. Thee do we worship, and Thine aid we seek. Shalawat and salam we always dedicate to our prophet Muhammad (pbuh), to his family, best friends and his faithful followers.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS/ABBREVIATION

Symblos/Abbreviation	Caption
AV	Acid value
BI	Browning index
CB	Cocoa butter
DSC	Differential scanning calorimeter
DPPH	2,2-diphenyl-1-picrylhydrazyl
FA	Fatty acid
FAME	Fatty acid methyl esters
F-RSF	Fermented *rambutan* seed fat
FR-RSF	Fermented-roasted *rambutan* seed fat
GC	Gas Chromatogrhapy
MRPs	Maillard reaction products
MUFA	Monounsaturated fatty acid
n FR-RSF	*n* days fermented-roasted *rambutan* seed fat
n F-RSF	*n* days fermented *rambutan* seed fat
PAV	p-anisidine value
PUFA	Polyunsaturated fatty acid
PV	Peroxide value
R-RSF	Roasted *rambutan* seed fat
RSA	Radical scavenging activity
RSF	*Rambutan* seed fat
SFA	Saturated fatty acid
SFI	Solid fat index
TAG	Triacylglycerol
TGA	Thermal gravimetric analysis
TPC	Total phenolic content
U-RSF	Untreated *rambutan* seed fat

KESAN PENGOLAHAN FERMENTASI DAN PEMANGGANGAN KE ATAS LEMAK BIJI *RAMBUTAN* (*Nephelium lappaceum*) DAN POTENSI PENGGUNAANNYA SEBAGAI LEMAK KONFEKSIONERI

ABSTRAK

Rambutan (*Nephelium lappaceum* L.) ialah buah eksotik yang berasal daripada Malaysia dan Indonesia, yang mana kebiasaanya digunakan dalam keadaan segar setelah dituai. Pada masa kini, rambutan telah dikomersialkan kepada pelbagai produk seperti buah-buahan dalam tin, jus, jem, jeli, dan marmalad. Dengan peningkatan dalam penghasilan produk rambutan, isu produk sampingan juga telah menjadi satu masalah yang perlu diselesaikan. Biji rambutan ialah salah satu produk sampingan yang mempunyai potensi untuk digunakan, terutama sekali sebagai lemak biji rambutan (RSF). Penyelidikan ini dijalankan bagi mengkaji prospek fermentasi dan pemanggangan keatas biji rambutan untuk meningkatkan sifat-sifat nilai tambah lemak biji rambutan. Biji-biji rambutan telah difermentasi selama 3, 6, 9, dan 12 hari serta diikuti dengan pemanggangan pada suhu 150˚C untuk 30 minit. Pengekstrakan RSF telah dilakukan secara fizikal menggunakan mesin tekanan skru. Pengendalian fermentasi dan/atau pemanggangan yang dijalankan keatas biji rambutan telah mengubah ciri-ciri fisikokimia lemak biji rambutan secara signifikan. Fermentasi tidak lebih daripada 9 hari diikuti dengan proses pemanggangan telah menghasilkan RSF yang mempunyai konsistensi, penghabluran, dan titik cair yang lebih tinggi, tetapi stabiliti termal yang rendah dibandingkan RSF yang tidak difermentasi. Pengendalian ini juga telah meningkatkan aktiviti antioksida RSF secara signifikan. Sebagai tambahan, sifat nilai tambah seperti perisa *pyrazine* yang dikehendaki juga telah diperhatikan untuk diperkembangkan melalui pengendalian fermentasi dan

pemanggangan. Proses fermentasi selama 6 hari yang diikuti dengan pemanggangan telah dinilai untuk menyerlahkan karakter RSF yang dikehendaki. Selain itu, kami dapati, ia berpotensi untuk digunakan sebagai pengganti mentega koko, yang mana membolehkan pencampuran dengan mentega koko dalam nisbah yang kecil. Namun begitu, ia juga boleh digunakan sebagai alternatif baru untuk menggantikan mentega koko dalam keadaan ketiadaaan mentega koko. Hasil penyelidikan mencadangkan fermentasi dan pemanggangan biji rambutan telah berjaya meningkatkan kualiti lemak biji rambutan yang mana mempunyai potensi untuk digunakan dalam bidang industri, terutama sekali dalam konfeksionari.

EFFECTS OF FERMENTATION AND ROASTING TREATMENT ON FAT OF *RAMBUTAN* (*Nephelium lappaceum*) SEED AND ITS POTENTIAL UTILIZATION AS CONFECTIONERY FAT

ABSTRACT

Rambutan (*Nephelium lappaceum* L.) is popular exotic fruit native to Malaysia and Indonesia which usually consumed fresh after harvested. Today, *rambutan* is already commercialized for the production of canned fruit, juice, jam, jellies, and marmalades. As the production of *rambutan* products increase, by-product issues also become one of the problems that need to be solved. *Rambutan* seed is one of *rambutan* by-product that has a potential to be utilized, especially as *rambutan* seed fat (RSF). This research was carried out to study the prospect of fermentation and roasting pretreatment on *rambutan* seed to increase the value-added properties of *rambutan* seed fat. *Rambutan* seeds were fermented for 3, 6, 9, and 12 days and followed by roasting at 150˚C for 30 minutes. The extraction of RSF was carried out physically using a screw-press machine. Fermentation and/or roasting treatment carried out on *rambutan* seed significantly altered the physicochemical properties of *rambutan* seed fat. Fermentation not more than 9 days followed by the roasting process resulted in RSF with higher consistency, crystallization and melting points but lower thermal stability than unfermented RSF. The treatments also significantly increased the antioxidant activity of RSF. In addition, value-added properties such as desirable pyrazine flavor were also observed to be developed by the fermentation and roasting treatment. Six days of fermentation process followed by roasting has been observed to exhibit desirable characteristic of RSF. In addition, RSF from 6 day fermented and roasted *rambutan* seed showed its potential to be

utilized as cocoa butter replacer which allows the mixing with cocoa butter in small ratios. However, it can also be utilized as new alternative of cocoa butter substitutes which can be used in the absence of cocoa butter. These results suggest that fermentation and roasting of *rambutan* seed successfully increase the quality of *rambutan* seed fat which can potentially be utilized in industry, especially in confectionery industries.

CHAPTER 1

INTRODUCTION

1.1 Background

Rambutan (*Nephelium lappaceum* L.) is popular exotic fruit native to Malaysia and Indonesia. It is usually consumed fresh after harvested. Currently, *rambutan* is amongst the priority commodities vastly grown in Malaysia, Indonesia, Thailand, Philippines, and Vietnam (Yaacob & Subhadrabandhu, 1995; Rasip *et al*. 1999). *Rambutan* is already industrialized into the production of juice, jam, jellies, and marmalades or canned in syrup (Morton, 1987; Poerwanto, 2009). As is, the case for manufacturing process of food products using fresh commodities as raw material, the occurrence of waste becomes a common issue that needs to be solved. The edible portion of *rambutan* is approximately 33-56% (fresh fruit basis) depending on the species. This means that a manufacturing process using *rambutans* will generate the rind (approx. 39-61%) and seeds (around 5-6% of fresh fruit) as by-products which are considered as wastes (Tindall, 1994). This value becomes noteworthy since cumulative production using *rambutans* by major producers such as Thailand, Indonesia, and Malaysia could reach as much as 1.5 million tons per year (BPS, 2013; FAO, 2007; Norlia *et al*, 2011).

Increasing concern on the zero waste processing system has inspired the fruit and vegetable industry to apply the waste-bioremediation system to produce potential value added products. This idea emerged due to the fact that fruit and vegetable processing industry could generate up to 60% of raw material as solid wastes. This solid waste was reported to possess enormous potential that can be utilized (Eipeson and Ramteke, 2003). Currently, the rind of *rambutan* fruit has been utilized as feed, source of antioxidant, and for medicinal purpose as an anti-hyperglycemic agent (Mulyanto, 1993; Palanisamy *et al*. 2008; Palanisamy *et al*. 2011, Perera *et al*. 2012). The seeds can conventionally be consumed after roasting or boiling. In addition, *rambutan* seed fats can be used in candles, soaps and fuel manufacturing (Morton, 1987), and latest research carried out by Solís-Fuentes *et al*. (2010) and Sirisompong *et al*. (2011) mentioned that edible *rambutan* fat has physical and chemical characteristics which makes it possible to be applied in cosmetics and food industries.

Among the food by-product processing methods, chemical free processing method is preferred due to the increasing public awareness on environmental and health issues (Febrianto & Yang, 2011). Solid-state fermentation is one of the biological methods that have been vastly applied into the production of microbial products such as feed, food, industrial chemicals and pharmaceutical products (Pandey, 2003). In the field of by-product processing, solid-state fermentation have been used for composting, production of bioactive compounds, and production of chemicals. However, several applications in particular agricultural products have led into the production of high value-added and commercially high-priced functional food product (Couto & Sanromán, 2006). For example, the proper fermentation of cocoa beans followed by roasting process can produce a higher quality and more valuable product than the unprocessed one (Reineccius *&* Henry, 2006; Bonvehi and Coll, 2002).

A roasting process is usually utilized to improve the palatability, to alter the sensory properties and to extend the range of tastes, aromas and textures of the food; at the same time it also destroys enzymes and micro-organism, lowers water activity to some extent, thus preserving the food (Fellows, 2000). In cocoa bean production, apart from improving the palatability, roasting is used to induce non-enzymatic browning reaction that generates the unique cocoa flavor. This step is very important in order to produce not only edible but also a high quality cocoa bean product (Bonvehi & Coll. 2002).

This thesis will discuss about the *rambutan* by-product especially *rambutan* seeds, along with the insight into the application of solid-state fermentation and roasting process to produce *rambutan* seed fat. This study is aimed to provide a better understanding of the effects of fermentation and roasting processes to the quality of *rambutan* seed fat including its physicochemical characteristic, antioxidant activity, flavor development and its possible application in the industry as confectionery fat.

1.2 Problem statement

1. *Rambutan* seed is an industrial by-product considered as waste, its utilization is possible to produce value-added product;
2. *Rambutan* seed fat is a potential material; however its application is still limited to unprocessed *rambutan* seed fat and subsequent processes are needed to improve the characteristics of *rambutan* seed fat;
3. Fermentation and roasting are quality improvement processes, but compared to other controlled enzymatic process, natural fermentation could produce varying results. More information on how the fermentation works and its effect on *rambutan* seed fat is needed to provide a better understanding of its application on rambutan seed fat characteristics.

1.3 Importance of study

1. There is no previous study and established data on lipid characteristic of *rambutan* seed which was subjected to fermentation and roasting process;
2. Product resulted from this research could be a newfound alternative of confectionery fat with possible application in industry;
3. Data established in this research could be relevant information for industrial application.

1.4 Objectives

The main objectives of this study are:

1. To improve the physicochemical characterisctics of *rambutan* seed fat by applying fermentation and roasting process on the seeds;
2. To determine the effect of fermentation and roasting on the antioxidant properties of *rambutan* seed fat;
3. To enhance the flavor properties of *rambutan* seed fat through fermentation and roasting process of *rambutan* seed;
4. To determine the application of *rambutan* seed fat as confectionery fat by incorporating it with cocoa butter.

1.5 Thesis outline

In this thesis, the prospect of utilizing fermentation and roasting process on *rambutan* seed to improve the characteristic of *rambutan* seed fat (RSF) will be discussed. This thesis consists of seven main chapters. The first and second chapter will provide general introduction of the study and literature information regarding the research. The following three chapters will discuss the effects of fermentation and roastion of *rambutan* seed on the fat properties including, physicochemical characteristic, antioxidant properties, and flavor development. The sixth chapter will discuss the possible application of fermented and/or roasted RSF to be applied as confectionery fat. Whereas, overall conclusions of the research and several recommendation for further studies are stated in last chapter. Briefly, the descriptions of each chapter are as follows:

CHAPTER 1 provides general introduction to this research, including the background of the study as well as the current development and its challenges. Main problems regarding *rambutan* seed that is still considered as industrial waste along with its potential development process for further utilization is discussed. The importance of this study is also described briefly, since current utilization of the subject of this research is still limited despite its potential.

CHAPTER 2 is aimed to provide general information of *rambutan* including its production and distribution, anatomy, and its by-product. This chapter also provides the information on the potential application of *rambutan* by-product in industry, as well as the potential utilization of solid-state fermentation and roasting that can be applied to improve the quality of *rambutan* seed fat.

CHAPTER 3 entitled "Character modification of *rambutan* seed fat by means of fermentation and roasting" will discuss the effects of fermentation and/or roasting process of *rambutan* seed on the physicochemical characteristic of RSF produced.

CHAPTER 4 entitled "Effect of fermentation time and roasting process on the antioxidant properties of *rambutan* (*Nephelium lappaceum*) seed fat" will discuss the antioxidant activity of RSF produced in this research.

CHAPTER 5 entitled "Identification of flavour compounds in fermented and/or roasted *rambutan* seed's fat determined by solid phase microextraction-gas chromatography" will provide the information on identification of the flavor compounds of *rambutan* seed fat developed as the effect of fermentation and roasting process of *rambutan* seed.

CHAPTER 6 entitled "Thermal behaviour, microstructure and texture properties of fermented *rambutan* seed fat and its mixtures with cocoa butter" will discuss the possible application of fermented and/or roasted RSF to be applied as cocoa butter alternatives (CBA) by incorporating RSF with cocoa butter.

CHAPTER 7 will provide the overall conclusion of the findings in this research as well as several recommendations for further studies regarding *rambutan* seed fat.

CHAPTER 2

LITERATURE REVIEW

This literature review is divided into 4 sections consisting of sections on (1) *rambutan*, (2) confectionery fat, (3) solid-state fermentation and (4) roasting. Section (1) is aimed to provide a general information of *rambutan* including its production and distribution, anatomy, and its by-product. Subsequent sections provide the information on the potential application of *rambutan* by-product in industry as well as the potential processing method that can be applied (Sections 3 and 4).

2.1 *Rambutan*

2.1.1 Rambutan production and distribution

Rambutan (*Nephelium lappaceum* L.) is native in Malaysia and Indonesia. It is suspected that *rambutan* is originally from the Malay Archipelago, from where it spread westwards to Thailand, Myanmar, Sri Lanka and India, whereas eastward distribution was probably to Vietnam, the Philippines and Indonesia (Delabarre, 1989; Tindall, 1994). '*Rambutan*' is the general name in Malaysia, Indonesia and the Philippines, which it derived from Malay word '*rambut*' means 'hair'. Aside from '*rambutan*', it is also known as '*usan*', '*usau*' or '*usare*' in the Philippines, '*ngo*' or '*phruan*' in Thailand, and '*ser mon*' or '*chle sao mao*' in Cambodia (Tindall, 1994).

Rambutan belongs to the family of *Sapindaceae* which covers about 125 genera and more than 1000 species including lychee, pulasan and longan (Popenoe, 1920; Bailey, 1949). The *rambutan* tree is a tropical medium-sized evergreen, varying in size from 12 to 25 meters with seasonal floral development (Popenoe, 1920; Tindall, 1994). *Rambutan* fruits are classified as non-climacteric fruits which will not continue to ripen once harvested. Usually, *rambutan* is harvested on the basis of its skin color which varies from yellow, orange, pink, and red when ripe depending on the cultivar. The fruit becomes fully mature approximately 15 weeks after fruit set, and the acceptable appearance is discovered between 16-28 days after fruit's color-break (O'Hare 1995; Tindall, 1994).

According to the Indonesian Ministry of Research and Technology (Ristek, 2000) and Rukmana & Yuniarsih (2002), there are 22 popular cultivars of *rambutan* in Indonesia, in which *Binjai, Rapiah, Lebak Bulus, Antalagi, Sibongkok, Sibatuk Ganal, Garuda* and *Nona* are cultivars that have relatively high economic value. It is also reported that the productivity of these cultivars are 40-68, 18-30, 50-100, 160-210, 175-225, 240-280, 200-270, and 20-22.5 kg $tree^{-1}$ $year^{-1}$, respectively. Whereas in Malaysia, *rambutan* cultivars are named with code letter 'R', and R3, R4, R99, R169, R170, RS6, and R191 (yield approximately 1.2 - 15 tons ha^{-1} $year^{-1}$) are the cultivar clones recommended by the Department of Agriculture (DOA, 2013).

Global production of tropical fruit has increased significantly from 60 million tons in 1999-2001 to more than 70 million tons in 2005 (FAO, 2007). However, exotic fruit such as durian, *rambutan* and guava are still considered as minor due to their low production which is less than 5 million tons a year (FAO, 2007). *Rambutan* is still mostly produced in ASEAN countries, in which Thailand, Indonesia and Malaysia are the major producers. In 2005, Thailand produced more than 700,000 tons of *rambutan,* whereas Indonesia

675,579 tons, and Malaysia up to 130,000 tons (BPS, 2013; FAO, 2007). In the latest reports of *rambutan* production recorded in 2011, Indonesia is reported to produce 811,909 tons of *rambutan*, while Malaysia 86,085 tons; whereas there are no report on *rambutan* production in Thailand, but it is predicted at a range of 700,000 tons (BPS, 2013; FAO, 2007).

Aside from the major producer countries of *rambutan*, there are also several attempts to cultivate *rambutan* in other countries, even though the yield is still not comparable. The Philippines produced 6,270 tons *rambutan* in 2011, whereas Vietnam is predicted to produce 500-650 tons *rambutan* since 2011. Non-Asian country such as Mexico and Australia has been developing *rambutan* with the production worth 7,000 tons and 1000 tons in 2011, respectively. These developments cannot be separated from the rise in demand from top importers such as United Arab Emirates, Korea and the Netherlands, and the increasing export opportunity to The United States, and European country (ITFN, 2013; DAFF, 2013; VietNamNet; 2013).

2.1.2 Rambutan fruit

The *rambutan* fruit consists of the skin, flesh and seed. *Rambutan* skin is covered in hair-like spinterns that can be colored similarly to the skin or remain green according to the cultivar, whereas the skin itself can vary in color from pink to deep crimson and from yellow to yellow-orange (Watson, 1988). The edible portion of *rambutan* is a fleshy, tanslucent-white sarcotesta which arises from integument surrounding the seed. In several cultivars, the sarcotesta and integument can be separated easily; while others are more difficult (O'Hare, 1994). Detailed schematics of *rambutan* fruit is presented in Figure 2.1

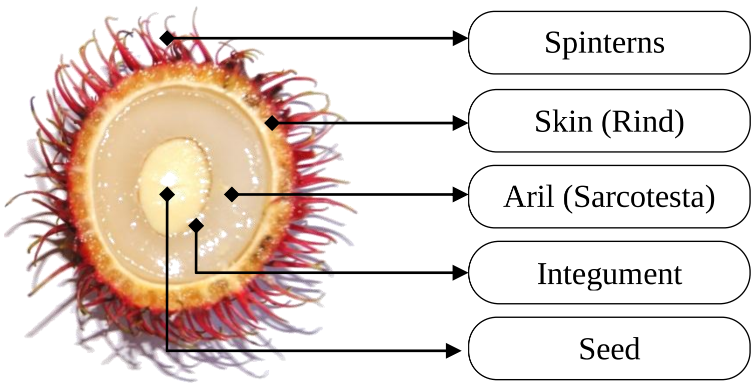

Figure 2.1 Cross-section of *rambutan* fruit (source: O'Hare, 1995)

According to Ng & Thamboo (1967), *rambutan* fruits are composed of 47.5 % skin, 46.1 % of aril and 6.3% of seed in which the edible portion (aril) is high in calcium and Vitamin C. However, the proportion of skin, oil and seed are different depending on the cultivar. Watson (1984) also mentioned that the proportion of aril could vary from 33% to 56% based on the cultivar, likewise the skin may also vary from 39% to 61%, and the seeds from 5% to 7%. The compositions of *rambutan* edible portion as reported by several researchers are summarized in Table 2.1.

Table 2.1 Composition of *rambutan* edible portion per 100 g

Component	Lam & Kosiyachinda (1987)	Rukmana & Yuniarsih (2002)	
		Broto (1981)	Indonesia Ministry of Health (1981)
Water (g)	82.10	80.40	80.50
Protein (g)	0.90	1.00	0.90
Fat (g)	0.30	0.30	1.00
Ash (g)	0.30	0.30	-
Carbohydrate (g)			18.10
Glucose (g)	2.80	2.80	-
Fructose (g)	3.00	3.00	-
Sucrose (g)	9.90	9.90	-
Mallic acid (g)	0.05	0.05	-
Citric acid (g)	0.31	0.31	-
Dietary fiber (g)	2.80	2.80	-
Vitamin C (mg)	70.00	66.75	58.00
Niacin (mg)	0.50	0.50	-
Thiamin (mg)	0.01	0.01	-
Riboflavin (mg)	0.07	0.07	-
Mineral (mg)			16.00
K (mg)	140.00	140.00	-
Na (mg)	2.00	2.00	-
Ca (mg)	15.00	13.00	-
Mg (mg)	10.00	10.00	-
Fe (mg)	0.10	0.80	0.50
Zn (mg)	-	0.60	-
P (mg)	0	16.00	16.00
Energy	297 kJ	297 kJ	69 kJ

(-): not analyzed

The aril (sarcotesta) of the *rambutan* have a refreshing flavor quite similar to a lychee, but it is less aromatic and the texture is firmer and less juicy (Ong *et al*., 1998). Until now, *rambutan* aril is still considered as the only part of *rambutan* that has economic value, and has already been industrialized into the production of juice, jam, jellies, and marmalades or canned in syrup (Morton, 1987; Poerwanto, 2009). During the *rambutan* canning process, *rambutan* fruit is cut mechanically to separate the *rambutan* pulp and seeds; however, it will produce *rambutan* seeds by-product which still covered by a small amount of *rambutan* pulp (Yang *et al*., 2012). Despite the minor applications of its byproducts such as *rambutan* rind for local medicine, and the utilization of *rambutan* seed in candles, soap, and fuel manufacturing, the rind and the seeds of *rambutan* is still considered as waste in a *rambutan* fruit processing.

2.1.3 By-products of rambutan fruit processing

(*a*) *Rambutan rind*

Rambutan rind has been reported to contain a toxic saponin, but in Java and Malaysia it is used as a local medicine after drying, whereas the fresh one is used as dyes (Tindall, 1994). In 1992, Chalimah (1992) reported that the rind of the several *rambutan* cultivars in Indonesia (*Silengkeng*, *Sinyonya* and *Lebakbulus*) consist of 25.54-33.73% of cell wall, 2.62-8.71% hemicellulose, 22.92-24.33% lignocellulose, 8.68-10.39% cellulose, and 8.43-10.04% lignin (dw). Following this work, Mulyanto (1993) reported the proximate composition of *rambutan* rind, which consists of ± 75% moisture (ww), and 7.81-8.80% protein, 2.04-4.01% fat, 13.18-16.72% fiber, 2.04-3.23% ash, and 70.37-71.35 carbohydrate in the dry matter. These reports also concluded that *rambutan* rind could be utilized as a feed source for ruminants.

Several researches have also reported the occurrence of anti-nutrients and vitamin content in *rambutan* rind. Fila *et al*. (2012) reported that 100 g fresh *rambutan* rind contains anti-nutrient such as saponin (0.53 mg), alkaloid (2.17 mg), tannin (1.35 mg), phytate (0.17 mg), phenol (0.31 mg), oxalate (0.12 mg), and flavonoids (88.84 mg); whereas Johnson *et al*. (2013) reported that 100 g fresh *rambutan* rind contains 10.60 μg carotene, 0.04 mg thiamine, 0.06 mg riboflavin, 0.31 mg niacin, and 7.43 mg ascorbic acid.

Furthermore, recent studies that focused on the effects of bioactive compound in *rambutan* rind has led into the possibilities of *rambutan* rind to be used in medicine as a source of antioxidant, antimicrobial, antiviral, anticancer, antiproliferative, antihyper-glycemic, and antihypertensive agents (Mohamed *et al*., 1994; Khonkarn *et al*., 2010; Sun *et al*., 2012; Wan Nur Hidayati *et al*., 2011; Kumar *et al*., 2012; Palanisamy *et al*., 2011; Maran *et al*., 2013). The list of several researches that has been carried out regarding the bioactive compound study is shown in Table 2.2.

The possible application of the *rambutan* rind extract in pharmaceutical cannot be separated from the role of geraniin, the major component of *rambutan* rind isolated from its ethanolic extract. Geraniin can be classified as polyphenol compound under the group of ellagitannins (hydrolyzed tannins). Geraniin has been reported to have a higher antioxidant activity compared to acarbose (carbohydrate hydrolysis inhibitor), quercetin (aldol reductase inhibitor) and green tea (AGE inhibitor). It also possess in vitro hypoglycemic activity (alpha-glucosidase inhibition :IC50=0.92 μg/ml and alpha-amylase inhibition: IC50=0.93 μg/ml). Recently, there is also reported that large-scale purification of geraniin from *rambutan* rind has been studied for its potential to produce high purity and high yield geraniin (Perera *et al*., 2012; Palanisamy *et al*., 2011).

Table 2.2 List of bioactive compound studies from *rambutan* rind

Focus of study	Extraction/isolation method	Performance assays	Researcher
Antibacterial activity	Petroleum ether, $CHCl_3$ and Ethanol extraction	Inhibition of gram positive and gram negative bacterial activity using agar diffusion method	Mohamed & others, 1994
Antioxidant & antiproliferative activities	Crude extract obtained by ethanol extraction, fractioned extracts obtained by consecutive extraction by hexane, ethyl acetate, butanol, and methanol	Total phenolic content ABTS assay FRAP assay Cytotoxicity assay	Khonkarn & others, 2010
Antioxidant activities	Microwave assisted extraction using water, 60% ethanol and 60% methanol.	Soluble phenolic content Reducing power assay, $DPPH^{\bullet}$ assay, Hydroxyl radical scavenging assay, Lipid peroxidation inhibition ability assay, Nitrite-scavenging ability assay	Sun & Others, 2012
Cancer chemopreventive agent	Ethanol extraction	Cell proliferation assay Cell cycle progression and apoptosis induction, Cytotoxicity assay	Wan Nur Hidayati & Others, 2011
Antioxidant and therapeutic effect	Ethanol extraction	Total phenolic content Histopathological assay & Immunohistochemistry assay using collagen-induced arthritis rats.	Kumar & others, 2012
Isolation of geraniin Antihypergly-cemic activity	Ethanolic extraction Geraniin isolation using acetonitrile and glass column packed with LiChroprep RP-18	Galvinoxyl and ABTS assay, Antihyperglycemic assay (alpha glucosidase, inhibitory activity, alpha-amylase inhibitory, aldose-reductase inhibitory and advanced glycation endproducts formation inhibitory.	Palanisamy & others, 2011
Antioxidant content	Ultrasound assisted distilled water extraction	Total anthocyanin content Total phenolic content Total flavonoid content	Maran & others, 2013

(*b*) *Rambutan seed*

Rambutan seed has been reported to contain 14.1% protein, 37.1-38.9% crude fat, 2.8-6.6% crude fiber and 2.6-2.9% ash on dry weight basis (Augustin & Chua, 1988). Compared to the other parts, *rambutan* seed possesses relatively high anti-nutrient compound; containing 0.98 mg of saponin, 0.82 mg alkaloid, 0.15 mg tannin, 0.40 mg phytate, 0.20 mg phenol, 0.26 mg oxalate, and 16.00 mg flavonoid for 100 g of fresh rambutan (Fila *et al*., 2012). In contrast, it contains much lower vitamin than the other parts; on the same basis it contains 3.42 µg carotene, 0.02 mg thiamine, 0.09 mg riboflavin, 0.08 mg niacin, and 4.69 mg ascorbic acid (Johnson *et al*., 2013).

Due to its poor nutrient value, *rambutan* seed is still not commonly used in food production; however, there is information that mentioned about the consumption of *rambutan* seeds as nut-like product after it is roasted or boiled (Thulaja, 2004). The economic value of *rambutan* seed most likely is its fat utilization in candles, soaps and fuel manufacturing (Morton, 1987). According to Tindall (1994), *rambutan* seed fat composed mostly of arachidic acid and oleic acid. It is also stated that *rambutan* seed fat is possible to be utilized as cooking ingredient, aside from soap manufacturing material.

Recent research on *rambutan* seed fat carried out by Solís-Fuentes *et al*. (2010) and Sirisompong *et al*. (2011), reported that *rambutan* seed fat has several characteristics that make it a potential ingredient in food, cosmetic and confectionery industries. They found that *rambutan* seed fat is mostly composed of saturated fatty acid and mono-unsaturated fatty acid, but with varying proportions depending on the cultivar and the location of the plantation. Detailed fatty acid composition of *rambutan* fat is shown in Table 2.3.

Table 2.3 Fatty acid composition of *rambutan* seed fat (g/100 g)

Fatty acid		Solís-Fuentes *et al*. (2010) (*Rambutan* from local plantation, Mexico)	Sirisompong *et al*. (2011) (*Rambutan* from Thailand)
Myristic acid	C14:0	-	0.02 ± 0.00
Palmitic acid	C16:0	6.10	4.69 ± 0.15
Stearic acid	C18:0	7.10	7.03 ± 0.08
Arachidic acid	C20:0	34.50	34.32 ± 0.01
Heneicosanoic acid	C21:0	-	0.05 ± 0.00
Behenic acid	C22:0	2.90	3.10 ± 0.04
Tricosanoic acid	C23:0	-	0.03 ± 0.01
Lignoceric acid	C24:0	-	0.33 ± 0.06
Total Saturated Fatty Acid	**SFA**	50.70	49.57 ± 0.14
Palmitoleic acid	C16:1ω7	1.50	0.49 ± 0.04
Trans-9-Elaidic acid	C18:1ω9t	-	0.03 ± 0.00
Cis-9-Oleic acid	C18:1ω9c	40.30	36.79 ± 0.16
Gondoic acid	C20:1ω9	6.30	-
Erucic acid	C22:1ω9	-	0.66 ± 0.03
Total Monounsaturated FA	**MUFA**	48.10	37.97 ± 0.22
Cis-9,12-Linoleic acid	C18:2ω6	-	1.37 ± 0.02
α-Linolenic acid	C18:3ω3	-	6.48 ± 0.03
Cis-11,14-Eicosadienoic acid	C20:2	-	0.04 ± 0.00
Total Polyunsaturated FA	**PUFA**	-	7.89 ± 0.01

Physicochemical analyses of *rambutan* seed fat carried out by Solís-Fuentes *et al*. (2010) and Sirisompong *et al*. (2011) suggested that *rambutan* seed fat is highly stable to oxidation due to its high SFA content; furthermore, apart from its considerable yield of fat that reach up to 37.35%, the origin of *rambutan* seed fat which is solid at room temperature could be used as a potential natural replacement of hydrogenated, partially-hydrogenated, and inter-esterified fat in industries. Moreover, the relatively simple fat phase behavior of fusion and crystallization, and the high thermal stability of *rambutan* seed fat may justify its prospect to be utilized in confectionery industries as confectionery fat.

2.2 Confectionery fat

Confectionery can be defined as a group of all sweets product, including fancy cakes, candy and chocolate. In trading, confectionery is divided into three classifications such as sugar confectionery, chocolate confectionery, and flour confectionery. *Sugar confectionery* includes boiled sweets, toffees, fudge, jellies, pastilles, fondants and others that not covered with chocolate and including biscuits or cakes. *Chocolate confectionery* includes much of sugar confectionery covered in chocolate and usually chocolate products. *Flour confectionery* includes baked fancy cakes in which ices or chocolate covered product also belong to this group (Minnifie, 1970).

Cocoa butter is one of the important fats used in confectionery product due to its unique physical characteristic contributed from its triacylglycerol composition (Wang *et al*., 2006). Cocoa butter is mostly composed of triglyceride (94%) consisting of palmitic acid (24.1-25.8%), stearic acid (33.3-37.4%) and oleic acid (32.9-34.6%) in a configuration of POP (Palmitic-Oleic-Palmitic: 13.8-16.4%), POS (Palmitic-Oleic-Stearic: 34.6-38.3%), and SOS (Stearic-Oleic-Stearic: 23.7-26.8%) (Podlaha *et al*., 1984; Francis, 1999; Lipp & Anklam, 1997).

Cocoa butter possesses unique characteristics that are suitable for use in chocolate-based confectionery product; it is appreciated for the taste, nutritional value, brittleness important to provide snaps features, and sharp melting point (melt in the mouth) with good mouth-feel and flavor release ability (Lipp & Anklam, 1997). With the increase in demand of the confectionery product such as its popular chocolate, the world demand on natural cocoa butter has increased from year to year. However, the world supply of cocoa is low (approximately 4,187,587 tons in 2010), fluctuating with high volatility (could vary from US$ 2,000-3,500 per ton) in prices (FAO 2012; ICCO 2012; Indexmundi, 2012). This condition has forced industries to seek for alternatives from other vegetable fats as substitute for cocoa butter. Nowadays, these alternative fats are classified under the general term of *Cocoa Butter Alternatives* (CBA) (Lipp & Anklam, 1997).

2.2.1 Cocoa butter alternatives

(*a*) *Cocoa butter equivalents*

Cocoa butter equivalent (CBE) is non-lauric plant fats that have similar properties physically and chemically to cocoa butter. CBE is classified into 2 subgroups; cocoa butter extender (CBEX) that is not mixable in every ratio of cocoa butter, and cocoa butter improvers (CBIs) that are used to improve the properties of soft cocoa butter due to its higher solid triglycerides (Lipp & Anklam, 1997; Francis, 1999). Palm oil, Illipe butter, Shea butter (*Vitellaria paradoxa*), Kokum butter (*Garcinia indica*) and Sal fat (*Shorea robusta*) are commonly used as a CBE. These fats mainly consist of palmitic acid, stearic acid, oleic acid, linoleic, and arachidic in the combination of POP, POS and SOS in triglyceride, where P represents palmitic acid; O, oleic acid and S, stearic acid (Brinkmann in Lipp & Anklam, 1997).

(*b*) *Cocoa butter replacer*

Cocoa butter replacer (CBR) accommodates the group of non-lauric fats that have a similar fatty acid composition with cocoa butter, but have a completely different structure of triglyceride and are only mixable in small ratios with cocoa butter (Lipp & Anklam, 1997). According to Brinkmann in Lipp & Anklam (1997), soya oil, rape seed oil, cotton oil, groundnut oil, and palm olein which mostly consist of elaidic acid, stearic acid, palmitic acid and linoleic acid (Palmitic-Elaidic-Elaidic & Stearic-Elaidic-Elaidic configuration) are commonly used as CBRs.

(*c*) *Cocoa butter substitutes*

Cocoa butter substitutes (CBS) are lauric plant fats that have some similar physical characteristic with cocoa butter in spite of their totally different chemical properties. It is suitable as substitute for cocoa butter up to 100%, and is usually produced from coconut oil, palm kernel oil and medium chain triglycerides (mostly lauric and palmitic acids in configuration Lauric-Lauric-Lauric, Lauric-Lauric-Myristic and Lauric-Myristic-Myristic) (Lipp & Anklam, 1997).

2.2.2 Recent development of cocoa butter alternatives

Aforementioned vegetable oils and fats are used in great quantities in confectionery product. However, natural form of oil and fats are not suitable for use without prior physical and chemical treatment. Usually the oil will be refined, deodorized and then subjected to a hardening process such as fractionation, hydrogenation, and interesterification (Minnifie, 1970; Lipp & Anklam, 1997).

The production of the cocoa butter alternatives (CBA) that involves hydrogenation and interesterification process with chemical has gained much attention due to its negative impact and adverse health effects (Sundram *et al*., 2007). Recent development of non-chemical and more eco-friendly CBA have been reported, such as the use of enzymatic interesterification carried out by Bootello *et al*. (2012), Çiftçi *et al*. (2009), and Shekarchizades *et al*. (2009). On the other hand, the development of CBA by using a natural product (that already have some similarities with cocoa butter characteristic) and modified by blending or fractionation are still reported as promising alternatives that can be utilized (Calliauw *et al*., 2005; Zaidul *et al*., 2007).

Currently, the development of CBA only focuses into the development on physical characteristics, but no attempts were made for the development of flavor compounds that is also an important factor of cocoa butter (Lips & Anklam, 1997). Cocoa flavor is unique and can only be achieved by doing proper post-harvest processing, in which the fermentation of cocoa beans and the roasting process is the most crucial part (Lopez, 1986; Puziah *et al.*, 1998).

2.3 Fermentation

2.3.1 Solid-state fermentation

Solid-state fermentation (SSF) can be defined as the microorganism cultivation on solid, moist substrate in the absence (or near absence) of free water. However, it still have to possess enough moisture for supporting the microorganisms growth and metabolism (Pandey, 1992, 1994; Pandey *et al*., 2000, 2001). In this type of fermentation, the solid matrix can be the source of nutrients (substrate) or it only utilized to support the development of micro-organism while impregnated with proper nutrients (Singhania *et al*., 2009).

SSF is widely applied in Asian countries as a state-of-the-art technology that has been applied mainly in food industries. However, the application of SSF in enzymes and metabolite production have also been recorded. In western countries, the application of SSF is less popular compared to submerged fermentation (SmF) since the breakthrough of penicillin production; in addition, SmF is found to be technically easier for large scale industrial rationalization and standardization (Hölker & Lenz, 2005; Pandey, 2003).

Compared to SmF, SSF is the resemblance of the natural habitat of microorganism. Due to the low moisture content and the absence of free flowing liquid, the fermentation is only performed by limited number of microorganisms, mainly yeast and fungi, although some bacteria have also been used (Pandey *et al*., 2000; Singhania *et al*., 2009). SSF is also simple technology that can utilize low-cost media to gain high productivity, provide better oxygen circulation in the fermentation system, requiring less effort in downstream processing thus requiring less energy and cost. However, for big scale or industrial application, SSF has been found to be difficult to scale-up due to its low mixing ability, difficulties in controlling process parameters (pH, heat, moisture, etc.), large amount of heat generated during process unbalance with its low thermal conductivity, all these resulting in product with high amount of impurities and high cost for product recovery (Couto & Sanromán, 2006).

There are several factors that effect the SSF including pH, aeration, water activity and moisture, temperature, nature of solid substrate, particle size, substrate pre-treatment, inoculum, supplementation and bed properties. Optimization studies of SSF regarding these factors are still being reported until now with its possibility for a wider range of application (Pandey *et al*., 2000; Singhania *et al*., 2009).

From the ecological aspect, SSF is considered as a more environmentally friendly process compared to SmF. SSF have gained much interest because its flexibility in the ability to utilize agricultural wastes as carbon and energy source. It is also requiring low water consumption and producing less effluent. In addition, there are also possibility of performing SSF in semi-sterile condition which negates the demand of the sterilization process and instrumentation (Hölker & Lenz, 2005; Couto & Sanromán, 2006). With the increase in the number of researches, more findings regarding more efficient and more optimum SSF reactors have been reported. These findings is not only advantages to solve aforementioned SSF problems, but it also broaden the scope of SSF application rather than merely in its small-scale conventional application (Couto & Sanromán, 2006).

2.3.2 Application of solid-state fermentation

(*a*) *Waste management*

SSF has been used to break down the organic matter and compost production as an alternative for environmentally-friendly waste management. It is commonly used for municipal waste, food waste and sewage sludge treatment and agro-industrial residues with the aim for bioremediation, biodegradation of hazardous compounds, biological detoxification, bioconversion, biopulping, and biotransformation for nutritional enrichment (Singhania *et al*., 2009; Couto & Sanromán, 2006).

According to Raimbault, (1998), there are several group of microorganism that are involved in composting process such as *Bacillus* sp., *Pseudomonas* sp., *Serratia* sp., *Streptococcus* sp., *Altemaria* sp., *Aspergillus* sp., *Fusarium* sp., *Monilia* sp., *Mucor* sp., *Rhizopus* sp., *Phanerochaette chrysosporium*, and *Trichoderma* sp. However, in addition to their use as pure microorganism culture, additives and fermentation residual (sewage sludge, digester sludge) also can be used as biocatalyst for SSF. Nonetheless, SSF can also be carried out naturally (Gabhane *et al*., 2012; Kim *et al*., 2011; Sanchez-Arias *et al*., 2008; Zayed & Abdel-Motaal, 2005; Matute *et al*., 2010).

During SSF of organic matter, secondary metabolite products such as gasses, enzymes, antibiotics, chemical, alkaloid, organic acids, biofuel, aroma compounds, mycopesticides, and biopesticides will be produced (Singhania *et al*., 2009). To avoid confusion, the SSF process on organic matter (even though from waste) that produces enzymes and chemicals will be separated and discussed later in *section 2.4.2(b)*. A list of several researches focusing on SSF application on waste management is presented in Table 2.4.

Table 2.4 List of researches regarding SSF application in waste management

Substrate	Focus of study	Culture & method summary	Researcher
Green waste: grass cutting & Fallen leaves	Composting	Dried and shredded material (1 kg) was placed in tray and sprayed with water and added with additives (surfactant tween 80 & biosurfactant rhamnolipid) and incubated at 25±3˚C for 3 days.	Gabhane & others, 2012
Food waste, paper waste & livestock waste	Anaerobic digestion Biogas production	Crushed material was mixed in different proportion. Seeding source were dewatered sludge cake and anaerobic digester sludge. Substrate was then placed into reactor.	Kim & others, 2011
Olive mill wastes & sewage sludge	Composting	Olive mill wastes & sewage sludge was added with ferrous sulphate waste and composted for 50 days.	Sánchez-Arias & others, 2008
Rice straw	Bio-active compost production	Microorganism: *Trichoderma viride* & *Aspergillus niger*. Rice straw was enriched with rock phosphate and inoculated with prepared inocula then incubated in pile for 105 days.	Zayed & Abdell-Motaal, 2005
Sunflower seed hulls, wheat straw, wheat bran	Composting	Ingredients were mixed then moistened (65-70%) with ammonium sulfate and urea solution. Composting was carried out in composter for total 16 days.	Matute & others, 2010