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BIOTRANSFORMATION OF RAMBUTAN (Nephelium lappaceum L.) SEED INTO A COCOA POWDER-LIKE PRODUCT

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BIOTRANSFORMATION OF RAMBUTAN (*Nephelium lappaceum* L.) SEED INTO A COCOA POWDER-LIKE PRODUCT

By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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BIOTRANSFORMATION OF RAMBUTAN (*Nephelium lappaceum* L.) SEED INTO A COCOA POWDER-LIKE PRODUCT

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CHAI KONG FEI

November 2017

Chairman: Professor Hasanah Mohd Ghazali, PhD
Faculty : Food Science and Technology

Rambutan (*Nephelium lappaceum* L.) is an exotic fruit originally found in Southeast Asia. There is a glut of rambutan every year which leads to wastage. A novel way to reduce wastage is to convert rambutan seeds into a cocoa powder-like product. Thus, the objectives of this study were to select the best rambutan variety for fermentation by comparing the physicochemical properties of pulp and seed of eleven varieties of rambutan fruit with those of cocoa pulp and seed, to determine the physicochemical properties of rambutan fruit sweatings, seed and seed fat during solid state fermentation at different fermentation times and turning intervals, to optimize the roasting process of rambutan seed from fermented and dried rambutan fruit and determine the physicochemical properties of rambutan seed powder, and to determine the toxicology safety of the roasted rambutan seed powder using brine shrimp lethality assay.

Eleven varieties of rambutan were examined in this study, and most of the physicochemical properties of a wild type rambutan, WT1, were found to be similar to those of cocoa bean. However, due to subsequent unavailability of the variety, rambutan Clone R4 was chosen to be the best variety to be investigated on the effect of fermentation as its titratable acidity was not significantly different from that of cocoa bean and the total sugar content of the pulp is comparable to that of cocoa pulp. Besides, the seed had the highest crude fat content (39.13 %) and lowest saponin content (14.27 mg soya saponin/100 g).

Peeled rambutan fruit Clone R4 was subjected to natural fermentation in covered perforated plastic boxes for 0, 1, 3, 5, 7 and 10 days at room temperature. Fermentation time significantly affected the physicochemical properties of sweatings (liquid released naturally from the fruit), seed and seed fat. The study showed that 8 days of fermentation was regarded as sufficient to produce well fermented seeds. The effect of

turning intervals during fermentation for 8 days did not lead to significant changes in the properties of sweatings. Also, there was no significant difference on most of the physicochemical properties of the fermented seeds, between 24 and 48 hours of turning intervals. Considering practical aspects of fermentation in both small and large scale contexts, turning at 48 hours intervals for eight days is recommended. Fermentation for 8 days is also recommended as it served to eliminate microorganisms that are potentially harmful.

Roasting conditions (time and temperature) of seeds after fermentation for 8 days with a 48-hour turning interval significantly affected the physicochemical properties of the fermented seeds. GCMS analysis showed that roasted seed powder contained pyrazines, indicating that the powder has aroma and flavor similar to that of cocoa powder. Results from the brine shrimp lethality assay showed that the LC_{50} of roasted rambutan seed powder extract was 7.07×10^4 µg/mL and is considered as non-toxic. The overall findings of the study indicate that it is possible to obtain a cocoa powder-like product from the seeds of rambutan that had undergone fermentation that is relatively safe for consumption.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia Sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

BIOTRANSFORMASI BIJI RAMBUTAN (*Nephelium lappaceum* L.*)* KEPADA PRODUK SEPERTI SERBUK KOKO

Oleh

CHAI KONG FEI

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Rambutan (*Nephelium lappaceum* L.) adalah sejenis buah eksotik yang berasal dari Asia Tenggara. Buah rambutan yang berlebihan telah menyebabkan pembaziran setiap tahun. Salah satu cara novel untuk mengurangkan pembaziran adalah dengan menghasilkan produk seperti serbuk koko dengan menggunakan biji rambutan. Oleh itu, objektif kajian ini adalah memilih varieti rambutan yang paling sesuai untuk proses fermentasi dengan membandingkan ciri-ciri fizikokimia isi dan biji sebelas varieti rambutan dengan isi dan biji koko, menentukan ciri-ciri fizikokimia cecair fermentasi, biji dan lemak biji rambutan semasa fermentasi substrat pepejal pada masa fermentasi dan selang pengacauan yang berbeza, mengoptimumkan proses pemanggangan biji rambutan daripada buah rambutan yang telah difermentasi dan dikeringkan dan menentukan ciri-ciri fizikokimia serbuk biji rambutan, serta menentukan toksikologi keselamatan serbuk biji rambutan yang telah dipanggang dengan menggunakan ujian ketosikan anak udang.

Sebelas varieti rambutan telah dikaji dan didapati bahawa kebanyakan ciri-ciri fizikokimia rambutan jenis liar, WT1, sama dengan koko. Akan tetapi, disebabkan ketidakdapatan WT1 pada musim tuai yang seterusnya, rambutan klon R4 telah dipilih sebagai varieti yang paling baik untuk dikaji kerana keasidan tertitratnya tidak berbeza secara signifikan dengan koko dan jumlah gula isinya sebanding dengan koko. Selain itu, biji klon R4 mempunyai kandungan lemak mentah yang tertinggi (39.13 %) dan kandungan saponin yang terendah (14.27 mg soya saponin/100 g).

Buah rambutan Klon R4 yang telah dikupas difermentasi secara semula jadi dalam bekas plastik selama 0, 1, 3, 5, 7 dan 10 hari pada suhu bilik. Masa fermentasi menjejaskan ciri-ciri fizikokimia cecair fermentasi, biji dan lemak biji secara signifikan. Keputusan menunjukkan fermentasi sebanyak 8 hari adalah memadai untuk

menghasilkan biji yang difermentasi dengan baik. Selang pengacausan tidak memberi kesan yang signifikan kepada ciri-ciri fizikokimia cecair fermentasi semasa buah difermentasi selama 8 hari. Di samping itu, tiada perbezaan signifikan antara selang pengacauan 24 dan 48 jam pada ciri-ciri fizikokimia biji yang difermentasi, maka selang pengacauan 48 jam untuk fermentasi sebanyak 8 hari adalah disyorkan. Fermentasi selama 8 hari adalah disyorkan kerana ia dapat menghapuskan mikroorganisma yang berbahaya.

Keadaan pemangganan (masa dan suhu) menjejaskan ciri-ciri fizikokimia biji yang telah defermentasi selama 8 hari dengan selang pengacauan 48 jam secara signifikan. Analisis GCMS menunjukkan serbuk biji yang telah dipanggang mengandungi pyrazines. Ini menunjukkan serbuk biji yang telah dipanggang mempunyai aroma dan rasa serupa dengan serbuk koko. Ujian ketoksikan anak udang menunjukkan LC_{50} ekstrak serbuk biji rambutan panggang ialah 7.07×10^4 μg/mL dan dianggap sebagai tidak toksik. Dapatan kajian menunjukkan produk seperti serbuk koko dapat dihasilkan daripada biji rambutan yang telah difermentasi dan ia adalah selamat untuk dimakan.

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I certify that a Thesis Examination Committee has met on 28 November 2017 to conduct the final examination of Chai Kong Fei on his thesis entitled "Biotransformation of Rambutan (*Nephelium lappaceum* L.) Seed Into a Cocoa Powder-Like Product " in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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This is to confirm that:

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LIST OF ABBREVIATIONS

AAB	acetic acid bacteria
ANOVA	analysis of variance
C	Celsius
CE	catechin equivalent
cm	centimeter
DSC	Differential Scanning Calorimeter
FFA	free fatty acid
FAME	fatty acid methyl ester
g	gram
GC	gas chromatography
HCl	hydrochloric acid
HPLC	high performance liquid chromatography
I.D.	internal diameter
IV	iodine value
kg	kilogram
KOH	potassium hydroxide
L	liter
LAB	lactic acid bacteria
LCMS	liquid chromatography mass-spectrometry
M	Molar
mg	milligram
min	minute
mL	milliliter
mm	millimeter
N	normality

N_2	nitrogen
NaOH	sodium hydroxide
ND	not detected
nm	nanometer
psi	pounds per square inch
rpm	revolutions per minute
SFI	solid fat index
TAG	triacylglycerol
UV-Vis	ultra violet-visible
v/v	volume/volume
w/v	weight/volume
µL	microliter

CHAPTER 1

INTRODUCTION

Rambutan (*Nephelium lappaceum* Linn.) is a popular tropical fruit from the Sapindaceae family. It is native to Malaysia and Indonesia (Issara, Zzaman, & Yang, 2014). The rambutan fruit has a colored skin and spinterns. The peel of the fruit is red, yellow or orange yellow in color. According to Sun et al. (2011), the fruit peel contains abundant of anthocyanins, which are mainly responsible for the red color of the ripe fruit. There are more than 187 varieties of rambutan are registered to the Department of Agriculture Malaysia and additional 25 varieties are found in Singapore, Indonesia, the Philippines, and Thailand (Zee, 1993). Figure 1.1 shows two common (red and yellow) skin colors of the rambutan fruit. The fruits are large (3-6 cm long and 3-4 cm broad) (Fila et al., 2012) and spherical in shape. Rambutan has two fruiting seasons (July-September and December-February) per year. A rambutan tree may produce more than 5000-6000 fruits per fruiting season (Ahmad & Alrozi, 2011). The fruits take 3 to 6 months to mature depending on growing climate and cultivar (Diczbalis, 2002).

Figure 1.1: Rambutan fruit with red, yellow and orange yellow skin colors

Thailand, Indonesia and Malaysia account for approximately 80 % of the total world production of rambutan and only Malaysia, Thailand and Indonesia export this fruit among the ASEAN member countries (Ahmad & Chua, 2013; Tindall, 1994). Fresh and processed rambutan products are exported from Thailand to Asian and European countries. In other Asian countries, the fruits are sold in the domestic markets (Paull & Duarte, 2012; Tindall, 1994). According to the Department of Statistics Malaysia (2016), Malaysia has sufficient rambutan fruit supply for domestic demands. In 2013, the self-sufficiency ratio of the fruits to demands in Malaysia was 98.2 % while the ratio in 2014 was 100.5 %. As advocated in the *Dasar Agromakanan Negara* 2011-2020 (Ministry of Agriculture and Agro-Based Industry Malaysia, 2011), the production of the domestic fruits especially star fruit, papaya, pineapple and rambutan should be increased commercially due to the increase of export market demand. Fruits production is expected to increase in line with the planting area from 9.6 metric ton per hectare in 2010 to 12.9 metric ton per hectare in 2020 (Ministry of Agriculture and Agro-Based Industry Malaysia, 2011).

The rambutan fruit is rich in carbohydrates, fiber, iron, potassium, magnesium, vitamin C and calcium as shown in Table 1.1 (P. Lee, Tan, Yu, Curran, & Liu, 2013; Sukasih & Setyadjit, 2015; Tindall, 1994; Wall, 2006a). The skin of the fruit contains flavonoids, saponin and tannin (Lestari, Djati, Rudijanto, & Fatchiyah, 2013) while the seeds have high fat content (38 %) (Manaf, Marikkar, Long, & Ghazali, 2013), antioxidant activity and high phenolic content (Thitilertdecha, Teerawutgulrag, & Rakariyatham, 2008a). All these nutrients and minerals allow this fruit to possess medicinal and therapeutic properties.

Table 1.1: Composition of rambutan per 100 g edible portion

Constituents	Value per 100 g
Energy	297 kJ[a]
Moisture	82.1 g[a]
Fat	0.9 g[a]
Protein	0.66 g[b]
Carbohydrate	19.66 g[b]
Dietary fiber	2.8 g[a]
Ash	0.3 g[a]
Starch	0.0 g[a]
Glucose	2.8 g[a]
Fructose	3.0 g[a]
Sucrose	9.9 g[a]
Malic acid	0.05 g[a]
Citric acid	0.31 g[a]
Niacin	0.5 mg[a]
Carotene	0.0 mg[a]
Vitamin C	70.0 mg[a]
Thiamine	0.01 mg[a]
Riboflavin	0.07 mg[a]
Phosphorus	8.8-18.8 mg[c]
Potassium	133.5-249.4 mg[c]
Calcium	6.8-8.7 mg[c]
Magnesium	13.3-17.2 mg[c]
Sodium	5.5-8.2 mg[c]
Iron	0.41-0.56 mg[c]
Manganese	0.07-0.38 mg[c]
Zinc	0.16-0.26 mg[c]
Copper	0.16-0.20 mg[c]
Boron	0.11-0.16 mg[c]

(Adapted from: [a]-Tindall, 1994; [b]-Fila, Itam, & Johnson, 2013; [c]-Wall, 2006)

Generally, rambutan fruits are consumed in the fresh state. The aril (flesh or pulp) is sometimes mixed with other fruits to prepare salads. Some rambutan varieties are suitable for canning, thus extending the period of consumption other than during the local fruiting seasons. In Malaysia and Thailand, rambutan is industrially produced into

juice, jam, jelly, marmalade and spread (Morton, 1987; Dapur Kayu Enterprise, 2017; Solís-Fuentes, Camey-Ortíz, Hernández-Medel, Pérez-Mendoza, & Durán-de-Bazúa, 2010). Besides, the fruits can be processed as rambutan stuffed with a chunk of pineapple and canned in syrup (Sirisompong, Jirapakkul, & Klinkesorn, 2011). Rambutan chips are also available in the market. Some popular rambutan-derived products are shown in Figure 1.2.

Figure 1.2: Some commercial rambutan products

Although rambutan is one of the most popular fruits in Malaysia as it is available throughout the country, it is seasonal and susceptible to deterioration (3-4 days at ambient temperature, or a few weeks at 12-13 °C and 90-95 % relative humidity) (Siriphanich, 2003). When supply is greater than demand, the fruits are often wasted. The massive amount of the excessive fruits is being disregarded, causing a severe problem in the community as they gradually ferment and release off-odors. This is uncommon to be seen in any other popular non-exotic fruits such as apple, carob, peach, grape and citrus as many food industries have been working on these fruits as well as their by-products in order to fully utilize them (Davidov-Pardo & McClements, 2015; Fernández-López, Sendra, Sayas-Barberá, Navarro, & Pérez-Alvarez, 2008; Loizzo et al., 2015; Raina et al., 2015; Sheng et al., 2016). However, to date, there is no large industry in Malaysia to diversify the rambutan fruit into various products. When rambutan fruit is canned, the fruits are deseeded during processing and the seeds (~ 4-9 g/100 g) become a waste by-product (Solís-Fuentes et al., 2010). Hence, in order to reduce wastage and increase the variation of food from rambutan fruit, there is a potential in converting the rambutan seeds into a cocoa powder-like product through the process of natural fermentation involving the whole fruit (Low, 2011). In this preliminary study, cocoa powder-like product was produced by fermenting and drying rambutan fruit as well as roasting and grinding the dried fermented seed.

To date, rambutan seed powder as a possible complement to cocoa powder has not been produced. It is relatively a new idea in product development. From the literature, much of the research has been confined to cocoa powder processing (Afoakwa, Paterson, & Fowler, 2007; Afoakwa, Paterson, Fowler, & Ryan, 2008; Afoakwa, 2015; Andres-Lacueva et al., 2008; Hofberger & Tanabe, 2007; Minifie, 1989; Tomas-Barberán et al.,

2007). Report has been made on rambutan fruit fermentation (Mehdizadeh, Lasekan, Muhammad, & Baharin, 2015) but not on the production of rambutan seed powder. Scientific analysis on rambutan seed powder has not been carried out and definitely there is still lacking of information on the rambutan seed powder processing. Thus, the main objective of this study was to produce a cocoa powder-like product from rambutan seeds which had undergone the process of natural fermentation followed by roasting, and to determine the toxicity of the powder. Based on this objective, the specific objectives were:

1. To select the best rambutan variety for fermentation by comparing the physicochemical properties of pulp and seed of rambutan fruit with those of cocoa pulp and seed.
2. To determine the physicochemical properties of rambutan fruit sweatings, seed and seed fat during solid state fermentation at different fermentation times and turning intervals.
3. To optimize the roasting process of rambutan seed removed from fermented and dried rambutan fruit, and to determine the physicochemical properties and toxicity of rambutan seed powder.

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