UNIVERSITI PUTRA MALAYSIA

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

CHAI KONG FEI

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BIOTRANSFORMATION OF RAMBUTAN (*Nephelium lappaceum* L.)
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By

CHAI KONG FEI

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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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

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

By

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 LC₅₀ 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

November 2017

Pengerusi: Profesor Hasanah Mohd Ghazali, PhD
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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 ketidakdapatannya 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 pengacauan tidak memberi kesan yang signifikan kepada ciri-ciri fizikokimia ceair 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 LC30 ekstrak serbuk biji rambutan panggang ialah 7.07×10^4 μg/mL dan dianggap sebagai tidak toksik. Dapat 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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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>i</td>
</tr>
<tr>
<td>ABSTRAK</td>
<td>iii</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>v</td>
</tr>
<tr>
<td>APPROVAL</td>
<td>vi</td>
</tr>
<tr>
<td>DECLARATION</td>
<td>viii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xv</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xix</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>xxiii</td>
</tr>
</tbody>
</table>

## CHAPTER 1

1 INTRODUCTION

## CHAPTER 2

2 LITERATURE REVIEW

### Rambutan
- Characteristics and origin of rambutan
- Exports of fresh and processed rambutan fruit
- Nutritional value of rambutan fruit and seed
- Applications of rambutan

### Cocoa
- Cocoa bean processing
  - Fermentation
    - Biochemical changes during fermentation
    - Factors affecting fermentation
    - Fermentation methods
    - Endpoint of fermentation
- Drying
- Roasting
  - Whole bean roasting
  - Nib roasting
  - Cocoa mass roasting
- Cocoa butter and cocoa butter alternatives
- Palm kernel oil and palm oil
- Mango seed kernel fat
- Kokum butter
- Shea butter
- Rambutan seed fat
- Relationship between rambutan and cocoa bean fermentation

## CHAPTER 3

3 PHYSICOCHEMICAL PROPERTIES OF DIFFERENT VARIETIES OF RAMBUTAN FRUIT PULP AND SEED

### Introduction
### Materials and Methods
  - Sample collection and sampling procedures
  - Determination of the proportion of different parts of fruit
  - Characterization of rambutan fruit pulp
Determination of pH, titratable acidity and total soluble solids 32
Determination of sugar composition 33
Determination of organic acid composition and ascorbic content 35
Characterization of rambutan seed and seed fat 36
Extraction and determination of crude fat content 36
Determination of iodine value and free fatty acid contents 36
Determination of fatty acid composition 37
Determination of triacylglycerol profile 37
Determination of thermal behavior 38
Determination of solid fat index 38
Determination of saponin content 39
Determination of tannin content 39
Statistical analysis 40
Results and Discussion 40
Proportion of different parts of rambutan fruit 40
Physicochemical properties of rambutan pulp 41
pH, titratable acidity and total soluble solids 41
Sugar composition and content 42
Organic acid composition and ascorbic acid content 44
Physicochemical properties of rambutan seed 50
Crude fat content, iodine value, free fatty acid content 50
Fatty acid composition 51
Triacylglycerol profile 52
Thermal profile 57
Solid fat index 58
Saponin and tannin contents 64
Comparison of the physicochemical properties of 11 varieties of rambutan with those of cocoa pulp and seed 65
Conclusion 68

4 PHYSICOCHEMICAL PROPERTIES OF SWEATINGS, SEED AND SEED FAT DURING SOLID SUBSTRATE FERMENTATION OF RAMBUTAN FRUIT
Introduction 69
Materials and Methods 70
Materials 70
Sample selection and collection for fermentation 70
Fermentation of rambutan fruit 70
Characterization of fermentation sweatings 72
Determination of pH, titratable acidity and total soluble solids of sweatings 72
Determination of sugar, organic acid and ascorbic acid contents of sweatings 72
Characterization of fermented fruit 72
Measurements of cut test 73
Fermentation index 120
Color 120
pH, titratable acidity and total soluble solids 124
Sugar composition and content 125
Organic acid and ascorbic acid contents 127
Crude fat and free fatty acid contents 130
Fatty acid composition 130
Triacylglycerol profile 131
Thermal profile 131
Solid fat index 135
Saponin and tannin contents 137
Microbiological analysis 138

Conclusion 139

6 PHYSICOCHEMICAL PROPERTIES, VOLATILE PROFILE AND TOXICITY OF ROASTED FERMENTED RAMBUTAN SEED
Introduction 141
Materials and Methods 142
Materials 142
Fermentation of rambutan fruit 142
Drying of fermented rambutan fruit 142
Roasting of dried fermented rambutan fruit 143
Experimental design 144
Optimization and validation procedures 145
Physicochemical properties of roasted rambutan seed 145
Percentage of seed and waste 146
Percentage of loss of moisture 146
Total phenolic content 146
Extraction of volatile compounds 147
Separation and identification of volatile compounds 147
Toxicological assessment using brine shrimp lethality assay 147
Statistical analysis 149
Results and Discussion 150
Effect of roasting time and temperature on L*, a*, b* values and total phenolic content of fermented rambutan seed 150
Percentage of seed and waste 163
Moisture loss 163
Crude fat content 166
Fatty acid composition of fat from rambutan seed powder 166
Triacylglycerol profile of fat from rambutan seed powder 170
Thermal profile of fat from rambutan seed powder 170
Solid fat index of fat from rambutan seed powder 176
Volatile compounds in roasted rambutan seed powder 179
Toxicological assessment using brine shrimp lethality assay 182
# Conclusion

## SUMMARY, GENERAL CONCLUSION AND RECOMMENDATIONS FOR FUTURE RESEARCH

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>REFERENCES</td>
<td>188</td>
</tr>
<tr>
<td>APPENDICES</td>
<td>215</td>
</tr>
<tr>
<td>BIODATA OF STUDENT</td>
<td>253</td>
</tr>
<tr>
<td>LIST OF PUBLICATIONS</td>
<td>254</td>
</tr>
</tbody>
</table>
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Composition of rambutan per 100 g edible portion</td>
<td>2</td>
</tr>
<tr>
<td>2.1</td>
<td>Supply and utilization accounts of rambutan fruits in Malaysia on 2013, 2014, and 2015</td>
<td>6</td>
</tr>
<tr>
<td>2.2</td>
<td>Harvested area and total yield of rambutan fruits in Thailand from 2008-2013</td>
<td>6</td>
</tr>
<tr>
<td>2.3</td>
<td>Export volume and value of rambutan fruits in Thailand from 2008-2013</td>
<td>8</td>
</tr>
<tr>
<td>2.4</td>
<td>Composition of cocoa pulp (g.100 g⁻¹ fresh weight pulp)</td>
<td>10</td>
</tr>
<tr>
<td>2.5</td>
<td>Bean composition of unfermented West African (Forastero) cocoa</td>
<td>10</td>
</tr>
<tr>
<td>2.6</td>
<td>Cocoa butter alternatives: examples, properties and composition</td>
<td>27</td>
</tr>
<tr>
<td>3.1</td>
<td>Relative percent of different parts of rambutan fruit</td>
<td>41</td>
</tr>
<tr>
<td>3.2</td>
<td>pH, titratable acidity and total soluble solids of rambutan fruit</td>
<td>42</td>
</tr>
<tr>
<td>3.3</td>
<td>Calibration parameters for different sugars standards</td>
<td>44</td>
</tr>
<tr>
<td>3.4</td>
<td>Sugar contents of rambutan fruit</td>
<td>44</td>
</tr>
<tr>
<td>3.5</td>
<td>Calibration parameters for different organic acids and ascorbic acid standards</td>
<td>46</td>
</tr>
<tr>
<td>3.6</td>
<td>Organic acid and ascorbic acid contents of rambutan fruit</td>
<td>47</td>
</tr>
<tr>
<td>3.7</td>
<td>Crude fat content, iodine value and free fatty acid contents of rambutan seed fats</td>
<td>51</td>
</tr>
<tr>
<td>3.8</td>
<td>Fatty acid composition of rambutan seed fats</td>
<td>53</td>
</tr>
<tr>
<td>3.9</td>
<td>Triacylglycerol composition of rambutan seed fats</td>
<td>55</td>
</tr>
<tr>
<td>3.10</td>
<td>Temperature of phase transition (T) and crystallization and melting enthalpies (ΔH) of rambutan seed fat</td>
<td>62</td>
</tr>
<tr>
<td>3.11</td>
<td>SFI of rambutan seed fat at human body temperature (37 °C)</td>
<td>64</td>
</tr>
<tr>
<td>3.12</td>
<td>Saponin and tannin contents in rambutan seed</td>
<td>65</td>
</tr>
<tr>
<td>3.13</td>
<td>Comparison of physicochemical properties of 11 varieties of rambutan to those of cocoa bean</td>
<td>67</td>
</tr>
</tbody>
</table>
4.1 Yield, pH, titratable acidity and total soluble solids, sugars, organic acids and ascorbic acid contents of rambutan sweatings during fermentation
4.2 Effect of fermentation time on surface color and cut test score of dried rambutan seeds
4.3 Effect of fermentation time on color fractions absorbance values and fermentation index of rambutan seeds
4.4 L*, a* and b* values of fermented and dried fermented rambutan seeds
4.5 pH, titratable acidity and total soluble solids of fermented rambutan seeds
4.6 Sugar contents of fermented rambutan seeds
4.7 Organic acid and ascorbic acid contents of fermented rambutan seeds
4.8 Crude fat, fat yield and free fatty acid contents of fermented rambutan seeds
4.9 Fatty acid composition of fermented rambutan seeds fat
4.10 Triacylglycerol composition of rambutan seeds fat at different fermentation times
4.11 Temperature of phase transition (T) and crystallization and melting enthalpies (ΔH) of fermented rambutan seeds fat
4.12 SFI of fermented seeds fat at human body temperature (37 °C)
4.13 Saponin and tannin contents of fermented rambutan seeds
5.1 Yield, pH, titratable acidity and total soluble solids of rambutan sweatings
5.2 Sugars in rambutan sweatings
5.3 Organic acids and ascorbic acid in rambutan sweatings
5.4 Effect of mixing intervals on surface color and cut test score of dried rambutan seeds
5.5 Effect of mixing intervals on color fractions absorbance values and fermentation index of rambutan seeds
5.6 L*, a* and b* values of fermented and dried fermented rambutan seeds as affected by mixing intervals
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.7</td>
<td>Effect of mixing intervals on pH, titratable acidity and total soluble solids of fermented rambutan seeds</td>
<td>124</td>
</tr>
<tr>
<td>5.8</td>
<td>Effect of mixing intervals on sugar contents of fermented rambutan seeds</td>
<td>126</td>
</tr>
<tr>
<td>5.9</td>
<td>Effect of mixing intervals on organic acid and ascorbic acid contents of fermented rambutan seeds</td>
<td>128</td>
</tr>
<tr>
<td>5.10</td>
<td>Crude fat content, fat yield and free fatty acid content of fermented rambutan seeds with different mixing intervals</td>
<td>130</td>
</tr>
<tr>
<td>5.11</td>
<td>Fatty acid composition of fermented rambutan seed fat with different mixing intervals</td>
<td>132</td>
</tr>
<tr>
<td>5.12</td>
<td>Triacylglycerol composition of fermented rambutan seed fat with different mixing intervals</td>
<td>133</td>
</tr>
<tr>
<td>5.13</td>
<td>Temperature of phase transition (T) and crystallization and melting enthalpies (∆H) of fermented rambutan seed fat</td>
<td>136</td>
</tr>
<tr>
<td>5.14</td>
<td>SFI of fermented seed fat at human body temperature (37 °C)</td>
<td>137</td>
</tr>
<tr>
<td>5.15</td>
<td>Saponin and tannin contents of fermented rambutan seeds</td>
<td>138</td>
</tr>
<tr>
<td>6.1</td>
<td>Central composite design for the independent variables</td>
<td>145</td>
</tr>
<tr>
<td>6.2</td>
<td>Effect of roasting time and temperature on the dependent variables</td>
<td>153</td>
</tr>
<tr>
<td>6.3</td>
<td>Regression coefficients, R², adjusted R², probability value and lack of fit for dependent variables</td>
<td>154</td>
</tr>
<tr>
<td>6.4</td>
<td>The significance of each independent variable effect indicated by F ratio and p value in the model</td>
<td>155</td>
</tr>
<tr>
<td>6.5</td>
<td>Experimental and predicted values for the response variables</td>
<td>164</td>
</tr>
<tr>
<td>6.6</td>
<td>Percentage of seed and waste, moisture loss and crude fat content of seed after roasted at different conditions</td>
<td>165</td>
</tr>
<tr>
<td>6.7</td>
<td>Fatty acid composition of fat from roasted rambutan seed powder</td>
<td>167</td>
</tr>
<tr>
<td>6.8</td>
<td>Triacylglycerol composition of fat from roasted rambutan seed powder</td>
<td>171</td>
</tr>
<tr>
<td>6.9</td>
<td>Temperature of phase transition (T) and crystallization and melting enthalpies (∆H) of roasted rambutan seed fat</td>
<td>174</td>
</tr>
<tr>
<td>6.10</td>
<td>SFI of roasted seed fat at human body temperature (37 °C)</td>
<td>179</td>
</tr>
</tbody>
</table>

xvii
6.11 Identification of volatile compounds of rambutan seed after roasting at different conditions 180
6.12 Brine shrimp toxicity of roasted rambutan seed powder 184
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Rambutan fruit with red, yellow and orange yellow skin colors</td>
<td>1</td>
</tr>
<tr>
<td>1.2</td>
<td>Some commercial rambutan products</td>
<td>3</td>
</tr>
<tr>
<td>2.1</td>
<td>Probable geographical distribution of rambutan from the centres of origin</td>
<td>7</td>
</tr>
<tr>
<td>2.2</td>
<td>Cocoa bean processing</td>
<td>11</td>
</tr>
<tr>
<td>2.3</td>
<td>Wet beans</td>
<td>12</td>
</tr>
<tr>
<td>2.4</td>
<td>Cocoa seed and its parts</td>
<td>12</td>
</tr>
<tr>
<td>2.5</td>
<td>Cross section of cocoa bean</td>
<td>13</td>
</tr>
<tr>
<td>2.6</td>
<td>Changes in the cocoa beans during fermentation related to the cocoa flavor</td>
<td>14</td>
</tr>
<tr>
<td>2.7</td>
<td>Schematic illustration of a microbial succession during cocoa bean fermentation</td>
<td>15</td>
</tr>
<tr>
<td>2.8</td>
<td>Fermentation products of pulp sugar</td>
<td>15</td>
</tr>
<tr>
<td>2.9</td>
<td>Heap method</td>
<td>19</td>
</tr>
<tr>
<td>2.10</td>
<td>Tray method</td>
<td>19</td>
</tr>
<tr>
<td>2.11</td>
<td>Box method</td>
<td>20</td>
</tr>
<tr>
<td>2.12</td>
<td>Sun drying</td>
<td>22</td>
</tr>
<tr>
<td>3.1</td>
<td>Different varieties of rambutan used in the study</td>
<td>34</td>
</tr>
<tr>
<td>3.2</td>
<td>HPLC chromatograms of sugar standards (A) and sugars present in rambutan pulp of Clone R4 (B)</td>
<td>43</td>
</tr>
<tr>
<td>3.3</td>
<td>HPLC chromatograms of organic acid standards (A) and organic acids present in rambutan pulp of Clone R4 (B)</td>
<td>45</td>
</tr>
<tr>
<td>3.4</td>
<td>Verification of peaks ‘a’ (A) and ‘b’ (B) fractionated from rambutan pulp extract of Clone R4</td>
<td>48</td>
</tr>
<tr>
<td>3.5</td>
<td>Total ion current (TIC) of blank (solvent) and unknown compounds ‘a’ and ‘b’ (A) and chromatograms of extracted compound of unknown compounds ‘a’ (B) and ‘b’ (C) in rambutan pulp extract of Clone R4 using LCMS</td>
<td>49</td>
</tr>
</tbody>
</table>

xix
3.6 Chemical structures of 2,3-dihydro-2-hydroxy-9-methoxy-7H-furo(3,2-g)(1)benzopyran-7-one (A) and 4-methylumbelliferyl acetate (B)

3.7 Representative HPLC chromatogram of triacylglycerols (TAG) in rambutan seed fat of Clone R10 (A) and palm olein (B)

3.8 Fatty acid profile of unknown TAG ‘a’ (A), ‘b’ (B), ‘c’ (C), ‘d’ (D) and ‘e’ (E) from Clone R4

3.9 Thermograms of seed fat of R3 (A), R4 (B), R7 (C), R10 (D), R153 (E), R156 (F), R169 (G), R191 (H), Sarjan (I), WT1 (J), WT2 (K)

3.10 SFI of seed fat of Clones R3, R4, R7 and R10 (A), Clones R153, 156, 169 and 191 (B) and Varieties of Sarjan, WT1 and WT2 (C)

4.1 Perforated fermentation container

4.2 Fermentation of rambutan in a perforated plastic box

4.3 Internal and external temperatures of fermentation mass during solid substrate fermentation

4.4 Sweatings of first day (A) and second day (B) collected in the lower container during fruit fermentation.

4.5 Effect of fermentation time on the concentrations of 2,3-dihydro-2-hydroxy-9-methoxy-7H-furo(3,2-g)(1)benzopyran-7-one and 4-methylumbelliferyl acetate (based on peak area) in the sweatings

4.6 Fermentation of rambutan fruit for 0 day (A), 1 day (B), 3 days (C), 5 days (D), 7 days (E) and 10 days (F)

4.7 Rambutan fruits after fermented for 10 days before drying (A) and after drying (B)

4.8 Lengthwise-cut dried rambutan seeds after 0 (A), 1(B), 3 (C), 5 (D), 7 (E) and 10 (F) days of fermentation

4.9 Rambutan seed powder from Portion 2 after 0 (A), 1 (C), 3 (E), 5 (G), 7 (I) and 10 (K) days of fermentation and dried rambutan seeds powder from Portion 1 after 0 (B), 1 (D), 3 (F), 5 (H), 7 (J) and 10 (L) days of fermentation

4.10 Effect of fermentation time on the concentrations of 2,3-dihydro-2-hydroxy-9-methoxy-7H-furo(3,2-g)(1)benzopyran-7-one and 4-methylumbelliferyl acetate (based on peak area) in the seeds

xx
4.11 Thermograms of rambutan seeds fat after 0 day (A), 1 day (B), 3 days (C), 5 days (D), 7 days (E) and 10 days (F) of fermentation

4.12 SFI of rambutan seeds fat after 0 (A), 1 (B), 3 (C), 5 (D), 7 (E) and 10 (F) days of fermentation

5.1 Internal temperatures of fermentation mass during solid substrate fermentation

5.2 External temperatures of fermentation mass during solid substrate fermentation

5.3 HPLC chromatogram of sugar standards (A) and typical HPLC chromatogram of sugars in rambutan sweatings with mixing at every 48 hours (B)

5.4 HPLC chromatogram of organic acids and ascorbic acid standards (A) and typical HPLC chromatogram of organic acids in rambutan sweatings with mixing at every 48 hours (B)

5.5 Effects of turning interval and fermentation time on the concentrations of 2,3-dihydro-2-hydroxy-9-methoxy-7H-furo(3,2-g)(1)benzopyr-an-7-one and 4-methylumbelliferyl acetate (based on peak area) in the sweatings

5.6 Rambutan fruits fermented for eight days without mixing (A), with mixing at every 24 (B), 48 (C) and 72 (D) hours

5.7 Lengthwise-cut dried rambutan seeds after 8 days of fermentation with no mixing (A), mixing at every 24 (B), 48 (C) and 72 (D) hours

5.8 Rambutan seed powder after 8 days of fermentation with no mixing (A), mixing at every 24 (C), 48 (E) and 72 (G) hours and dried rambutan seed powder after 8 days of fermentation with no mixing (B), mixing at every 24 (D), 48 (F) and 72 (H) hours

5.9 HPLC chromatogram of sugar standards (A) and typical HPLC chromatogram of sugars in rambutan seeds after 8 days of fermentation with mixing at every 48 hours (B)

5.10 HPLC chromatogram of organic acid and ascorbic acid standards (A) and typical HPLC chromatogram of organic acids and ascorbic acid in rambutan seeds after 8 days of fermentation with mixing at every 48 hours (B)

5.11 Effect of turning interval on the concentrations of 2,3-dihydro-2-hydroxy-9-methoxy-7H-furo(3,2-g)(1)benzopyr-an-7-one and 4-methylumbelliferyl acetate (based on peak area) in the seeds after eight days of fermentation

xxi
5.12 Typical thermogram of rambutan seed fat after 8 days of fermentation with mixing at every 48 hours

5.13 Solid fat index of rambutan seed fat after 8 days of fermentation with no mixing (A), mixing at every 24 (B), 48 (C) and 72 (D) hours

5.14 Evolution of bacteria in different medium during 8 days of rambutan fermentation with mixing at 48 hours intervals

6.1 Fermented fruits were spread on a tray prior to drying

6.2 Roaster for roasting

6.3 Mixture of brine shrimp eggs and simulated seawater (from the premix) aerated with air pump and provided with light source at 27±2 °C

6.4 Beaker with roasted rambutan seed powder and methanol sealed with cotton wool and aluminum foil accompanied by stirring using a magnetic stirrer

6.5 Rambutan seed powder with different roasting time and temperature as treated based on Table 6.1

6.6 A response surface plot for the effect of roasting time and temperature on L* value

6.7 A response surface plot for the effect of roasting time and temperature on a* value

6.8 A response surface plot for the effect of roasting time and temperature on b* value

6.9 A response surface plot for the effect of roasting time and temperature on total phenolic content

6.10 Response optimizer plot for interaction effect of roasting variables on color and total phenolic content of rambutan seed powder

6.11 Typical thermogram (heating and cooling curves) of roasted rambutan seed fat from treatment 14

6.12 SFI of rambutan seed fat after 8 days of fermentation, control (A), and roasting based on treatment 1 (B), 2 (C), 3 (D), 4 (E), 5 (F), 6 (G), 7 (H), 8 (I), 9 (J), 10 (K), 11 (L), 12 (M) and 13 (N), 14 (O)

6.13 Typical GCMS chromatogram of volatile compounds present in roasted rambutan seed powder from treatment 14
**LIST OF ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAB</td>
<td>acetic acid bacteria</td>
</tr>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>C</td>
<td>Celsius</td>
</tr>
<tr>
<td>CE</td>
<td>catechin equivalent</td>
</tr>
<tr>
<td>cm</td>
<td>centimeter</td>
</tr>
<tr>
<td>DSC</td>
<td>Differential Scanning Calorimeter</td>
</tr>
<tr>
<td>FFA</td>
<td>free fatty acid</td>
</tr>
<tr>
<td>FAME</td>
<td>fatty acid methyl ester</td>
</tr>
<tr>
<td>g</td>
<td>gram</td>
</tr>
<tr>
<td>GC</td>
<td>gas chromatography</td>
</tr>
<tr>
<td>HCl</td>
<td>hydrochloric acid</td>
</tr>
<tr>
<td>HPLC</td>
<td>high performance liquid chromatography</td>
</tr>
<tr>
<td>I.D.</td>
<td>internal diameter</td>
</tr>
<tr>
<td>IV</td>
<td>iodine value</td>
</tr>
<tr>
<td>kg</td>
<td>kilogram</td>
</tr>
<tr>
<td>KOH</td>
<td>potassium hydroxide</td>
</tr>
<tr>
<td>L</td>
<td>liter</td>
</tr>
<tr>
<td>LAB</td>
<td>lactic acid bacteria</td>
</tr>
<tr>
<td>LCMS</td>
<td>liquid chromatography mass-spectrometry</td>
</tr>
<tr>
<td>M</td>
<td>Molar</td>
</tr>
<tr>
<td>mg</td>
<td>milligram</td>
</tr>
<tr>
<td>min</td>
<td>minute</td>
</tr>
<tr>
<td>mL</td>
<td>milliliter</td>
</tr>
<tr>
<td>mm</td>
<td>millimeter</td>
</tr>
<tr>
<td>N</td>
<td>normality</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>------------------------------------</td>
</tr>
<tr>
<td>$N_2$</td>
<td>nitrogen</td>
</tr>
<tr>
<td>NaOH</td>
<td>sodium hydroxide</td>
</tr>
<tr>
<td>ND</td>
<td>not detected</td>
</tr>
<tr>
<td>nm</td>
<td>nanometer</td>
</tr>
<tr>
<td>psi</td>
<td>pounds per square inch</td>
</tr>
<tr>
<td>rpm</td>
<td>revolutions per minute</td>
</tr>
<tr>
<td>SFI</td>
<td>solid fat index</td>
</tr>
<tr>
<td>TAG</td>
<td>triacylglycerol</td>
</tr>
<tr>
<td>UV-Vis</td>
<td>ultra violet-visible</td>
</tr>
<tr>
<td>v/v</td>
<td>volume/volume</td>
</tr>
<tr>
<td>w/v</td>
<td>weight/volume</td>
</tr>
<tr>
<td>$\mu$L</td>
<td>microliter</td>
</tr>
</tbody>
</table>
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

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

(Adapted from: \textsuperscript{a}-Tindall, 1994; \textsuperscript{b}-Fila, Itam, & Johnson, 2013; \textsuperscript{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](image)

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 the 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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198


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204


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208


210


