ANTIOXIDANT PROPERTIES AND PHENOLIC COMPOUNDS
OF FERMENTED BAMBANGAN (*Mangifera pajang*)

CHAN SHET TENG

THESIS SUBMITTED IN PARTIAL FULFILLMENT FOR THE DEGREE OF
BACHELOR OF FOOD SCIENCE WITH HONOURS (FOOD SERVICE)

SCHOOL OF FOOD SCIENCE AND NUTRITION
UNIVERSITI MALAYSIA SABAH
2013
Universiti Malaysia Sabah

Borang Pengesahan Status Tesis

Judul: Antioxidant Properties and Phenolic Components of Fermented Bambangan (Mangifera pajang)

Ijazah: Bachelor of Food Science with Honours (Food Science)

Sesi Pengajian: 2009/2010

Saya Chan Chey Teng

(Huruf Besar)

Mengaku membencarkan tesis (LPS/Sarjana/Doktor Falsafah) ini di simpan di Perpustakaan Universiti Malaysia Sabah dengan syarat-syarat kegunaan seperti berikut:

1. Tesis adalah hakmilik Universiti Malaysia Sabah.
2. Perpustakaan Universiti Malaysia Sabah dibencarkan membuat salinan untuk tujuan pengajian sahaja.
3. Perpustakaan dibencarkan membuat salinan tesis ini sebagai bahan pertakaran antara institusi pengajian tinggi.
4. ** Sila tandakan (x) (Mengandungi maklumat yang berdarjah keselamatan atau kepentingan Malaysia seperti yang termaktub di dalam Akta Rahsia Rasmi 1972)

☐ SULIT

☐ TERHAD

☐ TIDAK TERHAD

Disahkan oleh

(TandaTangan Penulis)

Jamat Tetap: 21, Jalan Wawasan 5/8, Bandar Baru Ampang, 68000 Selangor

Tarikh: 17/10/2012

(TandaTangan Pustakawan)

Nama Penyelidik

 Assoc. Prof. Dr. Chye Fook Yee

Tarikh: 17/10/2012

Tatang: * Potong yang tidak berkencan.
* Jika tesis ini SULIT atau TERHAD, sila lampirkan surat daripada pihak berkuasa/organisasi berkencan dengan menyatakan selagi sebab dan tempoh tesis ini perlu dikelaskan sebagai SULIT dan TERHAD.
* Tesis dimaksudkan sebagai tesis bagi ijazah Doktor Falsafah dan Sarjana secara penyelidikan, atau disertasi bagi pengajian secara kerja kursus dan penyelidikan, atau Laporan Projek Sarjana Muda (LPSM).
DECLARATION

I hereby declare that the materials in this thesis is my own except for quotations, excerpts, equations, summaries and references, which have been duly acknowledged.

7 October 2013

CHAN SHET TENG
BN09110068
CERTIFICATION

NAME : CHAN SHET TENG
MATRIC NO. : BN09110068
TITLE : ANTIOXIDANT PROPERTIES AND PHENOLIC COMPOUNDS OF FERMENTED BAMBANGAN (Mangifera pajang)
DEGREE : BACHELOR OF FOOD SCIENCE WITH HONOURS (FOOD SERVICE)

DECLARED BY

1. SUPERVISOR
   (ASSOC. PROF. DR. CHYE FOOK YEE)

2. EXAMINER 1
   (Dr. AFSANEH FARHADIAN)

3. EXAMINER 2
   (Dr. MOHD ROSNI SULAIMAN)

4. DEAN
   (ASSOC. PROF. DR. SHARIFUDIN MD. SHAARANI)

SIGNATURE

iii
ACKNOWLEDGEMENT

I wish to express my deepest gratitude and appreciation to my supervisor, Assoc. Prof. Dr. Chye Fook Yee of the School of Food Science and Nutrition, Universiti Malaysia Sabah, for his excellent guidance, caring, and patiently corrected my thesis writing. Indeed, without his guidance, I would not be able to complete my thesis. I would like to like appreciate School of Food Science and Nutrition and our Dean (Assoc. Prof. Dr. Sharifudin Md. Shaarani) given me this opportunity for doing my thesis. I also like to thank Dr. Mohd Rosni Sulaiman and Dr. Afsaneh Farhadian for being examiners for my thesis who given me advice and guidance.

I would like to thank Science officer (Mr. Muhin Haji), lab assistant (Mr. Sahirun Salihin and Mr. Fritzgerald Andrew) who providing me excellent laboratory atmosphere for doing my lab work.

I would also like to thank to seniors, Mr. Ng Seah Young and Miss Ooi Pei Wan who always willing to help and giving me suggestion during my thesis. My thesis would not be able to finish without their guidance.

Lastly, I would like thank to my parents for their unconditional support, both financially and emotionally throughout my degree. I also would like to thank to my friends for their support and encouragement.

Chan Shet Teng
4 October 2013
ABSTRACT

ANTIOXIDANT PROPERTIES AND PHENOLIC COMPOUNDS OF FERMENTED BAMBANGAN (Mangifera pajang)

The objective of the present study was to determine the antioxidant activities and phenolic compounds during Bambangan fermentation and extended storage. Bambangan was kept at room temperature (28°C) and elevated temperature (35°C) during the process of fermentation before kept at room temperature and 4°C for storage. Samples were obtained from each interval during fermentation and storage for antioxidant activity determination by 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging capacity, ferric-ion-reducing antioxidant power (FRAP), ABTS radical cation assay, oxygen - radical absorbing capacity (ORAC) and also physiochemical test of pH, total lactic acidity and microbiological count determination. High performance liquid chromatography (HPLC) method was used to identify the presence of phenolic compounds in the fermented Bambangan. The pH values of Bambangan fermented at room temperature (28°C) and 35°C decreased slowly throughout the fermentation. Lactic acid bacteria counts had achieved the highest count on day-4 at both temperatures during the fermentation, with the Bambangan placed at 35°C (7.37 log cfu/g) was higher than the sample fermented at room temperature (28°C) (6.29 log cfu/g). Antioxidant activities of Bambangan fermented at room temperature (28°C) and 35°C increased during the fermentation, but slightly reduced in the later fermentation. However, Bambangan fermented at 35°C achieved the highest FRAP and ABTS values within the first four days (114.44±0.33 mM Fe(II)/g and 5.00±0.00 mmol TE/g) as compare to the Bambangan fermented at room temperature (28°C) required 10 days to achieve the highest activity of 149.32±0.00 mM Fe(II)/g and 6.09±0.01 mmol TE/g. DPPH radical scavenging activity was having the highest activity on day-8 for the Bambangan fermented at 35°C, but the same measurement was found continuously increasing up to day-14 for Bambangan kept at room temperature. The total phenolic content (TPC) of Bambangan was found increasing throughout the fermentation (within first two weeks) at room temperature with the highest value of 44.69±0.01 mg GAE/g, which is much higher than Bambangan that was kept at the elevated temperature (35°C) (26.69 mg GAE/g). During the storage period, no significant changes (p>0.05) on antioxidant activities and microbiological count was noticed for both fermentation temperatures. However, prolonged fermentation of Bambangan at room temperature (28°C) gave higher TPC than Bambangan kept at refrigerated temperature (4°C). Gallic acid, chlorogenic acid, vanillin, p-coumaric acid and rutin were the phenolic compounds identified during fermentation and storage of Bambangan. Chlorogenic acid (22.95 μg/g DW) was the phenolic compound obtained the highest concentration within 4 days and then followed by gallic acid (3.22 μg/g DW) on day-14 during Bambangan fermented at 35°C. Hence, elevated temperature (35°C) was observed to produce higher phenolic compounds than Bambangan fermented at room temperature (28°C). Antioxidant activity and phenolic compounds of fermented Bambangan were able to achieve faster at elevated temperature (35°C) and stable throughout the storage period at 4°C up to 90th day.
ABSTRAK

SIFAT ANTIOKSIDA DAN KOMPAUN FENOLIK FERMENTASI BAMBANGAN
(Mangifera pajang)

Objektif kajian ini adalah untuk menentukan aktiviti antioksidan dan kompaun fenolik semasa fermentasi dan penyimpanan Bambangan. Bambangan akan disimpan di dalam suhu bilik (28 °C) dan suhu 35 °C semasa proses fermentasi sebelum disimpan di dalam suhu bilik dan 4 °C untuk penyimpanan. Sampel akan diperolehi daripada setiap selang hari semasa fermentasi dan penyimpanan untuk menentukan aktiviti antioksidan dengan menggunakan cara 2,2-Diphenyl-1-picrylhydrazyl (DPPH), FRAP, ABTS kation radikal, kapasiti penyerapan radikal oksigen (ORAC) dan juga ujian physiochemical seperti pH, jumlah asid laktik dan penentuan kiraan mikrobiologi. Prestasi HPLC digunakan untuk mengenal pasti kehadiran kompaun fenolik di dalam fermentasi Bambangan. Nilai pH bagi Bambangan ferment pada suhu bilik (28°C) dan 35 °C adalah menurun secara perlahan-sejuk selama proses fermentasi. Aktiviti antioksidan Bambangan ferment pada suhu bilik (28°C) dan 35°C seperti FRAP assay dan ABTS assay kation radikal meningkat semasa fermentasi dan penyimpanan. Walaubagaimanapun, Bambangan ferment pada suhu 35°C memiliki aktiviti antioksidan tertinggi pada hari ke-4 pada kedua-dua suhu semasa proses fermentasi, dengan mencapai 149.32 ± 0.00 mM Fe (II)/g dan 6.09 ± 0.01 mmol TE/g semasa proses fermentasi. Aktiviti pemerangkap radikal terdapat aktiviti yang tinggi pada hari ke-8 bagi Bambangan ferment pada suhu 35°C, manakala, dengan pengukuran yang sama, Bambangan ferment pada suhu bilik (28°C) meningkat secara berterusan sehingga hari ke-14. Jumlah kandungan fenolik (TPC) bagi Bambangan ferment pada suhu bilik semakin meningkat sehingga hari ke-14 dengan nilai yang tertinggi 44.69 ± 0.01 mg GAE/g, ia lebih tinggi daripada sampel ferment pada suhu bilik (28°C) (6.68 ± 0.00 mg GAE/g) di dalam suhu 35°C. Dalam tempoh penyimpanan, fermentasi Bambangan yang menyimpan pada suhu bilik (28°C) dan suhu sejuk (4°C) menunjukkan tiada perubahan bagi aktiviti antioksidan dan kiraan mikrobiologi. Walaubagaimanapun, fermentasos Bambangan pada suhu bilik (28°C) adalah lebih tinggi daripada Bambangan yang disimpan pada suhu sejuk (4°C). Asid gallic, asid klorogenik, vanillin, asid p-coumaric dan rutin adalah kompaun fenol yang dapat dikenalpasti semasa proses fermentasi dan penyimpanan Bambangan dengan menggunakan HPLC. Asid klorogenik (22.95 μg/g DW) adalah kompaun fenolik yang diperolehi dengan kepekatan tertinggi dalam tempoh 4 hari dan kemudian diikuti oleh asid gallic (3.22 μg/g DW) pada hari ke-14 semasa Bambangan diferment pada 35 °C. Oleh itu, 35 °C diperhatikan dapat menghasilkan kompaun fenolik yang lebih tinggi daripada Bambangan yang diferment pada suhu bilik (28°C). Aktiviti antioksidan dan kompaun fenolik Bambangan difermentasi dapat mencapai lebih cepat pada suhu 35°C dan stabil sepanjang tempoh penyimpanan pada 4 °C sehingga hari ke-90.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>TITLE</td>
<td>i</td>
</tr>
<tr>
<td>DECLARATION</td>
<td>ii</td>
</tr>
<tr>
<td>CERTIFICATION</td>
<td>iii</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENT</td>
<td>iv</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>v</td>
</tr>
<tr>
<td>ABSTRAK</td>
<td>vi</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xi</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xii</td>
</tr>
<tr>
<td>LIST OF ABBREVIATION</td>
<td>xii</td>
</tr>
<tr>
<td>LIST OF APPENDIX</td>
<td>xv</td>
</tr>
<tr>
<td>LIST OF CONTENTS</td>
<td></td>
</tr>
<tr>
<td>CHAPTER 1 INTRODUCTION</td>
<td></td>
</tr>
<tr>
<td>CHAPTER 2 LITERATURE REVIEW</td>
<td></td>
</tr>
<tr>
<td>2.1 Food Fermentation</td>
<td></td>
</tr>
<tr>
<td>2.1.1 Classification of Fermented Food</td>
<td></td>
</tr>
<tr>
<td>2.1.2 Indigenous Fermented Food</td>
<td></td>
</tr>
<tr>
<td>2.1.3 Traditional Fermented Food in Malaysia</td>
<td></td>
</tr>
<tr>
<td>2.2 Benefits of Fermented Food</td>
<td></td>
</tr>
<tr>
<td>2.2.1 Bio – enrichment of Nutritional Values</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>11</td>
<td>11</td>
</tr>
</tbody>
</table>
a. Protein 11
b. Essential Amino Acids 12
c. Vitamins 13

2.2.2 Removal of Toxin and Anti – Nutrient Compounds 13

2.3 Biodiversity of Lactic Acid Bacteria 14
  2.3.1 Application of Lactic Acid Bacteria in Food Fermentation 15
  2.3.2 Benefit of Lactic Acid Bacteria to Human Health 17

2.4 Natural Antioxidant from Fermentation 18
  2.4.1 Flavonoids 20
  2.4.2 Phenolic Acids 22

2.5 Potential Fermentation as a Source of Biological Active Compounds 24
  2.5.1 Antihypertensive Peptides 25
  2.5.2 Maytansine 26
  2.5.3 Ganoderic Acid 27
  2.5.4 Antimicrobial Agent of Phenyllactic Acid 27

CHAPTER 3 MATERIALS AND METHODS

3.1 Materials 29

3.2 Sampling and Samples Preparation 30

3.3 Study on the Effect of Grated Kernel Content on the Antioxidant Properties of Bambangan Fermentation 31

3.4 Microbiological Analyses 31

3.5 Samples Extraction 32

3.6 Determination of Antioxidant Activity 32
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 2.1</td>
<td>Classification of fermented food</td>
<td>7</td>
</tr>
<tr>
<td>Table 2.2</td>
<td>Traditional fermented food in Malaysia</td>
<td>9</td>
</tr>
<tr>
<td>Table 2.3</td>
<td>Examples of fermented foods involving lactic acid bacteria (LAB)</td>
<td>16</td>
</tr>
<tr>
<td>Table 2.4</td>
<td>Metabolism of aromatic compounds by <em>L. plantarum</em> strains</td>
<td>23</td>
</tr>
<tr>
<td>Table 4.1</td>
<td>pH and acidity of <em>Bambangan</em> during process of fermentation and storage period</td>
<td>38</td>
</tr>
<tr>
<td>Table 4.2</td>
<td>Antioxidant activity of <em>Bambangan</em> during the fermentation and storage at room temperature, 28°C</td>
<td>45</td>
</tr>
<tr>
<td>Table 4.3</td>
<td>Antioxidant activity of <em>Bambangan</em> during the fermentation process at 35°C and storage at 4°C</td>
<td>46</td>
</tr>
<tr>
<td>Table 4.4</td>
<td>Total phenolic contents of <em>Bambangan</em> during the fermentation and storage</td>
<td>49</td>
</tr>
<tr>
<td>Table 4.5</td>
<td>Evolution of phenolic compounds during <em>Bambangan</em> fermentation and storage at room temperature, 28°C (μg/g DW)</td>
<td>54</td>
</tr>
<tr>
<td>Table 4.6</td>
<td>Evolution of phenolic compounds during <em>Bambangan</em> fermentation process at 35°C and storage (4°C) (μg/g DW)</td>
<td>55</td>
</tr>
</tbody>
</table>
**LIST OF FIGURES**

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 2.1</td>
<td>Groups of Phytochemicals</td>
<td>19</td>
</tr>
<tr>
<td>Figure 2.2</td>
<td>Basic chemical structure and number pattern of flavonoids</td>
<td>21</td>
</tr>
<tr>
<td>Figure 3.1</td>
<td><em>Bambangan</em> fermentation procedures</td>
<td>30</td>
</tr>
<tr>
<td>Figure 4.1</td>
<td>Microbial changes during <em>Bambangan</em> fermentation at room temperature, (28°C) and (b) 35°C</td>
<td>40</td>
</tr>
<tr>
<td>Figure 4.2</td>
<td>Microbial changes of fermented <em>Bambangan</em> during storage period at (a) room temperature (28°C) and (b) 4°C</td>
<td>43</td>
</tr>
<tr>
<td>Figure 4.3</td>
<td>HPLC chromatogram of extract of <em>Bambangan</em> fermented at room temperature (28 °C) on day-12 with the phenolic compounds of Gallic acid, Chlorogenic acid, Vanillin, α -coumaric acid and Rutin</td>
<td>52</td>
</tr>
<tr>
<td>Figure 4.4</td>
<td>HPLC chromatogram, of extract of <em>Bambangan</em> fermented at 35°C on day-4 with the phenolic compounds of Gallic acid, Chlorogenic acid, Vanillin, α-coumaric acid and Rutin</td>
<td>52</td>
</tr>
</tbody>
</table>
## LIST OF ABBREVIATION

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAPH</td>
<td>$2,2'$-azobis(2-amidinopropane) dihydrochloride</td>
</tr>
<tr>
<td>AEAC</td>
<td>Ascorbic acid Equivalent Antioxidant Capacity</td>
</tr>
<tr>
<td>DAD</td>
<td>Diode Array Detector</td>
</tr>
<tr>
<td>DPPH</td>
<td>1,1-diphenyl-2-picrylhydrazyl</td>
</tr>
<tr>
<td>FAO</td>
<td>Food Agriculture Organization</td>
</tr>
<tr>
<td>FRAP</td>
<td>Ferric Reducing/Antioxidant Power</td>
</tr>
<tr>
<td>FW</td>
<td>Fresh weight</td>
</tr>
<tr>
<td>GA</td>
<td>Ganoderic Acid</td>
</tr>
<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
</tr>
<tr>
<td>L.</td>
<td><em>Lactobacill</em></td>
</tr>
<tr>
<td>LAB</td>
<td>Lactic acid bacteria</td>
</tr>
<tr>
<td>Lb.</td>
<td><em>Lactobacillus</em></td>
</tr>
<tr>
<td>Leuc.</td>
<td><em>Leuconostoc</em></td>
</tr>
<tr>
<td>mmol</td>
<td>Milimoles</td>
</tr>
<tr>
<td>ORAC</td>
<td>Oxygen – radical absorbing capacity</td>
</tr>
<tr>
<td>P.</td>
<td><em>Pediococcus</em></td>
</tr>
<tr>
<td>PAD</td>
<td>Phenolic acid dehydrogenase</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffer saline</td>
</tr>
<tr>
<td>PLA</td>
<td>Phenyllatic Acid</td>
</tr>
</tbody>
</table>
S. \hspace{1cm} Saccharomyces

S.D. \hspace{1cm} Standard deviation

SPSS \hspace{1cm} Statistical Package for Social Science

TPTZ \hspace{1cm} 2,4,6-tripyridyl-s-triazine

Trolox \hspace{1cm} 2,5,7,8-tetramethychroman-2-carboxylic acid

UV \hspace{1cm} Ultraviolet

WHO \hspace{1cm} World Health Organization

β-PE \hspace{1cm} β-phycoerythrin

μg \hspace{1cm} Microgram

μl \hspace{1cm} Microliter

μM \hspace{1cm} Micromoles
# List of Appendix

<table>
<thead>
<tr>
<th>Appendix</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appendix A</td>
<td>Flow diagrams of <em>Bambangan</em> fermentation</td>
<td>78</td>
</tr>
<tr>
<td>Appendix B</td>
<td>Antioxidant Assays Standard Curve</td>
<td>79</td>
</tr>
<tr>
<td>Appendix C</td>
<td>Phenolic Compounds Calibration curve by using HPLC</td>
<td>80</td>
</tr>
<tr>
<td>Appendix D</td>
<td>Mixed Phenolic Compounds Standard by using Wavelength-265 nm</td>
<td>82</td>
</tr>
<tr>
<td>Appendix E</td>
<td>Chromatograms for Samples of Day 0 until Day 90 During the Fermentation Process and Storage Periods at Room Temperature (28°C)</td>
<td>84</td>
</tr>
<tr>
<td>Appendix F</td>
<td>Chromatograms for Samples of Day 0 until Day 90 During Fermentation Process at 35°C and Storage (4°C)</td>
<td>88</td>
</tr>
</tbody>
</table>
CHAPTER 1

INTRODUCTION

Antioxidants are compound present in low concentration which prevent or delays oxidation of an oxidizable substrate and classified as a compound able to prevent the biological oxidative damage (Brewer, 2011). Superoxide anion radicals, hydroxyl radicals and hydrogen peroxide are the potentially harmful reactive oxygen species that are generated by the metabolic processes of environment pollution and UV radiation (Sun et al., 2009). These reactive oxygen species are unstable, highly reactive and can be formed in the body and food system which may cause oxidative damage by oxidizing all cellular macromolecules such as proteins, carbohydrates, lipids and nucleic acid (Kumar, 2011). Oxidative damage may result in various chronic diseases such as cardiovascular disease, diabetes and cancer (Chu et al., 2002).

Carotenoids, tocopherols, ascorbates, lipoic acids and polyphenols are phytochemicals that consist of strong natural antioxidant with free radical scavenging which naturally occur in foods with specific protective effect against oxidative deterioration and obtain optimal health (Prakash et al., 2011).

Molecules containing a structure of benzene ring substituted by at least, one hydroxyl group is known as “Phenolic compound” and it is the important elements in food products of plant origin due to their health characteristics, principally related to antioxidant properties (Mannach et al., 2004). These compounds are divided into different groups based on their binding structural to one another such as phenolic acid, flavonoids, stilbenes and lignans (D'Archivio et al., 2007). Sensory properties of food such as colour, flavor, and astringency are mainly influence by phenolic compounds. Aroma of food products are mainly due to the presence of volatile phenol that produced by the hydrolysis of superior alcohols or metabolisms of microorganisms (yeasts and Lactic acid bacteria) (Rodriguez et al., 2009). Anthocyanins from the phenolic group of flavonoids are known as the natural pigments which influence the
colour changes of food products and could act as potentially natural food colorants (Vendramini and Trugo, 2004). Therefore, fruits and vegetables are the good sources of dietary polyphenols which are the bioactive phytochemicals with strong antioxidant activity that could be helpful to human health due to their different type of colours, flavors and might play an important role as body's natural defense to oxidative damage (Netzet et al., 2007; Dordevic et al., 2009).

Malaysia contain a rich diversity of underutilized fruits which might be important to the Malaysian diet. These fruits are usually less popular, grown in wild, seasonal and not cultivated in commercial scale. According to Emmy et al., (2009), nam-nam (Cynometra sp.), bacang (Mangifera sp.), jambu bol (Psidium sp.), durian (Durio sp.), bidara (Ziziphus sp.), mertajam (Leppisanthes sp.), longan (Dimorcarpus sp.), lenggeng serawak (Pometia sp.), belimbing buloh (Averrhoa sp.), assam kelubi (Salacca sp.), buah melaka (Phyllanthes sp.), assam gelugor (Garcinia sp.), remia (Boues sp.), sentol (Sendoricum sp.), rambai Sarawak (Baccaurea sp.) and pulasan (Nephelium sp.) are the underutilized fruits existed In Malaysia. Some of these certain underutilized fruits from the genera of Baccaurea, Durio, Garcinia and Mangifera are known as potential functional fruits (Ashraf, 2011; Eng, 2009).

Bambangan (Mangifera pajang) is a type of seasonal fruit within the group of mango and belongs to the family of Anarcardiaceae, which is an important underutilized fruit in East Malaysia. It is oval in shape with thick brownish skin, three times larger than commercial mango (Mangifera indica), while the pulp is fibrous, juicy, yellow in colour and having a strong aromatic flavor. Bambangan can be consumed in fresh or in salt-preserved form. Fermented Bambangan is a popular indigenous fermented fruit which is mainly consumed by the community of Kadazan Dusun in Sabah. Based on the empirical process of Bambangan fermentation, fresh bambangan pulp, grated seed and salt are mixed and left to ferment for 7 – 10 days under ambient temperature in a tightly closed container. This traditional lactic acid fermentation (Chin et al., 2008) is likes other vegetable fermentations such as olives, cabbages and cucumbers (Othman et al., 2009; Sun et al., 2009; Tamminen et al., 2004) which mainly depend on the naturally occurrence microorganisms in the raw materials and
processing environment. Hence, organic acid needed for preservation is beliefs to be produced by the presence of lactic acid bacteria. It can be the important factors influencing fermentation and final product quality as well as the changes of sensory attributes and texture of the fibrous pulp.

Phenolic compounds may alter during fermentation process due to the metabolism of microorganisms and endogenous enzymes which decrease the pH from 7 to 4. The growth of microorganisms is affected by the salt and acid concentration during fermentation process. Therefore, the important factors in influencing the quality of fermented foods are salt concentration, fermentation time and temperature (Rodriguez et al., 2009). Qualitative and quantitative changes in phenolic composition during fermentation may be due to the degradation of glycoside linkage causes different bioavailability compared to unfermented Chinese cabbage (Harbaum et al., 2008). Landete et al., (2010), reported acid and enzymatic hydrolysis of polymerized phenolic compounds cause release of simple phenolic compounds which are easily metabolized by microorganisms. For example, during olive fermentation, concentration of hydroxytyrosol increased due to the acid and enzymatic hydrolysis of oleuropein which is the important components in antioxidant activity (Othman et al., 2009).

Recently, several studies have reported Bambangan as a rich source of antioxidants, high level of phenolics and flavonoids (Abu Bakar et al., 2009; Al-Sheraji et al., 2012; Hassan et al., 2011). Furthermore, some of the phenolic compounds found in Bambangan are gallic acid, chlorogenic acid, protocatechuic acid, mangiferin, daidzein and genistein (Hassan et al., 2011; Prasad et al., 2011; Khoo and Ismail, 2008). However, there is still lack of information on the antioxidant properties and phenolic compounds of fermented Bambangan. Therefore, the present study aims to determine the antioxidant activities and phenolic compounds of Bambangan during fermentation and extended storage.
The Specific Objectives of the Present Study are:

1. To determine the antioxidant activities during the fermentation of Bambangan.
2. To identify the changes of phenolic compounds that are responsible for the antioxidant activities.
3. To determine the stability of antioxidant activity and phenolics in fermented Bambangan during storage.
CHAPTER 2

LITERATURE REVIEW

2.1 Food Fermentation

Food fermentation has historical, philosophical, archaeological and religious significance (Ray and Sivakumar, 2009). It can be traced back to thousands of years. It is thought that in this time period the art of cheese making was developed in the fertile Crescent between the Tigris and Euphrates Rivers in Iraq, at a time when plants and animals were just being domesticated (Abriouel et al., 2008). After that, alcoholic fermentations involved in winemaking and brewing are thought to have been developed during the 2000 – 4000 BC by the Egyptians and Sumerians (Ross et al., 2002). Through the ages, fermentation has had a major impact on nutritional habits and traditions, on culture and on the commercial distribution and storage of food (Holzapfel, 2002).

Fermentation is one of the oldest technologies and most economical methods used for food preservation. Hence, the main objectives of fermentation are extending the shelf-life of foods, with aspects such as wholesomeness, acceptability and overall quality (Marshall and Mejia, 2012). These fermentative activities have been utilized in the production of fermented foods and beverages, which are defined as those products that have been subordinated to the effect of microorganisms or enzymes to cause the desirable biochemical changes (Blandino et al., 2003). This definition means that the process will convert carbohydrate to alcohol and carbon dioxide or conversion of organic acid by yeast or bacteria under anaerobic condition.

Food supply has always been a problem in third world population, controlled and efficient fermentation enabled to decrease production costs and loss due to spoilage. Especially in African countries, fermentation play an important role in the nutrition of infants and young children as it is used for the preparation of complementary foods (Motarjemi, 2000). The major roles of fermentation are
preservation of food through formation of inhibitory metabolites such as organic acids (lactic acid, acetic acid, formic acid, propionic acid), ethanol, bacteriocins, often in combination with decrease of water activity (by drying or use of salt), Improving food safety through inhibition of pathogens or removal of toxic compounds, improving nutritional value and organoleptic quality of the food (Bourdichon et al., 2012).

There are two types of fermentation: spontaneous or natural fermentation is the raw materials fermented without using starter cultures by the natural microflora present raw ingredients or environment, while, controlled fermentation is using starter culture (Tamang and Kailasapathy, 2010). Back – slopping is a kind of traditional fermentation technique which this procedure was able to increase the initial number of desirable microorganisms and it is a primitive precursor of the starter culture method (Solieri and Giodici, 2009). In general, this technique is able to improve the growth of useful yeasts, while inhibiting growth of pathogenic microorganisms and reducing spoilage (Holzapfel, 2002). The spontaneous fermentation of vegetables and fruits may involve in the succession of hetero- and homo- fermentative lactic acid bacteria together with or without yeasts (Cagno et al., 2013).

Generally, the uses of starter cultures are related to the ‘pure culture’ technique and it could represent a potential source of spread of genes (Holzapfel, 2002). Hence, it is able to increase safety, stability and efficiency of the process and reduces production losses caused by uncontrolled fermentation, eliminating undesired features (Solieri and Giodici, 2009). For instance, during sauerkraut fermentation, the mixture of commercial starters containing – *Leuc. mesenteriodes*, *P. pentosaceus*, *L.plantarum* and *L.brevis* enabled to ferment cabbages with a result of rapidly decrease of the pH, improved the sensory properties and gave a firm texture (Cagno et al., 2013). Globally, there are large varieties of fermented food in various part of the world including edible and inedible raw materials. For example, vegetables lactic acid fermentations are: sauerkraut, cucumber pickles, olives in the Western world; Korean Kim – Chi; Chinese hum – choy and Malaysian tempoyak (Steinkraus, 2002).
### 2.1.1 Classification of Fermented Food

Fermented food can be classified in different ways to differentiate it from one to another. Campbell – Platt, (1987), has classified fermented foods based on the class, included beverages, cereal products, dairy products, fish products, fruit and vegetable products, legume, and meat product. On the other hands, Steinkraus, (1997), categorized according to the type of fermentation – fermentations producing textured vegetable protein meat substitutes in legume – cereal mixtures, high – salt meat – flavored amino acid – peptide sauce and paste fermentations, lactic acid fermentations, alcoholic fermentations, acetic acid fermentations, alkaline fermentations, leavened breads and flat unleavened breads. According to the Joint FAO / WHO Food Standards Programme, (2004), food had been categorized based on their commodities of products such as dairy products; fruits and vegetables; cereal and cereal products derived from cereal grains; roots and tubers, pulses and legumes; bakery wares; meat; fish products including molluscs, crustaceans and echinoderms; eggs; salt, spices, soups, sauces, salads, protein products and beverages (excluding dairy products). Classification of fermented food is listed in Table 2.1.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Beverages</td>
<td>Alcoholic fermentation</td>
<td>Beverages</td>
</tr>
<tr>
<td></td>
<td>Acetic fermentation</td>
<td></td>
</tr>
<tr>
<td>Dairy products</td>
<td>Lactic acid fermentation</td>
<td>Dairy products</td>
</tr>
<tr>
<td>Fruit and vegetable products</td>
<td>Fermentation producing textured vegetable protein meat substitutes in legume – cereal mixture</td>
<td>Fruit and vegetables products</td>
</tr>
<tr>
<td>Legume</td>
<td>Roots and tubers, pulses and legumes</td>
<td>Cereal and cereal products derived from cereal grains</td>
</tr>
<tr>
<td>Cereal products</td>
<td>Fish products including mollusks, crustaceans and echinoderms</td>
<td>Meat products</td>
</tr>
<tr>
<td>Fish products</td>
<td>High – salt meat – flavor amino acid</td>
<td>Egg, salt, spices, soups, sauces, salads, protein products</td>
</tr>
<tr>
<td>Meat products</td>
<td>Alkaline fermentation</td>
<td>Bakery wares</td>
</tr>
<tr>
<td></td>
<td>Leavened breads and unleavened breads</td>
<td></td>
</tr>
</tbody>
</table>
2.1.2 Indigenous Fermented Food

Indigenous food fermentation is embedded in the traditional cultures and rural life. It is widely practiced as household technology all over the world which believes that our ancestors used it to survive during the changes of seasons. All the indigenous fermented foods are originally fermented by natural microorganisms which have been handled down from generation to generation (Achi, 2005). During indigenous food fermentation preparation, the present of microorganisms naturally come from the sources of ingredients, plant or animals, utensils, containers and the environment.

Indigenous fermented foods play significant role in the diet of people in the developing world. Generally, they are prepared based on the experience gained through trial and error by consecutive generation of food producers and households without any training in microbiology or chemistry and often in unhygienic environments (Steinkraus, 2002). However, people had consumed their own cultural foods for generations. Globally, there are large varieties of indigenous fermented foods consumed in various part of the world including edible and inedible raw materials Porridge, beverages (alcoholic and non-alcoholic), breads, fermented meat, fish, vegetables, dairy products and condiments are the examples of indigenous fermented food. Most of these indigenous fermented foods are produced based on different cultural and different geographical areas. For example, Hwaijar (fermented soybean), Soibum/Saijim, Soidon (bamboo shoot products), Ngari, Hentak (fish products, Ziang Sang, Ziang Dui (mustard leaf extract) are the indigenous fermented food in Manipur (Jeyaram et al., 2009); Kenkey (fermented maize dough) in Ghana; Dahi, Gheu, Mohi (fermented cow milk), Philu (fermented mutton) in Himalaya (Thakur et al., 2004).

2.1.3 Traditional Fermented Food in Malaysia

Malaysia has a rich diversity of traditional fermented food from agricultural products as well as from animal and marine sources. Majority of these fermented foods are still being produce in traditional method on a cottage industrial scale. Techniques of fermentation were handled generation by generation. Traditional fermented food commonly consumed by local community in Malaysia has listed in Table 2.2.
REFERENCES


Amirdivani, S. and Baba, A.S. 2011. Changes in yogurt fermentation characteristics, and antioxidant potential and in vitro inhibition angiotensin-1 converting enzyme upon the inclusion of peppermint, dill and basil. LWT — Food Science and Technology, 44: 1458 – 1464


Brewer, M.S. 2011. Natural antioxidants: sources, compounds, mechanisms of action, and potential application. *Comprehensive Reviews in Food Science and Food Safety*. **10**: 221-247


Cagno, R.D., Coda, R., Angelis, M.D. and Gobbetti, M. 2013. Exploitation of vegetables and fruits through lactic acid fermentation. *Food Microbiology*. **33**: 1 – 10


Chen, Y.S. Yanagida, F. and Hsu, J.S. 2006. Isolation and characterization of lactic acid bacteria from suan-tsai (fermented mustard), a traditional fermented food in Taiwan. *Journal of Applied Microbiology*, **101**: 125 – 130

Chen, Y.S., Yanagida, F. and Hsu, J.S. 2006. Isolation and characterization of lactic acid bacteria from suan-tsai (fermented mustard), a traditional fermented food in Taiwan. *Journal of Applied Microbiology*, **101**: 125 – 130


UMS UNIVERSITI MALAYSIA SABAH

PERPUSTAKAAN

UNIVERSITI MALAYSIA SABAH


Marsilio, V. and Lanza, B. 1998. Characterization of an oleuropein degrading strain of Lactobacillus plantarum. combined effects of compounds present in olive fermenting brines (phenols, glucose and NaCl) on bacterial activity. *Journal of Science of Food and Agriculture*. **76**: 520 – 524


Motarjeml, Y. 2002. Impact of small scale fermentation technology on food safety in developing countries. International *Journal of Food Microbiology*. **75**: 213 – 229


Lactobacillus plantarum CECT 748. *Journal of Agricultural and Food Chemistry.* **56:** 3068 – 3072


Ruiz-Barba, J.L., Cathcart, D.P., Warner, P.J. and Jimenez-Diaz, R. 1994. Use of *Lactobacillus plantarum* LPCO 10, a bacteriocin producer, as a starter culture in Spanish-style green olive fermentations. *Applied and Environmental Microbiology,* **2059:** 2064


Schnurer, J. and Magnusson, J. 2005. Antifungal lactic acid bacteria as biopreservatives. *Trend in Food Science and Technology.* **16:** 70 – 78


73


Zhou, G.Y. 2006. *Investigation of the biological characteristics of lactic acid bacteria isolated from traditional fermented pickle samples in Sichuan Province*. Thesis. Sichuan Agricultural University, Chengdu, China.