

**BIOCHEMICAL COMPARISON OF RICE WINE
PRODUCED USING COMMON AND
GLUTINOUS RICE WITH THREE DIFFERENT
TRADITIONAL STARTER CAKES**



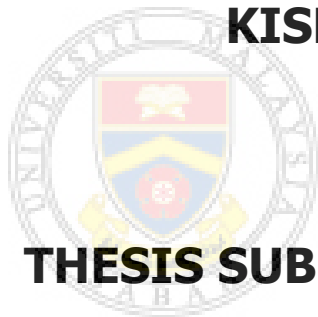
KISHNETH PALANIVELLOO

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**INSTITUTE FOR
TROPICAL BIOLOGY AND CONSERVATION
UNIVERSITI MALAYSIA SABAH
2010**

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PRODUCED USING COMMON AND
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KISHNETH PALANIVELOO



**THESIS SUBMITTED IN FULFILLMENT FOR
THE DEGREE OF MASTER OF SCIENCE**

**INSTITUTE FOR
TROPICAL BIOLOGY AND CONSERVATION
UNIVERSITI MALAYSIA SABAH**

2010

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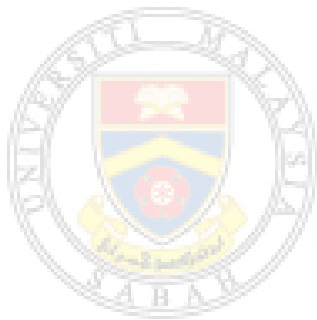
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VERIFICATION

NAME : KISHNETH PALANIVELOO
MATRIC NO : PP2007-8429
**TITLE : BIOCHEMICAL COMPARISON OF RICE WINE
PRODUCED USING COMMON AND GLUTINOUS RICE
WITH THREE DIFFERENT TRADITIONAL STARTER
CAKES.**
DEGREE : MASTER OF SCIENCE
VIVA DATE : 20 JULY 2010

VERIFIED BY

Signature

1. SUPERVISOR

(ASSOC. PROF. DR CHARLES S. VAIRAPPAN



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LIST OF FIGURES

	Page
Figure 4.1 Rice Wine Making process; (From Top Left) (A) Common and glutinous Rice, (B) Cooked rice, (C) Starter Cake, (D) Starter cake pounded, (E) Starter cake mixed evenly with rice, (F) Mixture filled into earthen jar, (G) Utensils used in village, (H) Earthen jar sealed, and (I) Produced rice wine	36
Figure 4.2 Yeast/Lactic Acid Bacteria counts of starter cake used in rice wine fermentation by the Kadazan Dusun community in Sabah	39
Figure 4.3 Yeast/LAB profile for fermentation by common rice and starter cake using DRBC and MRS agar (A-Bitter, B-Bitter Sweet, C-Sweet)	42
Figure 4.4 Yeast/LAB profile for fermentation by glutinous rice and starter cake using DRBC and MRS agar (A-Bitter, B-Bitter Sweet, C-Sweet)	42
Figure 4.5 Percentage reduction in wet weight of rice before and after fermentation for common and glutinous rice using three starters cakes (Com – Common Rice, Glu – Glutinous Rice; B-Bitter, BS-Bitter Sweet, S-Sweet)	43
Figure 4.6 Yield of wine produced by fermentation of common and glutinous rice with three different starter cakes.	45
Figure 4.7 pH values of rice wine produced by fermentation of common and glutinous rice with three different starter cakes	48
Figure 4.8 Scanning Electron Microscope (SEM) micrographs showing the surface of nonfermented rice grains and their cross section surface. (1) Common rice; A1-20x, B1-50x, C1-500x, D1-1,500x (2) Glutinous rice; A2-20x, B2-50x, C2-500x, D2-1,500x	51
Figure 4.9 SEM micrographs of fermented common rice grains and their cross section surface. [A1(20x), B1(10x), C1(20x); A2, B2, C2 – 500X; A3, B3, C3 – 1500X] A-Bitter, B-Bitter Sweet, C-Sweet	53
Figure 4.10 SEM micrographs of fermented glutinous rice grains and their cross section surface. [A1(20x), B1(250x), C1(20x);	

	A2,B2,C2 – 500X; A3,B3,C3 – 1500X] A-Bitter, B-Bitter Sweet, C-Sweet	54
Figure 4.11	Thin Layer Chromatography of starter Cakes (BS – Bitter Sweet, B – Bitter, S – Sweet)	55
Figure 4.12	Thin Layer Chromatography of; A) rice wine , and B) rice cake extracts (BS – Bitter Sweet, B – Bitter, S – Sweet)	57
Figure 4.13	Glucose content of rice wine produced by fermentation of common and glutinous rice with three different starter cakes	59
Figure 4.14	Glucose content of fermented rice produced by fermentation of common and glutinous rice with three different starter cakes	62
Figure 4.15	Alcohol percentage of rice wine produced by fermentation of common and glutinous rice with three different starter cakes	65
Figure 4.16	IC ₅₀ value for rice wine free radical scavenging produced by fermentation of common and glutinous rice with three different starter cakes compared to Ascorbic Acid. (Com – Common Rice, Glu – Glutinous Rice; B-Bitter, BS-Bitter Sweet, S-Sweet)	76
Figure 4.17	IC ₅₀ value for fermented rice free radical scavenging produced by fermentation of common and glutinous rice with three different starter cakes compared to Ascorbic Acid. (Com – Common Rice, Glu – Glutinous Rice; B-Bitter, BS-Bitter Sweet, S-Sweet)	77
Figure 4.18	Fibrinolytic enzyme assay inhibition for fermented rice extracts produced by fermentation of common and glutinous rice with three different starter cakes. (Com – Common Rice, Glu – Glutinous Rice; B-Bitter, BS-Bitter Sweet, S-Sweet)	80
Figure 4.19	Fibrinolytic Enzyme Activity of Fermented Common rice extracts produced by fermentation with three different starter cakes.	83
Figure 4.20	Fibrinolytic Enzyme Activity of Fermented Glutinous rice extracts produced by fermentation with three different starter cakes.	83
Figure D-1	H-NMR Spectrum of isolated common major compound of rice wine and fermented rice (DCSV#931)	106
Figure D-2	¹³ C-NMR Spectrum of isolated common major compound of	

	rice wine and fermented rice (DCSV#933)	107
Figure E-1	HPLC Chromatogram and Standard Curve of Sugar Standard	108
Figure E-2	HPLC Chromatogram of 10 mg/ml Glucose Standard	109
Figure E-3	HPLC Chromatogram of 50 mg/ml Glucose Standard	109
Figure E-4	HPLC Chromatogram of 100 mg/ml Glucose Standard	110
Figure E-5	HPLC Chromatogram of 200 mg/ml Glucose Standard	110
Figure E-6	HPLC Chromatogram for Common Bitter Sweet Wine	111
Figure E-7	HPLC Chromatogram for Common Bitter Wine	111
Figure E-8	HPLC Chromatogram for Common Sweet Wine	112
Figure E-9	HPLC Chromatogram for Glutinous Bitter Wine	112
Figure E-10	HPLC Chromatogram for Glutinous Bitter Sweet Wine	113
Figure E-11	HPLC Chromatogram for Glutinous Sweet Wine	113
Figure E-12	HPLC Chromatogram for Common Bitter Rice Cake	114
Figure E-13	HPLC Chromatogram for Common Bitter Sweet Rice Cake	114
Figure E-14	HPLC Chromatogram for Common Sweet Rice Cake	115
Figure E-15	HPLC Chromatogram for Glutinous Bitter Rice Cake	115
Figure E-16	HPLC Chromatogram for Glutinous Bitter Sweet Rice Cake	116
Figure E-17	HPLC Chromatogram for Glutinous Sweet Rice Cake	116
Figure F-1	Alcohol standard curve graph	117
Figure F-2	Gas Chromatographic Spectrum for Alcohol Standard 1 %	118
Figure F-3	Gas Chromatographic Spectrum for Alcohol Standard 10 %	118
Figure F-4	GC Alcohol Spectrum for Common Bitter fermentation	119
Figure F-5	GC Alcohol Spectrum for Common Bitter Sweet fermentation	119
Figure F-6	GC Alcohol Spectrum for Common Sweet fermentation	120
Figure F-7	GC Alcohol Spectrum for Glutinous Bitter fermentation	120

Figure F-8	GC Alcohol Spectrum for Glutinous Bitter Sweet fermentation	121
Figure F -9	GC Alcohol Spectrum for Glutinous Sweet fermentation	121
Figure G-1	GC Spectrum for Common Bitter Rice Wine Volatile Hydrocarbons	122
Figure G-2	GC Spectrum for Common Bitter Sweet Rice Wine Volatile Hydrocarbons	123
Figure G-3	GC Spectrum for Common Sweet Rice Wine Volatile Hydrocarbons	124
Figure G-4	GC Spectrum for Glutinous Bitter Rice Wine Volatile Hydrocarbons	125
Figure G-5	GC Spectrum for Glutinous Bitter Sweet Rice Wine Volatile Hydrocarbons	126
Figure G-6	GC Spectrum for Glutinous Sweet Rice Wine Volatile Hydrocarbons	127
Figure G-7	GC Spectrum for Common Bitter Rice Cake Volatile Hydrocarbons	128
Figure G-8	GC Spectrum for Common Bitter Sweet Rice Cake Volatile Hydrocarbons	129
Figure G-9	GC Spectrum for Common Sweet Rice Cake Volatile Hydrocarbons	130
Figure G-10	GC Spectrum for Glutinous Bitter Rice Cake Volatile Hydrocarbons	131
Figure G-11	GC Spectrum for Glutinous Bitter Sweet Rice Cake Volatile Hydrocarbons	132
Figure G-12	GC Spectrum for Glutinous Sweet Rice Cake Volatile Hydrocarbons	133
Figure H-1	Ascorbic Acid Standard Curve (Top and Bottom)	134
Figure H-2	Common Bitter Rice Cake Graph for Average Percentage Inhibition	135
Figure H-3	Common Bitter Sweet Rice Cake Graph for Average Percentage Inhibition	136

Figure H-4	Common Sweet Rice Cake Graph for Average Percentage Inhibition	137
Figure H-5	Glutinous Bitter Rice Cake Graph for Average Percentage Inhibition	138
Figure H-6	Glutinous Bitter Sweet Rice Cake Graph for Average Percentage Inhibition	139
Figure H-7	Glutinous Sweet Rice Cake Graph for Average Percentage Inhibition	140
Figure H-8	Common Bitter Rice Wine Graph for Average Percentage Inhibition	141
Figure H-9	Common Bitter Sweet Rice Wine Graph for Average Percentage Inhibition	142
Figure H-10	Common Sweet Rice Wine Graph for Average Percentage Inhibition	143
Figure H-11	Glutinous Bitter Rice Wine Graph for Average Percentage Inhibition	144
Figure H-12	Glutinous Bitter Sweet Rice Wine Graph for Average Percentage Inhibition	145
Figure H-13	Glutinous Sweet Rice Wine Graph for Average Percentage Inhibition	146

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Once again, THANKS! to all...

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ABSTRACT

BIOCHEMICAL COMPARISON OF RICE WINE PRODUCED USING COMMON AND GLUTINOUS RICE WITH THREE DIFFERENT TRADITIONAL STARTER CAKES.

Rice wine or 'tapai' is one of the traditional alcoholic beverages, which has been produced in Sabah for ages and is used in most of the cultural rituals and ceremonies. This study delves on the chemical characterization of rice wine produced from common and glutinous rice using three different traditional starter cakes; 1) bitter, 2) bitter sweet, 3) sweet. Each batch of fermentation involving one type of rice and starter cake lasted for 4 weeks and the wine collected were separated from its rice, and the fermented rice was extracted in ethanol to yield crude extract. Sweet starter cake had the highest yeast/LAB load followed by bitter sweet and bitter and accordingly resulted in and weight reduction of substrate at an average of 450 %, 230 % and 210 % respectively. The volume of wine produced by glutinous rice was twice the volume produced by common rice. pH of the wines was in the range of 4.3 and 4.7. Morphological changes in the rice grain during fermentation were observed through scanning electron micrographs (SEM) and the presence of yeast/LAB growth and changes to the rice grains were observed. High Performance Liquid Chromatography analysis of the wine revealed that wine produced from common rice with its respective starter cake contained much lower glucose content [1) Bitter : 120.643 ± 0.21 mg/ml, 2) Bitter-Sweet : 270.42 ± 0.32 mg/ml, 3) Sweet : 310.326 ± 5.83 mg/ml] as compared to glutinous rice [1)Bitter : 300.274 ± 0.28 mg/ml, 2) Sweet and bitter : 320.251 ± 0.00 mg/ml, 3) Sweet : 440.138 ± 29.97 mg/ml]. However, gas chromatography analysis showed that rice wine produced from common rice had comparatively higher alcohol percentage; 9 % to 12 % as compared to glutinous rice; 7 % to 8 %. The difference in wine taste could be attributed to the varying type and amounts of organic acids and metabolites contained in the starter cakes and produced during the fermentation process. Gas Chromatography analysis revealed details of this complex composition. Comparison of these chemicals between their starter cake, wine and fermented rice is discussed. Ethanolic extract of the respective fermented rice contained low glucose levels but showed strong antioxidant and potent fibrinolytic enzyme activities, with bitter sweet starter cake displaying the best of results. Antioxidant potential of fermented rice extracts of common rice displayed higher scavenging activity compared to glutinous rice, while common rice wine displayed higher antioxidant values compared to glutinous rice wine. Fibrinolytic enzyme activities were present in fermented rice extracts, and extracts of common rice showed double the activity by diameter as compared to glutinous rice for all three starters. In summary, fermentation using different rice types with different starter cakes confirms the difference in chemical properties as well as bioactive potentials of the rice wine and fermented rice.

ABSTRAK

Tapai atau 'rice wine' merupakan salah satu minuman beralkohol tradisional yang telah dihasilkan di Sabah sejak berkurun lamanya dan digunakan dalam kebanyakan upacara dan keramaian. Kajian ini melibatkan penyelidikan terhadap ciri-ciri kimia tapai yang dihasilkan daripada beras putih dan pulut menggunakan tiga ragi berbeza; 1) pahit, 2) pahit manis, 3) manis. Setiap set penapaian yang melibatkan salah satu beras dan ragi mengambil masa 4 minggu. Tapai yang dihasilkan telah diasingkan daripada nasi yang seterusnya diekstrak dengan ethanol untuk mendapatkan ekstraknya. Ragi manis mempunyai kandungan yis/LAB tertinggi diikuti ragi pahit manis serta ragi pahit dan dalam urutan sedemikian perubahan berat substrat untuk penapaian adalah dalam purata 450 %, 230 % dan 210 % masing-masing. Isipadu arak yang dihasilkan beras pulut adalah dua kali ganda arak yang dihasilkan oleh beras biasa. pH semua arak berada dalam julat 4.3 dan 4.7. Perubahan morfologi butiran nasi sewaktu penapaian diteliti melalui mikroskop pengimbasan elektron (SEM) dan kehadiran yis/LAB serta perubahan terhadap butiran nasi juga diteliti. Analisis Kromatografi Cair Kinerja Tinggi menunjukkan alkohol yang dihasilkan menggunakan beras biasa dengan ragi berlainan mengandungi kandungan glukosa yang lebih rendah [1) Pahit : 120.643 ± 0.21 mg/ml, 2) Pahit manis : 270.42 ± 0.32 mg/ml, 3) Manis : 310.326 ± 5.83 mg/ml] berbanding beras pulut [1) Pahit : 300.274 ± 0.28 mg/ml, 2) Pahit manis : 320.251 ± 0.00 mg/ml, 3) Manis : 440.138 ± 29.97 mg/ml]. Bagaimanapun, analisis kromatografi gas menunjukkan tapai yang dihasilkan dari beras biasa mempunyai kandungan alkohol yang lebih tinggi; 9 % hingga 12 % berbanding beras pulut; 7 % hingga 8 %. Perbezaan dari segi rasa pula boleh dikaitkan dengan jumlah dan kepelbagaian asid organik dan unsur-unsur yang terkandung dalam ragi dan yang dihasilkan semasa proses penapaian. Analisis kromatografi gas telah memberi data yang lebih mendalam berkenaan komposisi kompleks ini. Perbezaan kandungan kimia antara ragi, tapai dan nasi hasil penapaian juga telah dikaji. Ekstrak ethanol nasi hasil penapaian mengandungi kandungan glukosa yang rendah tetapi menunjukkan keupayaan antioksidan dan aktiviti enzim fibrinolitik yang kuat dengan hasil ragi pahit manis memberi keputusan terbaik. Keupayaan antioksidan ekstrak nasi biasa menunjukkan kebolehan 'scavenging' yang lebih tinggi berbanding ekstrak nasi pulut, manakala tapai beras biasa memberi bacaan antioksidan yang lebih tinggi berbanding hasil beras pulut. Aktiviti enzim fibrinolitik adalah ketara pada ekstrak nasi dan ekstrak nasi biasa menunjukkan aktiviti dua kali ganda lebih kuat dari segi diameter daripada ekstrak nasi pulut bagi ketiga-tiga ragi. Secara kesimpulan, penapaian menggunakan jenis beras berbeza dengan ragi berbeza pastinya menunjukkan perbezaan dari segi kandungan kimia dan potensi bioaktif tapai dan nasi hasil penapaian.

TABLE OF CONTENTS

	Page
TITLE	i
DECLARATION	ii
VERIFICATION	iii
ACKNOWLEDGEMENT	iv
ABSTRACT	v
ABSTRAK	vi
TABLE OF CONTENTS	vii
LIST OF TABLES	x
LIST OF FIGURES	xiii
LIST OF ABBREVIATIONS	xviii
LIST OF APPENDIX	xix
 CHAPTER 1: INTRODUCTION	 1
1.1 Food and Fermentation	1
1.2 Benefits of Fermentation	2
1.3 Rice Wine of Sabah	3
1.4 Objectives	4
1.5 Significance of Study	5
 CHAPTER 2: LITERATURE REVIEW	 6
2.1 Fermented Product	6
2.2 Types of Fermentation	6
2.2.1 High Salt Savory Flavoured Amino/ Peptide Sauces and Pastes	7
2.2.2 Lactic Acid Fermentation	7
2.2.3 Alcoholic Fermentation	8
2.2.4 Acetic Acid Fermentation	9
2.2.5 Alkaline Fermentation	9
2.2.6 Leavened Bread Fermentation	9
2.2.7 Mixed Acid Fermentation	10
2.3 Fermented Beverage	10
2.3.1 Japanese 'Sake'	11
2.3.2 Chinese Rice Wine	11
2.3.3 Red and White Wine	12
2.3.4 Other world beverages	13
2.4 Biochemical Studies on Fermented Beverage	14
2.4.1 Microbial Diversity	14
2.4.2 Chemical Profile	16
2.5 Health Benefits	18
2.5.1 Increased Bone Density	18
2.5.2 Reduced Cardiovascular Risk	19
2.5.3 Gastritis, Ulcer and Cancer	20
2.5.4 Antioxidant Potential	20
2.6 Fermented Product Market	21

2.7	Conversation of Traditional Knowledge	22
CHAPTER 3: METHODOLOGY		24
3.1	Pre-Fermentation	24
3.2	Post-Fermentation	25
3.3	Scanning Electron Microscope (SEM) Imaging	25
3.4	Chemical Extraction of Fermented Rice	26
3.5	Chemical Profiling of Rice Wine and Fermented Rice Extract	26
3.5.1	Thin Layer Chromatography (TLC)	26
3.5.2	Preparative Thin Layer Chromatography (PTLC)	27
3.5.3	High Performance Liquid Chromatography (HPLC)	27
3.5.4	Gas Chromatography Mass Spectrometer (GCMS)	28
3.5.5	Nuclear Magnetic Resonance (NMR)	28
3.6	Yeast/Lactic Acid Bacteria (LAB) Enumeration	29
3.6.1	Enumeration of Starter Cakes	29
3.6.2	Enumeration of Fermented Rice/Wine	29
3.6.3	Preparation of de Man, Rogosa, and Sharpe (MRS) Agar	30
3.6.4	Preparation of Dicloran Rose Bengal Choramphenicol (DRBC) Agar	30
3.7	Antibacterial Bioassay	30
3.7.1	Preparation of Nutrient Agar (NA)	31
3.7.2	Preparation of Nutrient Broth (NB)	31
3.8	Antioxidant Assay	31
3.9	Fibrinolytic Enzyme Assay	32
3.10	Statistical Analysis	32
CHAPTER 4: RESULTS AND DISCUSSION		33
4.1	Limitations of Study	33
4.2	Documentation of Traditional Knowledge in Rice Wine Making	33
4.3	Fermentation Profile	37
4.3.1	Yeast/Lactic Acid Bacteria Count for Starter Cakes	37
4.3.2	Yeast/Lactic Acid Bacteria Increment During Fermentation	39
4.3.3	Weight of Rice Before and After Fermentation	43
4.3.4	Yield of wine	44
4.3.5	pH	47
4.4	Scanning Electron Microscope (SEM) Imaging	50
4.5	Chemical Profiling	55
4.5.1	Thin Layer Chromatography (TLC) of Starter Cake	55
4.5.2	Thin Layer Chromatography (TLC) of Fermented Rice and Wine	56
4.6	Sugar Analysis	58
4.6.1	Rice Wine	58
4.6.2	Fermented Rice	61
4.7	Alcohol Content	64
4.8	Volatile Hydrocarbons	67
4.9	Biological Assay/ Bioactive Potential Evaluation	74
4.9.1	Antibacterial Assay	74
4.9.2	Antioxidant Test	75

4.9.3 Fibrinolytic Enzyme Assay	79
CHAPTER 5: CONCLUSION	84
REFERENCES	86
APPENDIX	94



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LIST OF TABLES

	Page
Table 2.1 Volatile compounds formed during cereal fermentation	18
Table 4.1 Retention factors (R_f) values of visible (blue) spots on TLC	56
Table 4.2 Retention factors (R_f) value for visible (blue) spots of common and glutinous rice wine.	58
Table 4.3 Retention factors (R_f) value for visible (blue) spots of common and glutinous rice extract.	58
Table 4.4 Volatile Compounds from Common Rice Wines of Different Starter Cakes based on GCMS analysis.	69
Table 4.5 Volatile Compounds from Glutinous Rice Wines of Different Starter Cakes based on GCMS analysis.	70
Table 4.6 Volatile Compounds from Common Rice Cakes of Different Starter Cakes based on GCMS analysis.	73
Table 4.7 Volatile Compounds from Glutinous Rice Cakes of Different Starter Cakes based on GCMS analysis.	74
Table A-1 Total Yeast/LAB Count for Starter Cakes	94
Table A- 2 Summary of Yeast/LAB Count for Starter Cakes	94
Table B-1 Total Yeast/LAB Count for Common Rice Fermentation (DRBC)	95
Table B-2 Summary of Yeast/LAB Count for Common Rice Fermentation (DRBC)	95
Table B-3 Total Yeast/LAB Count for Glutinous Rice Fermentation (DRBC)	96
Table B-4 Summary of Yeast/LAB Count for Glutinous Rice Fermentation (DRBC)	96
Table B-5 Total Yeast/LAB Count for Common Rice Fermentation (MRS)	97
Table B-6 Summary of Yeast/LAB Count for Common Rice Fermentation (MRS)	97

Table B-7	Total Yeast/LAB Count for Glutinous Rice Fermentation (MRS)	98
Table B-8	Summary of Yeast/LAB Count for Glutinous Rice Fermentation (MRS)	98
Table C-1	Summary of Rice Wine Data	99
Table C-2	Summary of Rice Cake Data	100
Table C-3	Summary of Fixed Factors	101
Table C-4	Raw Data for Rice Wine in Triplicates	101
Table C-5	Descriptive Statistics for Rice Wine	102
Table C-6	Tests of Between-Subjects Effects for Rice Wine	103
Table C-7	Raw Data for Fermented Rice in Triplicates	104
Table C-8	Descriptive Statistics for Fermented Rice	105
Table C-9	Tests of Between-Subjects Effects for Fermented Rice	106
Table F-1	Area below the graph values for 1 % and 10 % alcohol concentration	117
Table H-1	Absorbance Value and Percentage Inhibition of Ascorbic Acid	134
Table H-2	Absorbance Value and Percentage Inhibition of Common Bitter Rice Cake	135
Table H-3	Absorbance Value and Percentage Inhibition of Common Bitter Sweet Rice Cake	136
Table H-4	Absorbance Value and Percentage Inhibition of Common Sweet Rice Cake	137
Table H-5	Absorbance Value and Percentage Inhibition of Glutinous Bitter Rice Cake	138
Table H-6	Absorbance Value and Percentage Inhibition of Glutinous Bitter Sweet Rice	139
Table H-7	Absorbance Value and Percentage Inhibition of Glutinous Sweet Rice Cake	140
Table H-8	Absorbance Value and Percentage Inhibition of Common Bitter Rice Wine	141

Table H-9	Absorbance Value and Percentage Inhibition of Common Bitter Sweet Rice Wine	142
Table H-10	Absorbance Value and Percentage Inhibition of Common Sweet Rice Wine	143
Table H-11	Absorbance Value and Percentage Inhibition of Glutinous Bitter Rice Wine	144
Table H-12	Absorbance Value and Percentage Inhibition of Glutinous Bitter Sweet Rice Wine	145
Table H-13	Absorbance Value and Percentage Inhibition of Glutinous Sweet Rice Wine	146



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LIST OF ABBREVIATIONS AND SYMBOLS

C-B	Common Rice, Bitter Starter Cake
C-BS	Common Rice, Bitter Sweet Starter Cake
C-S	Common Rice, Sweet Starter Cake
G-B	Glutinous Rice, Bitter Starter Cake
G-BS	Glutinous Rice, Bitter Sweet Starter Cake
G-S	Glutinous Rice, Sweet Starter Cake
%	Percent
°C	Degree Celsius
CFU	Colony Forming Unit
AA	Ascorbic Acid
cm	centimeter
g	gram
GCMS	Gas chromatography Mass Spectrometry
HPLC	High Performance Liquid Chromatography
mg	milligram
mL	milliliter
µg	microgram
µL	microliter
NaCl	Sodium Chloride
RT	Retention Time
RI	Retention Index
R _f	mobility relative to front
PTLC	Preparative Thin Layer Chromatography
TLC	Thin Layer Chromatography
Tol	Toluene
UV	ultra violet
w/v	Weight over Volume

LIST OF APPENDIX

	Page
Appendix A Bacterial Enumeration for Starters	94
Appendix B Bacterial Enumeration for Fermentation	95
Appendix C Data Summary and Statistical Analysis	99
Appendix D Nuclear Magnetic Resonance (NMR) spectrum	106
Appendix E HPLC Sugar Analysis Spectrum	108
Appendix F GC Alcohol Analysis Spectrum	117
Appendix G GC Volatile Hydrocarbon Spectrum	122
Appendix H Antioxidant Assay	134



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CHAPTER 1

INTRODUCTION

1.1 FOOD AND FERMENTATION

Man had practiced the art of food fermentation for centuries even before its basic principle were really understood. The study of fermentation in a scientific approach only took place in the 1850s with the isolation of amyl alcohol by Louis Pasteur (El-Mansi *et al.*, 2007). According to Pasteur, fermentation is associated with the life and structural integrity of the yeast cells whereby yeasts cells play the role as the ferment. Various theories were proposed by various researches regarding fermentation but generally fermentation is understood as an enzyme catalyzed, energy producing process which generally uses sugar as substrate to transform it into products such as ethanol, lactic acid, hydrogen and at the same time resulting in the production of other minor organic acids (Cornish-Bowden, 1997). Fermentation was also described as a sequence of events whereby oxygen from sugar was transferred from one molecule of sugar to the other to form a highly oxidized and reduced product. Finally, the concept of fermentation was modified to support the idea that fermentation was a function of a living entity involving a chain of multiple reactions catalyzed by various enzymes produced by microorganisms (El-Mansi *et al.*, 2007). Therefore, fermentation can be considered as the breaking of food into small components for easy consumption and digestion as well as proper assimilation of nutrient by the body.

At present, there are various types of food that are fermented for human and animal consumption. Fermented food are the end product from biochemical processes caused by microorganisms whereby their enzymes hydrolyze polysaccharides, proteins and lipids to nontoxic products with better flavorings, aromas and texture so that it is much attraction for human consumption (Streinkraus, 2002). Originally fermented food was only restricted to household

consumption and was produced in limited quantity but as demand increased, these food production transformed to cottage industry and is now produced in large scale industrially.

1.2 BENEFITS OF FERMENTATION

Fermentation of food has been known to provide multiple benefits to consumers and animals. Fermentation has the potential to enrich the food by developing a variety of flavors, aromas, and textures from the various chemical processes and fine chemicals contributed by the microorganisms. In the production of wine, the final product can taste sweet, bitter or sour depending on the combination of ingredients used and produced.

Fermented food as a whole is much more nutritious as the process of fermentation enriches the end product biologically with vitamins, proteins, amino acids and fatty acids. As an example, starch from rice is converted into sugar then fermented into alcohol; there would be an increase in protein level and free amino acid content (Steinkraus, 2002). However, the variation in nutritional quality and quantity at the end of the fermentation process depend on the kind of microorganisms employed and the parameters governing the process.

During the process of fermentation, detoxification takes place through the continuous hydration of the raw material and by subjecting the materials to extreme condition (either acidic or alkaline), which removes potential toxins in food. At the same time, the specific extreme pH condition created by the yeast/Lactic acid bacteria eliminates unwanted microorganisms present that might spoil the fermented product, thus making it last longer. As such, fermentation also helps to preserve food so that it can be stored long without deteriorating its quality and taste. Preservation of food is best through lactic acid, alcohol, acetic acid and alkaline fermentations (Steinkraus, 2002).

On the contrary, though fermented food takes time to be produced very little energy is required to produce it. Fermented food is produced naturally with the reaction of microorganism under suitable conditions to provide hygienic, health

beneficial food ready for consumption. Therefore, fermentation is an environment friendly process as energy is conserved (Steinkraus, 2002).

1.3 RICE WINE OF SABAH

Today, indigenous fermented food has become a new research interest since these are believed to contain therapeutic properties beneficial to the health of non-traditional consumers (Chiang *et al.*, 2006). The application of fermentation is not restricted to a particular region but is practiced all over the world thus widespread research had been conducted to understand the benefits of fermented food. Some example of fermented food are the Indonesian "tempe", Chinese soy sauce, Malaysian "belacan" and "tempoyak", Cambodian "prahoc", Middle-Eastern yoghurts, Nigerian "gari", Indian jackfruit wine, Zambian maize beer and Kenyan "busaa", among the many varieties available today.

In this regard, the East Malaysian states in the island of Borneo are extremely rich in nature as well as culture with the most number of indigenous ethnic races with their unique cultural values. With the diversity of culture comes various traditional food and techniques for their preparation. Specifically, in Sabah, the local community has the knowledge to transform harvested rice into an alcoholic beverage. The rice wine which is locally known as 'tapai' is one of such product and has been produced for ages by the local community in small quantities, mainly for household consumption as well as cultural ceremonies and rituals. This wine has a variety of names, which differ with the ethnic races, for instance certain Dusun slangs call it 'hiing', whereas others call it 'kinomol', 'segantang', 'kinarung', 'kinopi', and 'linahas'.

The produced wine has an alcoholic aroma with a mixture of bitter sweet taste and a sparkling feel. The freshly produced wine comes in faded yellow color but turns bright golden yellow when stored for a long duration. This alcoholic beverage is the end product of rice fermentation with the aid of a starter cake or yeast/lactic acid bacteria, which is locally known as 'ragi' or 'sasad'. The starter cake, which is extremely important in the production of wines, is made from rice, spice and yeast. A variety of rice is used to make rice wine and the starter cake.