

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

KISHNETH PALANIVELOO

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

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

2010



UMS
UNIVERSITI MALAYSIA SABAH

UNIVERSITI MALAYSIA SABAH

BORANG PENGESAHAN STATUS TESIS

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

IJAZAH : SARJANA SAINS (S.Sn)

SAYA : KISHNETH PALANIVELOO SESI PENGAJIAN : 2008/2010

Mengaku membenarkan tesis (LPSM/Sarjana/Doktor Falsafah) ini disimpan di Perpustakaan Universiti Malaysia Sabah dengan syarat-syarat kegunaan seperti berikut :-

1. Tesis adalah hakmilik Universiti Malaysia Sabah.
2. Perpustakaan Universiti Malaysia Sabah dibenarkan membuat salinan untuk tujuan pengajian sahaja.
3. Perpustakaan dibenarkan membuat salinan tesis ini sebagai bahan pertukaran antara institusi pengajian tinggi.
4. Sila tandakan (/)

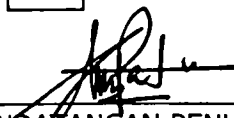
SULIT

(Mengandungi maklumat yang berdarjah keselamatan atau Kepentingan Malaysia seperti yang termaktub di dalam AKTA RAHSIA RASMI 1972)


TERHAD

(Mengandungi maklumat TERHAD yang telah ditentukan oleh organisasi/badan di mana penyelidikan dijalankan)

TIDAK TERHAD

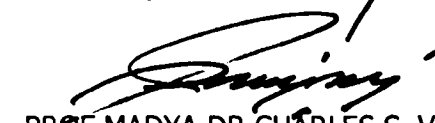


(TANDATANGAN PENULIS)

Disahkan Oleh
JAMIUN MICHEAL
PUSTAKAWAN
PERPUSTAKAAN
UNIVERSITI MALAYSIA SABAH


(TANDATANGAN PUSTAKAWAN)

Alamat Tetap: 133, Jalan Tanjung,
Taman Bunga Raya, 08000 Sungai
Petani, Kedah Darul Aman



PROF MADYA DR CHARLES S. VAIRAPPAN

Tarikh: 09-08-2010

Tarikh: 09-08-2010



DECLARATION

I declare that the work presented in this thesis is to the best of my knowledge and belief, original and my own work except as acknowledged in the text.

7 JUNE 2010


KISHNETH PALANIVELOO
PP2007-8429

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)**



ACKNOWLEDGEMENT

The completion of this research thesis involved the support and contribution of several parties along the way. Therefore as appreciation I would like to convey my heartfelt gratitude.

Firstly, I would like to express my heartfelt gratitude to my supervisor Associate Professor Dr. Charles Santhanaraju Vairappan for his undivided attention and guidance which led me to the successful completion of this thesis.

Next, I wish to thank my parents, Mr and Mrs Palaniveloo Sinayah for their neverending support being it financial or morally and patience for the 6 years spent away from home pursuing my studies at Universiti Malaysia Sabah.

I would like to extend my gratitude to the Director of Institute of Tropical Biology and Conservation (ITBC) for sufficient facilities made available for the completion of this research. Heartfelt thanks to its staffs as well for their voluntary participating in questionnaires pertaining to the research conducted and not forgetting a group of Conservation Biology students of the Batch 2006/2009 who contributed a handful in the process of obtaining the samples for this research. Special thanks to the Natural Products Chemistry lab assistant, Julianah Joseph for supplying starter cultures all the way from Tamparuli and to Madam Rinduh bte Lian of Kg.Tenghilan for her demonstration of traditional wine making which was the basis for this research.

Finally, I would also like to thank all those whose name has not been mentioned here but has played a role in contributing to my thesis. All the wonderful moments and learning experience with all of you will be deeply engraved in my heart.

Once again, THANKS! to all...

Kishneth Palaniveloo
7 June 2010

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 mikrograf 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 Food	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) Analysis	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
4.4.2	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

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

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

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 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

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.

REFERENCES

- Aa, E., Townsend, J.P., Adams, R.I., Nielsen, K.M., Taylor, J.W. 2006. Population structure and gene evolution in *Saccharomyces cerevisiae*. *FEMS Yeast Research* 6:702-715.
- Arnao, M.B. 2000. Some methodological problems in the determination of antioxidant activity using chromogen radicals: a practical case. *Trends in Food Science & Technology* 11:419-421.
- Ashipala, O.K., and He, Q. 2007. Optimization of fibrinolytic enzyme production by *Bacillus subtilis* DC-2 in aqueous two-phase system (poly-ethylene glycol 4000 and sodium sulfate) *Bioresource Technology* 99(10):4112-4119
- Atkinson, R. W. 1881. Chemistry of Sake Brewing. University of Tokyo.
- Azmi, A., Hasan, M., Mel, M., Cheng, N. 2009. Single-step Bioconversion of starch to Bio-ethanol by Coculture of ragi tapai and *Saccharomyces cerevisiae*. *Chemical Engineering Transactions* 18
- Bai, F.W., Anderson, W.A., Moo-Young, M. 2008. Ethanol fermentation technologies from sugar and starch feedstocks. *Biotechnology Advances* 26:89-105.
- Balaraman, K. and Prabakaran, G. 2007. Production & purification of a fibrinolytic enzyme (thrombinase) from *Bacillus sphaericus*. *Indian Journal of Medical Research* 126:459-464.
- Bergmen, C.J. and Xu, Z. 2003. Genotype and Environment Effects on Tocopherol, Tocotrienol, and γ -Oryzanol Contents of Southern U.S. *Rice Cereal Chemistry* 80(4):446-449
- Blandino, A., Al-Aseeri, M. E., Pandiella, S. S., Cantero, D. and Webb, C. 2003. Cereal-based fermented food and beverages. *Food Research International* 36:527-543.
- Camara, J.S., Marques, J.C., Alves, A. and Ferreira, A.C.S. 2003. Heterocyclic acetals in Madeira wines. *Analytical and Bioanalytical Chemistry* 375 : 1221-1224.
- Carneiro, A., Couto, J. A., Mena, C., Queiroz, J. and Hogg, T. 2008. Activity of wine against *Campylobacter jejuni*. *Food Control* 19:800-805.
- Carroll, G.C. and Wicklow. D.T. 1992. The Fungal community: its organization and role in the ecosystem. In: (M.J. Robert Nout, ed) *Ecological Aspects of Mixed-Culture Food Fermentations*. Marcel Dekker Inc. New York. pp 817-854



- Cavalieri, D., McGovern, P.E., Hartl, D.L., Mortimer, R. and Polsinelli, M. 2003. Evidence for *S. cerevisiae* Fermentation in Ancient Wine. *Journal of Molecular Evolution* 57:S226-S232.
- Chang, C.T., Fan, M.H., Kho, F.C. and Sung, H.Y. 2000. Potent Fibrinolytic Enzyme from a Mutant of *Bacillus subtilis* IMR-NK1. *Journal of Agricultural and Food Chemistry* 48:3210-3216.
- Chen, M. and Bergman, C.J. 2007. Rice grain vitamin-E homologs and g-Oryzanol: effects of grain development and cultural practices. *Rice Utilization Workshop Proceedings*.
- Chiang Y. W., Chye, F. Y. and Mohd Ismail, A. 2006. Microbial Diversity and Proximate Composition of Tapai, A Sabah's Fermented Beverage. *Malaysian Journal of Microbiology*, 2(1):1-6.
- Ciani, M., Beco, L. and Comitini, F. 2006. Fermentation behavior and metabolic interactions of multistarter wine yeast fermentations. *International Journal of Food Microbiology* 108:239-245.
- Clemente-Jimenez, J. M., Mingorance-Cazorla, L., Martinez-Rodriguez, S., Las Heras-Vazquez, F. J. and Rodriguez-Vico, F. 2005. Influence of sequential yeast mixtures on wine fermentation. *International Journal of Food Microbiology* 98:301– 308.
- Cocolin, L., Bisson, L.F. and Mills, D.A. 2000. Direct profiling of the yeast dynamics in wine fermentations. *FEMS Microbiology Letters* 189:81-87.
- Combina, M., Elia, A., Mercado, L., Catania, C., Ganga, A. and Martinez, C. 2005. Dynamics of indigenous yeast populations during spontaneous fermentation of wines from Mendoza, Argentina. *International Journal of Food Microbiology* 99:237– 243
- Cornish-Bowden, 1997. *New Beer in an Old Bottle: Eduard Buchner and the Growth of Biochemical Knowledge*. Universitat de València
- Di Maro, E., Ercolini, D. and Coppola, S. 2007. Yeast dynamics during spontaneous wine fermentation of the Catalanesca grape. *International Journal of Food Microbiology* 117:201-210.
- Dung, N.T.P., Rombouts, F.M. and Nout, M.J.R. 2005. Development of defined mixed- culture fungal fermentation starter granulate for controlled production of rice wine. *Innovative Food Science and Emerging Technologies* 6:429 – 441.
- Dung, N.T.P., Rombouts, F.M. and Nout, M.J.R. 2007. Characteristics of some traditional Vietnamese starch-based rice wine fermentation starters (*men*). *LWT* 40:130-135.

- Eijkhoff, P. 2000. Wine in China: Its History and Contemporary Developments. The Netherlands / Nederlands Wijn Gilde, Utrecht.
- El-Mansi, E.M.T., Bryce, C.F.A., Demain, A.L. and Allman, A.R. 2007. *Fermentation Microbiology and Biotechnology*, Taylor and Francis Group, CRC Press, United Kingdom.
- Ennouali, M., Elmoualdi, L., labioui, H., Ouhsine, M. and Elyachioui, M. 2006. Biotransformation of the fish waste by fermentation. *African Journal of Biotechnology* 5(19):1733-1737.
- Fei, Q., Linchun, M. and Xin, P. 2006. Antioxidant activities of Five Chinese rice wines and the involvement of phenolic compounds. *Food Research International* 39:581-587.
- Battcock, M. and Azam-Ali, S. 1998. Fermented Fruits and Vegetables. A Global Perspective. *Food and Agricultural Organization Agricultural Services Bulletin* 134. Food and Agricultural Organization of the United Nations, Rome.
- Fernandez-Novales, J., Lopez, M., Sanchez, M., Garcia, J. and Morales, J. 2008. A feasibility study on the use of a miniature fiber optic NIR spectrometer for the prediction of volumic mass and reducing sugars in the white wine fermentations. *Journal of Food Engineering* 89:325-329.
- Johannesen, R. 1991. Energy Efficiency & Environmental News : Alcohol Production from Biomass. Institute of Food and Agricultural Sciences, University of Florida. May Issue.
- Gandjar, I. 2003. Tapai from Cassava and Cereals. Paper presented at the First *International Symposium and Workshop on Insight into the World of Indigenous Fermented Foods for Technology Development and Food Safety*, Kasetsart University, August 13-17.
- Garabal, J.I. 2007. Biodiversity and the survival of autochthonous fermented products. *International Microbiology* 10:1-3.
- Giraffa, G. 2004. Studying the dynamics of microbial populations during food fermentation. *FEMS Microbiology Reviews* 28:251-260.
- Henkel, T.W. 2005. Parakari, an indigenous fermented beverage using amylolytic *Rhizopus* in Guyana. *Mycologia*, 97(1):1-11.
- Hesseltine, C.W., Rogers, R. and Winarno, F.G. 1988. Microbiological studies on amylolytic oriental fermentation starters. *Mycopathologia* 101:141-155.
- Herjavec, S., Podgorski, V., Redzepovic, S. and Mirosevic, N. 2003. Influence of *S. cerevisiae* Strains on the Quality of Wines, *Food Technology and Biotechnology* 41(1):77-81.

- Juhan-Vague, I. and Alessi, M. C. 1996. Fibrinolysis and risk of coronary artery disease. Invited review: Plenary Lecture at the International Congress on Fibrinolysis & Thrombolysis, Barcelona 1996. *Fibrinolysis* 10(3):127-136.
- Kelebek, H., Selli, S., Canbas, A. and Turgut, C. 2009. HPLC determination of organic acids, sugars, phenolic compositions and antioxidant capacity of orange juice and orange wine made from a Turkish cv. Kozan. *Microchemical Journal* 91:187-192.
- Kerr, S.L., Frow, M.T., Heller, A. and Whitnack, C. 2007. Engineering Wine. A Business Model.
- Kennedy, J. 2007. The Science of Red Wine. The New York Academy of Sciences Magazine. May/June:11-13.
- Kim, S.S., Lee, S.E., Kim, O.W. and Kim, D.C. 2000. Physicochemical Characteristics of Chalky Kernels and Their Effects on Sensory Quality of Cooked Rice. *Cereal Chemistry* 77(3):376-379.
- Kourkoutas, Y., Kanellaki, M., Koutinas, A.A. and Tzia, C. 2005. Effect of fermentation conditions and immobilization supports on the wine making. *Journal of Food Engineering* 69:115-123.
- Krishna, H.S., Prasanthi, K., Chowdary, G.V. and Ayyanna, C. 1998. Simultaneous saccharification and fermentation of pretreated sugar cane leaves to ethanol. *Process Biochemistry* 33(8):825-830.
- Kumar, K. S., Ganesan, K. and Rao, P. V. S. 2008. Antioxidant potential of solvent extracts of *Kappaphycus alvarezii* (Doty) Doty – An edible seaweed. *Food Chemistry* 107:289-295.
- Le Jeune, C., Emy, C., Demuyter, C. and Lollier, M. 2006. Evolution of the population of *Saccharomyces cerevisiae* from grape to wine in a spontaneous fermentation. *Food Microbiology* 23:709–716.
- Lilly, M., Lanbrechts, M. G. and Pretorius, I. S. 1999. Effect of Increased Yeast Alcohol Acetyltransferase Activity on Flavor Profiles of Wine and Distillates. *Applied and Environmental Microbiology*. 66:744–753.
- Llyod, B.J., Siebenmorgen, T.J. and Beers, K.W. 2000. Effects of Commercial Processing on Antioxidants in Rice Bran. *Cereal Chemistry* 77(5):551-555.
- Mateo, J.J., Jimenez, M., Pastor, A. and Huerta, T. 2001. Yeast tarter cultures affecting wine fermentation and volatiles. *Food Research International* 34:307-314.
- Matsubara, K., Sumi, H., Hori, K. and Miyazawa, K. 1998. Purification and characterization of Two Fibrinolytic Enzymes from a Marine Green Alga,

- Codium intricatum*. *Comparative Biochemistry and Physiology* 119B(1):177–181.
- Matsubara, K., Hori, K., Matsuura, Y. and Miyazawa, K. 2000. Purification and characterization of a fibrinolytic enzyme and identification of fibrinogen clotting enzyme in a marine green alga, *Codium diarticatum*. *Comparative Biochemistry and Physiology* 125B:137–143.
- McDonough, K. H. 2003. Antioxidant nutrients and alcohol. *Toxicology* 189:89-97.
- Mine, Y., Wong, A.H.K. and Jiang, B. 2005. Fibrinolytic enzymes in Asian traditional fermented foods. *Food Research International* 38:243–250.
- Miyasaka Brewing Company Ltd. 2007. Sake- Brewing Process.
- Morata, A., Gomez-Cordoves, M. C., Calderon, F. and Suarez, J. A. 2006. Effects of pH, temperature and SO₂ on the formation of pyranoanthocyanins during red wine fermentation with two species of *Saccharomyces*. *International Journal of Food Microbiology* 106:123 – 129.
- Murray, L. J., Lane, A. J., Harvey, I. M., Donovan, J. L., Nair, P. and Harvey, R. F. 2002. Inverse relationship between alcohol consumption and active *Helicobacter pylori* infection: the Bristol *Helicobacter* project. *The American Journal of Gastroenterology* 97:2750-2755.
- Navarro, A. R., Sepulveda, M. C. and Rubio, M.C. 2000. Bio-concentration of vinasse from the alcoholic fermentation of sugar cane molasses. *Waste Management* 20:581-585.
- Ocloo, F., C., K. and Ayernor, G. S. 2008. Physical, chemical and microbiological changes in alcoholic fermentation of sugar syrup from cassava flour. *African Journal of Biotechnology*. 7(2):164-168.
- Olsen, K. M. And Purugganan, M. D. 2002. Molecular Evidence on the Origin and Evolution of Glutinous Rice. *Genetics* 162: 941–950
- Ou, B., Huang, D., Hampsch-Woodill, M., Flanagan, J.A. and Deemer, E.K. 2002. Analysis of Antioxidant Activities of Common Vegetables Employing Oxygen Radical Absorbance Capacity (ORAC) and Ferric Reducing Antioxidant Power (FRAP) Assays : A Comparative Study. *Journal of Agricultural and Food Chemistry* 50:3122-3128.
- Ozturk M, Aydogmus-Ozturk F, Duru, M. E. and Topcu, G. 2006. Antioxidant activity of stem and root extracts of Rhubarb (*Rheum ribes*): An edible medicinal plant. *Food Chemistry* 103:623-30.
- Pardo, I., Garcia, M.J., Zuniga, M. and Uruburu, F. 1989. Dynamics of Microbial Populations during Fermentation of Wines from the Utiel-Requena Region of Spain. *Applied and Environmental Microbiology*, 55(2): 539-541.

- Parker, N. 2008. Are Craft Brewers Underaerating Their Wort? *Master Brewers Association of the Americas Technical Quarterly*. 45(4):352- 354.
- Petiot, E. 2007. The Next Generation of Fuel Ethanol. *Biotimes* 01/07.
- Polychroniadou, E., Kanellaki, M., Iconomopoulou, M., Koutinas, A.A., Marchant, R. and Banat, I.M. 2003. Grape and apple wines volatile fermentation products and possible relation to spoilage. *Bioresource Technology* 87:337-339.
- Pramanik, K. and Rao, E. 2005. Kinetic Study on Ethanol Fermentation of Grape Waste Using *Saccharomyces cerevisiae* Yeast Isolated from Toddy. *IE (I) Journal-CH* 85:53-58.
- Que, F., Mao, L. and Pan, X. 2006. Antioxidant activities of five Chinese rice wines and the involvement of phenolic compounds. *Food Research International* 39:581-587.
- Raimbault, M. 1998. Review Article : General and microbiological aspects of solid substrate fermentation. *Electronic Journal of Biotechnology*. 1(3):1-15.
- Rohrer, C.A. and Siebenmorgen, T.J. 2004. Nutraceutical Concentrations within the Bran of Various Rice Kernel Thickness Fractions. *Biosystems Engineering* 88(4):453-460.
- Roig, B. and Thomas, O. 2003. UV monitoring of sugars during wine making. *Carbohydrate Research* 338:79-83.
- Romano, P., Fiore, C., Paraggio, M., Caruso, M. and Capece, A. 2003. Function of yeast species and strains in wine flavor. *International Journal of Food Microbiology* 86:169- 180.
- Romano, P., Suzzi, G., Turbanti, L. and Polsinelli, M. 1994. Acetaldehyde production in *Saccharomyces cerevisiae* wine yeasts. *FEMS Microbiology Letters* 118:213-218
- Rong, X. G. and Fa, B. T. Grandiose Survey of Chinese Alcoholic Drinks and Beverages. (Downloaded from internet ; <http://www.sytu.edu.cn/zhqjiu/umain.htm>.)
- Roy, H. and Lundy, S. 2005. Resveratrol. *Pennington Nutrition Series* 7
- Sablayrolles, J. M. 2009. Control of alcoholic fermentation in winemaking: Current situation and prospect. *Food Research International* 42:418-424
- Sahana, P and Fauzia, H. 2003. Fermented Cereal from Indigenous Raw Materials. *Pakistan Journal of Nutrition* 2(5):289-291

- Sahlin, P. 1999. Fermentation as a Method of Food Processing. *Production of organic acids, pH-development and microbial growth in fermenting cereals*. Licentiate thesis. Division of Applied Nutrition and Food Chemistry, Center for Chemistry and Chemical Engineering, Lund Institute of Technology, Lund University.
- Salamon, A. 2006. Techniques to achieve moderate alcohol levels in South African wine. *Assignment submitted in partial requirement for the Cape Wine Master Diploma*
- Scott, R. and Sullivan, W.C. 2008. Ecology of Fermented Foods. *Human Ecology Reviews*. 15(1):25-31.
- Sekar, S. and Mariappan, S. 2007. Usage of traditional fermented products by Indian rural folks and IPR. *Indian Journal of Traditional Knowledge*. 6(1):111-120.
- Soufleros, E. H., Pissab, I., Petridisb, D., Lygerakisb, M., Mermelasb, K., Boukouvalasb, G. and Tsimitakisb, E. 2001. Instrumental analysis of volatile and other compounds of Greek kiwi wine; sensory evaluation and optimisation of its composition. *Analytical, Nutritional and Clinical Methods Section. Food Chemistry* 75:487-500.
- Steinkraus, K.H. 2002. Fermentation in World Food Processing. *Comprehensive reviews in Food Science and Food Safety*. 1:23-27.
- Striegler, R. K. and Morris, J. R. 1984. Yield and Quality of Wine Grape Cultivars in Arkansas. *American Journal of Enology and Viticulture* 35(4):216-219.
- Sugimoto, S., Fujii, T., Morimiya, T., Johdo, O. and Nakamura, T. 2007. The Fibrinolytic Activity of a Novel Protease Derived from a Tempeh Producing Fungus, *Fusarium* sp. BLB. *Bioscience Biotechnology Biochemistry*. 71(9):2184-2189.
- Sumi, H., Nakajima, N. and Yatagai, C. 1995. A unique strong fibrinolytic enzyme (katsuwokinase) in shipjack "Shiokara," a Japanese traditional fermented food. *Comparative Biochemistry and Physiology* 112B(3):543-437.
- Taillandier, P., Portugal, F.R., Fuster, A. and Strehaiano, P. 2007. Effect of ammonium concentration on alcoholic fermentation kinetics by wine yeasts for high sugar content. *Food Microbiology* 24:95-100.
- Techakriengkrai, I. and Surakarnkul, R. 2007. Analysis of benzoic acid and sorbic acid in Thai rice wines and distillates by solid-phase sorbent extraction and high-performance liquid chromatography. *Journal of Food Composition and Analysis*. 20(3-4):220-225.
- Torija, M.J., Beltran, G., Novo, M., Poblet, M., Guillamon, J.M., Mas, A. and Rozes, N. 2003. Effects of fermentation temperature and *Saccharaomyces* species

- on the cell fatty acid composition and presence of volatile compounds in wine. *International Journal of Food Microbiology* 85:127-136.
- Tronchoni, J., Gamero, A., Arroyo-López, F. N., Barrio, E. and Querol, A. 2009. Differences in the glucose and fructose consumption profiles in diverse *Saccharomyces* wine species and their hybrids during grape juice fermentation. *International Journal of Food Microbiology* 134:237–243.
- Vairappan, C. S. 2003. Potent antibacterial activity of halogenated metabolites from Malaysian red algae, *Laurencia majuscula* (*Rhodomelaceae Ceremiales*). *Biomolecular Engineering* 20:255-259.
- Vairappan, C. S., Suzuki, M., Ishii, T., Okino, T., Abe, T. and Matsuda, M. 2008. Antibacterial activity of halogenated sesquiterpenes from Malaysian *Laurencia* spp. *Phytochemistry*. 69:2490–2494.
- Wang, Q., Han, P., Zhang, M., Xia, M. and Zhu, H. 2007. Supplementation of black rice pigment fraction improves antioxidant and anti-inflammatory status in patients with coronary heart disease. *Asia Pacific Journal of Clinical Nutrition* 16(1):295-301.
- Windley, S. 2008. Benefits of red wine. www.PureHealthMD.com. Pure Health Corporation Fort Wayne, USA.
- Wittwer, G. and Rothfield, J. 2005. Projecting the world wine market from 2003 to 2010. *Australasian Agribusiness Review* 13(21). Adapted from http://www.agrifood.info/review/2005/Wittwer_Rothfield.html
- Wong, A.H.K. and Mine, Y. 2004. Novel Fibrinolytic Enzyme in Fermented Shrimp Paste, a Traditional Asian Fermented Seasoning. *Journal of Agricultural and Food Chemistry*. 52:980-986.
- <https://www.fas.usda.gov/info/foia/06foia.asp>. Foreign Agricultural Service Report 2006. United States Department of Agriculture.