

**IDENTIFICATION OF MICROBIAL
COMMUNITIES IN RICE WINE TRADITIONAL
STARTER (SASAD) AND FERMENTATION
OF RICE WINES**



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UNIVERSITI MALAYSIA SABAH

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**FACULTY OF FOOD SCIENCE AND NUTRITION
UNIVERSITI MALAYSIA SABAH
2023**

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COMMUNITIES IN RICE WINE TRADITIONAL
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KOAY MEIWEI



UMS

**THESIS SUBMITTED IN FULFILMENT OF THE
REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE**

**FACULTY OF FOOD SCIENCE AND NUTRITION
UNIVERSITI MALAYSIA SABAH
2023**

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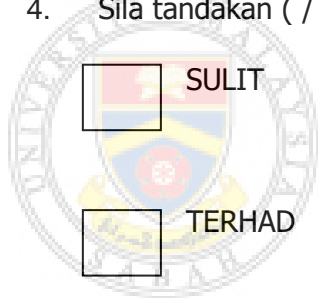
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DECLARATION

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

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Koay Meiwei
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ABSTRACT

Rice wine is a rice-based alcoholic beverage produced through the fermentation of cereal grains, mainly rice, with microbial starters. However, the starter cultures sold in the market are often prepared based on empirical knowledge and thus varied in microbial compositions. Their exact microbial contents are usually unknown, giving rise to the possibility of product quality and food safety issues. Although many researchers have identified the microbial communities in various rice wine starter cultures using molecular approaches, such information on sasad, a traditional starter culture for rice wine fermentation in Sabah, Malaysia, has not been published. Therefore, this research aims to identify the fungal and bacterial communities in sasad, study the physicochemical and microbiological changes in rice wines during fermentation, and determine the consumers' acceptance of rice wines. The fungal and bacterial communities were identified through amplicon-based next-generation sequencing (NGS). Physicochemical properties including total soluble solids (TSS), alcohol content, total titratable acidity (TTA), pH, and colour, as well as microbiological properties such as total yeast and mould count (TYMC) and total lactic acid bacteria (LAB) count were determined. In addition, consumers' acceptance of rice wines was determined through preference ranking and hedonic tests. A total of two fungal phyla (Mucoromycota and Ascomycota) with five genera (*Mucor*, *Rhizopus*, *Saccharomycopsis*, *Wickerhamomyces*, and *Kodamaea*) and two bacterial phyla (Proteobacteria and Firmicutes) with 10 genera (*Kosakonia*, *Weissella*, *Enterobacter*, *Lactococcus*, *Pseudomonas*, *Bacillus*, *Chromobacterium*, *Paludibacterium*, *Enterococcus*, and *Gluconobacter*) were identified as the core microbiota (relative abundance > 1.00%) in the sasad samples. Batch fermentation was successfully carried out using the sasad samples. The overall fermentation dynamics of rice wines (RWA, RWB, and RWC) produced using different sasad (SA, SB, and SC, respectively) exhibited similar trends. However, variations between the samples were still detected. After 28 days of fermentation, RWC showed the lowest TSS (22.9 ± 0.2 °Brix) and highest alcohol content ($15.95 \pm 0.07\%$), while RWA had the highest TTA ($0.68 \pm 0.04\%$ lactic acid) and lowest pH (3.60 ± 0.01), demonstrating the effect of microbial compositions of sasad on the quality of final rice wines. All rice wine samples were acceptable to the panellists, while rice wines with darker shades of yellow and higher alcohol content or stronger alcoholic aroma were preferable. However, there is no significant difference ($p > 0.05$) in liking scores for taste, mouthfeel, and overall acceptance among the rice wine samples. The variation in consumers' tastes suggested a potential for developing rice wines with various flavours to fulfil consumers' needs. This research provides important insights into the potential roles of core microbiota found in sasad, the fermentation dynamics of rice wines, and the consumers' acceptance of rice wines. The occurrence of opportunistic spoilage and pathogenic microorganisms in sasad emphasises the importance of developing sasad with well-defined microbial compositions for the consistent production of safe and high-quality rice wines.

Keywords: Rice wine, starter culture, fungal and bacterial communities, fermentation dynamics, consumer preference and acceptance

ABSTRAK

IDENTIFIKASI KOMUNITI MIKROB DALAM PEMULA TRADISIONAL WAIN BERAS (SASAD) DAN PENAPAIAN WAIN BERAS

Wain beras merupakan minuman beralkohol yang dihasilkan melalui penapaian bijirin-bijirin terutamanya beras dengan pemula mikrob. Namun, kultur pemula yang dijual di pasaran selalunya disediakan berdasarkan pengetahuan empirikal. Oleh itu, ia berbeza dalam komposisi mikrob dan kandungan mikrobnnya tidak diketahui. Hal ini menimbulkan isu kualiti produk dan keselamatan makanan. Walaupun ramai penyelidik telah mengenal pasti komuniti mikrob dalam pelbagai pemula wain beras menggunakan pendekatan molekul, di mana maklumat yang berkaitan dengan sasad (pemula tradisional wain beras di Sabah, Malaysia) belum diterbitkan. Oleh itu, penyelidikan ini bertujuan untuk mengenal pasti komuniti mikrob dalam sasad, mengkaji perubahan sifat fizikokimia dan mikrobiologi wain beras semasa penapaian, serta menentukan penerimaan pengguna terhadap wain beras. Komuniti kulat dan bakteria telah dikenal pasti melalui penjujukan generasi akan datang (NGS) berasaskan amplicon. Sifat fizikokimia termasuk jumlah pepejal larut (TSS), kandungan alkohol, jumlah keasidan yang boleh dititrasi (TTA), pH dan warna, serta sifat mikrobiologi seperti jumlah yis dan kulat (TYMC) dan jumlah bakteria asid laktik (LAB) telah ditentukan. Di samping itu, penerimaan pengguna terhadap wain beras telah ditentukan melalui ujian pemeringkatan keutamaan dan hedonik. Sebanyak dua filum kulat (*Mucoromycota* dan *Ascomycota*) dengan lima genus (*Mucor*, *Rhizopus*, *Saccharomycopsis*, *Wickerhamomyces* dan *Kodamaea*) dan dua filum bakteria (*Proteobacteria* dan *Firmicutes*) dengan 10 genus (*Kosakonia*, *Weissella*, *Enterobacter*, *Lactococcus*, *Pseudomonas*, *Bacillus*, *Chromobacterium*, *Paludibacterium*, *Enterococcus* dan *Gluconobacter*) telah dikenal pasti sebagai mikrobiota teras (kelimpahan relatif > 1.00%) dalam sampel sasad. Penapaian kelompok telah berjaya dijalankan menggunakan sampel sasad. Dinamik penapaian keseluruhan wain beras (RWA, RWB dan RWC) yang dihasilkan menggunakan sasad yang berbeza (SA, SB dan SC, masing-masing) menunjukkan trend yang sama, tetapi variasi antara sampel masih dapat dikesan. RWC menunjukkan TSS terendah (22.9 ± 0.2 °Brix) dan kandungan alkohol tertinggi ($15.95 \pm 0.07\%$), manakala RWA mempunyai TTA tertinggi ($0.68 \pm 0.04\%$ asid laktik) dan pH terendah (3.60 ± 0.01) selepas 28 hari penapaian. Hasil ini menunjukkan kesan komposisi mikrob sasad terhadap kualiti wain beras yang disediakan. Semua sampel wain beras boleh diterima oleh ahli panel, manakala wain beras dengan warna kuning yang lebih gelap dan aroma alkohol yang lebih kuat lebih digemari. Namun, skor kesukaan untuk rasa, rasa mulut dan penerimaan keseluruhan antara sampel tidak berbeza secara ketara ($p > 0.05$). Variasi cita rasa pengguna mencadangkan potensi untuk pengembangan wain beras dengan pelbagai perisa bagi memenuhi kehendak pengguna. Penyelidikan ini menunjukkan potensi peranan mikrobiota teras yang terdapat dalam sasad, dinamik penapaian wain beras dan penerimaan pengguna terhadap wain beras. Kemunculan patogen oportunistik dalam sasad menekankan kepentingan pembangunan kultur pemula dengan komposisi mikrob yang ditetapkan untuk pengeluaran wain beras yang berkualiti tinggi dan selamat untuk diambil.

Kata kunci: Wain beras, kultur pemula, komuniti kulat dan bakteria, dinamik penapaian, keutamaan dan penerimaan pengguna

LIST OF CONTENTS

	Page
TITLE	i
DECLARATION	ii
CERTIFICATION	iii
ACKNOWLEDGEMENTS	iv
ABSTRACT	v
ABSTRAK	vi
LIST OF CONTENTS	vii
LIST OF TABLES	xi
LIST OF FIGURES	xii
LIST OF EQUATIONS	xv
LIST OF SYMBOLS AND ABBREVIATIONS	xvi
LIST OF APPENDICES	xix
CHAPTER 1: INTRODUCTION	
1.1 Research Background	1
1.2 Problem Statement	3
1.3 Significance of Research	4
1.4 Research Objectives	6
CHAPTER 2: LITERATURE REVIEW	
2.1 Rice Wines	7
2.1.1 Raw Materials of Rice Wines	11
2.1.2 Compositions of Rice Wines	18
2.1.3 Health Benefits of Rice Wines	23

2.2	Processing of Handcrafted Rice Wines	26
2.2.1	Preparation of Handcrafted Rice Wines	26
2.2.2	Alcohol Production Process of Rice Wines	27
2.2.3	Quality and Safety Control of Rice Wines	36
2.3	Identification of Microbial Communities in Rice Wines and Starter Cultures	38

CHAPTER 3: MATERIALS AND METHODS

3.1	Raw Materials	44
3.2	Chemicals	44
3.3	Equipment	45
3.4	Research Experiment Outline	46
3.5	Comparative Study on DNA Extraction Methods for Fingerprinting of Fungal and Bacterial Communities by PCR-DGGE	49
3.5.1	Genomic DNA (gDNA) Extraction	49
3.5.2	Qualitative and Quantitative Assessment of Genomic DNA (gDNA)	51
3.5.3	Polymerase Chain Reaction (PCR) Amplification	53
3.5.4	Denaturing Gradient Gel Electrophoresis (DGGE)	55
3.5.5	DGGE Profiling	56
3.6	Identification of Fungal and Bacterial Communities Using Molecular Approach	57
3.6.1	Amplicon-Based Next-Generation Sequencing (NGS)	57
3.6.2	Amplicon Sequence Analysis	58
3.7	Batch Fermentation	58
3.8	Physicochemical Analysis	59
3.8.1	Total Soluble Solids (TSS)	59
3.8.2	Alcohol Content	59

3.8.3	Total Titratable Acidity (TTA)	60
3.8.4	pH	61
3.8.5	Colour	61
3.9	Microbiological Analysis	61
3.10	Sensory Evaluation	61
3.10.1	Ranking Test	62
3.10.2	Hedonic Test	62
3.11	Statistical Analysis	62
CHAPTER 4: RESULTS		
4.1	Comparative Study on DNA Extraction Methods for Fingerprinting of Fungal and Bacterial Communities by PCR-DGGE	64
4.1.1	Qualitative and Quantitative Assessment of Genomic DNA (gDNA)	64
4.1.2	Polymerase Chain Reaction (PCR) Amplification	66
4.1.3	DGGE Profiling	67
4.2	Microbial Communities in Rice Wine Starter Cultures (Sasad)	73
4.2.1	Fungal Communities in Rice Wine Starter Cultures (Sasad)	74
4.2.2	Bacterial Communities in Rice Wine Starter Cultures (Sasad)	80
4.3	Physicochemical Changes in Rice Wines during Fermentation	86
4.3.1	Total Soluble Solids (TSS)	86
4.3.2	Alcohol Content	87
4.3.3	Total Titratable Acidity (TTA)	88
4.3.4	pH	89
4.4	Microbiological Changes in Rice Wines during Fermentation	90
4.4.1	Total Yeast and Mould Count (TYMC)	90
4.4.2	Total Lactic Acid Bacteria (LAB) Count	91

4.5	Sensory Evaluation of Rice Wines	92
4.5.1	Ranking Test	92
4.5.2	Hedonic Test	93
4.6	Comparative Study on Physicochemical Properties of Final Rice Wines and The Most Preferred Marketed Rice Wine	94
CHAPTER 5: DISCUSSIONS		
5.1	Comparative Study on DNA Extraction Methods for Fingerprinting of Fungal and Bacterial Communities by PCR-DGGE	97
5.2	Microbial Communities in Rice Wine Starter Cultures (Sasad)	98
5.2.1	Fungal Communities in Rice Wine Starter Cultures (Sasad)	99
5.2.2	Bacterial Communities in Rice Wine Starter Cultures (Sasad)	106
5.3	Physicochemical and Microbiological Changes in Rice Wines during Fermentation	114
5.4	Sensory Evaluation of Rice Wines	116
CHAPTER 6: CONCLUSION		
REFERENCES		
APPENDICES		

LIST OF TABLES

	Page
Table 2.1 : Rice wines in different regions, along with their starch substrates and starter cultures used	8
Table 2.2 : Key differences between tapai and lihing	11
Table 2.3 : Compositions of cereal grains	12
Table 2.4 : Starter cultures in different regions, along with their respective base substrates and predominant microorganism	15
Table 2.5 : Alcohol content and pH of rice wines	19
Table 2.6 : Volatile compounds frequently found in rice wines	20
Table 2.7 : Reaction specificities of amylolytic enzymes in starch hydrolysis	30
Table 3.1 : Reagents needed in standard and sample assay tubes	53
Table 4.1 : Yield and purity of gDNA extracted from three types of sasad (SA, SB, and SC) using DNeasy PowerLyzer PowerSoil Kit (Qiagen) and phenol chloroform extraction	66
Table 4.2 : Alpha diversity indices of fungal communities in starter cultures (sasad)	74
Table 4.3 : Alpha diversity indices of bacterial communities in starter cultures (sasad)	80
Table 4.4 : Physicochemical properties of rice wine products	96

LIST OF FIGURES

	Page
Figure 2.1 : Overview of alcohol production processes	28
Figure 2.2 : Glycolysis (Embden-Meyerhof-Parnas pathway) and ethanol fermentation steps in yeast	31
Figure 3.1 : Research experiment outline	48
Figure 4.1 : gDNA extracted from three types of sasad (SA, SB, and SC), each with three biological triplicates using (a) DNeasy PowerLyzer PowerSoil Kit (Qiagen) and (b) phenol chloroform extraction. Lane L represents the 1 kb DNA ladder (Promega). Lanes SA1 to SA3, SB1 to SB3, and SC1 to SC3 were the biological replicates of SA, SB, and SC, respectively.	65
Figure 4.2 : DGGE profiles of the fungal communities in sasad samples (SA, SB, and SC). Lanes A1 to A3, B1 to B3, and C1 to C3 represent the fungal communities obtained using DNeasy PowerLyzer PowerSoil Kit (Qiagen) from biological triplicates of SA, SB, and SC, respectively. Lanes A4 to A6, B4 to B6, and C4 to C6 represent fungal communities obtained using chloroform extraction from biological triplicates of SA, SB, and SC, respectively.	68
Figure 4.3 : Diversity indices calculated from the DGGE profiles of fungal ITS2 amplicons amplified using gDNA extracted through different DNA extraction methods	69
Figure 4.4 : Dendrogram showing the similarities among DGGE profiles of fungal ITS2 amplicons obtained from gDNA extracted using (a) DNeasy PowerLyzer PowerSoil Kit (Qiagen) and (b) phenol chloroform extraction from sasad samples (SA, SB, and SC)	70
Figure 4.5 : DGGE profiles of the bacterial communities in sasad samples (SA, SB, and SC). Lanes A1 to A3, B1 to B3, and C1 to C3 represent the bacterial communities obtained using gDNA extracted via DNeasy PowerLyzer PowerSoil Kit (Qiagen) from biological triplicates of SA, SB, and SC, respectively. Lanes A4 to A6, B4 to B6, and C4 to C6 represent bacterial communities obtained using gDNA extracted via phenol chloroform extraction from biological triplicates of SA, SB, and SC, respectively.	71

Figure 4.6	: Diversity indices calculated from the DGGE profiles of bacterial 16S rRNA V3-V4 amplicons amplified using gDNA extracted through different DNA extraction methods	72
Figure 4.7	: Dendrogram showing the similarities among DGGE profiles of bacterial 16S rRNA V3-V4 amplicons obtained from gDNA extracted using (a) DNeasy PowerLyzer PowerSoil Kit (Qiagen) and (b) phenol chloroform extraction from sasad samples (SA, SB, and SC)	73
Figure 4.8	: Taxonomic identification and relative abundance of fungi detected in sasad A (SA)	76
Figure 4.9	: Taxonomic identification and relative abundance of fungi detected in sasad B (SB)	77
Figure 4.10	: Taxonomic identification and relative abundance of fungi detected in sasad C (SC)	78
Figure 4.11	: Dendrogram (UPGMA) based on weighted UniFrac distance matrices of fungal communities in sasad (SA, SB, and SC) along with their relative abundance at the family level	79
Figure 4.12	: Taxonomic identification and relative abundance of bacteria detected in sasad A (SA)	82
Figure 4.13	: Taxonomic identification and relative abundance of bacteria detected in sasad B (SB)	83
Figure 4.14	: Taxonomic identification and relative abundance of bacteria detected in sasad C (SC)	84
Figure 4.15	: Dendrogram (UPGMA) based on weighted UniFrac distance matrices of bacterial communities in sasad (SA, SB, and SC) along with their relative abundance at the family level	85
Figure 4.16	: Total soluble solids (TSS) of rice wines (RWA, RWB, and RWC) during fermentation	86
Figure 4.17	: Alcohol content of rice wines (RWA, RWB, and RWC) during fermentation	87
Figure 4.18	: Total titratable acidity (TTA) of rice wines (RWA, RWB, and RWC) during fermentation	88
Figure 4.19	: pH of rice wines (RWA, RWB, and RWC) during fermentation	89
Figure 4.20	: Total yeast and mould count (TYMC) in rice wines (RWA, RWB, and RWC) during fermentation	90

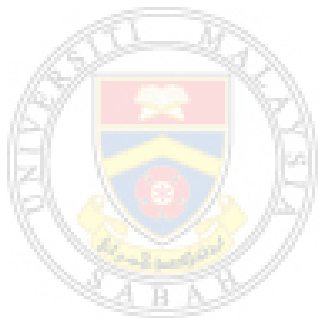
Figure 4.21	:	Total lactic acid bacteria (LAB) count in rice wines (RWA, RWB, and RWC) during fermentation	91
Figure 4.22	:	Ranking test for marketed rice wines (MRW1, MRW2, and MRW3)	93
Figure 4.23	:	Hedonic test for final rice wines (RWA, RWB, and RWC) and the most preferred marketed rice wine (MRW1) determined through the ranking test	94
Figure 4.24	:	Rice wines (MRW1, RWA, RWB, and RWC) in shot glasses	95



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LIST OF EQUATIONS

	Page
Equation 3.1 : DNA yield	52
Equation 3.2 : DNA purity (A_{260}/A_{280} ratio)	52
Equation 3.3 : DNA purity (A_{260}/A_{230} ratio)	52
Equation 3.4 : Shannon diversity index	56
Equation 3.5 : Evenness	57
Equation 3.6 : Total titratable acidity	60



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LIST OF SYMBOLS AND ABBREVIATIONS

%	-	Percent
× g	-	Times Gravity
°C	-	Degree Celcius
μ	-	Micro
ANOVA	-	Analysis of Variance
ATP	-	Adenosine Triphosphate
bp	-	Base Pair
CFU	-	Colony-Forming Units
CI	-	Chloroform:Isoamyl Alcohol
DGGE	-	Denaturing Gradient Gel Electrophoresis
dNTP	-	Deoxynucleotide Triphosphates
DRBC	-	Dichloran Rose-Bengal Chloramphenicol
E	-	Evenness
EtBr	-	Ethidium Bromide
g	-	Gram
GC-FID	-	Gas Chromatography with Flame Ionisation Detection
gDNA	-	Genomic Deoxyribonucleic Acid
GTE	-	Glucose-Tris-Ethylenediaminetetraacetic Acid
h	-	Hour
H'	-	Shannon Diversity Index
HCl	-	Hydrochloric Acid
HSD	-	Honestly Significant Difference
HTS	-	High-Throughput Sequencing
ITS	-	Internal Transcribed Spacers
kb	-	Kilo Base Pair
KDM	-	Kadazan-Dusun Murut
L	-	Litre

LAB	-	Lactic Acid Bacteria
m	-	Milli
M	-	Molar
MgCl₂	-	Magnesium Chloride
min	-	Minute
mol	-	Mole
MRS	-	Man Rogosa and Sharpe
n	-	Nano
N	-	Normality
NaOH	-	Sodium Hydroxide
NGS	-	Next-Generation Sequencing
OAV	-	Odour Activity Value
PCI	-	Phenol:Chloroform:Isoamyl Alcohol
PCR	-	Polymerase Chain Reaction
PTFE	-	Polytetrafluoroethylene
RFLP	-	Restriction Fragment Length Polymorphism
RNase A	-	Ribonuclease A
rRNA	-	Ribosomal Ribonucleic Acid
S	-	Richness
s	-	Second
SDS	-	Sodium Dodecyl Sulfate
SHF	-	Separate Hydrolysis and Fermentation
sp./spp.	-	Species
SSF	-	Simultaneous Saccharification and Fermentation
TAE	-	Tris-Acetate-Ethylenediaminetetraacetic Acid
TBE	-	Tris-Borate-Ethylenediaminetetraacetic Acid
TEMED	-	Tetramethylethylenediamine
TSS	-	Total Soluble Solids
TTA	-	Total Titratable Acidity

TYMC	-	Total Yeast And Mould Count
U	-	Unit
UPGMA	-	Unweighted Pair Group Method with Arithmetic Mean
UV-Vis	-	Ultraviolet-Visible
V	-	Volt
v	-	Volume
w	-	Weight
WGS	-	Whole Genome Sequencing
α	-	Alpha
β	-	Beta



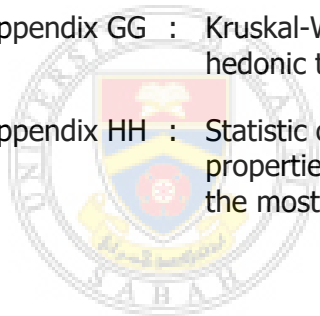
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LIST OF APPENDICES

	Page
Appendix A : Ranking Test Form	146
Appendix B : Hedonic Test Form	147
Appendix C : Independent Samples t Test for yield and purity of gDNA extracted using DNeasy PowerLyzer PowerSoil Kit (Qiagen) and phenol chloroform extraction	148
Appendix D : Fungal ITS2 amplicons amplified using gDNA extracted from three types of sasad (SA, SB, and SC) via (a) DNeasy PowerLyzer PowerSoil Kit (Qiagen) and (b) phenol chloroform extraction	151
Appendix E : Bacterial 16S rRNA V3-V4 amplicons amplified using gDNA extracted from three types of sasad (SA, SB, and SC) via (a) DNeasy PowerLyzer PowerSoil Kit (Qiagen) and (b) phenol chloroform extraction	152
Appendix F : Mann-Whitney U Test for diversity indices calculated from the DGGE profiles of fungal ITS2 amplicons amplified using gDNA extracted via different DNA extraction methods	153
Appendix G : Mann-Whitney U Test for diversity indices calculated from the DGGE profiles of bacterial 16S rRNA V3-V4 amplicons amplified from gDNA extracted using different DNA extraction methods	155
Appendix H : Statistic outputs for alpha-diversity indices of fungal communities in starter cultures (sasad)	157
Appendix I : One-way ANOVA and Tukey's HSD post hoc test for relative abundances of fungal phyla in starter cultures (sasad)	159
Appendix J : One-way ANOVA and Tukey's HSD post hoc test for relative abundances of core fungal genera in starter cultures (sasad)	161
Appendix K : One-way ANOVA and Tukey's HSD post hoc test for alpha-diversity indices of bacterial communities in starter cultures (sasad)	164
Appendix L : One-way ANOVA and Tukey's HSD post hoc test for relative abundances of bacterial phyla in starter cultures (sasad)	166

Appendix M	: One-way ANOVA and Tukey's HSD post hoc test for relative abundances of core bacterial genera in starter cultures (sasad)	170
Appendix N	: Total soluble solids (TSS) of rice wines (RWA, RWB, and RWC) during fermentation	175
Appendix O	: One-way ANOVA and Tukey's HSD post hoc test for changes in total soluble solids (TSS) of rice wines (RWA, RWB, and RWC) during fermentation	176
Appendix P	: One-way ANOVA and Tukey's HSD post hoc test for total soluble solids (TSS) of rice wines (RWA, RWB, and RWC) at different fermentation periods	178
Appendix Q	: Alcohol content of rice wines (RWA, RWB, and RWC) during fermentation	181
Appendix R	: One-way ANOVA and Tukey's HSD post hoc test for changes in alcohol content of rice wines (RWA, RWB, and RWC) during fermentation	182
Appendix S	: One-way ANOVA and Tukey's HSD post hoc test for alcohol content of rice wines (RWA, RWB, and RWC) at different fermentation periods	184
Appendix T	: Total titratable acidity (TTA) of rice wines (RWA, RWB, and RWC) during fermentation	187
Appendix U	: One-way ANOVA and Tukey's HSD post hoc test for changes in total titratable acidity (TTA) of rice wines (RWA, RWB, and RWC) during fermentation	188
Appendix V	: One-way ANOVA and Tukey's HSD post hoc test for total titratable acidity (TTA) of rice wines (RWA, RWB, and RWC) at different fermentation periods	190
Appendix W	: pH of rice wines (RWA, RWB, and RWC) during fermentation	193
Appendix X	: One-way ANOVA and Tukey's HSD post hoc test for changes in pH of rice wines (RWA, RWB, and RWC) during fermentation	194
Appendix Y	: One-way ANOVA and Tukey's HSD post hoc test for pH of rice wines (RWA, RWB, and RWC) at different fermentation periods	196
Appendix Z	: Total yeast and mould count (TYMC) of rice wines (RWA, RWB, and RWC) during fermentation	200

Appendix AA	: One-way ANOVA and Tukey's HSD post hoc test for changes in total yeast and mould count (TYMC) in rice wines (RWA, RWB, and RWC) during fermentation	201
Appendix BB	: One-way ANOVA and Tukey's HSD post hoc test for total yeast and mould count (TYMC) in rice wines (RWA, RWB, and RWC) at different fermentation periods	203
Appendix CC	: Total lactic acid bacteria (LAB) count of rice wines (RWA, RWB, and RWC) during fermentation	207
Appendix DD	: One-way ANOVA and Tukey's HSD post hoc test for changes in total lactic acid bacteria (LAB) count in rice wines (RWA, RWB, and RWC) during fermentation	208
Appendix EE	: One-way ANOVA and Tukey's HSD post hoc test for total lactic acid bacteria (LAB) count in rice wines (RWA, RWB, and RWC) at different fermentation periods	210
Appendix FF	: Kruskal-Wallis test and Dunn-Bonferroni post hoc test for ranking test	214
Appendix GG	: Kruskal-Wallis test and Dunn-Bonferroni post hoc test for hedonic test	216
Appendix HH	: Statistic outputs for comparative study on physicochemical properties of final rice wines (RWA, RWB, and RWC) and the most preferred marketed rice wine (MRW1)	219



CHAPTER 1

INTRODUCTION

1.1 Research Background

Fermentation is a traditional form of food processing implemented for preservation purposes. This technique has been employed for centuries to produce alcoholic beverages from various substrates such as fruits, vegetables, and grains. Rice wine is an alcoholic beverage produced by fermenting cereal grains, principally rice, with starter cultures (Rhee *et al.*, 2011). The consumption and production of rice wine is a global phenomenon, particularly prevalent in Asian nations, where it is commonly featured during cultural festivities and traditions. According to a report by Fortune Business Insights (2020), the global rice wine market is projected to grow, considering an increase in demand for rice wine in Western nations and the use of rice wine in culinary arts.

The naming of rice wines varies across their regions of production such as tapai in Sabah, sato or ou in Thailand, makgeolli in Korea, sake in Japan, brem in Indonesia, ruou nep in Vietnam, and Shaoxing rice wine in China (Chaijamrus & Mouthung, 2011; Chiang *et al.*, 2006; Dung *et al.*, 2007; Jeyaram *et al.*, 2008; Kim *et al.*, 2018; Luangkhlapho *et al.*, 2014; Miki *et al.*, 2005; Xie *et al.*, 2007). In addition, rice wines vary in their nutritional, biochemical, and organoleptic properties due to different starch substrates and starter cultures used. For example, tapai (Sabah, Malaysia) prepared using glutinous rice with a starter culture known as sasad or ragi, typically has a complex flavour profile that includes sweet, sour, and bitter notes, as well as an alcoholic aroma (Chiang *et al.*, 2006). Whereas makgeolli (Korea) produced