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Research Article

Biochemical properties of rice wine produced from three different starter cultures

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ABSTRACT. Starter cultures influence aroma and flavour in rice wine production. The biochemical characterization of rice wine produced from common and glutinous rice using three starter cultures; 1) bitter ($1.51x10^5$ CFU/g), 2) bitter-sweet ($5.5x10^4$ CFU/g), and 3) sweet ($4.47x10^5$ CFU/g), were investigated. The volume of wine, with pH ranging from 4.3 to 4.7, obtained from glutinous rice was twice the volume of common rice. Glucose levels of wine from glutinous rice ranged between 300.27±0.28 ~ 440.14±29.97 mg/ml, twice of that in common rice wine. The wine from common rice contained higher alcohol (9.96±0.08 ~ 12.53±1.35%) as compared to wine from glutinous rice. Volatile hydrocarbons in both rice wines were analysed and reported. Rice wine and fermented rice cakes were tested for their antioxidant and fibrinolytic activities. However, only fermented rice cakes from common rice displayed positive antioxidant and fibrinolytic activities. Best fibrinolytic activity was exhibited by a bitter-sweet starter with IC_{50} 4.67±0.51 mg/ml and 0.32±0.01 unit plasmin/mg.

Keywords: Rice wine, starter cultures, fermentation, antioxidant, fibrinolytic activity.

INTRODUCTION

Fermentation is the oldest transformation method used to preserve and enhance flavour, aroma and nutritive values of food (Steinkraus, 2002). In this regard, rice fermentation for the production of alcoholic beverage is an ancient process practiced in most Asian countries. Rice wine is widely consumed during social and cultural events, and is part of offerings for a good harvest, traditional medicine and post-natal recovery (Chiang *et al.*, 2006). The Chinese *Huangjiu*, Indian *Sonti*, Japanese *sake*, and Korean *Yakju* are a few examples of fermented rice wines (Steinkraus, 2002). In rice wine production, starch (sugar) from rice is converted into alcohol *via* fermentation by yeast, fungus and lactic acid bacteria (LAB) (Chuenchomrat *et al.*, 2008). The choice of raw material plays a vital role in producing wine of good quality and volume. Aroma determines the quality of wine produced through the volatile compounds produced during fermentation (Gobetti *et al.*, 1995). The use of different starter cultures with varying microbial content and rice variety has been associated with the production of wine with different tastes and flavours. Variety of *Saccharomyces cereviseae* has been reported to produce wine with a diverse flavour profile (Chen & Xu, 2010). In addition, the type of rice fermented can influence the quantity and quality of wine. Glutinous rice for instance is a rich source of starch, protein and various microelements that are used by microbes during the fermentation process to produce more wine (Que *et al.*, 2006). The taste and quality of wine produced can also be affected by local producers' recipe depending on availability of raw materials and starter cultures (Dung *et al.*, 2005).

In Borneo, rice wine is produced and used during cultural functions. It is produced using

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common and glutinous rice with a variety of starters based on preference. The brewers use three different starter cultures; 1) bitter, 2) bitter-sweet and 3) sweet. The microbial strains used in each of the cultures are similar but vary in density. The addition of spices such as cinnamon, pepper, dried chili and local herbs results in the unique flavour of these starters (Gandjar, 2003). However, no scientific information is available on the chemical qualities and nutritional properties of this local beverage. Hence, this report provides comparative data on the biochemical properties of rice wine and cake produced using three different starters currently unavailable locally so as to complement the available microbial data (Chiang *et al.*, 2006).

MATERIALS AND METHODS

Fermentation

A total of 3.3 kg glutinous and common rice obtained from Tenghilan, Sabah was used in each set of fermentation (4 L/jar, 3 jars/set). Rice was cooked (rice grain : water = 2:1) and cooled at room temperature (28~30°C). A total of 80 g of starter culture was mixed with the cooled white and glutinous rice. This mixture was put into jars without additional water and left to ferment for four weeks. Three starter cultures were used; 1) bitter, 2) bitter-sweet, and 3) sweet. After fermentation, rice wine and cake were separated through filtration. The wine was stored under refrigeration (< 4°C) prior to analysis and rice cake (1 kg) was extracted in ethanol for bioassay and chemical analysis.

Sugar Analysis - High Performance Liquid Chromatography (HPLC)

Rice wine and cake extracts were diluted (10x) in distilled water and analysed under these conditions: Shimadzu low pressure gradient, NH_2 Luna Phenomenex 5 µm 100A column (250 x 4.6 mm ID), RI detector, isocratic mode, mobile phase (80% acetonitrile) at flow rate of 3 ml/min, 10 µl injection volume, and column oven at 35°C.

Gas Chromatography Mass Spectrometer (GCMS) analysis

Alcohol content analysis of rice wine samples were done by direct injection of diluted rice wine (5x dilutions) into GCMS using a Q-Plot (30 m x 0.25 mm) column. Instrument conditions during analysis were as follows: GC temperature: 100-180°C at 5°C/min, helium carrier gas: 2.88 ml/min, the injector temperature: 250°C, interphase temperature: 280°C and mass range: 450 a.m.u. Analysis of volatile hydrocarbon was done by injection of concentrated hexane (solvent-solvent partition with rice wine and cake extract) into GCMS equipped with BPX 5 (30 m x 0.25 mm). Instrument conditions during analysis were as follows: GC temperature programme: 50-280°C at 3°C/min, helium carrier gas: 0.8 ml/min, injector temperature: 250°C, interphase temperature: 280°C and mass range: 450 a.m.u. Resulting peaks were analysed and compared to the National Institute of Standards and Technology (NIST) and Flavour, Fragrance, Natural and Synthetic Compounds (FFNSC) database.

Microbial enumeration for starter culture and fermentation

A total 1 g of finely pounded starter culture; 1) bitter, 2) bitter-sweet, and 3) sweet, was subjected to 5x dilutions and enumerated for total microbial count using spread plate method as described by Chiang *et al.* (2006). Two specific agars were used; 1) de Man, Rogosa, and Sharpe (MRS) agar, 2) Dichloran Rose Bengal Choramphenicol (DRBC) agar. Similar enumerations were also conducted on specimens of the 1^{st} and 28^{th} day of fermentation.

Antioxidant assay

DPPH (1,1-diphenyl-2-picryhydrazyl) assay (Kumar *et al.*, 2008) was used. Absorbance was measured at 517 nm using a micro-plate reader (Infinite M200, TECAN, Switzerland) and MAGELLAN software. The Free Radical Scavenging ability was calculated as IC_{50} and

expressed as ascorbic acid equivalent antioxidant capacity (AEAC) in mg AA/g amount as follows:

AEAC (mg AA/g) = [IC_{50} (ascorbate) / IC_{50} (extract)] x 10^5

Fibrinolytic enzyme assay

Fibrinolytic enzyme assay was carried out based on a method described by Mine *et al.* (2005). Fibrinogen solution consisting 0.8% (w/v) of bovine fibrinogen in 0.8% (w/v) of agarose solution at 37°C was prepared. The solution was mixed with 0.2% (v/v) of 1 unit/μL of thrombin protease (Amersham Pharmacia Biotech, Netherlands) and 10 ml was poured into a 6 cm glass Petri dish. The Petri dish was left at room temperature for two hours to form a fibrin clot layer. The sample was inserted into pre-punched hole (5 mm) prior to incubation for 17 hours at room temperature. The diameter of the clear zone was measured and expressed in plasmin units.

Statistical analysis

Experimental values were means ± SD of three replicates. A multivariate analysis of variance was performed using SPSS v11.5 for Windows. *P* values < 0.05 were regarded as significant.

RESULTS AND DISCUSSION

Fermentation dynamics

Total microbial load of starters

Total microbial load in starters were enumerated using DRBC and MRS agars due to their specificity to assist the growth of yeast and lactic acid bacteria, respectively (Chiang *et al.*, 2006). Total microbial load of yeast and lactic acid bacteria (LAB) in all three starters showed a consistent trend as shown in Table 1; sweet>bitter-sweet>bitter for both common and glutinous rice. Total microbial load in sweet starters were 196.1% and 712.7% more than bitter and bitter-sweet for yeast. Similarly, microbial load in sweet starters were 285.4% and 757.5% more than bitter and bitter-sweet for LAB.

The total microbial load in starters plays an important role in fermentation dynamics. Varying bacterial load has proven to affect the microbial growth during fermentation and its relation to the reduction in rice cake weight during fermentation, volume and pH of wine produced (Dung *et al.*, 2005). The microbial content of each starter culture varied by density but the diversity of strains was the same and similar to those as reported by Chiang *et al.* (2006). The differences in the density of microbes of the starters could be due to the influence of herbs as promoters or inhibitors of microbial dynamics (Gandjar, 2003). Spices and herbs are also known to improve aroma, taste and to prevent growth of unwanted microorganisms (Dung *et al.*, 2005). In this study, *Saccharomyces cereviseae* was the primary yeast in the starters and was the most dominant due to its ability to survive in low acidity levels (Gandjar, 2003). Among the other yeast found in the starter culture were *Candida krusei, C. pelliculosa, C. utilis, C. sphaerica, C. magnolia* and *Rhodoturula glutinis*. *Lactobacillus plantarum* and *L. brevis* were the major lactic acid bacteria detected in the starter cultures as reported by Chiang *et al.* (2006).

Microbial increment during fermentation

Comparative microbial increment during the 28 day fermentation for common and glutinous rice with the three starters is shown in Table 1. Rice fermented with sweet starter showed the highest increment of microbial growth, between 579.3±11.2% and 581.7±27.9% in common rice for yeast and LAB, respectively. A similar trend was also observed for glutinous rice at 543.5±34.2% and 559.6±19.7% for yeast and LAB, respectively. Fermentation using a bitter-sweet starter showed higher microbial growth in glutinous rice as compared to common rice, contrary to the trend showed by sweet and bitter starters. The overall percentage of microbial increment for bitter-sweet was almost 50% of that shown by the microbes in sweet starter fermentations. On the other hand, microbial growth profile in fermentation using bitter starter was the lowest for both types of rice.

Table 1. Microbial counts of starter cultures and their increment after fermentation using common and glutinous rice.

		DRBC (CFU/g)			MRS (CFU/g)		
		B	BS	S	B	BS	S
Starters	Microbial Load	(1.51 ± 0.42) x 10^5	(5.5 ± 0.85) x 10^4	(4.47 ± 3.27) x 10^5	(1.03 ± 0.26) x 10^5	(4.63 ± 0.36) x 10^4	(3.97 ± 2.98) x 10^5
Common Rice	Day 0	(2.63 ± 0.54) x 10^5	(6.14 ± 1.76) x 10^4	(3.15 ± 1.65) x 10^5	(2.3 ± 0.12) x 10^5	6.15 ± 1.30) x 10^4	(2.89 ± 1.48) x 10^5
	Day 28	(9.42 ±1.9) x 10^5	(1.91 ± 0.70) x 10^5	(2.15 ± 1.10) x 10^6	(8.18 ± 0.90) x 10^5	(2.58 ± 0.14) x 10^5	(1.86 ± 0.94) x 10^6
	% Increment	208.7 ± 22.5	224.1 ± 18.7	579.3 ± 11.2	229.1 ± 16.8	251.2 ± 10.4	581.7 ± 27.9
Glutinous Rice	Day 0	(2.37 ± 1.21) x 10^5	(7.35 ± 0.39) x 10^4	(4.18 ± 0.53) x 10^5	(2.15 ± 1.13) x 10^5	(5.43 ± 0.72) x 10^4	(3.32 ± 2.39) x 10^5
	Day 28	(5.82 ± 0.70) x 10^5	(2.33 ± 0.51) x 10^5	(2.69 ± 0.33) x 10^6	(6.13 ± 3.46) x 10^5	(2.53 ± 0.27) x 10^5	(2.22 ± 1.64) x 10^6
	% Increment	157.8 ± 13.6	278.2 ± 13.7	543.5 ± 34.2	185.1 ± 13.9	367.8 ± 20.9	559.6 ± 19.7

(Note: Values are means ± standard deviations (n=3).
(B - Bitter ; BS - Bitter - Sweet ; S - Sweet ; DRBC - Dichloran Rose Bengal Choramphenicol ; MRS - Man, Rogosa and Sharpe)

This variability can be related to the original microbial load of the starter. The microbial growth during the fermentation process undergoes the classic dynamics of increment during the early stages and eventually decreases towards the end of fermentation. The microbial growths were calculated to reflect the highest increment of the microbes in the fermentation process. The differences in microbial growth profiles could be associated to the additions of herbs and spices during the starter preparation (Gandjar, 2003). The sweet starter exhibited relatively higher microbial growth due to the addition of sugar during the starter preparation. Similarly, bitter and bitter-sweet starters were prepared using a combination of herbs that would have resulted in their interaction with the microbes incorporated in the starters. The differences in microbial dynamics between rice types could be attributed to the total starch content and rice type (Dung *et al.*, 2005).

Weight reduction in rice during fermentation

The efficiency of this fermentation could be predicted based on bacterial dynamics (Table 1) and the reduction rice weight (Table 2). The highest reduction was recorded for common and glutinous rice fermented with sweet starter culture. The average percentage of reduction was calculated to be 57.10±4.20% and 55.10±4.70%, respectively. This was followed by bitter-sweet starter culture with a reduction of 52.80±2.40% and 50.70±1.80% for common and glutinous rice, respectively. The lowest reduction in utilisation of rice was recorded for bitter starter cultures with a total reduction of 48.10±2.30% and 46.10±3.10%. Reduction in the weight of the rice cake showed a trend related to the microbial load of the starters. Starter culture with higher microbial load gave higher increment of microbes during the fermentation process and this caused significant reduction in rice weight (Wu *et al.* 2010). The reduction in weight of common rice was greater than of glutinous rice due to the higher content of starch in the latter. Starch is comprised of amylose and amylopectin, which degrades into simple sugar that can be utilised by microbes (Raimbault, 1998). In glutinous rice, amylopectin is much higher than amylose, compared to common rice. Since amylopectin is more soluble, glutinous rice is more capable of absorbing more water, thus adding to its weight (Raimbault, 1998). After 28 days of fermentation, common rice, being of lower starch level would have exhausted it amylopectin content resulting in greater weight loss (Dung *et al.*, 2007). However, total weight reduction in glutinous rice was more since there was plenty of starch for utilisation by microbes.

Rice wine volume, pH, alcohol and sugar profile

The rice wine volume, pH and sugar profiles are shown in Table 2. Fermentation with sweet starter culture produced the highest amount of wine, followed by bitter-sweet then bitter starter cultures. There was also a consistent trend that glutinous rice produced twice the wine volume of common rice. In bitter starter culture, glutinous rice wine was 209% more than common rice and 228% more when bitter-sweet starter culture was used. Fermentation using sweet starter culture produced 205% more wine. Relatively higher wine volume could be associated to the much higher starch content in glutinous rice comparatively (Que *et al.*, 2006).

The pH of the produced wines were in the range of 4.3 to 4.7 and in accordance to published reports (Chiang *et al.*, 2006). Wine produced using sweet starter cultures recorded pH of 4.5 and 4.7 for common and glutinous rice respectively. However, wine produced using bitter-sweet starter cultures had a slightly lower pH level of 4.3 and 4.4 for common and glutinous rice. The low pH level in the wine is contributed by the presence of alcohol, various organic acids and by products during the anaerobic process (Chiang *et al.*, 2006).

Sugar profiling in the produced rice wine was performed using High Performance Liquid Chromatography (HPLC). Resulting peak was compared to sugar standards; maltose, lactose, glucose, galactose and fructose. The sugar detected in the wine sample was glucose. Rice wine from common rice fermented with sweet starter culture contained the highest amount of

Table 2. Biochemical properties of rice wine by starter culture and rice type.

	pH	VOLUME (L)	ALCOHOL (%)	SUGAR (mg/ml)	DPPH		WEIGHT REDUCT (%)
					Ic_{50} (mg/ml)	AEAC (mgAA/ml)	
C - Bitter	4.27 ± 0.06	1.40 ± 0.10	12.53 ± 1.35	120.64 ± 0.21	21.00 ± 1.73	5.26 ± 0.42	48.10 ± 2.30
C - Bitter-Sweet	4.33 ± 0.20	0.73 ± 0.25	11.48 ± 1.40	270.42 ± 0.32	20.03 ± 0.12	5.49 ± 0.03	52.80 ± 2.40
C - Sweet	4.53 ± 0.08	2.00 ± 0.00	9.96 ± 0.08	310.33 ± 5.83	19.62 ± 0.58	5.61 ± 0.23	57.10 ± 4.20
G - Bitter	4.70 ± 0.23	3.00 ± 0.00	8.38 ± 0.00	300.27 ± 0.28	68.00 ± 0.01	1.62 ± 0.01	46.10 ± 3.10
G - Bitter-Sweet	4.37 ± 0.11	1.67 ± 0.58	7.92 ± 0.12	320.25 ± 0.00	61.21 ± 0.58	1.79 ± 0.04	50.70 ± 1.80
G - Sweet	4.72 ± 0.27	4.00 ± 0.00	7.09 ± 0.10	440.14 ± 29.97	70.00 ± 0.01	1.57 ± 0.01	55.10 ± 4.70

DPPH IC_{50} was calculated using 10 ml of rice wine.
(C - common, G - glutinous; Values are means ± standard deviations (n=3). Values significantly different (p<0.05) within rice)

glucose at 310.33±5.83 mg/ml followed by bitter-sweet and bitter starter cultures with 270.42±0.32 mg/ml and 120.64±0.21 mg/ml, respectively. The wine produced from the fermentation of glutinous rice had a similar pattern to that of common rice, with the highest amount of glucose in the wine produced using sweet starter culture at 440.14±29.97 mg/ml, bitter-sweet at 320.25±0.00 mg/ml, and bitter at 300.27±0.28 mg/ml.

Glucose concentration in rice cake was also quantified. Rice cake from common rice fermented with bitter-sweet starter culture contained the highest amount of sugar at 4.35±0.13 mg/ml followed by sweet and bitter starter cultures with 4.29±0.68 mg/ml and 4.28±0.49 mg/ml, respectively. The glutinous rice cake on the other hand recorded the highest concentration of glucose in rice cake produced using the bitter starter culture at 6.98±0.41 mg/ml followed by bitter-sweet at 5.85±0.56 mg/ml and finally bitter at 5.17±0.26 mg/ml.

Glutinous rice contained higher glucose concentration compared to common rice, while the sweet starter culture produced the sweetest wine. As for rice cake, glutinous rice contained a higher level of starch (amylose and amylopectin) for the microbes to utilise during fermentation. During primary stages of fermentation, total conversion of starch into sugar (glucose) takes place prior to alcohol conversion (Dung *et al.*, 2007). During sugar conversion into alcohol, when the fermentation is abruptly halted after four weeks, partially converted free sugars (glucose) will be abundantly present, directly contributing to the sweetness of the wine in addition to the presence of volatiles and non fermentable sugars (Navarro *et al.*, 2000). However, glucose levels in the rice cake was extremely low compared to wines since microbes utilise starch from rice and converts it into alcohol (Wu *et al.*, 2010). The initial microbial load in the starter cultures could affect the sugar levels in rice wine and rice cakes. The higher the load, more concurrent conversion of glucose into alcohol takes place and upon external interruption, non-convertable glucose molecules directly contribute to high glucose levels in the rice wine.

The presence of alcohol is a positive indicator of successful fermentation. Rice wines obtained from common rice contained much higher alcohol level (9.9-13.9%) and differ significantly with wines produced using glutinous rice (7.0-8.4%). The highest percentage of alcohol was displayed in rice wine produced with common rice and bitter starter combination (12.53±1.35%), fermentation with bitter-sweet starter contained 11.48±1.40%, while sweet starter combination produced 9.96±0.08% alcohol. Glutinous rice also displayed a similar trend with respect to the starter culture with alcohol levels 8.38±0.00%, 7.92±0.12% and 7.10±0.01%, for bitter, bitter-sweet and sweet, respectively.

The alcohol level in rice wine is inversely proportional to sugar content in rice cake. Alcohol is produced from the conversion of sugar by microbes (Dung *et al.*, 2007) and with sufficient substrate for microbes to use, sugar will continue to be consumed and alcohol produced (Pramanik & Rao, 2005). However, when sugar level is very high and reaches an equilibrium between alcohol percentage, this disrupts further transformation of sugar into alcohol (Navarro *et al.*, 2000). Comparison between rice types shows that common rice contained higher alcohol content compared to glutinous rice, suggesting that with low starch content, sugar levels were not high enough to halt alcohol production whereas in glutionous rice, the lower alcohol level could be due to higher sugar content in fermentation.

Volatile hydrocarbons

The quality, aroma and taste of wine is influenced by the volatile hydrocarbon composition and production of secondary metabolites during fermentation (Gobetti *et al.*, 1995). Table 4 shows the composition of volatile hydrocarbons in rice wine and cake. A total of 16 volatile organics were detected in rice wine samples and 21 volatiles were detected in rice cake. Volatile compounds in wine could be

Table 4. Volatile compound composition (%) in rice wine and cake produced using different starter culture and rice.

R Time	R Index	Volatile Compund	Common			Glutinous		
			B	BS	S	B	BS	S
RICE WINE	(%/ml)							
3.2min	729	1,3-Dioxolane	4.76 ± 0.12	3.05 ± 0.48	3.86 ± 0.32	3.29 ± 0.02	2.95 ± 0.05	3.12 ± 0.01
3.3min	730	Isoamyl Alcohol	16.30 ± 0.08	16.23 ± 0.22	12.20 ± 2.60	22.88 ± 2.40	20.67 ± 1.22	22.17 ± 2.22
3.4min	697	1-Butanol	8.43 ± 0.21	8.71 ± 0.60	8.37 ± 0.44	12.01 ± 0.80	9.75 ± 0.48	10.58 ± 0.58
4.8min	814	Lactic acid	14.24 ± 0.54	14.86 ± 0.24	14.59 ± 0.06	15.63 ± 0.05	13.25 ± 1.80	12.61 ± 1.15
4.8min	824	1,3-Butanediol	4.32 ± 0.25	4.07 ± 0.48	4.12 ± 0.04	n.d	n.d	n.d
16.6min	1113	Phenylethyl alcohol	10.85 ± 0.02	11.26 ± 1.18	10.64 ± 1.12	5.42 ± 1.13	4.55 ± 0.10	4.83 ± 0.06
24.7min	1300	Tridecane	3.97 ± 0.11	3.92 ± 0.52	5.05 ± 0.80	3.56 ± 1.14	5.04 ± 0.24	3.98 ± 0.12
25.2min	1189	undeca-1,3,5,7,9-pentaene	3.33 ± 0.80	3.32 ± 0.04	2.83 ± 0.06	3.21 ± 0.56	3.44 ± 0.05	3.23 ± 0.42
28.8min	1400	2-Tetradecene	3.16 + 0.12	3.66 ± 0.01	3.83 ± 0.06	3.29 ± 0.24	4.28 ± 0.05	3.89 ± 0.06
28.8min	1620	3-Hexadecene	n.d	n.d	n.d	3.20 ± 1.15	3.20 ± 0.10	3.50 ± 0.08
29.1min	1400	Tetradecane	4.86 ± 1.13	6.36 ± 1.12	6.07 ± 0.01	5.01 ± 0.64	4.34 ± 0.28	5.26 ± 0.13
33.3min	1500	Pentadecane	5.27 ± 0.58	5.41 ± 2.01	5.97 ± 0.22	4.87 ± 1.14	6.05 ± 0.01	5.45 ± 0.26
37.1min	1602	1-Hexadecane	4.85 ± 1.40	4.45 ± 1.21	5.32 ± 0.41	4.21 ± 0.24	5.32 ± 1.23	4.89 ± 0.05
37.3min	1600	Hexadecane	4.42 ± 0.06	4.26 ± 0.30	4.27 ± 1.12	3.33 ± 0.22	4.23 ± 0.80	4.00 ± 0.01
41.1min	1700	Heptadecane	6.60 ± 0.24	6.23 ± 0.04	8.06 ± 0.05	6.07 ± 0.06	7.27 ± 1.10	8.05 ± 0.01
44.5min	1818	5-Octadecene	4.64 ± 0.18	4.21 ± 0.20	4.82 ± 0.03	4.02 ± 0.01	5.66 ± 0.23	4.44 ± 0.50
RICE WINE	(%/ml)							
4.2min	743	2,3-Butanediol	6.06 ± 0.04	6.82 ± 0.30	6.92 ± 0.28	6.30 ± 0.42	7.34 ± 0.52	6.45 ± 0.42
4.8min	813	Acetic acid	4.85 ± 0.12	4.34 ± 0.15	4.38 ± 0.20	4.22 ± 0.22	4.22 ± 0.28	4.90 ± 0.21
16.4min	1136	Phenylethyl alcohol	3.43 ± 0.58	2.31 ± 0.02	4.32 ± 0.02	4.31 ± 0.20	3.54 ± 0.09	4.18 ± 0.23
19.8min	1204	1-Dodecene	3.56 ± 1.13	3.21 ± 0.01	2.84 ± 0.06	2.57 ± 0.12	1.52 ± 0.21	2.57 ± 0.34
20.1min	1200	Dodecane	n.d	n.d	n.d	3.51 ± 0.04	3.81 ± 0.34	3.23 ± 0.45
24.7min	1300	Tridecane	1.56 ± 0.24	1.21 ± 0.10	2.75 ± 0.04	4.12 ± 0.14	2.28 ± 0.84	3.72 ± 0.06
29.1min	1400	Tetradecane	1.95 ± 0.41	1.40 ± 0.50	2.69 ± 0.01	6.10 ± 0.06	7.55 ± 0.69	6.98 ± 0.12
33.3min	1500	Pentadecane	2.01 ± 0.03	1.53 ± 0.32	2.65 ± 0.01	3.30 ± 0.11	2.87 ± 0.32	3.65 ± 0.04
37.3min	1600	Hexadecane	1.03 ± 0.02	0.96 ± 0.01	1.23 ± 0.01	4.35 ± 1.11	3.27 ± 0.23	3.62 ± 0.01
37.1min	1620	3-Hexadecene	2.54 ± 0.01	1.23 ± 0.02	1.56 ± 0.02	7.34 ± 1.20	5.61 ± 0.14	6.03 ± 0.02
41.1min	1700	Heptadecane	2.42 ± 0.01	2.65 ± 0.04	3.35 ± 0.42	2.82 ± 0.32	1.88 ± 0.25	2.22 ± 0.01
44.5min	1801	1-Octadecene	1.50 ± 0.22	0.96 ± 0.01	2.26 ± 0.05	n.d	n.d	n.d
44.5min	1808	*E*-14-Hexadecenal	n.d	n.d	n.d	4.10 ± 0.21	3.94 ± 0.65	4.65 ± 0.23
44.6min	1794	Tetradecanoic acid	1.09 ± 0.10	1.43 ± 0.01	1.23 ± 0.01	n.d	n.d	n.d
51.2min	2017	3-Eicosene	n.d	n.d	n.d	3.88 ± 0.64	4.12 ± 0.82	3.02 ± 0.50
51.3min	1878	Hexadecanoic acid	9.84 ± 1.24	10.52 ± 1.02	9.21 ± 0.84	n.d	n.d	n.d
51.3min	1993	Palmitate	10.54 ± 2.20	12.27 ± 2.24	9.43 ± 0.46	12.98 ± 1.12	14.58 ± 2.22	13.10 ± 1.14
54.7min	2185	6-Octadecanoic acid	1.65 ± 0.14	2.84 ± 0.58	2.16 ± 0.06	n.d	n.d	n.d
56.5min	1976	9,12-Octadecadienoic acid	27.33 ± 0.41	28.01 ± 1.38	26.93 ± 2.81	18.23 ± 2.30	20.42 ± 2.80	19.34 ± 2.21
56.7min	2185	Ethyl oleate	18.64 ± 1.12	18.31 ± 2.01	16.12 ± 1.22	11.87 ± 1.58	13.05 ± 2.22	12.34 ± 2.20

n.d - Not detected in samples (Note: Values are means ± standard deviations (n=3), B - Bitter ; BS - Bitter - Sweet ; S - Sweet)

grouped into alcohol, heterocyclic acetals, alkane, alkene and organic acids. Alcohol present in wine samples were isoamyl alcohol (ca. 16%/ml, common rice; ca. 20%/ml, glutinous rice), 1-butanol (ca. 8%/ml, common rice; ca. 10%/ml, glutinous rice), and phenylethyl alcohol (ca. 10%/ml, common rice; ca. 54%/ml in glutinous rice). However, rice wine produced using common rice contained 1,3-butanediol (ca. 4%/ml) in addition to the ones stated above. Lactic acid was also detected in all wines produced with an average of 14%/ml composition. Among the other volatiles detected in the rice wine samples were hydrocarbons (3-8%/ml) and 1,3-dioxolane (ca. 4%/ml).

Volatile compounds detected in common and glutinous rice cakes also consists of esters, alcohols, organic acids, alkane, alkene and aldehydes. Common and glutinous rice cake extracts mainly comprised of hydrocarbons such as 1-dodeceane, tridecane, tetradecane, pentadecane, and heptadecane. Alcohols detected in both rice cakes were 2,3-butanediol and phenylethyl alcohol at a composition of ca. 6%/mg and ca. 4%/mg, respectively. In terms of organic acids, glutinous rice contained three of the six organic acids found in common rice cake. The three were acetic acid, palmitic acid and 9,12-octadecadienoic acid while common rice cake additionally contained 6-octadecanoic, hexadecanoic and tetradecanoic acids. 9,12-octadecadienoic acid was detected in the higest composition (> 18%/mg) in both rice cakes. The detection of acetic acid (ca. 4%/mg) was evident in both the analysed rice cake samples. Acetic acid explains the sour feeling in the rice cake. All rice cake samples contained ethyl oleate, while glutinous rice cakes had an additional aldehyde (14-hexadecenal) and 3-eicosene. Hydrocarbons were much lower in composition compared to rice wine samples, mainly ranging from 1-3%/ml. Differences in the volatile organics were significant between rice types rather than starter cultures.

Hydrocarbons produced in these fermentations are mainly influenced by the type of rice. The hydrocarbons formed during fermentation could have originated from the outer membranes of rice grains or intermediate products of fermentation. However, the presence of yeast and LAB which is introduced through the starter culture plays an important role since volatiles are byproducts of fermentation by these microbes (Chen & Xu, 2010). Detection of lactic acid is a good indicator of the presence and importance of LAB in these fermentations (Gobetti *et al.*, 1995). This is supported by the 14%/ml average composition of lactic acid detected in the rice wine samples.

In addition, butanediol in both rice wine and cake samples is a byproduct during the breakdown of pyruvate during ethanol production by yeast. This alcohol is a common product from the fermentation of sugar and can be detected in most alcoholic beverages undergoing anaerobic microbial fermentation. Mateo *et al.* (2001) also detected this compound in wine samples they tested. It is a common byproduct in anaerobic microbial fermentation. The other alcohols detected in the wine samples were isoamyl alcohol, 1-butanol and phenylethyl alcohol. Isoamyl alcohol is a byproduct produced by almost all strains of *S. cerevisiae* (Chuenchomrat *et al.*, 2008), while the presence of phenylethyl alcohol could explain the aroma in the wine produced (Chen & Xu, 2010). Phenylethyl alcohol was reported to be a compound produced by strains of commercial *S. cerevisiae* (Chen & Xu, 2010) and it gives out floral odour. On the other hand, 1,3-dioxolane detected in the rice wine samples is a product from the acetalisation of acetaldehydes and glycerol. This compound could be an indicator of the wine age (Camara *et al.*, 2003).

Antioxidant (DPPH) and fibrinolytic enzyme assays

DPPH radical scavenging activity of rice wine and cake extracts was expressed by the percentage reduction (IC_{50}) of the radical ion by samples. In rice wine, the best radical scavenging activity was observed by sweet starter (IC_{50}, 19.62±0.58 mg/ml), followed by bitter-sweet (IC_{50}, 20.03±0.12 mg/ml) and bitter (IC_{50}, 21.00±1.73 mg/ml). As for

glutinous rice wine, the activity was displayed using bitter-sweet starter (IC_{50}, 61.21±0.58 mg/ml), followed by bitter (IC_{50}, 68.00±0.01 mg/ml) and sweet (IC_{50}, 70.00±0.01 mg/ml). The radical scavenging potential of rice cakes were also tested and common rice cake fermented with bitter-sweet starter exhibited the best activity (IC_{50}, 1.87±0.20 mg/ml), followed by bitter (IC_{50}, 4.00±0.80 mg/ml) and sweet (IC_{50}, 4.48±0.20 mg/ml) starter. Meanwhile, glutinous rice cake fermented with bitter-sweet starter displayed the best activity (IC_{50}, 4.60±0.32 mg/ml) followed by sweet (IC_{50}, 10.80±1.15 mg/ml) and bitter (IC_{50}, 11.20±0.35 mg/ml). Radical scavenging activity of rice wine and cakes were compared to ascorbic acid (IC_{50}, 0.011±0.01 mg/ml) and is expressed in Ascorbic Acid Equivalent Antioxidant Capacity (AEAC). Data is displayed in Tables 2 and 3.

Based on these findings, both common rice wine and cake displayed better antioxidant properties compared to glutinous rice. The antioxidant activity can be related to the type of rice. Rice contains phytochemicals such as tocopherols, oryzanol and polyphenols (Rohrer & Siebenmorgen, 2004). Polyphenols, such as vanillic acid, quercetin and other phenolics are known to be potent antioxidants which have the potential to be protective against cardiovascular diseases (Que *et al.*, 2006). In addition, low molecular weight polysaccharides, proteins or peptides are also known to influence the antioxidant properties (Kumar *et al.*, 2007).

Fibrinolytic enzyme assay

Fibrinolytic enzyme is a major component in fermented food (Ashipala & He, 2008). Only rice cakes that showed activities and results are shown in Table 3. Fibrinolytic activity was expressed in plasmin units. Common rice cake extracts fermented with bitter-sweet starter displayed the best fibrinolytic activity (0.32±0.01 unit/mg), followed by sweet (0.28±0.01 unit/mg) and bitter (0.23±0.00 unit/mg). In glutinous rice cake, the strongest inhibition was observed using bitter-sweet starter (0.17±0.00 unit/mg) followed by sweet (0.16±0.00 unit/mg) and bitter (0.14±0.01 unit/mg). The use of a different starter did not influence the fibrinolytic enzyme activity. Instead, the choice of rice showed a clear difference with common rice displaying an activity twice, as shown by glutinous rice.

Fibrinolytic enzymes have been closely associated with fermentation related microorganisms from the genus *Bacillus* (Wong & Mine, 2004). The presence of fibrinolytic enzyme disrupts the formation of fibrin clots in the blood circulatory system, thus aiding in the restoration of blood clots. Since commercial enzymes are expensive, fermented food could be an alternative option. Oral administration of fermented food (rice cake) containing fibrinolytic enzyme could promote the introduction of plasminogen activator in humans as a means to disrupt the formation of fibrin clots (Wong & Mine, 2004), directly helping to reduce cardiovascular problems.

Table 3. Antioxidant and fibrinolytic enzyme activities of rice cake by starter culture and rice type.

	DPPH		FIBRIN (unit/mg)
	Ic50 (mg/ml)	AEAC (mgAA/g)	
C- Bitter	4.00 ± 0.80	(5.67 ± 1.16) x 10^3	0.23 ± 0.00
C - Bitter-Sweet	1.87 ± 0.20	(11.87 ± 1.37) x 10^3	0.32 ± 0.01
C - Sweet	4.48 ± 0.20	(4.93 ± 0.23) x 10^3	0.28 ± 0.01
G - Bitter	11.20 ± 0.35	(1.96 ± 0.06) x 10^3	0.14 ± 0.01
G - Bitter-Sweet	4.60 ± 0.32	(4.81 ± 0.34) x 10^3	0.17 ± 0.00
G - Sweet	10.80 ± 1.15	(2.03 ± 0.23) x 10^3	0.16 ± 0.00

DPPH IC_{50} was calculated using 2.5 g of rice cake.
(C- common, G- glutinous; Values are means ± standard deviations (n=3). Values significantly different ($p<0.05$) within rice)

CONCLUSION

Biochemical properties of rice wine were influenced significantly by the type of rice rather than the starter culture. Glutinous rice produced twice more wine compared to common rice and the wines were much sweeter due the presence of free sugar, glucose. The alcohol level is at least 40 ~ 50% higher in common rice compared to glutinous rice. It was also shown that bitter starters produced highest alcohol level but lowest volume of wine regardless of rice type. The reduced wine volume could have resulted in the higher alcohol level of wine produced in batches fermented with this starter. On the other hand, gas chromatographic analysis revealed that the volatile hydrocarbons were almost similar in composition. In addition, rice cake samples displayed positive antioxidant and fibrinolytic properties making it healthy for consumption. Therefore, through this study, it could be concluded that using different rice types and different starters produce significant differences in the quality of wine. It also produces rice cakes with high bioactive potentials that could be utilised as a functional food.

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