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# An overview of fermentation in rice winemaking

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# Abstract

Rice wine is an alcoholic beverage produced via the fermentation of cereals, primarily rice with starter cultures. It is produced and consumed globally, especially in Asian countries. With the growth of the global rice wine market, the development of high-quality rice wines is gaining increasing interest. This paper reviews and discusses the comprehensive research details of rice wines in different regions, including the selection of starch substrates, comparison of starter cultures' microbial compositions, compositions of rice wines and its health benefits. The simultaneous saccharification and fermentation (SSF) of rice wine, microorganisms involved in the fermentation, and factors affecting the fermentation process are discussed, thus providing an overview of the rice wine fermentation and the involved study perspectives.

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## 1. Introduction

Fermentation is an ancient food processing technique used for food preservation while the fermentation of cereal grains such as rice, wheat, and millet to produce alcoholic beverages has been practised for centuries. Rice wine is an alcoholic beverage produced via the fermentation of cereals, mainly rice with starter cultures (1). It is produced and consumed globally, especially in Asian countries during cultural events and celebrations.

The global rice wine market is forecasted to grow, owing to the growing demand for rice wine in western countries due to trade and globalization as well as the use of rice wine in novel food product development or as a cooking ingredient (2). In addition, the unique flavour and consumers' preference for traditionally brewed drinks significantly increased global rice wine consumption.

However, traditional rice wine fermentation remains empirical and raises food safety and quality concerns. This detailed and systematic review provides an overview of rice winemaking by revealing the properties of rice wines and their raw materials, principles underlying the fermentation process, and critical factors influencing it on top of addressing the future improvement and research needed, to benefit the food and biotechnology sectors, particularly the rice wine industry.

# 2. Rice Wines in Different Regions

Rice wines are produced and consumed worldwide, especially in Asian countries for centuries. However, their local names and organoleptic properties are distinct among different regions based on the availability of raw materials, starter cultures, and the

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manufacturing process. Table 1 shows a variety of rice wines in different regions along with their starch substrates and starter cultures used.

Rice wines	Starch substrates	Starter cultures	Regions	References
Apong / sai mod	Rice	Aopo pitha	India (Dhemaji / Lakhimpur / Jorha)	(3)
Atingba	Glutinous rice	Hamei	India (Manipur)	(4)
Brem	Black and white glutinous rice	Ragi tape	Indonesia (Bali Island)	(5)
Hong qu glutinous rice wine	Glutinous rice	Hong qu / bai qu / yao qu	China (Fujian)	(6,7)
Hor-alank	Rice	Thap	India (Karbi Anglong)	(3)
Jaanr / jaand	Rice, millet, maize, wheat	Marcha / murcha	India / Nepal / Bhutan / Tibet	(8,9)
Judima	Rice	Umhu / humao	India (Dima Hasao / Dimapur)	(3)
Maibra jou bishi	Glutinous rice	Angkur	India (Kokrajhar)	(3)
Makgeolli / takju / dongdongju / nongju	Rice (glutinous rice / non-glutinous rice)	Nuruk / koji	Korea	(10–13)
Matha jou bishi	Non-glutinous rice	Angkur	India (Kokrajhar)	(3)
Оро	Rice, burnt rice husk (ampe)	Siiyeh / opop	India (Arunachal Pradesh)	(3)
Ou	Glutinous rice	Loog-paeng / loog- pang	Thailand	(14,15)
Ruou can	Rice, maize, cassava	Men	Vietnam	(16)
Ruou de	Rice	Men	Vietnam	(16)
Ruou nep	Glutinous rice	Men	Vietnam	(16)
Ruou nep than	Purple glutinous rice	Men	Vietnam (Mekong Delta)	(16)
Sake	Rice	Којі	Japan	(17)
Sato	Glutinous rice	Loog-pang	Thailand	(18)
Shandong jimo millet wine	Millet	Jiu qu	China	(7,19)
Shanlan rice wine	Glutinous rice	Qiubing	China (Hainan)	(20,21)
Shaoxing rice wine	Rice, wheat	Wheat qu	China (Zhejiang)	(22)
Srapeang	White rice, red rice	Medomdae / dombea / mesra	Cambodia	(23,24)
Sujen	Rice	Perok kushi	India (Lakhimpur / Sibasagar / Dibrugarh / Tinsukia)	(3)
Тараі	Glutinous rice	Sasad	Malaysia (Sabah)	(25)
Тариу	Rice, red rice	Bubod	Philippines	(26,27)
Xaj pani / koloh pani	Rice	Vekur pitha	India (Sivasagar)	(3)
Zutho / litchumsu	Rice	Piazu	India (Dimapur / Kohima)	(3)

Tapai produced in Sabah, Malaysia from the fermentation of glutinous rice is characterized by a combination of sweet, sour, and bitter tastes with an alcoholic aroma (25). The glutinous rice can be replaced with other starch substrates such as rice, cassava,

13

maize, or pineapples in some parts of Sabah to provide a variety of flavours. Similarly, in Vietnam, glutinous rice and non-glutinous rice are used to produce ruou nep and ruou de, respectively, while rice, maize or cassava can be used to produce ruou can (28). Furthermore, purple glutinous rice was used to produce ruou nep than (purple glutinous rice wine) with a sherry-like taste and an appealing brown-red colour.

Other similar rice wines include makgeolli (Korea) which tastes sweet, sour, bitter, salty, and umami (11); Brem (Bali Island, Indonesia) which tastes sweet, sour, and alcoholic (5); and Tapuy (Philippines) which tastes sweet and acidic (27). Chinese rice wines from different provinces of China such as hong qu glutinous rice wine (Fujian), Shaoxing rice wine (Zhejiang), and Shandong jimo millet wine (Shandong) also have their distinct flavours, while hong qu rice wine is gaining popularity due to its bright red colour, subtle sweet flavour, and functional properties brought by hong qu (starter culture made from red yeast rice) (29).

### 3. Raw Materials of Rice Wines

The raw materials used to produce rice wine include starch substrates and starter cultures. The starch from the substrate will be converted into alcohol through fermentation by the microorganisms found in the starter culture (14).

### 3.1. Starch Substrates

Fermented alcoholic beverages can be produced from various substrates such as grains, fruits, and vegetables, while the fermentation of cereal grains such as rice, wheat, and millet to produce alcoholic beverages had been practised long ago. The selection of starch substrates used for the fermentation of rice wine is greatly depending on the regional preferences and the availability of agricultural starchy materials. The most used starch substrates for rice wine production are dehulled rice (*Oryza sativa* L.), glutinous rice (*Oryza sativa* var. *glutinosa*), and purple glutinous rice (30). Rice is grown on 161.62 million hectares globally, producing 487.35 million tons of milled rice, of which most of it is contributed by Asia (31).

The composition of rice is one of the major factors affecting the flavour and quality of rice wine (32). Rice contains carbohydrates, proteins, lipids, and various micronutrients, where carbohydrate is the major constituent, ranging from 70-90% or more, depending on the environment and variety of rice (33,34). According to Okonogi et al., glutinous rice, also known as sticky rice, sweet rice or waxy rice has a lower carbohydrate content but higher protein and lipid content than that of non-glutinous rice (34). Table 2 shows the compositions of various cereal grains used to produce rice wine.

Concelleration		Compositions (%)	
Cereal grains	Carbohydrate	Protein	Lipid
Glutinous rice	77.40	7.30	1.50
Non-glutinous rice	79.70	6.80	0.70
Maize	74.26	9.42	4.74
Millet	72.85	11.02	4.22
Wheat	75.90	11.31	1.71

Table 2. Compositions of cereal grains (35,36).

Glutinous rice varies from non-glutinous rice in its amylose and amylopectin content. Tester et al. found out that the amylose contents in glutinous, normal, and high-amylose rice grains were <15%, 16-35%, and >36%, respectively, indicating that glutinous rice has a lower amylose content than non-glutinous rice (37). A similar trend was discovered by Okonogi et al. who compared the amylose content of non-glutinous rice (21.8%), aromatic non-glutinous rice (17.5%), and glutinous rice (4.0-7.4%) (34).

Generally, glutinous rice is low in amylose and high in amylopectin compared to nonglutinous rice. Thus, glutinous rice consists of lesser long glucose chains and more branched, short glucose chains, which can easily be debranched by amylase to produce short chains and water-soluble sugar such as monosaccharides, disaccharides, and oligosaccharides, which may enhance the sweetness of the rice wine (38). In addition, Palaniveloo and Vairappan noticed that the yield of rice wine produced using glutinous rice was twice the volume of rice wine produced using non-glutinous rice (39). Thus, the use of glutinous rice is greatly preferred over non-glutinous rice in the preparation of rice wine (3,20).

Alcohol is the result of successful fermentation. Lai et al. found that the alcohol content of rice wine produced from glutinous rice (8.9%) was significantly higher than that of the rice wine produced from non-glutinous rice (6.5%) after four days of fermentation (38). However, the research run by Palaniveloo and Vairappan showed an opposite trend, in which the rice wine produced from glutinous rice has a lower alcohol content (7.0-8.4%) than that of the rice wine produced using non-glutinous rice (9.9-13.9%) after 28 days of fermentation (39).

It is believed that glutinous rice with higher amylopectin content is broken down more easily, resulting in earlier ethanol production than that of non-glutinous rice (38). Therefore, glutinous rice wine produced with a short fermentation period has a higher alcohol content compared to that of non-glutinous rice wine. However, Palaniveloo and Vairappan suggested that high sugar content from the hydrolysis of starch in glutinous rice may inhibit the alcohol fermentation process, resulting in glutinous rice wine with lower ethanol content compared to that of non-glutinous rice (39).

Other than that, rice proteins facilitate alcoholic fermentation and impart organoleptic characteristics to rice wines. Proteins are catabolized into short peptides and amino acids, which are the nitrogen source for microbial growth during fermentation, thus affecting the microbial metabolite composition such as alcohols, acids, and esters, which eventually affect the flavour and quality of rice wines (40–42). A recent study by Xie et al. concluded that an increase in the protein content of rice contributes to the improved taste of rice wine (41). Therefore, rice wine produced from glutinous rice which has a higher protein content may taste better than that from non-glutinous rice.

Most studies tended to focus on the effect of carbohydrates and proteins in rice on the rice wine qualities. However, the effect of rice lipids on the properties of rice wine has not been dealt with in-depth. Chen and Xu who conducted experiments on the growth of yeast cells during Chinese rice wine brewing using wheat Qu and mixed commercial enzymes showed that the death of yeast cells increased with the ethanol concentration of the fermentation mash, while fermentation using wheat Qu had a lower yeast cell death rate compared to those using mixed commercial enzymes (43). On top of that, they suggested that lipids in wheat Qu may account for the higher yeast activity in fermentation. Their assumptions seem to be well-grounded as extensive research were indicating that unsaturated fatty acids and sterols can increase the ethanol tolerance of yeast cells (44,45).

### 3.2. Starter Cultures

Starter cultures used in the rice wine fermentation comprise mixed cultures containing fungi and bacteria with starchy cereals as the base. These starter cultures are usually found in the form of dried powder, flattened cakes, or hard balls of various sizes (23,24). Traditional starter cultures are made up of various base substrates and microorganisms, greatly depending on the regions in which they are produced and are named differently worldwide. Table 3 summarized a variety of starter cultures in different regions, along with their respective base substrates used and predominant microorganisms.

Starter	Base		Predominant microorganisms	Regions	References
cultures Dombea	substrates Red rice	Yeast	: Saccharomyces sp., Saccharomycopsis	Cambodia	(24)
Dombea	Reuffice	Teast	sp.	Camboula	(24)
		Mould	: <i>Rhizopus</i> sp.		
			: Lactobacillus sp., Pediococcus sp.,		
			Leuconostoc sp., Weissella sp.,		
			Streptococcus sp.		
Hamei	Rice	Yeast	: Candida tropicalis, Candida montana,	India	(4)
			Candida parapsilosis, Pichia anomala,	(Manipur)	
			Pichia fabianii, Pichia guilliermondi,		
			Saccharomyces cerevisiae, Torulaspora		
			delbrueckii, Trichosporon sp.		
Hong qu /	Rice	Yeast	: Saccharomyces cerevisiae,	China	(7)
yao qu			Saccharomycopsis fibuligera	(Fujian)	
Loog-pang	Rice flour	Yeast	: Saccharomycopsis fibuligera	Thailand	(15)
		Mould	: Amylomyces sp., Mucor sp., Rhizopus		
			sp.		
		Bacteria	: Pediococcus pentosaceus,		
Marcha /	Glutinous	Voact	Gluconobacter sp. : Candida glabrata, Pichia anomala,	India /	(9)
murcha	rice	Yeast	Pichia burtonii, Saccharomyces bayanus,	Nepal /	(9)
marcha	nee		Saccharomycopsis capsularis,	Bhutan /	
			Saccharomycopsis fibuligera	Tibet	
Men	Rice flour,	Yeast	: Candida glabrata, Pichia anomala,	Vietnam	(16,30)
	cassava flour		Saccharomyces cerevisiae		(
		Mould	: Amylomyces rouxii, Rhizopus		
			oligosporus		
Nuruk	Rice, barley,	Yeast	: unspecified	Korea	(12)
	millet,	Mould	: Aspergillus oryzae, Emericella nidulans,		
	maize,		Lichtheimia corymbifera, Lichtheimia		
	soybean,		ramosa		
	rye, oats				
Sasad	Rice flour	Yeast	: Candida utilis, Candida krusei,	Malaysia	(25,30)
			Endomycopsis spp.	(Sabah)	
		Mould	: Amylomyces rouxii, Rhizopus spp.		
		Bacteria	: Lactobacillus paracasei, Lactobacillus		
			plantarum, Pediococcus pentosaceu,		
Wheat qu	Wheat	Voact	Lactococcus lactis : Candida tropicalis, Clavispora	China	(22)
wheat qu	vvileat	Yeast	lusitaniae, Pichia anomala,	(Zhejiang)	(22)
			Saccharomyces cerevisiae	(Zitejiarig)	
			Succial onlyces cerevisiae		

Table 3. Starter cultures in different regions, along with their respective base substrates used and predominant microorganisms.

Mould : Absidia corymbifera, Aspergillus fumigatus, Aspergillus niger, Aspergillus oryzae, Emericella nidulans, Rhizomucor pusillus, Rhizopus oryzae

Generally, the starter cultures are prepared in a similar manner, where a base substrate is inoculated with a previous starter culture or naturally occurring microflora from the plants, herbs, and spices, followed by the development of desired microflora for a short period before drying (8). The dried starter culture can be used for fermentation of rice wines or stored under room temperature for months in dry, airtight containers (4,18,46). However, traditional starters are often prepared based on empirical knowledge under uncontrolled and non-aseptic conditions in homes and villages, resulting in the inconsistency of rice wine quality and yield.

To produce sasad (a starter culture used to produce tapai in Sabah), rice flour is mixed with fresh plant materials (garlic and ginger), spices (peppers, red chillies, and cinnamon), coconut water, and water or sugar cane juice, moulded into small disks or circular flat cake, allowed to ferment for few days, and sun-dried (30,46). The addition of coconut water or sugar cane juice provides sugars and nutrients which promote microbial growth in the starter culture. Furthermore, plant materials, herbs, and spices are added during the preparation of starter culture to enhance the flavour of rice wine, inhibit the growth of undesirable microorganisms, as well as to bring health benefits such as improving blood flow and reducing muscle pain (23).

Rice wines produced using different starter cultures with different microbial contents have different yields, compositions, and flavours. Palaniveloo and Vairappan claimed that the rice wine produced using sweet starter culture (with higher total microbial load) has a higher yield, lower alcohol content, and higher sugar content compared to that of the bitter starter culture (with lower total microbial load) (39). In addition, Chim et al. mentioned that rice wine produced using medombae (a traditional starter culture used to produce rice wine in Cambodia) has a lower yield but a better taste and aroma compared to the rice wine produced using starter cultures imported from Vietnam (23).

Nevertheless, local rice winemakers in Vietnam claimed that the rice wine produced using a combination of different starter cultures is of better quality with a stronger sweet alcoholic taste together with a more appealing flavour than the rice wine produced using a single starter (30). Each starter culture has its strengths and weaknesses in the production of rice wine, depending greatly on its microbial content. Thus, a more systematic and theoretical analysis is required to determine the effect of each microorganism on the quality of rice wine.

### 4. Compositions of Rice Wines

Rice wine is a complex matrix containing alcohols as well as organic and inorganic compounds, such as carbohydrates, proteins, organic acids, volatile compounds, vitamins, and minerals (11,47,48). The complexity of the composition of rice wines gives rice wines their distinct flavours and nutritional components.

Ethanol or ethyl alcohol is the main alcoholic component in rice wines produced through fermentation, while the alcohol content of rice wine is greatly dependent on the manufacturing practices, raw materials used, and microorganisms employed. Alcohol, Tobacco Products and Firearms (27 CFR. § 4.21) stated that wine from fermentable

agricultural products such as rice can be classified into table wine and dessert wine with alcohol content not exceeding 14% (v/v) and 24% (v/v), respectively (49). According to section 375 (1) of Food Regulation 1985 (as at 5<sup>th</sup> May 2021), rice wine produced through alcoholic fermentation of rice or other grain shall contain 12-20%(v/v) of alcohol, which refers to ethanol (50). However, this regulation does not apply to rice wines prepared, produced, or packaged for export outside Malaysia. A growing body of literature has suggested that rice wines with various alcohol contents can be produced (Table 4).

Rice wines	Alcohol content %(v/v)	рН	References
Chinese rice wine	16.20 - 17.10	4.15 - 4.54	(51)
Chinese rice wine	12.60 - 13.90	3.50 - 3.70	(40)
Foxtail millet sake	10.87	3.53	(52)
Makgeolli	6.69 - 13.70	3.58 - 4.12	(11)
Ou	12.40 - 13.10	3.72 - 4.10	(53)
Ruou nep than	11.15 - 15.21	3.40 - 4.30	(16)
Sake	13.00 - 17.00	4.20 - 4.70	(54)
Sato	13.00 - 15.00	4.00	(18)
Тараі	12.30	4.00	(25)
Тариу	8.19 - 19.83	3.01 - 3.74	(27)
Тариу	10.60 - 12.90	4.65 - 5.00	(26)
Non-glutinous rice wine	9.96 - 12.53	4.27 - 4.53	(39)
Glutinous rice wine	7.09 - 8.38	4.37 - 4.72	(39)
Waxy non-pigmented rice wine	13.60	4.60	(55)
Waxy pigmented rice wine	13.50	4.30	(55)

#### Table 4. Alcohol content and pH of rice wines.

Apart from ethanol, flavour compounds such as higher alcohols, organic acids, esters, aldehydes and ketones which contribute to the delicate taste and aroma of rice wines were formed in various concentrations during alcoholic fermentation (40,51,56,57). Table 5 shows some of the volatile compounds frequently found in rice wines.

			Volatil	e compounds
		Acids	Alcohols	Esters
Rice wines	References	Hexanoic acid / caproic acid Ethanoic acid / acetic acid	Propan-1-ol 3-Methvlbutan-1-ol 2-Phenvlethan-1-ol 2-Methylpropan-1-ol	Ethyl octanoate Ethyl hexanoate Ethyl ethanoate / ethyl acetate Ethyl decanoate Ethyl butanoate Ethyl butanoate Ethyl 2-hydroxypropanoate / ethyl lactate 3-Methylbuty 2-Phenethyl acetate
Cambodian rice	(24)	V	$\vee$ $\vee$ $\vee$ $\vee$	√ √ √
wine	(51)		-1 -1 -1	
Chinese rice wine	(51)	$\vee$ $\vee$	$\sqrt{\sqrt{\sqrt{2}}}$	$\sqrt{\sqrt{\sqrt{\sqrt{\sqrt{\sqrt{\sqrt{\sqrt{\sqrt{\sqrt{\sqrt{\sqrt{\sqrt{\sqrt{\sqrt{\sqrt{\sqrt{\sqrt{$
Chinese rice wine	(57)	V V	$\vee$ $\vee$ $\vee$ $\vee$	$\vee$ $\vee$ $\vee$ $\vee$ $\vee$
Chinese rice wine	(56)	V	$\vee$ $\vee$ $\vee$ $\vee$	$\vee \vee \vee \vee \vee \vee \vee \vee \vee$
Chinese rice wine	(40)	VV	$\vee$ $\vee$ $\vee$	$\vee$ $\vee$ $\vee$ $\vee$ $\vee$ $\vee$
Huadiao Chinese	(58)	V V	$\sqrt{\sqrt{\sqrt{2}}}$	$\vee$ $\vee$ $\vee$ $\vee$ $\vee$ $\vee$
rice wine	( )			
Makgeolli	(11)		$\vee$ $\vee$ $\vee$ $\vee$	$\vee  \vee  \vee  \vee  \vee  \vee  \vee  \vee  \vee  \vee $
Ou	(14)	V	$\sqrt{\sqrt{\sqrt{2}}}$	$\vee$ $\vee$ $\vee$
Soto	(59)	V	$\vee$ $\vee$ $\vee$	$\vee$ $\vee$ $\vee$ $\vee$

#### Table 5. Volatile compounds frequently found in rice wines.

Organic acids prevent the growth of spoilage microorganisms (by lowering the pH) and significantly affect the flavour (by contributing to the sourness and decreasing the sweetness) of rice wines, while their concentrations vary with the types of rice wine, environmental factors, as well as microbial metabolism during fermentation and storage of rice wines (60). Yu et al. have recognized butanedioic acid (succinic acid), 2-hydroxypropanoic acid (lactic acid), and 2-hydroxypropane-1,2,3-tricarboxylic acid (citric acid) as the main organic acids found in 20 Chinese rice wines (42). However, ethanoic acid (acetic acid) which is associated with the sour vinegar taste was detected in most of the rice wines from different regions (Table 5).

On top of that, acids were gradually converted into aromatic esters through esterification with alcohols, especially during storage. For example, ethyl ethanoate (ethyl acetate) with a fruity smell and brandy note is present in all of the rice wines shown in Table 5 (61). Besides that, 3-methylbutyl ethanoate (3-methylbutyl acetate) with a fruity, banana, sweet, fragrant, powerful odour and a bittersweet taste reminiscent of pear; ethyl 2-hydroxypropanoate (ethyl lactate) with a buttery, cream, sweet, fruity odour; ethyl butanoate with a fruity odour, reminiscent of pineapples; and ethyl hexanoate with an apple, fruity, sweet, banana, strawberry aroma were found in most of the rice wines (61,62).

Odour activity value (OAV) defined as the ratio of a volatile compound's concentration to its detection threshold is often used to identify the volatile flavour compounds which contribute to the aroma of rice wines, where only compounds with OAVs≥1 can be detected

by the olfactory system and compounds with higher OAVs are potentially the characteristic flavour components (57). According to Chen et al., esters have a low odour detection threshold, which may contribute to the aroma profile of rice wine even in low concentrations, giving the rice wine a desirable fruity odour (58). This is in good agreement with previous findings by Chen and Xu, where the OAV of ethyl octanoate (12.50) is higher than that of 2-methylpropan-1-ol (2.40) although ethyl octanoate is present in a remarkably lower concentration ( $62\mu g/L$ ) than 2-methylpropan-1-ol (95,331 $\mu g/L$ ) in the Chinese rice wine because ethyl octanoate has a lower detection threshold ( $5\mu g/L$ ) compared to 2-methylpropan-1-ol (40,000 $\mu g/L$ ) (51).

Additionally, sugars may affect the taste of rice wines. Sugars are formed through saccharification of starch and are converted into ethanol and acids by fungi and bacteria during fermentation. The residual sugars which are not consumed by the microorganisms after fermentation is stopped may enhance the sweetness of the rice wines (38,63). According to Classification of Alcoholic Beverages (GB/T 17204-2008), rice wines can be classified based on their total sugar content into dry, semi-dry, semi-sweet, and sweet rice wines with total sugar content  $\leq 15.0$ g/L, 15.1-40.0g/L, 40.1-100.0g/L, and >100.0g/L, respectively (64). Yu et al. reported that glucose was the major sugar component in rice wine and was present in a higher concentration in rice wine compared to that of fruit wines such as grape wine, cherry wine, and orange wine, thus giving the rice wine a relatively sweeter taste (42).

Furthermore, amino acids produced through proteolysis during fermentation contribute to the flavour of rice wines. Amino acids usually taste sweet or bitter, whereas glutamic and aspartic acids taste sour (65). More recent evidence showed that alanine, proline, leucine, arginine, and glutamic acid were abundantly found in Chinese rice wines (42,66). In addition, Xie et al. who investigated the correlation between the protein content in glutinous rice and amino acid composition in rice wine suggested that rice wine produced from glutinous rice with higher total protein content contained higher amino acid content and has a better taste (41).

Besides that, amino acids can be converted into higher alcohols by fungi through the Ehrlich pathway. For example, 2-methylpropan-1-ol (with a sweet musty odour), 3-methylbutan-1-ol (with a whisky character and pungent odour), and 2-phenylethan-1-ol (with a rose-like odour) can be derived from the degradation of valine, leucine, and phenylalanine, respectively (57,61,67). Chen and Xu who investigated the effect of yeast strains on volatile flavour profiles of Chinese rice wine concluded that yeast strains greatly influenced the concentration of flavour compounds in Chinese rice wines, contributing to the flavour differences among the rice wines (51). They found that 2-methylpropan-1-ol (isobutanol), 3-methylbutan-1-ol (isoamyl alcohol), and 2-phenylethan-1-ol were the key aroma compounds that contribute to the flavour of the Chinese rice wines. This is in line with the previous literature (Table 5).

### 5. Health Benefits of Rice Wines

Rice wine is a highly nutritious functional alcoholic beverage that contains carbohydrates, proteins, organic acids, vitamins, minerals, and a variety of bioactive compounds that can confer health benefits to consumers (10,27,41). The fermentation process has increased the bioavailability of nutrients in rice wine, thus increasing its nutritional value.

Rice wine was consumed by patients and postnatal women for energy recovery due to its high-calorie content (25). Rice wine contains monosaccharides (formed through saccharification of starch) which can be utilised to produce energy or stored in the form of glycogen in the liver and muscles. Besides that, Zhao et al. showed that Chinese rice wine features antifatigue ability as it has significantly decreased the level of blood lactic acid, in which lactic acid decreases the pH in blood and muscles, leading to physical exhaustion (68).

Rice wine can promote blood circulation considering that ethanol decreases sympathetic nervous activity which consequently decreases cardiac contraction and heart rate while inducing vasodilation (dilates blood vessels) to improve blood flow, promoting cardiovascular health (69). Other than that, rice wine has anti-cancer, anti-tumour, and anti-inflammatory properties because it contains peptides and farnesol (70,71). In addition, peptides may contribute to anti-hypertensive, antimicrobial, antithrombotic, and antioxidant activities, while farnesol may relieve allergic asthma, atherosclerosis, obesity, hyperlipidaemia, and diabetes (72,73). Ha et al. compared the concentration of farnesol in various alcoholic beverages and found that the farnesol content in makgeolli (Korean rice wine) is notably higher than that of beer, sake, and wine analysed (71). Also, Choi et al. proved that makgeolli possesses anti-diabetic properties (10).

Several studies suggested that rice wines exhibited antioxidant properties which can scavenge free radicals and protect body cells against oxidation (27,39,53,74–77). Que et al. showed that Chinese rice wine contains phenolic compounds, predominantly syringic acid and (+)-catechin which are positively correlated with the antioxidant properties of the rice wines (77). Besides that, Hipol and Alma-in reported that tapuy (Philippine rice wine) and its concentrate (non-volatile fraction) contain a greater amount of phenolic compounds, thus exhibiting a greater antioxidant capacity than that of its volatile fraction, suggesting that the antioxidant compounds are present mainly in the non-volatile fraction of the rice wine (27).

The phenolic compositions and antioxidant properties of rice wines may depend on the raw materials used, including the starch substrates and starter cultures (39,74). Generally, rice wine produced from non-glutinous rice has higher antioxidant activity than that from glutinous rice, while rice wine produced from pigmented rice has higher antioxidant activity than that from non-pigmented rice. In addition, Palaniveloo and Vairappan demonstrated that the non-glutinous rice wine produced using sweet starter culture (with higher total microbial load) has the best radical scavenging ability, followed by the bitter-sweet and bitter starter cultures (with lower total microbial load), whereas, glutinous rice wine produced using bitter-sweet starter culture has the best radical scavenging ability, followed by bitter and sweet starter cultures (39). However, the relationship between starter cultures used and the antioxidant properties of rice wine is yet to be discovered.

Antioxidants in rice wine not only prevent atherosclerotic plaque formation which may lead to heart attack, stroke, or death, but also potentially prevent photoaging. Seo et al. studied the anti-aging effect of rice wine by applying rice wine onto hairless mice skin and suggested that rice wine is a potential anti-aging agent for the prevention and treatment of ultraviolet-induced skin aging because the topical application of rice wine improved skin barrier function and decreased skin wrinkling and epidermal thickening (78). In addition, Hirotsune et al. concluded that topical or oral ingestion of rice wine may have a positive effect on the skin (79). An increasing number of studies have found that rice wine may contain potential probiotic lactic acid bacteria (LAB) which can improve gastrointestinal health (25,80,81). Lactobacillus plantarum, Lactobacillus brevis, Lactobacillus paracasei, and Leuconostoc pseudomesenteroides are predominant LAB found in rice wines. According to Food Act 1983 (Act 281), section 26A(4), the probiotics shall remain viable and present in an adequate amount (viable probiotic count  $\geq 10^6$  CFU/mL or CFU/g) within the shelf life of probiotic rice wine to confer health benefits on the consumers (50). For instance, Yusmarini et al. produced tapai with total lactic acid bacteria ranging from  $10^8$ - $10^9$  CFU/mL and claimed it as a probiotic tapai (80).

Apart from that, rice wine has good solubility for various chemical compounds and good tissue penetration capability, thus it serves as a good organic solvent and is usually used as an ingredient in traditional Chinese medicine such as juhuanglian (*Rhizoma coptidis* steamed with rice wine) for treatment of diabetes (82). Rice wine can be used for steeping, boiling, and steaming herbs, or for making pills and medicated wine as it may improve drug efficacy and taste (83).

It is well acknowledged that rice wine is highly nutritious, provides health benefits, and improves drug efficacy and taste. However, excessive alcohol consumption may lead to health problems such as alcoholic liver disease and alcohol-induced pancreatitis (84). Therefore, any alcoholic beverages, including rice wine should be consumed in moderation. According to Dietary Guidelines for Americans 2015-2020, moderate alcohol consumption is defined as having up to one drink per day for women and up to two drinks per day for men, only by adults of legal drinking age (21 and above), where a standard drink is equal to 14.0g of ethanol (85).

### 6. Processing of Rice Wine

Rice wines are usually prepared under barely controlled and non-aseptic conditions in households or small-scale industries as an income source. Aside from starch substrates and starter cultures used, the processing of rice wine plays an important role in affecting the flavour and quality of rice wines.

#### 6.1. Preparation of Rice Wine

The preparation of rice wine varies among different regions, based on individual experiences and traditional practices, resulting in rice wines with different nutritional, biochemical, and organoleptic properties. However, the general method for rice wine preparation is similar. Generally, rice wines are produced through solid-state fermentation, where microorganisms grow in a solid matrix without a free-flowing aqueous phase, or/and submerged fermentation, where microorganisms grow in a liquid medium (86). Solid-state fermentation has gained increased attention in recent years probably due to the advantages it offers such as negligible waste produced, low capital investment, ease in handling, easy downstream processing, high yield, and high product concentration compared to submerged fermentation (87).

Rice wines such as tapai, ou, and Chinese sweet rice wine are produced through solidstate fermentation as follows: [1] clean, wash, and soak the rice; [2] cook the rice for starch gelatinization; [3] cool the cooked rice to approximately 30°C by spreading it over a clean surface; [4] sprinkle the grounded or powdered starter culture evenly onto the cooled rice; [5] mix all the ingredients thoroughly; [6] transfer the mixture into fermentation jar and seal it with the lid or a sheet of plastic to create an anaerobic condition for alcoholic fermentation (14,25,39,88). The mixture shall undergo fermentation for 21 days or more, depending on the manufacturing practices under room temperature to produce a matured rice wine with considerably good quality (25).

According to the local rice wine producers in Sabah, Malaysia, tajau (earthen jar) is traditionally used for the fermentation of tapai because the thick clay wall can facilitate temperature regulation in the jar. However, in the past decade, most rice winemakers preferred to use plastic containers as it is easier to clean.

On the other hand, rice wines such as sato, srapeang, and Chinese rice wine are produced through an initial solid-state fermentation as mentioned above for 2-3 days, followed by submerged fermentation, where the fermented rice is added with water to further ferment for 3-14 days or even longer depending on the manufacturing practices under room temperature (18,24,56,89).

The fermented product is then filtered using cheesecloth to collect the alcoholic liquid (rice wine) while the residual mash (rice wine lees) can be used for livestock feeding or as functional food ingredients (90,91). The rice wine can be pasteurized or sterilized before bottling to kill microorganisms and increase shelf life. However, the pasteurization and sterilization process may affect the organoleptic properties of rice wine (92,93).

### 6.2. Alcohol Production Process of Rice Wine

Rice wines are produced through simultaneous saccharification and fermentation (SSF), in which starch hydrolysis and alcoholic fermentation occur concurrently in the same vessel (38,51,94,95). Amylolytic starter cultures used to produce rice wines usually contain amylolytic fungi for starch hydrolysis and yeast for alcoholic fermentation. SSF is an alternative to separate hydrolysis and fermentation (SHF), where starch hydrolysis is carried out before alcoholic fermentation in two independent vessels (96).

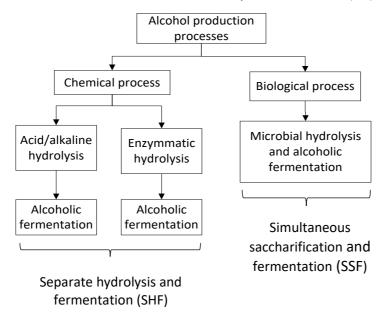


Figure 1. Overview of alcohol production processes (96).

One of the advantages of SSF is capital cost reduction because starch hydrolysis and fermentation are carried out in the same vessel (96). In SSF, sugars produced through

23

saccharification are immediately converted into ethanol by yeast, preventing sugar accumulation which may inhibit the amylolytic enzymes and yeast growth (51,97). Therefore, SSF improves starch hydrolysis and fermentation efficiencies, resulting in rice wines with relatively higher ethanol content compared to that of SHF. The rapid increase in ethanol content of rice wine produced through SSF may also reduce microbial contamination. However, the main disadvantage of SSF is its incompetency to independently optimize the enzymatic or microbial hydrolysis and alcoholic fermentation processes which vary in their optimum temperatures (98).

#### 6.2.1. Starch Hydrolysis

Starch is composed of amylose (linear polymers of glucose units, linked by  $\alpha$ -1,4-glycosidic bonds) and amylopectin (branched polymers composed of  $\alpha$ -1,4-linked glucose linear chains interconnected through  $\alpha$ -1,6-glycosidic bonds). It is the starting material for rice wine and needs to be converted into glucose before it can be utilized by yeast to produce ethanol (55). Hence, the production of rice wine involves an additional saccharification step before fermentation in contrast to fruit wines with saccharides (sucrose, fructose, and glucose) as the starting materials.

Starch can be hydrolysed into fermentable sugars through the chemical (acid or alkaline hydrolysis) or biological (enzymatic or microbial hydrolysis) process as illustrated in

Figure 1, while microbial hydrolysis is employed in rice winemaking. Microbial hydrolysis is specific, less expensive, and environment-friendly as it employs microorganisms that secrete amylolytic enzymes for the degradation of starch (98). Likewise, amylolytic microorganisms break down rice starch into glucose which can be utilized by ethanol-producing microorganisms to produce ethanol in rice wines.

Starch hydrolysis in the processing of rice wine involves gelatinization, liquefaction, and saccharification (99). Rice is cooked by heating with an excess of water before being inoculated with starter cultures for starch gelatinization to improve the availability of starch to amylolytic enzymes (24). The gelatinized starch is then liquefied and saccharified by amylolytic enzymes to release glucose for further fermentation. Table 6 summarized the reaction specificities of some amylolytic enzymes involved in the hydrolysis of rice starch for alcoholic fermentation.

Amylolytic enzymes	EC number	Cleavage sites (glycosidic bond)	End products
Endoamylases			
α-amylase	3.2.1.1	α-1,4	Glucose, maltose, maltotriose, α-limit dextrin, linear oligosaccharides
Exoamylases			
β-amylase	3.2.1.2	Second α-1,4	Maltose, β-limit dextrin
Glucoamylase	3.2.1.3	α-1,4 and α-1,6	Glucose
Debranching Enzymes			
Pullulanase type I	3.2.1.41	α-1,6	Maltotriose
Pullulanase type II	3.2.1.41	α-1,4 and α-1,6	Glucose, maltose, maltotriose
Isoamylase	3.2.1.68	α-1,6	Linear oligosaccharides

#### Table 6. Reaction specificities of amylolytic enzymes in starch hydrolysis (100).

Amylolytic enzymes can be categorized into [1] endoamylases ( $\alpha$ -amylase) which randomly cleave glycosidic bonds within the molecules; [2] exoamylases ( $\beta$ -amylase and glucoamylase) which degrade starch from the non-reducing ends of the chains; and [3] debranching enzymes (pullulanase and isoamylase). The main difference between the debranching enzymes and other amylases ( $\alpha$ -amylase,  $\beta$ -amylase and glucoamylase) is their greater affinity toward  $\alpha$ -1,6-glycosidic bonds and  $\alpha$ -1,4-glycosidic bonds, respectively (101).

Many existing studies in the broader literature have shown that fungi and bacteria can produce amylolytic enzymes for starch hydrolysis, demonstrating their importance in rice winemaking as they can hydrolyse rice starch and facilitate the fermentation process (100). Cai et al. isolated microorganisms from Chinese sweet rice wine starters from different parts of southern China and found several amylolytic fungal strains (*Rhizopus oryzae, Rhizopus microsporus, Aspergillus niger, Aspergillus candidus, Mucor indicus, Mucor circinelloides, Neurospora crassa*, and *Saccharomycopsis fibuligera*) and amylolytic *Bacillus strains* (*Bacillus subtilis, Bacillus amyloliquefaciens, Bacillus velezensis, Bacillus atrophaeus, Bacillus cereus, and Bacillus licheniformis*) (88). Furthermore, Limtong et al. isolated yeasts from Thai traditional fermentation starters (loog-pang) and showed that most of the yeasts revealed low amylolytic activity, while *Saccharomycopsis fibuligera* revealed relatively high amylolytic activity, proving its importance in converting starch to glucose for alcoholic fermentation (102).

#### 6.2.2. Alcoholic Fermentation

Glucose saccharified from starch is readily fermentable by yeast to form ethanol under anaerobic conditions. Glucose is converted into pyruvate through glycolysis via the Embden-Meyerhof-Parnas (EMP) pathway, while the pyruvate formed is converted into ethanol and carbon dioxide through alcoholic fermentation (98). The overall process is shown in Figure 2.

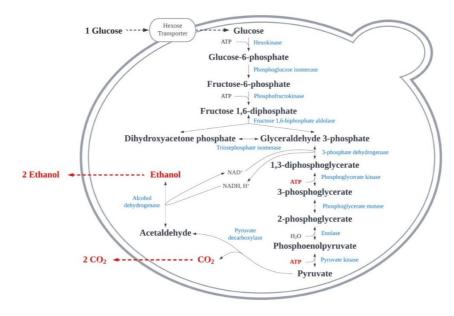


Figure 2. Glycolysis (Embden-Meyerhof-Parnas pathway) and ethanol fermentation steps in yeast (103,104).

First, glucose is transported across the plasma membrane of yeast cells by hexose transporter protein and converted into glucose-6-phosphate via hexokinase-catalysed phosphorylation with one molecule of adenosine triphosphate (ATP) as the phosphate donor. Next, glucose-6-phosphate is isomerized into fructose-6-phosphate by phosphoglucose isomerase and phosphorylated into fructose 1,6-diphosphate by phosphofructokinase. Fructose 1,6-diphosphate is then cleaved by fructose-1,6-bisphosphate aldolase into two triose phosphates, specifically glyceraldehyde-3-phosphate and dihydroxyacetone phosphate which are interconvertible by triosephosphate isomerase. Dihydroxyacetone phosphate can be converted into glycerol which may contribute to the sweetness and fullness of rice wine (18).

Meanwhile, glyceraldehyde-3-phosphate is converted into pyruvate for ethanol fermentation. Glyceraldehyde-3-phosphate is oxidized by NAD<sup>+</sup> (oxidized form of nicotinamide adenine dinucleotide) and phosphorylated by 3-phosphate dehydrogenase into 1,3-diphosphoglycerate which in turn, converted into 3-phosphoglycerate by phosphoglycerate kinase, yielding one molecule of ATP. The phosphate group in 3-phosphoglycerate is then relocated from C3 to C2 by phosphoglycerate mutase, resulting in 2-phosphoglycerate which is subsequently dehydrated by enolase to form phosphoenolpyruvate. The phosphoenolpyruvate is converted into pyruvate by pyruvate kinase and one molecule of ATP is yielded. Therefore, the glycolysis of one glucose molecule will give a net production of two pyruvate molecules and two ATP molecules.

Pyruvate is the precursor molecule for aerobic respiration (tricarboxylic acid (TCA) or Krebs cycle), alcoholic fermentation, and lactic acid fermentation (104). Under anaerobic conditions, yeast will carry out alcoholic fermentation, in which the pyruvate formed through glycolysis is decarboxylated by pyruvate decarboxylase into acetaldehyde and subsequently reduced by alcohol dehydrogenase to form ethanol. Overall, two molecules of each ethanol, carbon dioxide, and ATP are formed from one molecule of glucose through glycolysis and alcoholic fermentation (Glucose  $\rightarrow$  2 Ethanol + 2 CO<sub>2</sub> + 2 ATP).

Yeasts usually carry out alcoholic fermentation under anaerobic conditions and aerobic respiration in the presence of oxygen. However, high sugar concentration in the environment may inhibit aerobic metabolism and promotes alcoholic fermentation even in the presence of oxygen (104). This aerobic fermentation by yeast is known as the Crabtree effect. Verduyn et al. demonstrated the response of *Saccharomyces cerevisiae* towards different glucose concentrations under aerobic conditions and showed that there is no ethanol production when glucose concentration is below 150mg/L, whereas the rate of ethanol production was positively correlated to glucose concentration from 150- 1000mg/L, concluding that *Saccharomyces cerevisiae* is a Crabtree-positive yeast and exhibited Crabtree effect in glucose concentration above 150mg/L (105). In contrast, Crabtree-negative yeasts such as *Candida utilis* do not produce ethanol under aerobic conditions even in a high glucose environment (106). These yeasts carry out energy-efficient aerobic respiration in the presence of adequate oxygen and ferment mainly under anaerobic conditions.

Cai et al. isolated several ethanol-producing yeasts such as *Saccharomyces cerevisiae*, *Pichia burtonii*, and *Candida glabrata* from Chinese sweet rice wine starters from different parts of southern China (88). Besides that, Limtong et al. isolated yeasts from Thai traditional fermentation starters (loog-pang) and showed that *Saccharomyces cerevisiae*, *Pichia burtonii*, *Pichia anomala*, *Torulaspora globosa*, and *Issatchenkia orientalis* (also known as *Candida krusei* or *Pichia kudriavzevii*) produced a high concentration of ethanol, proving their importance in alcoholic fermentation (102). Whereas most isolates of *Saccharomycopsis fibuligera* produced a relatively low concentration of ethanol while *Rhodotorula philyla* and *Trichosporon asahii* could not ferment but might contribute to the flavour of rice wines.

Theoretically, amylolytic yeasts can be used for direct alcoholic fermentation of starch as they can saccharify starch and carry out alcoholic fermentation. However, the amylolytic yeasts which can efficiently hydrolyse starch are limited (97). This is probably the reason why traditional starters usually comprise a mixed culture of fungi and bacteria. Tsuyoshi et al. isolated yeasts from marcha or murcha (traditional amylolytic starter used to produce jaanr, a sweet and sour rice wine in Sikkim, India) and found that all marcha samples analysed contained both amylolytic and ethanol-producing yeasts (9). Most of the yeasts identified were ethanol-producing yeasts without amylolytic activity, where *Saccharomyces bayanus* showed the highest ethanol productivity and *Candida glabrata* showed moderate ethanol productivity. In contrast, *Saccharomycopsis fibuligera, Saccharomycopsis capsularis*, and *Pichia burtonii* isolated showed high amylolytic activities but produced no or negligible amounts of ethanol. Based on the collective data, yeasts can either hydrolyse starch or produce ethanol, but not both, efficiently. Therefore, both amylolytic and ethanolproducing fungi shall be employed for the efficient fermentation of rice wine.

#### 6.2.3. Factors Affecting the Fermentation Process

On top of starch substrates (3.1 above) and starter cultures (3.2 above) used, factors such as fermentation period, temperature, pH, and substrate concentration may affect the ethanol content of rice wines. Fermentation temperature affects the enzyme reaction rate in starch hydrolysis, yeast growth which influences ethanol production, and bacterial growth which influences the production of by-products such as organic acids (94).

According to Lin et al., yeast growth increased exponentially at the beginning of fermentation and eventually entered a stationary phase, while this exponential growth shortens when fermentation temperature is increased (107). Thus, the fermentation rate increases with fermentation temperature. However, high fermentation temperature (33°C) may accelerate cellular aging, while yeast growth was inhibited at 50°C due to denaturation of yeast proteins, resulting in decreased ethanol production (63,107). Fakruddin et al. found a similar trend where ethanol production by Saccharomyces cerevisiae increased when fermentation temperature increased from 25-30°C and decreased significantly at higher temperatures (33-35°C), indicating that the optimum fermentation temperature for Saccharomyces cerevisiae was 30°C (108). Nevertheless, Liu et al. obtained the highest ethanol production at 23°C using Saccharomyces cerevisiae and Chinese wheat qu (63); Zohri et al. concluded that the optimum temperature for ethanol production by Saccharomyces cerevisiae ranged between 30-40°C (109); while Lin et al. observed maximum yeast growth and ethanol production between 33-45°C (107). It is believed that the variation in optimum fermentation temperatures among several studies is due to the difference in yeast strains and microorganisms used.

Besides that, high fermentation temperature might cause excessive organic acid production, which can affect the flavour of rice wines (94). Liu et al. found that the concentrations of lactic acid, acetic acid, and tartaric acid in Chinese rice wine fermented at 33°C were significantly higher than those fermented at lower temperatures (18-28°C) (63).

This is because yeast and bacteria in the starter culture have different optimum growth temperatures. For instance, *Lactobacillus* spp. which produced lactic acid grew well at a higher temperature (33°C) while *Saccharomyces cerevisiae* usually grows optimally at 30°C. Therefore, rice wines fermented under higher temperatures are more likely to have lower ethanol contents and higher acid contents.

Moreover, pH can affect the fermentation process because microorganisms grow best at their optimum pH. According to Zohri et al., pH 4.5-5.5 is suitable for ethanol production by *Saccharomyces cerevisiae*, while ethanol production decreased with a further increase in pH because yeasts tend to produce acid under alkaline conditions (109). However, Lin et al. observed a decrease in ethanol production when pH was below 4.0 and above 5.0, suggesting that the optimum pH range for anaerobic ethanol fermentation is between 4.0-5.0 (107). The variation in optimum pH between studies is probably due to the difference in yeast strains tested.

Furthermore, ethanol production can be increased by increasing the substrate concentrations, whereas high sugar concentration will increase the osmotic pressure on yeast, which eventually inhibits yeast growth and ethanol production (107,109). However, glucose concentration in rice wine during simultaneous saccharification and fermentation (SSF) is maintained low as glucose produced through saccharification is immediately fermented by yeast, thus preventing the inhibitory effect of glucose on fermentation (51,97).

### 7. Quality and Safety Control of Rice Wine

Traditional rice wines are produced through spontaneous fermentation based on individual experiences and traditional practices in different regions under non-aseptic or barely controlled conditions. In recent years, there has been growing interest in the quality, safety, process optimization, and modern industrial development of rice wines (89). The traditional methods of rice wine processing were lack of standardization of raw materials and a properly controlled fermentation process, giving rise to problems such as inconsistent rice wine quality and food safety issues (23,24).

The variations in starter cultures used among rice wine producers lead to the production of rice wines with remarkably different biochemical and organoleptic properties (23). Researchers have isolated and identified microorganisms from traditional starter cultures (Table 3). A vast diversity of fungi and bacteria were found. It is believed that these microorganisms play an important role in the alcohol and aroma production in rice wines (6). Microorganisms present in traditional stater cultures varied in diversity and density, which in turn produce different metabolite compositions, resulting in flavour variation between rice wines of different batches (39).

Furthermore, stater cultures produced under insufficient quality control may contain undesirable and pathogenic microorganisms. More recent evidence showed that pathogenic species such as *Aspergillus nomius*, *Clostridium* sp., *Enterobacter* sp., *Escherichia coli*, *Fusarium culmorum*, *Penicillium georgiense*, and *Pseudomonas* sp. were identified from hong qu and bai qu or yao qu (traditional fermentation starters used for the production of hong qu glutinous rice wine) in China (6). Therefore, it is important to develop a defined starter culture for the consistent production of high-quality rice wines with desirable flavour and safe for consumption.

Over the last few years, the safety of rice wines is gaining increasing attention as deaths caused by consumption of methanol contaminated fermented alcoholic beverages have been reported globally. World Health Organization (WHO) urged to raise awareness on methanol poisoning by showing numerous methanol poisoning outbreaks in several countries (110). Methanol is a colourless liquid with an odour like ethanol, thus it was often added into alcoholic beverages by unethical producers to increase profit (111). Methanol is metabolized in the liver to methanoic acid or formic acid which has a lower elimination rate compared to ethanol, hence accumulates and causes toxic effects with symptoms such as dizziness, headache, nausea, abdominal pain, hyperventilation, blindness, convulsion, coma, and death (110). According to Regulation (EU) 2019/787 of the European Parliament and of the Council, the maximum level of methanol in 100% ethanol of agricultural origin is 0.3g/L (112). Methanol can be found in fermented alcoholic beverages in trace amounts but high methanol concentrations were found in illegally produced alcoholic beverages (113). Ohimain believed that pectinase-producing microorganisms inoculated through spontaneous fermentation were responsible for the production of methanol in traditional fermented alcoholic beverages (114).

Therefore, starter cultures with fixed microbial compositions can be formulated to produce rice wines with desirable ethanol content and consistent flavour as well as to minimize the production of methanol by contaminating microorganisms. However, current literature on the development of consistent starter culture for high-quality rice wine is inadequate. Although a growing body of literature has studied the microbial compositions of certain starter cultures, the microbial compositions of most starter cultures and their roles in rice wine fermentation such as sasad for tapai production in Sabah remained uncertain.

### 8. Conclusions

In conclusion, this paper has reviewed the key areas of rice wine fermentation. Rice wines in different regions have different local names and organoleptic properties, which greatly depend on the availability of raw materials, starter cultures, and the manufacturing process. The composition of rice and the microbial composition of starter cultures are the major factors affecting the yield, composition, and flavour of rice wines. The complexity of the rice wines' compositions, including the alcohol contents and a variety of organic and inorganic compounds, such as carbohydrates, proteins, organic acids, volatile compounds, vitamins, and minerals give rise to their distinct flavours and nutritional components. Rice wine is highly nutritious, provides health benefits, and improves drug efficacy and taste.

Rice wines are produced through simultaneous saccharification and fermentation (SSF) using amylolytic starter cultures containing both amylolytic and ethanol-producing fungi and bacteria. On top of starch substrates and starter cultures used, factors such as fermentation period, temperature, pH, and substrate concentration may affect the fermentation process and the final properties of rice wines.

These traditional rice wines are usually produced through spontaneous fermentation based on individual experiences and traditional practices in different regions under barely controlled conditions, giving rise to problems such as inconsistent rice wine quality and food safety issues. Therefore, future research on the development of starter cultures with fixed microbial compositions is vital for the consistent production of high-quality rice wines.

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# **Author Contributions**

M.K., H.Y.F. and C.M.V.L.W. conceptualized the content of this review paper; M.K. wrote the paper: C.M.V.L.W and H.Y.F. contributed to the article correction and publication.

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# **Conflicts of Interest**

The authors declare no conflict of interest.

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