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Original Article

Material Changes and Flavors Formation of Jiangxi Rice Wine during Fermentation

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Abstract:
Jiangxi rice wine is a traditional rice wine in China, which is rich in nutrients and greatly beneficial to health, while its flavors profiles and microbiota changes are unclear. We investigated the dynamics of microbial communities, physicochemical properties and flavor components during the fermentation of Jiangxi rice wine. The volatile compounds were detected by gas chromatography, and the bacterial community compositions were revealed by 16S sequencing. The results clarified that the core clades of rice wine during fermentation were *Proteobacteria* and *Firmicutes*. At the genus level, *Acinetobacter* was the dominant bacterium. A total of 62 volatile substances were assessed, esters and alcohols played key roles during the fermentation. Glutamic acid contributed the most to the taste of Jiangxi rice wine. While esters contribute significantly to the flavor. This study provides a guidance in the aroma and tastes regulation on Jiangxi rice wine fermentation.

Key words: Jiangxi rice wine, microbial communities, volatile compound, flavor, fermentation

Introduction

Rice wine is also known as wine brew, or mash, as one of the oldest alcoholic beverages in the world.(Jin et al., 2021) Because of pure flavor and rich nutrition, it is very popular to the Chinese people(Liang et al., 2020). Jiangxi rice wine is made from fermentation of Jiangxi glutinous rice with local microbiota, containing rich nutrients, including protein, fat, carbohydrates, calcium, iron, VB_1, VB_2, niacin, etc(L. Jiang, Su, Mu, & Mu, 2020). After the glutinous rice is steamed and pasted, large molecules such as starch, protein and fat are degraded into small molecules(T. Chen et al., 2020) such as glucose and other monosaccharides, oligosaccharides, peptides, amino acids and organic acids(Q. Xiong et al., 2022). These contribute to the unique nutritional and flavor characteristics of rice wine(G.-M. Chen et al., 2021).

The fermentation of traditional Jiangxi rice wine is a complex process, including soaking, cooking, cooling, inoculation, cultivation, saccharification and fermentation(Yijin Yang et al., 2023). The entire brewing process involves a range of strains of bacteria obtained from the brewery, the raw materials and the environment(Wang et al., 2014). Therefore, the brewing process of Jiangxi rice wine is associated with a large number of bacteria, which further influence the unique aroma, flavor and color of the rice wine.(C. Zhao, Su, Mu, Jiang, & Mu, 2020)

However, few studies focused on the microbiota succession during the fermentation of traditional Jiangxi rice wine. Recent studies showing that bacterial diversity is more abundant than fungi and plays a dominant role in fermentation(Lin et al., 2022; K. Xiong et al., 2021; Zhang et al., 2021). During the fermentation of traditional rice wine, Ren et al. revealed the changing pattern of bacteria in rice wine and predicted the relationship between metabolites and bacteria,(Ren et al., 2020) demonstrated that bacteria play a key role in the flavor formation of rice wine.

Flavor is composed of odor and flavor molecules of volatile and non-volatile compounds that play a critical role in food quality(Y. Chen et al., 2021). Headspace solid-phase micro extraction gas chromatography-mass spectrometry (HS-SPME-GC-MS) and gas chromatography-ion mobility spectrometry (GC-IMS) can accurately separate and characterize volatile compounds from complex samples.(Y. Chen et al., 2021; Guo, Schwab, Ho, Song, & Wan, 2021) And has been widely used in food testing such as tea(Y. Xiao et al., 2022), wine(S. Zhao et al., 2022) and fermented milk(A. Liu et al., 2022). Besides flavor, taste is a complex interaction with human senses, effected the quality of a rice wine product as well. It requires a variety of physical and chemical properties and sensory attributes to measure(Y. Yang et al., 2022).

For microbiota succession detection, high-throughput sequencing (HTS) can overcome the limitations of traditional molecular methods. Research has shown that HTS enables a more in-depth and accurate assessment of microbiota while reducing its cost and assessment time.(Lee et al., 2017) It has been widely used for the comprehensive analysis of microorganisms in various fermented foods, such as rice wine(Tian, Zeng, Zhou, & Du, 2022), traditionally homemade paocai and Chinese spicy cabbage(Z. Liu et al., 2019), tea(Jia et al., 2022), etc.

In this study, we monitored the dynamics of physicochemical properties, bacterial communities and volatile compounds during the fermentation of Jiangxi rice wine, discovering the correlation between core bacteria and volatile compounds.

2. Materials and Methods

2.1. Brewing and Collection of Jiangxi Rice Wine

Glutinous rice was purchased from a fresh market in Nanchang City, Jiangxi Province, China. Jiuqu was provided by Jiangxi Yaoshang Wine Co., Ltd. The ingredients were soaked and washed, then steamed. Then rinse the rice with water to make it cool down quickly to about 25℃，Then add 0.3% distiller's yeast and stir well, The rice is fermented for 5d after 48h of saccharification by rice cultivation bacteria. Then the lees were filtered and subjected to secondary fermentation for 48h（Figure.1）.

During the fermentation process, 10 ml of rice wine was randomly collected daily under aseptic conditions for DNA extraction and HTS. In addition, 500 mL of rice wine and less were collected at the same time point under aseptic conditions to identify the physicochemical properties and flavor components. After collection, all samples were transported to the laboratory for storage at -80°C until analysis.(Y. Jiang et al., 2019).

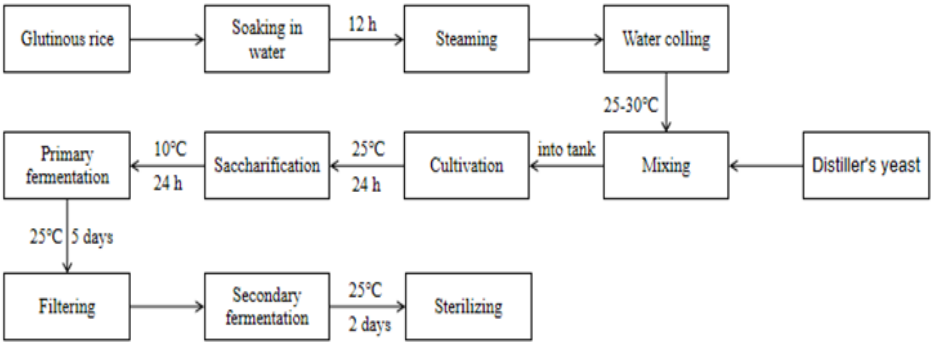

Figure. 1 Process of jiangxi rice wine

2.2. Physical and chemical property analysis

The physicochemical properties include pH, total acid, amino acid nitrogen, total sugar, alcohol and starch, crude protein and crude fat of the lees of rice wine.

The pH of rice wine samples was measured with a pH meter(PHS-3C; Fangzhou Technology, China);Total acidity (in terms of lactic acid) and amino nitrogen content were simultaneously determined by titration using standard NaOH solution (C = 0.1 mol/L) (GB/T 13662-2018). Total sugars were determined by arbitration law (GB/T 13662-2018). Alcohol degree was determined by distilling rice wine and got through the alcohol meter (GB 5009.225-2016).Starch was determined by acid hydrolysis method (GB 5009.9-2016).Crude fat was determined by Soxhlet extraction method (GB 5009.6-2016). Crude protein was determined by Kjeldahl nitrogen determination method (GB 5009.5-2016).

2.3 Free Amino acid Analysis

The extraction and measurements of Free amino acid (FAA) followed the methods described by Xiao et al(M. Xiao et al., 2022)，The samples were pre-treated by sulfosalicylic acid and filtered through a 0.45 μm membrane, The processed sample was injected into L-8900 automatic amino acid analyzer (Hitachi, Tokyo, Japan). The content of FAAs in rice wine samples was calculated by calibration with standard amino acids.

2.4 HS-SPME for Volatile Compounds

The samples were first transferred to a 40 ml headspace vial, the tube was sealed with a screw cap equipped with Teflon/silicone septum and incubated in a water bath at 60 °C. A conditioned HS-SPME fiber coated with divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS, 50/30 μm) was maintained 2 cm above the liquid, and exposed to the headspace for 40 min. After the extraction, the fiber was inserted into the injection port of the GC–MS and desorbed at 250 °C for 5 min.(Yu, Xie, Xie, Ai, & Tian, 2019)

2.5 GC–MS Analysis

GC–MS analysis was performed on an Agilent 7890B-7000D gas chromatograph, equipped with an Agilent 5975 mass spectrometer (Agilent Technologies, Santa Clara, USA). Helium was used as a carrier gas at a constant flow rate of 1.5 mL/min. The HS-SPME sample, chemical desorption was carried out in the GC injector at 250 °C for 5 min; the HS-SPME samples were loaded in splitless mode and analyzed on an Agilent VF-Waxms column (60 m × 0.25 mm × 0.5μm). The oven temperature program was started at 50 °C, and maintained for 4 min, Then raised to 150 °C at 6 °C/min, Continue for 6 min, and finally ramped to 250 °C at 9 °C/min and held for 6 min. The MS was operated in an electron ionization (EI) mode at 70 eV. The mass scan range was set from 40 to 550 m/z. The volatiles were identified by comparing their mass spectra with MS spectra libraries (NIST05.L, wiley7n.L, and W8N08.L) and the aforementioned authentic standards(C. Zhao et al., 2020).

2.6 Gas Chromatography-Ion Mobility Spectrometry (GC-IMS) Analysis

The GC-IMS analysis system was a FlavourSpec® system (Gesellschaft für Analytische Sensorsy stemembH, Dortmund, Germany) equipped with an autosampler unit (CTC Analytics AG, Zwingen, Switzerland). MXT-WAX chromatographic column, with a length of 30 m and an inner diameter of 0.53 mm and provided by G.A.S was used in the system. A

1 ml sample was placed in a 20 ml headspace glass sampling vial and incubated at 60℃ for 10 min. After incubation, 100 µL of the headspace sample was automatically injected into the sampler through an 85℃ injection needle. The sample was passed through the MXT-WAX column under the presence of nitrogen. The carrier gas flowed according to the following program: 2 ml/min for 0-2 min, 10 ml/min for 2-10 min, and 100 ml/min for 10-40 min. Analytes were eluted and separated at 45℃ Then ionized in an IMS ionization chamber by a 3H ionization source (300 MBq activity) in positive ion mode. 9.8 cm drift tubes were run at a constant voltage (5 kV) at 45℃ with a nitrogen flow of 150 ml/min. The retention index (RI) of the volatile compounds was calculated using N-ketones C4-C9 (Sinopharm Chemical Reagent Beijing Co., Ltd., Beijing, China) as external references. The National Institute of Standards and Technology (NIST) database and IMS database built-in GC × IMS Library Search software were used to characterize the volatile compounds based on RI and the drift time (DT)(Y. Chen et al., 2021).

2.7 Microbial Analysis

Total genomic DNA was extracted using HiPure Soil DNA Kit B (Magen, Guangzhou, China) according to the manufacturer’s protocol. DNA was also quantified by Nanodrop (Thermo Scientific, USA) and the quality of DNA extraction was detected by 1.2% agarose gel electrophoresis. After polymerase chain reaction amplification, purification and fluorescence quantification of amplified products. Sequencing libraries were prepared using Illumina's TruSeq Nano DNA LT Library Prep Kit.T. Sequencing of 16S rDNA in the 16S V3-V4 region on the NovaSeq 6000 (Illumina, San Diego, CA, USA) by Personal Biotechnology Co., Ltd. (Shanghai, China). All three independent replicates were analyzed.

2.8 Statistical Analysis

Physicochemical measurements were measured in triplicate and expressed as mean values ± standard deviation (SPSS statistics 26). Analysis of variance (ANOVA) with Duncan’s multiple comparison was used to assess the significance of sample effects ($p < 0.05$).

3. Results and discussion

3.1. Physicochemical properties

The levels of total sugar, total acid, pH, alcohol and amino acid nitrogen during fermentation are important quality parameters that affect by microbial growth and accumulation of microbial metabolites(Wu et al., 2015). These are often used to determine the state and extent of fermentation and can also reflect the state of the microorganisms in the fermentation mash(C. Zhao et al., 2020).

As shown in Table 1, during the brewing period, the pH of Jiangxi rice wine decreased and finally remained stable, which is consistent with the results of previous study(Yijin Yang et al., 2018). The amino acid nitrogen content increased at first, it decreased in the secondary fermentation stage, due to the nutrients being consumed by microorganisms after the lees were filtered. The total sugar content of the rice wine decreased significantly after the 4th day, from a maximum of 136.51 ± 4.34 g/L to 65.01 ± 1.11 g/L. The yeasts in the fermentation mash reproduce and grow through the utilization of fermentable sugars, causing the total sugar content decreased rapidly(C. Zhao et al., 2023).

Table 1 Changes in physicochemical properties during Jiangxi rice wine fermentation.

sample	pH	Total acid (g/L)	Total sugar (g/L)	Alcohol content (v/v, %)	Amino acid nitrogen (g/L)
RW 0	3.48±0.01	2.09±0.07	46.91±1.30	1.37±0.06	0.09±0.00
RW 1	3.44±0.01	2.54±0.03	62.32±1.36	1.87±0.12	0.09±0.00
RW 2	3.40±0.02	3.18±0.05	95.20±0.61	2.67±0.06	0.14±0.01
RW 3	3.38±0.01	3.48±0.05	136.51±4.34	3.30±0.10	0.16±0.00
RW 4	3.35±0.01	3.78±0.09	100.00±2.08	4.67±0.23	0.19±0.01
RW 5	3.33±0.01	4.26±0.10	91.43±2.61	6.27±0.12	0.21±0.00
RW 6	3.31±0.01	4.35±0.05	78.47±1.47	9.68±0.23	0.15±0.00
RW 7	3.31±0.01	4.38±0.05	65.01±1.11	11.92±0.14	0.16±0.00

Values are presented as mean ± standard error (n = 3).

Similarly, the amounts of lees nutrients can reflect the microbial growth and metabolism, which is one of the important quality parameters in the fermentation process. As shown in Table 2, the starch content in the lees first decreased rapidly during the brewing process, then slowed down on the 5th day and finally stabilized; the protein content decreased on the 2nd day, then gradually increased and then stabilized; the fat content rose to the highest value on the 4th day. These indicate that during the fermentation process of Jiangxi rice wine, microbial growth is metabolically intense in the early stages and consumes mainly starch, and stabilizes in the later stages of fermentation.

Table 2 Nutrients in lees during the brewing process of Jiangxi rice wine

Samples	Starch content (%)	Protein content (%)	Fat content (%)
WL 0	78.75±2.09	8.31±0.03	0.82±0.02
WL 1	72.03±0.68	7.72±0.02	0.76±0.01
WL 2	54.44±1.00	13.97±0.00	0.86±0.00
WL 3	32.63±0.23	23.91±0.00	2.24±0.06
WL 4	19.87±0.31	24.20±0.03	2.86±0.10
WL 5	15.50±0.10	23.33±0.10	1.83±0.12
WL 6	12.55±0.11	27.58±0.06	1.92±0.01
WL 7	11.13±0.13	27.23±0.02	2.07±0.05

Values are presented as mean ± standard error (n = 3).

3.2. Changes in FAAs

The contents of free amino acids (FAAs) can influence the taste of certain foods directly and contribute to their unique flavor(C. J. Zhao, Schieber, & Ganzle, 2016). Here, 17 FAAs were quantified in rice wine fermentation, include seven essential FAAs (Thr, Val, Met, Ile, Leu, Phe and Lys). As shown in Figure 2, during the fermentation process of rice wine, the content of total FAAs in rice wine first increases and then decreases. The initial concentration of total free FAAs was 332.32mg/kg, which gradually increased and reached a maximum value of 727.51mg/kg on the fifth day. Thereafter, the concentration of FAAs gradually decreased to 531.87mg/kg at the end of fermentation. The overall decreasing trend of FAAs was also reported by Rao et al(Rao et al., 2020) in traditional fermented foods. This is because microorganisms convert FAAs into smaller flavor molecules(A.-j. Chen et al., 2019).

These 17 FAAs can be categorized into umami, sweet, and bitter FAAs groups according to previous researches(Y. Yang et al., 2022). In Figure 2, it can be seen that during the fermentation, the content of bitter FAAs is the highest on(H. Gao et al., 2021). The content of bitter FAAs is higher than the total amount of sweet and umami FAAs, which plays an important role in the unique and delicious taste of fermented foods(A.-j. Chen et al., 2019). The coexistence of bitter FAAs and Glu can have a synergistic effect on taste(Fuke & Shimizu, 1993), and flavoring activity of bitter FAAs can be masked by sweet and umami substances. In fact, the total content of umami and sweet amino acids increased from 58.03 mg/kg to 91.52 mg/kg after fermentation, while the bitter amino acids increased from 332.32 mg/kg to 531.87 mg/kg (shown in Table S1)

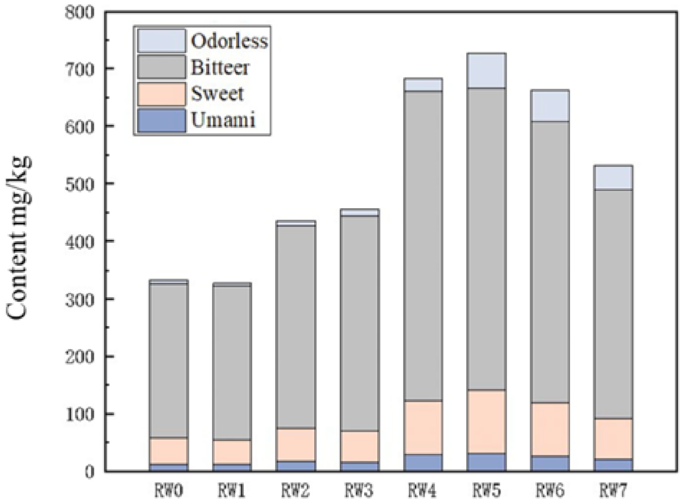

Figure. 2 Compositions of FAAs during the fermentation of Jiangxi rice wine in groups of umami, sweet, bitter, and odorless amino acid

3.3. Bacterial Diversity

After quality control, a total of approximately 583425 high-quality bacterial tags were obtained in our study. The biodiversity richness, biodiversity index, and Good's coverage are listed in Table 3. These results showed that the richness and diversity levels of the bacterial communities fluctuated during Jiangxi rice wine fermentation. According to the α diversity analysis, in RW4 the abundance of Observe species were highest, Good's coverage and rarefaction curves are indicators of sample integrity. Good's coverage was equal to 100%, and the rarefaction curve (Figure.3) results indicated that the samples exhibited sufficient bacterial diversity.

Table 3 The α diversity analysis

Sample	Chao1	Good's coverage	Observed species	Shannon	Simpson
DY1	214.53	100%	211.50	4.74	0.93
RW0	335.77	100%	310.40	3.73	0.82
RW1	308.72	100%	291.60	3.59	0.81
RW2	411.57	100%	381.60	3.78	0.83
RW3	397.61	100%	369.80	3.90	0.85
RW4	531.44	100%	494.30	4.39	0.87
RW5	456.77	100%	425.20	4.26	0.87
RW6	396.30	100%	364.60	4.34	0.90
RW7	451.27	100%	431.70	4.74	0.91

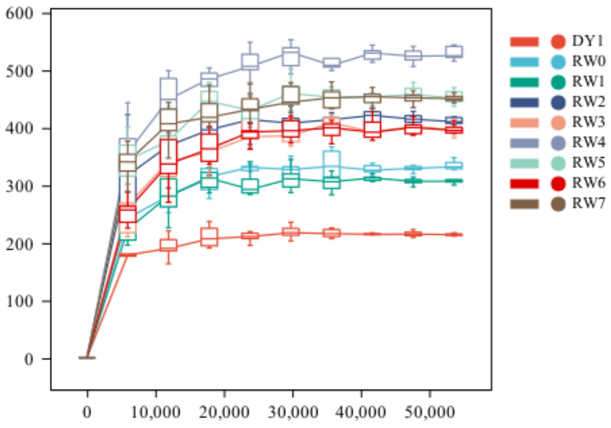

Figure. 3 Rarefaction curve analysis of the sequences of Jiangxi rice wine and Jiuqu samples.

3.4. Bacterial population and dynamics

Next, we categorize the sequencing data at the phylum and genus levels to investigate community succession during fermentation. A total of 12 phyla and 181 genera were obtained from the samples. The relative abundance of the bacterial communities at the phylum and genus levels is shown in Fig 4 A and Fig 4 B. The core phylum was *Proteobacteria*, followed by *Firmicutes*. During the fermentation of Jiangxi rice wine, *Proteobacteria* and *Firmicutes* together accounted for more than 98% of the total bacterial flora abundance in rice wine samples, consistent with previous findings(Li et al., 2020). *Proteobacteria* dominated the early stages of fermentation, while their abundances decreased as that of *Firmicutes* increased during fermentation. *Firmicutes* can produce carbohydrate-active enzymes and promote carbohydrate hydrolysis(El Kaoutari, Armougom, Gordon, Raoult, & Henrissat, 2013). Therefore, the abundance of *Firmicutes* is high in the early stage of rice wine fermentation and decreases in the late stage of rice wine fermentation due to the decrease of raw material starch content and the increase of *Proteobacteria* activity.

At the genus level, the top ten core genera in abundance were *Acinetobacter*, *Bacillu*, *Enterobacter*, *Pantoea*, *Pseudomonas*, *Enhydrobacter*, *Leuconostoc*, *Kluyvera*, *Staphylococcus*, and *Cronobacter*. The variation of these 10 genera of bacteria during the fermentation of the wine mash and rice wine is shown in Figure 4 B. During the fermentation of rice wine, *Acinetobacter* had the highest abundance (41.82%~65.82%) and was the dominant bacterium. It had a trend of increasing and then decreasing during the fermentation process, which may be due to the increase in alcohol content at the later stage of fermentation having a certain inhibitory effect on the bacterial growth of this genus. *Bacillus*, as a functional microorganism, can secrete a large amount of hydrolytic enzymes to promote flavor formation in rice wine. Its abundance increased during the cultivation and saccharification stages, and then showed an overall decreasing trend. This may be due to the fact that this group of bacteria is mostly aerobic, and the anoxic conditions of post-fermentation inhibited its growth, resulting in a decreasing trend of its abundance. Bokulich concluded that the oxygen content was the main factor affecting *Bacillus* when he studied the microbial changes in sake(Bokulich, Ohta, Lee, & Mills, 2014). Moreover, higher alcohol content also inhibits the growth of Bacillus, leading to a decrease in its abundance during the post-fermentation stage of rice wine(S. P. Liu et al., 2015).

Leuconostoc belongs to the order *Lactobacillus* and has a tendency to decrease and then increase during the fermentation process. Most of the bacteria in this genus are parthenogenic anaerobes, capable of secreting lactic acid, making them tolerant of acidic environments and suitable for growth under low oxygen pressure. *Enhydrobacter* showed an overall decreasing trend in relative abundance during fermentation. *Pseudomonas* showed an overall increasing trend in abundance during the fermentation process and decreased in abundance until the post-fermentation stage, but then increased again. *Enterobacter* and *Pantoea* showed an overall increasing trend throughout the fermentation process, and the relative abundance of *Enterobacter* reached 21.26% at the end of fermentation. These results were also supported by a genus heatmap (Fig. 4 C) plotted according to OTU levels(G.-M. Chen et al., 2021).

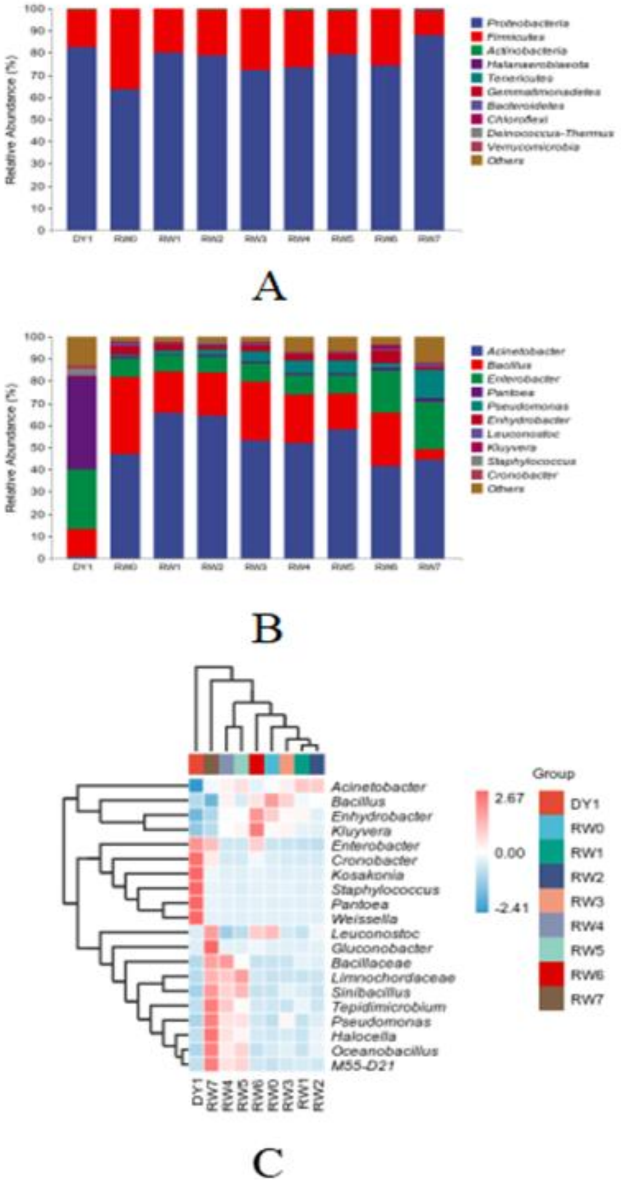

Figure. 4 The relative abundances of phyla (A) and genera (B) and a genus heatmap (C) of different samples

3.5. Dynamics of Volatile Flavor Compounds

HS-SPME-GC-MS was used to detect volatile flavor substances in rice wine samples during fermentation.

A total of 62 volatile flavor substances were detected (Table S2). They are mainly composed of esters, alcohols, acids, aldehydes, ketones, alkanes, olefins and other compounds. The types and relative contents of compounds in the rice wine samples varied at different fermentation times, and the result was shown in Figure 5. The volatile fractions were low at the beginning of fermentation, with only 14 flavor substances detected, and slowly increased as fermentation progressed. The two volatile flavor substances with the highest relative content in rice wine throughout the fermentation process were alcohols and esters, and the flavor substances in rice wine changed dynamically during the fermentation period.

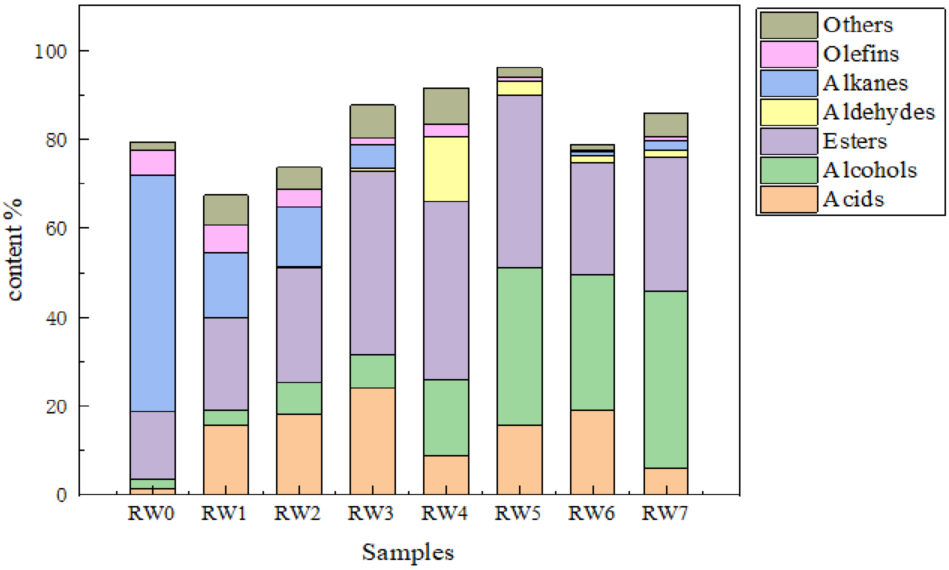

Figure. 5 Composition of volatile flavor substances during fermentation of Jiangxi rice wine

Esters are probably the most important flavor compounds in wine. Most esters have floral and fruity aromas that can increase the complexity and typicality of the wine aroma and play a very important role in the flavor of the wine(Ebeler & Thorngate, 2009). A total of 23 esters were detected in the rice wine samples

of this experiment, among which ethyl caprylate, phenethyl acetate, ethyl decanoate, ethyl laurate, ethyl isochorismate, ethyl myristate, ethyl 9-hexadecenoate, ethyl palmitate, ethyl oleate, and ethyl linoleate were the major esters. The contents of ethyl caprylate, ethyl oleate and ethyl linoleate showed an overall increasing trend during the fermentation process and reached the highest level at the end of fermentation. Phenethyl acetate, ethyl caprate, ethyl laurate and ethyl 9-hexadecenoate were found only at the end of fermentation, indicating that these flavor substances were mainly formed at the end of fermentation by yeast metabolism and esterification reactions. Ethyl isocholate was highest at the beginning of fermentation and showed an overall decreasing trend as fermentation progressed, while the relative levels of ethyl myristate and ethyl palmitate increased and then decreased during fermentation. Probably due to the fact that the rate of total volatile flavor substances produced in the later stage of fermentation was greater than those of these esters.

Alcohols are the most important aroma substances in wine, mainly presenting fruit and floral aromas. The appropriate concentration of these substances can release the ester aroma and promote the coordination of aroma, which are important aroma substances in wine. Phenylethyl alcohol is the main alcohol in Jiangxi rice wine during fermentation, and its relative content increases all the time.

Some other important compounds (aldehydes, ketones, olefins) are also important for the flavor of rice wine. Benzaldehyde and phenylacetaldehyde are important volatile compounds. Both of them are produced after a period of rice wine fermentation, and their relative content tends to increase and then decrease during the fermentation process. Benzaldehyde has a bitter almond flavor, and Genovese et al. concluded that benzyl alcohol can be oxidized to produce benzaldehyde(Genovese, Gambuti, Piombino, & Moio, 2007). Some studies have shown that aldehydes and ketones in fermented wine are mainly due to oxidation of linoleic acid by yeast.

3.6. GC-IMS analysis of volatile flavor substances in rice wine

GC-IMS is a detection technology that combines ion mobility spectroscopy and gas chromatography. Results showed that 31 typical volatile compounds were identified in the GC-IMS library, including 11 esters, 6 alcohols, 1 acid, 8 aldehydes, 3 ketones, and 2 ethers. (Table 4). Fig. 6 is GC-IMS spectra of volatile flavor substances and gallery Plot of volatile flavor substances in Jiangxi rice wine.

Table 4 Qualitative analysis of volatile flavor compounds in in Jiangxi rice wine

Count	Compound	CAS#	Formula	MW	RI	Rt [sec]	Dt [a.u.]	Content
1	Decanoic acid ethyl ester	C110383	C12H24O2	200.3	1608.3	2164.309	1.61502	1.13
2	Benzaldehyde	C100527	C7H6O	106.1	1476.1	1309.42	1.15345	0.94
3	Acetic acid(M)	C64197	C2H4O2	60.1	1450.5	1187.826	1.04951	7.51
4	Acetic acid(D)	C64197	C2H4O2	60.1	1449.9	1185.063	1.15035	1.26
5	Ethyl octanoate	C106321	C10H20O2	172.3	1427.8	1089.308	1.48074	1.23
6	3-Methylbutan-1-ol	C123513	C5H12O	88.1	1212.9	676.663	1.48992	16.88
7	Ethyl hexanoate(M)	C123660	C8H16O2	144.2	1242.9	716.974	1.34239	0.78
8	Ethyl hexanoate(D)	C123660	C8H16O2	144.2	1240.3	713.356	1.80485	0.67
9	2-Methyl-1-propanol	C78831	C4H10O	74.1	1099.7	483.618	1.36325	13.86
10	1-Propanol(M)	C71238	C3H8O	60.1	1048.4	420.83	1.25534	3.03
11	1-Propanol(D)	C71238	C3H8O	60.1	1048.4	420.83	1.38323	1.28
12	Butanoic acid ethyl ester	C105544	C6H12O2	116.2	1048.8	421.348	1.55863	2.10
13	2-Methyl-1-propyl acetate	C110190	C6H12O2	116.2	1026.4	396.754	1.61435	1.49
14	Acetic acid propyl ester	C109604	C5H10O2	102.1	991.5	362.323	1.48372	0.87
15	Ethyl 2-methyl propionate	C97621	C6H12O2	116.2	980.0	353.78	1.56411	0.06
16	Ethyl propanoate	C105373	C5H10O2	102.1	973.6	349.121	1.45266	1.87
17	3-Methyl butanal	C590863	C5H10O	86.1	931.7	320.126	1.40698	0.28
18	Acetic acid ethyl ester	C141786	C4H8O2	88.1	901.5	300.71	1.34304	12.83
19	Butanal	C123728	C4H8O	72.1	919.8	312.36	1.29188	1.24
20	2-Butanone	C78933	C4H8O	72.1	919.3	312.053	1.24435	0.17
21	Dimethyl sulfide	C75183	C2H6S	62.1	793.1	240.292	0.95702	0.69
22	Acetaldehyde	C75070	C2H4O	44.1	763.1	225.816	0.95741	0.04
23	Acetone	C67641	C3H6O	58.1	850.2	270.449	1.11433	2.63
24	Propanal(M)	C123386	C3H6O	58.1	835.1	262.119	1.07018	0.73
25	Propanal(D)	C123386	C3H6O	58.1	835.9	262.525	1.14622	0.56
26	2-Methyl propanal	C78842	C4H8O	72.1	845.9	268.011	1.28603	0.48
27	1-Pentanal	C110623	C5H10O	86.1	997.2	366.849	1.42725	0.10
28	Ethanol	C64175	C2H6O	46.1	942.1	327.119	1.13817	11.93
29	Tetrahydrofuran(M)	C109999	C4H8O	72.1	857.3	274.441	1.06025	0.09
30	Tetrahydrofuran(D)	C109999	C4H8O	72.1	856.7	274.066	1.22702	0.05
31	Ethyl 2-hydroxypropanoate	C97643	C5H10O3	118.1	1353.9	891.122	1.14348	0.30
32	3-Methyl-3-buten-1-ol	C763326	C5H10O	86.1	1258.3	738.632	1.17154	0.08
33	3-Hydroxy-2-butanone(M)	C513860	C4H8O2	88.1	1292.4	788.831	1.06302	0.49
34	3-Hydroxy-2-butanone(D)	C513860	C4H8O2	88.1	1292.1	788.357	1.33058	0.41
35	1-Butanol(M)	C71363	C4H10O	74.1	1155.5	578.163	1.1771	0.26
36	1-Butanol(D)	C71363	C4H10O	74.1	1153.9	575.172	1.37809	0.17
37	Isoamyl acetate(M)	C123922	C7H14O2	130.2	1137.2	545.259	1.30722	1.09
38	Isoamyl acetate(D)	C123922	C7H14O2	130.2	1135.4	542.111	1.75485	7.75
39	1-Octanal	C124130	C8H16O	128.2	1291.4	787.287	1.39897	0.33

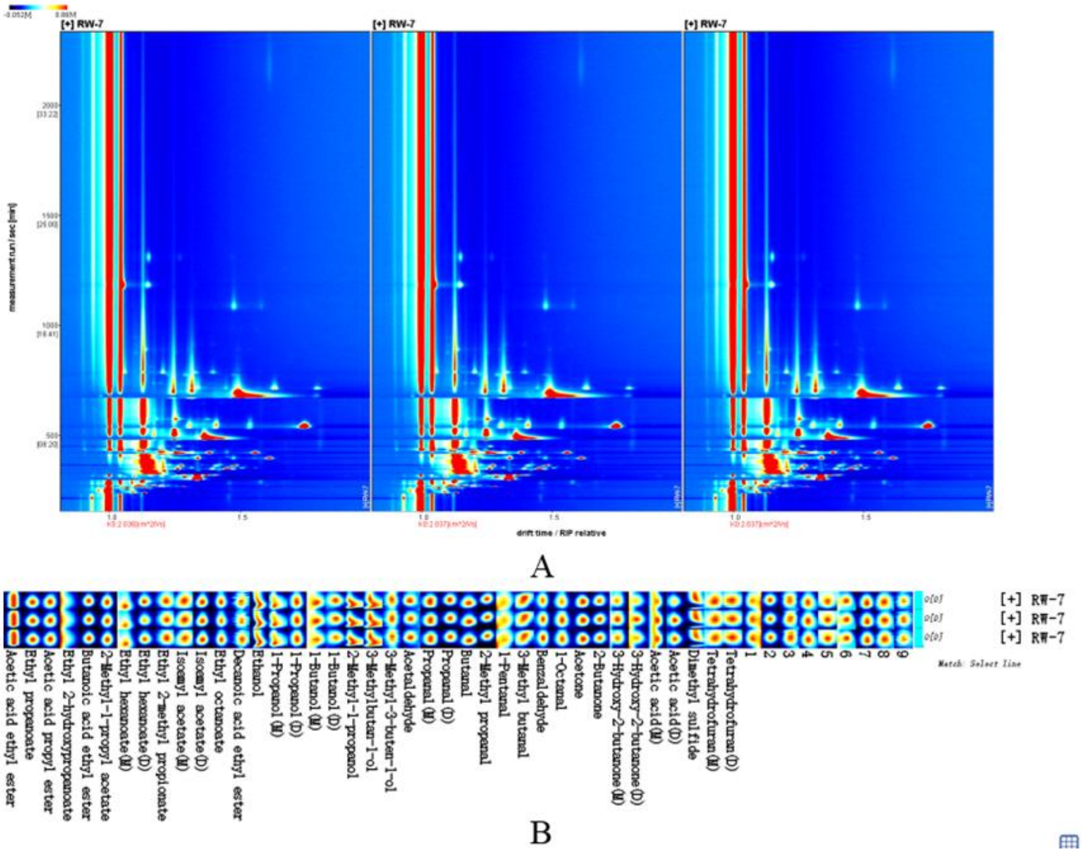

Figure 6 GC-IMS spectra of volatile flavor substances (A) and gallery Plot of volatile flavor substances (B) in Jiangxi rice wine

Volatile flavor substances have an important influence on the sensory characteristics of wine and are an important component of wine quality. The ROAV value of volatile flavor substances was used to further illustrate the magnitude of their contribution to the overall aroma of rice wine. Substances with ROAV ≥ 1 were regarded as characteristic volatile substances of the wine; substances with 0.1 ≤ ROAV < 1 had a modifying effect on the overall flavor, and the larger the ROAV, the greater the contribution of the substance to the overall flavor.

In Table 5, ROAV was calculated based on the threshold values of reported volatile flavor substances and their characterization(W. Gao, Fan, & Xu, 2014; Gu, Zhang, Wang, Wang, & Du, 2021; He et al., 2022; Ma, Gao, Chen, & Meng, 2020; D. Zhao et al., 2018). Nine volatile components were found in rice wine with great contribution to the body (ROAV ≥ 1), namely ethyl isobutyrate, isobutyl acetate, 3-hydroxy-2-butanone, propionaldehyde, ethyl butyrate, 3-methylbutyraldehyde, ethyl caproate, isoamyl acetate, and ethyl caprylate. Other nine volatile components were found in rice wine with modifying effect on the flavor of the wine (ROAV ≥ 0.1), namely ethyl propionate, 1-butanol, 1-pentanal, benzaldehyde, propyl acetate, isobutanol, ethyl acetate, butyraldehyde, and ethyl decanoate.

Table 5 ROAV of volatile components in Jiangxi rice wine

No.	Aroma active compound	Formula	Odor threshold（μg/L）	Odor description	ROAV values
1	Ethyl propionate	$C_5H_{10}O_2$	19019.33	Sweet, floral	0.1
2	1-Butanol	$C_4H_{10}O$	2730	Fruity	0.16
3	1-Pentanal	$C_5H_{10}O$	500	Malt, grass	0.22
4	Benzaldehyde	C_7H_6O	4203	Nutty, woody	0.25
5	Propyl acetate	$C_5H_{10}O_2$	2700	Sweet ,fruity	0.34

6	Isobutanol	$C_4H_{10}O$	40000	Malt, fruity	0.36
7	Ethyl acetate	$C_4H_8O_2$	32600	Grass, floral	0.41
8	Butyraldehyde	C_4H_8O	2900	Sweet	0.45
9	Ethyl decanoate	$C_{12}H_{24}O_2$	1122.3	Grape	0.99
10	Ethyl isobutyrate	$C_6H_{12}O_2$	57.47	Sweet, fruity	1.23
11	Isobutyl acetate	$C_6H_{12}O_2$	922	Sweet, apple	1.71
12	3-Hydroxy-2-butanone	$C_4H_8O_2$	259	Floral, fruity	3.76
13	Propanal	C_3H_6O	200	Grass, fruity	6.68
14	Ethyl butyrate	$C_6H_{12}O_2$	206	Fruity, floral	10.71
15	3-Methyl butanal	$C_5H_{10}O$	16.51	Grass	17.17
16	Ethyl caproate	$C_8H_{16}O_2$	55	Banana, fruity	27.79
17	Isopentyl acetate	$C_7H_{14}O_2$	93.93	Banana, fruity	99.07
18	Ethyl caprylate	$C_{10}H_{20}O_2$	13	Pineapple	100

4. Conclusion

During the fermentation of Jiangxi rice wine, the core bacterial communities of rice wine during fermentation are *Proteobacteria* and *Firmicutes*. At genus level, *Acinetobacter* is the dominant bacterium, had the greatest abundance. Glutamic acid, alanine, valine, arginine and histidine contributed the most to the taste of Jiangxi rice wine. While esters, such as ethyl caproate, isoamyl acetate and ethyl caprylate contribute significantly to the flavor.

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Declaration of interest statement

This is the first submission of this manuscript and no parts of this manuscript are being considered for publication elsewhere. All authors have approved this manuscript. No author has financial or other contractual agreements that might cause conflicts of interest.

Data availability statement

The data is authentic and reliable (no applicable).

Disclosure Statement

The authors report there are no competing interests to declare

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Note: Supplymentary Mterials