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Dynamic changes of microbial community structure and flavor compounds formation during Qingzhi compound-flavor Baijiu fermentation

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Abstract

A Qingzhi compound-flavor Baijiu (QZCF Baijiu) was developed and the physiochemical factors and microbial community structure in Jiupei (fermented grains) during Baijiu fermentation were revealed. Physiochemical factors of ethanol, moisture and acidity significantly increased in the fermentation, while starch and reducing sugar decreased. The results of high-throughput sequencing showed that *Weissella*, *Pediococcus*, *Bacillus*, *Pantoea*, *Streptococcus*, *Sphingobacterium*, *Saccharomyces*, *Rhizopus*, *Issatchenkia* and *Wickerhamomyces* were the dominant microorganisms in Jiupei. Moreover, correlation analysis between the physicochemical factors, the dominant microorganisms and the important flavor compounds in QZCF Baijiu production highlighted that the changes of physiochemical factors would lead to the corresponding changes of core microbiota and important flavor substances. Physiochemical factors of starch, reducing sugar and temperature could significantly affect the microbial community structure at the early stage of fermentation, while the influences of ethanol content and acidity became greater at the later stage, resulting in the differences of flavor compounds in Jiupei. Therefore, the growth and metabolism of microbial communities could be regulated by purposefully adjusting the physiochemical factors, thereby improving the flavor and quality of Baijiu. This work enriched the flavor types of Baijiu and provided a reference for the Baijiu-making industries to manage their production process.

Keywords: qingzhi compound-flavor baijiu; physicochemical factors; microbial community structure; correlation analysis.

Practical Application: Deep understanding of the relationship between the physicochemical factors, the dominant microorganisms and the important flavor compounds is the basis of monitoring and optimizing the fermentative condition in Qingzhi compound-flavor Baijiu making and therefore, help to guarantee the quality and quantity of Baijiu.

1 Introduction

Baijiu is a unique liquor in China with thousands of years history, and it is also one of the most consumed distilled spirits in the world (Sakandar et al., 2020). In 2019, Baijiu production in China reached 78.6 million liters, with sales exceeding 87.8 billion dollars (Wang et al., 2022). As the continuous improvement of consumers' requirements on Baijiu at present, they have paid more attention to the taste and quality of Baijiu, as well as the multi-aroma compound flavor. Therefore, developing a new compound-flavor Baijiu can not only enrich the Baijiu category, but also meet the market demands. Traditionally, Baijiu is produced through solid state fermentation of sorghum, corn, paddy and the other grains, which is a complex fermentation system with the cooperative participation of multiple strains, including fungi and bacteria (Tan et al., 2019). The diversity and dynamic changes of microbial community structure in Baijiu fermentation can directly affect the yield and flavor of the products, which have brought great challenges to the regulation of Baijiu quality. Therefore, it is of great value to analyze the microbial community structure and their evolution rules in Baijiu fermentation for regulating the production process and improving the product quality (Zhang et al., 2021b; Zhao et al., 2022).

Research on brewing microbial community includes the determination of species diversity, the functions of individual organisms, the interactions between microorganisms, and the prediction of microbial functions at the community scale. The most common method of microbial isolation and purification is the traditional streak plate method. Zhao et al. (2017) used this method to screen a high ethyl caproate producing strain of *Bacillus cereu* (2.15 g/L), which could increase the content of ethyl caproate in Jiupei and thus improve the flavor of strong-flavor Baijiu. Fan et al. (2019) isolated an abnormal *Wickerhamomyces Anomalus* Y3604 from Gujinggong Daqu (a fermentation starter) by traditional streak plate method, which ethyl acetate yield could reach 16.92 g/L under the optimal conditions. Although the traditional streak plate method plays an important role in the isolation and identification of microorganisms, it can only isolate the microorganisms that are easy to culture, thus it is unsuitable to comprehensively and efficiently understand the

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microflora in Baijiu fermentation. The other common method, PCR-denaturing gradient gel electrophoresis (PCR-DGGE) technology can only detect the major microorganisms with the abundance above 1%, and its result is highly dependent on analysis (Xiong et al., 2020). As an alternative, high-throughput sequencing technology has the advantages of the higher detection flux and lower detection limit, compared with the streak plate method and PCR-DGGE. It has been widely used in the study of microorganisms because of its high sensitivity, high accuracy and large amount of data processing (Cao et al., 2022; Fan et al., 2022). More importantly, high-throughput sequencing can be used to analyze the abundance and diversity of microorganisms, so as to reveal the microbial community structure and their evolution rules during Baijiu fermentation. Wang et al. (2021a) used high-throughput sequencing to study the microbial community in the Maotai-flavor Baijiu fermentation, and *Bacillus, Lentibacillus, Kroppenstendtia* and *Lactobacills* were identified as the core microorganisms in the seven rounds. Hu et al. (2021a) investigated the changes of microbial composition in the rice-flavor Baijiu production by high-throughput sequencing and found that parts of the major microorganisms came from the environment and raw rice materials.

In the Baijiu fermentation, the diversity and structure of microbial community are affected by a variety of environmental factors, such as the temperature, acidity, moisture and substrates (Song et al., 2017; Wang et al., 2018). It is meaningful to study the driving factors of microbial community structure succession for regulating the Baijiu fermentation process. Zhang et al. (2021a) investigated the community structure of bacteria and fungi in Maotai-flavor Baijiu fermentation and the results showed that *Pichia pastoris* was the main fungal genus, and the interaction of temperature, ethanol and *Pichia pastoris* could synergistically drive the transformation of microbial community structure in Jiupei. Guan et al. (2020) revealed the effects of physicochemical factors on microbiota dynamics in Strong-flavor Baijiu fermentation and reported that starch and pH of Jiupei were the most important factors influencing both bacterial and fungal communities at the initial stage, characterized by increasing microbial diversity and evenness. Nevertheless, the influencing factors of flavor substances production and their relationships with microbial community structure during Baijiu fermentation are still unknown.

In this study, a Qingzhi compound-flavor Baijiu (QZCF Baijiu) was developed with the sensory of both Qing aroma and sesame aroma (roasted aroma). The changes of physicochemical factors during Baijiu fermentation were analyzed and the flavor substances composition in Jiupei was detected by the headspace solid phase microextraction (HS-SPME) combined with gas chromatography-mass spectrometry (GC-MS). Meanwhile, high-throughput sequencing was used to reveal the microbial community structure in QZCF Baijiu fermentation and the influence of physicochemical factors on the microbiota dynamics was systematically elucidated. Moreover, the microbial species and physicochemical factors with the significant effects on the flavor difference of QZCF Baijiu were identified according to the Spearman correlation analysis. This study improved the understanding of the brewing mechanism of QZCF Baijiu, with benefit to the production regulation.

2 Materials and methods

2.1 QZCF Baijiu production

In the QZCF Baijiu production, sorghum was used as raw material. Qing-flavor Daqu and Sesame-flavor Daqu were employed as fermentation starters to produce QZCF Baijiu through solid state fermentation. As shown in Figure 1, sorghum was soaked in hot water at 85 °C for 15 h. Subsequently, the soaked sorghum was steamed at 1.5 MPa for 30 min after removing the soaking water, followed by adding 80 °C water over the sorghum's surface and holding for 15 min. The water was then removed and the sorghum was steamed at 1.5 MPa for 20 min. After that, the steamed sorghum was spread and cooled down to 28-32 °C. Approximately 11.6% (w/w) Sesame-flavor Daqu (provided by Shandong Xuxu Jiuqu Co., Ltd., China) and 6.0% (w/w) of wheat bran were added in the sorghum, and then stacked as a pile and cultured for 45 h in room temperature. When the Sesame-flavor grain had been cultured for 19 h, the preparation of the sorghum (soaking and steaming) was repeated. Differently, about 8.0% (w/w) Qing-flavor Daqu (provided by Shandong Xuxu Jiuqu Co., Ltd., China) and 6.0% (w/w) wheat bran were mixed with the prepared grain. Then, the mixture was spread with a thickness of 10-15 cm and saccharified for 21 h in room temperature. Finally, the cultured Sesame-flavor grain and the saccharified Qing-flavor grain were mixed at a ratio of 52.8: 47.2 (w/w) before taking into the fermentation tank (1 m^3, SSC1100, Yantai Liangrong Machinery Precision Industry Co., Ltd., China). The cultures were conducted at 20-25 °C prior to obtain the QZCF Baijiu through a distillation process. The QZCF Baijiu with ethanol content more than 50%vol was collected and preserved for further analysis.

2.2 Sample collection

Samples from the stacking process during QZCF Baijiu production was collected at 0 d and 2 d (signed as Ss0 and Ss1, respectively), while samples from the saccharification process was taken at 0 d and 1 d (signed as Sl0 and Sl1, respectively). In the fermentation process, samples were taken from the central point and four corners of the tank at 0 d, 1 d, 3 d, 6 d, 9 d, 13 d, and 18 d, and the depths of collection points included 10 cm, 30 cm, and 50 cm (Figure 2). Subsequently, the samples from

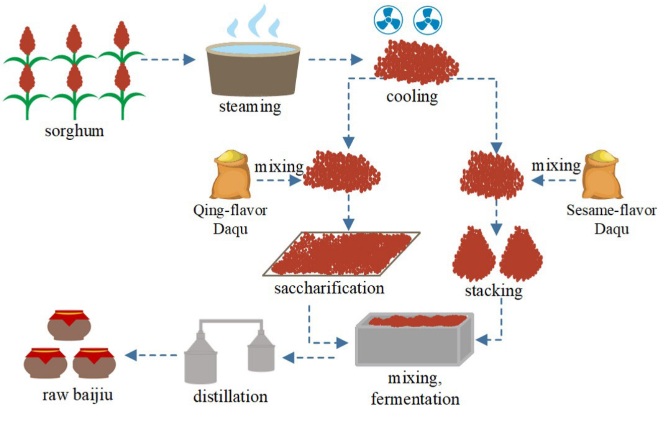

Figure 1. Flow chart of QZCF Baijiu production process.

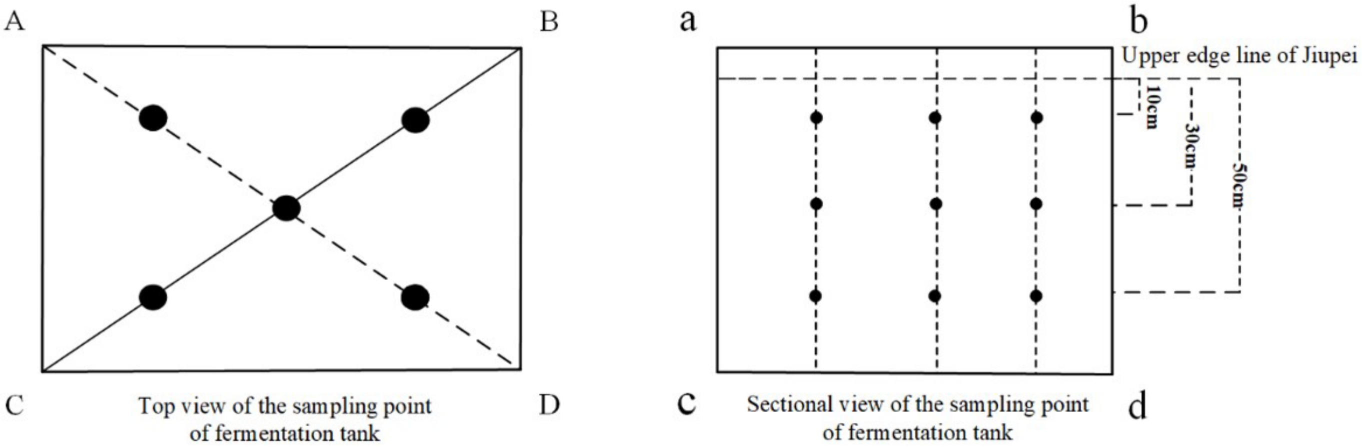

Figure 2. Schematic diagram of the sampling process in QZCF Baijiu fermentation. The letters A, B, C, D, a, b, c, d represent the four corners of the fermentation tank in the top view and sectional view.

different points and depths were thoroughly mixed and signed as F0, F1, F3, F6, F9, F13 and F18, respectively. All collected samples were preserved at -80 °C prior to analysis.

2.3 High-throughput sequencing

The dynamic change of microbial community structure in Jiupei during QZCF Baijiu fermentation was investigated by the Illumina NovaSeq 6000 sequencing platform (Beijing Nuohezhiyuan Bioinformatics Technology Co., Ltd., China). After the total DNA of the sample was extracted, the 16S V3-V4 region and ITS2 region amplicons were sequenced and analyzed, respectively. The data of each sample was normalized and used for alpha diversity analysis (Zhou et al. 2022). Afterwards, QIIME pipeline (Version 1.9.1) was used to calculate the Observed species, Chao1, Shannon, Simpson, ACE, and Goods-coverage index. The physicochemical factors were sorted and analyzed by the function in the vegan package, and the factors with significant impact were selected for distance-based redundancy analysis (db-RDA). Effective tags of all samples were clustered by Uparse software (Uparse v7.0.1001), in which the sequence with the highest frequency in OTUs was considered as the representative sequence of OTUs.

2.4 Analytical methods

In the QZCF Baijiu fermentation, temperature, moisture, acidity, starch, reducing sugar and ethanol contents of Jiupei were detected according to the previous study by You et al. (2021). The volatile compounds in Jiupei during the fermentation and the main aroma components in the product of QZCF Baijiu were analyzed by headspace solid phase microextraction combined with gas chromatography-mass spectrometer (HS-SPME-GC-MS). Prior to analysis, the Jiupei and Baijiu samples were pretreated according to the methods described by Wang et al. (2021c) and Du et al. (2021), respectively. For the sample of Jiupei, approximately 5 g sample and 20 mL mixed salt solution (0.85% NaCl and 1% $CaCl_2$, w/v) were added to a 50 mL centrifuge tubes, and then the mixture was ultrasonically treated in ice-bath for 30 min. Subsequently, the mixture was centrifuged at 4°C, 8000×g for 5 min and the supernatant was pipetted 8 mL into 30 mL headspace bottle before adding 3 g NaCl. Meanwhile, 10 μL tert-amyl alcohol (0.94 g/L), 10 μL amyl acetate (0.97 g/L), and 10 μL 2-ethylbutyric acid (0.94 g/L) were accurately supplemented as the internal standard prior to analysis by HS-SPME-GC-MS. For the sample of QZCF Baijiu, the ethanol content was reduced to 14%vol by adding ultrapure water and then the diluted sample was pipetted 8 mL into 30 mL headspace bottle with 3 g NaCl addition. Afterwards, the internal standard was supplemented prior to analysis by HS-SPME-GC-MS. Odor activity value (OAV) of the flavor substances in Baijiu samples were calculated according to the threshold value reported in the references (Sun et al., 2018; You et al., 2021).

2.5 Statistical analysis

Physicochemical factors and flavor compounds in Jiupei, and flavor substances in QZCF Baijiu were determined in triplicate and each result was presented as means or mean ± standard deviation. Statistical significances of the differences on these results were distinguished by one-way analysis of variance (ANOVA) and $p < 0.05$ was considered significant. The heatmap was performed using the R software (Version 3.6.2) with pheatmap package, while the correlation coefficients between the physicochemical factors, the microflora and the flavor compounds were visualized by the software cytoscape (Version 3.7.2).

3 Results and discussion

3.1 Physicochemical factors and volatile compounds of the Jiupei in QZCF Baijiu fermentation

Changes of physicochemical factors of the Jiupei during QZCF Baijiu fermentation were shown in Figure 3. The temperature of Jiupei was considered as a direct indicator to reflect the microbial activity, because the bioheat produced by the flourishing growth of the microbial community was the main reason for the temperature changes (Xiao et al., 2017; Zhang et al., 2020a). In QZCF Baijiu fermentation, the temperature increased at first and then slowly decreased at the later of the fermentation (Figure 3A), which trend was in accordance with the found by

Hao et al. (2021). The moisture increased rapidly in the early stage, and then reached a relatively stable state until 6 d. This phenomenon might be caused by a small amount of oxygen contained in the fermentation tank initially, which was consumed by microorganisms for aerobic respiration, resulting in the water generation. The acidity of Jiupei increased after stacking and saccharification process, and the improved acid production was also found in the early fermentation stage, indicating that there were many aerobic or facultative anaerobic acid-producing microorganisms in Jiupei (Figure 3B). This result was consistent with the reports by Tan et al. (2019) who highlighted that the acidity of Jiupei could increase to 4° in first 30 d of Strong-flavor Baijiu fermentation.

As shown in Figure 3B, a small amount of ethanol was produced during the stacking and saccharification process. The center of Jiupei was a basically anaerobic environment in stacking process which led to the higher ethanol production, compared with that of the saccharification process. In the fermentation stage, the ethanol content significantly increased in first 6 d and then reached a relatively stable state until the end of the fermentation. This result was in accordance with the findings by Hu et al. (2021b) who reported that ethanol and moisture in Jiupei markedly increased at the early stage of Strong-flavor Baijiu fermentation. The change of starch content was contrary to that of the reducing sugar content during the stacking and saccharification stage (Figure 3C). Moreover, the starch content decreased rapidly in first 6 d, which could be caused by the reason that in the early stage, the mold could use the oxygen remained in the tank to grow and produce amylase to decompose the starch. However, the rate of starch decomposition was decreased as a large number of molds and enzymes losing their vitalities at the later stage. The reducing sugar of Jiupei was decreased from 5.65% to 3.91% in first 3 d and then increased slightly after the 4 d (Figure 3C). This phenomenon might be due to the fact that a small amount of oxygen entered into the fermentation system during the sampling process, which made the glucoamylase-producing microbes regrown and improved the sugars production, resulting in the accumulation of reducing sugar.

Changes of volatile compounds in Jiupei during QZCF Baijiu fermentation were analyzed by GC-MS and the results were shown in Figure 4. A total of 39 volatile compounds were identified in QZCF Baijiu production, which could be divided into six groups, including esters (17), alcohols (9), aldehydes and ketones (5), phenols (5), and the others (3). Most of them were

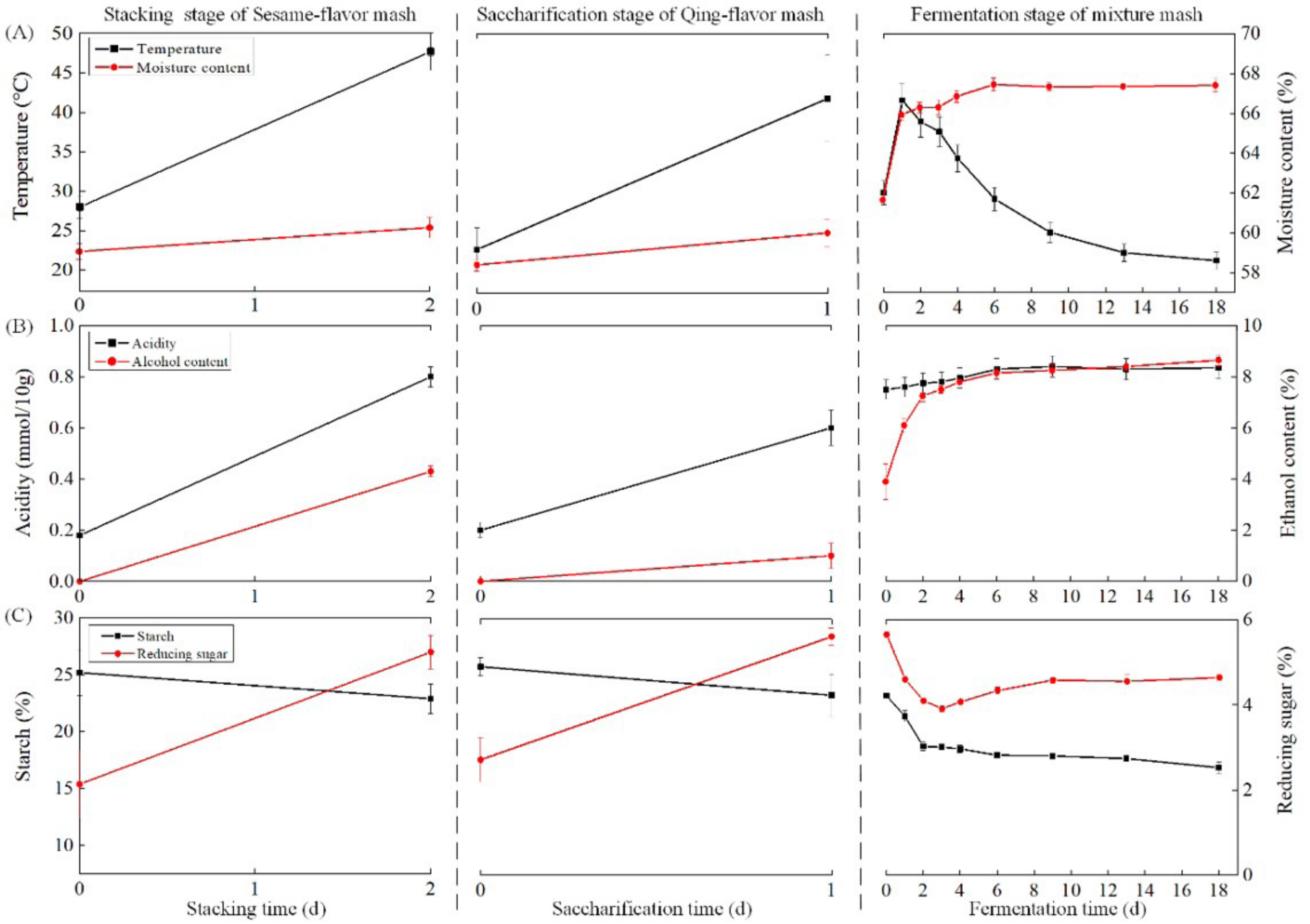

Figure 3. Changes of (A) temperature and moisture, (B) acidity and ethanol, (C) starch and reducing sugar contents in Jiupei during the QZCF Baijiu fermentation.

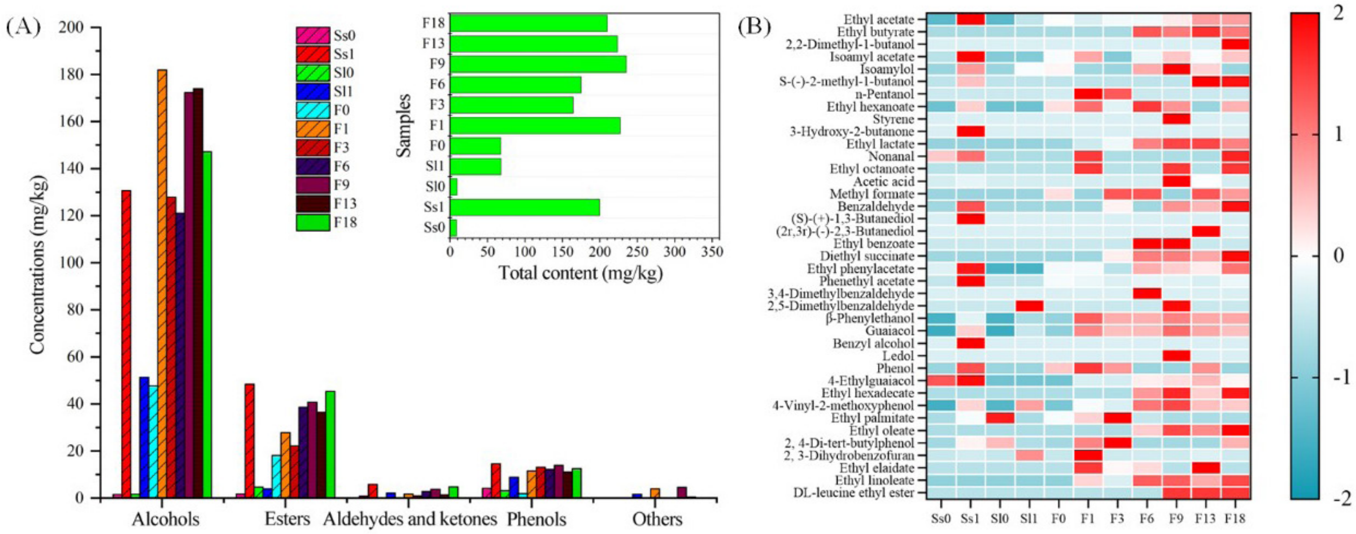

Figure 4. Changes of flavor substances in Jiupei during QZCF Baijiu fermentation. (A) Flavor substances concentrations, and (B) heatmap of flavor substances.

important flavor substances and had been widely distributed in different flavor types of Baijiu (Jin et al., 2017). Alcohols had the highest concentration in almost all samples, and the second were esters and phenols, while aldehydes and ketones had the lowest concentration. Ethyl acetate is one of the characteristic flavor substances in Qing-flavor Baijiu and its high concentration has been regarded as the marker of the quality Baijiu (Pang et al., 2018). In this study, ethyl acetate was the most abundant ester in Jiupei and reached 10.00 mg/kg at the end of fermentation, which would provide a Qing-aroma for QZCF Baijiu. Isoamyl alcohol and 2-phenylethanol were widespread with the higher contents among alcohols in QZCF Baijiu fermentation. The highest content of phenols was 4-vinyl-dimethoxyphenol (7.09 mg/kg), followed by 2, 4-di-tert-butylphenol (2.84 mg/kg), 4-ethylguaiacol (2.08 mg/kg) and guaiacol (0.58 mg/kg). Sun et al. (2018) reported that 4-ethylguaiacol and guaiacol were the key odorants in Sesame-flavor Baijiu and contributes to the sesame flavor. Therefore, the higher contents of 4-ethylguaiacol and guaiacol in Jiupei would provide a Sesame-aroma for QZCF Baijiu.

According to the heatmap, large amounts of volatile compounds accumulated in the stacking process, and their varieties and quantities were higher than that of the saccharification stage (Figure 4B). However, parts of these substances were decreased at the later stage which could be the reason of being reused as the precursors to produce the other compounds. Actually, most of volatile compounds were produced during the fermentation stage, especially at the later stage of fermentation (9-18 d). This result is expectable as it is widely known that the solid state fermentation of Baijiu has been divided into three stages, including the saccharification stage, the ethanol production stage and the flavor substances production stage, in which the flavor substances production stage is mainly occurred at the later stage of fermentation (Zhang et al., 2020b).

3.2 Dynamic changes of microbial community structure in QZCF Baijiu fermentation

Changes of microbial community structure in Jiupei during QZCF Baijiu fermentation were studied by high-throughput sequencing, and the results were shown in Table 1. After the quality control of sequencing data, 3976 bacterial OTUs and 711 fungal OTUs were obtained totally. Meanwhile, the Coverage values of all samples were above 99%, which proved that the sequencing data had good credibility.

It is well-known that the Shannon index and the Chao 1 index can be used to evaluate the diversity and richness of microbial communities (Xue et al., 2022). For the bacteria in this study, the Shannon index decreased after the stacking and saccharification process, which indicated that the species diversity of the bacterial community decreased. Meanwhile, the diversity of bacterial microorganisms had always fluctuated during the fermentation stage. The Chao1 index of Sl1 was the largest, followed by Sl0, which demonstrated that the bacterial community had a higher distribution and richness in Qing-flavor Jiupei. During the stacking process, the species richness of bacteria in Sesame-flavor Jiupei decreased, which was probably because some heat-sensitive bacteria were killed by the high temperature generated in this stage. The abundance of bacterial community increased during the saccharification process of Qing-flavor Jiupei, which could be due to the fact that microorganisms in the environment were entered into the fermentation system.

Similar to that of the bacterial community, the Shannon index of fungal microorganisms decreased after the stacking and saccharification process, and then gradually declined until the end of QZCF Baijiu fermentation. The Chao1 and ACE indexes of F0 were the largest, followed by Ss1, which highlighted that the richness of fungal community reached the highest at the end of stacking process and at the beginning of

Table 1. Alpha diversity statistics of the microbial communities.

Sample	Bacteria						Fungi					
	Observed species	Shannon	Simpson	Chao1	ACE	Goods coverage	Observed species	Shannon	Simpson	Chao1	ACE	Goods coverage
Ss0	676	3.280	0.786	840.2	872.0	0.996	27	1.521	0.617	28.1	30.1	1.00
Ss1	592	2.975	0.758	735.6	763.0	0.996	113	1.471	0.503	126.5	130.0	1.00
Sl0	907	3.476	0.793	1077.7	1077.4	0.995	29	1.397	0.532	31.7	33.8	1.00
Sl1	1018	3.090	0.723	1196.7	1242.8	0.995	38	1.329	0.516	41.4	43.5	1.00
F0	777	2.558	0.670	916.1	961.7	0.995	155	1.470	0.454	173.9	175.9	1.00
F1	549	2.169	0.640	678.2	713.5	0.996	75	1.254	0.456	79.7	83.8	1.00
F3	523	2.763	0.697	620.2	647.9	0.997	31	1.599	0.622	35.7	39.0	1.00
F6	448	3.082	0.741	516.2	534.5	0.998	84	1.718	0.583	95.4	96.9	1.00
F9	494	2.978	0.746	594.0	614.3	0.997	20	1.226	0.523	23.2	27.1	1.00
F13	494	2.589	0.726	618.3	688.2	0.997	53	1.576	0.505	59.9	61.5	1.00
F18	353	3.193	0.766	422.5	432.5	0.998	21	1.289	0.461	22.4	26.1	1.00

ACE: abundance-based coverage estimator.

fermentation. Environmental microorganisms were considered as the important microbial source and driving force for Baijiu solid-state fermentation (Chen et al., 2014; Wang et al., 2020). The abundance of fungi significantly increased during the stacking and saccharification process, which the Chao1 indexes raised from 28.1 and 31.7 to 126.5 and 41.4, respectively. It suggested that a large number of fungi in the environment had entered into the Jiupei and participated in the QZCF Baijiu fermentation.

According to the results of species annotation, 10 species with the highest abundance at genus taxonomic levels were selected to make the bar plots. As shown in Figure 5, the microbial community structure significantly changed during the stacking process of Sesame-flavor Jiupei (Ss0-Ss1). For the bacterial community, the genus *Bacillus* (48.98%) was the most abundant microorganism at the early stage of stacking process (Ss0), followed by *Sphingobacterium* (8.56%), *Weissella* (3.38%), *Streptococcus* (1.57%) and *Pediococcus* (1.20%). At the later stage (Ss1), the dominate genus had changed to *Weissella* (18.52%), *Pediococcus* (9.23%), *Streptococcus* (7.06%), *Bacillus* (2.26%). Overall, the relative abundance of the dominant bacteria decreased after the stacking process, which was consistent with the decrease of Chao1 index in Table 1. The structure of fungal community also changed greatly during the stacking process (Figure 5B). The dominant fungi in Jiupei before the stacking process were *Rhizopus* (66.71%), *Issatchenkia* (15.24%), *Saccharomyces* (7.40%), *Wickerhamomyces* (7.16%) and *Aspergillus* (1.49%), while it had evolved to *Issatchenkia* (70.67%), *Rhizopus* (14.02%), *Saccharomyces* (12.00%) and *Wickerhamomyces* (3.11%) at the end of stacking process. It could be found that the relative abundance of *Issatchenkia* had been greatly enhanced in the stacking process, showing the excellent heat stability. Combining with the improvement of ethanol content in Jiupei, the genus *Issatchenkia* might also have an outstanding ability to produce ethanol, which had been verified by Kwon et al. (2011) and Zwirzitz et al. (2021). At the saccharification stage (Sl0-Sl1), the initially dominant genera of bacterial community in Jiupei were *Bacillus* (20.31%), *Weissella* (19.85%), *Streptococcus* (6.02%), *unidentified Mitochondria* (2.72%) and *Pediococcus* (1.63%), while *Weissella* (44.04%) became the most dominant bacterial genus after 1 d, followed by *Streptococcus* (9.85%), *Bacillus* (5.59%) and *Pediococcus* (2.09%). In addition, *Saccharomyces, Rhizopus* and *Issatchenkia* were the largest groups of fungal genera after the saccharification process.

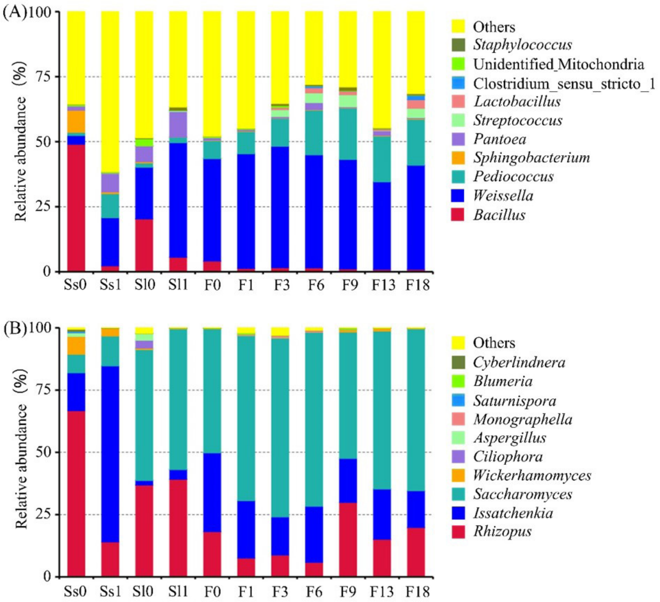

Figure 5. Bar plots of microbial structure at genus taxonomic level. (A) Bacteria, (B) Fungi.

At the fermentation stage (F0-F18), *Weissella* and *Pediococcus* were the dominant genera of bacterial community (Figure 5A). The relative abundance of *Pediococcus* gradually increased in QZCF Baijiu fermentation, while a slight fluctuation of *Weissella* was found. At the later stage of fermentation, the relative abundance of *Lactobacillus* had been predicted to increase due to the improvement of acidity in Jiupei, which was in agreement with the found by Cui et al. (2018) who reported that the genus *Lactobacillus* augmented after the 5 d of Baijiu fermentation. The dominant fungal genus was generally stable, and the proportion of the microbial genera changed slightly (Figure 5B). *Saccharomyces* was the most abundant fungi throughout the fermentation stage, although its abundance slightly declined during the 9-18 d.

3.3 Driving factors of dynamic changes of flavor substances and microbes in Jiupei

The microbial community structure in Jiupei could be influenced by the interaction of different microorganisms and environmental factors (Wu et al., 2021). Analysis of the distribution of the dominant microorganisms in Jiupei could be helpful to understand the microbial community composition in QZCF Baijiu production. In this study, microorganisms with the average relative abundance more than 1.0% were defined as the dominant microbial genus, including *Weissella*, *Pediococcus*, *Bacillus*, *Pantoea*, *Streptococcs*, *Sphingobaum*, *Saccharomys*, *Rhizopus*, *Issatchenkia* and *Wickerhamomyces* (Table 2). Among these, *Weissella*, *Pediococcus*, *Saccharomys*, *Rhizopus* and *Issatchenkia* were widely distributed in all samples throughout the Baijiu production. *Weissella* and *Saccharomyces* were the bacterial and fungal microorganisms with the highest relative abundance, accounting for 34.12% and 51.38%, respectively. The average relative abundances of *Pediococcus, Bacillus, Pantoea, Rhizopus* and *Issatchenkia* were 10.23%, 8.04%, 2.86%, 23.80% and 21.52% respectively, and their relative abundances were above 1.0% in more than 50% of the samples. The average relative abundance of *Streptococcus* was 1.38%, and its abundance was greater than 1.0% in the middle and later stage of fermentation, while the average relative abundance of *Sphingobacterium* was 1.02% and the abundance above 1.0% was only found at the initial stage of stacking process. The abundance of *Wickerhamomyces* ranged from 0.13% to 7.16%, and its abundance greater than 1.0% occurred in the stacking process and the 9 d, 13 d of Baijiu fermentation.

Table 2. The abundance and distribution frequency of the dominant microbes in QZCF Baijiu production.

Genus	Abundance range (%)	Average relative abundance (%)	Distribution frequency (%)
Weissella	3.38-46.70	34.12	100
Pediococcus	1.20-19.73	10.23	100
Bacillus	0.88-48.98	8.04	81.82
Pantoea	0.34-9.85	2.86	54.55
Streptococcs	0.00-4.60	1.38	36.36
Sphingobaum	0.13-8.55	1.02	9.09
Saccharomys	7.40-71.76	51.38	100
Rhizopus	5.84-66.71	23.80	100
Issatchenkia	1.83-70.67	21.52	100
Wickerhamomyces	0.13-7.16	1.28	36.36

The physicochemical factors in fermentation could affect the species distribution of microbial community, and also play a key role in selecting the functional microbes and inhibiting the growth of spoilage bacteria and harmful microorganisms (Ciani & Comitini, 2015; Kastman et al., 2016). Study of the relationships between the physicochemical factors and the microbial community succession would provide a theoretical basis to improve the Baijiu quality by appropriately adjusting the production parameters (Guan et al., 2020). Therefore, db-RDA was performed to reveal their relationships based on OTUs in this study and the results were shown in Figure 6. The explanatory rates of physicochemical factors of samples on the distribution of bacterial and fungal communities were 85.68% and 95.22%, respectively. This result indicated that physicochemical factors had an important impact on microbial succession change (Hao et al., 2021). The distribution of bacterial community at the initial stage of Baijiu fermentation (F0, F1) was positively correlated with the physicochemical factors, such as the reducing sugar, temperature and starch content. Among these, the starch content had the strongest correlation (Figure 6A). At the later stage (F9, F18), the distribution of bacterial community was positively correlated with the acidity, moisture and ethanol content. As for the fungal community, the starch and reducing sugar in Jiupei had the positive correlation for its distribution at the initial stage, while the acidity and ethanol content became the mainly driving factors later (Figure 6B). This result was

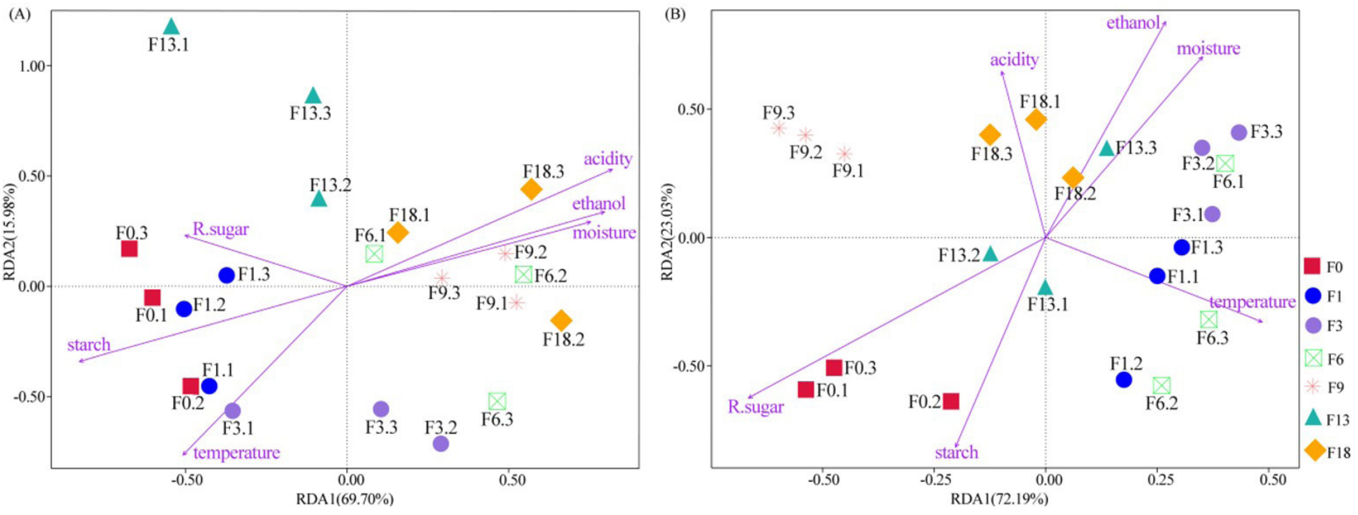

Figure 6. Distance based-redundancy analysis between the microbial composition and physicochemical factors during QZCF Baijiu fermentation. (A) Bacteria, (B) Fungi.

similar to the findings by Guan et al. (2020) who reported that starch content was one of the most positive factors influencing both bacterial and fungal communities at the start-up stage of Strong-flavor Baijiu fermentation (30-years cellar), while acidity and moisture, acidity and glucose became the mainly positive factors for bacterial and fungal communities at the later stage, respectively.

3.4 Analysis of the main flavor substances in QZCF Baijiu and its production regulation

After the fermentation and distillation process, the QZCF Baijiu was obtained and its main flavor substances was analyzed by GC-MS. A total of 36 compounds were identified in QZCF Baijiu together with their retention time (RT) and concentrations, including esters (20), alcohols (7), phenols (2), and the others (7). Among these, alcohols had the highest concentration (1673482.48 μg/L) in QZCF Baijiu and the second was esters (1622405.70 μg/L) (data not shown). OAV has been suggested as an evaluating value of the contribution of an aroma component to the overall aroma, which is calculated by the ratio of the odor compound concentration to its odor threshold values in an appropriate matrix (Niu et al., 2020). OAV greater than 1 not only indicates that the content of odor compound in the matrix is relatively high or the recognition threshold is low, but also reveals the compound can directly influence the overall flavor. Wang et al. (2021b) studied 19 main flavor compounds in Baijiu and proved that evaluating the contribution of the single flavor compounds to the overall aroma of Baijiu, OAV values should be taken into account. Zheng et al. (2016) successfully reconstructed the flavor characteristics of sesame-flavor Baijiu by identifying 26 important aromatic compounds from 56 substances based on OAV values. In general, OAV plays an important role in the evaluation of odor intensity of odorant foods. In this study, OAV of the flavor compounds in QZCF Baijiu were calculated according to the reported threshold value and the detected results by GC-MS. A total of 14 flavor compounds with OAV greater than 1 were found, including 8 esters, 4 alcohols, and 2 phenols (Table 3). Esters had the highest OAV (1191.82), which mainly contributed to the fruity, floral and sweet flavor for QZCF Baijiu. This result was in agreement with the findings by Wang et al. (2021d) who reported that esters were crucial flavor compounds, and total esters contributed to Baijiu aroma greatly. The total OAV of alcohols was 261.22 which mainly contributed to the aroma of flower, roast and malt. The OAV of phenols was 175.12 which contributed to the smoky and woody flavor. Moreover, ethyl caprylic acid had the highest OAV of 1030.79, indicating the great contribution for Baijiu flavor. The second were nonyl alcohol and guaiacol which OAV were higher than 100, while the OAV of ethyl acetate, ethyl butyrate, ethyl caproate, ethyl decanoate, ethyl laurate and 4-vinyl-2-methoxyphenol were above 10. The OAV of isobutanol, hexanol, ethyl phenylacetate, phenylethyl acetate and 2-phenylethanol ranged from 1.65 to 6.85, which were the important components of QZCF Baijiu flavor. Notably, ethyl acetate as one of the characteristic flavor substances in Qing-flavor Baijiu had the OVA of 43.93, which could provide a Qing-aroma for QZCF Baijiu. Isobutanol had the OVA of 1.65, which might be responsible for the Sesame-aroma of QZCF Baijiu (Wang et al., 2019). Moreover, guaiacol with the OAV of 147.12 could also contribute to the sesame flavor (Sun et al., 2018).

Correlation analysis between the important flavor compounds (Table 3), the dominant microorganisms (Figure 5), and the physiochemical factors (Figure 3) during QZCF Baijiu production were calculated, and the correlation indexes greater than 0.6 were summarized in Figure 7. The results showed that the main flavor compounds in QZCF Baijiu were closely related to various microorganisms. For example, *Issatchenkia* and *Wickerhamomyces* affected the production of phenethyl acetate, while *Pediococcus* and *Weissella* had significant effects on the production of ethyl acetate, ethyl butyrate, ethyl hexanoate, guaiacol, 4-vinyl-2-methoxyphenol, ethyl phenylacetate and 2-phenylethanol. The genus *Pantoea* could negatively influence the 2-phenylethanol and ethyl caprylate production, which contributed to the fruity and floral flavor. These results

Table 3. Flavor compounds with OAV greater than 1 in QZCF Baijiu.

No.	RT[a]	Compounds	Concentration (μg/L)	Threshold value (μg/L)	OAV	Aroma description (Wang et al., 2019)
1	0.86	Ethyl acetate	1430000.00	32551.60	43.93	nail polish-like, fruity, alcoholic
2	2.60	Isobutanol	66111.60	40000.00	1.65	fruity, malty, roast, nut-like, wine
3	3.28	Ethyl butyrate	1549.86	81.50	19.02	fruity, sweet
4	6.86	Ethyl hexanoate	11782.61	55.33	49.57	fruity
5	11.05	Hexanol	27183.14	5370.00	5.06	floral, green
6	13.47	Ethyl caprylate	13266.22	12.87	1030.79	fruity, fatty
7	19.32	Ethyl caprate	21738.68	1122.30	19.37	fruity, floral
8	20.16	Nonanol	18952.01	75.00	252.69	grassy
9	23.72	Ethyl phenylacetate	2788.48	406.83	6.85	rosy, fruity, sweet
10	24.74	Phenylethyl acetate	5207.27	908.83	5.73	floral, rose, honey
11	25.74	Ethyl laurate	6624.82	400.00	16.56	medicinal, grassy, sweet, fruity
12	26.18	Guajacolum	1972.87	13.41	147.12	smoky, woody
13	27.93	2-phenylethanol	52632.53	28922.73	1.82	rose, honey, rose-like, floral, rosy
14	36.45	4-vinyl-2-methoxyphenl	5860.31	209.30	28.00	smoky

[a] RT, retention time.

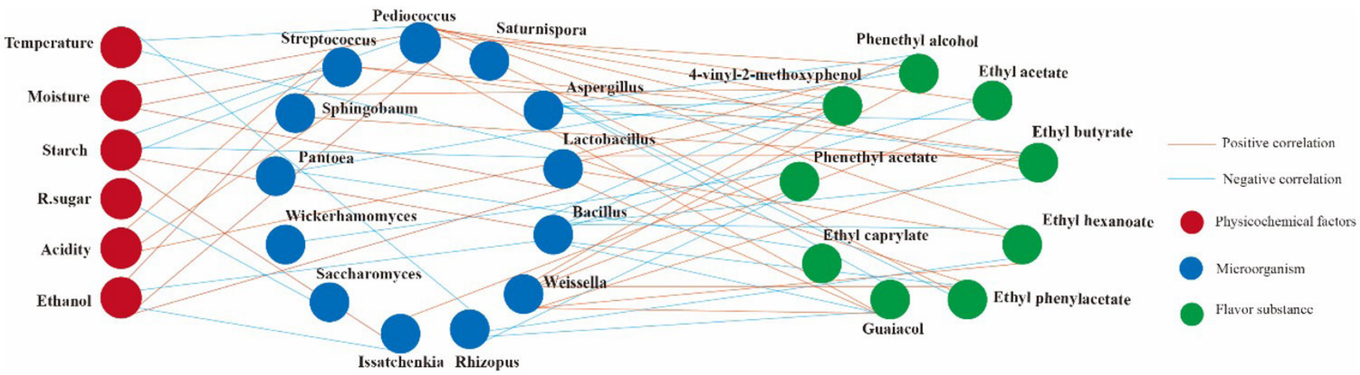

Figure 7. Correlation analysis between the dominant microorganisms, the physiochemical factors and the important flavor compounds in QZCF Baijiu production.

indicated that improving the abundance or metabolic activity of the specific microorganisms might change the contents of the important flavor compounds in QZCF Baijiu. Physiochemical factors, i.e. temperature, reducing sugar, pH, moisture, ethanol and starch content, played an important role in the growth and metabolism of microorganisms in Jiupei. As shown in Figure 7, *Pediococcus* was negatively correlated with temperature and starch content, and positively correlated with moisture, acidity and ethanol content. This result indicated that *Pediococcus* might be the heat-sensitive microorganism with the capacity of producing acids, which had been proved by Hu et al. (2021a). It is well-known that acids are the precursor substances for the synthesis of ester compounds, and esters have been considered as the key indicator to evaluate the Baijiu quality (Pang et al., 2020). Therefore, it could be foreseen that appropriate decrease of temperature and starch content or increase the moisture content of Jiupei in QZCF Baijiu fermentation might increase the relative abundance of *Pediococcus* to accumulate the acids, resulting in the improvement of esters production and Baijiu quality. *Rhizopus* can produce a large amount of amylase, which converts starch into fermentable sugar and provides carbon source for the growth and metabolism of the other microorganisms, promoting the Baijiu fermentation (de Barros Ranke et al., 2020). In QZCF Baijiu production, *Rhizopus* existed in the whole process and was only negatively correlated with temperature. This result indicated that appropriate decrease of the fermentation temperature or supplement of the heat-resistant *Rhizopus* would be meaningful to improve the utilization rate of raw materials and increase the Baijiu yield. Therefore, it should be highlighted that purposefully regulating the physicochemical factors in QZCF Baijiu fermentation could directionally change the microbial community structure in Jiupei, influencing the flavor compounds production and Baijiu quality.

4 Conclusions

In this study, the changes of physicochemical factors, microbial community structure and flavor components in QZCF Baijiu production were investigated and their correlations were calculated according to the Spearman correlation analysis. It should be highlighted that the change of physicochemical factors in Baijiu making process could drive the succession of microbial community structure, resulting in the flavor difference of QZCF Baijiu. Therefore, the directional change of flavor components in QZCF Baijiu could be realized by purposefully regulating the physicochemical factors. This study not only provided a substantial basis for the improvement of QZCF Baijiu production, but also laid a foundation for the research of Baijiu flavor formation and the relationship between microorganisms.

Conflicts of interest

The authors declare no conflict of interest.

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Author contributions

Conceptualization, J.X., Y.Z., Y.J. and J.W.; Methodology, J.X., Y.Z. and Y.J.; Software, Y.Z. and Y.J.; Validation, R.Z., F.C. and Z.Z.; Formal Analysis, J.X., Y.Z., Y.J., J.C. and Q.Y.; Investigation, J.X., Y.Z. and Y.J.; Resources, J.X., Z.L. and J.W.; Data Curation, J.X., Y.Z. and Y.J.; Writing-Original Draft Preparation, J.X., Y.Z. and Y.J.; Writing-Review and Editing, J.X. and J.W.; Visualization, J.X., Y.Z. and Y.J.; Supervision, J.W.; Project Administration, J.W.; Funding Acquisition, J.X. and J.W. All authors have read and agreed to the published version of the manuscript.

References

Cao, J. H., Zheng, Y. L., Zhao, T., Mao, H., Fang, S. L., Chen, M. B., & Liu, S. L. (2022). Changes in the microbial community structure during the digitally managed fermentation of medium-temperature Daqu. *Food Science and Technology (Campinas)*, 42, e87122. http://dx.doi.org/10.1590/fst.87122.

Chen, B., Wu, Q., & Xu, Y. (2014). Filamentous fungal diversity and community structure associated with the solid state fermentation of Chinese Maotai-flavor liquor. *International Journal of Food Microbiology*, 179, 80-84. http://dx.doi.org/10.1016/j.ijfoodmicro.2014.03.011. PMid:24742997.

Ciani, M., & Comitini, F. (2015). Yeast interactions in multi-starter wine fermentation. *Current Opinion in Food Science*, 1, 1-6. http://dx.doi.org/10.1016/j.cofs.2014.07.001.

Cui, K., Wu, Q., & Xu, Y. (2018). Biodegradation of ethyl carbamate and urea with *Lysinibacillus sphaericus* MT33 in Chinese liquor fermentation. *Journal of Agricultural and Food Chemistry*, 66(6), 1583-1590. http://dx.doi.org/10.1021/acs.jafc.7b05190. PMid:29359925.

de Barros Ranke, F. F., Shinya, T. Y., de Figueiredo, F. C., Fernández Núñez, E. G., Cabral, H., & de Oliva, P., No. (2020). Ethanol from rice byproduct using amylases secreted by *Rhizopus* microsporus var. *oligosporus*. Enzyme partial purification and characterization. *Journal of Environmental Management*, 266, 110591. http://dx.doi.org/10.1016/j.jenvman.2020.110591. PMid:32392142.

Du, H., Ji, M., Xing, M., Wang, X., & Xu, Y. (2021). The effects of dynamic bacterial succession on the flavor metabolites during Baijiu fermentation. *Food Research International*, 140, 109860. http://dx.doi.org/10.1016/j.foodres.2020.109860. PMid:33648178.

Fan, G., Teng, C., Xu, D., Fu, Z., Minhazul, K. A., Wu, Q., Liu, P., Yang, R., & Li, X. (2019). Enhanced production of ethyl acetate using co-culture of *Wickerhamomyces anomalus* and *Saccharomyces cerevisiae*. *Journal of Bioscience and Bioengineering*, 128(5), 564-570. http://dx.doi.org/10.1016/j.jbiosc.2019.05.002. PMid:31175063.

Fan, Y., Ma, C., & Wang, R. (2022). Deciphering bacterial community succession patterns and their correlations with physicochemical factors in solid-state fermentation of high-quality jujube vinegar. *Food Science and Technology (Campinas)*, 42, e05022. http://dx.doi.org/10.1590/fst.05022.

Guan, T., Lin, Y., Chen, K., Ou, M., & Zhang, J. (2020). Physicochemical factors affecting microbiota dynamics during traditional solid-state fermentation of Chinese strong-flavor Baijiu. *Frontiers in Microbiology*, 11, 2090. http://dx.doi.org/10.3389/fmicb.2020.02090. PMid:33013762.

Hao, F., Tan, Y., Lv, X., Chen, L., Yang, F., Wang, H., Du, H., Wang, L., & Xu, Y. (2021). Microbial community succession and its environment driving factors during initial fermentation of Maotai-Flavor Baijiu. *Frontiers in Microbiology*, 12, 669201. http://dx.doi.org/10.3389/fmicb.2021.669201. PMid:34025626.

Hu, X., Tian, R., Wang, K., Cao, Z., Yan, P., Li, F., Li, X., Li, F., & He, P. (2021a). The prokaryotic community, physicochemical properties and flavors dynamics and their correlations in fermented grains for Chinese strong-flavor Baijiu production. *Food Research International*, 148, 110626. http://dx.doi.org/10.1016/j.foodres.2021.110626. PMid:34507770.

Hu, Y., Lei, X., Zhang, X., Guan, T., Wang, L., Zhang, Z., Yu, X., Tu, J., Peng, N., Liang, Y., & Zhao, S. (2021b). Characteristics of the microbial community in the production of Chinese Rice-flavor Baijiu and comparisons with the microflora of other flavors of Baijiu. *Frontiers in Microbiology*, 12, 673670. http://dx.doi.org/10.3389/fmicb.2021.673670. PMid:33995338.

Jin, G., Zhu, Y., & Xu, Y. (2017). Mystery behind Chinese liquor fermentation. *Trends in Food Science & Technology*, 63, 18-28. http://dx.doi.org/10.1016/j.tifs.2017.02.016.

Kastman, E. K., Kamelamela, N., Norville, J. W., Cosetta, C. M., Dutton, R. J., & Wolfe, B. E. (2016). Biotic interactions shape the ecological distributions of *Staphylococcus* species. *mBio*, 7(5), e01157-e16. http://dx.doi.org/10.1128/mBio.01157-16. PMid:27795388.

Kwon, Y. J., Wang, F., & Liu, C. Z. (2011). Deep-bed solid state fermentation of sweet sorghum stalk to ethanol by thermotolerant *Issatchenkia orientalis* IPE 100. *Bioresource Technology*, 102(24), 11262-11265. http://dx.doi.org/10.1016/j.biortech.2011.09.103. PMid:22014707.

Niu, Y., Zhu, Q., & Xiao, Z. (2020). Characterization of perceptual interactions among ester aroma compounds found in Chinese Moutai Baijiu by gas chromatography-olfactometry, odor intensity, olfactory threshold and odor activity value. *Food Research International*, 131, 108986. http://dx.doi.org/10.1016/j.foodres.2020.108986. PMid:32247502.

Pang, X. N., Han, B. Z., Huang, X. N., Zhang, X., Hou, L. F., Cao, M., Gao, L. J., Hu, G. H., & Chen, J. Y. (2018). Effect of the environment microbiota on the flavour of light-flavour Baijiu during spontaneous fermentation. *Scientific Reports*, 8(1), 3396. http://dx.doi.org/10.1038/s41598-018-21814-y. PMid:29467508.

Pang, X. N., Huang, X. N., Chen, J. Y., Yu, H. X., Wang, X. Y., & Han, B. Z. (2020). Exploring the diversity and role of microbiota during material pretreatment of light-flavor Baijiu. *Food Microbiology*, 91, 103514. http://dx.doi.org/10.1016/j.fm.2020.103514. PMid:32539964.

Sakandar, H. A., Hussain, R., Farid Khan, Q., & Zhang, H. (2020). Functional microbiota in Chinese traditional Baijiu and Mijiu Qu (starters): a review. *Food Research International*, 138(Pt B), 109830. http://dx.doi.org/10.1016/j.foodres.2020.109830. PMid:33288161.

Song, Z., Du, H., Zhang, Y., & Xu, Y. (2017). Unraveling core functional microbiota in traditional solid-state fermentation by high-throughput amplicons and metatranscriptomics sequencing. *Frontiers in Microbiology*, 8, 1294. http://dx.doi.org/10.3389/fmicb.2017.01294. PMid:28769888.

Sun, J., Li, Q., Luo, S., Zhang, J., Huang, M., Chen, F., Zheng, F., Sun, X., & Li, H. (2018). Characterization of key aroma compounds in Meilanchun sesame flavor style baijiu by application of aroma extract dilution analysis, quantitative measurements, aroma recombination, and omission/addition experiments. *RSC Advances*, 8(42), 23757-23767. http://dx.doi.org/10.1039/C8RA02727G. PMid:35540260.

Tan, Y., Zhong, H., Zhao, D., Du, H., & Xu, Y. (2019). Succession rate of microbial community causes flavor difference in strong-aroma Baijiu making process. *International Journal of Food Microbiology*, 311, 108350. http://dx.doi.org/10.1016/j.ijfoodmicro.2019.108350. PMid:31614280.

Wang, H., Huang, Y., & Huang, Y. (2021a). Microbiome diversity and evolution in stacking fermentation during different rounds of Jiang-flavoured Baijiu brewing. *LWT*, 143, 111119. http://dx.doi.org/10.1016/j.lwt.2021.111119.

Wang, J., Huang, M. Q., Zhang, J., & Wu, J. (2019). Flavor chemistry in baijiu with sesame flavor: a review. In B. Guthrie, J. D. Beauchamp, A. Buettner, S. Toth & M. C. Qian (Eds.), *Sex, smoke, and spirits: the role of chemistry*. Washington, DC: American Chemical Society. http://dx.doi.org/10.1021/bk-2019-1321.ch013.

Wang, J., Kong, B., Feng, J., Wang, H., Zhang, R., Cai, F., Yu, Q., Zhu, Z., Cao, J., & Xu, J. (2022). A novel strategy for comprehensive utilization of distillers' grain waste towards energy and resource recovery. *Process Biochemistry*, 113, 141-149. http://dx.doi.org/10.1016/j.procbio.2021.12.030.

Wang, L., Zhu, L., Zheng, F., Zhang, F., Shen, C., Gao, X., Sun, B. G., Huang, M., Li, H., & Chen, F. (2021b). Determination and comparison of flavor (retronasal) threshold values of 19 flavor compounds in Baijiu. *Journal of Food Science*, 86(5), 2061-2074. http://dx.doi.org/10.1111/1750-3841.15718. PMid:33884627.

Wang, S., Xiong, W., Wang, Y., Nie, Y., Wu, Q., Xu, Y., & Geisen, S. (2020). Temperature-induced annual variation in microbial community changes and resulting metabolome shifts in a controlled fermentation system. *mSystems*, 5(4), e00555-e20. http://dx.doi.org/10.1128/mSystems.00555-20. PMid:32694129.

Wang, W., Xu, Y., Huang, H., Pang, Z., Fu, Z., Niu, J., Zhang, C., Li, W., Li, X., & Sun, B. (2021c). Correlation between microbial communities and flavor compounds during the fifth and sixth rounds of sauce-flavor baijiu fermentation. *Food Research International*, 150(Pt A), 110741. http://dx.doi.org/10.1016/j.foodres.2021.110741. PMid:34865760.

Wang, X., Du, H., Zhang, Y., & Xu, Y. (2018). Environmental microbiota drives microbial succession and metabolic profiles during Chinese liquor fermentation. *Applied and Environmental Microbiology*, 84(4), e02369-e17. http://dx.doi.org/10.1128/AEM.02369-17. PMid:29196296.

Wang, Z., Wang, Y., Zhu, T., Wang, J., Huang, M., Wei, J., Ye, H., Wu, J., Zhang, J., & Meng, N. (2021d). Characterization of the key odorants and their content variation in Niulanshan Baijiu with different storage years using flavor sensory omics analysis. *Food Chemistry*, 376, 131851. http://dx.doi.org/10.1016/j.foodchem.2021.131851. PMid:34973637.

Wu, Q., Zhu, Y., Fang, C., Wijffels, R. H., & Xu, Y. (2021). Can we control microbiota in spontaneous food fermentation?—Chinese liquor as a case example. *Trends in Food Science & Technology*, 110, 321-331. http://dx.doi.org/10.1016/j.tifs.2021.02.011.

Xiao, C., Lu, Z. M., Zhang, X. J., Wang, S. T., Ao, L., Shen, C. H., Shi, J. S., & Xu, Z. H. (2017). Bio-heat is a key environmental driver shaping the microbial community of medium-temperature Daqu. *Applied and Environmental Microbiology*, 83(23), e01550-e17. http://dx.doi.org/10.1128/AEM.01550-17. PMid:28970223.

Xiong, Z. Q., Li, Y. Y., Xiang, Y. W., Xia, Y. J., Zhang, H., Wang, S. J., & Ai, L. Z. (2020). Dynamic changes in bacterial diversity during the production of powdered infant formula by PCR-DGGE and high-throughput sequencing. *Journal of Dairy Science*, 103(7), 5972-5977. http://dx.doi.org/10.3168/jds.2019-18064. PMid:32331873.

Xue, B., Yu, J., Zhang, J., Hao, F., Zhang, X., Dong, J., Sun, J., & Xue, J. (2022). Microbial diversity analysis of vineyard son the eastern foothills of the Helan Mountain region using high-throughput sequencing. *Food Science and Technology (Campinas)*, 42, e66320. http://dx.doi.org/10.1590/fst.66320.

You, L., Zhao, D., Zhou, R., Tan, Y., Wang, T., & Zheng, J. (2021). Distribution and function of dominant yeast species in the fermentation of strong-flavor baijiu. *World Journal of Microbiology & Biotechnology*, 37(2), 26. http://dx.doi.org/10.1007/s11274-020-02988-y. PMid:33427975.

Zhang, H., Wang, L., Tan, Y., Wang, H., Yang, F., Chen, L., Hao, F., Lv, X., Du, H., & Xu, Y. (2021a). Effect of Pichia on shaping the fermentation microbial community of sauce-flavor Baijiu. *International Journal of Food Microbiology*, 336, 108898. http://dx.doi.org/10.1016/j.ijfoodmicro.2020.108898. PMid:33129005.

Zhang, H., Wang, L., Wang, H., Yang, F., Chen, L., Hao, F., Lv, X., Du, H., & Xu, Y. (2021b). Effects of initial temperature on microbial community succession rate and volatile flavors during Baijiu fermentation process. *Food Research International*, 141, 109887. http://dx.doi.org/10.1016/j.foodres.2020.109887. PMid:33641943.

Zhang, W., Si, G., Du, H., Li, J., Zhou, P., & Ye, M. (2020a). Directional design of a starter to assemble the initial microbial fermentation community of Baijiu. *Food Research International*, 134, 109255. http://dx.doi.org/10.1016/j.foodres.2020.109255. PMid:32517943.

Zhang, W., Li, J., Rao, Z., Si, G., Zhang, X., Gao, C., Ye, M., & Zhou, P. (2020b). Sesame flavour baijiu: a review. *Journal of the Institute of Brewing*, 126(3), 224-232. http://dx.doi.org/10.1002/jib.614.

Zhao, C., Yan, X., Yang, S., & Chen, F. (2017). Screening of *Bacillus* strains from Luzhou-flavor liquor making for high-yield ethyl hexanoate and low-yield propanol. *LWT*, 77, 60-66. http://dx.doi.org/10.1016/j.lwt.2016.11.035.

Zhao, L., Mo, X., Zhang, C., Yang, L., & Wang, X. (2022). Community diversity and succession in fermented grains during the stacking fermentation of Chinese moutai-flavored liquor making. *Food Science and Technology (Campinas)*, 42, e61521. http://dx.doi.org/10.1590/fst.61521.

Zheng, Y., Sun, B., Zhao, M., Zheng, F., Huang, M., Sun, J., Sun, X., & Li, H. (2016). Characterization of the key odorants in Chinese zhima aroma-type baijiu by gas chromatography-olfactometry, quantitative measurements, aroma recombination, and omission studies. *Journal of Agricultural and Food Chemistry*, 64(26), 5367-5374. http://dx.doi.org/10.1021/acs.jafc.6b01390. PMid:27263543.

Zhou, X., Guan, Q., Qin, Y., Qin, Z., Du, B., & Lin, D. (2022). Dynamic changes in physic-chemical properties and bacterial community during natural fermentation of tomatoes. *Food Science and Technology (Campinas)*, 42, e63520. http://dx.doi.org/10.1590/fst.63520.

Zwirzitz, A., Alteio, L., Sulzenbacher, D., Atanasoff, M., & Selg, M. (2021). Ethanol production from wheat straw hydrolysate by *Issatchenkia orientalis* isolated from waste cooking oil. *Journal of Fungi (Basel, Switzerland)*, 7(2), 121. http://dx.doi.org/10.3390/jof7020121. PMid:33562172.