



# The mechanism of microbial structure and flavor characteristics in Qing-Jiang-flavor Jiupei regulated by different fermentation seasons

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## ABSTRACT

The physicochemical characteristics of *Jiupei* are crucial in regulating the metabolism of microbial communities and the flavor profile of Baijiu during the fermentation process. This study systematically monitored the physicochemical characteristics of Qing-Jiang-flavor Baijiu *Jiupei* (QJFJ) and analyzed its microbial community structure and flavor compounds. Results indicated that dominant bacteria were significantly enriched in Summer- and Autumn-*Jiupei* (Spring: Summer: Autumn: Winter = 1.00: 1.40: 1.29: 1.21), while dominant fungi were significantly enriched in Spring- and Autumn-*Jiupei* (Spring: Summer: Autumn: Winter = 1.45: 1.00: 1.35: 1.31). Sequentially, reducing sugars (day 0), temperature (day 5 - day 10), moisture (day 15), and acidity (day 20 - day 25) in *Jiupei* affected the succession pattern of the microbial community, regulating the abundance of *Saccharomyces*, *Staphylococcus*, *Cyberlindnera*, and *Lactobacillus*, individually. Alcohol and acid compounds are considered seasonal differential compounds in QJFJ. This study will provide a theoretical basis for Baijiu production across different seasons.

## 1. Introduction

The Chinese Baijiu, with a history of over 7000 years, holds significant importance in Chinese cuisine, culture, and economic development (Wang, Huang, Hu, & Li, 2021). Different Baijiu flavor profiles are mainly influenced by raw materials, fermentation processes, and physicochemical characteristics during the brewing process (Jin, Zhu, & Xu, 2017). The physicochemical characteristics differ across various brewing seasons, while the physicochemical characteristics drove the succession of microbial communities (Hao et al., 2021; Tan, Zhong, Zhao, Du, & Xu, 2019; Wang et al., 2021; Wang, Huang, & Huang, 2021). For example, studies on Maotai-flavor Baijiu have shown that acidity and temperature are the main factors in the succession of microbial communities during pit fermentation (Hao et al., 2021). Meanwhile, during the long-term fermentation process, key microbes and their metabolic activities regulated the flavor characteristics of Baijiu (Li et al., 2020). For instance, *Bacillus* exerted its role in flavor regulation by synthesizing hexanoate and propanol (Zhao, Yan, Yang, & Chen, 2017). *Lactobacillus* (*Lactobacillus*, *Enterococcus*, *Pediococcus*) were demonstrated to be the main contributors of methyl and ethyl esters (Jin et al., 2019).

*Saccharomyces* converts glucose into ethanol and produces higher alcohols such as isobutanol, 2-methyl-1-butanol, and 3-methyl-1-butanol (Xu et al., 2022). Therefore, a comprehensive grasp of the physicochemical characteristics during the Baijiu brewing process and their seasonal variations impact on the microbial community helps to regulate fermentation and Baijiu flavor tendency. This plays a significant role in stabilizing Baijiu quality.

Different regions produced Baijiu with distinctly different flavors, which was due to the significant differences in brewing conditions across regions, such as temperature, humidity, microbial communities, and other factors (Wei, Zou, Shen, & Yang, 2020). For instance, the Qing-flavor Baijiu from Chinese Shanxi Province was the most representative, boasting a flavor distinctly different from the Qing-flavor type Baijiu of other regions, which was closely related to the natural climate conditions of the area (Zheng & Han, 2016). The core production area of Jiang-flavor Baijiu was in the Chinese Chishui River basin, where the outstanding water quality and subtropical microclimate were conducive to the growth, reproduction, and metabolism of microorganisms suitable for brewing. Such a microbial environment was extremely favorable for the production of Jiang-flavor Baijiu (Gong, Ma, Li, Cheng, & Huang,

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2023). The region for producing *Qing-Jiang*-flavor Baijiu (QJFB) is found in mid-to-high altitude areas, where distinctive brewing conditions impart it with the refined qualities of *Qing*-flavor Baijiu, as well as the full-bodied characteristics of *Jiang*-flavor Baijiu. This has sparked a keen interest in the research of the unique flavor profile of QJFB within the spirits industry. However, current research on QJFB is still limited to the compositional analysis of its unique flavor (High total ester content, moderate total acidity, and low fusel oil) (Ma & Huang, 2019). The intrinsic mechanisms influencing flavor characteristics, particularly the microbiological structural composition, remain unclear. Moreover, the role of brewing physicochemical characteristics that impact the microbial community structure is not well defined.

Open-style Baijiu brewing is characterized by its continuity. The climatic conditions during different brewing seasons impacted the physicochemical characteristics of the Baijiu brewing system, which in turn affected the microbial community structure and metabolic activities in *Jiupei* (Xu et al., 2022). Therefore, the seasonal factors during fermentation have become a dynamic driving force that significantly influences the microbial ecosystem in *Jiupei* (Wang et al., 2020). Our previous research indicated that the capability of Daqu in saccharification, fermentation, and aroma production was affected by seasonal drivers. This suggested a close association with microbial differences caused by the diversity of seasonal environmental characteristics (Wang, Huang, & Huang, 2021). Thus, for the continuous brewing of Baijiu, it is crucial to consider the characteristics of the microbial community structure in *Jiupei* which are influenced by the brewing season, as well as the impact of microbial metabolism on flavor compounds. This will help regulate the quality stability of continuous production of Baijiu.

Due to the variations in physicochemical characteristics of *Jiupei* during different brewing seasons, it is possible for seasonal characteristics to emerge in the microbial composition and flavor profile of the Baijiu. Therefore, this study aims to reveal the differences in microbial communities and flavor compound compositions of QJFB across different seasons, as well as analyze the mechanisms behind seasonal fermentation differences. This research will contribute to a better understanding of the seasonal variations in microbial communities and flavor profiles during Baijiu fermentation. It will provide valuable guidance for optimizing the quality of Baijiu in different fermentation seasons.

## 2. Materials and methods

### 2.1. Preparation of QJFB samples

The *Jiupei* samples were collected from Guizhou Yanbo Distillery Co., Ltd. (with the altitude of 1960 m at 104°74' E and 25°99' N, a representative QJFB producer). The *Jiupei* samples were collected during different seasons: Spring (April 1st to 26th), Summer (July 1st to 26th), Autumn (October 1st to 26th), and Winter (January 1st to 26th). The sampling points were taken at 0, 5, 10, 15, 20, and 25 days of fermentation for each respective season. The *Jiupei* samples are sampled as upper, middle, and lower layers according to the cellar. In each layer, the *Jiupei* samples is collected from the middle and four corner positions. After sampling from a total of 15 points in the three layers, the samples are mixed to form one fermentation *Jiupei* sample for a fermentation tank (Fig. S1). Totally, 72 mixed composite *Jiupei* samples (18 mixed composite samples for each season, 1080 collected samples) were collected and stored at -80 °C for subsequent physicochemical characteristics and microbial analysis.

### 2.2. Measurement of physicochemical characteristics during QJFB fermentation

The temperature, moisture, acidity, and reducing sugar of the *Jiupei* were monitored. Temperature detection was carried out using probe-type electronic thermometers at each sampling point. The content of

moisture was calculated through drying to constant weight at 105 °C. The acidity of each *Jiupei* sample was detected by calculating the volume of NaOH (0.1 mol/L) used during titration, with the endpoint of titration at pH 8.0 (This method is derived from the standard methods specified in the analytical method for brewing Daqu in China (QB/T4257-2011)). The content of reducing sugars was determined using the 2,4-dinitrophenylhydrazine colorimetric method (Wang, Huang, & Huang, 2021).

### 2.3. Analysis of microbial community structure in QJFB

The genomic DNA of the bacterial community from 72 *Jiupei* samples were respectively extracted using the E.Z.N.A. Soil DNA Kit, Omega Biotek, USA. The hypervariable region (V3-V4) of bacterial 16S rRNA genes were amplified via the universal primer pairs 338F (5'-ACTCCTACGG-GAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'), and ITS-1R (5'-GCTGCGTCTTCATCGATGC-3') were used to amplify the ITS1 region of fungi (Wang, Huang, & Huang, 2021). The PCR system for amplification was performed with a 20 µL mixture reaction, containing DNA template (50 mM, 2 µL), primer pairs (each 0.8 µL for forward and reverse primer), 5 × FastPfu buffer (4 µL), dNTP (2.5 mM, 2 µL), FastPfu polymerase (0.4 µL), as well as sterile ddH<sub>2</sub>O (10 µL) (Zuo, Huang, & Guo, 2020). The PCR amplification was applied through the following conditions: firstly, an initial denaturation was performed at 95 °C for 3 min; then, 35 cycles were followed containing denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 45 s; finally, a single extension at 72 °C for 10 min was applied (Zuo et al., 2020). The PCR products were detected and separated by 1% agarose gel electrophoresis, purified via the gel extraction kit from Axygen Biosciences, as well as quantified through the Quantus™ Fluorometer from Promega (Wang, Huang, & Huang, 2021). The PCR amplification reactions were replicated in three time. After individual quantification, the equimolar amplicons were pooled and subjected to paired-end sequencing (2 × 250 bp) using the Illumina NovaSeq platform provided by Hangzhou Biotech Co., Ltd. (Hangzhou, China).

### 2.4. Qualitative and quantitative analysis of volatile flavors compounds in QJFB

Add 5 g of *Jiupei* to 20 mL of ultrapure water, then add 0.85% NaCl and 1% CaCl<sub>2</sub>. Shake thoroughly to mix, and then sonicate at 0 °C for 30 min. Finally, centrifuge at 4 °C and 8000 g for 5 min, and collect the supernatant. Take 3 g of NaCl and 8 mL of the pre-treated supernatant and add them to a headspace vial. Then, add 20 µL of pure menthol (99.99% purity) as an internal standard. Apply the headspace solid-phase microextraction combined with gas chromatography-mass spectrometry method for the detection of volatile flavor compounds (Wang, Huang, & Huang, 2021). The headspace solid-phase microextraction combined with gas chromatography-mass spectrometry is equipped with a TG-5MS chromatographic column (TSQ 8000 Evo, Trace MS/GC, Thermo Fisher Scientific, Waltham, Massachusetts, United States) and a flame ionization detector (30 m × 0.25 mm × 0.25 µm, J&W Scientific, Fullerton, California, United States). The temperature of the gas chromatography oven is maintained at 40 °C for 3 min, then ramped up to 100 °C at a rate of 2 °C /min and maintained for 5 min. It is then further ramped up to 150 °C at a rate of 2 °C /min and maintained for 2 min. Finally, it is ramped up to 230 °C at a rate of 10 °C /min and maintained for 5 min. The carrier gas flow rate of helium is 1 mL/min. The mass spectrometry is generated under 70 eV electron impact ionization, with a full scanning range of 30-400 amu. By matching the spectra with the NIST05 spectral database, flavor chemicals are identified.

### 2.5. Statistical analysis

Detailed data analysis methods are provided in the supplementary materials.

### 3. Results and discussion

#### 3.1. The changes of physicochemical characteristics in QJFJ of different fermentation seasons

The average temperature of the QJFJ in the brewing process shows seasonal rhythmic variations due to the influence of different fermentation seasons. However, the overall temperature shows a pattern of initially increasing (day 0 - day 5) and then decreasing (day 5 - day 25), which is similar to the trend observed in *Jiang*-flavor Baijiu. This conforms to the characteristics of solid-state fermentation (Hao et al., 2021) (Fig. 1A). The temperature of the four-seasons-*Jiupei* reaches the peak on the 5th day of fermentation ( $p < 0.05$ , Fig. 1A), with values of  $32.90 \pm 0.40$  °C,  $35.37 \pm 2.08$  °C,  $35.00 \pm 0.40$  °C, and  $32.50 \pm 0.20$  °C respectively. This is caused by the release of a large amount of heat from microbial decomposition of organic substrates (Wei, Wang, Hassan, & Xie, 2018). Diverging from the results of this study, the temperature peak of *Jiang*-flavor Baijiu fermentation occurs around the 10th day (Hao et al., 2021). The possible reason is that the Daqu used for brewing QJFB is not entirely composed of high-temperature Daqu (co-fermentation of four types of Daqu), which results in a fermentation microbial structure in QJFB that differs from that of *Jiang*-flavor Baijiu. The acidity of the QJFJ significantly increases between the 10 - 15th day of fermentation ( $p < 0.05$ , Fig. 1B). While, the Winter-*Jiupei* acidity increases slowly, this may be attributed to the low fermentation environment temperature and the temperature at which the *Jiupei* enters the cellar, which are not favorable for promoting microbial activity and the establishment of the initial fermentation temperature within *Jiupei*.

Sharp increases in acidity were observed during the later stage of *Jiupei* fermentation (day 25, Fig. 1B), resulting in a highly acidic environment that promotes the growth and metabolism of acid-tolerant microorganisms. For example, acid-tolerant microorganisms such as *Lactobacillus* proliferate during fermentation and become dominant microorganisms. They participate in the process and release organic acids, thus increasing the acidity of *Jiupei* (Hao et al., 2021). During the fermentation process of the four-seasons-QJFJ, the moisture content increases sharply in the early stage (day 0 - day 5) of brewing, while it shows a slow fluctuating pattern in the middle and late stages (day 5 - day 10) (Fig. 1C). This reflects the phenomenon of a significant accumulation of microorganisms in the early stages of fermentation, while the dominant role of specific microorganisms leads to a minor fluctuation in the overall microbial community of the *Jiupei* in the middle to later stages (Ma et al., 2022). In this study, the moisture content of QJFJ is higher than that of *Jiang*-flavor *Jiupei* (Hao et al., 2021), suggesting that the alcohol metabolism of QJFJ remains relatively high in the later stages of fermentation in the cellar pits. QJFJ had its highest reducing sugar content in the early stages of fermentation, significantly decreasing on the 5th day ( $p < 0.05$ , Fig. 1D). However, as fermentation progresses, the content of reducing sugar in *Jiupei* gradually increases (Fig. 1D). The saccharification action of microorganisms or biological enzymes on starch in the *Jiupei* can promote the continuous accumulation of reducing sugars (Hao et al., 2021), indicating a reciprocal pattern of consumption and accumulation of reducing sugars during the fermentation process. This indirectly indicates that this stage is the period of alcohol metabolism transition. Compared to the cellar fermentation of *Jiang*- and *Qing*-flavor Baijiu, the onset of alcohol

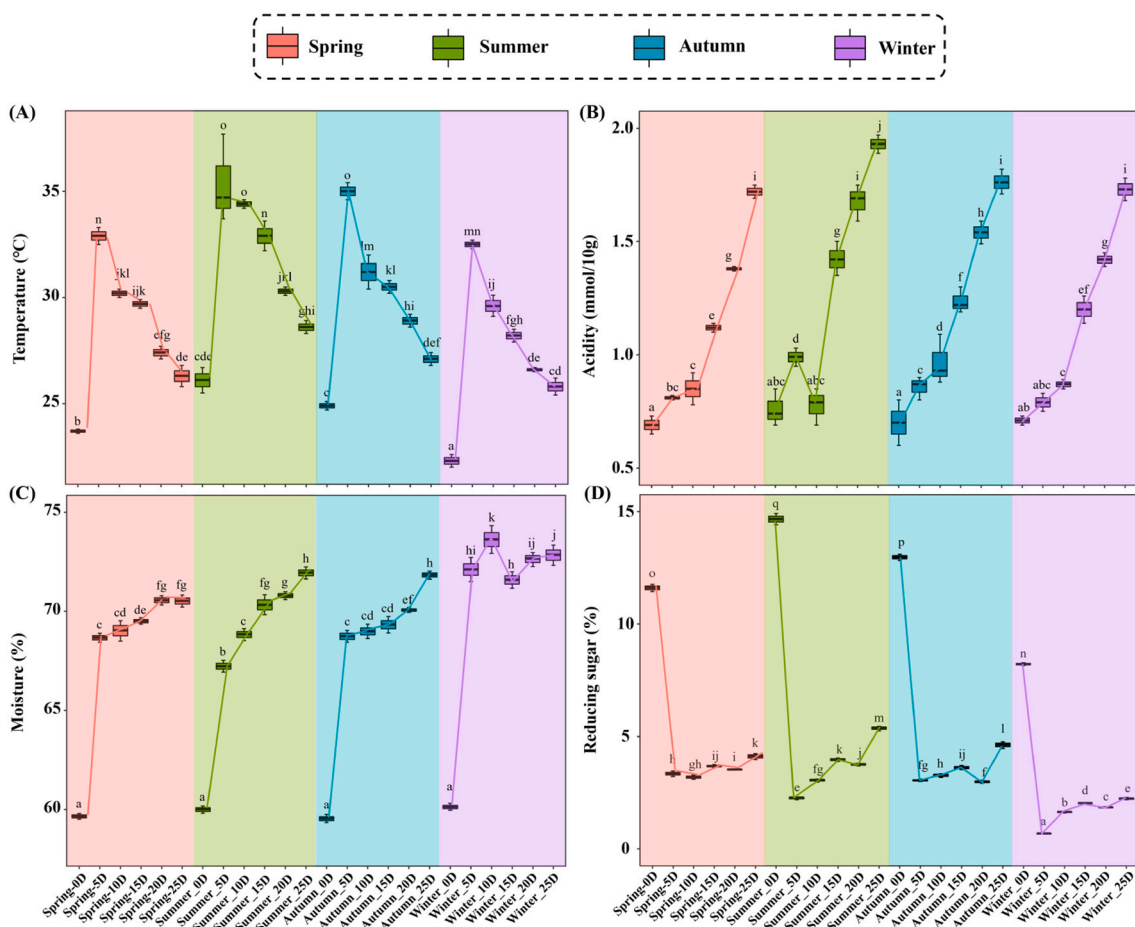


Fig. 1. The evolution of (A) temperature, (B) acidity, (C) moisture, and (D) reducing sugar of *Jiupei* during the fermentation process under different seasons. Means with different letters in figure showed significant difference at  $p < 0.05$ .

metabolism in QJFB occurs earlier. Moreover, its slow fermentation process allows for a longer duration of alcohol metabolism, forming distinct characteristics that differ from other Baijiu productions.

The fermentation temperature and moisture content of the QJFJ fluctuate significantly during the Winter brewing process, while acidity and reducing sugar content fluctuate the most during the Summer brewing process. Previous studies have indicated that the fluctuations of physicochemical characteristics during Baijiu fermentation process have a significant impact on the fermentation microecology of Baijiu (Wang, Huang, Hu, & Li, 2021). Therefore, this study conjecture that the microbial structure of QJFJ may be influenced by seasonally varying physicochemical characteristics, resulting in unique seasonal microbial community characteristics.

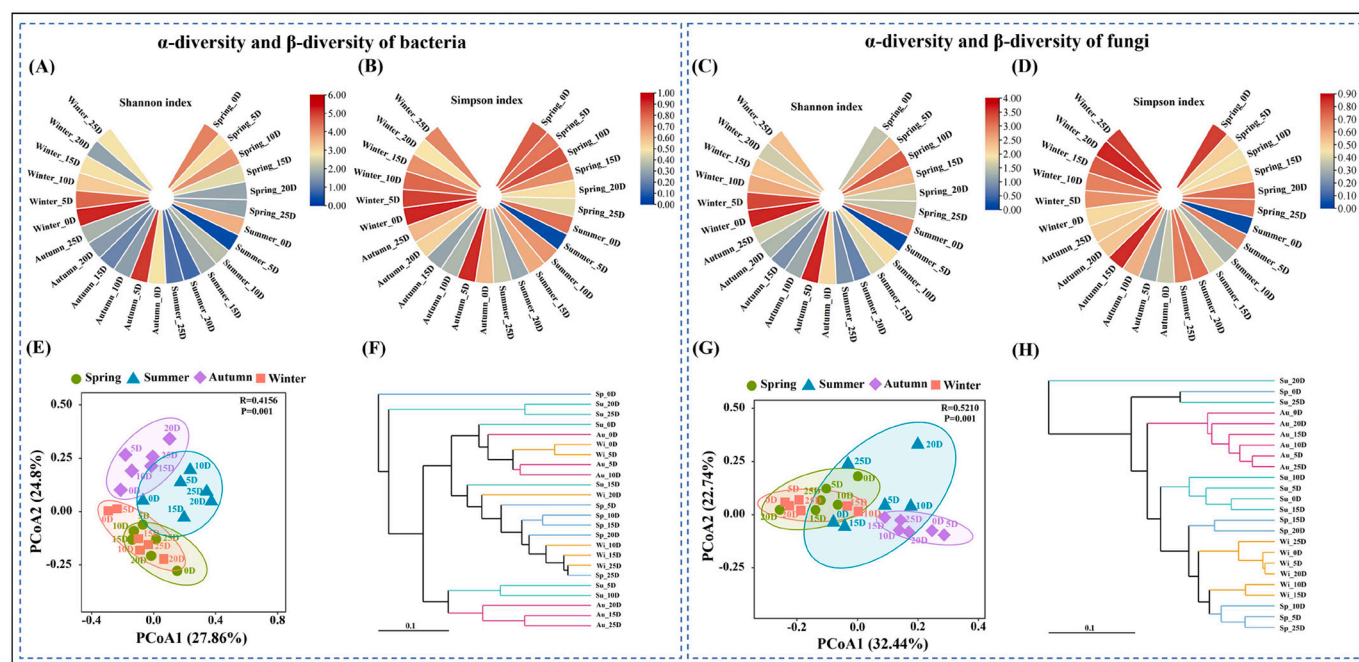
### 3.2. Analysis of $\alpha$ -diversity and $\beta$ -diversity in the microbial community

The  $\alpha$ -diversity trends of bacterial and fungal communities in QJFJ were found to be similar. Additionally, the  $\alpha$ -diversity of microbial communities in *Jiupei* during Spring and Winter was higher than that during Summer and Autumn (Fig. 2A - D, Table. S1-2). This phenomenon may be attributed to the higher temperatures experienced in Summer- and Autumn-*Jiupei*, which allow active microorganisms to transition more rapidly into fermentation modes that are regulated by dominant microbial structures. The results of  $\beta$ -diversity also indicate that the bacterial and fungal communities in QJFJ exhibit seasonal similarities (Fig. 2E - H). The similarity in microbial community structures between Spring- and Winter-*Jiupei*, as well as between Summer- and Autumn-*Jiupei*, indicated that the microbial community structure during fermentation had distinct seasonal characteristics (Fig. 2E - H). Higher diversity of bacterial communities was observed in the middle to later stages (day 10 - day 15) of Baijiu fermentation, while higher diversity of fungal communities occurred in the early to middle stages (day 0 - day 10) of the fermentation process (Fig. 2A - D). This is also direct evidence of the difference in the succession patterns of bacterial and fungal communities during the fermentation process. Bacteria (during the middle to later stage of fermentation) and fungi (during the early to

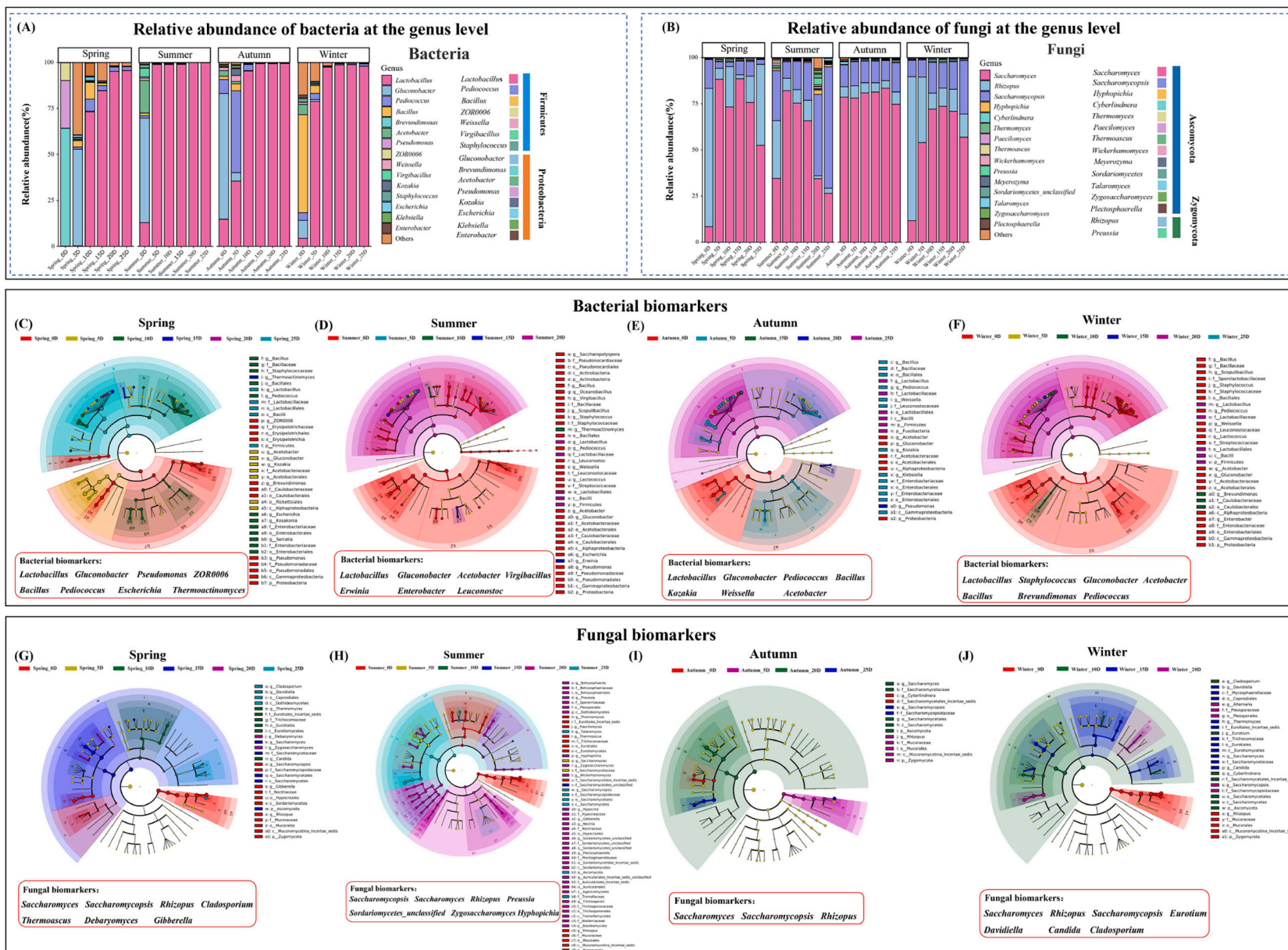
middle stage of fermentation) provide sequential fermentation impetus for the production of QJFB, regulating the flavor quality of Baijiu body by enriching hydrolytic enzymes and metabolic products (flavor compounds) (Qiao, Wang, Wang, Zhang, & Zheng, 2023).

### 3.3. Succession of microbial communities and biomarkers in QJFJ

The operational taxonomic units (OTU) number of seasonal *Jiupei* samples was calculated and showed in Fig. S2 at phylum and genus levels after clustering analysis and classification, respectively. A total of 23 bacterial phyla (Fig. S3A) and 497 bacterial genera (Fig. 3A) were detected in the QJFJ. Firmicutes and Proteobacteria were the dominant bacterial phyla in the QJFJ (Fig. 3A, Fig. S3A), which was similar to the reported results for Qing- and Jiang-flavor Baijiu (Hu et al., 2021; Wang, Du, Zhang, & Xu, 2018; Wang, Huang, Hu, & Li, 2021). As fermentation progresses, Firmicutes becomes the dominant bacterial phylum, while the abundance of Proteobacteria gradually decreases. This is influenced by the metabolic production of organic acids during the fermentation process of *Jiupei* (Li et al., 2020). This corresponds to the gradual increase in acidity observed in the *Jiupei* throughout the fermentation process in this study (Fig. 1B). *Lactobacillus* (LDA = 5.68,  $p < 0.05$ ), *Gluconobacter* (LDA = 5.44,  $p < 0.05$ ), *Pseudomonas* (LDA = 5.13,  $p < 0.05$ ), and *Bacillus* (LDA = 5.14,  $p < 0.05$ ) (Fig. 3C - F) are the bacteria with the greatest seasonal differences in the microbial community of QJFJ (bacterial biomarkers). They possess acid resistance characteristics and are functional bacteria that produce high levels of proteinase, metabolize acids, alcohols, esters, and other substances. These bacteria facilitate the effective metabolism of Baijiu flavor compounds in *Jiupei* even under the high-acid fermentation environment during the later stages of fermentation (Jin et al., 2019; Li et al., 2020). Consistent with previous research, the predominant biomarkers are mainly represented by *Lactobacillus* in the middle (day 10 - day 15) and late fermentation stages (day 20 - day 25) (Hu et al., 2021; Wang et al., 2015). While, seasonal variation was revealed in the relative abundance of *Lactobacillus*. Summer-*Jiupei* exhibited higher average (85.01%) and maximum (99.98%) abundance compared to other fermentation seasons, possibly



**Fig. 2.** The bacterial  $\alpha$ -diversity indices of *Jiupei* samples as (A) Shannon and (B) Simpson, while the fungal  $\alpha$ -diversity indices were represented by (C) Shannon and (D) Simpson. (E) Principal coordinate analysis (PCoA) and (F) Unweighted pair group method with arithmetic mean (UPGMA) were used for the analysis of bacterial community  $\beta$ -diversity during the *Jiupei* fermentation process. Similarly, the fungal community  $\beta$ -diversity was presented in (G) and (H). Sp: Spring, Su: Summer, Au: Autumn, and Wi: Winter. The R value represents the difference between-groups and within-groups, while the P value indicates the reliability of the data analysis.



**Fig. 3.** The relative abundance of (A) bacterial communities and (B) fungal communities at the genus level in the *Jiupai* fermentation process during different seasons. The evolutionary cluster was analyzed by LDA Effect Size (LEfSe) to identify the statistically significant biomarkers (LDA > 3.50) among treatment groups. Bacterial biomarkers in *Jiupai* during (C) Spring, (D) Summer, (E) Autumn, and (F) Winter; Fungal biomarkers in *Jiupai* during (G) Spring, (H) Summer, (I) Autumn, and (J) Winter. There are significant differences between biomarkers (LDA > 3.50,  $p < 0.01$ ).

attributable to the increased acidity observed in Summer-*Jiupei* (Fig. 1B). Therefore, it is recommended to closely monitor and regulate the abundance of *Lactobacillus* and the acidity of *Jiupei* during Baijiu fermentation in the Summer. This will help control the formation patterns of Baijiu flavor.

The 6 fungal phyla (Fig. S3B) and 251 fungal genera (Fig. 3B) were collectively detected in the QJFJ. Ascomycota and Zygomycota are dominant fungal phyla in the QJFJ (Fig. 3B, Fig. S3B), consistent with the microbial results of the Qing- and Jiang-flavor *Jiupei* (Hu et al., 2021; Luo et al., 2023; Wang, Huang, & Huang, 2021). *Saccharomyces* (LDA = 5.58,  $p < 0.05$ ), *Rhizopus* (LDA = 5.47,  $p < 0.05$ ) and *Saccharomycopsis* (LDA = 5.04,  $p < 0.05$ ) (Fig. 3G - J) are dominant fungal genera in the QJFJ, and they contribute the most to the fungal community differences in the QJFJ during different fermentation seasons (fungal biomarkers). *Saccharomyces* traverses through the fermentation process in different seasonal *Jiupei*, serving as the source of alcohol metabolism in solid-state brewing, capable of producing large amounts of ethanol and higher ester compounds. The abundance of *Saccharomyces* in the QJFJ was most prominent during Autumn, which contributed to the high liquor yield in Autumn (Stribny, Gamero, Pérez-Torrado, & Querol, 2015; Yan, Tong, & Guang, 2019). *Rhizopus* was a biomarker of Spring- and Winter-*Jiupei*, with higher abundance in the early stage of fermentation (day 0 - day 5). The saccharification fermentation effect of *Rhizopus* is notable (Kang et al., 2022), providing the necessary carbon source for other microbial communities. *Saccharomycopsis* was a fungal biomarker in Summer- and Autumn-*Jiupei*, possessing strong abilities to produce amylase and metabolize esters (Wang, Gao, Fan, & Xu, 2011). It makes significant contributions to the alcohol-producing capacity and flavor structure of Summer- and Autumn-QJFJ.

Seasonal difference is a characteristic of the microbial structure and succession in the *Jiupei* during the brewing process of QJFB. From the perspective of the succession of microbial community structure in the QJFJ, dominant bacterial genera (*Lactobacillus*, *Gluconobacter*) significantly enriched in Summer- and Autumn-*Jiupei*, while dominant fungal genera (*Saccharomyces*, *Rhizopus*) were notably enriched in Spring- and Autumn-*Jiupei*. In the continuous brewing process of QJFB, Autumn-*Jiupei* exhibited a rich microbial community of both bacteria and fungi, making a significant contribution to the higher alcohol yield and better quality of the Baijiu produced in the Autumn. This also aligned well with the practical results of the brewing production. It is suggested to strengthen the monitoring of microbial communities in Autumn during the fermentation process of QJFB. Real-time understanding of the microbial community structure and changes in the fermentation *Jiupei* is important to ensure the stability of Baijiu quality.

### 3.4. Microbial succession driven by physicochemical characteristics and the correlation relationship

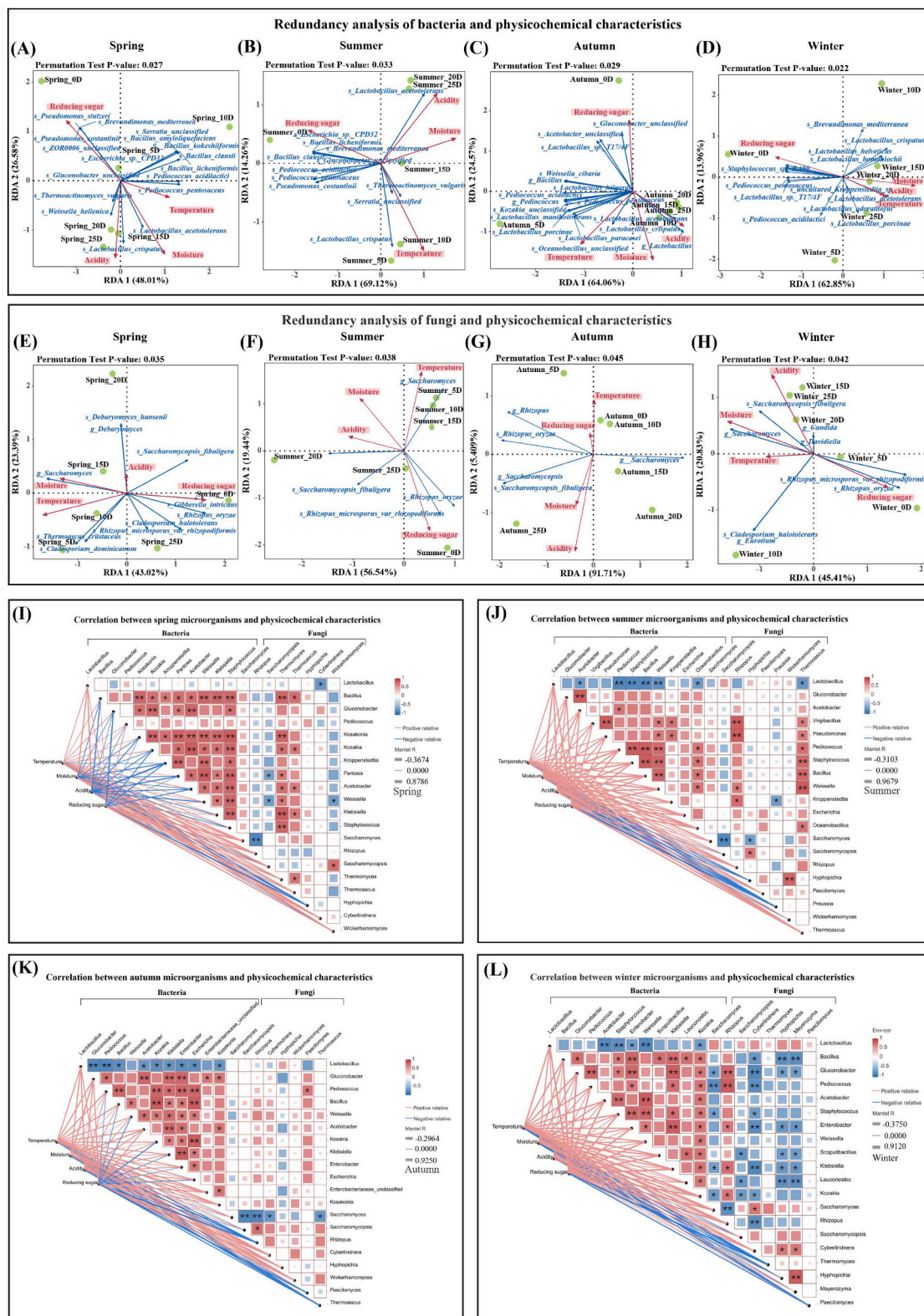
During the early and middle phases of *Jiupei* fermentation, the physicochemical characteristics affecting the structure of bacterial and fungal communities are, in sequence: reducing sugars (day 0) and temperature (day 5- day 10) (Fig. 4A - 4H). This result is mainly attributed to: (1) During the initial fermentation of QJFJ, the content of reducing sugars is the highest (Fig. 1D). Therefore, it is during this stage that the regulatory impact of reducing sugars on the growth and metabolism of microorganisms is particularly pronounced. (2) During the 5th to 10th day of fermentation of QJFJ, the temperature remains at a relatively high level (Fig. 1A). During that stage, the impact of temperature on the growth rate and metabolic activity of microorganisms was most significant.

While, in the middle and late stages of fermentation, physicochemical characteristics that regulate the structure of bacterial and fungal communities show variations (Fig. 4A - 4H). Bacteria are sequentially influenced by moisture (day 15) and acidity (day 20 - day 25), whereas fungi are sequentially influenced by acidity and temperature/acidity (day 20). From the 15th day onwards, the moisture content continues to

increase and reaches a higher level (Fig. 1C), which is closely related to the survival and interactions of microorganisms. Furthermore, the gradually increases of acidity lead to the dominant role of acid-tolerant bacteria. The growth and metabolism of non-acid-tolerant microorganisms are inhibited in the high-acidity environment. Therefore, the impact on the bacterial community structure is manifested as a transition from the regulatory role of moisture to the regulating effect of acidity. However, in comparison with the bacterial community in the *Jiupei*, the fungal community lacks acid-tolerant species (Hao et al., 2021). Thus, the regulatory effect of the acidity of the *Jiupei* on the fungal community became more prominent in the later stages of the fermentation of QJFB. The structure of the fungal community during this phase was mainly characterized by the regulation of acidity. In general, the regulation of microorganisms by physicochemical characteristics in the Baijiu fermentation process was characterized by a transition from dominance of a single factor in the early fermentation stage to overall regulation by multiple factors in the later fermentation stage. Therefore, conducting physicochemical characteristics detection becomes particularly crucial. This will aid in early warning of potential issues and optimizing process parameters, further refining the microbial community structure and metabolic activities.

During the fermentation process of Spring-QJFJ, moisture was the most important regulatory factor, playing a significant role in regulating both bacterial and fungal communities. For example, moisture showed a highly significant positive correlation with dominant biological markers (*Lactobacillus*, *Cyberlindnera*) ( $p < 0.01$ ,  $|\rho| > 0.6$ ). In primary, acidity regulates the bacterial community, showing a significant positive correlation with *Lactobacillus* ( $p < 0.05$ ,  $|\rho| > 0.6$ ). While, temperature and reducing sugars primarily regulated the fungal community, showing significant positive correlations with *Thermoascus*, *Cyberlindnera*, *Saccharomyces*, and *Rhizopus*, respectively ( $p < 0.05$ ,  $|\rho| > 0.6$ , Fig. 4I). Acidity was the most significant regulatory factor in the community structure of bacteria and fungi in the Summer-QJFJ, significantly impacting 9 bacterial genera and 2 fungal genera. For example, *Lactobacillus* (average relative abundance: 85.01%,  $p < 0.01$ ,  $|\rho| > 0.6$ ). Moisture significantly regulates 8 bacterial genera and 1 fungal genus. Temperature significantly regulated the abundance of 5 bacterial genera and 2 fungal genera. Reducing sugars played a regulatory role in 3 bacterial genera and 1 fungal genus (Fig. 4J). *Staphylococcus* showed a significant positive correlation with temperature, moisture, acidity, and reducing sugars ( $p < 0.01$ ,  $|\rho| > 0.6$ ), which was a unique characteristic of the Summer-QJFJ. Although *Staphylococcus* can promote the catabolism of branched-chain amino acids (leucine, isoleucine, and valine), thereby regulating the formation of alcohols and acids (Yang et al., 2022). However, there were also reports stating that *Staphylococcus* was a pathogenic bacterium in fermented foods (Bencardino, Amagliani, & Brandi, 2021), so its functional role in Baijiu brewing needs to be further analyzed. Therefore, when brewing QJFB in Summer, it is necessary to monitor the physicochemical characteristics of *Jiupei* in a timely manner to control the abundance of *Staphylococcus*.

During the fermentation process of the QJFB in Autumn, moisture was the most important regulatory factor, playing a crucial role in regulating both bacterial and fungal communities. It had a significant positive correlation with 3 bacterial genera and 2 fungi. Temperature and acidity only exerted significant regulatory effects on bacteria. For instance, 5 bacterial genera such as *Gluconobacter* and *Pediococcus* showed a significant positive correlation with temperature ( $p < 0.05$ ,  $|\rho| > 0.6$ ), while 3 bacterial genera, including *Lactobacillus*, exhibit an extremely significant positive correlation with acidity ( $p < 0.01$ ,  $|\rho| > 0.6$ ). Reducing sugars only regulated the fungal community. The microbial community structure of Winter-QJFJ was mainly influenced by temperature, reducing sugars and moisture (Fig. 4D). Temperature and reducing sugars regulated both bacterial and fungal communities, showing a significant positive correlation with 11 bacterial genera and 2 fungal genera. Acidity only significantly regulated the abundance of *Saccharomycopsis* ( $p < 0.05$ ,  $|\rho| > 0.6$ ).



**Fig. 4.** Redundancy analysis of major bacteria in *Jiubei* during (A) Spring, (B) Summer, (C) Autumn, and (D) Winter with respect to temperature, acidity, moisture, and reducing sugar; Redundancy analysis of major fungal in *Jiubei* during (E) Spring, (F) Summer, (G) Autumn, and (H) Winter with respect to temperature, acidity, moisture, and reducing sugar. The relationships are indicated by the length and angle of the arrows, with the values of axis 1 and axis 2 representing the respective axis percentages. All data passed the permutation test with a  $p < 0.05$ . Mantel test of major bacterial and fungal with physicochemical characteristics during (I) Spring, (J) Summer, (K) Autumn, and (L) Winter. The figure displays the correlation between the relative abundance of bacterial and fungal genera, represented by a color gradient. The R value represents the strength of the correlation between two correlation matrices. The R closer to 1 indicates a highly positive correlation, while the R closer to -1 indicates a highly negative correlation. \*:  $p < 0.05$ , \*\*:  $p < 0.01$ .

The impact of physicochemical characteristics in the fermentation environment on the microbial community structure was primarily manifested through intervening in the growth and metabolism of microorganisms. Microorganisms in the complex fermentation environment engage in mutual cooperation and competition, thereby establishing close connections that influence the trends in microbial community structure and their roles in the fermentation process (Li et al., 2024). Under the influence of seasonal drivers, the microbial community structure during the brewing process of QJFJ exhibited seasonal differences, displaying characteristics sequentially regulated by reducing sugars, temperature, moisture, and acidity. Seasonal differences manifested as moisture being the primary factor regulating microbial communities in Spring and Autumn, while acidity and temperature played a more significant role in regulating microbial communities during Summer and Winter.

Driven by physicochemical characteristics, totally 57 pairs of significant correlations ( $p < 0.05$ ,  $|\rho| > 0.6$ ) were identified from the microbial community in the Spring-*Jiupei* (Fig. 4I, Fig. S4). Among which, the most prominent is that 38 pairs of significant positive correlations (66.67%,  $p < 0.05$ ,  $|\rho| > 0.6$ ) existed within the bacterial community. The interactions among the microbes in the Summer-*Jiupei* were the weakest among the four-seasons-*Jiupei*, with a total of 42 pairs of significant correlations identified (Fig. 4J, Fig. S4). Among them, the most significant were still the positive correlations between bacteria (17 pairs, 40.48%,  $p < 0.05$ ,  $|\rho| > 0.6$ ). A total of 46 pairs of significant correlations were identified within the microbial community of the Autumn-*Jiupei*. Different from the Spring- and Summer-*Jiupei*, both the positive and negative correlations among bacteria were prominent in the Autumn-*Jiupei* (Fig. 4K, Fig. S4): 31 pairs of significant positive correlations (47.39%,  $p < 0.05$ ,  $|\rho| > 0.6$ ) and 9 pairs of significant negative correlations (19.57%,  $p < 0.05$ ,  $|\rho| > 0.6$ ) between the bacterial community. The interactions within the microbial community of the Winter-*Jiupei* are the most intricate among the four-seasons-*Jiupei*, totally 72 pairs of significant correlations were identified (Fig. 4L, Fig. S4): the most notable are the 30 pairs of significant positive correlations between bacteria (41.67%,  $p < 0.05$ ,  $|\rho| > 0.6$ ) and the 27 pairs of significant negative correlations between bacteria and fungi (37.5%,  $p < 0.05$ ,  $|\rho| > 0.6$ ).

Under the driving force of physicochemical characteristics, the interactions between microbial communities in *Jiupei* of different seasons were mainly dominated by bacterial interactions. However, there were significant differences in the interaction intensity between microbial communities in different seasons QJFJ. The interaction among microorganisms was stronger in the hypothermal Winter- and Spring-*Jiupei* compared to the hyperthermal Summer- and Autumn-*Jiupei*. It indicated that the microbial community structure stability of the QJFJ in Winter and Spring is higher than that in Summer and Autumn. This was corroborated by the higher microbial diversity observed in the fermentation of *Jiupei* in Spring and Winter (Fig. 2). Highly diverse and closely interconnected microbial communities possess greater resistance to disturbances and metabolic capabilities, allowing them to rapidly adapt to changes in environmental physicochemical characteristics while maintaining normal metabolic functions (Li et al., 2024).

The symbiotic microorganisms in *Jiupei* not only maintained interactions between microbes but also promoted the production of flavor compounds and drive the entire fermentation process. For instance, *Saccharomyces*, known for its acid tolerance and recognized as a crucial ethanol-producing strain, is widely present in Baijiu production. When fermenting in cellars, it primarily metabolizes ethanol (Sukpipat, Komeda, Prasertsan, & Asano, 2016), playing a pivotal regulatory role in flavor formation (Lu, Wu, Zhang, & Xu, 2015). *Lactobacillus*, as a core functional microorganism in acidification, produces lactic acid, ethanol, and acetic acid through heterolactic fermentation (Song, Du, Zhang, & Xu, 2017). Previous studies on fermented products found that *Saccharomyces* and *Lactobacillus* can jointly drive the development of microbial communities (Bourrie, Willing, & Cotter, 2016). In conclusion, the

formation of the QJFB was the result of the combined action of physicochemical characteristics and microorganisms. Studying the interactions between physicochemical characteristics and microorganisms in *Jiupei* during different seasons will provide guiding significance for the actual production of Baijiu.

### 3.5. Changes of flavor compounds in seasonal QJFJ

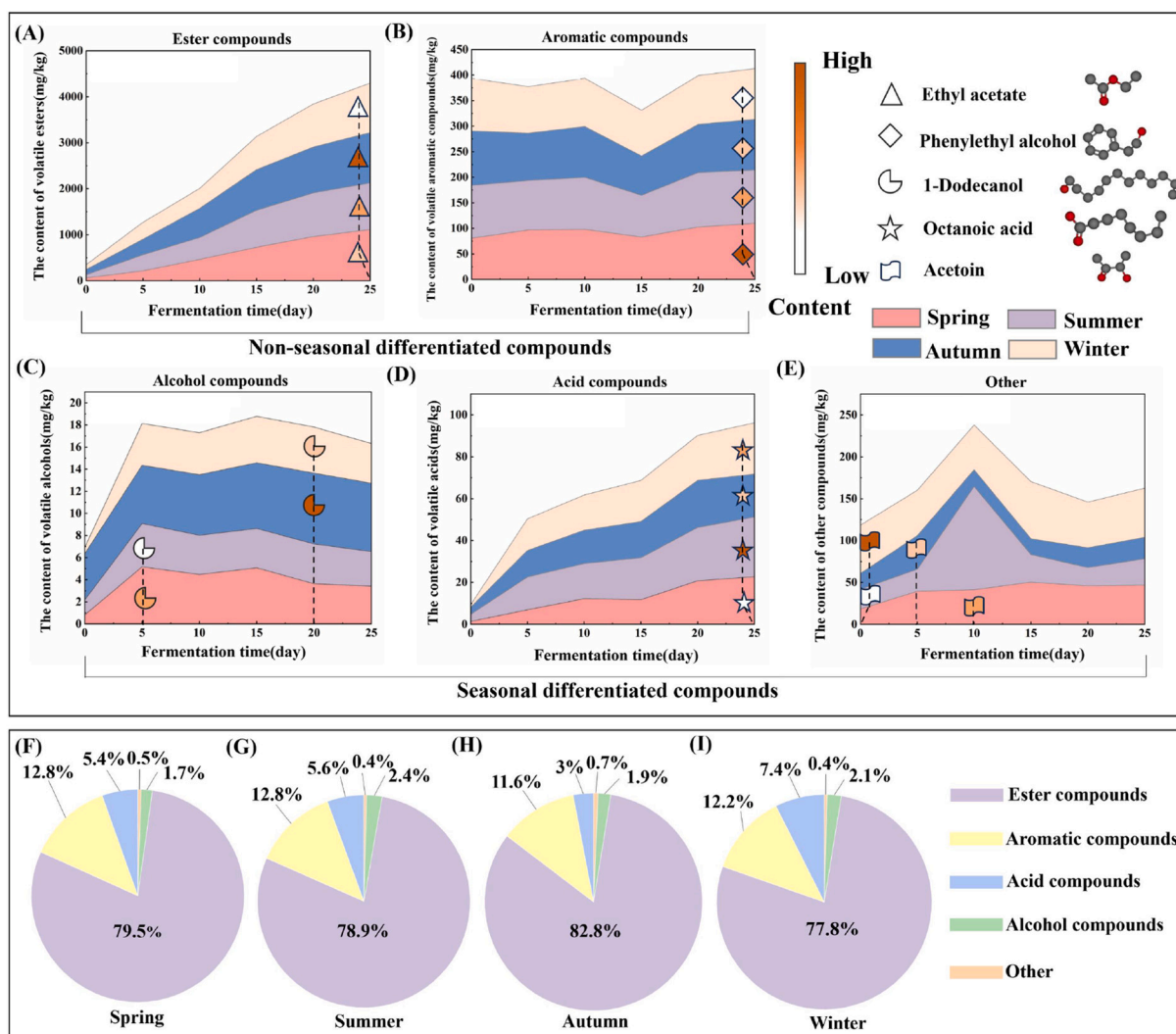
The structural characteristics of the main functional flavor compounds in the QJFB were as follows: esters had the highest content (27 types, 77.8% - 82.8%), followed by aromatic compounds (9 types, 11.6% - 12.8%), acid compounds (5 types, 3% - 7.4%), and alcohol compounds (7 types, 1.7% - 2.4%) (Fig. 5). This differs from Qing-flavor Baijiu (in the order of esters, organic acids, alcohols, carbonyl compounds, furans, and pyridines) (Wei et al., 2020), and Jiang-flavor Baijiu (the content of organic acids, aldehydes, ketones, alcohols, amino compounds, and nitrogen compounds was high, whereas the content of ester compounds was low) (Wei et al., 2020). The characteristic flavor structure was not only related to the unique production process of QJFB, but also closely linked to the microbial communities with geographical dependence in its production region. The production region of QJFB is located at latitude 25°N, which is lower than the production regions of Qing-flavor Baijiu (latitude 36°N - 37°N) and Jiang-flavor Baijiu (latitude 27°N - 28°N) (Tan et al., 2022). The resulting microbial communities with geographical dependence played an important role in contributing to the unique flavor structure of QJFB.

Ester compounds were the most abundant volatile flavor compounds in the QJFJ (Fig. 5), primarily including ethyl acetate (282.65–346.27 mg/kg), ethyl lactate (334.96–344.73 mg/kg), and ethyl propionate (57.35–69.38 mg/kg), among others. Previous studies have pointed out that the ratio of ethyl acetate, ethyl lactate, ethyl hexanoate, and ethyl butyrate had a significant impact on the flavor of Baijiu (Wei et al., 2020). The ratio of these esters in QJFB (59.06: 54.73: 8.81: 1.00) falls between Jiang-flavor Baijiu (4.04: 4.19: 1.86: 1.00) and Qing-flavor Baijiu (171: 157: 0.45: 1.00) (Xu et al., 2022), forming the unique ester flavor structure of QJFB. However, the content of ester compounds in four-seasons-*Jiupei* showed a trend of increasing gradually as the fermentation proceeded. This was because most of the ester compounds are formed through esterification reactions between fatty acids and alcohol compounds present in the brewing raw materials after fermentation and aging (Kruis et al., 2017). In addition, there was no significant difference in the content of main ester compounds in the QJFJ during different seasons (Fig. 5A, F-I), indicating that ester compounds were not the main factor causing seasonal differences in the flavor characteristics of QJFJ.

Phenylethyl alcohol, 4-ethylguaiacol, and 4-hydroxy-3-methoxystyrene were the main aromatic compounds during the brewing process of QJFJ in different seasons. The content of aromatic compounds exhibited a fluctuating trend in the four-seasons-*Jiupei*, but there was no significant difference in their total content at the end of fermentation (Fig. 5B, F-I). Phenylethyl alcohol is the aromatic compound with the highest content in the QJFJ. Similarly, it was also a characteristic flavor substance in Qing- and Jiang-flavor Baijiu (Zha, Sun, Wu, Yin, & Wang, 2018). Phenylethyl alcohol has a higher content during the fermentation process of the Autumn-*Jiupei* ( $71.38 \pm 0.65$  mg/kg), which was related to the presence of *Saccharomyces* that produces high levels of phenethyl alcohol in the Autumn-*Jiupei* (Fig. 3B) (Zha et al., 2018). The content of 4-ethylguaiacol was relatively high at the initial stage (day 0) of Winter-*Jiupei* fermentation. This was mainly driven by the high abundance of *Bacillus* at this stage (Fig. 3A), as *Bacillus* can convert ferulic acid in the brewing materials into 4-ethylguaiacol via a decarboxylation reaction (Sun, Lv, Yu, Li, & He, 2018).

The total content of alcohol compounds in QJFJ (mainly including 2,3-butanediol, 2-octanol, 1-dodecanol, and 3-methyl-1-butanol) showed seasonal variations, with the content ranking from high to low as follows: Autumn-, Spring-, Winter-, and Summer-*Jiupei* (Fig. 5C).





**Fig. 5.** Trends in the content variations of (A) esters, (B) aromatic compounds, (C) alcohols, (D) acids, and (E) other compounds in *Jiupai* across four seasons. Different shapes represent different compounds, and colors from dark to light represent compound content from high to low. The labeled positions indicate the fermentation time points with the highest content of the respective compounds. Distribution of flavor compound content in *Jiupai* during (F) Spring, (G) Summer, (H) Autumn, and (I) Winter.

Research indicates that the higher alcohol 3-methyl-1-butanol significantly contributed to the flavor of Baijiu (He et al., 2021). Thus, although the content of 3-Methyl-1-butanol was relatively low in the QJFJ (in the order of Autumn > Winter > Spring > Summer), it played a crucial role in regulating and balancing the overall flavor. This suggested that the more harmonious flavor of Autumn Baijiu was related to 3-Methyl-1-butanol. The acid compounds mainly found in the QJFJ include octanoic acid (8.24–9.53 mg/kg), acetic acid (7.49–9.23 mg/kg), capric acid (3.55–5.57 mg/kg), 3-methylvaleric acid (0.43–3.84 mg/kg), and hexanoic acid (0.26–0.35 mg/kg). The content of acid compounds detected in *Jiupai* was highest in Summer, followed by Winter, Autumn, and Spring (Fig. 5D). The content of acid compounds in different seasons gradually increased as fermentation progresses (Fig. 5D), which was positively correlated with the trend of gradually increasing acidity during the fermentation process of the *Jiupai* (Fig. 1B). Thus, by controlling the acidity level during the fermentation process of *Jiupai*, it was possible to achieve targeted and controllable levels of acidic compounds in the final product of Baijiu, which was particularly important for the flavor characteristics of Baijiu. In the brewing process of QJFB, flavor compounds such as tetramethylpyrazine, 2,3,5-trimethylpyrazine, acetoin, octanal, acetaldehyde, furfural, hexanal, and cyclohexanone were also detected in *Jiupai*. In general, changes in the

brewing season had a significant impact on the metabolism of flavor substances in the QJFJ. This result was closely associated with the physicochemical characteristics and microbial community structure characteristics of *Jiupai* in different seasons.

### 3.6. Analysis of the correlation between seasonal differential flavor compounds and microbial community

The accumulation of flavor compounds was usually driven and regulated by the metabolism of microorganisms in the fermentation *Jiupai* system (Jin et al., 2017). The seasonal variation in the content of alcohol compounds was mainly due to differences in the relative abundance of *Saccharomyces* in *Jiupai* and variations in fermentation temperatures during different seasons. The analysis results showed that the abundance of *Saccharomyces* showed a significantly positive correlation with the content of 2-Octanol, 1-Dodecanol, and Furfuryl alcohol ( $p < 0.01$ ,  $|\rho| > 0.6$ ), and a significant positive correlation with the content of 2-Butanol, 1-Propanol, and 3-Methyl-1-butanol ( $p < 0.05$ ,  $|\rho| > 0.6$ ). Highlighted the contribution of *Saccharomyces* to the accumulation of these alcohol compounds (Fig. 6A-D). However, although the seasonal differences in the relative abundance of *Saccharomyces* (relative abundance of *Saccharomyces*: Autumn > Spring > Winter > Summer) had an

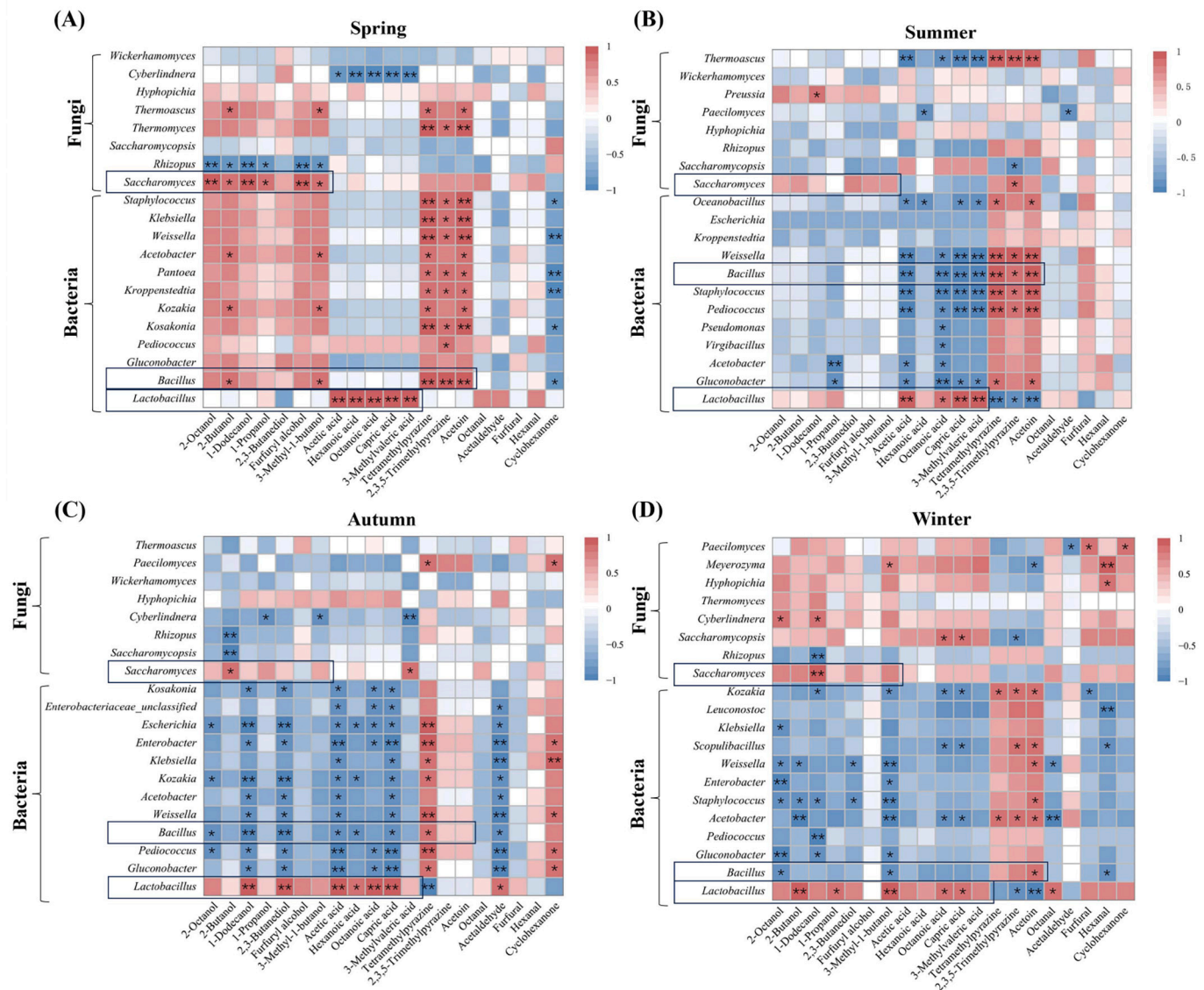


Fig. 6. Correlation analysis of major microbials with seasonal differential flavor compounds in *Jiupei* during (A) Spring, (B) Summer, (C) Autumn, and (D) Winter. \*:  $p < 0.05$ , \*\*:  $p < 0.01$ .

impact, the content of alcohol compounds followed the same changing pattern. The Mantel test analysis revealed a significant positive correlation between the relative abundance of *Saccharomyces* in *Jiupei* during Summer and Winter with temperature ( $p < 0.05$ ,  $|\rho| > 0.6$ , Fig. 4J, L). The average temperatures in *Jiupei* during Summer and Winter were  $31.28 \pm 3.58$  °C and  $27.50 \pm 3.48$  °C, respectively (Fig. 1A), which deviated from the optimal temperature range for *Saccharomyces* (30 °C) (Zha et al., 2018). From this, it indicated that the ability of *Saccharomyces* to metabolize and produce flavor compounds in the Summer- and Winter-*Jiupei* was weaker compared to that in Autumn- and Spring-*Jiupei*.

The main reason for the differences in the content of acid compounds between seasons was the variation in the relative abundance of *Lactobacillus* and acidity in *Jiupei* during different seasons. *Lactobacillus* metabolized fermentable sugars into organic acids, which were then enriched and converted into acid compounds in *Jiupei* (Wang, Huang, Hu, & Li, 2021). In addition, the relative abundance of *Lactobacillus* was significantly positively correlated with the content of acetic acid, hexanoic acid, octanoic acid, capric acid, and 3-Methylvaleric acid, further confirming this result ( $p < 0.01$ ,  $|\rho| > 0.6$ , Fig. 6A - D). The Mantel test analysis revealed that the relative abundance of *Lactobacillus* was

significantly positively correlated with acidity in the Spring-, Summer-, and Autumn-*Jiupei* ( $p < 0.05$ ,  $|\rho| > 0.6$ , Fig. 4). Additionally, the acidity of the *Jiupei* in Summer was greater than that in Autumn and Spring (Fig. 1B), confirming that the content of acid compounds in the *Jiupei* during Summer was higher than in Autumn and Spring.

The seasonal differences in the presence of tetramethylpyrazine, 2,3,5-trimethylpiperazine, and acetoin in the QJFJ were as follows: Winter > Spring > Autumn > Summer. Tetramethylpyrazine, 2,3,5-trimethylpiperazine, and acetoin were metabolites produced by *Bacillus* (Liu et al., 2023). They show significant ( $p < 0.05$ ,  $|\rho| > 0.6$ ) or highly significant ( $p < 0.01$ ,  $|\rho| > 0.6$ ) positive correlations with *Bacillus* in four-seasons-*Jiupei* (Fig. 6A - D). The seasonal variation trends in their levels were consistent with the changes in the relative abundance of *Bacillus* (Fig. 3A). The relative abundance of *Bacillus* in the Winter-*Jiupei* showed a significant positive correlation with the content of reducing sugars ( $p < 0.05$ ,  $|\rho| > 0.6$ , Fig. 4L), and the ample reducing sugar matter in the Winter-*Jiupei* significantly drove the growth and metabolism of *Bacillus*. The results indicated that the differences in the relative abundance of *Bacillus* in the fermentation *Jiupei* and the significant driving effect of reducing sugars on the growth and metabolism of *Bacillus* in different seasons were the primary reasons for the seasonal variations in

tetramethylpyrazine, 2,3,5-trimethylpiperazine, and acetoin.

#### 4. Conclusion

This study indicated that the microbial communities and the flavor profile in QJFJ exhibited distinct characteristics during different fermentation seasons. Moisture was the primary factor regulating the microbial community of Spring- and Autumn-QJFJ, while acidity and temperature played a more significant role in the regulation of Summer- and Winter-QJFJ. During Summer- and Autumn-Jiupi, dominant bacteria such as *Lactobacillus* and *Gluconobacter* were significantly enriched, whereas dominant fungi like *Saccharomyces* and *Rhizopus* were mainly enriched during Spring- and Autumn-Jiupi. Furthermore, the high content of ester compounds, along with low levels of aromatic, acidic, and alcoholic compounds, characterizes the QJFB. Under the regulation of *Saccharomyces*, *Lactobacillus*, and *Bacillus*, as well as temperature, acidity, and reducing sugars, alcohol and acid compounds manifested as seasonal differential compounds in the QJFJ. The results of this study on seasonal brewing microbiota and flavor profile could provide a targeted control formula for continuous brewing in the Baijiu industry.

#### CRedit authorship contribution statement

**Lijuan Lu:** Writing – original draft, Data curation. **Qiancheng Zuo:** Writing – review & editing. **Yuxin Cheng:** Resources, Methodology, Funding acquisition. **Yongguang Huang:** Writing – review & editing, Methodology, Investigation, Funding acquisition.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Data availability

Data will be made available on request.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fochx.2024.101392>.

#### References

- Bencardino, D., Amagliani, G., & Brandi, G. (2021). Carriage of *Staphylococcus aureus* among food handlers: An ongoing challenge in public health. *Food Control*, 130, Article 108362. <https://doi.org/10.1016/j.foodcont.2021.108362>
- Bourrie, B. C. T., Willing, B. P., & Cotter, P. D. (2016). The microbiota and health promoting characteristics of the fermented beverage kefir. *Frontiers in Microbiology*, 7, 647. <https://doi.org/10.3389/fmicb.2016.00647>
- Gong, J. X., Ma, Y., Li, L. L., Cheng, Y. X., & Huang, Y. G. (2023). Comparative characterization and contribution of key aroma compounds in the typical base liquor of Jiang-flavor Baijiu from different distributions in the Chinese Chishui River basin. *Food Chemistry: X*, 20, Article 100932. <https://doi.org/10.1016/j.fochx.2023.100932>
- Hao, F., Tan, Y. W., Lv, X. B., Chen, L. Q., Yang, F., Wang, H. Y., ... Xu, Y. (2021). Microbial community succession and its environment driving factors during initial fermentation of Maotai-flavor Baijiu. *Frontiers in Microbiology*, 12, Article 669201. <https://doi.org/10.3389/fmicb.2021.669201>
- He, F., Duan, J. W., Zhao, J. W., Li, H. H., Sun, J. Y., Huang, M. Q., & Sun, B. G. (2021). Different distillation stages Baijiu classification by temperature-programmed headspace-gas chromatography-ion mobility spectrometry and gas chromatography-olfactometry-mass spectrometry combined with chemometric strategies. *Food Chemistry*, 365, Article 130430. <https://doi.org/10.1016/j.foodchem.2021.130430>
- Hu, Y. N., Huang, X. N., Yang, B., Zhang, X., Han, Y., Chen, X. X., & Han, B. Z. (2021). Contrasting the microbial community and metabolic profile of three types of light-flavor Daqu. *Food Bioscience*, 44, Article 101395. <https://doi.org/10.1016/j.fbio.2021.101395>
- Jin, G. Y., Zhu, Y., & Xu, Y. (2017). Mystery behind Chinese liquor fermentation. *Trends in Food Science & Technology*, 63, 18–28. <https://doi.org/10.1016/j.tifs.2017.02.016>
- Jin, Y., Li, D. Y., Ai, M., Tang, Q. X., Huang, J., Ding, X. F., ... Zhou, R. Q. (2019). Correlation between volatile profiles and microbial communities: A metabonomic approach to study Jiang-flavor liquor Daqu. *Food Research International*, 121, 422–432. <https://doi.org/10.1016/j.foodres.2019.03.021>
- Kang, J. M., Zheng, X. W., Yang, X., Li, H. R., Cheng, J., Fan, L., ... Han, B. Z. (2022). Contrasting summer versus winter dynamic microbial communities and their environmental driving factors in the solid-state saccharification process of Fuyu-flavor Baijiu. *Food Research International*, 154, Article 111008. <https://doi.org/10.1016/j.foodres.2022.111008>
- Kruis, A. J., Levisson, M., Mars, A. E., van der Ploeg, M., Garcés Daza, F., Ellena, V., ... Weusthuis, R. A. (2017). Ethyl acetate production by the elusive alcohol acetyltransferase from yeast. *Metabolic Engineering*, 41, 92–101. <https://doi.org/10.1016/j.ymben.2017.03.004>
- Li, K. M., Chen, Y. R., Liu, T., Deng, M. F., Xu, Z. W., Fu, G. M., ... Zheng, F. P. (2020). Analysis of spatial distribution of bacterial community associated with accumulation of volatile compounds in Jiupi during the brewing of special-flavor liquor. *LWT-Food Science and Technology*, 130, Article 109620. <https://doi.org/10.1016/j.lwt.2020.109620>
- Li, M. L., Lao, F., Pan, X., Yuan, L., Zhang, D. H., & Wu, J. H. (2024). Insights into the mechanisms driving microbial community succession during pepper fermentation: Roles of microbial interactions and endogenous environmental changes. *Food Research International*, 179, Article 114033. <https://doi.org/10.1016/j.foodres.2024.114033>
- Liu, Y. B., Li, M. K., Hong, X. F., Li, H. D., Huang, R. N., Han, S., ... Pan, C. M. (2023). Screening and identification of high yield tetramethylpyrazine strains in Nongxiangxing liquor Daqu and study on the mechanism of tetramethylpyrazine production. *Journal of the Science of Food and Agriculture*, 104, 4. <https://doi.org/10.1002/jsfa.12773>
- Lu, X. W., Wu, Q., Zhang, Y., & Xu, Y. (2015). Genomic and transcriptomic analyses of the Chinese Maotai-flavored liquor yeast MT1 revealed its unique multi-carbon co-utilization. *BMC Genomics*, 16, 1064. <https://doi.org/10.1186/s12864-015-2263-0>
- Luo, L. J., Song, L., Han, Y., Zhen, P., Han, D. Y., Zhao, X., ... Bai, F. Y. (2023). Microbial communities and their correlation with flavor compound formation during the mechanized production of light-flavor Baijiu. *Food Research International*, 172, Article 113139. <https://doi.org/10.1016/j.foodres.2023.113139>
- Ma, S. Y., Luo, H. B., Zhao, D., Qiao, Z. W., Zheng, J., An, M. Z., & Huang, D. (2022). Environmental factors and interactions among microorganisms drive microbial community succession during fermentation of Nongxiangxing daqu. *Bioresource Technology*, 345, Article 126549. <https://doi.org/10.1016/j.biortech.2021.126549>
- Ma, Y., & Huang, Y. G. (2019). Volatile components and aroma characteristics of Fen-Maotai-Flavored Liquor. *Food Science*, 40, 241–248. <https://doi.org/10.7506/spkx1002-6630-20180730-363>
- Qiao, L. N., Wang, J., Wang, R. F., Zhang, N., & Zheng, F. P. (2023). A review on flavor of Baijiu and other world-renowned distilled liquors. *Food Chemistry: X*, 20, Article 100870. <https://doi.org/10.1016/j.fochx.2023.100870>
- Song, Z. W., 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. <https://doi.org/10.3389/fmicb.2017.01294>
- Stribny, J., Gamero, A., Pérez-Torrado, R., & Querol, A. (2015). *Saccharomyces kudriavzevii* and *Saccharomyces uvarum* differ from *Saccharomyces cerevisiae* during the production of aroma-active higher alcohols and acetate esters using their amino acidic precursors. *International Journal of Food Microbiology*, 205, 41–46. <https://doi.org/10.1016/j.ijfoodmicro.2015.04.003>
- Sukpipat, W., Kameda, H., Prasertsan, P., & Asano, Y. (2016). Purification and characterization of xylitol dehydrogenase with L-arabitol dehydrogenase activity from the newly isolated pentose-fermenting yeast *Meyerozyma caribbica* 5XY2. *Journal of Bioscience and Bioengineering*, 123, 20–27. <https://doi.org/10.1016/j.jbiosc.2016.07.011>
- Sun, L. H., Lv, S. W., Yu, F., Li, S. N., & He, L. Y. (2018). Biosynthesis of 4-vinylguaiacol from crude ferulic acid by *Bacillus licheniformis* DLF-17056. *Journal of Biotechnology*, 281, 144–149. <https://doi.org/10.1016/j.jbiotec.2018.07.021>
- Tan, Y. W., Du, H., Zhang, H. X., Fang, C., Jin, G. Y., Chen, S., ... Xu, Y. (2022). Geographically associated fungus-bacterium interactions contribute to the formation of geography-dependent flavor during high-complexity spontaneous fermentation. *Microbiology Spectrum*, 10, Article 01844-22. <https://doi.org/10.1128/spectrum.01844-22>
- Tan, Y. W., Zhong, H. P., 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, Article 108350. <https://doi.org/10.1016/j.ijfoodmicro.2019.108350>
- Wang, H., Huang, Y. G., & Huang, Y. L. (2021). Microbiome diversity and evolution in stacking fermentation during different rounds of Jiang-flavoured Baijiu brewing.

- LWT- Food Science and Technology, 143, Article 111119. <https://doi.org/10.1016/j.lwt.2021.111119>
- Wang, H. Y., Gao, Y. B., Fan, Q. W., & Xu, Y. (2011). Characterization and comparison of microbial community of different typical Chinese liquor Daqus by PCR-DGGE. *Letters in Applied Microbiology*, 53, 134–140. <https://doi.org/10.1111/j.1472-765X.2011.03076.x>
- Wang, L., Huang, Y. G., Hu, X. X., & Li, Y. Y. (2021). The impact of environmental factors on the environmental bacterial diversity and composition in the Jiang-flavoured Baijiu production region. *LWT- Food Science and Technology*, 149, Article 111784. <https://doi.org/10.1016/j.lwt.2021.111784>
- Wang, L., Wang, Y. Y., Wang, D. Q., Xu, J., Yang, F., Liu, G., ... Jin, T. (2015). Dynamic changes in the bacterial community in Moutai liquor fermentation process characterized by deep sequencing. *Journal of the Institute of Brewing*, 121, 603–608. <https://doi.org/10.1002/jib.259>
- Wang, S. L., Xiong, W., Wang, Y. Q., Nie, Y., Wu, Q., Xu, Y., ... Lindemann, S. R. (2020). Temperature-induced annual variation in microbial community changes and resulting metabolome shifts in a controlled fermentation system. *mSystems*, 5, 00555-20. <https://doi.org/10.1128/mSystems.00555-20>
- Wang, W. H., Xu, Y. Q., Huang, H. Q., Pang, Z. M., Fu, Z. L., Niu, J. L., ... Sun, B. G. (2021). Correlation between microbial communities and flavor compounds during the fifth and sixth rounds of sauce-flavor Baijiu fermentation. *Food Research International*, 150, Article 110741. <https://doi.org/10.1016/j.foodres.2021.110741>
- Wang, X. S., Du, H., Zhang, Y., & Xu, Y. (2018). Environmental microbiota drives microbial succession and metabolic profiles during Chinese liquor fermentation. *Applied and Environmental Microbiology*, 84, 02369-17. <https://doi.org/10.1128/AEM.02369-17>
- Wei, H. W., Wang, L. H., Hassan, M. H., & Xie, B. (2018). Succession of the functional microbial communities and the metabolic functions in maize straw composting process. *Bioresource Technology*, 256, 333–341. <https://doi.org/10.1016/j.biortech.2018.02.050>
- Wei, Y., Zou, W., Shen, C. H., & Yang, J. G. (2020). Basic flavor types and component characteristics of Chinese traditional liquors: A review. *Journal of Food Science*, 85, 4096–4107. <https://doi.org/10.1111/1750-3841.15536>
- Xu, Y. Q., Zhao, J. G., Liu, X., Zhang, C. S., Zhao, Z. G., Li, X. T., & Sun, B. G. (2022). Flavor mystery of Chinese traditional fermented Baijiu: The great contribution of ester compounds. *Food Chemistry*, 369, Article 130920. <https://doi.org/10.1016/j.foodchem.2021.130920>
- Yan, S. B., Tong, Q. Q., & Guang, J. Q. (2019). Yeast dynamics and changes in volatile compounds during the fermentation of the traditional Chinese strong-flavor Daqu. *LWT- Food Science and Technology*, 106, 57–63. <https://doi.org/10.1016/j.lwt.2019.02.058>
- Yang, P., Zhong, G. X., Yang, J. C., Zhao, L. Y., Sun, D., Tian, Y. Q., ... Rong, L. Y. (2022). Metagenomic and metabolomic profiling reveals the correlation between the microbiota and flavor compounds and nutrients in fermented sausages. *Food Chemistry*, 375, Article 131645. <https://doi.org/10.1016/j.foodchem.2021.131645>
- Zha, M. S., Sun, B. G., Wu, Y. P., Yin, S., & Wang, C. T. (2018). Improving flavor metabolism of *Saccharomyces cerevisiae* by mixed culture with *Wickerhamomyces anomalus* for Chinese Baijiu making. *Journal of Bioscience and Bioengineering*, 126, 189–195. <https://doi.org/10.1016/j.jbiosc.2018.02.010>
- Zhao, C. Q., Yan, X. L., Yang, S. T., & Chen, F. F. (2017). Screening of *Bacillus* strains from Luzhou-flavor liquor making for high-yield ethyl hexanoate and low-yield propanol. *LWT- Food Science and Technology*, 77, 60–66. <https://doi.org/10.1016/j.lwt.2016.11.035>
- Zheng, X. W., & Han, B. Z. (2016). Baijiu, Chinese liquor: History, classification and manufacture. *Journal of Ethnic Foods*, 3, 19–25. <https://doi.org/10.1016/j.jef.2016.03.001>
- Zuo, Q. C., Huang, Y. G., & Guo, M. (2020). Evaluation of bacterial diversity during fermentation process: A comparison between handmade and machine-made high-temperature Daqu of Maotai-flavor liquor. *Annals of Microbiology*, 70, 1–10. <https://doi.org/10.1186/s13213-020-01598-1>