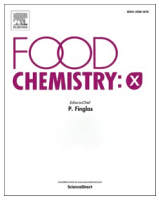
Food Chemistry: X 26 (2025) 102307

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



Multi-omics analysis of the dynamic changes in aroma compounds and microbial communities during the fermentation of Shanxi broomcorn millet Huangjiu

Qi Li [a,1], Linhua Cui [a,1], Jiaying Zhu [a], Ting Zhang [a], Guoqiang Gao [b], Yunlong Li [a,*]

[a] Shanxi Institute for Functional Food, Shanxi Agricultural University, Taiyuan 030000, China
[b] Shanxi Daixian Guixi Wine Co., Ltd., Xinzhou 034000, China

ARTICLE INFO

Keywords:
Broomcorn millet Huangjiu
High-throughput sequencing
Metabolomics
Fermentation
Relationship

ABSTRACT

Shanxi broomcorn millet Huangjiu (SXHJ) is a typical representative of northern Huangjiu. This study employed high-throughput sequencing and untargeted metabolomics to conduct an in-depth analysis of the key aroma components and microbial community during the traditional fermentation process of SXHJ. The research identified 16 amino acids and 72 key aroma compounds, with a wide variety of esters and a high concentration of alcohols. *Weissiella*, *Enterococcus*, *Paucibacter*, *Saccharomyces*, *Aspergillus*, *Candida*, *Mortierella*, *Pichia*, *Hygrocybe*, *Thermoascus*, and *Clavispora* were identified as core microorganisms. Notably, certain specific microorganisms, such as *Weissella* and *Saccharomyces*, were found to be strongly associated with the production of specific aroma compounds. Further analysis revealed a significant correlation between bitter amino acids and most microorganisms, with the exception of *Pichia* and Limosilactobacillus, suggesting unique interactions among microorganisms during fermentation. These insights are instrumental in guiding the regulation of SXHJ aromatic properties.

1. Introduction

Chinese Huangjiu, as one of the oldest fermented beverages in the world, boasts a cultural heritage spanning thousands of years (Liu et al., 2023; Mao et al., 2023; Peng, Meng, et al., 2023; Zhao, Liu, et al., 2022). It adopts the unique brewing process of "making wine with Jiuqu, bilateral fermentation", and the raw and auxiliary materials, brewing techniques and production environments used in the brewing process are different, resulting in a variety of varieties of Chinese Huangjiu with distinctive regional characteristics (Wang, 2023). Geographically, Chinese Huangjiu is delineated into southern and northern categories, with the former primarily employing rice and the latter utilizing broomcorn millet as the cornerstone ingredient. Broomcorn millet Huangjiu boasts an illustrious history in ancient China, having been revered as a ceremonial libation in imperial courts since the Shang and Zhou dynasties. Furthermore, in contrast to southen Huangjiu, broomcorn millet Huangjiu is enriched with a superior concentration of phenolic compounds and amino acids, and its sensory profile—comprising both flavor and aroma—markedly diverges from that of its southern counterparts (Han et al., 2019). Consequently, northern Huangjiu has remained a cherished beverage throughout the ages. Shanxi, a pivotal epicenter for broomcorn millet cultivation in the northern domain, yields Shanxi broomcorn millet Huangjiu (SXHJ), which serves as the quintessential archetype of northern Huangjiu. SXHJ is distinguished by its extensive suite of health-promoting attributes, including gastrothermal, splenetic, myorelaxant, hemodynamic, and fatigue-ameliorative effects, thereby underscoring its venerable stature within the corpus of Chinese gastronomic heritage.

It is widely acknowledged that the flavor and aroma of Huangjiu are pivotal indicators for assessing its quality and determining consumer preferences. Esters, alcohols, acids, and aldehydes constitute the foundational framework of Huangjiu's aromatic profile (Chen et al., 2013). The brewing process of SXHJ is intricate, encompassing stages such as soaking, steaming, cooling, mixing with Qu, and fermentation (Wang et al., 2020). This fermentation process operates within a natural open fermentation system, involving multi-strain mixed fermentation (Chen et al., 2019). The multi-strain fermentation fosters a complex and unique microbial community structure, which ultimately shapes the distinctive

* Corresponding author at: No. 79 Longcheng Street, Xiaodian District, Taiyuan, 030000, China.
E-mail address: liyunlong125@126.com (Y. Li).
[1] These authors have contributed equally to this work and share first authorship.

https://doi.org/10.1016/j.fochx.2025.102307
Received 5 January 2025; Received in revised form 11 February 2025; Accepted 20 February 2025
Available online 24 February 2025

aroma, flavor, and color of Huangjiu (Peng et al., 2022). Consequently, the complex metabolic processes of microorganisms are pivotal in shaping the flavor and aroma profile of Huangjiu (Liu et al., 2019). Research indicates that *Saccharomyces cerevisiae* is responsible for alcohol production and contributes to the flavor compounds of Chinese Huangjiu (Tian et al., 2022). *Lactobacillus*, *Neisseria*, *Staphylococcus*, *Thauera*, and *Bifidobacterium* are identified as the functional core bacteria for the production of higher alcohols in Huangjiu (Yan et al., 2022). *Weissella* and *Pediococcus* are known to enhance the production of volatile acids, alcohols, and esters in Shaoxing Huangjiu (Chen et al., 2020). Molds, renowned for their high amylase activity, such as *Rhizopus*, are closely associated with various organic acids like valeric, nonanoic, and octanoic acids (Wang et al., 2023). Additionally, *P. pentosaceus* and *R. emersonii* are primary producers of aldehydes in Baijiu Daqu (Zhang et al., 2021). While Wang et al. (Wang et al., 2020; Wang et al., 2022) have explored the temporal changes in aroma components during the production of SXHJ and identified key aroma compounds using sensory-directed flavor analysis, the exact interactions between microorganisms and flavor compounds remain partially unclear.

This investigation holistically characterized the microbial community succession during fermentation by leveraging high-throughput sequencing technology, integrated with untargeted metabolomics to dynamically follow the changes in flavor compounds. It successfully identified key functional microbial taxa and elucidated their significant correlations with pivotal aroma substances. These findings provide a robust scientific foundation for the precise regulation of SXHJ fermentation processes and the optimization of flavor quality.

2. Materials and methods

2.1. Materials

The fermentation samples employed in this research were obtained at designated time points during the traditional SXHJ production procedure at Guixi Wine Co., Ltd., located in Dai County, Shanxi Province. The sampling points were designated as follows: Day 1 (D1), Day 2 (D2), Day 3 (D3), Day 4 (D4), Day 5 (D5), Day 6 (D6), Day 7 (D7), Day 28 (D28), Day 49 (D49), Day 70 (D70), Day 100 (D100), and Day 130 (D130) (as displayed in Fig. S1).

2.2. Determination of physiochemical properties

In accordance with the GB/T 13662–2018 standard, the following parameters were determined: pH, total acidity, total sugar, amino acid nitrogen, and alcohol content.

2.3. Quantitative analysis of amino acids

The measurement of free amino acid content was performed based on the approach outlined by Li et al. (Li et al., 2015), incorporating minor adjustments. Specifically, 1 mL of the sample was combined with 9 mL of 1 % sulfosalicylic acid. The blend was then centrifuged at 10,000 rpm lasting 15 min. The resultant supernatant was then gathered and passed through a 0.22 μm filter membrane. The sample's free amino acid composition and content were then examined using an S—433D automatic amino acid analyzer (from Sykam, Munich, Germany).

2.4. Volatile profiles analyses

A 30 mg sample was placed in a 2 mL EP tube, mixed with 0.24 mL extraction solution (Vmethanol: VdH_2O = 3:1), and spiked with 10 μL ribitol (1 mg/mL stock in dH_2O) as an internal standard, followed by vortexing for 30 s. After adding ceramic beads, the mixture was homogenized at 45 Hz for 4 min and sonicated for 5 min in an ice-water bath. The sample was then centrifuged at 4 °C, 13,000 rpm for 15 min. The supernatant (0.18 mL) was transferred to a GC/MS vial and dried under vacuum. The dried metabolites were derivatized with 30 μL methoxyamine hydrochloride in pyridine (20 mg/mL) and incubated at 80 °C for 30 min, followed by addition of 50 μL BSTFA (1 % TMCS, *v*/v) and incubation at 70 °C for 1.5 h. After cooling, 5 μL FAMEs (Standard mixture of fatty acid methyl esters, C8-C16:1 mg/mL; C18-C24:0.5 mg/mL in chloroform) was added.

All samples were analyzed using a gas chromatography system coupled with a Pegasus HT time-of-flight mass spectrometer (GC-TOF-MS). The system utilized a DB-5MS column (30 m × 250 μm internal diameter, 0.25 μm film thickness; J&W Scientific, Folsom, CA, USA) with splitless injection mode. Helium was used as the carrier gas at a flow rate of 1 mL/min. The initial temperature was maintained at 50 °C for 1 min, then increased to 310 °C at a rate of 10 °C/min, and held at 310 °C for 8 min. The temperatures of the injection port, transfer line, and ion source were set at 280 °C, 270 °C, and 220 °C, respectively. Electron ionization energy was 70 eV. Data were acquired in scan mode with a mass range of *m/z* 35–500.

Raw data were processed using Chroma TOF 4.3× software and the LECO-Fiehn Rtx5 database for peak analysis, baseline correction, alignment, deconvolution, identification, and integration. Metabolite identification was based on mass spectral and retention index matching. Peak areas were normalized to total peak area for further analysis.

2.5. DNA extraction, amplification and sequencing

Total DNA was extracted from samples according to the TGuide S96 Magnetic Bead Soil/Fecal Genomic DNA Extraction Kit protocol. DNA concentration was quantified using the Qubit dsDNA HS Assay Kit and a fluorometer. The V3-V4 region of the bacterial 16S rRNA gene and the fungal ITS1 region were amplified using universal primers 338F/806R and ITS/ITS1F, respectively. Following PCR purification and quantification (Tian et al., 2022), the amplified products were sent to Beijing Biomarker Technologies Co., Ltd. for sequencing on the Illumina Novaseq6000 platform.

Raw sequencing data were filtered using Trimmomatic (version 0.33). Primer sequences were identified and removed with Cutadapt (version 1.9.1). PE reads were assembled using USEARCH (version 10), and chimeras were removed with UCHIME (version 8.1). OTU classification and annotation were performed using the Naive Bayes classifier in QIIME2, with the SILVA database (version 132) at a confidence threshold of 70 %.

2.6. Statistical analysis

Statistical analysis and data visualization were conducted using Excel-2019 (Microsoft Office, USA) and Origin 2019 (OriginLab, USA). Data were subjected to analysis of variance (ANOVA) using IBM SPSS Statistics 26.0 (SPSS Inc., Chicago, Ill, USA). Volatile compounds were analyzed by Principal Component Analysis (PCA), and Orthogonal Partial Least Squares-Discriminant Analysis (OPLS-DA) using SIMCA14.1 software (UMETRICS, Sweden). Pearson correlation was employed to calculate the relationships between key microorganisms and amino acids, as well as between microorganisms and volatile compounds, with visualization performed using Cytoscape 3.5.1 (NIGMS, Seattle, USA) and the chiplot cloud platform (https://www.chiplot.online/t).

3. Results and discussion

3.1. Variations in physicochemical properties throughout the fermentation process of SXHJ

Throughout the fermentation stage of Huangjiu, physicochemical parameters play a pivotal role in assessing quality and monitoring the process. The changes in various physicochemical indices are depicted in Fig. 1. Total sugar content, as an indicator, reflects the changes in

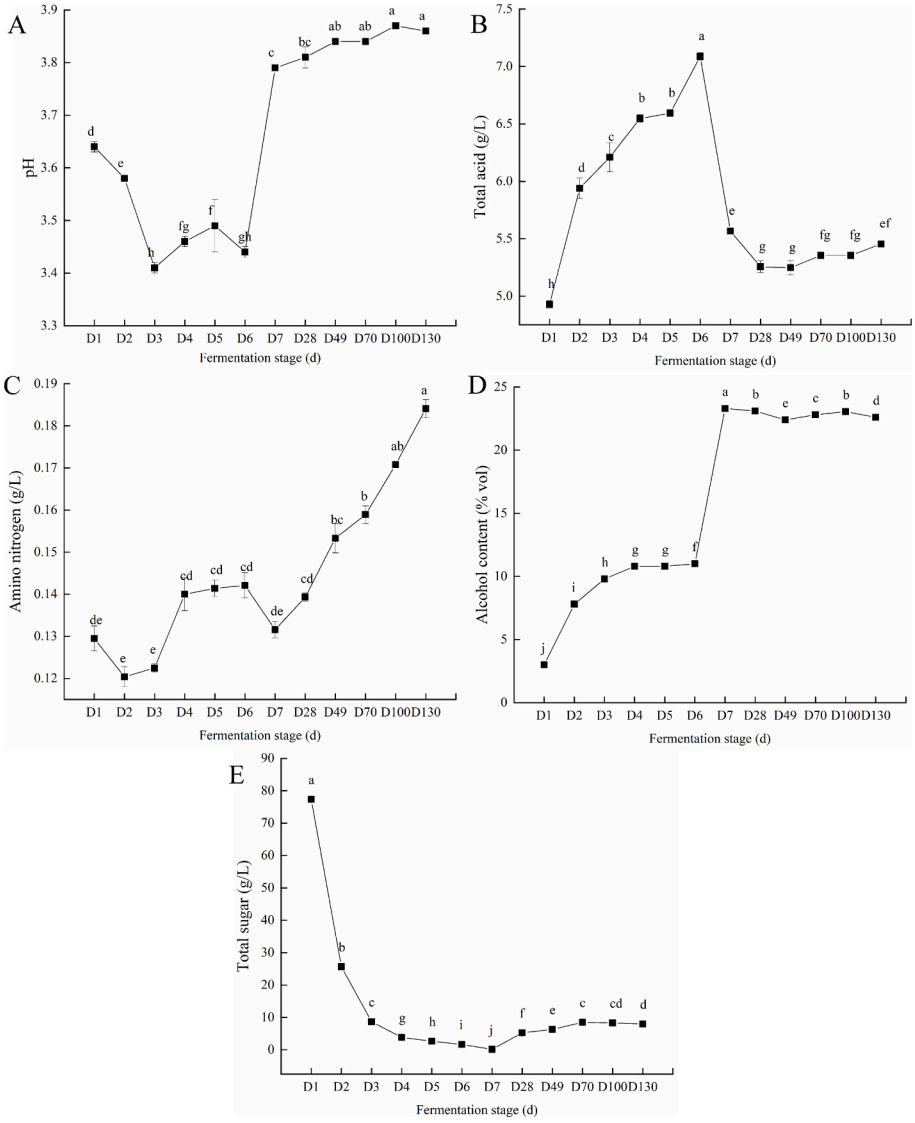


Fig. 1. Variations in physicochemical properties across samples:(A) pH, (B)total acidity, (C) amino acid nitrogen, (D) alcohol content, and (E)total sugar. Significant disparities are represented by varied lowercase letters ($P < 0.05$).

carbohydrates to some extent (Zhao et al., 2024). Fig. 1D and E illustrate that during the initial fermentation period, the total sugar level dropped dramatically from 77.35 g/L to 8.65 g/L, whereas the ethanol concentration notably surged to 7.8 % vol. This observation suggests the swift breakdown of starch in the raw materials by microbial activity and its subsequent transformation into ethanol during the initial fermentation stages, signifying the effective commencement and smooth advancement of SXHJ fermentation (Yang et al., 2024). The alcohol content increased sharply on D7 due to the addition of Baijiu at the end of the pre-fermentation stage (D6), which inhibited further fermentation of Huangjiu and was beneficial for the formation of its flavor profile. Acidity, in its role as a vital environmental factor, directly manifests and impacts the growth and metabolic dynamics of microorganisms (Luo et al., 2022). Over the course of the fermentation process, the total acidity content initially rose before declining (Fig. 1B), resulting in a corresponding decline followed by an increase in the pH value (Fig. 1A). The amount of amino acid nitrogen (Fig. 1C) incrementally climbed from 0.12 g/L to 0.18 g/L. All these indices are in compliance with the GB/T 13662–2018.

3.2. Variations in amino acid content throughout the SXHJ fermentation process

Amino acids significantly contribute to the growth and reproductive processes of bacteria and fungi during the fermentation of Chinese Huangjiu. The types and proportions of amino acids undergo dynamic changes influenced by microbial metabolic activities, environmental conditions, and fermentation stages. An amino acid analyzer was employed to analyze the free amino acids in SXHJ, identifying 8 bitter amino acids, 5 sweet amino acids, 2 umami amino acids, and 1 astringent amino acid. As indicated in Table 1, the content of each amino acid group increased as fermentation progressed, with a significant rise in total amino acid content in the post-fermentation phase. In SXHJ, the concentration of amino acids that determine the taste profile follows the order: bitter > sweet > umami > astringent. During the pre-fermentation stage, sweet amino acids were the most abundant. However, as fermentation continued, the proportion of bitter amino acids gradually increased. In the post-fermentation stage, the proportion of bitter amino acids is 47.46 %, sweet amino acids 40.98 %, umami amino acids 7.64 %, and astringent amino acids 3.91 %. Bitter amino acids predominated, reaching 0.2045 g/L at the D130 stage, accounting for 55.8 % of the total amino acid content. Chinese Huangjiu exhibits a

Table 1
Amino acid profiles in SXHJ samples across various fermentation phases.

Amino acid (g/L)	Code	D1	D2	D3	D4	D5	D6	D7	D28	D49	D70	D100	D130
Glutamicacid	Glu	0.0068	0.0060	0.0185	0.0148	0.0210	0.0007	0.0168	0.0119	0.0102	0.0113	0.0115	0.0125
Asparticacid	Asp	0.0015	0.0015	0.0021	0.0018	0.0026	–	0.0018	0.0050	0.0073	0.0091	0.0108	0.0122
Umami Amino Acids	UAA	0.0083	0.0075	0.0206	0.0166	0.0236	0.0007	0.0186	0.0169	0.0175	0.0204	0.0223	0.0247
Threonine	Thr	0.0005	0.0009	0.0013	0.0010	0.0015	0.0168	0.0010	0.0033	0.0051	0.0063	0.0072	0.0090
Serine	Ser	0.0014	0.0017	0.0018	0.0020	0.0022	–	0.0016	0.0049	0.0065	0.0073	0.0103	0.0119
Glycine	Gly	0.0017	0.0020	0.0051	0.0021	0.0068	0.0221	0.0055	0.0076	0.0106	0.0122	0.0138	0.0153
Alanine	Ala	0.0019	0.0019	0.0101	0.0039	0.0149	0.0002	0.0131	0.0200	0.0253	0.0285	0.0326	0.0383
Proline	Pro	0.0363	0.0507	0.0462	0.0491	0.0468	0.0071	0.0354	0.0388	0.0410	0.0414	0.1456	0.0466
Sweet Amino Acids	SAA	0.0417	0.0571	0.0646	0.0580	0.0722	0.0463	0.0565	0.0746	0.0884	0.0957	0.2094	0.1211
Valine	Val	0.0002	–	0.0016	0.0009	0.0018	0.0007	0.0011	0.0041	0.0061	0.0080	0.0118	0.0167
Isoleucine	Ile	0.0004	0.0004	0.0008	0.0005	0.0007	0.0015	0.0006	0.0025	0.0041	0.0056	0.0066	0.0081
Leucine	Leu	0.0014	0.0014	0.0024	0.0011	0.0022	0.0051	0.0022	0.0147	0.0238	0.0292	0.0339	0.0415
Phenylalanine	Phe	0.0076	0.0028	0.0047	0.0025	0.0034	0.0110	0.0029	0.0125	0.0125	0.0180	0.0193	0.0280
Histidine	His	0.0011	0.0007	0.0018	0.0018	0.0024	0.0033	0.0021	0.0042	0.0059	0.0070	0.0080	0.0095
Tryptophan	Try	0.0016	0.0009	0.0005	0.0008	0.0009	0.0000	0.0000	0.0010	0.0000	0.0001	–	–
Lysine	Lys	0.0025	0.0031	0.0035	0.0037	0.0052	0.0067	0.0038	0.0187	0.0269	0.0318	0.0378	0.0413
Arginine	Arg	0.0047	0.0061	0.0101	0.0095	0.0149	0.0218	0.0105	0.0272	0.0398	0.0460	0.0530	0.0595
Bitter Amino Acids	BAA	0.0194	0.0154	0.0255	0.0206	0.0315	0.0501	0.0231	0.0849	0.1192	0.1457	0.1703	0.2045
Tyrosine	Tyr	0.0005	0.0007	0.0023	0.0012	0.0031	0.0077	0.0025	0.0068	0.0095	0.0132	0.0136	0.0160
Astringent Amino Acids	AAA	0.0005	0.0007	0.0023	0.0012	0.0031	0.0077	0.0025	0.0068	0.0095	0.0132	0.0136	0.0160
Total Amino Acids	TAA	0.0699	0.0807	0.1130	0.0964	0.1303	0.1047	0.1008	0.1832	0.2346	0.2750	0.4157	0.3663

Note: The symbol "-" indicates that amino acid content cannot be detected.

higher total content of bitter amino acids compared to other amino acids, consistent with our observations (Liang, Lin, et al., 2020). The main components of amino acids in SXHJ include arginine, proline, leucine, lysine, alanine, and phenylalanine.

3.3. Variations in aroma compounds throughout the fermentation process of SXHJ

In the SXHJ fermentation samples, we detected a total of 828 metabolites. Among these, 187 metabolites were annotated in the HMDB database, encompassing 50 lipid and lipid-like molecules, 34 benzene ring compounds, 31 organic oxygen-containing compounds, 26 organic acids and their derivatives, 15 hydrocarbons, 14 organic heterocyclic compounds, 8 homogeneous non-metal compounds, 4 organic nitrogen-containing compounds, and 5 other compounds. The pie chart illustrating the classification of metabolites based on their quantities, as per the HMDB categories, is presented in Fig. 2.

Principal Component Analysis (PCA) reveals significant differences among the samples during the fermentation process of SXHJ (Fig. 3A). The samples from the early fermentation stages are relatively concentrated, while those from the later stages are more dispersed. Notably, samples from D7, D28, D49, and D70 are distinctly separated from the other fermentation time points, indicating active metabolic activities and a higher presence of differential metabolites during these periods. Fig. 3C hierarchical clustering analysis further demonstrates that SXHJ samples from different fermentation time points are divided into three clusters: D1-D6, D7, and D28-D130. These results suggest that there are pronounced differences in the aroma composition of SXHJ at various fermentation stages. To further investigate, a supervised Orthogonal Partial Least Squares-Discriminant Analysis (OPLS-DA) model was utilized to pinpoint volatile compounds with significant changes. With R^2X and Q^2 values of 0.812 and 0.664, the model demonstrates strong explanatory and predictive performance. To validate the model's dependability, it was subjected to 200 permutation tests, as illustrated in Fig. 3D. The intersection of the Q^2 regression line with the vertical axis below 0 confirms the absence of overfitting, affirming the model's

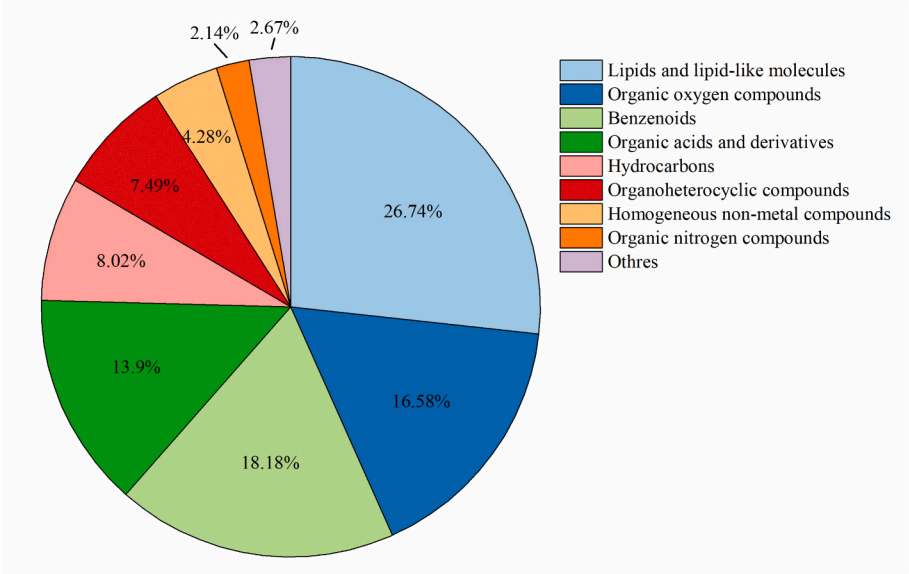


Fig. 2. Pie Chart: metabolite classification based on HMDB database.

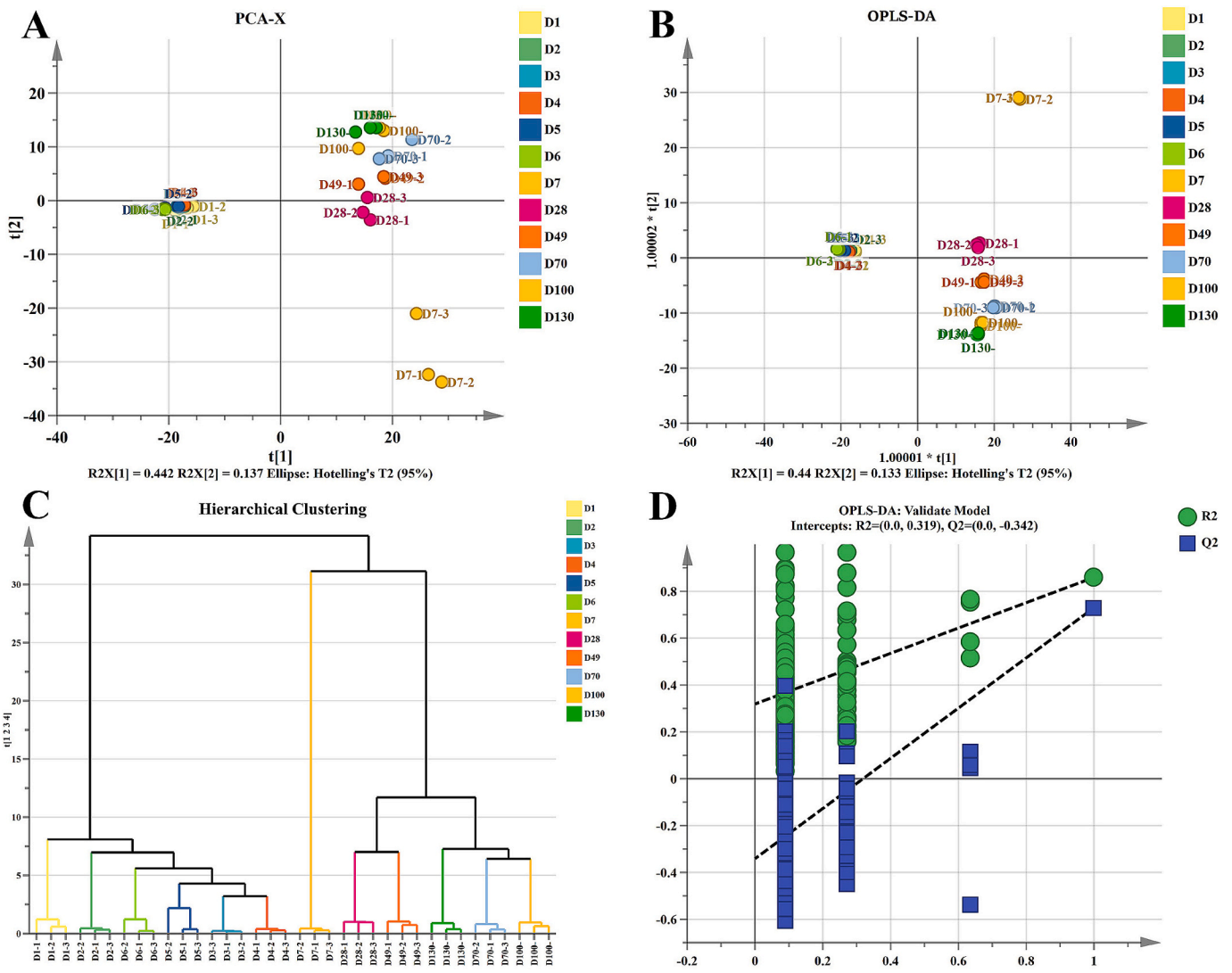


Fig. 3. Aroma compounds during the fermentation process of SXHJ. (A) PCA, (B) OPLS-DA, (C) Hierarchical Clustering Analysis (HCA), (D) validation plot of the OPLS-DA model.

reliability.

Variable Importance in Projection (VIP) was used to identify significant variables. Metabolites exhibiting a VIP value of ≥1.0 and a *P*-value of <0.01 were deemed significant differential metabolites. Based on these criteria, we identified 72 significant differential aroma compounds (Fig. 4), including 25 esters, 18 alcohols, 8 ketones, 6 aldehydes, 3 acids, 4 phenols, 2 aromatic compounds, 1 lactone, 3 alkenes, and 2 furans (Table S1). This profile is similar but not identical to the aroma compounds identified in Jimo Huangjiu (Zhou et al., 2024). Among the identified aroma compounds, esters exhibit the most diversity, while alcohols have the highest content, a pattern also observed in other Huangjiu (Jiang et al., 2020; Yu et al., 2023). Notably, the compounds with higher concentrations include methyl benzeneethanol, 3-methyl-1-butanol, phenylethyl alcohol, ethanol, (*S*)-(−)-ethyl lactate, 2-methyl-1-butanol, dimethyl silanediol, acetic acid 2-phenylethyl ester, butanedioic acid diethyl ester, and acetic acid. These aroma compounds are primarily produced during the mid to late stages of SXHJ fermentation, indicating that the post-fermentation phase is the key period for the production of flavor compounds in SXHJ, a characteristic shared with black rice wine (Tang & Peng, 2024).

Esters constitute the predominant class of compounds in Huangjiu, imparting fruity and floral aromas. The primary esters present during the brewing process of SXHJ include (*S*)-(−)-ethyl lactate, acetic acid 2-phenylethyl ester, butanedioic acid diethyl ester, 2-hydroxy-4-methylpentanoic acid ethyl ester, 3-methyl-1-butanol acetate, and hexanoic acid ethyl ester. These six esters account for approximately 89 % of the total ester content. These esters are predominantly formed during the post-fermentation stage of SXHJ and exhibit an overall increasing trend, rising from 76.78 μg/L to 6786.97 μg/L. (*S*)-(−)-ethyl lactate, as the most abundant and representative bacterial product in many Huangjiu fermentation processes (Wang et al., 2022), imparts cream, butter, and fruity aromas to the wine (Jin et al., 2021). It is predominantly generated during the post-fermentation phase, reaching its highest concentration of 3669.36 μg/L at the D130 stage, thereby markedly improving the aroma and flavor profile of the Huangjiu.

Alcohols are the most abundant organic compounds in Huangjiu, with higher alcohols constituting the main aroma framework of the beverage. Higher alcohols are formed through microbial metabolism, either via the Ehrlich pathway from amino acids present in the raw materials or directly from sugar degradation, also known as the Harris pathway (Yan et al., 2022). In this study, alcohols began to appear at D1 and were primarily formed during the initial fermentation stage. The main alcohol compounds in SXHJ include methyl benzeneethanol, 3-methyl-1-butanol, phenylethyl alcohol, ethanol, 2-methyl-1-butanol, and dimethyl silanediol, which together account for approximately 99.8 % of the total alcohols. Among these, methyl phenylethanol and

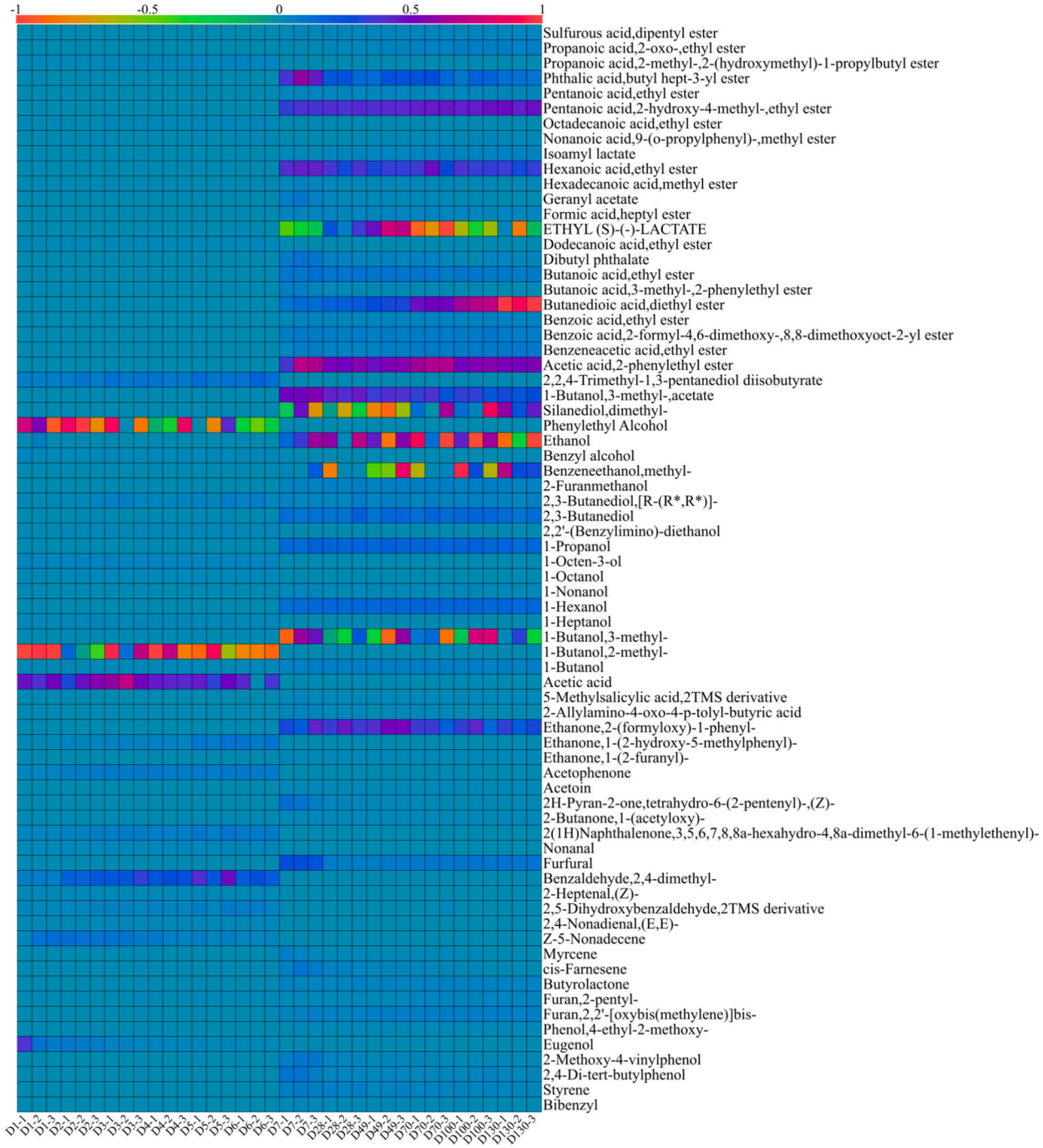


Fig. 4. Heatmaps of 72 major aroma compounds with VIP value ≥1.0 and *P*-value <0.01.

ethanol reached their highest concentrations at D100, while phenylethyl alcohol peaked at D4. phenylethyl alcohol, which possesses rose and honey aromas, is an important higher alcohol in Huangjiu and is significantly influenced by the raw materials used (Oscar et al., 2018). Ethanol constitutes the primary constituent of Huangjiu, with *Saccharomyces* serving as the predominant ethanol-producing microorganism (Liu et al., 2019). 3-Methyl-1-butanol, derived from the corresponding leucine, contributes to the malty aroma (Atsumi et al., 2008).

Acids, distinguished by their characteristic acidic aroma, constitute one of the principal aroma compounds integral to the flavor profile of Huangjiu. The concentration levels of these acids significantly impact sensory attributes such as softness and overall harmony. In the present study, acetic acid emerged as the most prevalent volatile organic acid. An optimal concentration of acetic acid contributes to a desirable taste in Huangjiu, whereas an overabundance leads to a pronounced acidic flavor. Furthermore, acetic acid consistently ranks as the organic acid with the highest content across various types of Huangjiu (Wang et al., 2020; Wang et al., 2022). In this research, acetic acid reached a relatively high concentration at the D3 stage. As the fermentation duration extended, its concentration slowly reduced, resulting in a progressively softer taste.

Other aroma compounds that significantly influence the flavor profile of Huangjiu include aldehydes and ketones, which contribute to the softness and harmony of the wine's aromatic bouquet. The majority of aldehydes are generated through the deamination and decarboxylation of amino acids (Wang et al., 2020). Within the aromatic profile of

Huangjiu, aldehydes are pivotal in modulating the emanation of the Huangjiu's aromatic compounds (Cheng et al., 2011). Furfural, for instance, contributes notable almond and caramel nuances (Cao et al., 2010). 2,4-dimethyl benzaldehyde is identified as the most abundant aldehyde, with its concentration gradually diminishing as the fermentation process advances. Despite this decline, its relatively low sensory threshold ensures that it remains perceptible even at low concentrations, imparting distinct jasmine or orange blossom aromas to the Huangjiu. Among ketones, 2-(formyloxy)-1-phenylethanone is the most prevalent, contributing a spectrum of fruity (such as apple and pear) and floral notes to the Huangjiu's aromatic profile.

In summary, methyl benzeneethanol, 3-methyl-1-butanol, phenylethyl alcohol, ethanol, (*S*)-(−)-ethyl lactate, 2-methyl-1-butanol, dimethyl silanediol, acetic acid 2-phenylethyl ester, butanedioic acid diethyl ester, acetic acid, 2-hydroxy-4-methyl-pentanoic acid ethyl ester, 3-methyl-1-butanol acetate, hexanoic acid ethyl ester, furfural, 2,4-dimethyl benzaldehyde, and 2-(formyloxy)-1-phenylethanone are the key aroma components found in SXHJ, which make significant

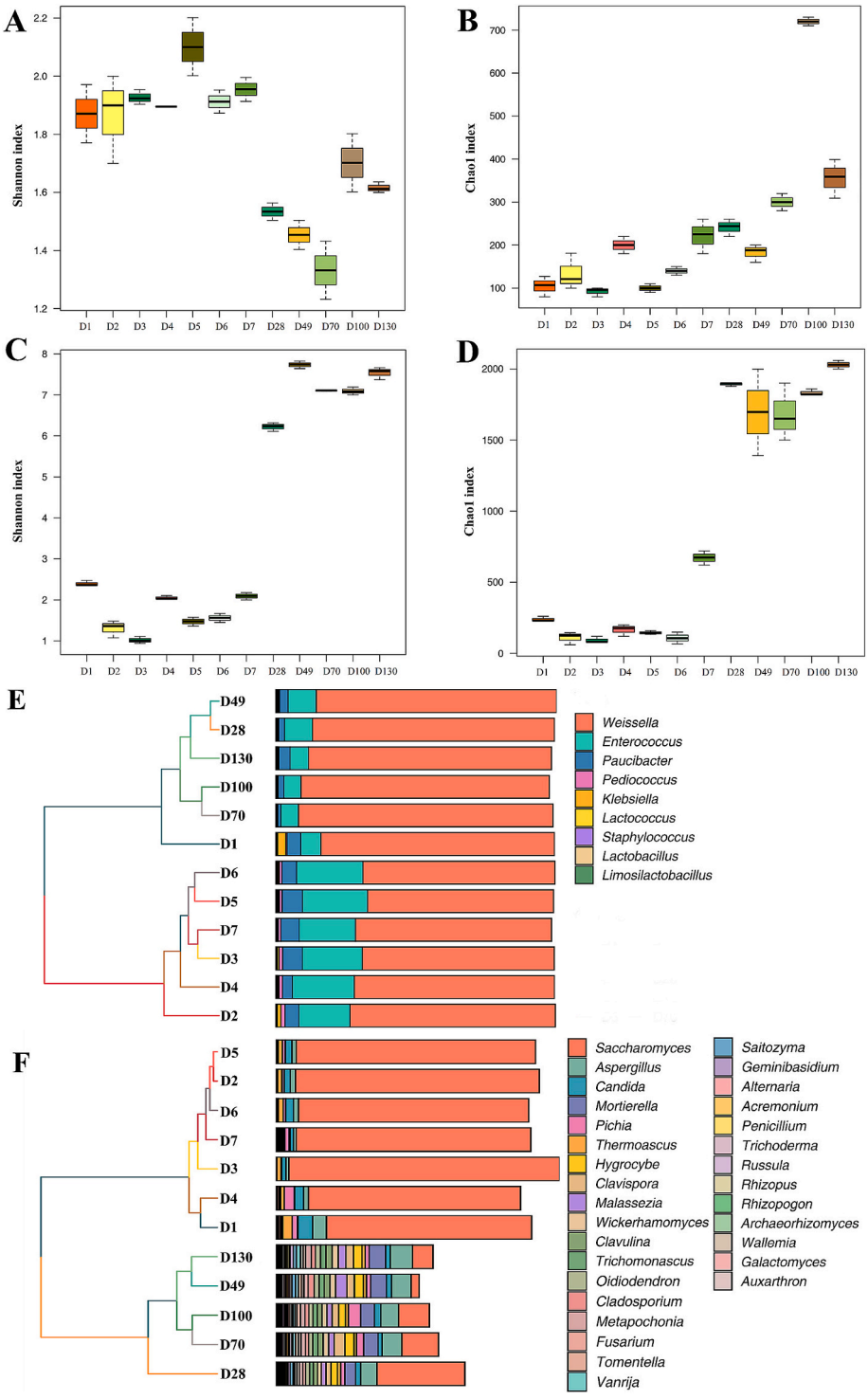


Fig. 5. Shannon index for bacteria (A) and fungi (C), Chao1 index for bacteria (B) and fungi (D), UPGMA dendrograms depicting the composition of bacterial (E) and fungal (F) communities in SXHJ.

contributions to the flavor profile of SXHJ.

3.4. Variations in microbial composition throughout the fermentation process of SXHJ

Through the analysis of microbial diversity, the succession of microbial communities throughout the fermentation process of SXHJ was effectively evaluated. Alpha diversity indices, including the Chao1 index and the Shannon index, were employed for this assessment. Fig. 5A reveals that, at the bacterial level, the Shannon index initially increases and then decreases, reaching its peak at D5, indicating the highest species diversity at this stage. As fermentation progresses, species diversity gradually declines. Throughout the fermentation process, the bacterial species diversity in the pre-fermentation samples is higher than that in the post-fermentation samples. At the fungal level, Fig. 5C and D show that both the Shannon index and the Chao1 index rapidly increase at D7 and D28 stages and subsequently stabilize. This indicates that the fungal population and richness are significantly greater in the post-fermentation stage compared to the pre-fermentation stage. Moreover, at the end of fermentation, the fungal population does not decrease. During the fermentation process of SXHJ, bacterial diversity gradually decreases while fungal diversity increases. This phenomenon is related to the tolerance of microorganisms in the fermentation starter. As fermentation progresses, the alcohol content rises, inhibiting the growth of some bacteria, while alcohol-tolerant fungi continue to proliferate. This results in a diminished bacterial diversity and an augmented fungal diversity, a trend that has been consistently documented in numerous studies (Tang & Peng, 2024; Yu et al., 2023).

Throughout the fermentation process of SXHJ, a comprehensive analysis revealed the presence of 9 bacterial genera and 31 fungal genera, with a relative abundance of $\geq$0.1 % (Fig. 5). The dominant bacterial genera (relative abundance $\geq$1 %) include *Weissella*, *Enterococcus*, and *Paucibacter*, belonging to the phyla *Firmicutes* and *Proteobacteria*. *Weissella* is the predominant genus throughout the fermentation process, with its abundance initially decreasing and then increasing, aligning with the observations reported by Zhao et al. (Zhao et al., 2020). This is attributed to *Weissella* being facultative anaerobes with strong acid tolerance, thus thriving in low-oxygen and low pH environments (Zhao et al., 2020). *Enterococcus* utilizes the nutrients in the fermentation broth efficiently during the early stages, leading to a rapid increase in its population, which then stabilizes. As the alcohol concentration rises during post-fermentation, some of the population dies off, resulting in a gradual decrease, a pattern also observed by Gao et al. (Gao et al., 2024). *Paucibacter* is associated with flavor compounds such as total phenols, D-erythronolide, and pyrazines (Ye et al., 2023), and its abundance in the SXHJ fermentation process first increases and then decreases, peaking on the fifth day.

The dominant fungal genera (abundance $\geq$1 %) are *Saccharomyces*, *Aspergillus*, *Candida*, *Mortierella*, *Pichia*, *Thermoascus*, *Hygrocybe*, and *Clavispora*, belonging to the phyla *Ascomycota*, *Basidiomycota*, and *Mortierellomycota*. *Saccharomyces* and *Aspergillus* are also the primary fungal genera that characterize Shaoxing Huangjiu (Yu et al., 2023). *Saccharomyces* is pivotal in alcoholic fermentation, characterized by its efficient sugar conversion, the generation of diverse flavor esters, and the contribution of ethanol along with various organic acids (Wu et al., 2013). The relative abundance of *Saccharomyces* decreases markedly compared to the pre-fermentation stage due to the higher alcohol concentration in the post-fermentation environment, which inhibits the fermentation function, activity, and survival rate of yeast cells. *Aspergillus* can secrete amylase and protease into the fermentation environment, thereby promoting the hydrolysis of residual starch and protein after fermentation (Yang et al., 2020), making significant contributions to the formation of fermentation flavors (Narzary et al., 2021). Cluster analysis indicates that microbial communities present at the same fermentation stage are more similar to each other.

3.5. Correlation analysis between microorganisms with physicochemical properties/ amino acid/ aroma compounds

Microbial community structure is intricately linked to environmental factors (Peng, Zheng, et al., 2023). Redundancy Analysis (RDA), an advanced extension of PCA, offers the distinct advantage of elucidating regression relationships between multiple microbial taxa and diverse environmental variables (Zhou et al., 2023). This approach is employed to delineate potential relationship between microbial flora and physicochemical characteristics. Results indicate that the microbial community is influenced by pH, total acid, amino acid nitrogen, total sugar, and alcohol content. In terms of bacterial community composition (Fig. 6A), the microbial community is most significantly influenced by pH and total acidity, followed by total sugar, amino acid nitrogen, and alcohol content. *Weissella* and *Lactobacillus*, both of which are lactate-producing bacteria, exhibit positive correlations with pH, amino acid nitrogen, and alcohol content, whereas they demonstrate negative correlations with total sugar and total acid concentrations. This aligns with the metabolic pattern of lactate-producing bacteria, which convert sugars into ethanol, degrade proteins into amino acids, and produce acidic compounds (Liu et al., 2020). For fungi (Fig. 6B), the microbial community is most profoundly influenced by alcohol content and total sugar, followed by pH, amino acid nitrogen, and total acidity. Similar results were observed in Fangxian Huangjiu (Gao et al., 2024). *Saccharomyces* is correlated positively with total acid and negatively with ethanol concentration. Notably, ethanol constitutes the primary constituent of Huangjiu, with yeast being the pivotal ethanol-producing microorganism (Liu et al., 2019). However, the results show that *Saccharomyces* is negatively correlated with ethanol, which may be due to the addition of alcohol during the SXHJ fermentation process, which has an impact on *Saccharomyces*, which is consistent with the results in 3.4.

Amino acids present in Huangjiu function dualistically, not solely as nutritional components but also as precursors of flavor compounds, thereby shaping the aroma and taste profile of the Huangjiu via microbial-mediated transformation or degradation (Wang et al., 2022). Amino acids derived mainly from the protease enzymatic degradation of the protein in the raw material, while autolysis of yeast and some other microorganisms were also the source of amino acids in Huangjiu fermentation. Some amino acids were used by yeast as nutrients, some (isoleucine, leucine, valine and phenylalanine) could be transformed into corresponding hydroxyamino acid, and the residual became part of composition improved Huangjiu quality (Zhao, Qian, et al., 2022). Network analysis, grounded in the correlation coefficients between microorganisms and amino acids, elucidates the intricate interrelationships that exist among these entities. Fig. 6C illustrates that a total of 11 positive correlations were detected for bacteria and 48 for fungi, with 29 negative correlations observed (18 blue lines for bacteria and 11 for fungi). The study reveals that fungi interact more frequently with amino acids than bacteria, a finding also observed in the research on Xiecun Huangjiu (Zhao et al., 2024). In comparison, *Weissella*, *Aspergillus*, *Mortierella*, *Thermoascus*, and *Clavispora* showed significant positive correlations with amino acids, while *Enterococcus*, *Paucibacter*, and *Saccharomyces* exhibited significant negative correlations. Bitter-tasting amino acids were significantly correlated with all microorganisms except *Pichia* and *Limosilactobacillus*. The Wuyi Hong Qu Huangjiu study also found that bitter amino acids have a strong positive correlation with *Saccharomyces*, *Pediococcus*, *Rhizopus*, *Monascus*, *Meyerozyma*, and *Bacillus* (Liang, Su, et al., 2020).

To elucidate the underlying correlations between the microbial community and pivotal aroma compounds during the traditional fermentation of SXHJ, a correlation heatmap (Fig. 7) was constructed. This heatmap was generated using the Pearson correlation coefficient ($|R| > 0.6$, $p < 0.05$), which quantified the relationships between 72 key aroma substances and 11 core microorganisms, comprising 8 bacterial and 3 fungal species. As can be seen from Fig. 7, many microorganisms and flavor substances are related. Among them, *Weissella*, *Aspergillus*,

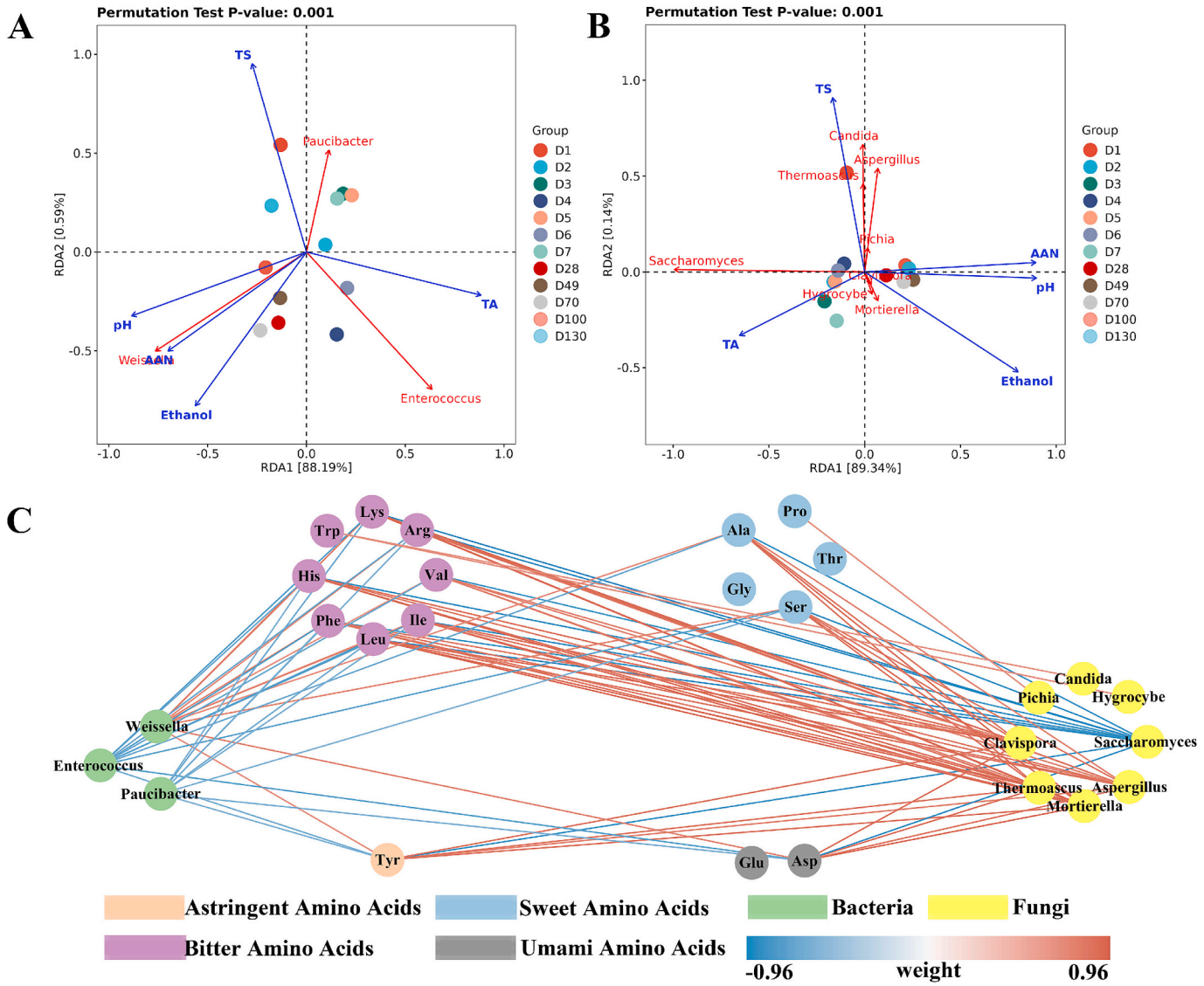


Fig. 6. RDA analysis of physicochemical indices with bacteria (A) and fungi (B) in SXHJ. Ethanol: alcohol content, AAN: amino acid nitrogen, TS: total sugar, TA: total acid. Relationship between amino acids and microorganisms in SXHJ (C).

Mortierella, *Thermoascus*, and *Clavispora* are positively correlated with most aroma substances, while *Paucibacter*, *Enterococcus*, *Thermoascus*, *Candida*, and *Saccharomyces* are negatively correlated with flavor substances, which is the same as the research results of black rice wine (Tang & Peng, 2024).

Among them, 9 microbial genera are highly positively correlated with 57 aroma substances ($|R| > 0.8$, $p < 0.05$), indicating that these 9 genera are very important in the flavor formation of SXHJ fermentation process. A correlation network diagram (Fig. 8) is drawn based on the correlation coefficient. Among them, *Saccharomyces*, which has the highest relative abundance in fungi, is significantly positively correlated with acetophenone, acetic acid, nonanal, (Z)-2-heptenal, 1-octen-3-ol, 1-octanol, and 1-heptanol. *Saccharomyces* contributes alcohol dehydrogenase and aldehyde dehydrogenase, which is involved in the conversion of alcohol-aldehyde-ester (Luo et al., 2024). *Aspergillus* is highly positively correlated with four esters, namely sulfurous acid dipentyl ester, 2-oxo-propanoic acid ethyl ester, isoamyl lactate, and (*S*)-(−)-ethyl lactate. *Aspergillus* is involved in the production of esterase (Xu et al., 2016), and previous studies on Fangxian Huangjiu have shown that *Aspergillus* has a significant contribution to ester synthesis (Gao et al., 2024). *Candida* and *Thermoascus* are significantly positively correlated with eugenol. However, *Mortierella*, *Hygrocybe*, and *Clavispora*, which have relatively low abundance, are significantly positively correlated with most aroma substances. In bacterial genera, *Weissella* is correlated with four esters, the same as *Aspergillus*. Research findings indicate that *Weissella* species are capable of augmenting the levels of esters and organic acids (Hu et al., 2021). *Enterococcus* is significantly positively correlated with dodecanoic acid ethyl ester, acetophenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)-2(1H) naphthalenone, 2,4-dimethyl-benzaldehyde, 2,2′-(benzylimino)-diethanol and 2-methyl-1-butanol. In light of the aforementioned findings, it can be deduced that certain microorganisms are pivotal in the biosynthesis of diverse compounds. Furthermore, the generation of specific aroma components is likely attributable to the synergistic interactions among multiple microbial species.

To investigate the metabolic pathways of key metabolites during the fermentation process of SXHJ, a metabolic network diagram was constructed using the KEGG database (Fig. 9). Central to this intricate metabolic network are key intermediates such as glucose, phosphoenolpyruvate, pyruvate, and acetyl-CoA, which are generated via the metabolic pathways encompassing starch and sucrose metabolism (ko00500), glycolysis (ko00010), and pyruvate metabolism (ko00620).

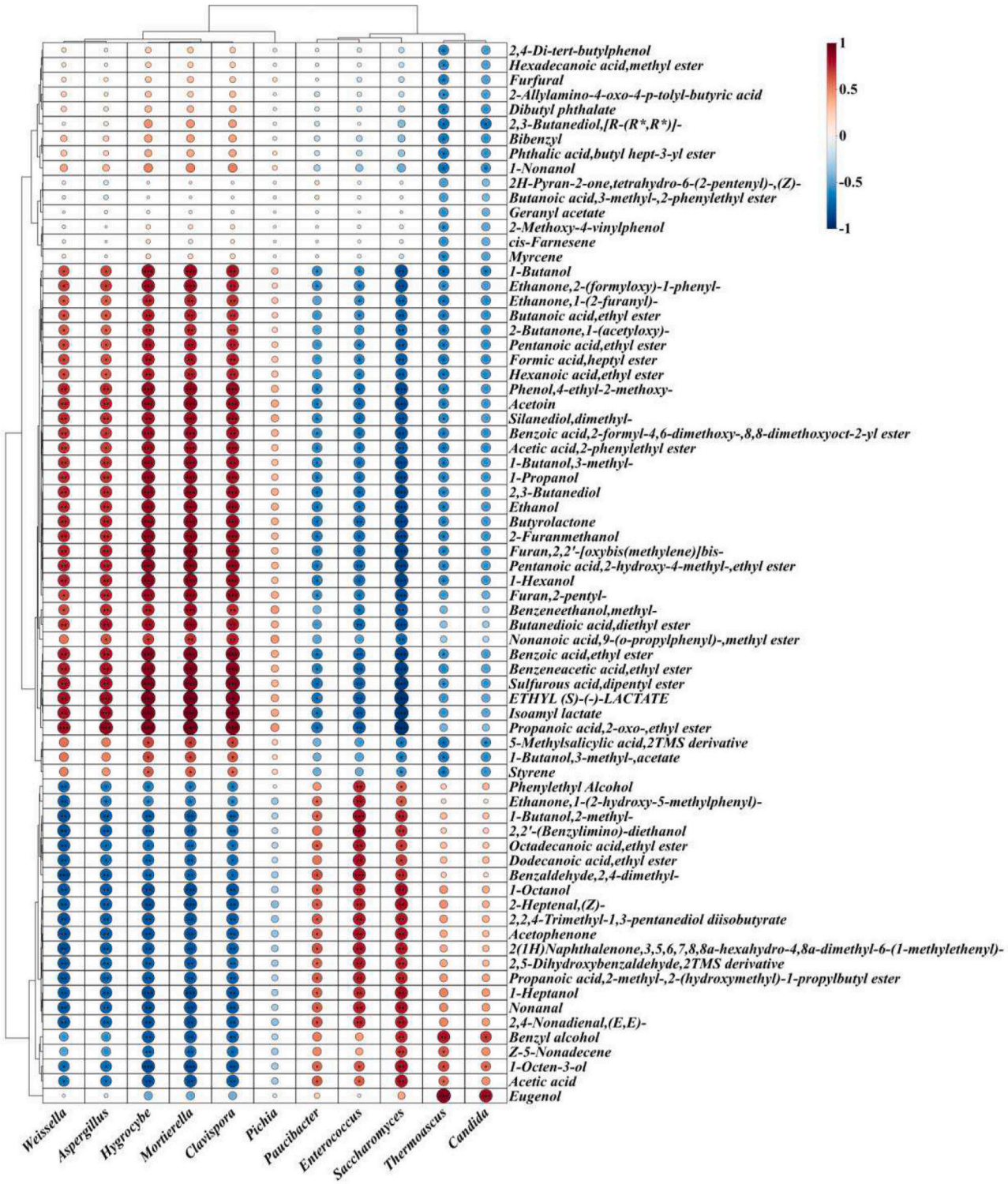


Fig. 7. Correlation heatmap of microorganisms and essential aroma compounds in SXHJ.

Glucose is pivotal in the synthesis of histidine, facilitated by the pentose phosphate pathway (ko00030) and the histidine metabolism pathway (ko00340). Additionally, glucose contributes to the generation of phosphoenolpyruvate through the glycolytic pathway (ko00010). Phosphoenolpyruvate serves as a crucial precursor for phenylalanine and tyrosine through the ko00400 pathway. These amino acids subsequently undergo transformations to yield phenylethyl alcohol via the ko00360 pathway and gentisatealdehyde via the ko00350 pathway, respectively. Pyruvate metabolism plays a pivotal role in the biosynthesis of a diverse array of flavor compounds. This includes the production of serine, glycine, threonine, isoleucine, and tryptophan through the ko00260 pathway, as well as alanine via the ko00470 pathway. Acetyl-CoA integrates into the TCA cycle (ko00020), thereby generating a spectrum of organic acids that are instrumental in defining the taste profile. Within the TCA cycle, 2-oxo-glutarate serves as a precursor for the synthesis of glutamic acid through the ko00220 pathway, as well as for the production of proline via the ko00330 pathway. Furthermore, aspartic acid plays a significant role in the interconversion

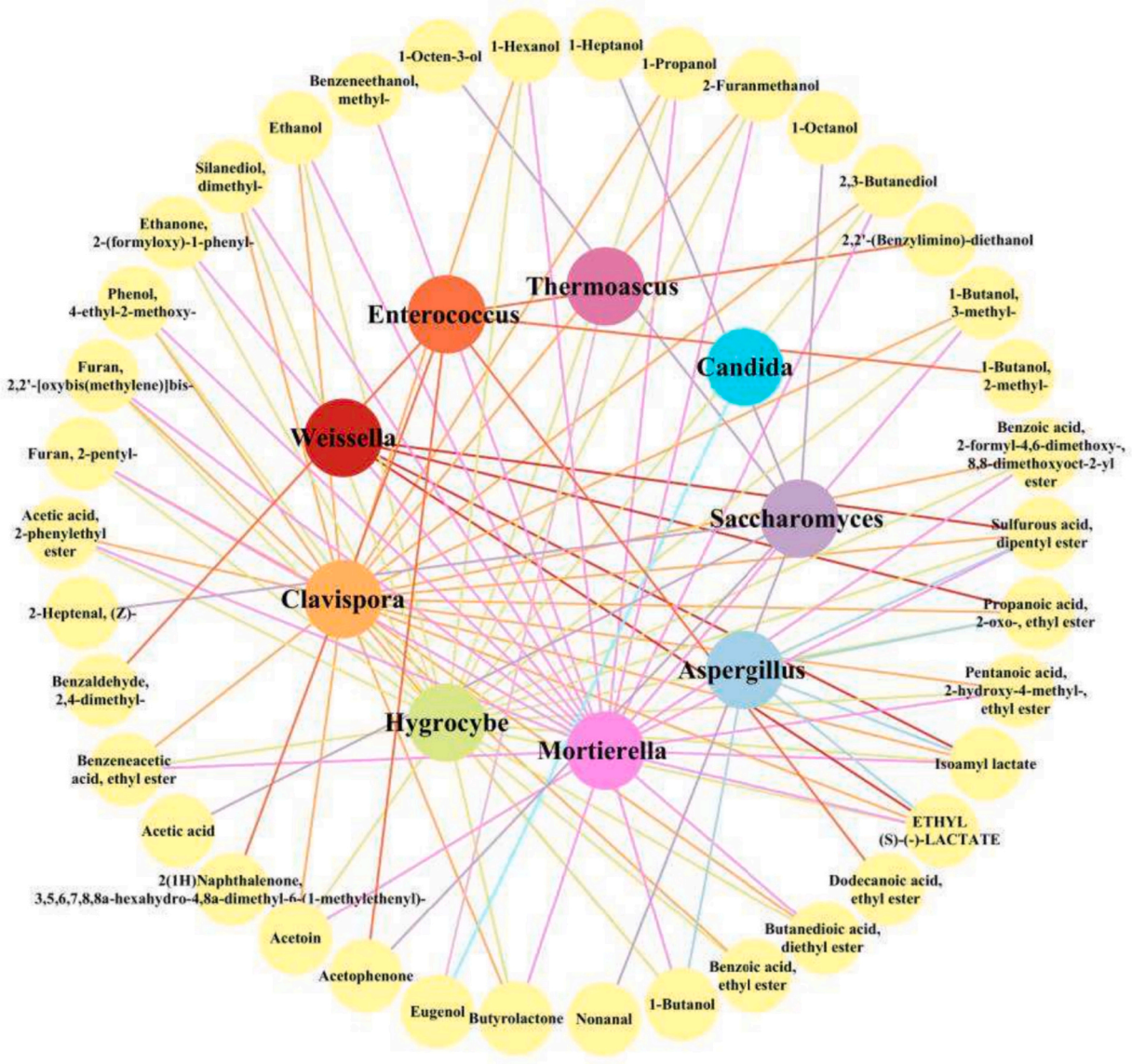


Fig. 8. Correlation network diagram of microorganisms and essential aroma compounds in SXHJ.

of lysine, arginine, proline, and alanine, facilitated by the pathways ko00250, ko00300, and ko00220. This intricate metabolic interplay is fundamental to establishing the primary amino acid profile inherent in SXHJ. Acetyl-CoA is intricately involved in the metabolism of acetic acid, acetaldehyde, and ethanol through the ko00620 pathway. Additionally, the pathways ko00061, ko00065, ko00062, ko00640, and ko00010 collectively contribute to the synthesis of octadecanoic acid, hexadecanoic acid, butanoic acid, formic acid, propionic acid, and lactic acid. These acids serve as the precursors for the synthesis of various esters, including ethyl stearate, methyl palmitate, and ethyl lactate. Acetyl-CoA emerges as one of the most pivotal factors influencing ester production (Yang et al., 2024), which is indispensable for the characteristic aroma profile of SXHJ.

The intricate interplay between environmental factors and microbial communities plays a pivotal role in the flavor development of SXHJ. Various microbial strains are instrumental in the biosynthesis of multiple compounds, with specific aroma components often arising from synergistic interactions among different microbial species. A profound understanding of the complex metabolic pathways involving key intermediates such as glucose, phosphoenolpyruvate, pyruvate, and acetyl-CoA is crucial for optimizing the fermentation process and enhancing the flavor and aromatic profile of SXHJ. Gaining mastery over these dynamic changes enables meticulous regulation of the fermentation process, thereby significantly enhancing the quality of SXHJ.

4. Conclusion

In conclusion, a total of 16 amino acids, 25 esters, 18 alcohols, 8 ketones, 6 aldehydes, 4 phenols, 2 aromatic compounds, 3 acids, 1 lactone, 3 alkenes, and 2 furans were identified throughout the fermentation process. Proline emerged as the amino acid with the highest abundance, and key aroma compounds included methyl benzeneethanol, 3-methyl-1-butanol, phenylethyl alcohol, ethanol, (*S*)-(−)-ethyl lactate, 2-methyl-1-butanol, dimethyl silanediol, acetic acid 2-phenylethyl ester, butanedioic acid diethyl ester, and acetic acid. Core microorganisms identified were *Weissella*, *Enterococcus*, *Paucibacter*, *Saccharomyces*, *Aspergillus*, *Candida*, *Mortierella*, *Pichia*, *Hygrocybe*, *Thermoascus*, and *Clavispora*, with 9 of these showing strong correlations with 57 aroma substances. Additionally, physicochemical indices were significantly correlated with microorganisms such as *Weissella*, *Lactobacillus*, *Saccharomyces*, and *Aspergillus*. Bitter amino acids showed significant correlations with all microorganisms except *Pichia* and

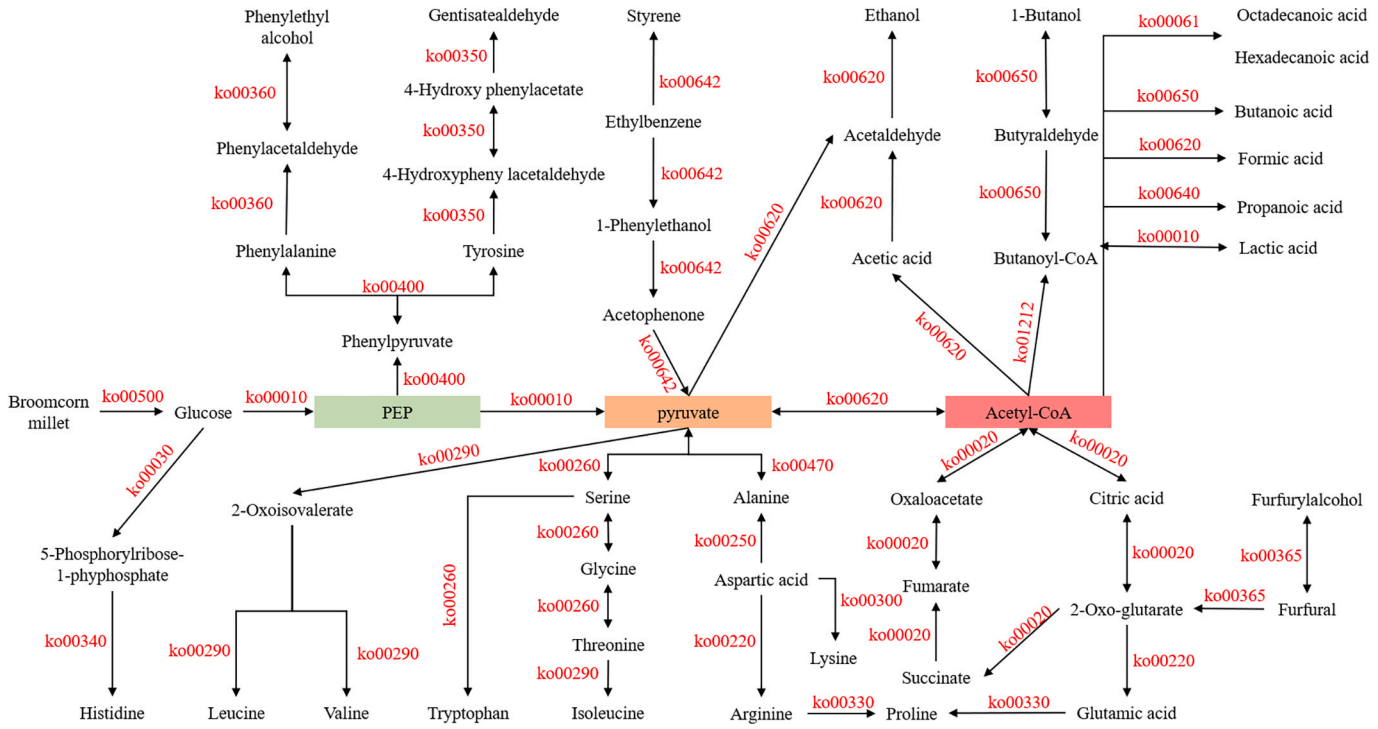


Fig. 9. Metabolic network of key aroma substances in SXHJ.

Limosilactobacillus. This research reveals the complex interplay between microbial communities and key aroma substances throughout the fermentation of SXHJ, providing an important theoretical and practical foundation for understanding the fermentation process, optimizing production techniques, enhancing product quality, protecting traditional brewing techniques, and advancing the sustainable progression of the Huangjiu industry.

CRediT authorship contribution statement

Qi Li: Writing – review & editing, Supervision, Project administration, Funding acquisition. **Linhua Cui:** Writing – original draft, Visualization, Methodology, Data curation. **Jiaying Zhu:** Investigation, Formal analysis. **Ting Zhang:** Visualization, Software. **Guoqiang Gao:** Resources, Methodology. **Yunlong Li:** Validation, Funding acquisition, Conceptualization, Resources.

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.

Acknowledgments

This work was supported by the Basic Research Foundation of Shanxi Province of China (No. 202303021212102), the earmarked fund for Modern Agro-industry Technology Research System of China (No. 2024CYJSTX03–04), and the science and technology innovation and promotion project of Shanxi Agricultural University of China (No. CXGC2023093).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2025.102307.

References

Atsumi, S., Hanai, T., & Liao, J. C. (2008). Non-fermentative pathways for synthesis of branched-chain higher alcohols as biofuels. *Nature, 451*(7174), 86–89. https://doi.org/10.1038/nature06450

Cao, Y., Xie, G., Wu, C., & Lu, J. (2010). A study on characteristic flavor compounds in traditional Chinese Rice wine — Guyue Longshan Rice wine. *Journal of the Institute of Brewing, 116*(2), 182–189. https://doi.org/10.1002/j.2050-0416.2010.tb00416.x

Chen, C., Liu, Y., Tian, H., Ai, L., & Yu, H. (2020). Metagenomic analysis reveals the impact of JIUYAO microbial diversity on fermentation and the volatile profile of Shaoxing-jiu. *Food Microbiology, 86*, Article 103326. https://doi.org/10.1016/j.fm.2019.103326

Chen, C., Liu, Y., Tian, H., & Yu, H. (2019). Research progress on the microbial community and their relationship with flavor formation in the brewing process of Chinese rice wine. *Journal of Food Safety and Quality, 10*(15), 4856–4863.

Chen, S., Xu, Y., & Qian, M. C. (2013). Aroma characterization of chinese rice wine by gas chromatography-olfactometry, chemical quantitative analysis, and aroma reconstitution. *Journal of Agricultural and Food Chemistry, 61*(47), 11295–11302. https://doi.org/10.1021/jf4030536

Cheng, T., Li, M., Zhang, Y., He, K., & Xia, Y. (2011). Application of principal component analysis in quality evaluation of Chinese strong-flavor liquors. *China Brewing, 01*, 89–90. https://doi.org/10.3969/j.issn.0254-5071.2011.01.026

Gao, R., Peng, P., Yu, L., Wan, B., Liang, X., Liu, P., Miao, & L.. (2024). Metagenomic analysis reveals the correlations between microbial communities and flavor compounds during the brewing of traditional Fangxian huangjiu. *Food. Bioscience, 58*, Article 103816. https://doi.org/10.1016/j.fbio.2024.103816

Han, H., Li, X., Geng, J., Xiao, Y., Wu, D., Li, M., & Wang, Z. (2019). Esearch status and prospects of Huangjiu brewing raw materials and production process. *Biotic Resources, 41*(01), 87–93. https://doi.org/10.14188/j.ajsh.2019.01.013

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

Jiang, L., Su, W., Mu, Y., & Mu, Y. (2020). Major metabolites and microbial Community of Fermented Black Glutinous Rice Wine with Different Starters. *Frontiers in Microbiology, 11*, 593. https://doi.org/10.3389/fmicb.2020.00593

Jin, Z., Cai, G., Wu, C., Hu, Z., Xu, X., Xie, G., Lu, & J.. (2021). Profiling the key metabolites produced during the modern brewing process of Chinese rice wine. *Food Research International, 139*, Article 109955. https://doi.org/10.1016/j.foodres.2020.109955

Li, X., Feng, T., Zhou, F., Zhou, S., Liu, Y., Li, W., Yang, & Y.. (2015). Effects of drying methods on the tasty compounds of Pleurotus eryngii. *Food Chemistry, 166*, 358–364. https://doi.org/10.1016/j.foodchem.2014.06.049

Liang, Z., Lin, X., He, Z., Su, H., Li, W., & Guo, Q. (2020). Comparison of microbial communities and amino acid metabolites in different traditional fermentation starters used during the fermentation of Hong Qu glutinous rice wine. *Food Research International, 136*, Article 109329. https://doi.org/10.1016/j.foodres.2020.109329

Liang, Z., Su, H., Lin, X., He, Z., Li, W., & Deng, D. (2020). Microbial communities and amino acids during the fermentation of Wuyi Hong Qu Huangjiu. *Lwt, 130*, Article 109743. https://doi.org/10.1016/j.lwt.2020.109743

Liu, S., Chen, Q., Zou, H., Yu, Y., Zhou, Z., Mao, J., & Zhang, S. (2019). A metagenomic analysis of the relationship between microorganisms and flavor development in Shaoxing mechanized huangjiu fermentation mashes. *International Journal of Food Microbiology, 303*, 9–18. https://doi.org/10.1016/j.ijfoodmicro.2019.05.001

Liu, S., Zhang, H., Peng, J., Yao, Z., Han, X., Liu, T., Mao, & J.. (2023). Effects of degree of milling on japonica and glutinous rice used for huangjiu brewing physicochemical properties. *Lwt, 183*, Article 114825. https://doi.org/10.1016/j.lwt.2023.114825

Liu, Z., Wang, Z., Sun, J., & Ni, L. (2020). The dynamics of volatile compounds and their correlation with the microbial succession during the traditional solid-state fermentation of Gutian Hong Qu glutinous rice wine. *Food Microbiology, 86*, Article 103347. https://doi.org/10.1016/j.fm.2019.103347

Luo, A., Yang, N., Yang, J., Hao, J., Zhao, J., Shi, S., & Hu, B. (2022). Effects of microbial interspecies relationships and physicochemical parameters on volatile flavors in sorghum-based fermented grains during the fermentation of Shanxi light-flavored liquor. *Food Science & Nutrition, 11*(3), 1452–1462. https://doi.org/10.1002/fsn3.3185

Luo, Y., Zhang, C., Liao, H., Luo, Y., Huang, X., Wang, Z., & Xiaole, X. (2024). Integrative metagenomics, volatilomics and chemometrics for deciphering the microbial structure and core metabolic network during Chinese rice wine (Huangjiu) fermentation in different regions. *Food Microbiology, 122*, Article 104569. https://doi.org/10.1016/j.fm.2024.104569

Mao, X., Yue, S., Xu, D., Fu, R., Han, J., Zhou, H., & Tang, Y. (2023). Research Progress on flavor and quality of Chinese Rice wine in the brewing process. *ACS Omega, 8*(36), 32311–32330. https://doi.org/10.1021/acsomega.3c04732

Narzary, D., Boro, N., Borah, A., Okubo, T., & Takami, H. (2021). Community structure and metabolic potentials of the traditional rice beer starter 'emao'. *Scientific Reports, 11*(1), 14628. https://doi.org/10.1038/s41598-021-94059-x

Oscar, M., Antoni, S., Xavier, F., & Raquel, B. (2018). Bioprocesses for 2-phenylethanol and 2-phenylethyl acetate production: Current state and perspectives. *Applied Microbiology and Biotechnology, 102*(23), 9991–10004. https://doi.org/10.1007/s00253-018-9384-8

Peng, Q., Meng, K., Yang, X., Zheng, H., Li, S., Chen, G., & Xie, G. (2023). Identification of geographical indication protected Huangjiu by metabonomics combined with flavor reconstruction. *Food Bioscience, 56*, Article 103216. https://doi.org/10.1016/j.fbio.2023.103216

Peng, Q., Zheng, H., Meng, K., Yu, H., Xie, G., Zhang, Y., Fu, & J.. (2022). Quantitative study on core bacteria producing flavor substances in Huangjiu (Chinese yellow rice wine). *Lwt, 168*, Article 113900. https://doi.org/10.1016/j.lwt.2022.113900

Peng, Q., Zheng, H., Yu, H., Meng, K., Cheng, Y., Yang, X., Zheng, & X.. (2023). Environmental factors drive the succession of microbial community structure during wheat Qu fermentation. *Food. Bioscience, 56*, Article 103169. https://doi.org/10.1016/j.fbio.2023.103169

Tang, A., & Peng, B. (2024). Metatranscriptomics reveals microbial community function succession and characteristic flavor formation mechanisms during black rice wine fermentation. *Food Chemistry, 457*, Article 140428. https://doi.org/10.1016/j.foodchem.2024.140428

Tian, S., Zeng, W., Fang, F., Zhou, J., & Du, G. (2022). The microbiome of Chinese rice wine (Huangjiu). *Current Research in Food Science, 5*, 325–335. https://doi.org/10.1016/j.crfs.2022.01.007

Wang, J., Yu, Y., Gao, X., Jiang, X., Huang, M., Ye, H., Wu, & Q.. (2022). Succession patterns of aroma components during brewing process of broomcorn millet (Panicum miliaceum L.) Huangjiu. *Food Research International, 154*, Article 110982. https://doi.org/10.1016/j.foodres.2022.110982

Wang, J., Yuan, C., Gao, X., Kang, Y., Huang, M., Wu, J., Zhang, & Y.. (2020). Characterization of key aroma compounds in Huangjiu from northern China by sensory-directed flavor analysis. *Food Research International, 134*, Article 109238. https://doi.org/10.1016/j.foodres.2020.109238

Wang, K., Wu, H., Wang, J., & Ren, Q. (2023). Microbiota composition during fermentation of broomcorn millet Huangjiu and their effects on flavor quality. *Foods (Basel, Switzerland), 12*(14), 2680. https://doi.org/10.3390/foods12142680

Wang, M. (2023). Research on flavor characteristics and the key aroma compounds of Hong Qu Huangjiu. *Master's Thesis, Jiangnan University*.. https://doi.org/10.27169/d.cnki.gwqgu.2023.001648

Wu, Q., Chen, L., & Xu, Y. (2013). Yeast community associated with the solid state fermentation of traditional Chinese Maotai -flavor liquor. *International Journal of Food Microbiology, 166*(2), 323–330. https://doi.org/10.1016/j.ijfoodmicro.2013.07.003

Xu, N., Liu, Y., Hu, Y., Zhou, M., Wang, C., & Li, D. (2016). Autolysis of aspergillus oryzae mycelium and effect on volatile flavor compounds of soy sauce. *Journal of Food Science, 81*(8), C1883–C1890. https://doi.org/10.1111/1750-3841.13396

Yan, Y., Sun, L., Xing, X., Wu, H., Lu, X., Zhang, W., … Q.. (2022). Microbial succession and exploration of higher alcohols-producing core bacteria in northern Huangjiu fermentation. *AMB Express, 12*(1), 79. https://doi.org/10.1186/s13568-022-01418-6

Yang, Y., Hu, W., Xia, Y., Mu, Z., Tao, L., Song, X., … L.. (2020). Flavor formation in Chinese Rice wine (Huangjiu): Impacts of the flavor-active microorganisms, raw materials, and fermentation technology. *Frontiers in Microbiology, 11*, Article 580247. https://doi.org/10.3389/fmicb.2020.580247

Yang, Y., Li, H., Xia, Y., Li, S., Wang, G., Ni, L., & Ai, L. (2024). Unraveling physicochemical characteristics, microbial community structure and functional enzymes governing fatty acid ethyl esters formation during Chinese rice wine brewing. *Lwt, 198*, Article 116035. https://doi.org/10.1016/j.lwt.2024.116035

Ye, X., Zhang, X., Hao, L., Lin, Q., & Bao, Y. (2023). Effects of different fermentation methods on the quality and microbial diversity of passion fruit wine. *Fermentation-Basel, 9*(5), 439. https://doi.org/10.3390/fermentation9050439

Yu, H., Li, Q., Guo, W., Chen, C., Ai, L., & Tian, H. (2023). Dynamic analysis of volatile metabolites and microbial community and their correlations during the fermentation process of traditional Huangjiu (Chinese rice wine) produced around winter solstice. *Food Chemistry: X, 18*, Article 100620. https://doi.org/10.1016/j.fochx.2023.100620

Zhang, J., Liu, S., Sun, H., Jiang, Z., Xu, Y., Mao, J., Mao, & J.. (2021). Metagenomics-based insights into the microbial community profiling and flavor development potentiality of baijiu Daqu and huangjiu wheat Qu. *Food Research International, 152*, Article 110707. https://doi.org/10.1016/j.foodres.2021.110707

Zhao, C., Su, W., Mu, Y., Jiang, L., & Mu, Y. (2020). Correlations between microbiota with physicochemical properties and volatile flavor components in black glutinous rice wine fermentation. *Food Research International, 138(pb)*, Article 109800. https://doi.org/10.1016/j.foodres.2020.109800

Zhao, W., Qian, M., Dong, H., Liu, X., Bai, W., Liu, G., & Lv, X. (2022). Effect of Hong Qu on the flavor and quality of Hakka yellow rice wine (Huangjiu) produced in southern China. *Lwt, 160*, Article 113264. https://doi.org/10.1016/j.lwt.2022.113264

Zhao, Y., Li, M., Zhan, P., Wang, P., He, W., & Tian, H. (2024). A quality comparison for Xiecun Huangjiu with different aging stages based on chemical profile, aroma composition and microbial succession. *Food Chemistry: X, 21*, Article 101132. https://doi.org/10.1016/j.fochx.2024.101132

Zhao, Y., Liu, S., Han, X., Zhou, Z., & Mao, J. (2022). Combined effects of fermentation temperature and Saccharomyces cerevisiae strains on free amino acids, flavor substances, and undesirable secondary metabolites in huangjiu fermentation. *Food Microbiology, 108*, Article 104091. https://doi.org/10.1016/j.fm.2022.104091

Zhou, C., Zhou, Y., Liu, T., Li, B., Hu, Y., Zhai, X., … Z.. (2023). Effects of protein components on the chemical composition and sensory properties of millet Huangjiu (Chinese millet wine). *Foods (Basel, Switzerland), 12*(7), 1458. https://doi.org/10.3390/foods12071458

Zhou, Z., Jiang, Z., Zhang, Z., Liu, S., Ji, Z., Han, J., & Mao, J. (2024). Identification and interaction of the key odorants in Jimo Huangjiu by means of molecular sensory science. *Journal of Food Composition and Analysis, 135*, Article 106695. https://doi.org/10.1016/j.jfca.2024.106695