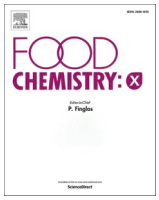
Food Chemistry: X 35 (2026) 103811

Contents lists available at ScienceDirect

Food Chemistry: X

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



Integration of volatile flavor metabolomics and metagenomics reveals microbial-enzymatic pathways governing key aromatic volatile compound biosynthesis in *Hongqujiu* fermentation

Guimei Chen [a,b,c], Suwen Tang [a,b], Hao Wang [a,b], Zihua Liang [a,b], Xucong Lv [a,b,c,*], Jinzhi Han [a,b], Li Ni [a,b,c,*]

[a] Institute of Food Science and Technology, College of Biological Science and Engineering, Fuzhou University, Fuzhou, Fujian 350108, PR China
[b] Food Nutrition and Health Research Center, School of Advanced Manufacturing, Fuzhou University, Jinjiang, Fujian 362200, PR China
[c] College of Chemical Engineering, Fuzhou University, Fuzhou, Fujian 350108, PR China

ARTICLE INFO

Keywords:
Hongqujiu
Volatile flavor compounds
Microbial-enzymatic pathways
Flavoromics
Metagenomics

ABSTRACT

The anabolic pathways of key volatile flavor compounds (VFCs) in *Hongqujiu* (HQJ) remain insufficiently elucidated. In this study, dynamic changes in volatile flavor profiles and microbial communities throughout HQJ brewing, were systematically investigated using an integrated multi-omics strategy combining metabolomics, flavoromics and metagenomics. The results demonstrated that the ethanol content, titratable acidity, amino nitrogen and higher alcohols increased progressively throughout fermentation. Quantitative flavor metabolomic profiling identified 18 key VFCs, maining comprising ethyl esters, acetate esters and higher alcohols. Metagenomic sequencing revealed that *Weissella*, *Lactobacillus*, *Saccharomyces*, *Aspergillus*, *Talaromyces* and *Monascus* were the predominant microbal genera throughout HQJ fermentation. Functional gene annotation further indicated that key enzymes involved in flavor metabolism are primarily associated with *Lactobacillus*, *Aspergillus*, *Talaromyces*, *Saccharomyces*, *Cyberlindnera* and *Monascus*. Overall, this study elucidates the microbial–enzymatic basis of VFC biosynthesis and establishes a comprehensive flavor metabolic framework for HQJ fermentation, providing a theoretical foundation for aroma quality improvement.

1. Introduction

Huangjiu (Chinese rice wine) is one of the oldest traditional fermented alcoholic beverages in China. According to the type of fermentation starter, it is generally classified into *Hongqujiu* (HQJ) and *Maiqujiu* (MQJ). HQJ is brewed using *Hongqu* as both the saccharification and fermentation starter and represents a distinctive subtype of *Huangjiu*. *Hongqu* is a traditional starter culture characterized by a complex microbial consortium. Previous studies have shown that its dominant bacterial genera include *Bacillus*, *Weissella*, *Pediococcus*, *Burkholderia* and *Gluconobacter*, while the dominant fungal genera include *Aspergillus*, *Monascus, Rhizopus*, *Meyerozyma* and *Saccharomyces* (Liang et al., 2020; Liu et al., 2020; Li et al., 2019). This diverse microbial community provides a rich enzymatic system that supports starch hydrolysis, ethanol production, and aroma compound formation during HQJ fermentation.

HQJ is celebrated for its distinctive flavor profile, characterized by a complex interplay of aromatic volatiles that contribute to its rich sensory appeal. Over recent years, HQJ has gained increasing popularity among consumers both domestically and internationally, owing to its unique taste, health-beneficial properties, and cultural significance. The traditional brewing process of HQJ involves multiple sequential stages, including soaking and steaming glutinous rice, inoculation with *Hongqu* (a natural fermentation starter containing diverse fungi and bacteria), saccharification, alcoholization, filtration, sterilization, and prolonged aging (Chen, Ren, et al., 2021a).

The quality of HQJ is largely determined by volatile flavor compounds (VFCs), including esters, higher alcohols, fatty acids, aldehydes, phenolic compounds, and ketones (Chen, Ren, et al., 2021a). These compounds are mainly generated during fermentation (saccharification and alcoholization), mainly due to the metabolic activities of different types of microbial species (Yang et al., 2020). During brewing, microorganisms secrete a variety of enzymes that hydrolyze macromolecules such as starch and proteins into monosaccharides, amino acids, and

* Corresponding authors at: Institute of Food Science and Technology, College of Biological Science and Engineering, Fuzhou University, Fujian 350108, PR China.
E-mail addresses: xucong1154@163.com (X. Lv), nili-fzu@qq.com (L. Ni).

https://doi.org/10.1016/j.fochx.2026.103811
Received 30 September 2025; Received in revised form 6 March 2026; Accepted 1 April 2026
Available online 3 April 2026

peptides (Qian et al., 2023). These metabolites subsequently serve as substrates for the biosynthesis of VFCs. Therefore, VFCs formation in HQJ is essentially a microbially driven metabolic process regulated by community structure and functional gene expression.

The microbial community during *Huangjiu* fermentation is highly dynamic and region-specific. Among the dominant microbial population, lactic acid bacteria (LAB), especially *Lactobacillus*, are particularly prevalent and contribute significantly to acidification and flavor development (Chen, Ren, et al., 2021b). Furthermore, metagenomic analysis revealed that *Saccharomyces cerevisiae* and LAB are key contributors to the production of oxidoreductases, transferases, and isomerases throughout *Huangjiu* fermentation process. These enzymes are crucial for the biosynthesis of ethyl esters, acetate esters and other flavor-active compounds (Yang et al., 2024). Luo et al. (2024) further identify 13 microbial species as regional markers of *Huangjiu* fermentation, highlighting the potential of metagenomics to elucidating microbial contributions to regional flavor differentiation. Collectively, these studies emphasize the importance of integrating metabolomics, flavoromics and metagenomics to unravel complex microbial dynamics and metabolic pathways underlying *Huangjiu* production, thereby advancing fermentation science and product quality. However, most existing studies focus on microbial composition or volatile profiles independently, while the direct linkage between microbial functional genes, metabolic pathways, and key VFCs formation in HQJ remains insufficiently elucidated. In particular, systematic reconstruction of microbial-driven metabolic networks responsible for aroma compound biosynthesis in HQJ is still lacking. This knowledge gap limits the mechanistic understanding of flavor formation and restricts precision regulation of fermentation.

Therefore, the present study aimed to systematically elucidate the microbial-driven metabolic pathways responsible for key VFCs in HQJ. Metagenomic sequencing combined with gas chromatography–mass spectrometry (GC–MS) was employed to characterize the temporal dynamics of microbial communities and volatile flavor profiles. Furthermore, KEGG pathway analysis was used to reconstruct the metabolic network of key VFCs and to, link specific genes and enzymes to functional microbial taxa. This study provided mechanistic insights into flavor formation in HQJ and established a scientific foundation for the targeted regulation and quality optimization of HQJ.

2. Materials and methods

2.1. Samples collection and preparation

Hongqu (fermentation starter) utilized for HQJ brewing was obtained from Fumao Wine Group (Jian'ou, Fujian Province, China). Premium glutinous rice (*Oryza sativa* var. glutinosa) served as the primary fermentation substrate. Prior to fermentation, the glutinous rice underwent standard preparatory steps. It was submerged in distilled water and soaked overnight (~12 h) at ambient temperature (approximately 25 °C). The soaked rice was then steamed for 30 min using atmospheric steam to achieve complete gelatinization. Following steaming, the rice was cooled to room temperature (approximately 25 °C), and its water absorption ratio was determined gravimetrically.

Solid-state fermentation was initiated by mixing the steamed rice with the *Hongqu* starter and distilled water. The substrate composition was precisely controlled at a ratio of raw rice: distilled water: *Hongqu* = 10 kg: 15 L: 1 kg (*w/v*/w). To ensure uniform inoculation, the *Hongqu* and steamed rice were layered alternately within sterile glass fermentation vessels (40 L capacity). Distilled water was then added to the mixture to initiate the fermentation process. The fermentation vessels were covered with sterile gauze to permit oxygen exchange. This phase was conducted at 18.0 °C (temperature control precision: ±0.5 °C) for 10 days to facilitate microbial growth, saccharification, and initial flavor development. After the aerobic phase, the gauze covers were replaced with airtight stoppers to create anaerobic conditions conducive to alcohol production (Supplementary Materials-Fig. S1). This phase continued at 18.0 °C (temperature control precision: ±0.5 °C) for an additional 35 days, culminating in a total fermentation duration of 45 days. The entire fermentation process was meticulously monitored and maintained at 18.0 ± 0.5 °C. All fermentations were performed in triplicate using independent batches to account for biological variability.

Representative samples of the fermenting mash were aseptically collected at critical time points throughout the 45-day fermentation period. Sampling intervals were designated as days 1, 2, 3, 5, 7, 10, 15, 20, and 45. Samples were taken from each of the three replicate fermentation vessels at each time point. Collected mash samples underwent immediate processing to separate microbial biomass from the liquid matrix for downstream analyses. Each mash sample was diluted 1:9 (weight/volume) with sterile physiological saline solution (0.85% *w/v* NaCl). The diluted suspension was vortexed vigorously to achieve homogeneity and then subjected to centrifugation (8000 ×*g*, 10 min, 4 °C). The resulting supernatant was carefully aliquoted and stored at −20 °C. This fraction was reserved for subsequent analyses, including physicochemical characterization (e.g., ethanol content, titratable acidity, amino nitrogen) and comprehensive flavor metabolite profiling. The microbial pellet obtained after centrifugation was promptly frozen and stored at −80 °C. This pellet was used for the extraction of metagenomic DNA to analyze the microbial community structure and functional potential.

2.2. Detections of the oenological parameters and higher alcohols

The concentrations of key oenological parameters including reducing sugar, titratable acidity, and amino nitrogen were determined using established methodologies (Chen et al., 2022). Reducing sugar content was quantified employing the 3,5-dinitrosalicylic acid (DNS) colorimetric method, while titratable acidity and amino nitrogen concentrations were measured according to the protocols outlined in the Chinese National Standard GB/T 13662–2011 (Method for Analysis of *Huangjiu*), ensuring standardized assessment of these critical fermentation indices.

Ethanol and specific higher alcohols (namely, n-propanol, isobutanol, 3-methylbutan-1-ol and phenylethyl alcohol) were quantified using gas chromatography coupled with a flame ionization detector (GC-FID, Agilent 7890 A, USA). Sample separation was achieved on an HP-INNOWAX capillary column (30.0 m × 0.25 mm i.d. × 0.25 μm film thickness, Agilent, USA). The injector temperature was maintained at 250 °C, and samples (1 μL) were injected in split mode (split ratio 10:1). The oven temperature program was optimized as follows: initial temperature 40 °C (held for 1 min), ramped to 120 °C at 5 °C/min (held for 1 min), and subsequently increased to 220 °C at 10 °C/min. Carrier gas (H_2) and detector auxiliary gases (air) flow rates were set at 30 mL/min and 400 mL/min, respectively. To ensure analytical reliability and accuracy within the complex fermented wine matrix, the GC-FID method underwent comprehensive validation prior to sample analysis. All fermentation samples were analyzed in triplicate to provide robust mean values and assess analytical precision.

2.3. Volatile profile analysis

The dynamic profile of VFCs throughout the HQJ fermentation process was analyzed using headspace solid-phase microextraction (HS-SPME) coupled with gas chromatography–mass spectrometry (GC–MS, Agilent 7890B/5977 A system). This technique was selected for its sensitivity and suitability for capturing the complex aroma profile of fermented beverages without extensive sample preparation. For each analysis, 6 mL of appropriately diluted wine sample (diluted tenfold with saturated NaCl solution to minimize matrix effects and enhance headspace partitioning) was transferred to a 20 mL SPME vial. To this, 2 g of sodium chloride (NaCl) and 10 μL of a 2-octanol solution (10 mg/mL

in ethanol, serving as the internal standard) were added. The addition of NaCl saturated the solution, elevating the ionic strength and thereby improving the extraction efficiency of volatile analytes into the headspace. Samples were equilibrated at 60 °C for 10 min under continuous magnetic stirring (500 rpm) to establish vapor-liquid equilibrium. Subsequently, a preconditioned divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) SPME fiber (50/30 μm film thickness, Supelco) was exposed to the sample headspace for 45 min at 60 °C under continuous agitation.

Following extraction, analytes were thermally desorbed from the SPME fiber in the GC injection port at 250 °C for 5 min in splitless mode. Chromatographic separation was achieved using an HP-INNOWAX capillary column (60% nitroterephthalic acid modified polyethylene glycol, 30 m × 0.25 mm i.d. × 0.25 μm film thickness), which is highly selective for polar volatile compounds. The GC oven temperature program was optimized as follows: initial temperature 40 °C (held for 5 min), ramped to 120 °C at 5 °C/min, then increased to 240 °C at 10 °C/min (held for 5 min). Ultra-high purity helium (99.999%) was used as the carrier gas at a constant flow rate of 1.0 mL/min. Compounds eluting from the chromatographic column were detected using electron ionization (EI) mass spectrometry at 70 eV. The mass spectrometer was operated in full scan mode over a mass-to-charge ratio (m/z) range of 35 to 350 to capture comprehensive spectra for compound identification. Key MS parameters were set as follows: GC–MS interface temperature, 280 °C; ion source temperature, 230 °C; quadrupole temperature, 150 °C. Tentative identification of VFCs was based on two primary criteria: (i) Comparison of experimental mass spectra with reference spectra in the NIST 2017 mass spectral library (similarity index ≥80% considered a good match). (ii) Calculation of experimental linear retention indices (LRIexp) using a homologous series of n-alkanes (C7-C40) analyzed under identical chromatographic conditions. LRIexp values were compared against published LRI values in established databases (NIST Chemistry WebBook, Flavornet) and relevant literature for compound assignment. Compounds identified by both matching mass spectra and LRI values within ±10 units of reference values were considered reliably identified.

Absolute quantification of identified VFCs was performed using the internal standard (2-octanol) method with experimentally determined Relative Response Factors (f). Standard solutions of authentic reference compounds (purity ≥98%) were analyzed under identical HS-SPME-GC–MS conditions to calculate f for each analyte relative to 2-octanol using the following equation:

$$f = \frac{m_{IS} \times A_{std}}{A_{IS} \times m_{std}}$$

where m_{std} and m_{IS} represent the masses of the analyte standard and internal standard added to the vial, respectively; A_{std} and A_{IS} are the chromatographic peak areas of the analyte standard and the internal standard, respectively.

The concentration of each target VFC (μg L^{-1}) in the wine samples was then calculated according to the following equation:

$$C = f \times \frac{m_1 \times A_2}{A_1 \times V}$$

where C is the concentration of the target VFCs (μg L^{-1}); A_1 and A_2 are the peak area of the internal standard and the target compound, respectively; m_1 is the mass of the internal standard (mg); V is the volume of the *undiluted* wine sample analyzed (L); and f is the relative response factor for the target compound.

To evaluate the sensory contribution of each VFC to the overall HQJ aroma profile, the Odor Activity Value (OAV) was calculated as the ratio of the compound concentration (μg L^{-1}) to its orthonasal odor threshold concentration in an appropriate matrix (typically hydroalcoholic solution or water), as reported in the literatures (Chaves et al., 2007; Culleré et al., 2011; Isogai et al., 2005; Moyano et al., 2002; Moyano et al., 2010; Ma et al., 2025; van Gemert, 2011; Poisson & Schieberle, 2008). VFCs with OAV ≥ 1 were considered to contribute significantly to the perceived aroma. Odor descriptors were assigned based on existing literature and databases such as Flavornet and the Good Scents Company database.

2.4. DNA extraction and sequencing analysis

Genomic DNA was extracted from HQJ fermentation samples using the Mag-Bind® Soil DNA Kit (Omega Bio-tek, Norcross, GA, USA), strictly following the manufacturer's protocol. DNA concentration and purity were determined spectrophotometrically using NanoDrop (e.g., A260/A280 and A260/A230 ratios), and DNA integrity was confirmed by 1% (w/v) agarose gel electrophoresis to ensure suitability for sequencing. Subsequently, high-quality DNA was fragmented to an average size of ~400 bp using a Covaris M220 focused-ultrasonicator (Covaris, Woburn, MA, USA). Standard paired-end sequencing libraries were prepared using the NEXTFLEX Rapid DNA-Seq Kit (Bioo Scientific, Austin, TX, USA). This process included end-repair, adapter ligation (to blunt-ended fragments), and PCR amplification. Sequencing was performed on an Illumina NovaSeq 6000 platform (Illumina Inc., San Diego, CA, USA) utilizing the NovaSeq 6000 S4 Reagent Kit v1.5 (300 cycles) at Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China), following the manufacturer's recommended procedures, generating 150-bp paired-end reads.

Raw sequencing reads underwent stringent quality control and preprocessing using fastp (version 0.23.0) (Chen et al., 2018). This included: (i) removal of adapter sequences, (ii) trimming of low-quality bases from read ends (quality threshold of <20 in a sliding window of 4 bp), (iii) discarding reads with an average quality score < 20 over >40% of their length, (iv) removal of reads shorter than 50 bp after trimming, and (v) removal of reads containing ambiguous bases ('N') exceeding 5%. Only high-quality paired-end reads passing all filters were retained for downstream analysis. High-quality reads from all samples were co-assembled de novo into contigs using MEGAHIT (version 1.2.9) (Li et al., 2015) with the meta-sensitive preset and a k-mer range of 21 to 121 (in steps of 10). Contigs shorter than 500 bp were discarded to improve assembly quality and reduce spurious predictions. Open reading frames (ORFs) were predicted on the assembled contigs using MetaGeneMark (version 3.38) (Zhu et al., 2010) with the default parameters for metagenomic sequences. A non-redundant gene catalog was constructed using CD-HIT (version 4.8.1) (Fu et al., 2012) with a sequence identity threshold of 95% and alignment coverage of 90%. Functional annotation of the predicted genes was performed using eggNOG-mapper (version 2.1.9) against the eggNOG 5.0 database. Additional annotation against the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (release 107.0, May 2023) and the Carbohydrate-Active enZYmes (CAZy) database (version 10, 2023) was conducted using Diamond (version 2.1.6) (BLASTp mode, e-value <1e-5). To estimate gene abundance, high-quality reads from each sample were mapped back to the non-redundant gene catalog using Bowtie2 (version 2.5.1) (Langmead & Salzberg, 2012) in end-to-end sensitive mode. Gene counts were generated using SAMtools (version 1.17) (Danecek et al., 2021) and normalized to transcripts per million (TPM) to account for gene length and sequencing depth variations across samples. Key functional genes involved in flavor metabolism were identified based on KEGG/CAZy annotations and their relative abundance profiles throughout fermentation. The raw sequencing data have been deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under the accession number PRJNA1194995.

2.5. Statistical and visual analysis

To ensure analytical robustness, the fermentation experiments were conducted in three independent biological replicates (n = 3) for each

sampling time point. GraphPad Prism version 8.0.2.263 (GraphPad Software, San Diego, CA, USA) was employed to visualize variations in oenological parameters and the concentrations of higher alcohols. All relevant parameters monitored during the HQJ brewing process, including ethanol content, acidity, amino nitrogen and higher alcohols, are expressed as Mean ± standard deviation (SD). Principal Component Analysis (PCA) in SIMCA-14.1 was used to identify clustering patterns in volatile profiles and microbial community, with permutation testing ($n = 200$) to validate model significance ($R^2/Q^2 > 0.5$). The distance between samples in the PCA score plot reflects their similarity/dissimilarity based on the entire volatile profiles and microbial community. Spearman's rank correlation was utilized to establish relationships between microbial taxa and key VFCs. Comprehensive data visualization, including heatmaps for VFC and microbial dynamics, Spearman's rank correlation, and the relative contributions of microbial taxa to enzymes, was performed using R software (version 4.3.1) with appropriate packages (heatmap, scatterpie, reshape2). These multivariate statistical approaches facilitated a deeper understanding of the relationships between microbial communities, enzymatic functions, and key flavor-related metabolites.

3. Results and discussion

3.1. Variation of oenological parameters during HQJ brewing

The oenological parameters (ethanol, reducing sugar, acidity, amino nitrogen) exhibited distinct temporal dynamics throughout HQJ fermentation process, reflecting the active metabolism of the microbial consortium involved. As illustrated in Fig. 1A, the ethanol content increased progressively, eventually reaching a final concentration of 15.59 ± 0.44% (*v*/v) on day 45. The most rapid increase occurred during the first 10 days, after which the rate of ethanol accumulation slowed down. This deceleration may be attributed to a combination of factors, including nutrient depletion, ethanol-induced stress, and acid inhibition, which collectively constrained the activity of yeast and other fermentative microbes (Longo et al., 2021). As shown in Fig. 1B, the content of reducing sugar showed a biphasic trend of first increasing and then rapidly decreasing. A noticeable rise was observed from day 1 to day 3, which was followed by a sharp decrease after day 3. By day 10, the sugar level stabilized at a lower concentration and remained relatively constant for the remainder of the fermentation period. This initial surge can be attributed to the enzymatic conversion of starch in glutinous rice into glucose during the early stage of fermentation. However, as fermentation progressed, yeast, lactic acid bacteria (LAB), and other microbial species metabolize glucose to produce ethanol, lactic acid, and various by-products, thereby causing a steep decline in reducing sugar level. As shown in Fig. 1C and D, both titratable acidity and amino nitrogen content exhibited continuous increases throughout the fermentation period. Titratable acidity rose sharply during the first 10 days, followed by a more gradual upward trend. Similarly, amino nitrogen content showed a steady increase, suggesting enhanced proteolytic activity or microbial nitrogen metabolism. A previous study reported a strong positive correlation between the production of organic acids and the metabolic activity of LAB in rice wine fermentation (Wang et al., 2014), emphasizing the critical role of LAB in the acidification process.

3.2. Formation of higher alcohols during HQJ brewing

Higher alcohols, including n-propanol, isobutanol, 3-methylbutan-1-ol and phenylethyl alcohol, are essential flavor precursors and the main contributors to the sensory complexity and aromatic characteristics of rice wine. At moderate concentrations, these compounds enhance the richness and harmony of the final product. However, excessive accumulation may lead to undesirable fusel oil-like sensory attributes and has been associated with adverse physiological effects such as dizziness and hangover symptoms. As illustrated in Fig. 2A, the total concentration of higher alcohols exhibited a significant increase during the early phase of fermentation and subsequently reached a plateau on the 20th

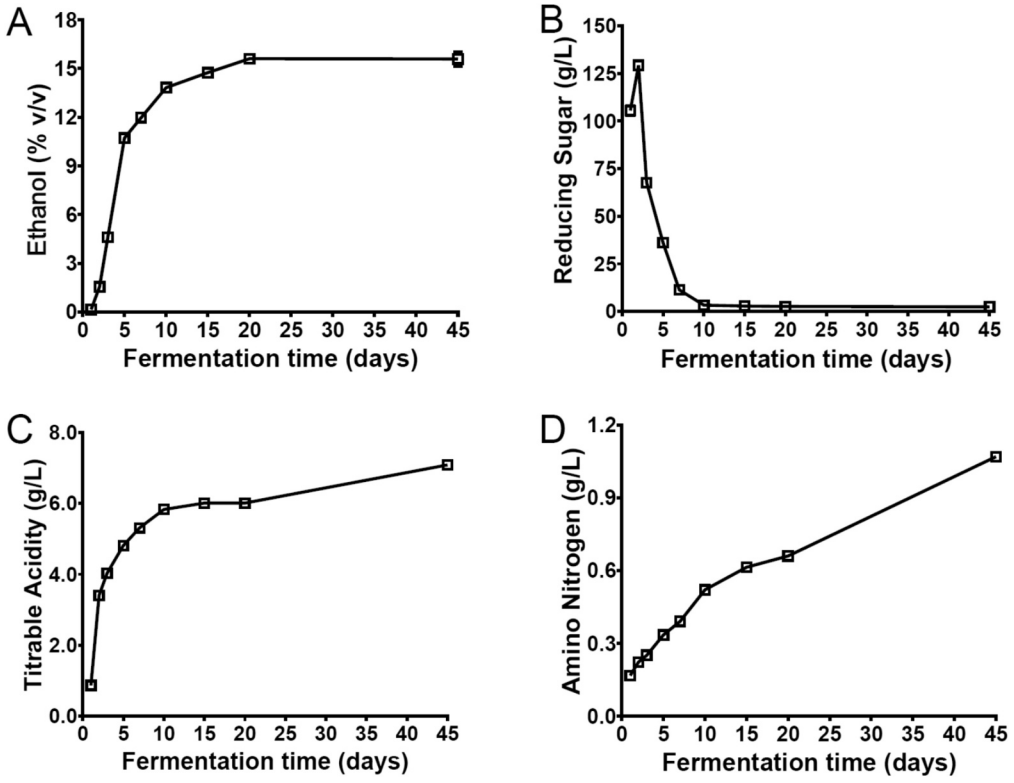


Fig. 1. The dynamic variation trend of oenological parameters during *Hongqujiu* (HQJ) brewing. (A) Ethanol, (B) Reducing sugar, (C) Titratable acidity, (D) Amino nitrogen. Results are presented as means of three independent fermentation batches (n = 3), error bars represent standard deviation.

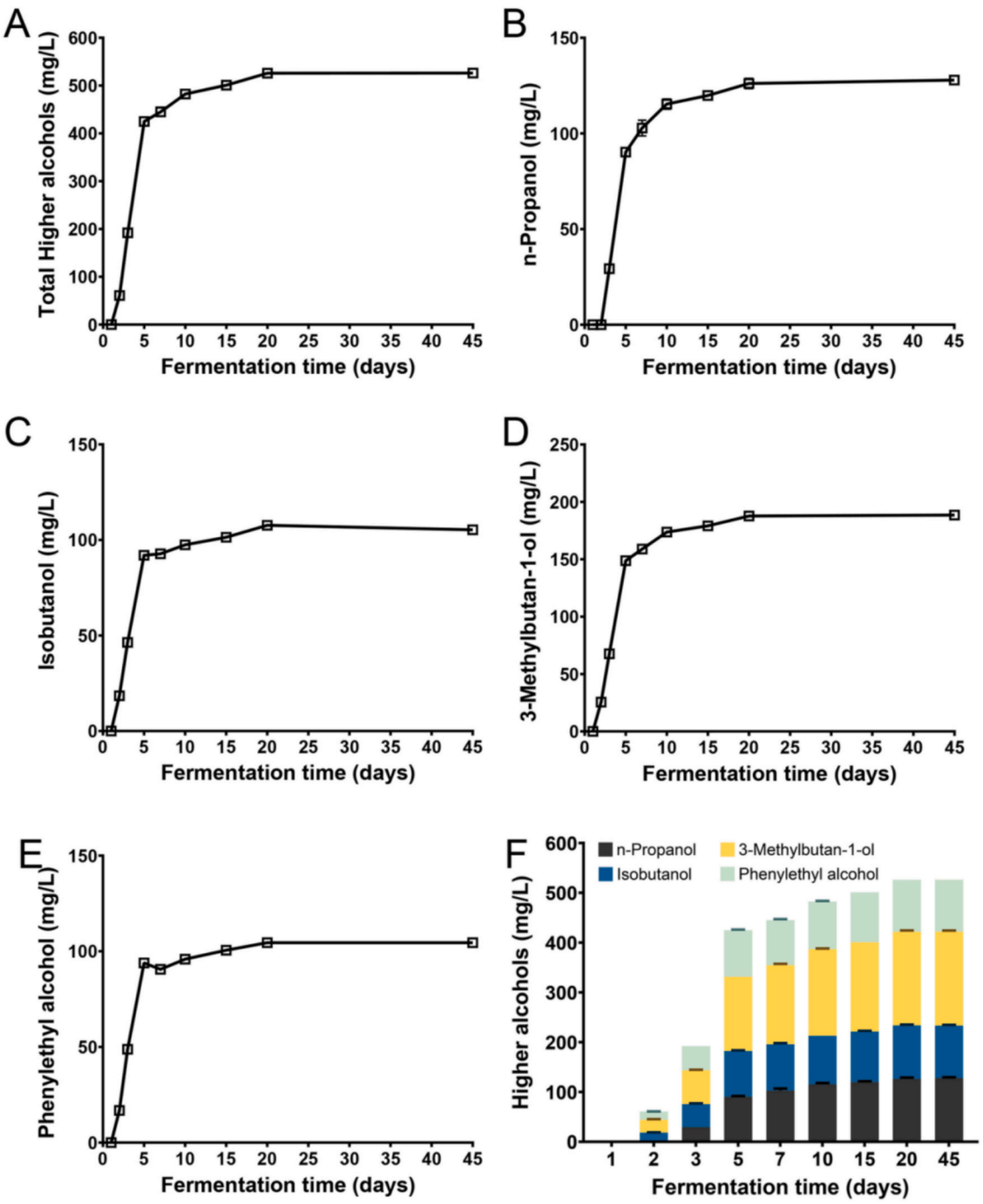


Fig. 2. The dynamic variation trend of higher alcohols content during *Hongqujiu* (HQJ) brewing. (A) Total higher alcohols, (B) n-Propanol, (C) Isobutanol, (D) Isoamyl alcohol, (E) Phenylethyl alcohol, (F) Proportion of different higher alcohols. Results are presented as means of three independent fermentation batches ($n = 3$), error bars represent standard deviation.

day. By the end of the brewing process, the total concentration of higher alcohols had escalated to a substantial level of 526.29 mg/L. Specifically, four major higher alcohols (n-propanol, isobutanol, 3-methylbutan-1-ol and phenylethyl alcohol) were detected during HQJ fermentation (Fig. 2B-E). Among these, 3-methylbutan-1-ol was found to reach the highest concentration, peaking at 188.45 ± 2.34 mg/L on day 45. The final concentrations of n-propanol, isobutanol, and phenylethyl alcohol were recorded as 127.90 ± 1.55 mg/L, 105.32 ± 1.36 mg/L, and 105.51 ± 0.00 mg/L, respectively (Fig. 2F). The biosynthesis of higher alcohols is predominantly attributed to the Ehrlich pathway in *S. cerevisiae*, wherein amino acids are transaminated and then decarboxylated to form corresponding aldehydes, which are subsequently reduced to higher alcohols (Lachenmeier et al., 2008). These compounds not only affect flavor characteristics but also interact with other VFCs, forming the overall sensory experience of fermented beverages. Therefore, controlling their formation and final content is crucial for achieving balance and the ideal flavor in traditional rice wine.

3.3. Formation of VFCs during HQJ brewing

The extended fermentation period of HQJ not only contributes to a gradual increase in ethanol content, but also plays a crucial role in refining its sensory attributes, including color, aroma, and flavor complexity (Chen, Ren, et al., 2021a). The profile of VFCs. serves as a

pivotal indicator for evaluating the fermentation dynamics and final quality of HQJ. In this study, a comprehensive analysis of VFCs was conducted using heatmap visualization and principal component analysis (PCA) throughout the brewing process (Fig. 3). The results revealed a progressive increase in the diversity and abundance of VFCs as fermentation progressed (Fig. 3A). A total of 67 distinct VFCs were identified, which were classified into six major categories: 4 acids, 17 alcohols, 5 aldehydes, 26 esters, 6 phenolic compounds, and 9 others. The integration of heatmap, PCA and hierarchical clustering enabled the delineation of the fermentation process into three distinct phases based on the evolving volatile compound profiles (Fig. 3A-D). Phase I (Day 1–3) marked the initial stage of fermentation, during which the volatile profile was relatively simple and less intense. Notable compounds during this stage included naphthalene (C38), 2-hydroxyethyl methacrylate (C43), 1-pentanol (C9), octanal (C10), 1-hexanol (C14), and 2-heptanone (C6). However, these compounds exhibited a marked decrease in concentration as fermentation advanced. Phase II (Day 7–10) represented the middle stage, characterized by a significant increase in both the diversity and concentration of VFCs. This period was marked by the active formation of alcohols and esters, which are essential contributors to the aromatic complexity of the brew. Phase III (Day 20–45) corresponded to the later stage of fermentation, during which the volatile flavor profile became increasingly enriched and mature. This phase culminated in the development of a distinctive and well-rounded aroma that is characteristic of HQJ, indicating the successful completion of the fermentation process in terms of flavor development.

3.4. Identification of key VFCs in HQJ

The characterization of aroma-active compounds is fundamental to understanding the sensory profile of fermented beverages. The Odor Activity Value (OAV), defined as the ratio of a compound's concentration to its odor detection threshold (ODT), is a robust quantitative metric used to identify VFCs that significantly contribute to the overall aroma perception. Compounds with OAV > 1 are generally considered key contributors to the characteristic flavor. In this study, OAV analysis was systematically applied to pinpoint the key VFCs responsible for the distinctive sensory attributes of HQJ. As detailed in Table 1, a total of 18 key VFCs were identified, including ethyl acetate, ethyl butanoate, isobutanol, isoamyl acetate, 3-methylbutan-1-ol, ethyl caproate, ethyl lactate, nonanal, ethyl octanoate, 1-octen-3-ol, ethyl caprate, ethyl 2-phenylacetate, hexanoic acid, guaiacol, phenylethyl alcohol, 5-

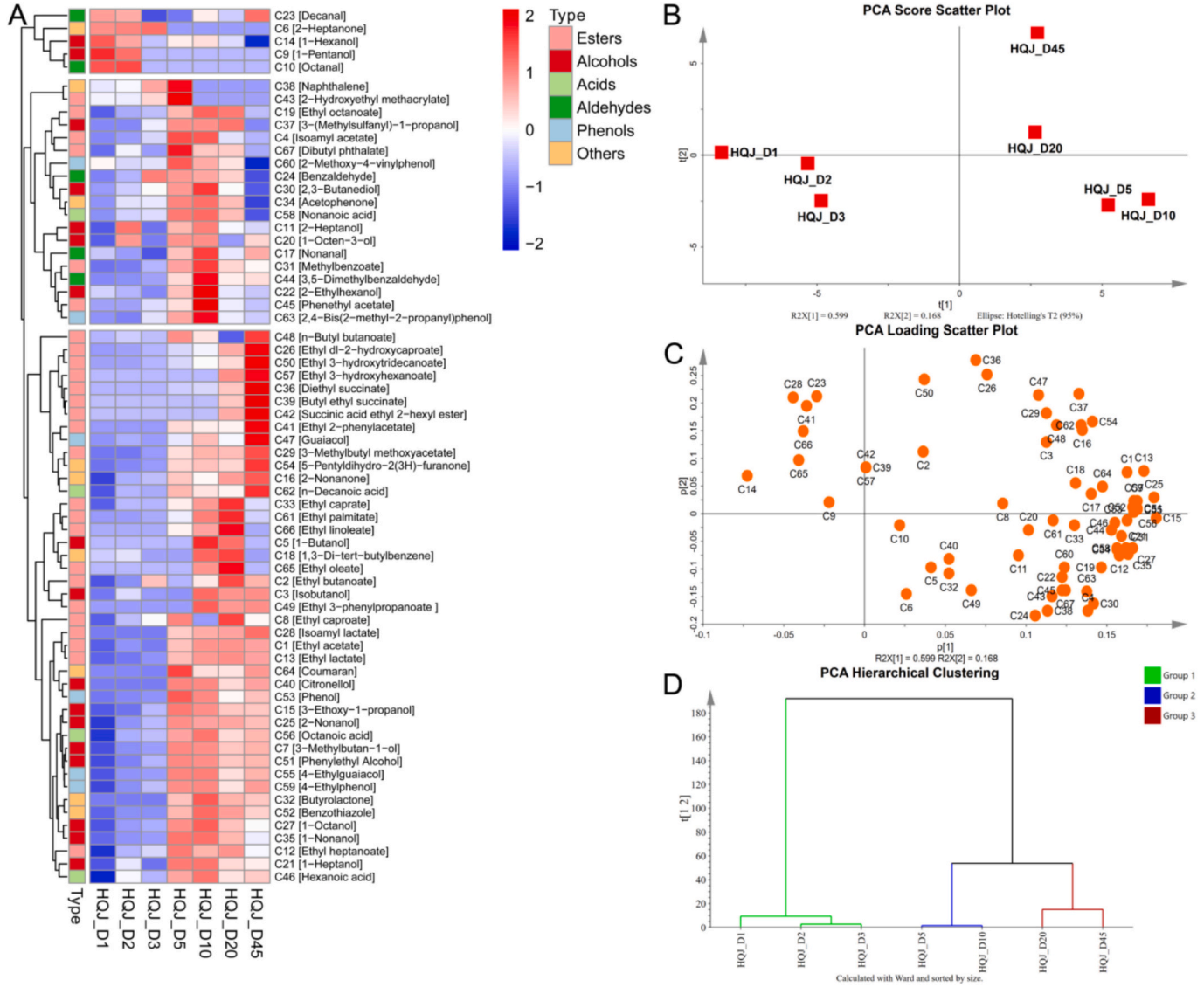


Fig. 3. The dynamic variation trend of volatile flavor compounds (VFCs) during *Hongqujiu* (HQJ) brewing. (A) Heatmap, (B) PCA score plot, (C) PCA loading plot, (D) Hierarchical clustering. Results are presented as means of three independent fermentation batches (n = 3).

Table 1
The key volatile flavor compounds (VFCs) with OAV > 1 in *Hongqujiu* (HQJ) revealed by metabolomic and flavoromic profiling.

VFCs	CAS	RI	Sensory Description	Threshold (mg/L)	Concentration (mg/L)	OAV	Ref
Ethyl acetate [C1]	141–78-6	888.73	Aromatic, Brandy, Grape	7.5	124.87 ± 6.56	16.65	Moyano et al.; 2010
Ethyl butanoate [C2]	105–54-4	1034.78	Apple, Butter, Cheese, Pineapple	0.02	0.7 ± 0.03	34.9	Moyano et al.; 2010
Isobutanol [C3]	78–83-1	1089.58	Apple, Bitter, Cocoa, Wine	40	77.1 ± 8.02	1.93	Moyano et al.; 2010
Isoamyl acetate [C4]	123–92-2	1110.55	Apple, Banana, Pear	0.03	0.22 ± 0.01	7.28	Moyano et al.; 2010
3-Methylbutan-1-ol [C7]	123–51-3	1194.62	Burnt, Cocoa, Floral, Malt	65	155.15 ± 25.79	2.39	Moyano et al.; 2002
Ethyl caproate [C8]	123–66-0	1222.38	Apple Peel, Brandy, Fruit Gum, Overripe Fruit, Pineapple	0.005	3.12 ± 0.19	624.75	Moyano et al.; 2010
Ethyl lactate [C13]	97–64-3	1330.01	Cheese, Floral, Fruit, Pungent, Rubber	100	178.36 ± 20.74	1.78	Moyano et al.; 2010
Nonanal [C17]	124–19-6	1376.18	Fat, Floral, Green, Lemo	0.0025	0.05 ± 0.01	20.99	Culleré et al.; 2011
Ethyl octanoate [C19]	106–32-1	1418.14	Apricot, Brandy, Fat, Floral, Pineapple	0.002	2.65 ± 0.19	1324.68	Moyano et al.; 2010
1-Octen-3-ol [C20]	3391-86-4	1436.29	Cucumber, Earth, Fat, Floral, Mushroom	0.02	0.02 ± 0	1.23	Ma et al.; 2025
Ethyl caprate [C33]	110–38-3	1619.94	Brandy, Grape, Pear	0.51	1.64 ± 0.15	3.21	Chaves et al.; 2007
Ethyl 2-phenylacetate [C41]	101–97-3	1764.49	Floral, Fruit, Honey, Rose	0.1	0.26 ± 0.05	2.61	Isogai et al.; 2005
Hexanoic acid [C46]	142–62-1	1826.21	Cheese, Oil, Pungent, Sour	3	46.52 ± 8.25	15.51	Chaves et al.; 2007
Guaiacol [C47]	90–05-1	1836.91	Burnt, Phenol, Wood	0.0092	0.15 ± 0.03	16.56	Poisson & Schieberle; 2008
Phenylethyl alcohol [C51]	60–12-8	1885.87	Fruit, Honey, Lilac, Rose, Wine	10	38.62 ± 2.69	3.86	Moyano et al.; 2010
5-Pentyldihydro-2(3H)-furanone [C54]	104–61-0	2003.19	Coconut	0.021	0.2 ± 0.04	9.4	Poisson & Schieberle; 2008
Octanoic acid [C56]	124–07-2	2039.89	Cheese, Fat, Grass, Oil	8.8	40.68 ± 6.96	4.62	Chaves et al.; 2007
Ethyl palmitate [C61]	628–97-7	2235.88	Wax	1	2.58 ± 0.09	2.58	Moyano et al.; 2002

Odor threshold values were selected from published literature (van Gemert, 2011) based on evaluation medium and relevance to fermented alcoholic beverages, with preference given to values most comparable to Huangjiu.

pentyldihydro-2(3H)-furanone, octanoic acid and ethyl palmitate.

Among these, esters are particularly significant in shaping the aromatic profile of *Huangjiu*, known for their fruity, floral, and sweet sensory characteristics (Hu et al., 2018). Ethyl esters, in particular, are widely recognized for their pleasant aroma contributions and are essential in enhancing the complexity and richness of the overall flavor (Yu et al., 2019). For instance, ethyl acetate enhances the wine with floral and fruity notes (Kang et al., 2016), although concentrations exceeding 200 mg/L may lead to off-flavors or sensory imbalance (Zhao et al., 2022). Ethyl lactate plays a crucial role in the fermentation of *Huangjiu*, especially in rice-flavored baijiu and broomcorn millet *Huangjiu* (Wang et al., 2022), contributing creamy, buttery, and fruity undertones (Jin et al., 2021). Ethyl palmitate, with its mild fruity aroma, has also been recognized for its positive influence on the sensory quality of fermented foods and beverages (Chen et al., 2023). Moreover, the sensory impact of HQJ aroma may be influenced by synergistic interactions among multiple VFCs. For example, the co-occurrence of ethyl octanoate and ethyl acetate may lead to aroma superimposition and mutual enhancement, thereby intensifying the fruity and sweet notes of the final product.

In addition to esters, higher alcohols, such as isobutanol and 3-methylbutan-1-ol, are important by-products of ethanol fermentation and play a dual role in the flavor profile of *Huangjiu*. While they can contribute to a full-bodied mouthfeel and complex aroma when present at moderate levels, excessive accumulation may result in harsh solvent-like or fusel oil off-flavors, thereby deteriorating sensory quality (Lachenmeier et al., 2008). Furthermore, high levels of higher alcohols have been associated with potential adverse physiological effects, including neurological discomfort and hangover symptoms after consumption (Yang et al., 2014). Therefore, controlling higher alcohol formation during fermentation is essential not only for flavor optimization but also for improving product safety and consumer acceptance.

Moreover, fatty acids, including hexanoic acid, octanoic acid, and n-decanoic acid, serve as important precursors in the biosynthesis of ethyl esters. These compounds participate in esterification reactions catalyzed by enzymes such as alcohol acyltransferases, thereby enriching the aroma profile. However, although moderate levels contribute indirectly to ester formation, excessive fatty acid accumulation may impart rancid, cheesy, or sweaty off-odors and suppress overall aroma harmony (Chen, Ren, et al., 2021a; Wang et al., 2020). High concentrations may also disrupt the acid–ester balance, limiting esterification efficiency and leading to sensory defects. Therefore, maintaining a balanced acid-ester equilibrium is crucial for achieving a harmonious and desirable flavor profile in HQJ.

The odor thresholds used for OAV calculation in this study (Table 1) were selected from recent literature specific to alcoholic beverages or widely accepted reference values in flavor chemistry. While thresholds determined specifically in a complex rice wine matrix are limited, the chosen values allow for meaningful comparative analysis with numerous other studies on fermented products (e.g., wine, beer, spirits), fulfilling the primary objective of identifying the key VFCs within the HQJ system based on their relative contribution potential. It is noted that certain compounds, particularly potent esters like ethyl caproate and ethyl octanoate, exhibited high OAV values. Such values are not uncommon in studies of distilled spirits and aromatic fermented beverages, reflecting the low odor thresholds and significant aromatic potency of these key esters. However, the interpretation of these high OAVs warrants consideration of potential influencing factors: matrix effects, synergistic or antagonistic interactions.

3.5. Dynamic evolution of microbial communities during HQJ brewing

The α-diversity indices revealed clear temporal shifts in the microbial community during HQJ fermentation (Table 2). The richness estimators (Chao and ACE) gradually decreased from D1 (632) to D45 (603), indicating a progressive reduction in community richness as fermentation advanced. The Shannon index also declined overall, from 2.46 (D1) to 1.57 (D45), suggesting a decrease in microbial diversity, although a slight rebound was observed at D10. In contrast, the Simpson index

Table 2
Analysis of α-diversity of microbial communities in the brewing process of HQJ.

Sample	Ace Index	Shannon Index	Simpson Index	Chao Index	Coverage Index
HQJ_D1	632	2.456275	0.176041	632	1
HQJ_D3	626	1.952381	0.332718	626	1
HQJ_D5	612	2.304155	0.227485	612	1
HQJ_D10	616	2.414933	0.197955	616	1
HQJ_D20	613	1.92001	0.347702	613	1
HQJ_D45	603	1.570146	0.46267	603	1

increased from 0.176 (D1) to 0.463 (D45), reflecting enhanced dominance and reduced evenness in the later fermentation stages. The coverage value was 1.00 for all samples, confirming sufficient sequencing depth and reliable representation of the microbial communities. Collectively, these results indicate that microbial richness and diversity gradually declined, whereas community dominance increased during HQJ fermentation. As shown in the principal component analysis (PCA) plots of microbial community composition at the genus level (Fig. 4A&B), both the bacterial and fungal community compositions undergo significant changes during HQJ fermentation process, with particularly pronounced changes occurring during the early fermentation stage (Day 1 to Day 10).

HQJ brewing involves a complex co-fermentation of multiple microorganisms, resulting in a dynamic and structured microbial ecosystem. The characteristic flavor profile of HQJ is shaped by the intricate interactions among various microbial components, including molds, yeasts, and bacteria, whose metabolic activities collectively define the sensory attributes of the final product (Sun et al., 2025). In this study, metagenomic sequencing was employed to monitor the temporal dynamics of microbial communities throughout fermentation. As illustrated in Fig. 5A and Fig. 6A, *Weissella*, *Lactobacillus*, *Enterobacter* and *Kosakonia* were the dominant bacterial genera in the entire fermentation of HQJ. On the initial day of fermentation, the microbial community was enriched in *Enterobacter*, *Kosakonia*, *Klebsiella* and *Pantoea*. However, the relative abundance of these genera decreased as fermentation progressed. In contrast, the relative abundance of *Lactococcus* exhibited fluctuations, with an overall increasing trend, particularly evident on Day 3 and 20–45. Notably, *Weissella* maintained the highest relative abundance throughout the entire fermentation process, followed closely by *Lactobacillus*, whose abundance gradually increased over time, indicating its pivotal role in flavor development and fermentation stability. Lactic acid bacteria (LAB), including *Weissella* and *Lactobacillus*, are crucial microbial consortia in fermented food systems. They contribute to flavor enhancement; the synthesis of antimicrobial compounds (e.g., bacteriocins and organic acids), and the production of bioactive metabolites. These compounds not only inhibit the growth of spoilage and pathogenic microorganisms, but also enhance the sensory attributes and flavor quality of fermented products (Cappello et al., 2017; Xiao et al., 2022). For instance, in the *Weissella*, *Weissella confusa* is the most abundant species (Fig. S2A). Relevant studies have demonstrated that the combined and sequential fermentation of mulberry fruit juices by *W. confusa* and *Pichia kudriavzevii* significantly increases esters by 61.06%, endowing the juice with fruity and floral aromas (Cai et al., 2026). Among the *Lactobacillus* genus, *Lactobacillus plantarum* is particularly significant (Fig. S2A). As a potential probiotic, it is widely present in various fermented foods. According to the research by Peng et al. (2024), the inoculation of *L. plantarum* promotes the increase of VFCs such as diethyl succinate, ethyl benzoate, and ethyl caprate in *Huangjiu*, thereby enhancing the richness and complexity of its flavor profile. Consequently, LAB play a dual role in maintaining fermentation stability and enriching the flavor profile of HQJ.

In terms of fungal communities, the primary fungal genera involved in HQJ fermentation included *Saccharomyces*, *Aspergillus*, *Talaromyces*, *Monascus*, and *Millerozyma* (Fig. 5B and Fig. 6B). Among these, *Aspergillus* was particularly abundant in the early stages of fermentation but showed a declining trend as the process progressed, which correlated with the reduction in available reducing sugars. In terms of further breakdown at the species level, *Aspergillus niger*, *Aspergillus awamori* and *Aspergillus welwitschiae* displayed decreasing trends, whereas *Aspergillus oryzae* showed an initial increase during the first 10 days followed by a decline (Fig. S2B). Similarly, *Monascus* exhibited an increase from Day 1 to Day 10, then gradually decreased, possibly due to rising alcohol levels that inhibit its metabolic activity beyond this stage. *Saccharomyces* maintained consistently high relative abundance (>35%) throughout HQJ fermentation, peaking on Day 3, slightly declining on Day 5, and then sharply increasing during the later stages (Days 20 and 45). This dominance can be attributed to its robust ethanol-producing capacity, which enables it to thrive in high-alcohol environments typical of advanced fermentation stages. Moreover, *Saccharomyces* is a major contributor to VFCs, playing a central role in shaping the sensory profile of fermented beverages through the synthesis of esters, higher alcohols, and other aromatic volatiles (Chen & Xu, 2010; Jung et al., 2012). These compounds not only enhance aroma complexity but also contribute to the overall flavor balance and mouthfeel of the final product (Xu et al., 2022). In addition to *Saccharomyces*, non-*Saccharomyces* yeasts such as *Millerozyma*, *Clavispora*, and *Meyerozyma* exhibited fluctuating abundance patterns, initially decreasing and then increasing again after Day 10 (Fig. 6B). These yeasts are increasingly recognized for their significant contributions to aroma generation, particularly in the early and mid-stages of fermentation (Cao et al., 2024; Ge et al., 2022). Their fluctuating abundance patterns suggest a dynamic interplay with the evolving chemical environment and flavor compound profiles, indicating their importance in shaping the final sensory characteristics of HQJ.

3.6. Metabolic networks of starch and glucose during HQJ brewing

During traditional HQJ production, the semi-open and solid-state fermentation process triggers dynamic changes in both VFCs and microbial community (Liu et al., 2020). The fermentation environment supports the growth of diverse microorganisms that utilize glutinous rice

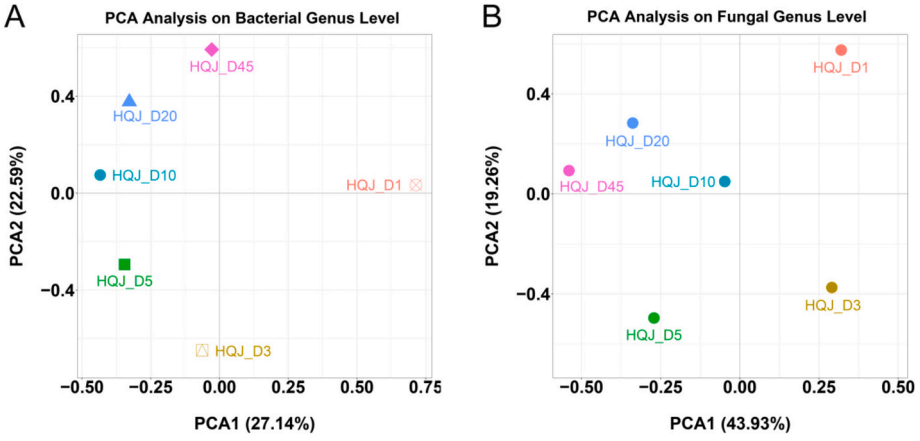


Fig. 4. Principal Component Analysis (PCA) of the composition of microbial community in the brewing process of *Hongqujiu* (HQJ). (A) Bacterial community, (B) Fungal community. Results are presented as means of three independent fermentation batches (n = 3).

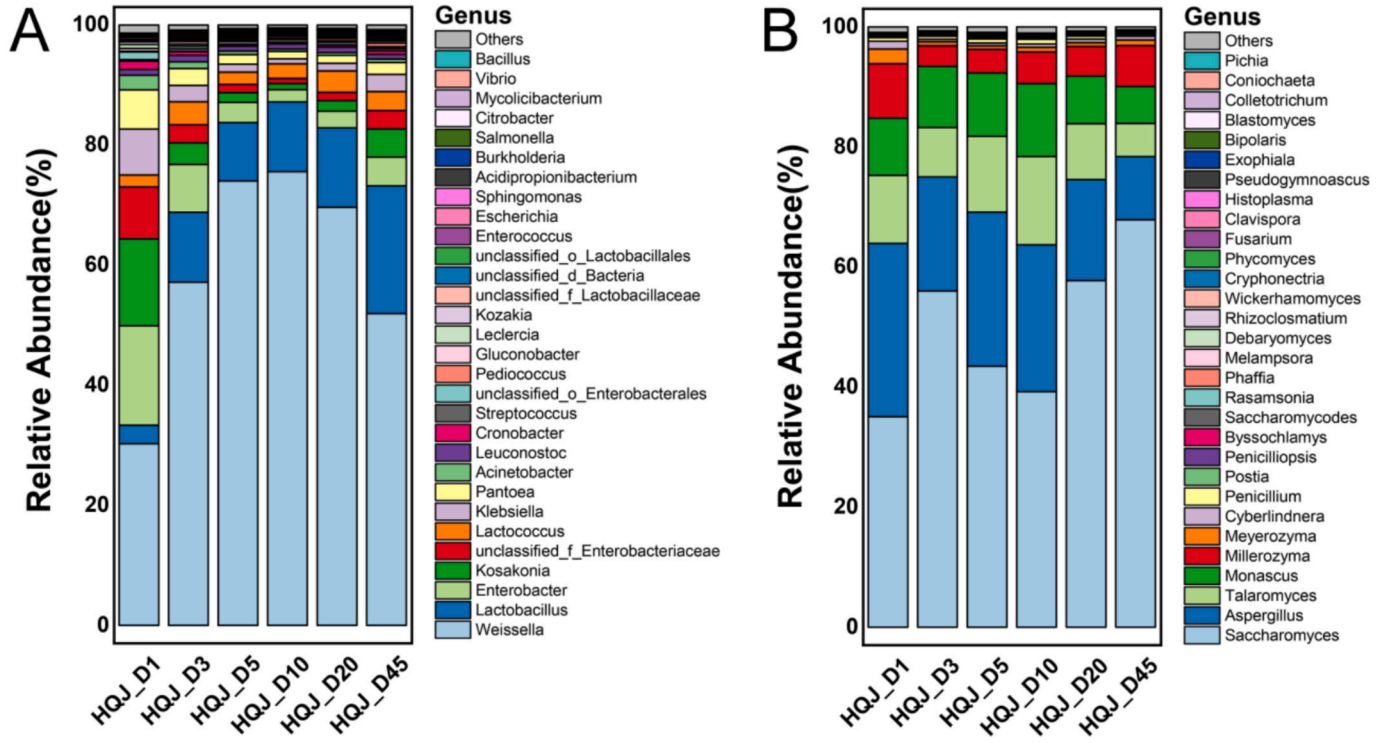


Fig. 5. Stacked histogram of the relative abundance of microbial genera during *Hongqujiu* (HQJ) brewing. (A) Bacterial community, (B) Fungal community. Results are presented as means of three independent fermentation batches (n = 3).

as the primary substrate, resulting in the formation of various metabolites. To explore the potential metabolic pathways associated with key VFCs biosynthesis, metagenomic sequencing data were analyzed using KEGG annotation (Fig. 7 and Fig. 8). A total of 147 enzyme-encoding genes related to key VFCs metabolism were identified, originating from 183 genera of fungi and bacteria. It should be noted that these annotations reflect the genetic potential for enzymatic functions rather than direct measurements of catalytic activity.

Fig. 7 illustrates the reconstructed metabolic pathways associated with starch and glucose degradation, encompassing starch hydrolysis, glycolysis, pyruvate metabolism, the tricarboxylic acid (TCA) cycle, and the pentose phosphate pathway. Fig. 8 further presents the contribution of different microbial genera to these enzymes. Among them, *Aspergillus* showed a high abundance of genes encoding carbohydrate-active hydrolases, suggesting its potential role in starch degradation, which is consistent with previous reports (Yamakawa et al., 2012; Zeng et al., 2021; Gan et al., 2019). Additionally, genes encoding alcohol dehydrogenase (EC 1.1.1.1), a key enzyme involved in ethanol formation, were widely distributed among *Weissella*, *Lactobacillus*, and *Saccharomyces* (Fig. 9). This distribution indicates that these genera may contribute to ethanol production during HQJ fermentation, although actual catalytic activity was not directly measured in this study.

3.7. Anabolic pathway of higher alcohols during HQJ brewing

This study further delineated the synthetic metabolic pathways of key VFCs at both the enzymatic and microbial genus levels, as illustrated in Fig. 8 and Fig. 9. The VFCs in *Huangjiu* are primarily derived from raw materials and microbial metabolites during brewing (Chen et al., 2020; Shen et al., 2024; Tian et al., 2022). Therefore, the sensory characteristics of the final product are closely associated with the diversity, abundance, and metabolic potential of the microbial community. To further strengthen the linkage between microbial taxa and higher alcohol formation, Spearman correlation analysis was performed (Supplementary Fig. S3). The results showed that genera such as *Streptococcus*, *Streptomyces* and *Lactobacillus* were positively correlated with higher alcohols, including isobutanol, 3-methylbutan-1-ol and phenylethyl alcohol, providing additional statistical support for their potential contribution to higher alcohol biosynthesis.

Pyruvate, acetyl-CoA, glycerate-3P, and phosphoenolpyruvate were identified as important precursor metabolites in the anabolic of the key VFCs. Higher alcohols, an essential group of VFCs, are primarily synthesized via two major metabolic pathways: the Ehrlich pathway (amino acid degradation) and the Harris pathway (pyruvate synthesis) (Cui et al., 2023). Glycerate-3P serves as a precursor for amino acid biosynthesis. Metagenomic annotation identified genes encoding phosphoglycerate dehydrogenase (EC 1.1.1.95), phosphoserine transaminase (EC 2.6.1.52), and phosphoserine phosphatase (EC 3.1.3.3), suggesting the potential for serine-family amino acid biosynthesis. These amino acids may subsequently be converted into propionaldehyde through reactions associated with annotated 3-hydroxy acid dehydrogenase (EC 1.1.1.381) and glycerol dehydrogenase (EC 1.1.1.6) (Fig. 8). The transformation of propanal into 1-propanol involves multiple enzymatic steps catalyzed by alcohol dehydrogenase (EC 1.1.1.1, primarily produced by *Weissella*, *Lactobacillus*, and *Saccharomyces*), alcohol dehydrogenase (NADP+) (EC 1.1.1.2, attributed to unclassified_f_*Enterobacteriaceae*, *Saccharomyces*, and *Pantoea*), and 1,3-propanediol dehydrogenase (EC 1.1.1.202, mainly contributed by *Lactobacillus*, unclassified_o_*Enterobacterales*, and *Pediococcus*) (Fig. 9). Notably, *Pediococcus* has been recognized as a key player in flavor development in rice wine (Chen et al., 2020).

The biosynthesis of phenylalanine from phosphoenolpyruvate involves multiple enzymatic steps. Genes related to these enzymes were annotated in *Weissella*, *Saccharomyces*, and *Lactobacillus*, suggesting their potential role in aromatic amino acid biosynthesis. Phenylalanine is subsequently converted into phenylacetaldehyde via reactions associated with aromatic-L-amino-acid decarboxylase (EC 4.1.1.28), catalase-peroxidase (EC 1.11.1.21), and amidase (EC 3.5.1.4), whose encoding genes were detected in *Talaromyces*, *Aspergillus*, *Monascus*, *Weissella* and *Saccharomyces* (Fig. 9). Further dehydrogenation of

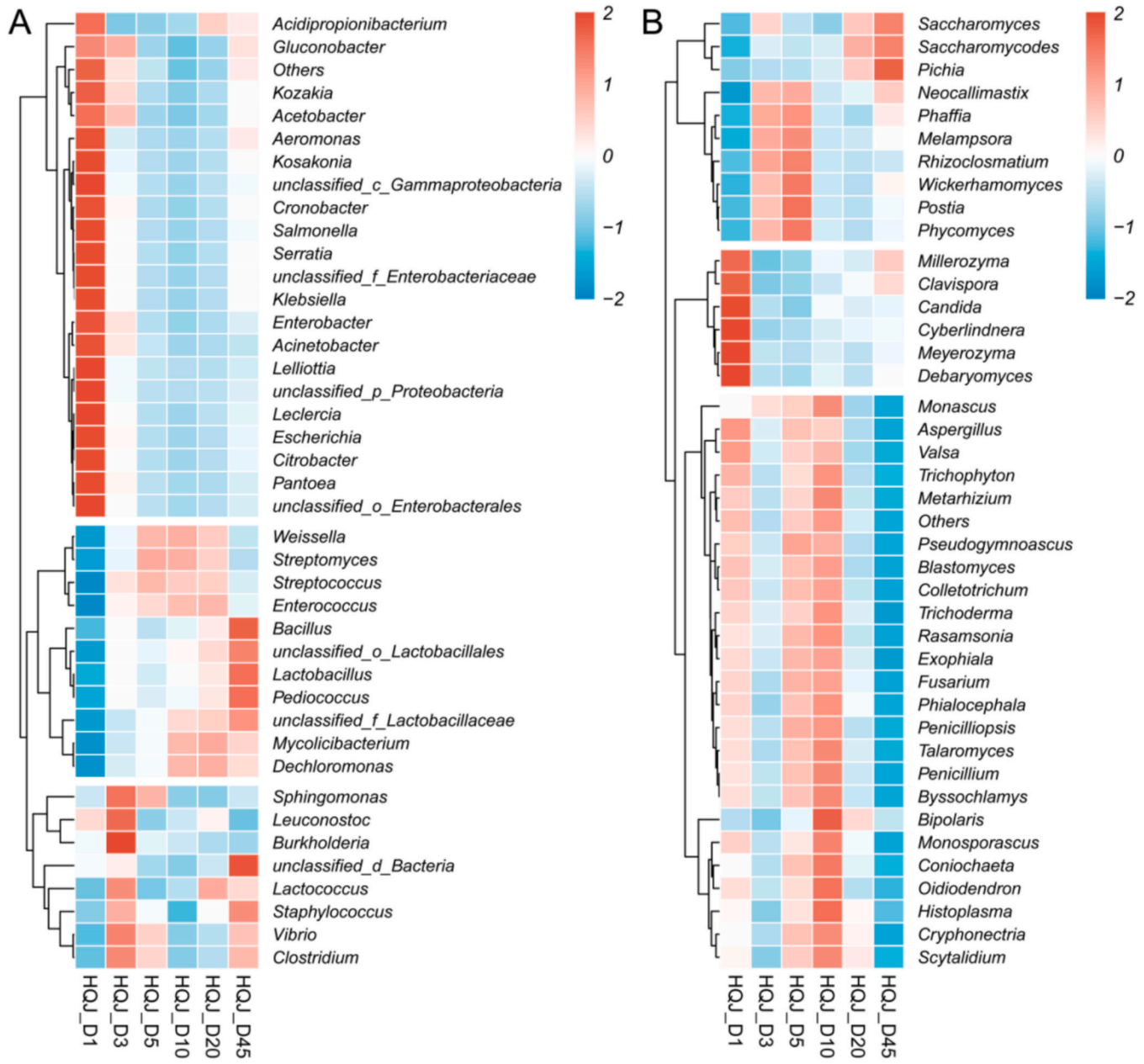


Fig. 6. Heatmap of the relative abundance of microbial genera during *Hongqujiu* (HQJ) brewing. (A) Bacterial community, (B) Fungal community. Results are presented as means of three independent fermentation batches (n = 3).

phenylacetaldehyde, catalyzed by alcohol dehydrogenase (EC 1.1.1.1), aryl-alcohol dehydrogenase (EC 1.1.1.90), and alcohol dehydrogenase (NADP+) (EC 1.1.1.2), leads to the formation of phenylethylalcohol. The presence of corresponding annotated genes suggests a metabolic potential for phenylethanol production rather than confirming actual catalytic activity.

Simultaneously, phenylalanine may be transformed into benzoate via benzoate 1,2-dioxygenase (EC 1.14.12.10), 1,6-dihydroxycyclohexa-2,4-diene-1-carboxylate dehydrogenase (EC 1.3.1.25), and catechol *O*-methyltransferase (EC 2.1.1.6). Genes encoding these enzymes were primarily annotated in unclassified_f_*Enterobacteriaceae*, *Acinetobacter*, and *Klebsiella,* suggesting a possible contribution of these genera to guaiacol-related metabolic routes. *Acinetobacter* has previously been associated with distinctive flavor metabolite formation in rice wine fermentation (Qian et al., 2023).

In addition, pyruvate serves as a precursor for the biosynthesis of valine and leucine, which are involved in isobutanol and 3-methylbutan-1-ol production. Genes encoding branched-chain-amino-acid transaminase (EC 2.6.1.42) and pyruvate decarboxylase (EC 4.1.1.1) were detected, indicating the potential for α-keto acid formation. These α-keto acids may subsequently be reduced to isobutanol and 3-methylbutan-1-ol by alcohol dehydrogenases, contributing to the aromatic complexity of HQJ. It should be noted that these pathway reconstructions are based on gene annotation and represent inferred metabolic potential rather than direct evidence of in situ enzymatic activity.

3.8. Anabolic pathway of fatty acids in HQJ during brewing

Fatty acids, including caproic acid, caprylic acid, and n-capric acid, are important contributors to the sensory profile of *Huangjiu.* At elevated concentrations, they may impart rancid or undesirable odors (Wang et al., 2020; Chen, Huang, et al., 2021). However, beyond their direct sensory impact, these compounds also serve as essential precursors for ester synthesis and therefore play a pivotal role in shaping the final

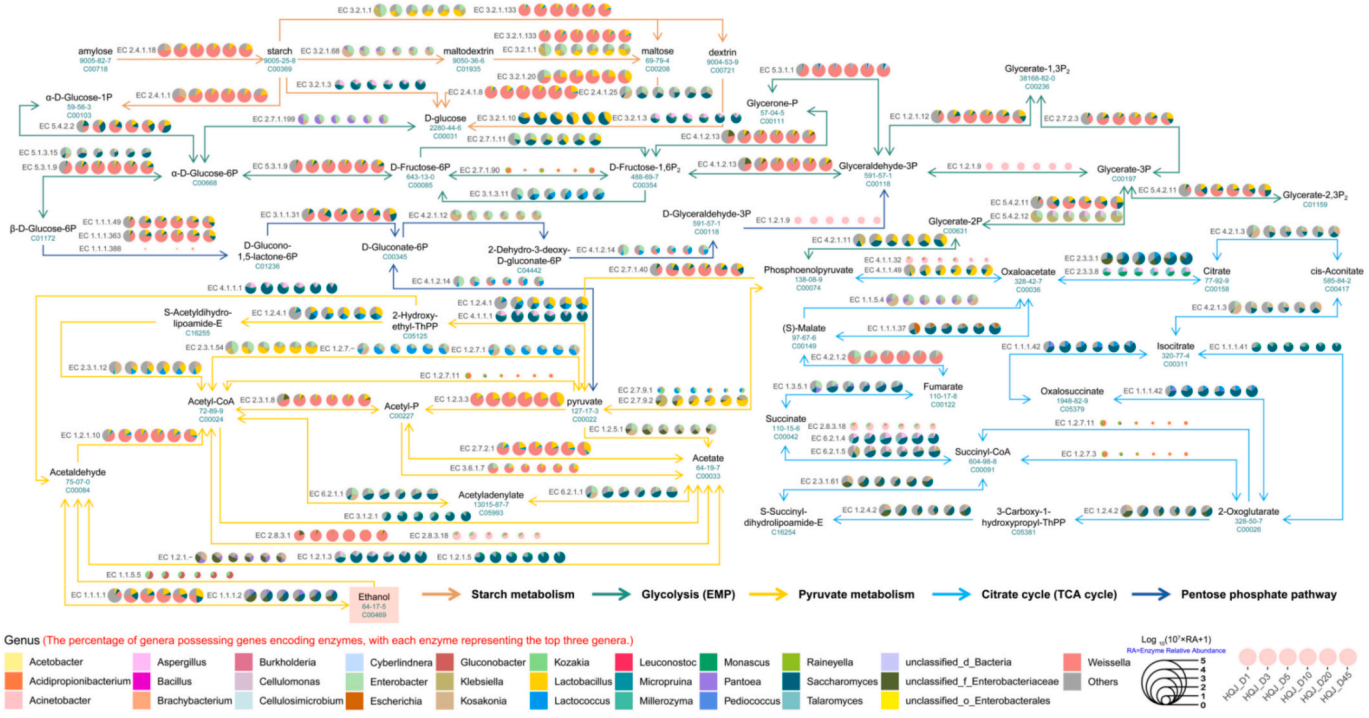


Fig. 7. The metabolic pathway network of starch degradation and glucose glycolysis during the brewing process of *Hongqujiu* (HQJ).

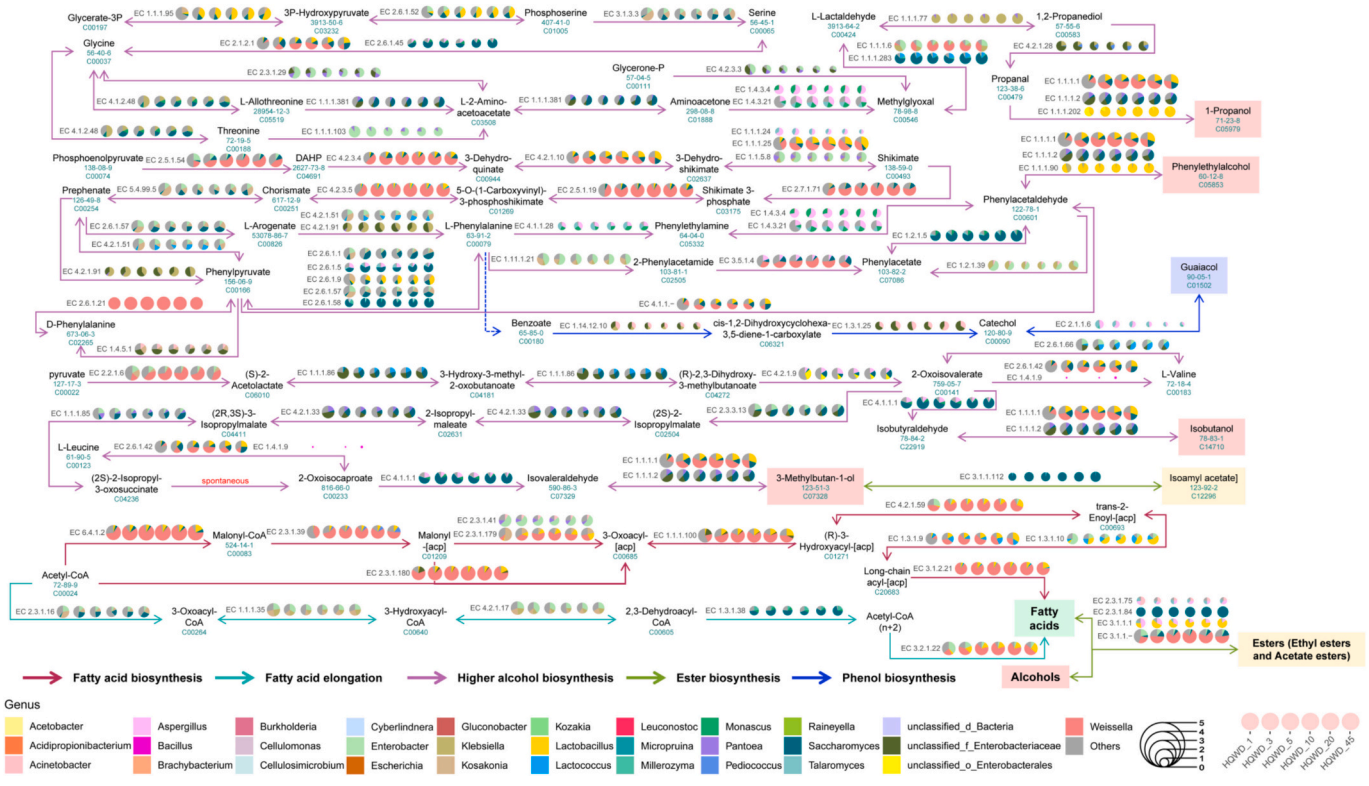


Fig. 8. The metabolic pathway network of key volatile flavor compounds (VFCs) during the brewing process of *Hongqujiu* (HQJ).

aroma profile of HQJ. In this study, several fatty acids were identified as the key VFCs, prompting further investigation into their potential anabolic pathways during fermentation.

The synthesis of fatty acids commences with acetyl-CoA, which is converted to malonyl-CoA by acetyl-CoA carboxylase (EC 6.4.1.2) (Fig. 8). This step is followed by a cascade of enzymatic reactions involving acyl-carrier proteins (ACPs), culminating in the production of various fatty acids. Metagenomic analysis revealed that multiple microbial genera, including *Weissella*, *Lactobacillus* and *Lactococcus*, harbor genes encoding enzymes associated with fatty acid biosynthesis (Fig. 9). Spearman correlation analysis (Supplementary Fig. S3) further demonstrated that genera such as *Weissella* was positively correlated with the

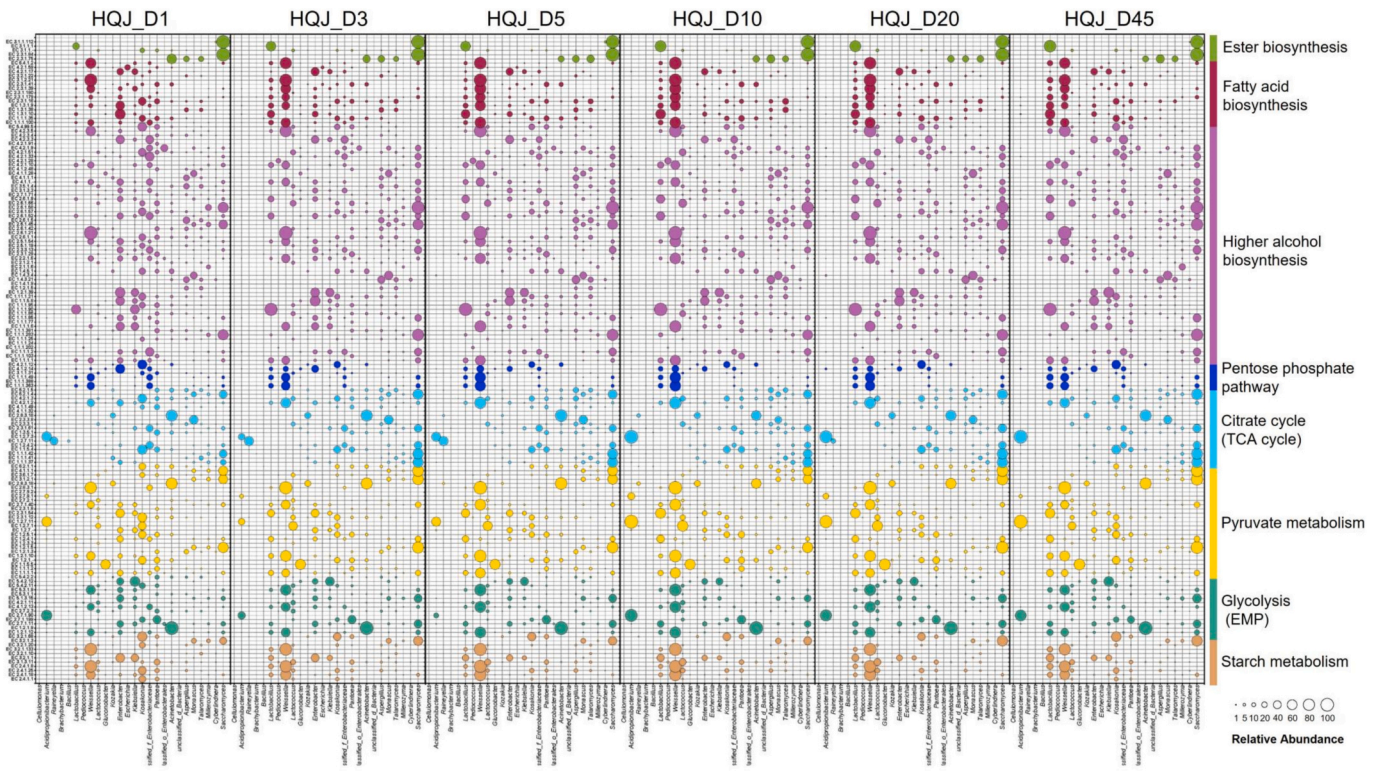


Fig. 9. Visualization of the contribution of key microorganisms to the key functional genes in the glycolysis and volatile flavor compounds (VFCs) synthesis pathways during the brewing process of *Hongqujiu* (HQJ). The size of the bubbles in the figure represents the contribution (in percentage) of specific microbial taxa to the relevant functional genes in the flavor metabolism pathway.

formation of hexanoic acid and octanoic acid. These results suggest that lactic acid bacteria (LAB) possess the genetic potential to participate in fatty acid anabolism during HQJ fermentation, which is consistent with the findings from a previous study (Wang et al., 2021).

The elongation of fatty acid chains, facilitated by the enzymatic conversion of acetyl-CoA into various CoA derivatives, is crucial for fatty acid synthesis. Genes encoding key enzymes involved in this process were annotated in diverse microbial genera, including *Kosakonia*, *Saccharomyces*, *Enterobacter*, *Klebsiella*, *Millerozyma*, *Monascus*, *Weissella* and *Lactobacillus* (Fig. 9). The presence of these annotated genes indicates the potential metabolic capacity of these microorganisms to contribute to fatty acid synthesis. These microorganisms play an integral role in the biosynthesis and accumulation of fatty acids during HQJ fermentation, not only contributing to the metabolic network but also influencing the formation of esters and other aroma-enhancing compounds.

The formation of acyl-CoA derivatives represents a critical metabolic node linking fatty acid biosynthesis to downstream ester production. Once synthesized, fatty acids can be activated to their corresponding acyl-CoA forms and subsequently participate in esterification reactions catalyzed by alcohol acyltransferases and related enzymes during the alcoholization stage. Therefore, fatty acid synthesis not only contributes directly to flavor formation but also establishes a metabolic precursor pool for ester biosynthesis. This connection provides a mechanistic basis for the coordinated regulation of fatty acids and esters discussed in Section 3.9, thereby completing the fatty acid–ester metabolic pathway chain in HQJ fermentation.

3.9. Anabolic pathway of esters in HQJ during brewing

As an extension of fatty acid anabolism described in Section 3.8, ester biosynthesis in HQJ fermentation relies on fatty acids as key precursors. Medium- and long-chain fatty acids generated during fatty acid synthesis provide acyl donors for ester formation through alcohol acyltransferase–mediated reactions. In the presence of corresponding alcohols, these acyl-CoA intermediates are converted into ethyl esters and acetate esters, which represent major contributors to the characteristic aroma of HQJ. Esters are among the most crucial VFCs in HQJ, playing a fundamental role in defining its characteristic flavor profile. Their formation primarily occurs during fermentation and aging through enzymatic esterification reactions between alcohols (e.g., ethanol, higher alcohols) and fatty acids (e.g., acetic acid, butyric acid, lactic acid). Metagenomic annotation identified multiple ester-related enzyme genes within the microbial community, including long-chain-alcohol O-fatty-acyltransferase (EC 2.3.1.75), alcohol *O*-acetyltransferase (EC 2.3.1.84), carboxylesterase (EC 3.1.1.1) and carboxylic-ester hydrolases (EC 3.1.1.-) (Fig. 8). The presence of these genes suggests a genetic potential for ester synthesis and transformation. However, it should be noted that gene annotation reflects functional potential rather than direct evidence of in situ catalytic activity.

Genes annotated as long-chain-alcohol O-fatty-acyltransferase were primarily affiliated with *Acinetobacter*, *Aspergillus* and *Talaromyces*. Similarly, the genes encoding alcohol *O*-acetyltransferase were primarily assigned to *Saccharomyces* and *Cyberlindnera*, suggesting their potential involvement in acetate ester formation. Although less frequently emphasized than *Saccharomyces*, *Cyberlindnera* has been reported to exhibit strong ester-synthesizing capacity through alcohol *O*-acetyltransferase activity, thereby contributing to acetate ester accumulation and aroma complexity (Liang et al., 2024). Furthermore, *Cyberlindnera* species are known to be proficient in nitrogen metabolism, potentially influencing amino acid availability and the formation of higher alcohols and other flavor precursors. Their presence and metabolic activity, potentially interacting with *Saccharomyces* and *Lactobacillus*, could play a synergistic role in shaping the overall flavor profile of HQJ. Genes annotated as Carboxylesterase (EC 3.1.1.1) were mainly affiliated with *Lactobacillus*, *Aspergillus* and *Talaromyces*, while genes encoding

carboxylic-ester hydrolases (EC 3.1.1.-) were predominantly generated by *Weissella*, *Enterobacter* and *Saccharomyces* (Fig. 9). Since these enzymes participate in both ester synthesis and hydrolysis, the results suggest that ester metabolism in HQJ involves a dynamic balance between formation and degradation.

Isoamyl acetate, a key ester responsible for apple-, banana-, and pear-like aromas, was strongly correlated with genes annotated as isoamyl acetate esterase (EC 3.1.1.112), primarily affiliated with *Saccharomyces* and *Cyberlindnera*. Nevertheless, these associations indicate potential functional links rather than direct enzymatic confirmation. Previous studies have reported close relationships between *Lactococcus*, *Saccharomyces*, *Aspergillus*, *Monascus* and VFCs biosynthesis (Liu et al., 2020), which aligns with our metagenomic observations. Collectively, these results suggest that ester formation in HQJ is supported by a genetically equipped microbial consortium. However, these findings suggest that ester formation in HQJ is supported by a genetically equipped and metabolically interactive microbial consortium. Further transcriptomic or enzymatic analyses would be required to validate actual catalytic activity and expression dynamics.

Moreover, Spearman correlation analysis (Supplementary Fig. S3) revealed significant associations between key ester compounds and dominant genera, indicating that *Lactococcus*, *Pediococcus*, *Bacillus*, *Weissella*, *Streptococcus*, *Streptomyces*, *Enterococcus*, *Mycolicibacterium* and *Dechloromonas* were closely linked to ester biosynthesis within the fermentation system. These results imply that ester production in HQJ is likely driven by cooperative metabolism among multiple microbial genera rather than by individual taxa alone. Lactic acid bacteria (e.g., *Weissella* and *Lactobacillus*) provide organic acids that serve as esterification substrates for yeasts such as *Saccharomyces* and *Cyberlindnera*. At the same time, the presence of similar ester-related enzymes across different genera suggests functional redundancy. Such cross-feeding and syntrophic interactions may enhance both the stability and complexity of ester formation during HQJ fermentation.

4. Conclusion

This study employed an integrative multi-omics strategy combining metabolomics and metagenomics to comprehensively investigate the evolution of volatile flavor metabolites and microbial communities throughout HQJ fermentation. Quantitative flavor metabolomic profiling successfully identified 18 key VFCs, including ethyl acetate, isobutanol, hexanoic acid, and guaiacol, which are critical determinants of HQJ's sensory profile. Concurrent metagenomic sequencing revealed a dynamic microbial consortium dominated by bacterial genera such as *Weissella*, *Lactobacillus*, and *Enterobacter*, alongside fungal genera *Saccharomyces*, *Aspergillus*, *Talaromyces*, *Cyberlindnera* and *Monascus*. The abundance of key enzymes involved in flavor compound biosynthesis and their primary microbial contributors were evaluated from a genomic perspective. KEGG-based pathway analysis facilitated the construction of a metabolic network elucidating the biosynthetic routes of key VFCs in HQJ. Functional annotation further revealed that the biosynthesis of these flavor compounds is mediated by a suite of key enzymes, including alcohol dehydrogenase (NADP+), aryl-alcohol dehydrogenase, O-fatty-acyltransferase, alcohol *O*-acetyltransferase, carboxylesterase, carboxylic-ester hydrolases, and catechol *O*-methyltransferase. These enzymes were primarily associated with microbial genera such as *Weissella*, *Lactobacillus*, *Saccharomyces*, *Cyberlindnera*, *Aspergillus* and *Talaromyces*. Collectively, these findings provide significant mechanistic insights into the complex interplay between microbial metabolism and the biosynthesis of key VFCs in HQJ fermentation. By linking specific microbial taxa to the genomic potential for aromatic volatile production, this research establishes a comprehensive framework for understanding flavor development in this traditional beverage. While this study significantly advances our understanding of flavor formation in HQJ, it is important to acknowledge its limitations. The metagenomic functional annotations used are inherently predictive, indicating genomic potential rather than confirmed in situ enzyme activity or expression levels. The correlations established between microbial taxa, genes, and VFCs, while strongly suggestive, do not definitively prove causal relationships within the complex fermentation ecosystem. Therefore, future research should prioritize integrating transcriptomic and proteomic approaches to validate gene expression and enzyme activity, providing a more direct link between microbial function and flavor compound synthesis.

CRediT authorship contribution statement

Guimei Chen: Writing – original draft, Validation, Methodology, Investigation. **Suwen Tang:** Writing – original draft, Validation, Investigation. **Hao Wang:** Writing – original draft, Validation, Software. **Zihua Liang:** Writing – original draft, Validation, Software. **Xucong Lv:** Writing – review & editing, Supervision, Project administration, Funding acquisition. **Jinzhi Han:** Writing – review & editing, Supervision. **Li Ni:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

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.

Acknowledgements

This research was funded by National Natural Science Foundation of China (No. 32472295), Fujian Provincial “Eagle Plan” for Young Elite Talents Project (Project No.: Min Ren She Wen [2024] No. 130).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2026.103811.

Data availability

Data will be made available on request.

References

Cai, C., Yan, F., Li, J., Du, B., Sun, Q., & Han, X. (2026). Differences in volatility and organoleptic characteristics of mixed and sequential fermentation of mulberry fruit juices by weissella confusa and pichia kudriavzevii using metabolomics analysis. *Journal of Future Foods, 6*(2), 307–318. https://doi.org/10.1016/j.jfutfo.2024.08.016

Cao, K., Wu, J., Wan, X., Hou, Y., Zhang, C., Wang, Y., Zhang, L., Yang, W., He, Y., & Wu, R. (2024). Impact of non-Saccharomyces yeasts derived from traditional fermented foods on beer aroma: Analysis based on HS-SPME-GC/MS combined with chemometrics. *Food Research International, 187*, Article 114366. https://doi.org/10.1016/j.foodres.2024.114366

Cappello, M. S., Zapparoli, G., Logrieco, A., & Bartowsky, E. (2017). Linking wine lactic acid bacteria diversity with wine aroma and flavour. *International Journal of Food Microbiology, 243*, 16–27. https://doi.org/10.1016/j.ijfoodmicro.2016.11.025

Chaves, M., Zea, L., Moyano, L., & Medina, M. (2007). Changes in color and odorant compounds during oxidative aging of pedro ximinez sweet wines. *Journal of Agricultural and Food Chemistry, 55*(9), 3592–3598. https://doi.org/10.1021/jf063506v

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 104205. https://doi.org/10.1016/j.fm.2019.103326

Chen, G., Huang, Z., Wu, L., Wu, Q., Guo, W., Zhao, W., … Sun, B. (2021). Microbial diversity and flavor of chinese rice wine (huangjiu): An overview of current research and future prospects. *Current Opinion in Food Science, 42*, 37–50. https://doi.org/10.1016/j.cofs.2021.02.017

Chen, G., Li, W., Tong, S., Qiu, Y., Han, J., Lv, X., … Ni, L. (2022). Effects of the microbial community on the formation of volatile compounds and biogenic amines during the traditional brewing of hongqu rice wine. *Current Research in Food Science, 5*, 1433–1444. https://doi.org/10.1016/j.crfs.2022.08.020

Chen, L., Ren, L., Li, D., & Ma, X. (2021a). Fermentation characteristics for flavor compounds production by quality yeast strains isolated from rice wine starters. *Food Science, 42*(6), 142–149. https://doi.org/10.7506/spkx1002-6630-20191212-134

Chen, L., Ren, L., Li, D., & Ma, X. (2021b). Analysis of microbiomes in three traditional starters and volatile components of the chinese rice wines. *Food Science and Biotechnology, 30*, 87–96. https://doi.org/10.1007/s10068-020-00839-y

Chen, L., Xiang, W., Liang, X., Liu, J., Zhu, H., Cai, T., … Tang, J. (2023). Fungal biomarkers in traditional starter determine the chemical characteristics of turbid rice wine from the rim of the Sichuan basin, China. *Foods, 12*(3), Article 585. https://doi.org/10.3390/foods12030585

Chen, S., & Xu, Y. (2010). The influence of yeast strains on the volatile flavour compounds of chinese rice wine. *Journal of the Institute of Brewing, 116*(2), 190–196. https://doi.org/10.1002/j.2050-0416.2010.tb00417.x

Chen, S., Zhou, Y., Chen, Y., & Gu, J. (2018). Fastp: An ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics, 34*(17), i884–i890. https://doi.org/10.1093/bioinformatics/bty560

Cui, D., Liu, L., Zhang, X., Lin, L., Li, X., Cheng, T., … Zhang, C. (2023). Using transcriptomics to reveal the molecular mechanism of higher alcohol metabolism in *saccharomyces cerevisiae*. *Food Bioscience, 51*, Article 102227. https://doi.org/10.1016/j.fbio.2022.102227

Culleré, L., Ferreira, V., & Cacho, J. (2011). Analysis, occurrence and potential sensory significance of aliphatic aldehydes in white wine. *Food Chemistry, 127*(3), 1397–1403. https://doi.org/10.1016/j.foodchem.2011.01.133

Danecek, P., Bonfield, J. K., Liddle, J., Marshall, J., Ohan, V., Pollard, M. O., … Li, H. (2021). Twelve years of SAMtools and BCFtools. *GigaScience, 10*(2), Article giab008. https://doi.org/10.1093/gigascience/giab008

Fu, L., Niu, B., Zhu, Z., Wu, S., & Li, W. (2012). CD-HIT: Accelerated for clustering the next-generation sequencing data. *Bioinformatics, 28*(23), 3150–3152. https://doi.org/10.1093/bioinformatics/bts565

Gan, S. H., Yang, F., Sahu, S. K., Luo, R. Y., Liao, S. L., Wang, H. Y., Jin, T., Wang, L., Zhang, P. F., Liu, X., Xu, J., Xu, J., Wang, Y. Y., & Liu, H. (2019). Deciphering the composition and functional profile of the microbial communities in Chinese Moutai liquor starters. *Frontiers in Microbiology, 10*. https://doi.org/10.3389/fmicb.2019.01540. article 1540.

Ge, Q., Guo, C., Yan, Y., Sun, X., Ma, T., Zhang, J., … Yuan, Y. (2022). Contribution of non-*saccharomyces* yeasts to aroma-active compound production, phenolic composition and sensory profile in Chinese vidal icewine. *Food Bioscience, 46*, Article 101152. https://doi.org/10.1016/j.fbio.2021.101152

van Gemert, L. J. (2011). *Compilations of odour threshold values in air, water and other media* (Second eEnlarged and Revised ed.) Oliemans Punter & Partners BV.

Hu, K., Jin, G. J., Mei, W. C., Li, T., & Tao, Y. S. (2018). Increase of medium-chain fatty acid ethyl ester content in mixed h. uvarum/S. Cerevisiae fermentation leads to wine fruity aroma enhancement. *Food Chemistry, 239*, 495–501. https://doi.org/10.1016/j.foodchem.2017.06.151

Isogai, A., Utsunomiya, H., Kanda, R., & Iwata, H. (2005). Changes in the aroma compounds of sake during aging. *Journal of Agricultural and Food Chemistry, 52*(10), 4118–4123. https://doi.org/10.1021/jf047933p

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

Jung, M.-J., Nam, Y.-D., Roh, S. W., & Bae, J.-W. (2012). Unexpected convergence of fungal and bacterial communities during fermentation of traditional korean alcoholic beverages inoculated with various natural starters. *Food Microbiology, 30*(1), 112–123. https://doi.org/10.1016/j.fm.2011.09.008

Kang, H.-R., Hwang, H.-J., Lee, J. E., & Kim, H. R. (2016). Quantitative analysis of volatile flavor components in korean alcoholic beverage and japanese sake using SPME-GC/MS. *Food Science and Biotechnology, 25*(4), 979–985. https://doi.org/10.1007/s10068-016-0159-7

Lachenmeier, D. W., Haupt, S., & Schulz, K. (2008). Defining maximum levels of higher alcohols in alcoholic beverages and surrogate alcohol products. *Regulatory Toxicology and Pharmacology, 50*(3), 313–321. https://doi.org/10.1016/j.yrtph.2007.12.008

Langmead, B., & Salzberg, S. L. (2012). Fast gapped-read alignment with bowtie 2. *Nature Methods, 9*(4), 357–359. https://doi.org/10.1038/nmeth.1923

Li, D., Liu, C. M., Luo, R., Sadakane, K., & Lam, T. W. (2015). MEGAHIT: An ultra-fast single-node solution for large and complex metagenomics assembly via succinct de bruijn graph. *Bioinformatics, 31*(10), 1674–1676. https://doi.org/10.1093/bioinformatics/btv033

Li, L., Lv, Y., Guo, W., Pan, Y., Hong, J., Zhao, L., … Lv, X. (2019). Analysis of microbial flora and volatile flavor components in traditional fermentation starters for hongqu glutinous rice wine. *Food Science, 40*(2), 79–84. https://doi.org/10.7506/spkx1002-6630-20180420-269

Liang, J., Yuan, H., Fei, Y., Wang, H., Qu, C., Bai, W., & Liu, G. (2024). Effects of saccharomyces cerevisiae and cyberlindnera fabianii inoculation on Rice-flavor baijiu fermentation. *Foods, 13*(19), 3175–3183. https://doi.org/10.3390/foods13193175

Liang, Z. C., Lin, X. Z., He, Z. G., Su, H., Li, W. X., & Guo, Q. 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

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

Longo, R., Carew, A., Sawyer, S., Kemp, B., & Kerslake, F. (2021). A review on the aroma composition of vitis vinifera l. pinot noir wines: Origins and influencing factors. *Critical Reviews in Food Science and Nutrition, 61*(10), 1589–1604. https://doi.org/10.1080/10408398.2020.1762535

Luo, Y., Zhang, C., Liao, H., Luo, Y., Huang, X., Wang, Z., & Xia, 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

Ma, Y., Wang, Y., Li, J., Qang, B., Li, M., Ma, T., … Zhang, B. (2025). Volatile organic compound dynamics in ugni blanc and vidal wines during fermentation in the hexi corridor (China): Insights from e-nose, GC-MS, GC-IMS, and multivariate statistical models. *LWT, 217*, Article 117440. https://doi.org/10.1016/j.lwt.2025.117440

Moyano, L., Zea, L., Moreno, J., & Medina, M. (2002). Analytical study of aromatic series in sherry wines subjected to biological aging. *Journal of Agricultural and Food Chemistry, 50*(25), 7356–7361. https://doi.org/10.1021/jf020645d

Moyano, L., Zea, L., Moreno, J., & Medina, M. (2010). Evaluation of the active odorants in amontillado sherry wines during the aging process. *Journal of Agricultural and Food Chemistry, 58*(11), 6900–6904. https://doi.org/10.1021/jf100410n

Peng, Q., Li, J., Fang, S., Zhang, Y., Zheng, H., Meng, K., … Xie, G. (2024). Enhancing the flavor quality of chinese rice wine (huangjiu) through inoculation with lactobacillus plantarum and leuconostoc mesenteroides. *LWT - Food Science and Technology, 211*, Article 116935. https://doi.org/10.1016/j.lwt.2024.116935

Poisson, L., & Schieberle, P. (2008). Characterization of the key aroma compounds in an american bourbon whisky by quantitative measurements, aroma recombination, and omission studies. *Journal of Agricultural and Food Chemistry, 56*(14), 5820–5826. https://doi.org/10.1021/jf800383v

Qian, M., Ruan, F., Zhao, W., Dong, H., Bai, W., Li, X., … Li, Y. (2023). The dynamics of physicochemical properties, microbial community, and flavor metabolites during the fermentation of semi-dry hakka rice wine and traditional sweet rice wine. *Food Chemistry, 416*, Article 135844. https://doi.org/10.1016/j.foodchem.2023.135844

Shen, C., Yu, Y., Zhang, X., Zhang, H., Chu, M., Yuan, B., … Xu, X. (2024). The dynamic of physicochemical properties, volatile compounds and microbial community during the fermentation of Chinese rice wine with diverse cereals. *Food Research International, 198*, Article 115319. https://doi.org/10.1016/j.foodres.2024.115319

Sun, X., Yu, L., Xiao, M., Zhang, C., Zhao, J., Narbad, A., … Tian, F. (2025). Exploring core fermentation microorganisms, flavor compounds, and metabolic pathways in fermented rice and wheat foods. *Food Chemistry, 463*(1), Article 141019. https://doi.org/10.1016/j.foodchem.2024.141019

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, P., Mao, J., Meng, X., Li, X., Liu, Y., & Feng, H. (2014). Changes in flavour characteristics and bacterial diversity during traditional fermentation of chinese rice wines from Shaoxing region. *Food Control, 44*, 58–63. https://doi.org/10.1016/j.foodcont.2014.03.018

Wang, Y., Wu, J., Lv, M., Shao, Z., Hungwe, M., Wang, J., … Geng, W. (2021). Metabolism characteristics of lactic acid bacteria and the expanding applications in food industry. *Frontiers in Bioengineering and Biotechnology, 9*, Article 612285. https://doi.org/10.3389/fbioe.2021.612285

Xiao, R., Chen, S., Wang, X., Chen, K., Hu, J., Wei, K., … Lu, F. (2022). Microbial community starters affect the profiles of volatile compounds in traditional Chinese xiaoqu rice wine: Assement via high-throughput sequencing and gas chromatography-ion mobility spectrometry. *LWT- Food Science and Technology, 170*, Article 114000. https://doi.org/10.1016/j.lwt.2022.114000

Xu, Z., Lin, L., Chen, Z., Wang, K., Sun, J., & Zhu, T. (2022). The same genetic regulation strategy produces inconsistent effects in different saccharomyces cerevisiae strains for 2-phenylethanol production. *Applied Microbiology and Biotechnology, 106*(11), 4041–4052. https://doi.org/10.1007/s00253-022-11993-0

Yamakawa, S., Yamada, R., Tanaka, T., Ogino, C., & Kondo, A. (2012). Repeated fermentation from raw starch using saccharomyces cerevisiae displaying both glucoamylase and α-amylase. *Enzyme and Microbial Technology, 50*(6–7), 343–347. https://doi.org/10.1016/j.enzmictec.2012.03.005

Yang, D., Luo, X., & Wang, X. (2014). Characteristics of traditional chinese shanlan wine fermentation. *Journal of Bioscience and Bioengineering, 117*(2), 203–207. https://doi.org/10.1016/j.jbiosc.2013.07.010

Yang, Y., Hu, W., Xia, Y., Mu, Z., Tao, L., Song, X., … Ai, 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- Food Science and Technology, 198*, Article 116035. https://doi.org/10.1016/j.lwt.2024.116035

Yu, H., Xie, T., Xie, J., Ai, L., & Tian, H. (2019). Characterization of key aroma compounds in chinese rice wine using gas chromatography-mass spectrometry and

gas chromatography-olfactometry. *Food Chemistry, 293*, 8–14. https://doi.org/10.1016/j.foodchem.2019.03.071

Zeng, C., Tagawa, Y., Yoshizaki, Y., Wang, T., Yamaguchi, M., Kadooka, C., … Takamine, K. (2021). The expression profiles of acid-stable α-amylase and acid-labile α-amylase of *aspergillus luchuensis* Mut. Kawachii effect on the microstructure of koji and alcohol fermentation. *LWT- Food Science and Technology, 139*, Article 110580. https://doi.org/10.1016/j.lwt.2020.110580

Zhao, Y., Liu, S., Yang, Q., Han, X., Zhou, Z., & Mao, J. (2022). *Saccharomyces cerevisiae* strains with low-yield higher alcohols and high-yield acetate esters improve the quality, drinking comfort and safety of huangjiu. *Food Research International, 161*, Article 111763. https://doi.org/10.1016/j.foodres.2022.111763

Zhu, W., Lomsadze, A., & Borodovsky, M. (2010). Ab initio gene identification in metagenomic sequences. *Nucleic Acids Research, 38*(12), Article e132. https://doi.org/10.1093/nar/gkq275