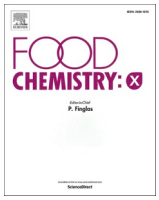
Food Chemistry: X 27 (2025) 102486

Contents lists available at ScienceDirect

Food Chemistry: X

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



Study on the correlation between microbial community succession and main flavor substances in the mashes of Tanggou liquor

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ARTICLE INFO

Keywords:
Tanggou liquor
Strong-flavor baijiu
Microbial community
High-throughput sequencing
Non-targeted metabolomics
GC-IMS

ABSTRACT

This study employed integrated omics to investigate microbial dynamics and flavor development in Tanggou wine fermentation. High-throughput sequencing identified 182 bacterial and 82 fungal OTUs, with late-stage DLD samples exhibiting peak diversity, including 49 unique bacteria and 14 fungi. *Lactobacillus* dominated bacterial communities at 99.14–99.85%, creating acidic conditions that enhanced lactic acid synthesis and ester stability. *Kazachstania* prevailed in fungal communities at 73.28–97.72%, mediating β-glucosidase-driven terpene liberation. Metabolomics revealed a 12.7-fold increase in tricarboxylic acids during DLD phase, while GC-IMS detected 77 volatiles dominated by esters, notably ethylhexanoate and phenylethanol. Four strong-flavor Baijiu signature esters peaked in DLD base liquor through stage-specific accumulation. Microbial network analysis demonstrated *Lactobacillus-Kazachstania* synergy in ethyl lactate production, while thermophilic actinomycetes modulated ethyl hexanoate/pyrazine ratios via lipase activity. These findings highlight microbial consortium engineering as an effective strategy for flavor optimization in traditional liquor manufacturing.

1. Introduction

Chinese Baijiu, a grain-based distilled spirit with millennia-old heritage, employs Daqu (a saccharification-fermentation starter) as its biochemical catalyst (Huang et al., 2017). The production process encompasses five critical stages: steaming, solid-state fermentation, distillation, aging, and blending, yielding distinct flavor categories including Sauce-, Strong-, and Sesame-aroma types (Zhang, Hou, et al., 2023). Dominating over 51% of the domestic market, Strong-aroma Baijiu achieves commercial success through its natural fermentation paradigm. Tanggou liquor, a prime exemplar, utilizes premium sorghum and a wheat-barley-pea Daqu formulation. Its innovative "five-stratum fermentation architecture" optimizes continuous fermentation via spatial compartmentalization: Return Mash (HZ), Large Mash (DC), Secondary Mash (EC), Small Mash (XC), and Multi-cycle Base (DLD). This stratification enables sequential reuse, with HZ discarded post-distillation while other layers undergo iterative fermentation cycles, ultimately producing differentiated raw liquor grades.

The Brewing Science and Enzyme Technology Center at Jiangnan University has conducted a comprehensive study on the flavor composition of Tanggou liquor. Their analysis identified 391 volatile compounds that collectively create its signature rich and mellow profile, characterized by a smooth aftertaste. Notably, 111 ester compounds were detected, a diversity comparable to that found in premium Chinese liquors. These esters, particularly the balanced combination of short-chain ethyl esters (C2-C6), serve as the key components shaping Tanggou liquor's distinctive flavor characteristics through multi-cycle fermentation processes (Zhang, Ma, et al., 2023).

The core mechanism of liquor fermentation resides in microbial interactions, with microorganisms primarily derived from three sources: fermentation mashes, cellar pits, and production environments (Xie et al., 2021). As critical bioreactors, mashes serve as both nutritional substrates and metabolic conduits for microbial communities, directly determining liquor quality and constituting a pivotal control point in Strong-flavor Baijiu production (Li et al., 2021). Recent advances in microbial profiling reveal distinct community structures: Cheng, Chen,

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https://doi.org/10.1016/j.fochx.2025.102486
Received 26 January 2025; Received in revised form 12 April 2025; Accepted 17 April 2025
Available online 24 April 2025

et al. (2024) identified *Aspergillus*, *Pichia pastoris*, and *Rhizopus* as dominant fungi, while *Lactobacillus* and *Bacillus* prevailed in mashes, with *Methanosarcina* and *Clostridium* sensu stricto *12* dominating cellar mud through amplicon sequencing. Complementary research by Zhao et al. (2022) employing Illumina MiSeq sequencing demonstrated *Firmicutes*, *Ascomycota*, and *Euryarchaeota* as keystone taxa in cellar ecosystems, with bacterial networks exhibiting greater structural complexity than fungal and archaeal counterparts.

Chinese Baijiu of different aroma types exhibits distinct microbial communities and metabolic characteristics. Sauce-flavor Baijiu employs high-temperature Daqu production at 65–70 °C and triple-round fermentation, fostering thermophilic microbial communities dominated by *Bacillus* and *Thermoascus*. These systems generate characteristic pyrazines such as 2,3,5-trimethylpyrazine through Maillard reactions under thermal stress (Shi et al., 2024). Light-flavor Baijiu utilizes low-temperature Daqu (45–50 °C) and short fermentation cycles of 28 days, maintaining simplified microbial consortia dominated by *Lactobacillus* and *Saccharomyces*, with ethyl acetate constituting 75–85% of total esters (Huang et al., 2020). Strong-flavor Baijiu relies on multi-grain layered fermentation and pit mud ecosystems, developing dynamic symbiotic systems between *Lactobacillus* and *Kazachstania* yeast. The anaerobic *Caproiciproducens* in pit mud synthesizes hexanoic acid, which is esterified into signature ethyl hexanoate (Qiu et al., 2024). These differences stem from process parameters such as temperature, fermentation cycles, and raw materials, which selectively shape microbial communities and subsequently drive aroma-specific metabolic pathways.

Recent advancements in high-throughput omics technologies have revolutionized microbial community analysis, enabling systematic characterization of microbiota in critical fermentation components including Daqu starters, fermentation mashes, and cellar ecosystems (Zhang et al., 2024). Concurrently, microbial metabolomics has undergone a paradigm shift - from initial focus on individual metabolite dynamics to current emphasis on community-level metabolic interplay, facilitated by advancements in mass spectrometry (Kuhlisch & Pohnert, 2015). Non-targeted metabolomics serves as a powerful discovery engine, prioritizing novel metabolite identification and pathway elucidation through advanced bioinformatics pipelines (Pang & Hu, 2023). GC-IMS demonstrates distinct advantages in microbial metabolome studies, combining superior separation resolution with high sensitivity, complemented by extensive NIST database compatibility for rapid compound annotation (Parastar & Weller, 2023).

In this study, high-throughput sequencing technology was employed to analyze the diversity of microbial communities in various mashes of Tanggou liquor. Additionally, LC-MS and GC-IMS were utilized to examine the relationship between microbial metabolism and flavor compounds in the original liquor. The composition and succession of microorganisms in Tanggou mashes, along with the connections between metabolites and active substances, were scientifically analyzed. This research provides theoretical support for the specialized production and brewing of Tanggou liquor and offers insights for further refining the brewing process.

2. Materials and methods

2.1. Sample collection

Samples were systematically collected from Jiangsu Tanggou Winery's historic cellar employing the optimized "five-stratum fermentation architecture" process. Five characteristic fermentation layers were targeted: HZ, XC, EC, DC, and DLD. Each 500 g specimen was aseptically extracted from the central zone of respective layers, representing distinct fermentation phases and vertical microenvironmental gradients. Samples were immediately cryopreserved at −77 °C to maintain microbial viability. Layer selection followed the dynamic fermentation cycle: HZ (previous cycle's XC), XC (upcycled EC), EC (half-retained DC), DC (core ferment), and DLD (extended-cycle base), ensuring comprehensive coverage of microbial succession patterns across temporal-spatial dimensions.

2.2. Microbial community analysis

Three replicates (10 g each) were aseptically dispensed into sterile 50 mL conical tubes from typical fermentation layers (HZ, DC, EC, XC, DLD). Genomic DNA was extracted using the Rapid DNA SPIN Kit (Shanghai Solabo Biotechnology Co., Ltd.), and the nucleic acid concentration and A260/A280 purity ratio were subsequently quantified using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA). DNA integrity was verified by 1% (*w/v*) agarose gel electrophoresis. The bacterial 16S rRNA gene was amplified using primer pair 338F (5'-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5'-GGACTACHVGGGTWTCTAAT-3′), while the fungal 18S rRNA gene was amplified using primers ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2R (5'-GCTGCGTTCTTCATCGATGC-3′) following established protocols (Xu et al., 2023). Barcoded primers were custom synthesized based on predefined hypervariable regions. PCR amplicons were purified using the AxyPrep DNA gel extraction kit (Axygen Biosciences, CA, USA) and quality controlled by 2% agarose electrophoresis after elution (Tris-HCl buffer). Raw sequencing data underwent strict quality control, including adapter trimming, chimera removal via Vsearch, and sequence orientation correction using the Silva database. Potential contaminating OTUs were filtered through negative controls and machine-learning-based decontam analysis. Subsequently, qualified libraries from the five experimental groups were subjected to paired-end sequencing on the Illumina MiSeq platform (Shanghai Majorbio Bio-Pharm Technology Co., Ltd.). Retained OTUs met stringent criteria: detection in ≥2/3 biological replicates, average relative abundance >0.01%, and taxonomic validation against the Greengenes database (confidence ≥80%).

2.3. Non-targeted metabolomics detection

Following the methodology described by Li et al. (2020), frozen fermentation mash samples (−20 °C) were processed through standardized metabolite extraction procedures. Each aliquot was combined with 100 μL of internal standard working solution and 400 μL of methanol, followed by 1 min vortex homogenization. Phase separation was achieved via centrifugation (12,000 r/min, 10 min, 4 °C). The supernatant was lyophilized under vacuum and subsequently reconstituted in 150 μL of 80% methanol solution (−20 °C). After thorough mixing and repeat centrifugation under identical conditions, the clarified extract was filtered through 0.22 μm membranes. Processed samples were analyzed using LC-MS at Shanghai Majorbio Bio-Pharm Technology Co., Ltd., with operational parameters maintained as per manufacturer specifications.

2.4. GC-IMS detection

The volatile compounds in the raw liquor samples corresponding to the five mashes were detected and analyzed. Referring to the method of Zhang et al., 100 mL of liquor sample was taken from each sample, placed in a 20 mL headspace bottle, incubated at 60 °C for 10 min, and analyzed by GC-IMS (GCMS-QP2020 Gas Chromatography Mass Spectrometer, Shimadzu Corporation). To mitigate matrix effects, samples were diluted 1:10 (*v*/v) with ultrapure water, and 2-octanol (50 μg/L) was added as an internal standard for retention index calibration and signal normalization. In the GC-IMS detection, the samples were separated using a WAX-CB-130 mID:0.53 mm column, with a column temperature of 60 °C and a time of 30 min. Headspace injection was used, with an injection volume of 100 μL, a needle temperature of 85 °C, a rotation speed of 500 r/min, and incubation at 60 °C for 10 min. Both the carrier gas and the drift gas were nitrogen (N2), the drift tube temperature was 45 °C, and the drift gas flow rate was 150 mL/min

(Zhang, Xiao, et al., 2023). All samples across fermentation stages were standardized by adjusting ethanol content to 50% (*v*/v).

2.5. Data Analysis

Microbial community analysis was performed using processed high-throughput sequencing data, and sequences were clustered into OTUs and chimeras were removed at a 97% similarity threshold. Alpha diversity metrics (Chao, Shannon, Simpson) and coverage were calculated using Mothur, while beta diversity was assessed by principal coordinate analysis (PCoA) based on Bray-Curtis distance. Bacterial 16S rRNA and fungal ITS sequences were taxonomically annotated using QIIME2–2023.2 and Silva138 and UNITE v10.0 databases (70% confidence threshold). For metabolomics, LC-MS data were processed by Progenesis QI v3.0 for peak alignment, annotation, and OPLS-DA modeling. GC-IMS spectra were normalized and aligned using the Lab Analysis Viewer. Genus-level correlations with flavor compounds (ethylhexanoate, ethylbutyrate, acetate, ethyllactate) were determined by Pearson analysis ($|r| > 0.6$, FDR-adjusted $p < 0.01$) and visualized by heatmap (pheatmap v1.0.12). Data processing and plotting were performed using Excel 2010, SPSS statistics 26, and Origin 2021.

3. Results and discussion

3.1. Microbial community analysis

3.1.1. Analysis and annotation of microbial flora OUTs sequences

High-resolution taxonomic profiling of microbial communities across five fermentation stages (HZ, DC, XC, EC, DLD) was performed using the Illumina Nova sequencing platform. Bacterial sequencing revealed 182 operational taxonomic units (OTUs) with progressive taxonomic resolution: 1 kingdom, 1 phylum, 12 classes, 58 orders, 81 families, 115 genera, and 152 species (Fig. 1A). Fungal analysis identified 82 OTUs with analogous taxonomic distribution (1 kingdom, 1 phylum, 5 classes, 13 orders, 20 families, 52 genera, 68 species; Fig. 1B). Improved genus/species-level resolution provides a key basis for dissecting the functional microbiota driving fermentation bioprocesses.

To reveal the overlap of common and unique OTUs in different mash samples, a Venn diagram was drawn based on the OTUs annotation results. The results are shown in Fig. 1C and D. Each color in the figure

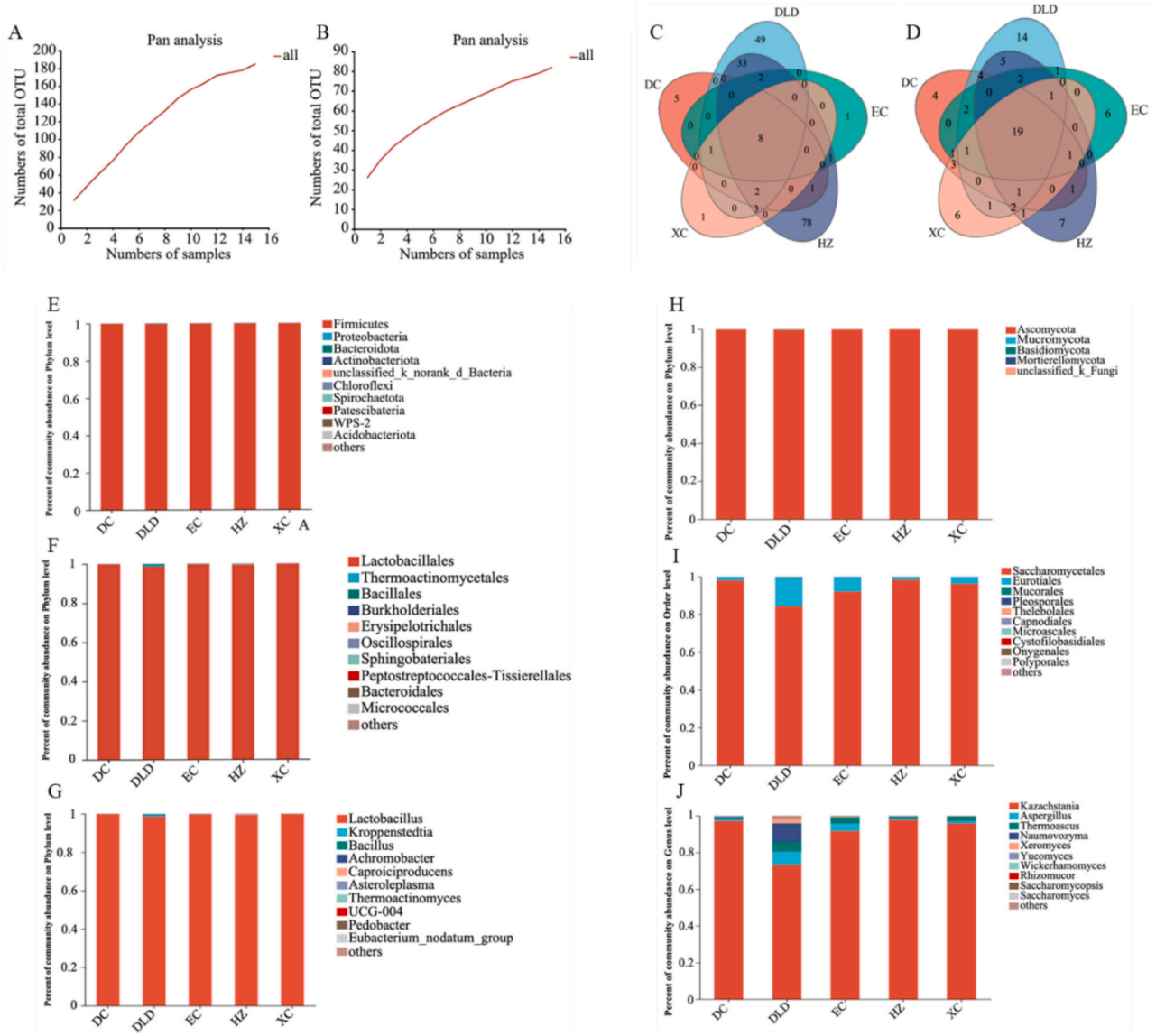

Fig. 1. OUT sequences analysis and species annotation of microbial flora during the fermentation of mashes.
Note: A-B: The number of OTU sequences, A: Bacteria; B: Fungi. C-D: The Venn diagram, C: Bacteria; D: Fungi. E-G: Distribution of bacteria in different samples at phylum (E) order (F) and genus (G) level. H-J: Distribution of fungi in different samples at the level of phylum (H), order (I) and genus (J).

represents a group of samples, and its number indicates the number of strains unique to or shared by different samples. As can be seen from Fig. 1C, at different fermentation stages, all samples had a total of 8 bacterial species. It is worth noting that the DC sample contained 5 unique strains, while the XC and EC samples each had 1 unique strain. In sharp contrast, the unique bacterial species of the DLD sample increased significantly, totaling 49 species, and the unique bacterial strains of the HZ sample reached 79 strains. This trend indicates that bacterial diversity expands dramatically as fermentation progresses, especially in the later stages (DLD and HZ). The continuous accumulation of unique bacterial species suggests that there may be specific microbial communities in these stages that contribute to the fermentation process, which may affect the flavor and quality of the final product.

During the initial fermentation phases (DC, XC, EC), fungal diversity remained limited with only 6, 6, and 4 unique fungal species detected respectively. A striking shift occurred in later stages, as DLD samples showed 14 unique fungal species, followed by 7 in HZ (Fig. 1D). This progression reveals distinct phases of fungal community development. The dramatic rise in fungal diversity during DDL suggests this stage creates favorable growth conditions, likely influenced by bacterial activity altering nutrients or metabolic products. The expanded fungal population during this phase could enhance flavor development through interactions that generate diverse aromatic compounds critical for Tanggou liquor's characteristic profile. Interestingly, fungal diversity growth slowed in HZ (7 species), signaling community stabilization. Two key factors may drive this change: (1) Depleted nutrients limiting new fungal colonization, and (2) Dominant species like Saccharomyces outcompeting others through faster resource consumption. This natural selection process leads to simpler but more resilient fungal communities as fermentation progresses.

3.1.2. Analysis of α-diversity of mashes

Alpha diversity analysis of the microbial communities at different fermentation stages provided valuable insights into the richness and diversity of bacterial and fungal populations. As shown in Table S1, the richness and diversity of the bacterial communities varied among the five sample groups. The coverage index for all samples was close to 1, indicating that the sequencing coverage was high and accurately reflected the microbial composition in the samples.

Quantitative analysis revealed significant microbial community variations across fermentation stages. For bacterial assemblages, the ACE (DLD:70.32 vs EC:9.77, 7.2-fold increase) and Chao (DLD:66.52 vs XC:11.33, 5.9-fold increase) indices demonstrated substantially enhanced richness in DLD and HZ phases. While bacterial Shannon diversity remained low overall (range 0.01–0.25), the DLD stage maintained relative predominance (0.25 vs HZ:0.09), suggesting increased ecological complexity supporting niche differentiation.

Fungal communities exhibited parallel patterns, with peak richness indices in DLD (ACE:37.71; Chao:35.78) and HZ (ACE:30.37) phases, representing 1.6–2.3× higher values than other stages. Notably, fungal Shannon diversity in DLD (1.50) significantly surpassed other stages (0.97–1.08, $p < 0.05$), indicating this critical phase facilitates fungal community maturation through enhanced species evenness and functional guild establishment.

3.1.3. Microbial community structure distribution

Bacterial communities serve as primary contributors to flavor compound synthesis in strong-flavor Baijiu fermentation. Taxonomic classification using SILVA 138 and UNITE databases revealed remarkable phylogenetic conservation across fermentation stages. At the phylum level, *Firmicutes* constituted >99% relative abundance in all samples (Fig. 1E), with *Lactobacillales* maintaining >98% dominance at the order level (Fig. 1F). Genus-level analysis showed persistent *Lactobacillus* predominance across stages (HZ:99.14%, DC:99.85%, EC:99.74%, XC:99.85%, DLD:98.58%), demonstrating remarkable community stability despite fermentation progression (Fig. 1D). Notably, the DLD stage exhibited enhanced diversity with secondary genera *Kroppenstedtia* (0.74%) and *Bacillus* (0.32%) emerging, aligning with microbial succession patterns reported in Baijiu fermentation systems (Sun et al., 2016; Wang et al., 2018).

The ecological dominance of Lactobacillus establishes a protective fermentation microenvironment through acidification and ethanol synthesis. These metabolites create synergistic antimicrobial conditions that suppress competitors while supporting *Saccharomyces* proliferation (Ding et al., 2015). Metabolic cross-feeding between *Lactobacillus* and yeast enhances ethyl lactate synthesis, a key aroma compound in Baijiu (Moens et al., 2014). However, excessive acidification risks impairing yeast viability and flavor balance, necessitating precise microbial management during later fermentation phases.

Fungal community dynamics exhibited hierarchical conservation with stage-specific specialization during Baijiu fermentation. *Ascomycota* maintained phylum-level dominance (>99% relative abundance across all stages, Fig. 1H), while the terminal DLD stage displayed increased phylogenetic complexity with four detected phyla (*Ascomycota*, *Mucoromycota*, *Basidiomycota*, *Mortierellomycota*), contrasting with 2–3 phyla in earlier stages. This progression corresponds to functional specialization requirements for starch conversion and ester synthesis during late fermentation (Hu et al., 2023). Order-level analysis revealed Saccharomycetales as the persistent dominant group (>92% in DC, EC, XC, and HZ stages), decreasing to 84% in DLD as *Eurotiales* increased to 15% (Fig. 1I). The complementary abundance patterns between these orders reflect microbial succession dynamics inherent to solid-state fermentation systems. Genus-level transitions demonstrated *Kazachstania*'s adaptive dominance throughout fermentation (97.72% in HZ to 73.28% in DLD), consistent with its documented role in flavor and alcohol metabolism (Piraine et al., 2022). The DLD stage showed increased diversity with *Naumovozyma* (10.1%), *Thermoascus* (5.31%), and *Aspergillus* (7.12%) emerging as secondary taxa. These shifts align with the ecological restructuring caused by iterative microbial inoculation (Daqu addition) and product removal in traditional fermentation processes (Lv et al., 2015). *Thermoascus*, a recognized esterification catalyst in koji systems (Ge et al., 2024), and *Aspergillus*, known for enhancing liquefaction and esterification capacities (Mu et al., 2024), collectively contribute to flavor refinement during terminal fermentation stages. This successional pattern corroborates previous observations of thermotolerant microbial proliferation in later fermentation phases (Wang et al., 2019).

The microbial ecosystem of strong-flavor Baijiu demonstrates striking specialization, with *Lactobacillus* achieving near-exclusive dominance (99.1–99.9% abundance) across fermentation stages—surpassing the 70–85% levels typical of standard fermentation pits. This exceptional dominance arises from the Tanggou process's layered fermentation geometry and five-grain formula, which optimize nutrient release while suppressing competitor proliferation. Late fermentation stages introduce specialized genera like *Kroppenstedtia*, known for breaking down grain husks, and *Bacillus*, which contributes subtle umami precursors, reflecting succession patterns distinct from Sauce-flavor Baijiu where *Bacillus* dominates earlier under high-temperature stress.

Fungal communities exhibit parallel specialization, with *Kazachstania* maintaining 73–98% dominance through unique adaptations absent in other Baijiu types: unlike Sauce-flavor systems where *Saccharomyces* thrives in high heat (50–65 °C), *Kazachstania* excels in moderate temperatures (25–35 °C) by simultaneously metabolizing lactic acid and ethanol—a trait critical for balancing acidity and ester production. Comparatively, Light-flavor Baijiu shows earlier microbial diversity peaks driven by *Pediococcus* and *Acetobacter*, while Sauce-flavor systems prioritize thermophilic *Thermoascus* and *Bacillus* for pyrazine synthesis. The Tanggou process uniquely recruits *Thermoascus* and *Aspergillus* in later stages as "flavor refiners," leveraging their esterification capabilities at lower temperatures than their Sauce-flavor counterparts. These contrasts highlight how fermentation architecture and temperature regimes fundamentally reshape microbial roles across

Baijiu subtypes, positioning layered fermentation as a key innovation for targeted flavor engineering.

3.1.4. Analysis of microbial interactions

The fermented mash constitutes a dynamic microbial ecosystem where interspecies interactions critically shape community structure and metabolic outputs. Focused analysis of dominant species through univariate association networks revealed key microbial relationships (Anjum et al., 2014; Yan et al., 2015). Spearman correlation analysis of the top 10 abundant bacterial genera demonstrated *Lactobacillus*' consistent negative interactions with all co-occurring taxa across five fermentation stages (Fig. 2A). This ecological dominance stems from its competitive advantages: (1) preferential nutrient utilization through high population density (98.58–99.85% relative abundance), and (2) microenvironment modification via lactic acid production, creating pH conditions that selectively inhibit acid-sensitive competitors. These findings align with established models of *Lactobacillus*' ecological succession in fermentation systems, particularly its documented acid tolerance and competitive exclusion mechanisms (Moens et al., 2014).

Within fungal communities, *Kazachstania* emerged as the keystone genus, demonstrating distinct correlation patterns with co-occurring species (Fig. 2B). This dominant fungus (73.28–97.72% relative abundance) exhibited positive association with *Yueomyces sinensis* while maintaining negative interactions with *Xeromyces*, *Thermoascus*, *Aspergillus*, *Rhizomucor*, and *Saccharomycopsis*. *Kazachstania*'s ecological significance manifests through two principal mechanisms: (1) Metabolic coordination via lactic acid assimilation and glucuronic acid hydrolysis, establishing cross-feeding networks that support microbial consortia (Decimo et al., 2017); (2) Flavor compound biosynthesis through specialized metabolism of small-molecule carbohydrates, particularly generating isopropanol and isopentanol - signature aroma components in strong-flavor Baijiu (Jood et al., 2017). These dual functional roles position *Kazachstania* as a critical determinant of both microbial community dynamics and final product sensorial quality.

During the fermentation of strong-flavor Baijiu, microbial communities exhibit highly specialized interaction patterns. *Lactobacillus* establishes dominance through dual strategies: rapidly depleting glucose and maltose to induce nutrient deprivation, while continuously

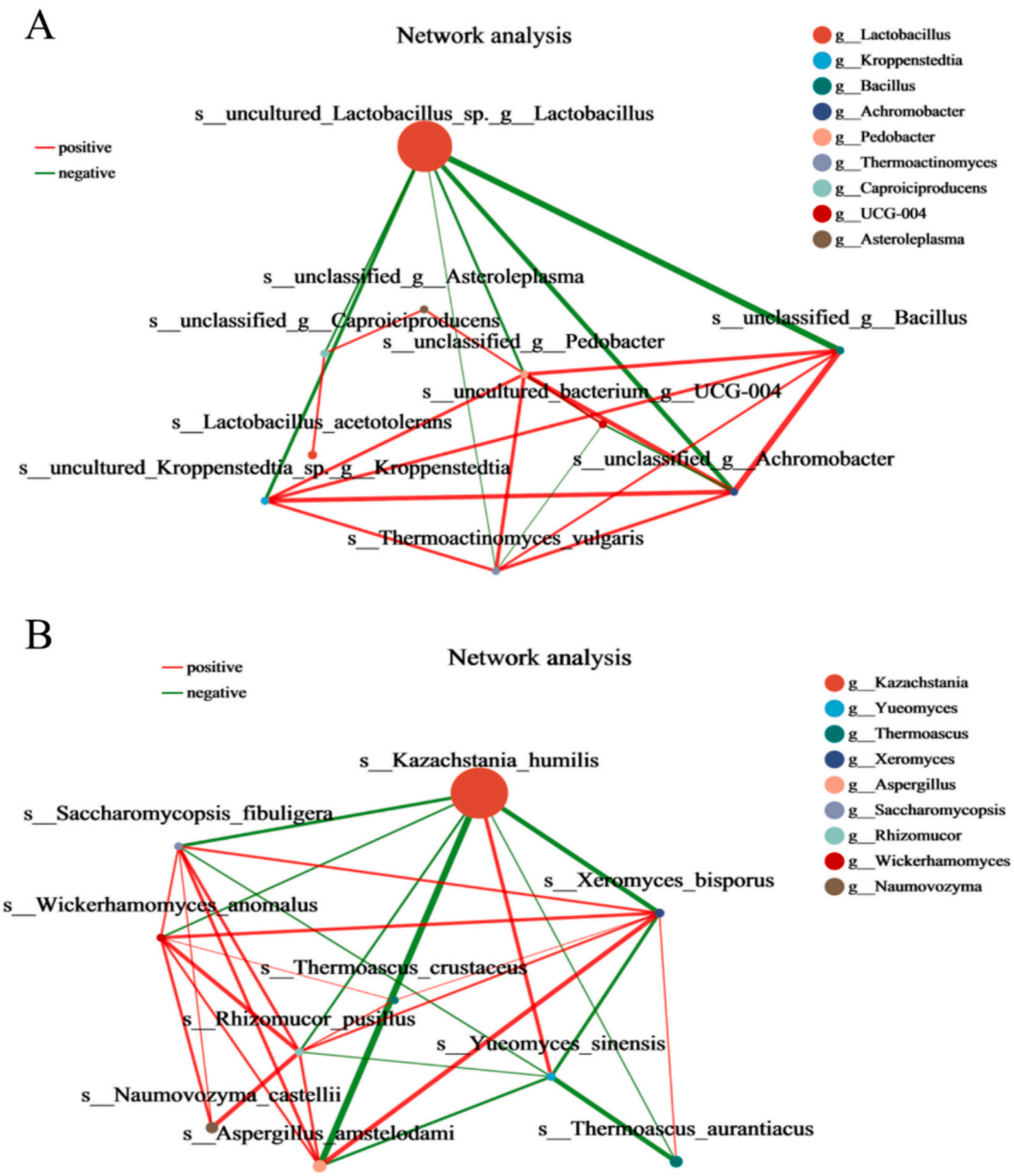

Fig. 2. Interaction analysis of bacteria (A) and fungi (B) in different samples (abundance top10).

secreting lactic acid to significantly suppress acid-sensitive bacteria such as *Bacillus* and *Enterobacter* (Yan et al., 2015). This near-monopolistic dominance contrasts sharply with the pH-mediated symbiotic partitioning observed between Lactobacillus and Acetobacter in light-flavor Baijiu systems.

The fungal community is governed by *Kazachstania*, which maintains ecological balance through metabolic network regulation. On one hand, it hydrolyzes β-glucuronides to provide carbon sources for the symbiotic yeast *Yueomyces sinensis*; on the other, it secretes serine proteases to degrade hyphal tip proteins of *Aspergillus* and *Rhizomucor*, thereby suppressing colonization by contaminating microbes. This dual regulatory strategy of "synergism-inhibition" enables *Kazachstania* to constitute over 97% of the fungal community during mid-to-late fermentation, while permitting limited thermophilic fungi to contribute to ester synthesis. Compared to the stress-induced mutualism between *Saccharomyces* and *Thermoascus* under high-temperature conditions in sauce-flavor Baijiu (Zhang et al., 2021), the strong-flavor system achieves precise regulation at moderate temperatures, reducing ethyl hexanoate concentration variability to 8.2–11.3% (versus 15.6–22.7% in traditional sauce-flavor processes), thereby significantly enhancing flavor stability.

3.1.5. Analysis of differences and similarities in microbial communities

To characterize microbial community dynamics during fermentation, principal coordinates analysis was conducted as shown in Fig. S1. Bacterial communities exhibited stage-dependent clustering patterns, with the first two principal coordinates PC1 and PC2 explaining 85.41% and 11.9% of total variance respectively, collectively accounting for 97.31%. The DLD-stage samples distinctly occupied the PC1-positive region, demonstrating substantial divergence from DC, EC, and XC

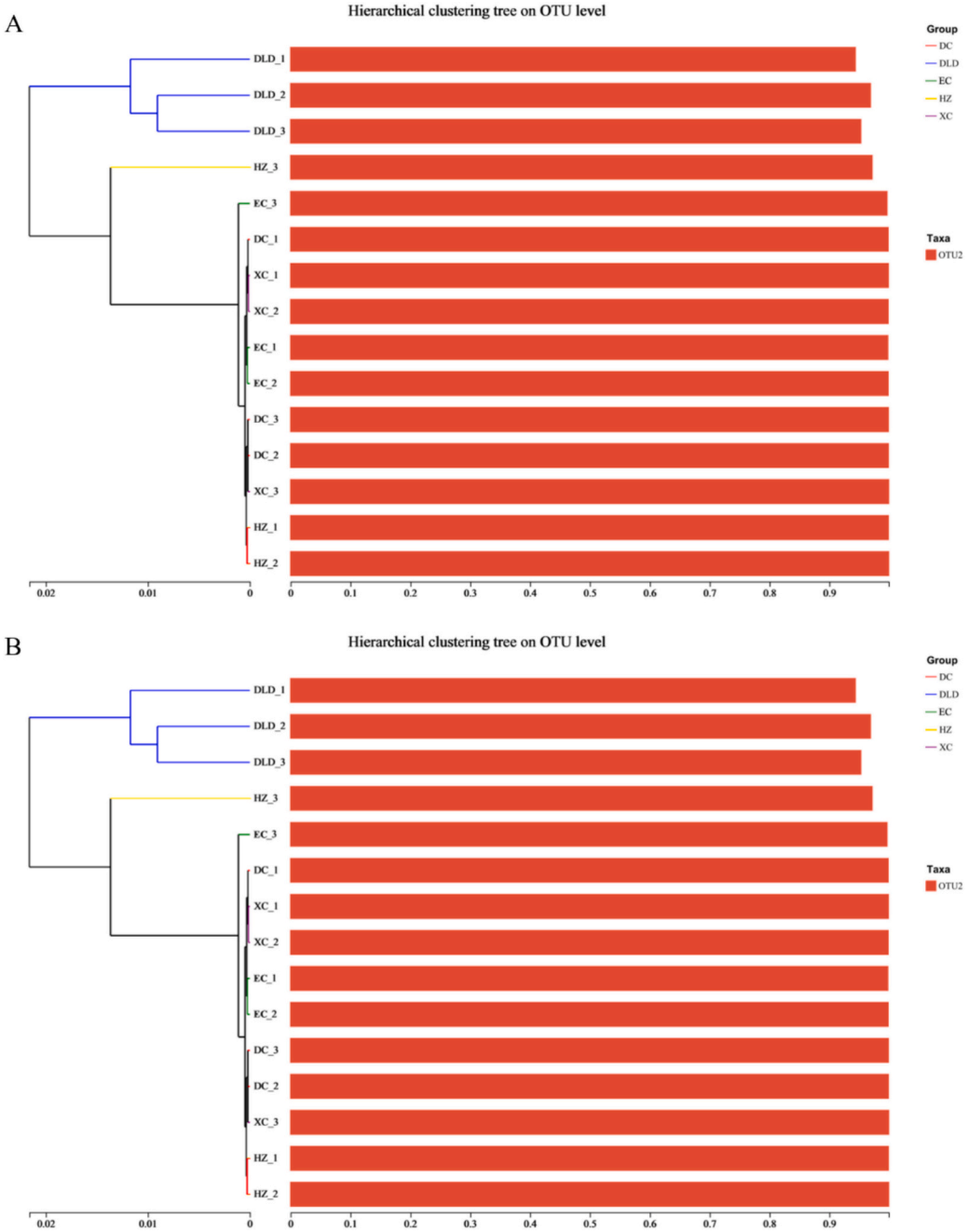

Fig. 3. Hierarchical cluster analysis of bacteria (A) and fungi (B) in different samples (percentage < 0.05 combined).

stages clustered in the PC1-negative region. Notably, DC, EC, and XC samples showed high compositional similarity, suggesting conserved microbial profiles during early fermentation phases. This uniformity may arise from shared substrate components and fermentation parameters in initial stages. Fungal communities displayed similar spatial patterns, where PC1 contributed 86.49% and PC2 accounted for 6.08% of variance, with a combined explanation of 92.37%. The DLD stage consistently formed a separate cluster in the PC1-positive region, mirroring bacterial community differentiation. These parallel patterns between bacterial and fungal communities highlight the DLD stage as a

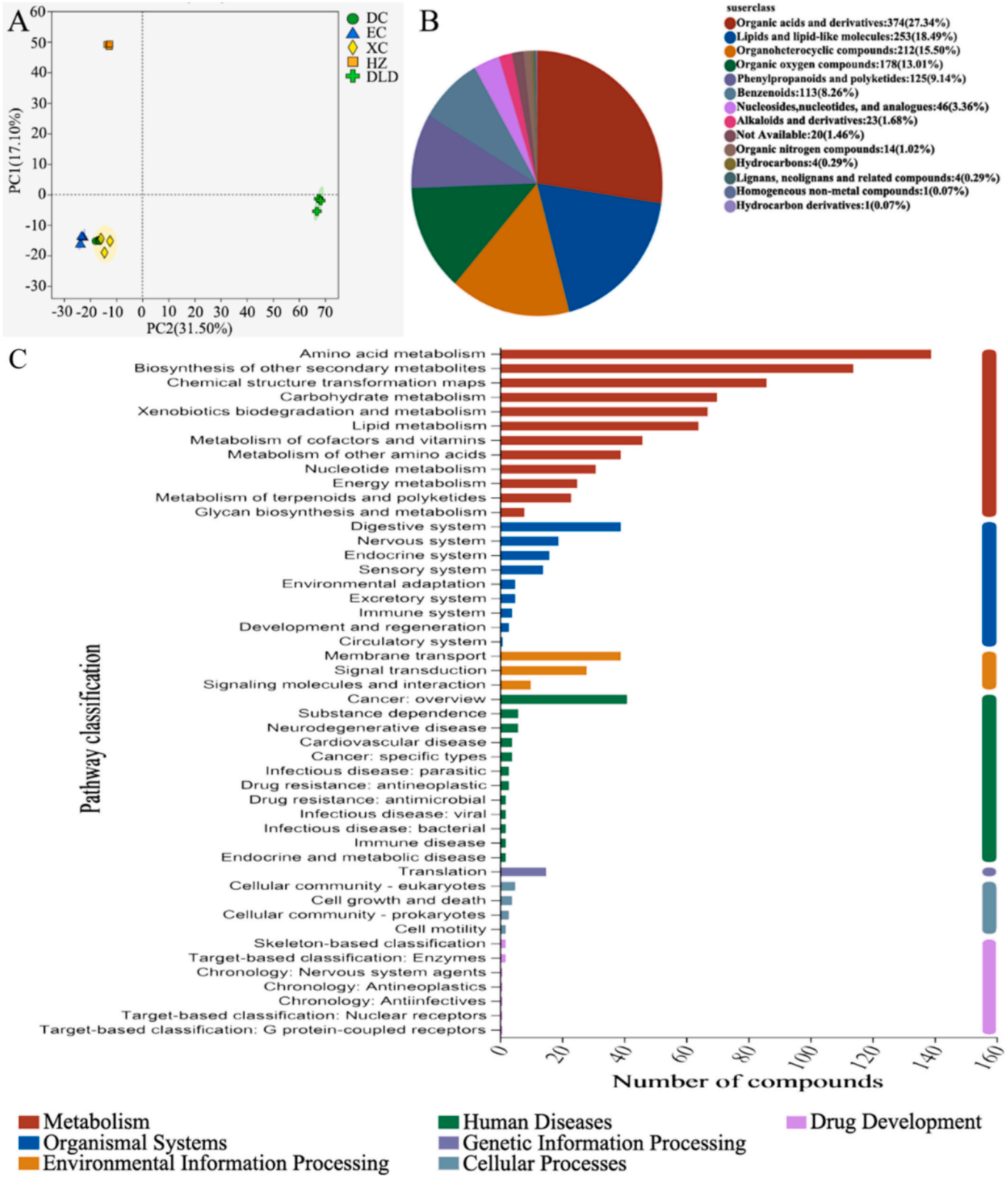

Fig. 4. PCA analysis of metabolites in different samples (A). HMDB Compound Classification Pie Chart (B). Metabolic pathway (C).

critical transitional phase with unique microenvironmental features.

Complementing the PCoA findings, hierarchical clustering analysis further characterized microbial community dynamics across fermentation stages (Fig. 3). Dendrogram topology revealed DC, EC, XC, and HZ stages forming a cohesive cluster within the same branch, demonstrating high compositional homology. This spatial configuration aligns with PCoA results, confirming conserved microbial profiles from early to mid-fermentation phases. The observed clustering patterns suggest these stages maintain shared metabolic functionality, where microbial consortia potentially operate through coordinated biochemical pathways. Such multistage consistency establishes fundamental microbial stability critical for Strong-flavor liquor production, as sustained microbial interactions and metabolic continuity directly support the biosynthesis of characteristic flavor compounds. This microbial equilibrium likely serves as the biochemical foundation for developing ideal organoleptic properties during liquor maturation.

3.2. Non-targeted metabolomics analysis

3.2.1. Analysis of metabolites and metabolic pathways

Metabolomic profiling through PCoA revealed stage-specific biochemical signatures during fermentation (Fig. 4A). The DLD stage exhibited distinct metabolic divergence, clustering exclusively in the PC1-positive quadrant (52.8% explained variance), while other stages occupied the PC1-negative region. Notably, DC, EC, and XC stages demonstrated metabolic convergence with overlapping coordinates, suggesting shared biochemical processes during intermediate fermentation phases. This spatial configuration aligns with microbial community dynamics observed in previous analyses, confirming system-level biological continuity (Cheng, Lan, et al., 2024). Chemical characterization identified four dominant metabolite classes in Tanggou distiller's grains: amino acids (21.93%) forming the largest fraction, followed by peptide analogs (9.06%), carbohydrate derivatives (5.19%), and fatty acid-carbon complexes (2.41%) (Fig. 4B). Pathway enrichment analysis delineated two-tier metabolic architecture (Fig. 4C): primary metabolism dominated by amino acid transformation pathways (38.2% activity), supported by secondary processes including xenobiotic degradation (14.7%) and lipid remodeling (9.3%). Membrane transport mechanisms accounted for 62.4% of environmental information processing activities, indicating active cellular-material exchange during fermentation maturation.

These findings elucidate the complex biochemical processes occurring during Tanggou lees fermentation. The distinct metabolic profiles at different fermentation stages can provide a reference for optimizing fermentation conditions and improving the quality of the final product. Future studies should focus on elucidating the specific pathways and enzymatic processes that lead to these metabolic changes, which may further enhance our understanding of fermentation kinetics and product characteristics in lees production.

Metabolomic investigations demonstrate that Tanggou liquor's characteristic flavor profile originates from coordinated microbial and biochemical interactions during fermentation. Dominant *Lactobacillus* species drive lactic acid production, modulating environmental pH to suppress competing microbial activity, thereby stabilizing key esters such as ethyl hexanoate—a major contributor to fruity aromas. Concurrently, yeast-mediated Ehrlich pathways efficiently convert branched-chain amino acids into aromatic compounds such as isoamyl acetate, known for its fruity notes, and phenylethanol, characterized by floral tones (Lutskova & Martirosyan, 2021). Cross-metabolic activity between *Bacillus* and *Lactobacillus* further generates trace pyrazines, enriching the liquor's flavor complexity (Tang et al., 2023). Under controlled mesophilic fermentation conditions, this microbial consortium achieves superior flavor consistency compared to other Baijiu styles, with reduced volatility in organic acid profiles.

3.2.2. Comparison of differences among metabolites

The variable importance in projection (VIP) score effectively characterizes intergroup metabolic disparities, where higher values denote stronger discriminatory power of metabolites in sample classification. Building upon established evidence of maximal microbial diversity in the DLD fermentation stage, we employed DLD as the reference group for comparative metabolite profiling. The tripeptide derivative 2-(3-carboxy-3-aminopropyl)-L-histidine exhibited the most pronounced divergence between DLD and other fermentation groups. Its elevated VIP score not only underscores its classification significance but also suggests a critical function in pH modulation and microbial population dynamics during fermentation. Fig. 5A demonstrates marked differential accumulation of 1,10-phenanthroline and phloretin between DC and DLD groups, implying microbial community-dependent biosynthesis under stage-specific fermentation conditions. Previous studies have established the association of such compounds with aromatic component synthesis, highlighting microbial consortia's regulatory effects on flavor development. Furthermore, Fig. 5B reveals substantial variations in 1,10-phenanthroline and piceatannol levels between EC and DLD groups, reinforcing their functional relevance to fermentation biochemistry. Comparative analyses across fermentation stages (Figs. 5C and D) confirm dynamic metabolite abundance patterns, particularly between HZ and DLD groups. The observed flux variations in 1,10-phenanthroline and phloretin correlate with microbial community succession, indicating progressive replacement of initial dominant populations by late-stage specialists, thereby driving metabolic network restructuring.

Cluster analysis of the top 50 metabolites (Fig. 5E) showed increasing accumulation patterns with fermentation duration, reflecting enhanced microbial activity over time. This progression provides biochemical prerequisites for flavor development. DC, EC, and XC stages displayed similar metabolite profiles, likely due to stable environmental conditions and shared core microbiota (*Lactobacillus* and *Kazachstania*). In contrast, the HZ stage exhibited unique metabolic features, including new detection of glucose 1-phosphate and D-galactose 1-phosphate, suggesting microbial adaptation through substrate recycling. The DLD stage showed marked metabolic enhancement, with 3-phenyllactic acid, atrolactic acid, p-tolualdehyde, and tricarballylic acid concentrations significantly exceeding earlier stages. This shift corresponded to microbial community evolution - early acid-producing *Lactobacillus* populations transitioned to late-stage ester-forming *Pichia* dominance, driving metabolic diversification through ecological niche specialization.

Metabolomic analysis revealed stage-specific biochemical dynamics during Tanggou liquor fermentation, with VIP scores identifying 2-(3-carboxy-3-aminopropyl)-L-histidine as the most discriminatory metabolite between the DLD stage and other fermentation phases. This tripeptide derivative contributes to pH stabilization (3.5–3.8 range) through its carboxyl groups while potentially regulating Lactobacillus quorum sensing via histidine-mediated signaling, creating favorable conditions for ester preservation. Differential accumulation of 1,10-phenanthroline and phloretin across stages (Fig. 5A–B) reflects microbial community-regulated secondary metabolism, where phenanthroline enhances yeast-driven Ehrlich pathway activity for isoamyl acetate synthesis, and phloretin stabilizes glycosylated aroma precursors. Cluster analysis (Fig. 5E) demonstrated metabolic progression aligned with microbial succession: early-stage Lactobacillus acidification preserved precursors, mid-stage Kazachstania activated phenolic transformations, and late-stage Pichia dominance drove esterification via tricarballylic acid-mediated CoA stabilization. Key pathways include TCA cycle shunts (12.7-fold 3-phenyllactic acid increase in DLD vs. DC) and cross-kingdom interactions producing β-phenylethyl alcohol and pyrazines. These coordinated microbial-metabolic mechanisms explain Tanggou liquor's layered flavor profile, balancing fruity esters, floral alcohols, and nutty aromas through phased biochemical regulation.

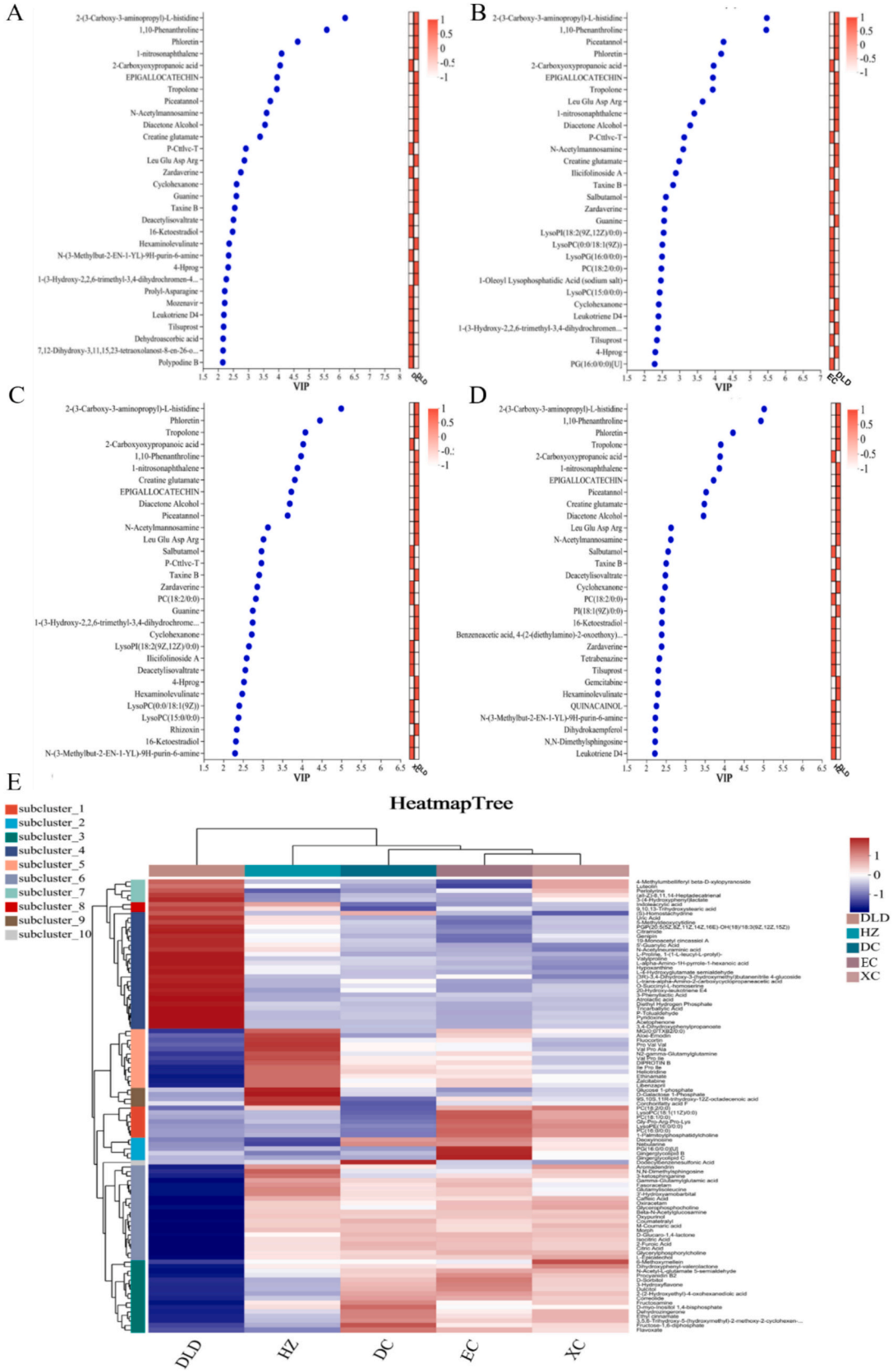

Fig. 5. VIP analysis between the DLD and other groups (A: DC; B: EC; C: XC; D: HZ). Heatmap tree (E).

3.2.3. Correlation analysis between microorganisms and metabolites

The association analysis between microorganisms and metabolites demonstrated the profound influence of distinct microbial communities on metabolic profiles during liquor fermentation. From the bacterial perspective (Fig. 6A), the robust correlation network of *Lactobacillus* with multiple metabolites establishes its central role in the fermentation process. This genus drives lactic acid biosynthesis through carbohydrate fermentation, serving as the essential precursor for ethyl lactate formation – a dual-functional process that simultaneously enhances wine mellowness and imparts characteristic acidity. Furthermore, *Lactobacillus* orchestrates the production of diverse organic acids, modulating both flavor complexity and gustatory balance. The functional significance of *Thermoactinomyces*, *Kroppenstedtiag*, and *Bacillus* was evidenced through their promotion of key metabolites. *Thermoactinomyces* regulates ester metabolism via lipase and phosphatase synthesis, effectively controlling ethyl lactate accumulation while generating aroma-enhancing compounds such as ethyl hexanoate and phenylethanol (Chen et al., 2022). *Kroppenstedtiag* demonstrates enzymatic versatility in organic substrate conversion, particularly critical for developing the characteristic profile of sesame-flavored liquors. *Bacillus* synthesizes pyrazine derivatives under thermophilic and acidic conditions, contributing distinctive nutty-roasted aromatic notes that amplify olfactory complexity (Fu et al., 2021). Notably, *Caproiciproducens*, *Eubacterium*, and *Asteroleplasma* participate in phosphorylated carbohydrate metabolism, producing glucose 1-phosphate and D-galactose 1-phosphate. These metabolites not only fuel downstream biochemical pathways but potentially initiate flavor precursor synthesis (Li et al., 2011). Fungal analysis (Fig. 6B) revealed *Kazachstania*'s exceptional metabolic influence, significantly enhancing β-*N*-acetylglucosamine, citric acid, oxypurinol, and glycerylphosphorylcholine production. The yeast's β-glycosidase activity facilitates glycoside hydrolysis, synergistically augmenting aroma dimensionality and persistence (Pozo-Bayón et al., 2012). While *Yueomyces* mirrors *Kazachstania*'s capacity in phosphorylated sugar synthesis, *Saccharomycopsis* exhibits contrasting inhibitory effects on these pathways. This suppression likely relates to *Saccharomycopsis*-mediated acidification (particularly acetic acid production) that alters microbial community dynamics, as evidenced by pH-dependent metabolic studies (Li et al., 2023). Despite its enzymatic contributions to fermentation efficiency, *Saccharomycopsis*' metabolic constraints may compromise flavor diversity (Wang et al., 2011).

The microbial-metabolite network revealed distinct functional roles of bacterial and fungal communities in shaping flavor profiles during Baijiu fermentation. Among bacteria, *Lactobacillus* exhibited unique metabolic correlations, notably promoting the production of citric acid, glycerylphosphorylcholine, and L-epicatechol—a flavonoid with antioxidant properties comparable to those observed in wine systems. This genus drives lactic acid production, serving dual roles: (1) providing precursors for ethyl lactate synthesis to enhance mellow mouthfeel and (2) modulating acid-base balance through organic acid secretion. *Thermoactinomyces* demonstrated critical flavor regulation by simultaneously suppressing ethyl lactate accumulation and generating aroma-enhancing compounds, including ethyl hexanoate and pyrazines. *Kroppenstedtiag*, a thermotolerant actinobacterium dominant in high-temperature Daqu, facilitated organic substrate conversion through multi-enzyme systems, particularly enhancing 3-phenyllactic acid (3-PLA) production to improve antimicrobial stability. *Bacillus* spp. generated roasted-nut aromas via Maillard reaction-derived pyrazines, such as 4-methylpyrazine and 2,6-dimethylpyrazine, under high-temperature conditions (Sun et al., 2024). The *Caproiciproducens-Eubacterium-Asteroleplasma* consortium maintained phosphorylated sugar pools, producing glucose 1-phosphate and D-galactose 1-phosphate as critical intermediates for UDP-glucose regeneration.

Fungal communities displayed complementary metabolic specialization. *Kazachstania* enhanced β-*N*-acetylglucosamine, a signaling molecule involved in microbial interactions, while its β-glucosidase activity liberated bound aroma compounds (e.g., terpenes) to improve olfactory complexity. *Yueomyces* synergistically amplified phosphorylated sugar synthesis, whereas *Saccharomycopsis* suppressed these pathways through acidification. Notably, 3-PLA and tricarballylic acid showed strong positive correlations with non-dominant fungal species, suggesting late-stage microbial succession drives secondary metabolite diversification. The dominance of Lactobacillus in bacterial communities created an acidic microenvironment that stabilized esterase-sensitive compounds while indirectly enabling fungal-derived flavor layering through pH-modulated enzymatic activities.

3.3. Analysis of GC-IMS results

3.3.1. Analysis of volatile substances

The dynamic changes of volatile organic compounds (VOCs) during liquor fermentation were systematically investigated using GC-IMS. Comparative analysis focused on five critical fermentation stages (HZ, XC, EC, DC, and DLD) revealed significant compositional variations. As illustrated in Fig. 7A, the characteristic reactive ion peak (RIP) was detected at a migration time of 1.0 ms. The flanking regions of the RIP peak correspond to distinct VOCs, with color intensity gradients reflecting compound concentration differences. Notably, multi-cycle base wine samples exhibited markedly higher color intensity compared to other groups, indicative of enhanced volatile compound accumulation throughout the extended fermentation process.

Comparative analysis of microbial diversity across fermentation stages revealed that the DLD and HZ stages exhibited significantly greater microbial complexity than DC, EC, and XC stages. This biodiversity pattern directly correlated with volatile compound profiles, as evidenced by the DLD-stage base liquor containing substantially more volatile substances than other stages. Such observations underscore the intrinsic connection between microbial metabolic activity and flavor compound biosynthesis during fermentation. Chromatographic heatmap analysis using HZ-stage base liquor as the reference (white background) demonstrated distinct concentration gradients of flavor compounds (Fig. 7B). The DLD samples showed predominant red zones indicating higher flavor intensity compared to HZ, while DC, EC, and XC groups displayed blue zones reflecting reduced concentrations. Notably, the DLD group's enhanced flavor profile suggests optimized microbial metabolic networks facilitating efficient flavor synthesis. In contrast, the limited flavor development in DC/EC/XC groups implies potential constraints in microbial diversity or metabolic pathway regulation. Temporal analysis revealed progressive flavor enrichment throughout fermentation. Extended duration promoted microbial community succession and enzymatic activation, enabling complete transformation of macromolecular precursors into diverse volatile compounds including alcohols, aldehydes, and acids. This time-dependent metabolic refinement enhanced both flavor complexity and sensory attributes through three synergistic mechanisms: sustained proliferation of flavor-producing microbiota, accumulation of Maillard reaction derivatives, and dynamic equilibrium of esterification processes.

Comprehensive fingerprint analysis of VOCs was conducted through spectral matching against the VOC library, revealing stage-specific chemical profiles in fermentation mashes. The chromatographic profiling identified 77 distinct VOCs across five chemical classes: 43 esters (55.8%), 18 alcohols (23.4%), 9 aldehydes (11.7%), 5 ketones (6.5%), and 2 miscellaneous compounds (2.6%), establishing esters as the dominant chemical class (Fig. 7C). These esterification products, formed through microbial-mediated acid-alcohol condensation reactions, constitute the aromatic foundation of Chinese liquor, with sensorially critical compounds including ethyl hexanoate (fruity, pineapple-like aroma) and ethyl butyrate (sweet, apple notes) demonstrating peak concentrations in multi-round fermentation samples. Advanced fermentation stages exhibited enhanced chemical diversity, with characteristic markers such as hexanal (fresh green notes), 3-methyl-2-butanol (herbaceous complexity), and heptanol (floral

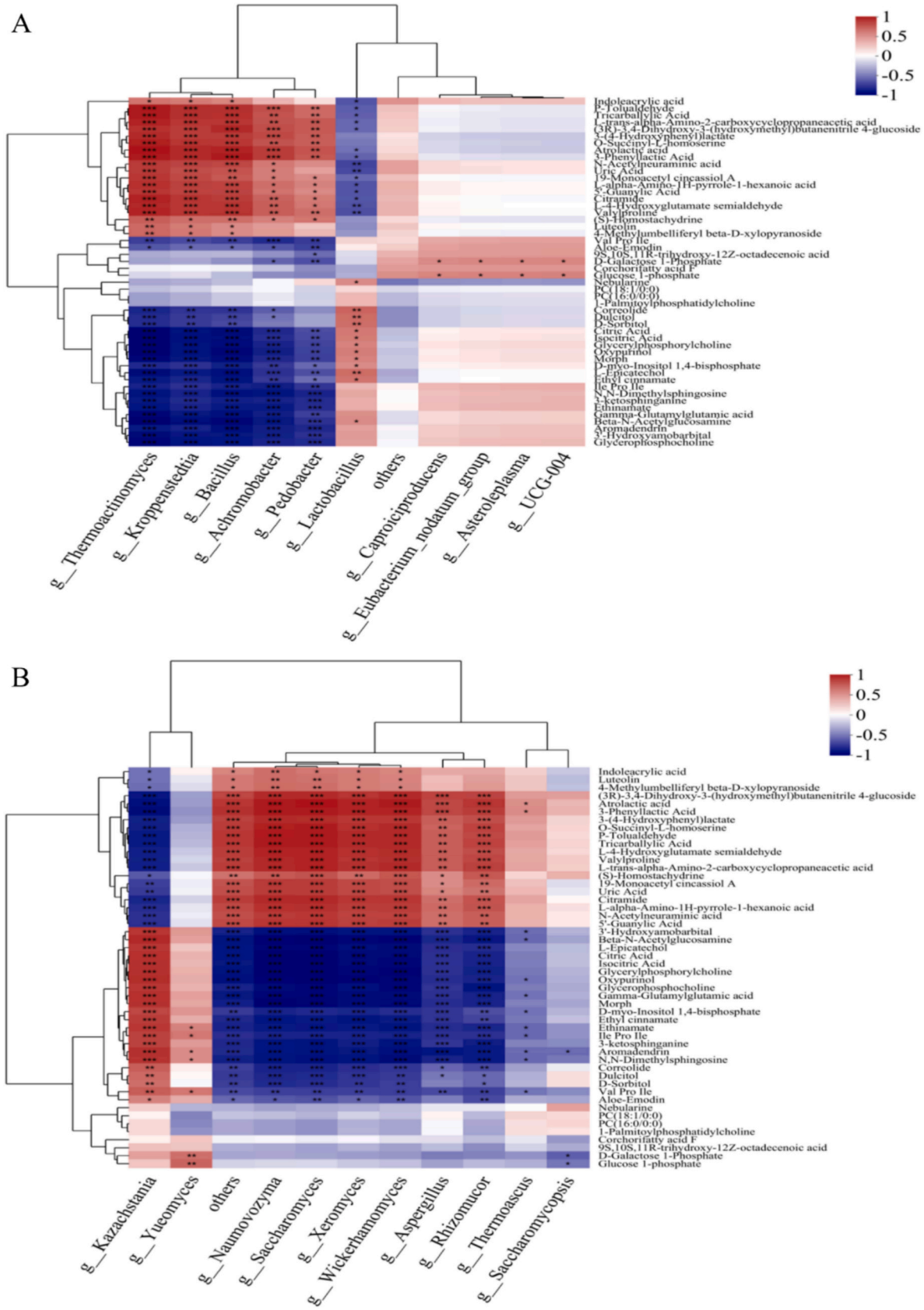

Fig. 6. Relationship between bacteria and metabolites (A). Relationship between fungi and metabolites (B).

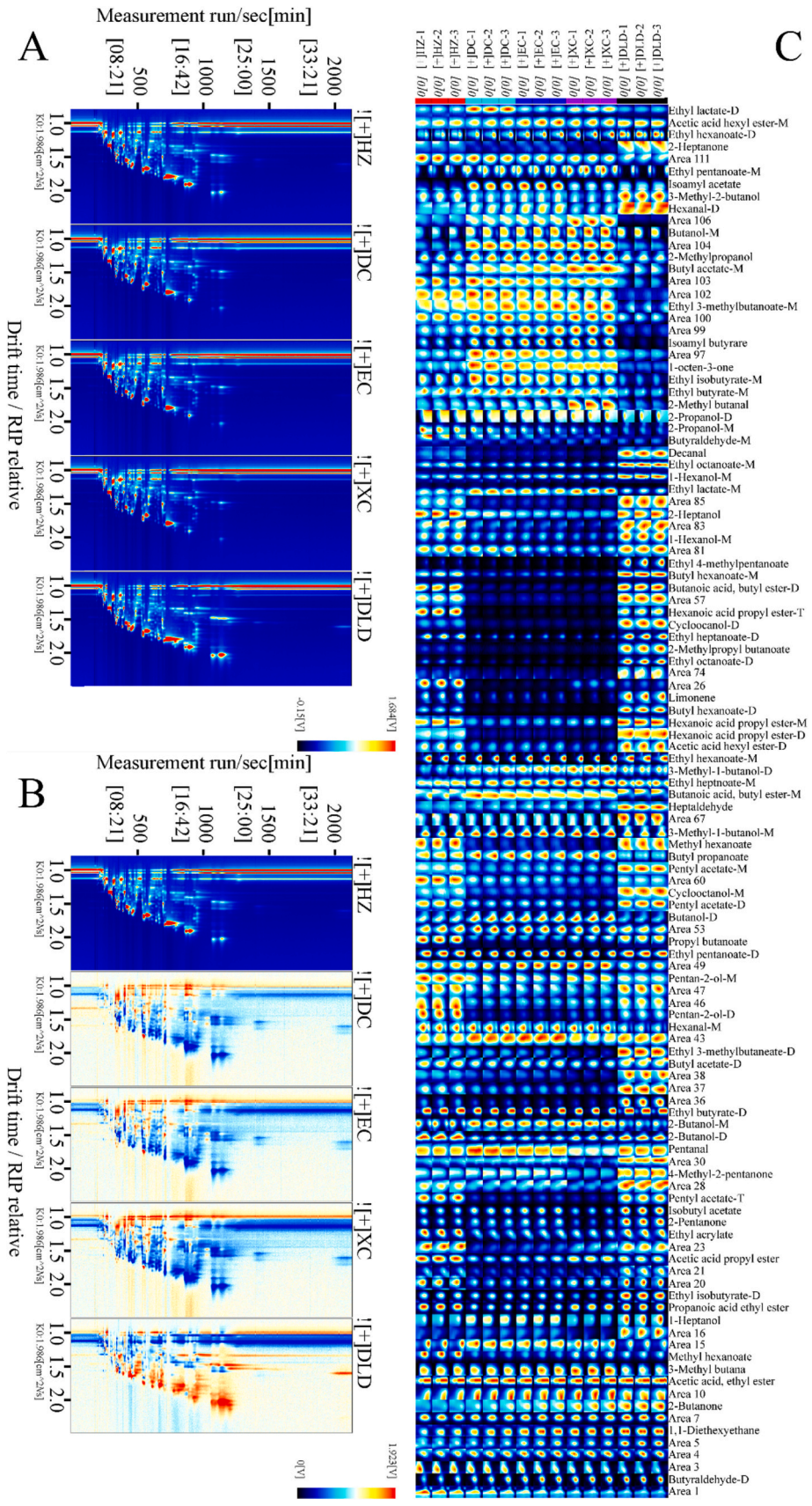

Fig. 7. GC-IMS spectrum of volatile organic compounds in mashes. A: top view; B: difference map; C: fingerprint of volatile organic compounds.

undertones) showing significantly higher concentrations compared to initial stages. This chemical evolution correlates with microbial consortia dynamics - elevated biodiversity in mature fermentation phases drives expanded metabolic networks, facilitating precursor conversion and secondary metabolite synthesis. The progressive accumulation of these organoleptic compounds critically determines the liquor's sensory signature, with hexanal contributing freshness while higher alcohols enhance structural depth, collectively elevating product differentiation in competitive markets.

3.3.2. Microbial association analysis of the four main esters

Strong-flavor Baijiu derives its characteristic aroma profile from hundreds of ester compounds, with ethyl caproate, ethyl butyrate, ethyl acetate, and ethyl lactate serving as the principal aromatic determinants that define its distinctive sensory identity (Wang et al., 2022). Analytical tracking of these four key esters across fermentation stages revealed distinct metabolic patterns (Fig. 8A). The base liquor from the DLD stage contained the highest concentrations of ethyl caproate, ethyl butyrate, and ethyl acetate, while exhibiting minimal ethyl lactate content. Ethyl caproate demonstrated stage-dependent variation, peaking during the DC phase before progressively declining through subsequent EC, XC, and HZ stages. Conversely, ethyl acetate and ethyl butyrate exhibited continuous concentration escalation across the DC to HZ phases. Ethyl lactate maintained relative stability after its DC-stage maximum, showing negligible fluctuations in later stages. The hierarchical distribution of ester content followed DLD > HZ > XC > DC ≈ EC, indicating temporal progression of aromatic compound synthesis. This fermentation chronology correlates with microbial metabolic activation - extended process duration enhances enzymatic esterification efficiency and precursor conversion rates, ultimately intensifying the liquor's aromatic complexity. The DLD stage particularly demonstrated superior ester accumulation, confirming its critical role in flavor development. Notably, environmental factors such as pH (3.8–4.2) and layered oxygen gradients were mechanistically linked to microbial metabolic specialization, though their direct quantitative impacts require further multivariate analysis.

PCoA was employed to assess flavor compound similarities across fermentation stages. Post-dimensionality reduction processing, the cumulative variance contribution rate of principal components exceeded 90%, effectively capturing the intrinsic characteristics of the original dataset. As illustrated in Fig. 8B, biological replicates exhibited high intra-stage clustering fidelity, while inter-stage samples demonstrated distinct spatial separation. Notably, DC, EC, and XC stage samples formed a proximate cluster in the principal component space, suggesting comparable flavor profiles during these phases. In contrast, DLD-stage samples occupied a statistically distinct quadrant, displaying maximal separation from other fermentation stages. This spatial differentiation confirms substantial divergence in flavor compound composition, particularly enhanced chemical diversity and concentration in HZ-stage samples. The observed metabolic progression aligns with fermentation ecology dynamics: DLD-phase samples, originating from the fermentation pit's anaerobic basal zone, benefit from extended microbial succession cycles (48–72 days). This prolonged incubation facilitates functional coordination between acid-producing bacteria and esterifying yeasts, driving accelerated precursor biotransformation and flavor compound deposition. The spatial-temporal pattern of PCoA coordinates directly correlates with microbial community restructuring events, substantiating the critical role of microenvironment-driven metabolic specialization in flavor development.

Significant functional correlations emerged between microbial communities and volatile flavor compounds, as demonstrated in Fig. 8C. Distinct microbial taxa exhibited specialized catalytic roles in flavor biosynthesis pathways. Fungal species including *Wickerhamomyces*, *Thermoascus*, and *Aspergillus*, alongside bacterial genera *Bacillus* and *Achromobacter*, significantly enhanced ethyl hexanoate production, whereas *Kazachstania* demonstrated inhibitory effects. Complementary metabolic functions were observed in *Naumovozyma* and *Rhizomucor*, which substantially promoted acetic acid and ethyl ester formation, revealing microbial niche specialization in flavor compound synthesis. Pearson correlation mapping (Fig. 8D) established robust linkages between fungal/bacterial consortia and key esters (ethyl hexanoate, ethyl butyrate, ethyl acetate, ethyl lactate) at genus-level resolution. *Lactobacillus-Kazachstania* synergism drove ethyl lactate biosynthesis, likely through lactic acid bacterial conversion of glucose-derived lactate under anaerobic, acidic conditions (pH 3.8–4.2). This metabolic partnership simultaneously suppressed competitive bacterial taxa, explaining ethyl lactate's inverse correlation with other esters and its peak accumulation during lees maturation. The majority of microbial communities, excluding *Lactobacillus*, *Rhizomucor*, *Naumovozyma*, *Yueomyces*, and *Kazachstania*, functioned as positive regulators for ethyl hexanoate and ethyl butyrate biogenesis. Similarly, all fungal species except *Kazachstania* and *Yueomyces* enhanced acetic acid-ethyl ester formation. The DLD stage's diverse microbial community, operating through extended fermentation (60–75 days) under layered oxygen conditions, drove synergistic metabolic processes that maximized production of acetic acid derivatives and key ethyl esters. These findings systematically establish microbial consortia engineering as a strategic approach for modulating Baijiu flavor profiles through targeted community manipulation. Future studies integrating in situ monitoring of temperature, redox potential, and pH dynamics are needed to resolve microenvironmental mediation of microbial-flavor networks.

4. Conclusion

This study elucidates the dynamic interplay between microbial succession and flavor development during the solid-state fermentation of Tanggou liquor. The fermentation process progresses through distinct functional phases governed by microbial guilds. Initially, lactic acid bacteria dominate, rapidly acidifying the environment to establish microbial selectivity while creating biochemical conditions conducive to ester stabilization. During the mid-fermentation phase, yeast populations emerge as key contributors, utilizing enzymatic mechanisms to release bound aromatic precursors and synergizing with bacterial metabolism to drive ester biosynthesis. In later stages, thermophilic microorganisms become active, generating characteristic nitrogen-containing heterocyclic compounds that enrich flavor complexity. Central to this process is the spatiotemporal coordination of microbial activities—bacterial acidification primes the system for yeast-driven aroma development, followed by thermophile-mediated flavor refinement. The layered fermentation strategy enhances process controllability by orchestrating this microbial succession pattern, effectively reducing batch-to-batch variability of core flavor components compared to traditional methods. These findings advance the understanding of microbial community engineering in traditional fermentation systems, providing a framework for optimizing flavor consistency while preserving the artisanal essence of heritage liquor production.

This study transcends the conventional static analysis framework of Baijiu microbiota research, systematically elucidating for the first time the coupling mechanism between microbial community succession and flavor stratification. The findings reveal that lactic acid bacteria establish metabolic foundations through environmental acidification during early fermentation, followed by yeast populations achieving spatiotemporal coordination of aroma precursor release and ester synthesis via enzymatic activity synchronization. Thermophilic microbial communities subsequently mediate cross-stage metabolic network reconstruction, transforming precursors into characteristic flavor compounds. The research uncovers the critical roles of Lactobacillus-yeast synergistic effects and thermophile-mediated metabolic bridging during microbial functional relay, establishing a dynamic microbial regulation-based theoretical model for flavor formation. This provides novel methodological support for targeted flavor design and process optimization in traditional fermentation systems. In addition, this study was funded by

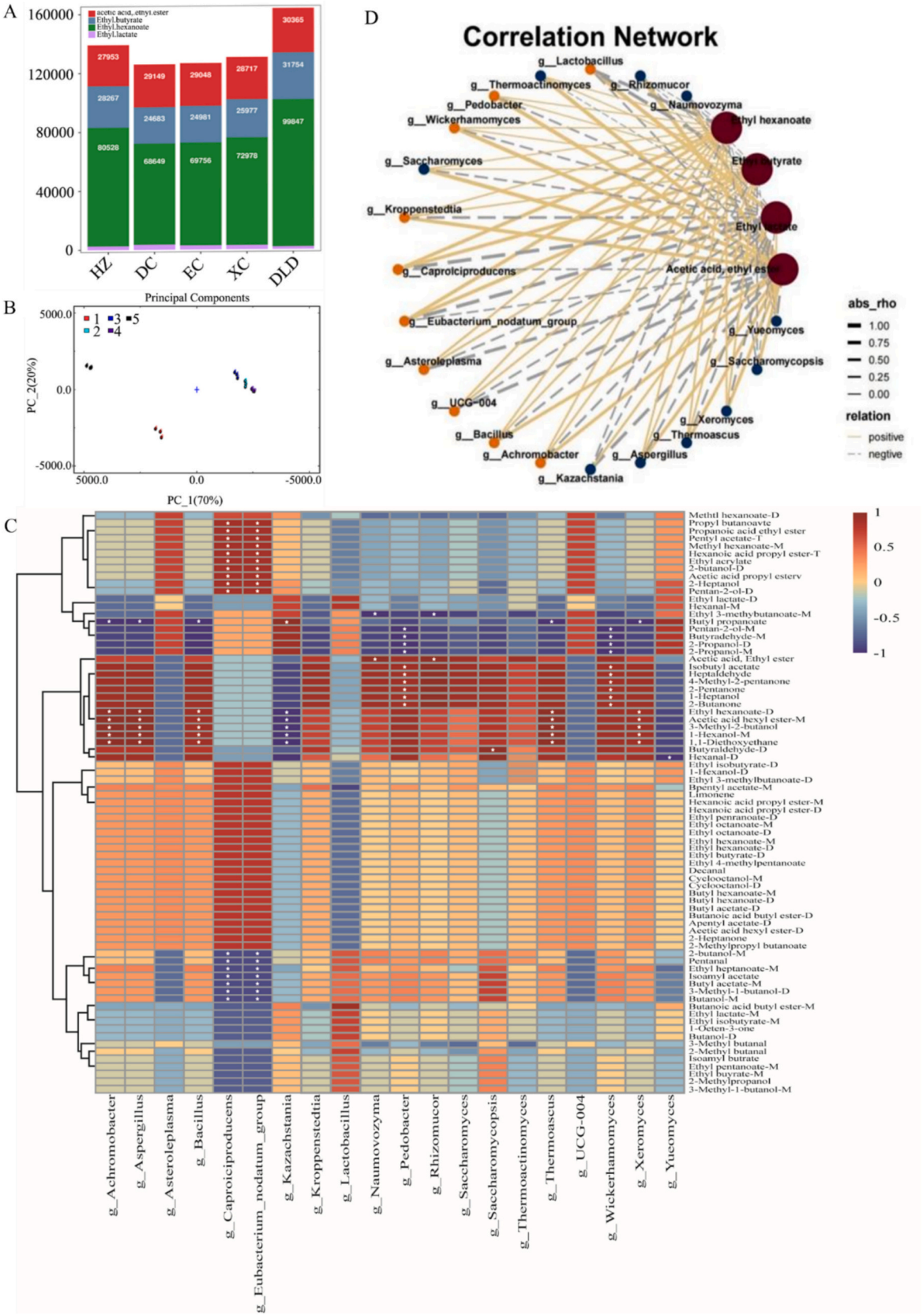

Fig. 8. Correlation analysis results between microorganisms and four lipids. A: Lipid content in different liquor samples; B: PCA analysis of flavor substances in different liquor samples; C: Correlation analysis between genus-level microorganisms and volatile odors; D: Correlation analysis between four lipids and microorganisms.

the Lianyungang "521 Project" scientific research project-Research on measures to improve the quality of 5000 tons of technologically upgraded famous and high-quality liquors, and the relevant results can also provide a theoretical basis for the implementation of this project.

CRediT authorship contribution statement

Antuo Hu: Writing – original draft. **Yaohui Zhu:** Conceptualization. **Zhan Gao:** Data curation. **Jun Zheng:** Formal analysis. **Dazhong Zhou:** Methodology. **Liang Zhang:** Resources. **Saikun Pan:** Writing – review & editing. **Jie Yang:** Validation, Supervision. **Sheng Xu:** Supervision.

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.

Acknowledgments

This work was supported by the Jiangsu Tanggou Lianghe Liquor Industry Co., Ltd., Enterprise Project (grant number: JOUH23243), Jiangsu University "Qinglan Project", Lianyungang "521 Project" scientific research project (grant number: LYG06521202330), Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2025.102486.

Data availability

Data will be made available on request.

References

Anjum, N., Maqsood, S., Masud, T., Ahmad, A., Sohail, A., & Momin, A. (2014). *Lactobacillus acidophilus*: Characterization of the species and application in food production. *Critical Reviews in Food Science and Nutrition, 54*(9), 1241–1251.

Chen, C., Yang, H., Liu, J., Luo, H., & Zou, W. (2022). Systematic review of actinomycetes in the baijiu fermentation microbiome. *Foods, 11*(22), 3551.

Cheng, W., Chen, X., Xue, X., Lan, W., Zeng, H., Li, R., & Yang, H. (2024). Comparison of the correlations of microbial community and volatile compounds between pit-mud and fermented grains of compound-flavor baijiu. *Foods, 13*(2), 203.

Cheng, W., Lan, W., Chen, X., Xue, X., Liang, H., Zeng, H., & Yang, H. (2024). Source and succession of microbial communities and tetramethylpyrazine during the brewing process of compound-flavor baijiu. *Frontiers in Microbiology, 15*, Article 1450997.

Decimo, M., Quattrini, M., Ricci, G., Fortina, M. G., Brasca, M., Silvetti, T., & Casiraghi, M. C. (2017). Evaluation of microbial consortia and chemical changes in spontaneous maize bran fermentation. *AMB Express, 7*, 1–13.

Ding, X., Wu, C., Huang, J., & Zhou, R. (2015). Interphase microbial community characteristics in the fermentation cellar of Chinese Luzhou-flavor liquor determined by PLFA and DGGE profiles. *Food Research International, 72*, 16–24.

Fu, G., Deng, M., Chen, K., Chen, Y., Cai, W., Wu, C., & Wan, Y. (2021). Peak-temperature effects of starter culture (Daqu) on microbial community succession and volatile substances in solid-state fermentation (Jiupei) during traditional Chinese special-flavour baijiu production. *Lwt, 152*, Article 112132.

Ge, D., Cai, W., Guo, Z., Wang, B., Liu, M., Shan, C., & Wang, Y. (2024). Comparative analysis of bacterial community structure and physicochemical quality in high-temperature Daqu of different colors in Qingzhou production area. *Heliyon, 10*(11).

Hu, P., Wang, J., Ali, U., Aziz, T., Sameeh, M. Y., & Feng, C. (2023). Comparative study on physicochemical properties, microbial composition, and the volatile component of different light flavor Daqu. *Food Science & Nutrition, 11*(9), 5174–5187.

Huang, X., Fan, Y., Lu, T., Kang, J., & Chen, J. (2020). Composition and metabolic functions of the microbiome in fermented grain during light-flavor baijiu fermentation. *Microorganisms, 8*(9), 1281.

Huang, Y., Yi, Z., Jin, Y., Zhao, Y., & Zhao, H. (2017). New microbial resource: Microbial diversity, function and dynamics in chinese liquor starter. *Rep, 7*(1).

Jood, I., Hoff, J. W., & Setati, M. E. (2017). Evaluating fermentation characteristics of *Kazachstania* spp. and their potential influence on wine quality. *World Journal of Microbiology and Biotechnology, 33*, 1–11.

Kuhlisch, C., & Pohnert, G. (2015). Metabolomics in chemical ecology. *Natural Product Reports, 32*(7), 937–955.

Li, H., Han, X., Liu, H., Hao, J., Jiang, W., & Li, S. (2021). Silage fermentation on sweet sorghum whole plant for fen-flavor baijiu. *Foods, 10*(7), 1477.

Li, J., Zhang, Q., & Sun, B. (2023). Chinese baijiu and whisky: Research reservoirs for flavor and functional food. *Foods, 12*(15), 2841.

Li, J., Zhao, Y., Qin, Y., & Shi, H. (2020). Influence of microbiota and metabolites on the quality of tobacco during fermentation. *BMC Microbiology, 20*, 1–15.

Li, X. R., Ma, E. B., Yan, L. Z., Meng, H., Du, X. W., Zhang, S. W., & Quan, Z. X. (2011). Bacterial and fungal diversity in the traditional Chinese liquor fermentation process. *International Journal of Food Microbiology, 146*(1), 31–37.

Lutskova, V., & Martirosyan, I. (2021). Influence of harvest date and grape variety on sensory attributes and aroma compounds in experimental icewines of Ukraine. *Fermentation, 7*(1), 7.

Lv, X. C., Chen, Z. C., Jia, R. B., Liu, Z. B., Zhang, W., Chen, S. J., & Ni, L. (2015). Microbial community structure and dynamics during the traditional brewing of Fuzhou Hong Qu glutinous rice wine as determined by culture-dependent and culture-independent techniques. *Food Control, 57*, 216–224.

Moens, F., Lefeber, T., & De Vuyst, L. (2014). Oxidation of metabolites highlights the microbial interactions and role of Acetobacter pasteurianus during cocoa bean fermentation. *Applied and Environmental Microbiology, 80*(6), 1848–1857.

Mu, Y., Huang, Y., Li, D., Zhu, Z., Yu, S., & Xie, F. (2024). Revealing the comprehensive effect of mechanization on sauce-flavor Daqu through high-throughput sequencing and multi-dimensional metabolite profiling. *Food Research International, 191*, 114645.

Pang, H., & Hu, Z. (2023). Metabolomics in drug research and development: The recent advances in technologies and applications. *Acta Pharmaceutica Sinica B, 13*(8), 3238–3251.

Parastar, H., & Weller, P. (2023). Towards greener volatilomics: Is GC-IMS the new Swiss army knife of gas phase analysis? *TrAC Trends in Analytical Chemistry, 170*, 117438.

Piraine, R. E. A., Nickens, D. G., Sun, D. J., Leite, F. P. L., & Bochman, M. L. (2022). Isolation of wild yeasts from Olympic National Park and *Moniliella megachiliensis* ONP131 physiological characterization for beer fermentation. *Food Microbiology, 104*, Article 103974.

Pozo-Bayón, M.Á., Monagas, M., Bartolome, B., & Moreno-Arribas, M. V. (2012). Wine features related to safety and consumer health: An integrated perspective. *Critical Reviews in Food Science and Nutrition, 52*(1), 31–54.

Qiu, F., Du, B., Zhang, C., Zhu, L., Yan, Y., Li, W., et al. (2024). Effects of saccharomyces cerevisiae on microbial community and flavor metabolites in solid-state fermentation of strong-flavor baijiu. *Food Bioscience, 59*(000), 10.

Shi, W., Chai, L. J., Zhao, H., Song, Y. N., Mei, J. L., He, Y. X., et al. (2024). Deciphering the effects of different types of high-temperature daqu on the fermentation process and flavor profiles of sauce-flavor baijiu. *Food Bioscience, 61*(000), 13.

Sun, J., Zhang, C., Song, Y., Chu, B., Wang, M., Zhang, Z., & Wang, X. (2024). Characterization of key aroma compounds and main contributing amino acids in hot-pressed oil prepared from various peanut varieties. *Molecules, 29*(9), 1947.

Sun, W., Xiao, H., Peng, Q., Zhang, Q., Li, X., & Han, Y. (2016). Analysis of bacterial diversity of Chinese Luzhou-flavor liquor brewed in different seasons by Illumina Miseq sequencing. *Annals of Microbiology, 66*, 1293–1301.

Tang, Q., Chen, X., Huang, J., Zhang, S., Qin, H., Dong, Y., & Zhou, R. (2023). Mechanism of enhancing pyrazines in Daqu via inoculating *Bacillus licheniformis* with strains specificity. *Foods, 12*(2), 304.

Wang, D., Chen, L., Yang, F., Wang, H., & Wang, L. (2019). Yeasts and their importance to the flavour of traditional Chinese liquor: A review. *Journal of the Institute of Brewing, 125*(2), 214–221.

Wang, H. Y., Gao, Y. B., Fan, Q. W., & Xu, Y. (2011). Characterization and comparison of microbial community of different typical Chinese liquor Daqus by PCR–DGGE. *Letters in Applied Microbiology, 53*(2), 134–140.

Wang, J., Chen, H., Wu, Y., & Zhao, D. (2022). Uncover the flavor code of strong-aroma baijiu: Research progress on the revelation of aroma compounds in strong-aroma baijiu by means of modern separation technology and molecular sensory evaluation. *Journal of Food Composition and Analysis, 109*, Article 104499.

Wang, J., Zhong, Q., Yang, Y., Li, H., Wang, L., Tong, Y., & Liao, Z. (2018). Comparison of bacterial diversity between two traditional starters and the round-koji-maker starter for traditional cantonese chi-flavor liquor brewing. *Frontiers in Microbiology, 9*, 1053.

Xie, G., Zheng, H., Qiu, Z., Lin, Z., Peng, Q., Dula Bealu, G., & Liu, G. (2021). Study on relationship between bacterial diversity and quality of Huangjiu (Chinese Rice wine) fermentation. *Food Science & Nutrition, 9*(7), 3885–3892.

Xu, Y., Wu, M., Zhao, D., Zheng, J., Dai, M., Li, X., & Sun, B. (2023). Simulated fermentation of strong-flavor baijiu through functional microbial combination to realize the stable synthesis of important flavor chemicals. *Foods, 12*(3), 644.

Yan, S., Wang, S., Wei, G., & Zhang, K. (2015). Investigation of the main parameters during the fermentation of Chinese Luzhou-f lavour liquor. *Journal of the Institute of Brewing, 121*(1), 145–154.

Zhang, G., Xiao, P., Yuan, M., Li, Y., Xu, Y., Li, H., & Sun, B. (2023). Roles of sulfur-containing compounds in fermented beverages with 2-furfurylthiol as a case example. *Frontiers in Nutrition, 10*, Article 1196816.

Zhang, H., Wang, L., Tan, Y., Wang, H., Yang, F., Chen, L., & Xu, Y. (2021). Effect of Pichia on shaping the fermentation microbial community of sauce-flavor baijiu. *International Journal of Food Microbiology, 336*, Article 108898.

Zhang, J., Hou, Y., Liu, Q., Zhang, Y., Gao, B., Zou, W., & Zhang, K. (2023). Fortified Jiuqu of the Chinese baijiu: A review on its functional microorganisms, strengthening effects, current challenges, and perspectives. *Food. Bioscience, 55*, 103045.

Zhang, P., Liu, Y., Li, H., Hui, M., & Pan, C. (2024). Strategies and challenges of microbiota regulation in baijiu brewing. *Foods, 13*(12), 1954.
Zhang, Q., Ma, J., Yang, Y., Deng, J., Zhu, K., Yi, Y., & Laghi, L. (2023). Effects of S. Cerevisiae strains on the sensory characteristics and flavor profile of kiwi wine based on E-tongue, GC-IMS and 1H-NMR. *Lwt, 185*, Article 115193.
Zhao, L., Wu, Y., Zhao, Y., Li, X., Zhang, M., Li, X., & Gu, S. (2022). Deciphering the intra- and inter-kingdom networks of microbiota in the pit mud of Chinese strong-flavor liquor. *Lwt, 165*, Article 113703.