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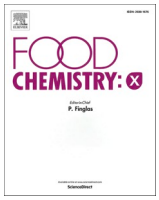

Study of microbial communities, sensory characteristics, volatile flavor compounds and the correlation during the storage of Xiangyang fresh Huangjiu

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ABSTRACT

Xiangyang fresh Huangjiu is a Chinese regional specialty fermented beverage and an intangible cultural heritage. The microbial community, sensory characteristics and volatile flavor compounds during the storage of two typs of Xiangyang fresh Huangjiu were investigated, and a correlative analysis was conducted between the microbial community and sensory characteristics as well as volatile flavor compounds. 14 dominant bacterial genera and 7 dominant fungal genera were indentified in both types of Huangjiu. The pronounced sourness in both types of Huangjiu during storage was strongly positively associated with *Saccharomyces*. The predominant flavor profile of 1-octen-3-one was significantly negatively correlated with *Gluconobacterium*, and the increase of *Wickerhamomyces* and *Millerozyma*, along with the decline of *Enterobacter*, *Komagataeibacter*, *Pseudomonas*, and *Saccharomycopsis*, contributed to the reduction of the pungent volatile compounds of dimethyl trisulfide and 2-methyl butanal. The results offer insights for quality enhancement, storage and consumption in the standardized industrial production of Xiangyang fresh Huangjiu.

1. Introduction

Huangjiu (Chinese rice wine), a time-honored traditional brewed alcoholic beverage in China (Mao et al., 2023), stands alongside beer and wine as one of the world's three ancient fermented drinks. It is highly favored by consumers for its unique taste, rich nutritional value, and cultural depth. Due to variations in raw materials, specific fermentation agents, environmental conditions, and brewing techniques, China boasts a multitude of Huangjiu varieties. Among them, Xiangyang fresh Huangjiu is a culinary calling card and an intangible cultural heritage of Xiangyang. Located north of the Nanxiang Basin and south of the Jianghan Plain, Xiangyang's advantageous natural conditions and geographical location have created a prosperity characterized by "the granary of Jiangnan and the fish and rice of Jingxiang", providing abundant raw materials for the brewing of Xiangyang fresh Huangjiu. Moreover, Xiangyang fresh Huangjiu adheres to the ancient method of natural brewing, which does not require high-temperature inactivation and can be consumed directly. As a result, it contains a large number of fermenting microorganisms, which continue to ferment during storage, offering a range of drinking flavors. Thus, while inheriting the excellent quality of traditional yellow wine, Xiangyang fresh Huangjiu also possesses unique regional characteristics in terms of brewing technology and flavor profile, promising a favorable market consumption prospect. Currently, the production of Xiangyang fresh Huangjiu is predominantly workshop-based, and the quality of the Huangjiu relies heavily on the brewer's experience. To realize the commercialization of Xiangyang fresh Huangjiu, it is necessary to further quantify the flavor quality and related influencing factors to lay the foundation for the standardized industrial production.

Chromatographic techniques, including Gas Chromatography-Mass Spectrometry-Olfactometry (GC-MS-O) and Gas Chromatography-Ion Mobility Spectrometry (GC-IMS), in conjunction with electronic sensory analysis technologies such as the electronic nose (*E*-nose) and electronic tongue (E-tongue), have provided effective methods for

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analyzing the flavor profiles of alcoholic beverages (Y. Hao et al., 2024). Chen et al. (2022) applied electronic nose and tongue techniques to evaluate the flavor of tea wine, which provided a reference for the optimization of tea wine fermentation process. Yin et al. (2024)used GC-MS, electronic nose and electronic tongue to analyze flavor characteristics of different types of Qingke Baijiu (QKBJ), which provided a reference for the fast distiguishment of different types of QKB J. Tang et al. (2024)have revealed the flavor differences between black rice wine and glutinous rice wine using GC-MS, GC-IMS, HPLC, and electronic sensory analysis. The unique flavors of Huangjiu are related to its multi-strain mixed natural open fermentation system, with microorganisms playing a significant role in regulating the volatile flavor compounds (Mao et al., 2023). Liang et al.(2020)indicated that *Saccharomyces cerevisiae*, *Pediococcus*, *Rhizopus*, *Monascus*, *Aspergillus*, and *Bacillus* are key microorganisms in the production of bitter amino acids during the fermentation process of Huangjiu by high-throughput sequencing technology. Therefore, the use of high-throughput sequencing technology, chromatographic techniques, and electronic sensory analysis can effectively analyze the microbial communities and flavor characteristics of Xiangyang fresh Huangjiu, as well as their potential relationships during the storage process, which have't been investigated.

In this study, the changes in microbial communities and dominant microorganisms during the storage fermentation period of Xiangyang fresh Huangjiu were firstly analyzed using high-throughput sequencing technology. The sensory characteristics of Xiangyang fresh Huangjiu during the storage fermentation period were explored using electronic sensory analysis, and key aroma compounds affecting the flavor of Xiangyang fresh Huangjiu were identified by GC-IMS analysis. The correlation analysis between the microbial communities and flavor characteristics of Xiangyang fresh Huangjiu was conducted to clarify the microbial communities that affect the flavor of Xiangyang fresh Huangjiu. The results could provide a useful reference for quality enhancement, storage and consumption in the standardized industrial production of Xiangyang fresh Huangjiu.

2. Materials and methods

2.1. Sample preparation

Two brands of Xiangyang fresh Huangjiu that are well-established in the local market and have distinct production processes and market shares was provided by local manufacturers, branded as A (XYFA) and B (XYFB). Since the recommended storage condition is refrigeration and the recommended consumption time based on market practice and the general quality perception of consumers in the Xiangyang area is around 20 days, the two types of Huangjiu samples were stored at 4 °C, and were divided into three categories, including control group without storage, one group stored for 10 days, and another group stored for 22 days. Three samples under each condition were used for the determination of microbial communities, electronic tongue characteristics, electronic nose characteristics, and the volatile compounds, respectively, and the average value was used for further analysis. Finally, a total of 72 samples were used.

2.2. Microbial communities analysis

DNA extraction, PCR amplification, and Illumina MiSeq sequencing were used to explore bacterial and fungal communities in Xiangyang fresh Huangjiu during storage. Approximately 20 mL of sample was collected and centrifuged at 8000 rpm/min for 10 min at 4 °C to obtain the sediment for DNA extraction. Then metagenomic DNA was extracted using an E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, Norcross, USA). The V3-V4 region of the bacterial 16S rRNA gene and the fungal ITS1 region were amplified using primers 338F/806R and ITS1F/ITS2R, respectively. The bacterial 16S rRNA gene was amplified with the primers 338F (5′-ACTCCTACGGGAGGCAGAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). The fungal ITS region was amplified using the primer pair ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS2 (5′-GCTGCGTTCTTCATCGATGC-3′). PCR amplification and sequencing were further performed under the protocol of the Illumina MiSeq PE300 platform (Illumina, San Diego, USA) and Majorbio Bio-Pharm Technology Co Ltd. (Shanghai, China). Operational taxonomic units (OTUs) were identified from the valid sequences using the UCLUST method based on a 97 % similarity threshold. Representative sequences for each OTU were selected and compared with sequences in the SILVA database to determine their taxonomic status (J. Li et al., 2023). Microbial community composition, alpha diversity, and beta diversity analysis were conducted to assess the bacterial community structure, species diversity, and significant differences between groups in samples based on OTU abundance and taxonomic status (Lin Wang et al., 2021).

2.3. Sensory evaluation

Electronic sensory analysis offered immediate advantages in terms of real-time, continuous monitoring, high-precision quantification, and being free from subjective influence, which is widely used for the sensory characteristic analysis of alcoholic beverages (J. Wang et al., 2024). Overall, electronic sensory analysis has proven to be a scientifically effective technique for analyzing sensory characteristics, which is crucial for providing practical and actionable guidance for future production of alcoholic beverages. Thus, electronic nose and tongue have been used for the sensory evaluation.

2.3.1. Electronic nose analysis

The odor characteristics of Huangjiu samples were analyzed using an electronic nose system (cNose-18, Shanghai Baosheng Industrial Development Co., Ltd., China) with 28 semiconductor metal oxide gas sensors. The specifications of each sensor was provided in the reference (Q. Sun et al., 2024). The test conditions were set as follows: an injection flow rate of 400 mL/min, the sampling duration of 90 s, and the cleaning time of 60 s.

2.3.2. Electronic tongue analysis

The taste of Huangjiiu samples was analyzed using the electronic tongue system (TS-5000Z, Intelligent Sensor Technology, Inc., Kanagawa, Japan) equipped with five lipid-membrane sensors, and each sensor generates specific electrical signals based on its corresponding distinct responsive substances, thereby acquiring quantified values of various taste characteristics. Specifically, the measurement cycle of a sample consists of measuring the reference solution, followed by measuring the sample solution, performing a brief cleaning step, then measuring the aftertaste, and finally conducting a final cleaning step. The sampling time was set to 120 s with the frequency was 1 time/s, and subsequent to each experimental trial, the sensors were subjected to a cleansing procedure utilizing a 10 % (volume/volume) ethanol solution. Finally, tastes including sourness, bitterness, astringency, umami, saltiness, were measured by determining the change in membrane potential caused by the adsorption of substances onto the lipid membranes corresponding to specific tastes after measuring the sample solution. The after taste of bitterness (aftertaste-B), the after taste of astringency (aftertaste-A) and the after taste of umami (richness) were measured by determining the change in membrane potential caused by the adsorption of substances onto the lipid membranes corresponding to specific tastes after the brief cleaning step (Woertz et al., 2011).

2.4. Isolation and identification of the volatile compounds

HS-GC-IMS has been widely applied in the investigation of volatile flavor compounds in alcoholic beverage (Y. Gao et al., 2024; Gu et al., 2021). Thus, the volatile compounds of Huangjiu samples were detected using the GC-IMS instrument (Flavourspec®, G.A.S, Dortmund, Germany) equipped with an autosampler (CTC Analytics AG, Zwingen,

Switzerland). Specifically, 2 mL of sample was placed into a 20 mL headspace vial (Wuhan Feiyang Biotechnology Co., Ltd., China) and incubated at an incubation temperature of 60 °C with an incubation rate of 500 rpm for 10 min (Yu et al., 2021). Nitrogen was used as the carrier and drift gas at a drift tube temperature of 45 °C. The linear voltage within the drift tube was set to 500 V cm^{-1}, and the length of the drift tube was 5.3 cm. For the injection process, the needle was maintained at a temperature of 85 °C, and a precise volume of 200 μL was introduced. The drift gas were set at a flow rate of 150 mL/min, and the detector was thermostatically controlled at 60 °C. The initial flow rate of the carrier gas was set at 2 mL/min, which was then maintained for 2 min before being incrementally increased to a final rate of 150 mL/min within 10 min. Finally, the volatile compounds were accurately identified by comparing the two-dimensional information of target compounds in Huangjiu samples with that of standard substances (gas chromatography retention times and ion mobility spectrometry drift times) in the software LAV version 2.0.0 from G.A.S.(Dortmund, Germany) with the flavor analysis instrument and the instrument's embedded databases. The relative contents of the volatile compounds were finally calculated by the peak area normalization method. Moreover, aroma descriptors could be obtained from Flavornet database (http://www.flavornet.org). The relative odor activity value (ROAV) is a indicator to quantify the contribution of individual volatile compounds to the overall aroma profile, and it can be expressed by the following formula (Zhang et al., 2022):

$$\mathrm{ROAV}_A = 100 \times \frac{C_A}{OT_A} \times \frac{OT_{max}}{C_{max}} \quad (1)$$

Where C_A is the relative content of the volatile compound, and OT_A is the odor threshold of the the volatile compound. C_{max} and OT_{max} are the maximum of C_A /OT_A among all the compounds in the sample.

Consequently, the ROAV for all components is ≤100, and the higher the ROAV, the greater the contribution of the component to the overall flavor profile of the sample. The ROAV of the volatile compound that make the greatest contribution to the overall flavor of the sample is set at 100. Volatile compounds with ROAV ≥1 are considered to have a direct impact on the aroma style and are identified as key aroma compounds. Compounds with 0.1 ≤ ROAV <1 are regarded as important compounds for modifying the aroma style and play a crucial role in facilitating aroma formation, thus being significant as supporting aroma constituents (K. Zhang et al., 2022).

2.5. Statistical analysis and data visualization

Experimental data were compiled and organized using Excel 2016 software, and the results are expressed as the mean ± standard deviation of three replicate experiments. Statistical differences between data sets were analyzed in SPSS Statistics 26.0 software (SPSS Inc., Chicago, IL, USA) using one-way analysis of variance (ANOVA) followed by Duncan's post-hoc test at $p \leq 0.05$. Figures of microbial community composition, alpha diversity, and beta diversity analysis were created using the I-Sanger platform (https:// www.majorbio.com/). Principal component analysis (PCA) plot of electronic sensory characteristics, figures of sensory characteristics analysis, GC-IMS substance statistical pie charts and cluster analysis diagrams, and correlative analysis between different indicators of Xiangyang fresh Huangjiu were generated using Origin 2021 software (OriginLab Corporation, Northampton, MA, USA). The GC-IMS fingerprint and differential plots were produced by the LAV-plugin-Gallery component.

3. Results and discussion

3.1. Microbial communities of Xiangyang fresh Huangjiu during storage

Based on Illumina MiSeq sequencing, a total of 962,000 optimized bacterial sequences and 1,129,262 optimized fungal sequences were obtained from Huangjiu samples. Using a 97 % sequence similarity threshold, these optimized sequences were clustered into 438 bacterial OTUs and 114 fungal OTUs.

3.1.1. Microbial community diversity analysis

The succession of microbial communities during the storage of Xiangyang fresh Huangjiu was investigated by analyzing microbial diversity, and the commonly used alpha diversity indexes, including Ace, Chao1, Shannon, and Simpson indexes were used for evaluation. Ace and Chao1 indexes revealed that higher values correspond to greater microbial species richness. Additionally, Shannon, and Simpson indexes reflect microbial communities diversity, and higher values of Shannon index are indicative of greater diversity, while lower values of simpson indexes are associated with higher levels of diversity (Paudel et al., 2023). The variation trends of these indexes for bacterial and fungal communities during the storage of Xiangyang fresh Huangjiu are presented in Fig. 1. The bacterial richness and diversity of XYFA Huangjiu gradually decreased during the storage. In contrast, XYFB Huangjiu demonstrated an upward trend in bacterial richness, and the diversity of bacterial communities followed an initial increase and a subsequent decrease, which may reflect complex interactions and shifts in the microbial composition over the fermentation during storage. Additionally, there were no significant differences in the species richness and diversity between the bacterial communities of the two types of Huangjiu ($P < 0.05$). The fungal richness and diversity of XYFA Huangjiu during storage showed a similar decreasing trend with those of bacterial communities, but the richness and diversity of fungus were lower than those of bacteria. The fungal richness of XYFB Huangjiu during storage also exhibited a decreasing trend, but the fungal diversity showed a trend of initial decrease followed by an increase. Overall, the change trends of fungal richness and diversity in XYFB Huangjiu were inversely related to those of bacteria, with fungal richness and diversity being lower than those of bacterial communities. Besides, the fungal richness and diversity of XYFB Huangjiu were higher that that of XYFA Huangjiu, especially a significant difference was observed between the fungal diversity of the two types of Huangjiu. Thus, both the quantity and variety of bacteria and fungi decreased during the storage of XYFA Huangjiu. For XYFB Huangjiu, there has a reduction in the the quantity and variety of bacteria, while the quantity of fungi has decreased, accompanied by an increase in fungal variety.

3.1.2. Microbial community composition analysis

The community composition of bacteria and fungi during the storage of Huangjiu were further investigated. Species with a relative abundance of >1 % at the phylum and genus levels were defined as "dominant", whereas taxonomic groups with a relative abundance of <1 % were categorized as "others"(Lei, Cai, Guo, Shan, & Wang, 2024; Lei, Cai, Wang, Wang, et al., 2024). Fig. 2 (a) and (b) show the bacterial community composition of XYFA and XYFB Huangjiu at the phylum and genus levels, respectively. Three dominant bacterial phyla were identified in both XYFA and XYFB Huangjiu, including *Firmicutes*, *Proteobacteria* and *Bacteroidota*, which was similar with the previous research (Lei, Cai, Guo, et al., 2024), especially *Firmicutes* and *Proteobacteria* accounted for a significant proportion. During storage, the relative abundance of *Firmicutes* in XYFA Huangjiu gradually increased, while its proportion in XYFB Huangjiu initially decreased before subsequently increasing. The relative abundance of *Proteobacteria* in the two types of Huangjiu exhibited opposite trends to that of *Firmicutes*. At the genus level, 14 dominant bacterial genera in both XYFA and XYFB Huangjiu were identified, including *Leuconostoc*, *Lacticaseibacillus*, *Lactiplantibacillus*, *Levilactobacillus*, *Acetobacter*, *Streptococcus*, *Enterobacter*, *Lactococcus*, *Gluconobacter*, *Lactobacillus*, *Komagataeibacter*, *Acinetobacter*, *Pseudomonas*, *Chryseobacterium*. In XYFA Huangjiu, *Lacticaseibacillus, Lactiplantibacillus, Levilactobacillus, Acetobacter* constituted the most predominant genus. As the storage duration increased, *Levilactobacillus* emerged as the dominant genus, reaching a proportion of 42.95 %.

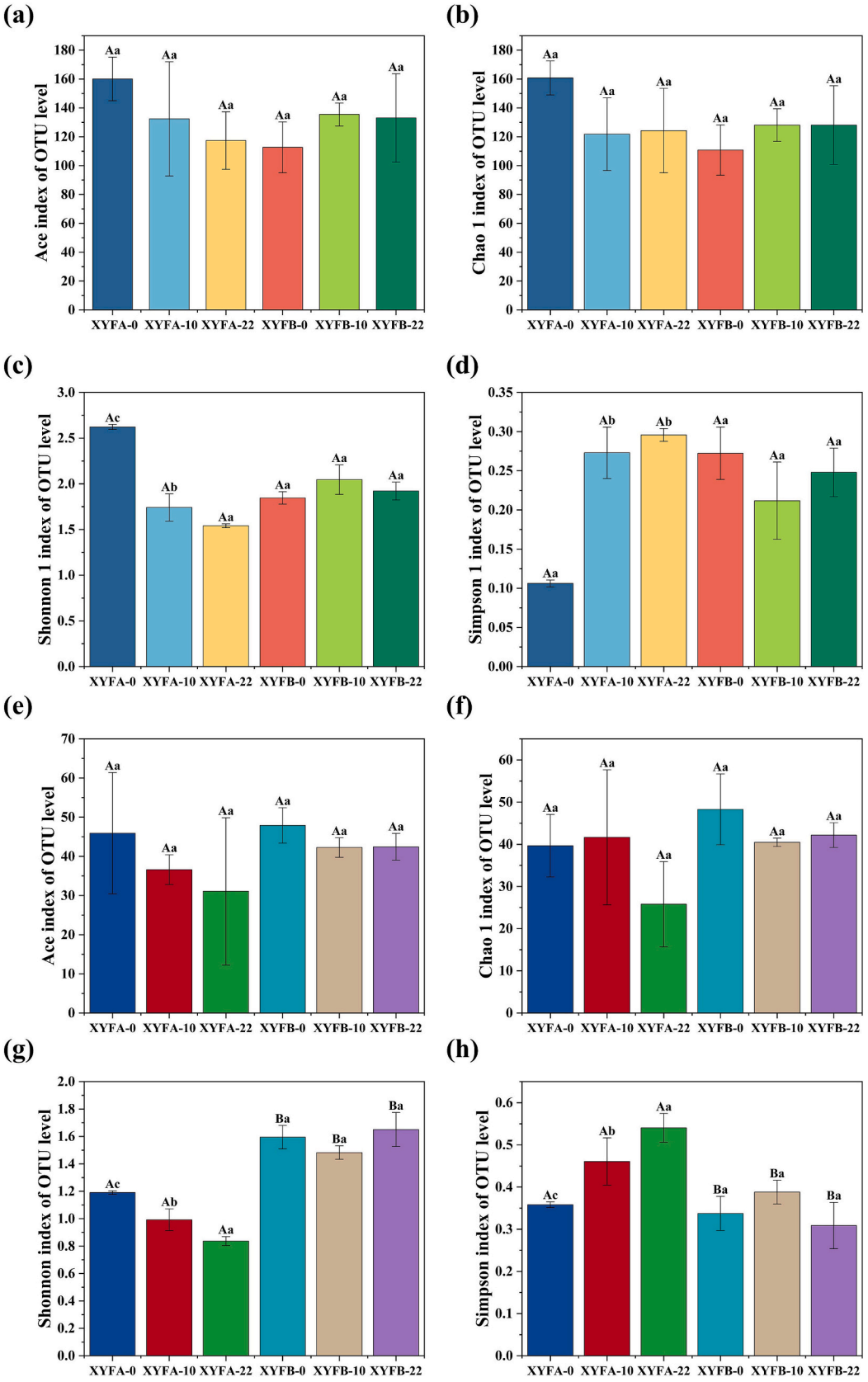

Fig. 1. Alpha diversity of microbial communities in Xiangyang fresh Huangjiu during storage. (a) Ace index for bacterial communities. (b) Chao1 index for bacterial communities. (c) Shannon index for bacterial communities. (d) Simpson index for bacterial communities. (e) Ace index for fungal communities. (f) Chao1 index for fungal communities. (g) Shannon index for fungal communities. (h) Simpson index for fungal communities.

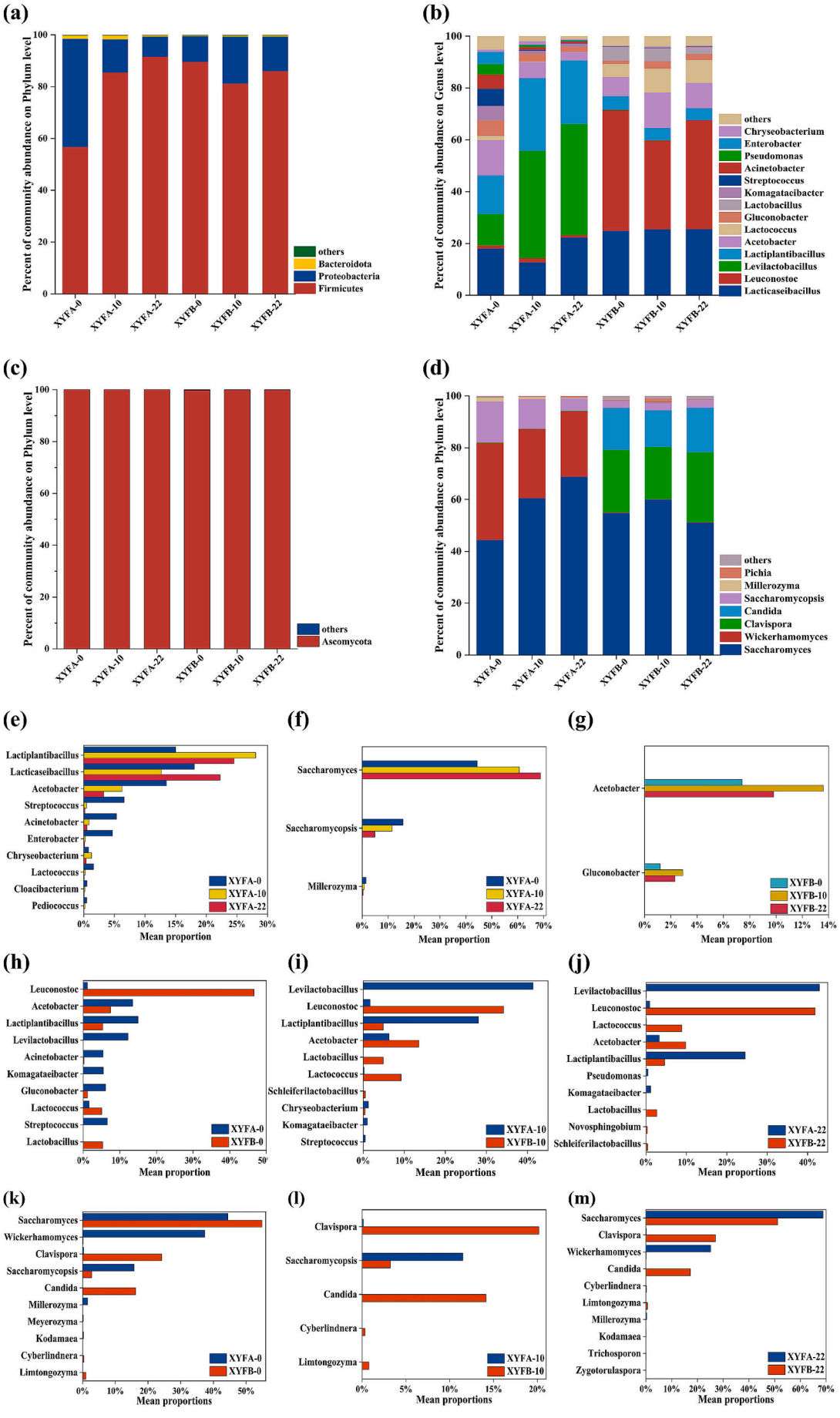

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Fig. 2. Changes in the microbial community of Xiangyang fresh Huangjiu during storage. (a) Composition of bacterial phyla. (b) Composition of bacterial genera. (c) Composition of fungal phyla. (d) Composition of fungal genera. (e) Bacterial genera with significant differences during the storage of XYFA Huangjiu. (f) Fungal genera with significant differences during the storage of XYFA Huangjiu. (g) Bacterial genera with significant differences during the storage of XYFB Huangjiu. (h) Bacterial genera with significant differences in XYFA and XYFB Huangjiu. (i) Fungal genera with significant differences in XYFA and XYFB Huangjiu. (j) Bacterial genera with significant differences in XYFA-22 and XYFB-22 Huangjiu. (k) Fungal genera with significant differences in XYFA-0 and XYFB-0 Huangjiu. (l) Fungal genera with significant differences in XYFA-10 and XYFB-10 Huangjiu. (m) Fungal genera with significant differences in XYFA-22 and XYFB-22 Huangjiu.

Meanwhile, *Leuconostoc* was the most abundant genus throughout the storage of XYFB Huangjiu, with its proportion ranging from 46.64 % to 41.91 %. The fungal community composition of the two types of Huangjiu at the phylum and genus levels are presented in Fig. 2 (c) and (d), respectively. *Ascomycota* was the only dominant fungal phylum in the two types of Huangjiu, and there were 7 dominant fungal genera in both XYFA and XYFB Huangjiu, including *Saccharomyces*, *Wickerhamomyces*, *Clavispora*, *Candida*, *Saccharomycopsis*, *Millerozyma*, and *Pichia*, and the result was similar with the previous research (Lei, Cai, Wang, Guo, & Shan, 2024). *Saccharomyces* was emerged as the most prevalent dominant fungal genus in the two types of Huangjiu, which played a pivotal role in enhancing the taste and flavor profile of Huangjiu.

To further investigate the differences in microbial community composition between two types of Huangjiu and their storage durations, β-diversity analysis was further performed using Welch's test and Kruskal-Wallis test, respectively. Fig. 2 show the microbial genera that exhibit significant differences during the storage of the two types of Huangjiu. During the storage of XYFA Huangjiu, there was a considerable shift in bacterial genera, with 10 genera showing significant differences ($P < 0.05$), including 7 dominant bacterial genera of *Lactiplantibacillus*, *Lacticaseibacillus*, *Acetobacter*, *Streptococcus*, *Enterobacter*, *Chryseobacterium* and *Lactococcus* and *Acinetobacter*, *Cloacibacterium* and *Pediococcus*. Among them, the abundance of the *Lactiplantibacillus* and *Chryseobacterium* reached its maximum on the 10th day of storage, while the abundance of the *Lacticaseibacillus* was at its minimum on the same day. Additionally, the abundance of other bacterial genera significantly decreased with the increse of storage time. Three dominant fungal genera had significant differences ($P < 0.05$) during the storage, including *Saccharomyces*, *Saccharomycopsis* and *Millerozyma*, and the abundance of the most prevalent dominant fungal genus of *Saccharomyces* significantly increased, while the abundance of *Saccharomycopsis* and *Millerozyma* significantly decreased. During the storage of XYFB Huangjiu, only two bacterial genera of *Acetobacter* and *Gluconobacter* were found to have significant differences ($P < 0.05$), and their abundance reached its maximum on the 10th day of storage. Moreover, no significant differences in fungal genera were detected during the storage. In summary, the microbial changes during the storage of the two types of Huangjiu were distinct, and the microbial community in XYFB Huangjiu exhibited relative stability. The microbial genera that exhibited significant differences between the two types of Huangjiu during storage were further investigated, as shown in Fig. 2(h-m). There were 10 dominant bacterial genera with significant differences in the two typs of Huangjiu ($P < 0.05$), including *Leuconostoc*, *Acetobacter*, *Lactiplantibacillus*, *Levilactobacillus*, *Gluconobacter*, *Lactococcus*, *Streptococcus*, *Acinetobacter*, *Komagataeibacter*, and *Lactobacillus*, which have undergone alterations after storage. After 10 days of storage, no significant differences were observed in the *Gluconobacter* and *Acinetobacter*. Instead, significant differences were detected between *Chryseobacterium* and *Schleiferilactobacillus* ($P < 0.05$). After 22 days of storage, significant differences were noted in *Pseudomonas* and *Novosphingobium* ($P < 0.05$), rather than *Chryseobacterium* and *Streptococcus*. It's worth noted that the abundance of the primary *Leuconostoc* in XYFB Huangjiu was always much higher than that in XYFA Huangjiu, while *Lactiplantibacillus* was more abundant during the storage of XYFA Huangjiu. Concurrently, 10 fungal genera with significant differences were also found in the two types of Huangjiu without storage ($P < 0.05$), including 6 dominant fungal genera of *Saccharomyces*, *Wickerhamomyces*, *Clavispora*, *Saccharomycopsis*, *Candida*, *Millerozyma* and *Limtongozyma*, *Cyberlindnera*, *Kodamaea* and *Meyerozyma*. After 10 days of storage, significant differences were only observed in *Clavispora*, *Saccharomycopsis*, *Candida*, *Limtongozyma* and *Cyberlindnera*. After 22 days of storage, significant differences were observed again in *Saccharomyces*, *Wickerhamomyces*, *Millerozyma*, and *Kodamaea*, *Trichosporon*, *Zygotortulaspota* also exhibited statistically significant differences ($P < 0.05$). Moreover, the abundance of *Clavispora* and *Candida* were much higher in XYFB Huangjiu, in contrast to the greater prevalence of *Saccharomyces*, *Wickerhamomyces* and *Saccharomycopsis* observed in XYFA Huangjiu.

3.2. Electronic sensory characteristics of Xiangyang fresh Huangjiu during storage

The electronic nose analysis results of the two types of Huangjiu during storage are given in Fig. 3 (a). The electronic nose sensor response values for XYFA Huangjiu on the 10th and 22nd days of storage were similar, higher than that of freshly stored Huangjiu. The results indicated that odor characteristics of XYFA Huangjiu underwent a significant enhancement during the initial phase of storage, and then exhibited a tendency towards stabilization after the 10th day of storage. Among the 28 sensors of the electronic nose, the response intensity of sensors S4, S5, S6, S7,S8, S10, S11, S12, S13, S14, S16, S18, S21, S22, S24, S25, S26, and S27 which are sensitive to sulfide, hydrogen sulfide, nitride, ammonia, organic gases, benzones, aldehydes, aromatic compounds, short chain alkanes, allyl sulfide, ketones, alcohols, methane, combustible gases, propane, butane, amines, sulfur-smelling gases, aliphatic hydrocarbons, alicyclic hydrocarbons, halogenated hydrocarbons, alkanes, olefins, organic acid esters, terpenes, sterols, exhibited an upward trend with increasing storage time. Moreover, the response values of sensors S8, S11, and S14 remained relatively high throughout the storage process of XYFA Huangjiu, indicated that the responsive substances of these sensors, including alkanes, alcohols and esters, were the main odor characteristics of XYFA Huangjiu during storage.

In contrast to XYFA Huangjiu, the electronic nose sensor response values for XYFB Huangjiu on the 0th and 10th days of storage were similar, whereas it was notably higher on the 22nd day of storage, indicating a more intense aroma. Among the 28 sensors of the electronic nose, the response values of sensors S4, S5, S6, S14, S15, S19, S20, and S28 showed a regular increase with storage time. Among them, sensors S15, S19, S20 are sensitive to volatile organic compounds, abnormal odor, short-chain alkanes, methane, alcohols, methanol, respectively. Moreover, the response values of sensors S8, S11, and S14 remained relatively high throughout the storage process of XYFB Huangjiu, which was consistent with XYFA Huangjiu. However, the overall sensor response values of XYFB Huangjiu were greater than those of XYFA Huangjiu. Fig. 3 (b) shows the principal component analysis (PCA) results based on the electronic nose sensor response values of two types of Huangjiu during storage, and it's worth noted that the flavor profiles of the fresh XYFA and XYFB Huangjiu differed significantly, whereas the flavors of the two types of Huangjiu became relatively similar after storage.

Fig. 3 (c) shows the electronic tongue analysis results of the two types of Huangjiu during storage, and the electric potential (EP) values of each sensor were used to assess the intensity of the corresponding taste. During the storage of XYFA Huangjiu, there was a significant increase in the intensity of sourness, astringency, aftertaste-A and saltiness. The

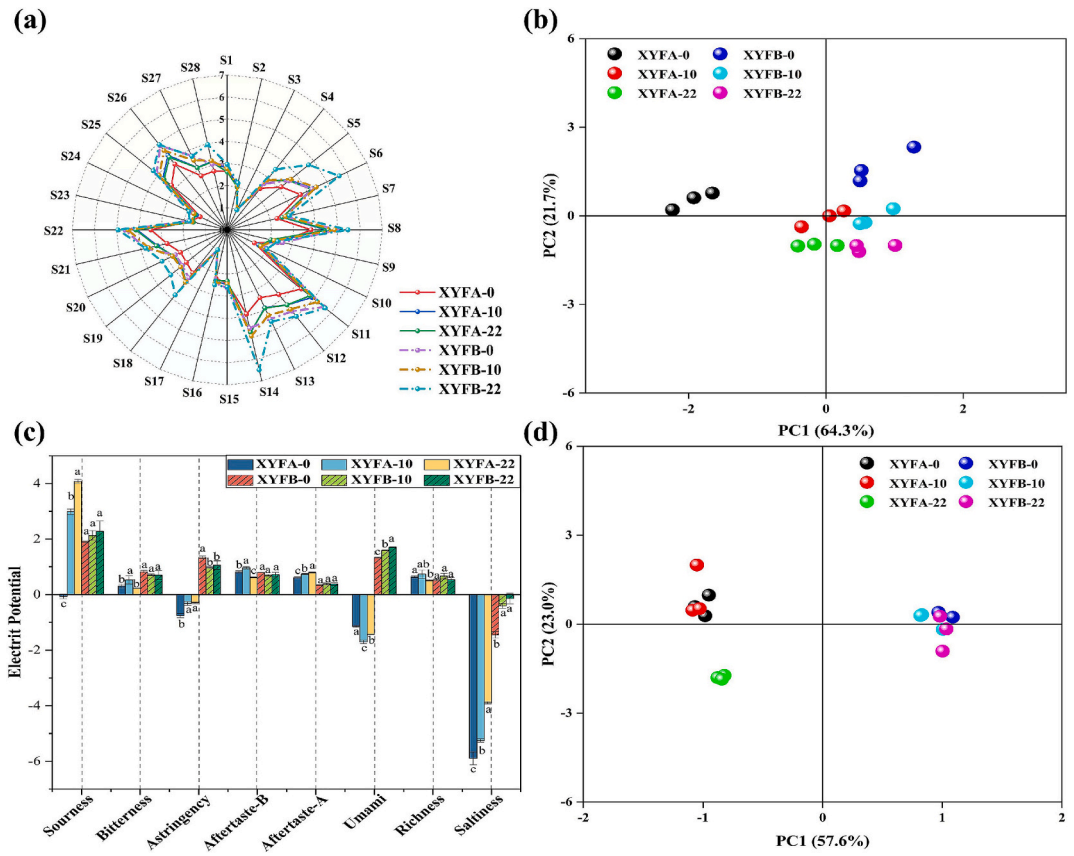

Fig. 3. Electronic sensory analysis of Xiangyang fresh Huangjiu during storage. (a) Radar graph for the *E*-nose analysis. (b) PCA biplot graph for the E-nose analysis. (c) The electric potential of each E-tongue sensor. (d) PCA biplot graph for the E-tongue analysis.

intensity of bitterness, aftertaste-B and richness exhibited a trend of initial increase followed by a decrease, peaking at the 10th day of storage, while the intensity of umami showed an opposite trend. Overall, aftertaste-B was most pronounced in the fresh XYFA Huangjiu, with sourness dominated in intensity after 10 days of storage. Additionally, astringency and umami flavors, as well as the intensity of saltiness, were relatively lower. During the storage of XYFB Huangjiu, the intensity of umami and saltiness significantly increased, while the intensity of astringency markly decreased. The intensity of sourness, bitterness, aftertaste-B, aftertaste-A and richness remained relatively stable. Throughout the storage period, sourness, umami and astringency were the predominant flavors. In general, XYFB Huangjiu exhibited a richer taste, especially the astringency and umami were significantly more pronounced than those in XYFA Huangjiu. Besides, sourness was emerging as a predominant taste in both types of Huangjiu during the storage period, which was in line with the results of previous research (Tang & Peng, 2024). Fig. 3 (d) gives the principal component analysis (PCA) results based on the EP values of electronic tongue sensor of two types of Huangjiu during storage. It's obvious that the two types of Huangjiu were distinctly differentiated. Besides, the taste of XYFA Huangjiu stored for 0 and 10 days were generally more similar, whereas the taste of XYFB Huangjiu remained relatively stable throughout the storage period.

3.3. *Volatile flavor compounds of Xiangyang fresh Huangjiu during storage*

To further analyze the aroma characteristics of XYFA and XYFB Huangjiu, GC-IMS detection was conducted, and 44 volatile flavor compounds and their relative concentrations were identified, which are presented in Table 1. Most signals were observed within a drift time range of 4 to 12 and a retention time interval of 100 to 1000 s. Relative concentrations of 44 volatile compounds were directly obtained by analyzing the peak volume of the compound using the LAV software accompanying the GC-IMS instrument, and the significance analysis of the relative concentrations of volatile compounds in XYFA and XYFB Huangjiu at different storage times was carried out. 44 volatile flavor compounds include 19 esters, 4 alcohols, 4 aldehydes, 5 ketones, 3 furans, 3 terpenes, 2 acids, 1 amine, 1 pyridine, 1 ether, and 1 monocyclic aromatic hydrocarbon, and the proportion of each category are presented in Fig. 4(a). Esters were the most abundant volatile flavor compounds in the two types of Huangjiu, followed by aldehydes, alcohols, ketones and terpenes. During storage, the content of esters and alcohols in XYFA Huangjiu gradually increased from 33.6 % and 7.8 % to 38.7 % and 13.5 %, respectively, while the content of aldehydes, ketones and terpenes progressively decreased from 31.4 %, 12.2 % and 8.8 % to 28.4 %, 6.4 % and 5.2 %, respectively. Conversely, the volatile compounds in XYFB Huangjiu remained relatively stable. To provide a more detailed and explicit visualization of the change of the volatile compounds during the storage of the two typs of Huangjiu, the difference comparison topographic plots were obtained using the topographic plot of fresh Huangjiu as a reference, as shown in Fig. 4 (b) and (c). In the topographic plot of fresh Huangjiu, dots on sides of the RIP peak (the reaction ion peak at 6.0 of drift time) represented volatile compounds, with deeper colors indicating higher concentrations. White and red colors denoted low and high concentrations, respectively. In the differential comparison topographic plot, volatile compound concentrations in stored Huangjiu that were similar to fresh Huangjiu were marked in white. Concentrations higher or lower than those in fresh Huangjiu were indicated by red and blue, respectively, with darker shades signifying greater deviations in concentration. It's obvious that concentrations of a variety of compounds increased or decreased during storage. The smell

Table 1
Volatile compounds and their relative concentrations in Xiangyang fresh Huangjiu during storage indentified by GC-IMS.

No	Label	CAS#	MW	Retention index	Retention Time/s	Drift time [RIPrel]	Relative content in XYFA-0 (%)	Relative content in XYFA-10 (%)	Relative content in XYFA-22 (%)	Relative content in XYFB-0 (%)	Relative content in XYFB-10 (%)	Relative content in XYFB-22 (%)
1	1-Octen-3-one	C4312996	126.2	980.1	230.258	1.28236	489.26 ± 6.75^{c}	668.24 ± 19.33^{b}	755.74 ± 6.78^{a}	781.31 ± 14.22^{a}	684.56 ± 22.04^{b}	693.34 ± 8.24^{b}
2	Alpha-Pinene	C80568	136.2	1041.2	274.575	1.27339	1954.5 ± 3.49^{a}	1915.63 ± 7.65^{b}	1739.4 ± 13.78^{c}	1885.49 ± 17.93^{a}	1899.79 ± 38.15^{a}	1884.92 ± 6.96^{a}
3	2-Methyl butanal	C96173	86.1	962.3	219.711	1.39901	107–5.87 ± 6.47^{a}	855.87 ± 17.72^{b}	841.15 ± 6.85^{b}	754.23 ± 16.31^{c}	784.44 ± 14.37^{b}	832.12 ± 11.33^{a}
4	2-Pentylfuran	C3777693	138.2	1224.4	494.132	1.25119	2021.95 ± 50.71^{a}	1507.85 ± 53.99^{b}	1228.12 ± 13.89^{c}	983.83 ± 25.05^{a}	1024.72 ± 35.55^{a}	989.95 ± 16.71^{a}
5	Dimethyl trisulfide	C3658808	126.3	1357.1	713.417	1.29874	295.98 ± 11.75^{c}	703.64 ± 5.51^{b}	1072.01 ± 29.38^{a}	1748.56 ± 46.07^{a}	1855.07 ± 95.71^{a}	1779.12 ± 14.95^{a}
6	(E)-2-hexen-1-al	C6728263	98.1	1213.8	477.203	1.54301	18,157.44 ± 168.09^{b}	18,528.08 ± 30.38^{a}	18,157.75 ± 82.51^{b}	19,603.93 ± 288.22^{a}	19,283.92 ± 119.64^{ab}	19,167.12 ± 152.75^{b}
7	2-Octanone	C111137	128.2	1294.8	622.912	1.32598	6018.69 ± 207.04^{a}	2677.13 ± 64.99^{b}	1244.57 ± 45.06^{c}	1699.04 ± 55.13^{b}	1888.43 ± 38.21^{a}	1784.35 ± 34.54^{b}
8	3-Methyl butyl acetate	C123922	130.2	1128	359.101	1.74289	2496.26 ± 125.75^{a}	2080.21 ± 37.04^{b}	1798.21 ± 19.76^{c}	1413.92 ± 34.95^{a}	1376.55 ± 56.54^{ab}	1299.28 ± 10.52^{b}
9	Ethyl butanoate	C105544	116.2	1041.4	274.692	1.55907	1698.33 ± 39.28^{c}	2082.37 ± 13.84^{b}	3335.09 ± 30.1^{a}	2817.17 ± 51.06^{ab}	2787.07 ± 137.69^{b}	2974.73 ± 50.84^{a}
10	Ethyl 3-methylbutanoate	C108645	130.2	1056.9	287.626	1.26741	521.64 ± 7.8^{a}	456.16 ± 4.01^{b}	430.76 ± 32.37^{b}	809.83 ± 52.34^{a}	760.49 ± 67.61^{a}	751.6 ± 50.97^{a}
11	Ethyl octanoate	C106321	172.3	1435.1	845.204	1.48359	267.33 ± 8.24^{b}	911.5 ± 33.04^{a}	994.15 ± 72.46^{a}	293.36 ± 18.65^{b}	337.39 ± 12.89^{a}	351 ± 15.68^{a}
12	Ethyl pentanoate	C539822	130.2	1149.3	385.387	1.27254	881.75 ± 27.16^{c}	1090.93 ± 14.84^{b}	1363.63 ± 7.08^{a}	1051.34 ± 43.65^{a}	927.01 ± 24.4^{b}	927.52 ± 19.51^{b}
13	n-pentanal	C110623	86.1	981.2	230.908	1.411	830.62 ± 17.88^{a}	582.1 ± 10.13^{b}	536.97 ± 4.17^{c}	564.51 ± 16.24^{a}	555.94 ± 14.13^{a}	562.55 ± 13.09^{a}
14	(Z)-3-Hexenyl butyrate	C16491364	170.3	1208	468.233	1.40405	2271.24 ± 13.13^{b}	2255.29 ± 35.23^{b}	2361.46 ± 16.46^{a}	2843.13 ± 38.95^{b}	3048.27 ± 53.65^{a}	3053.22 ± 6.67^{a}
15	2- Butanol	C78922	74.1	1013.5	252.864	1.33996	2601.93 ± 23.1^{a}	2411.49 ± 58.65^{b}	2627.23 ± 12.2^{a}	3451.88 ± 109.52^{ab}	3269.08 ± 156.48^{b}	3591.46 ± 68.84^{a}
16	3-Hydroxy-2-butanone。	C513860	88.1	1260.9	557.115	1.33114	850.31 ± 9.15^{b}	1156.67 ± 75.3^{a}	1202.77 ± 31.81^{a}	1733.72 ± 47.59^{b}	1716 ± 28.14^{b}	1855.17 ± 36.23^{a}
17	2-Ethylfuran	C3208160	96.1	970.8	224.682	1.31792	198.58 ± 6.58^{c}	247.59 ± 10.4^{b}	310.09 ± 1.27^{a}	462.92 ± 4.64^{a}	426.97 ± 9.33^{b}	475.71 ± 8.88^{a}
18	2-Hexanol	C626937	102.2	1239.8	519.805	1.57256	92.69 ± 3.95^{c}	181.68 ± 2.89^{b}	224.19 ± 17.57^{a}	237.27 ± 2.07^{c}	277.85 ± 15.69^{b}	314.7 ± 12.35^{a}
19	2-Hexanone	C591786	100.2	1073.4	302.047	1.49558	74.51 ± 5.03^{b}	70.16 ± 1.24^{b}	83.75 ± 5.93^{a}	336.03 ± 15.95^{b}	352.79 ± 55.14^{ab}	424 ± 36.05^{a}
20	2-Hydroxy-2-methyl-4-pentanone	C123422	116.2	1356.1	711.829	1.52835	280.56 ± 8.96^{c}	893.33 ± 12.31^{b}	1650.4 ± 89.8^{a}	4492.67 ± 181^{a}	4524.26 ± 112.29^{a}	4390.43 ± 115.55^{a}
21	2-Methyl butanoic acid ethyl ester	C7452791	130.2	1072.2	300.995	1.64844	31.4 ± 3.13^{a}	30.71 ± 1.1^{a}	39.99 ± 7.08^{a}	185.96 ± 17.32^{b}	216.21 ± 43.29^{ab}	269.71 ± 28.84^{a}
22	2-Methyl propanal	C78842	72.1	833	156.321	1.27705	2345.55 ± 73.86^{a}	1867.67 ± 10.66^{b}	497.12 ± 21.51^{c}	825.42 ± 124.49^{b}	1039 ± 14.4^{a}	816.49 ± 33.8^{b}
23	2-Methylheptanoic acid	C1188029	144.2	1135.1	367.666	1.39886	712.07 ± 24.78^{c}	1151.73 ± 55.91^{b}	1381.11 ± 23.15^{a}	1403.24±15.76^{a}	1062.68±35.86^{b}	1080.1±33.21^{b}
24	3-Ethylpyridine	C536787	107.2	965.6	221.639	1.50604	733.59 ± 19.52^{a}	670.67 ± 9.49^{b}	720.7 ± 7.95^{a}	1368.78±14.27^{b}	1374.57±47.57^{b}	1449.63±15.32^{a}
25	3-Methyl-2-pentanone	C565617	100.2	1019.5	257.391	1.47553	1263.67 ± 25.06^{a}	1152.79 ± 27.76^{b}	1234.39 ± 3.93^{a}	1734.81±41.47^{b}	1740.68±62.34^{b}	1901.22±41.37^{a}
26	3-Methylbutyric acid	C503742	102.1	824.4	152.84	1.48142	75.33 ± 5.92^{a}	71.85 ± 2.46^{a}	78.35 ± 14.94^{a}	105.92±24.82^{a}	258.08±210.36^{a}	390.64±195.9^{a}

(*continued on next page*)

Table 1 (*continued*)

No	Label	CAS#	MW	Retention index	Retention Time/s	Drift time [RIPrel]	Relative content in XYFA-0 (%)	Relative content in XYFA-10 (%)	Relative content in XYFA-22 (%)	Relative content in XYFB-0 (%)	Relative content in XYFB-10 (%)	Relative content in XYFB-22 (%)
27	Beta-Elemene	C515139	204.4	1358.1	715.005	1.42716	111.11 ± 1.05^{c}	198.12 ± 9.16^{b}	270 ± 7.67^{a}	564.39±29.37^{c}	680.08±9.25^{a}	640.11±12.1^{b}
28	Butyl 2-methylbutanoate	C15706737	158.2	1041.5	274.809	1.38114	563.65 ± 5.64^{c}	657.77 ± 6.25^{b}	770.33 ± 4.67^{a}	801.26±4.58^{a}	687.54±7.02^{b}	749.03±2.75^{c}
29	Butyl butanoate	C109217	144.2	1223.1	491.999	1.33026	1381.12 ± 41.25^{c}	2053.08 ± 62.96^{b}	2351.68 ± 23.83^{a}	2948.59±48.35^{a}	2639.06±46.89^{b}	2747.27±52.83^{c}
30	Butyl pentanoate	C591684	158.2	1096.7	323.659	1.39064	8001.55 ± 86.05^{a}	7638.03 ± 80.48^{b}	7043.99 ± 108.99^{c}	8655.8±185.38^{a}	8083.74±177.51^{b}	8249.14±130.97^{b}
31	Butyl propionate	C590012	130.2	909.9	191.389	1.28428	490.09 ± 15.3^{a}	387.35 ± 6.07^{b}	239.01 ± 6.01^{c}	423.37±7.41^{a}	405.09±8.99^{b}	396.03±0.64^{b}
32	Ethyl 2-methylpropanoate	C97621	116.2	969.8	224.078	1.56172	478.05 ± 6.25^{a}	400.64 ± 6.47^{b}	411.58 ± 8.23^{b}	2250.34±14.1^{ab}	2213.53±106.51^{b}	2346.11±18.46^{a}
33	Ethyl hexanoate	C123660	144.2	1239.1	518.679	1.7892	196.89 ± 14.91^{c}	780.88 ± 10.41^{b}	967.05 ± 139.1^{a}	431.79±22.24^{b}	500.83±50.65^{b}	576.35±33.58^{a}
34	Ethyl propanoate	C105373	102.1	962.3	219.687	1.45132	2083.09 ± 28.55^{c}	2406.29 ± 8.02^{b}	2754.01 ± 21.63^{a}	2597.1±55.54^{a}	2603.41±50.65^{a}	2665.45±29.78^{a}
35	Hexyl acetate	C142927	144.2	1240.2	520.481	1.40646	143.84 ± 6.35^{c}	278.43 ± 2.69^{b}	329.31 ± 10.07^{a}	294.91±2.14^{b}	308.14±12.23^{b}	364.6±10.61^{a}
36	Linalool oxide	C60047178	170.3	1064.7	294.338	1.80613	2596.44 ± 55.76^{b}	2588.63 ± 440.13^{b}	5068.16 ± 1531.89^{a}	3536.48 ±1215.71^{a}	3389.24 ±1761.62^{a}	2794.63 ±1250.62^{a}
37	Maltol	C118718	126.1	1125.2	355.852	1.53647	933.79 ± 26.22^{b}	930.76 ± 13.99^{b}	989.08 ± 20.62^{a}	1443.55±15.35^{a}	1395.08±20.22^{a}	1426.17±37.15^{a}
38	Methyl acetate	C79209	74.1	842.3	160.198	1.19753	1647.32 ± 17.06^{a}	1643.54 ± 10.47^{a}	1338.02 ± 19.66^{b}	2272.45±51.98^{a}	2257.12±70.43^{a}	2127.6±34.9^{b}
39	Phenylacetic acid butyl ester	C122430	192.3	1434.6	844.41	2.02258	58.61 ± 6.32^{c}	113.65 ± 2.67^{b}	165.47 ± 20.66^{a}	54.86±3.84^{a}	62.72±13.77^{a}	59.63±0.61^{a}
40	Propyl acetate	C109604	102.1	979.9	230.095	1.47916	798.14 ± 16.63^{a}	587.28 ± 13.97^{b}	563.02 ± 5.68^{b}	593.99±13.94^{a}	563.45±28.76^{a}	602.97±9.86^{a}
41	Styrene	C100425	104.2	1240.1	520.256	1.43775	38.98 ± 2.42^{c}	124.62 ± 12.47^{b}	182.73 ± 14.71^{a}	130.1±8.36^{a}	101.16±6.17^{b}	127.01±3.78^{a}
42	Terpinolene	C586629	136.2	1297.1	626.088	1.21118	4246.43 ± 88.33^{a}	3871.61 ± 40.56^{b}	1677.96 ± 9.8^{c}	2069.02 ±149.59^{a}	1975.63±145.56^{a}	1974.93±64.09^{a}
43	THF	C109999	72.1	828.7	154.604	1.22971	348.85 ± 102.82^{b}	529.69 ± 8.2^{a}	429.22 ± 11.96^{ab}	356.78±92.31^{a}	354.5±48.31^{a}	293.3±33.81^{a}
44	Triethylamine	C121448	101.2	796.6	142.06	1.46413	36.81 ± 1.15^{ab}	32.13 ± 4.15^{b}	39.21 ± 3.18^{a}	95.07±23.6^{a}	142.18±88^{a}	223.93±59.3^{a}

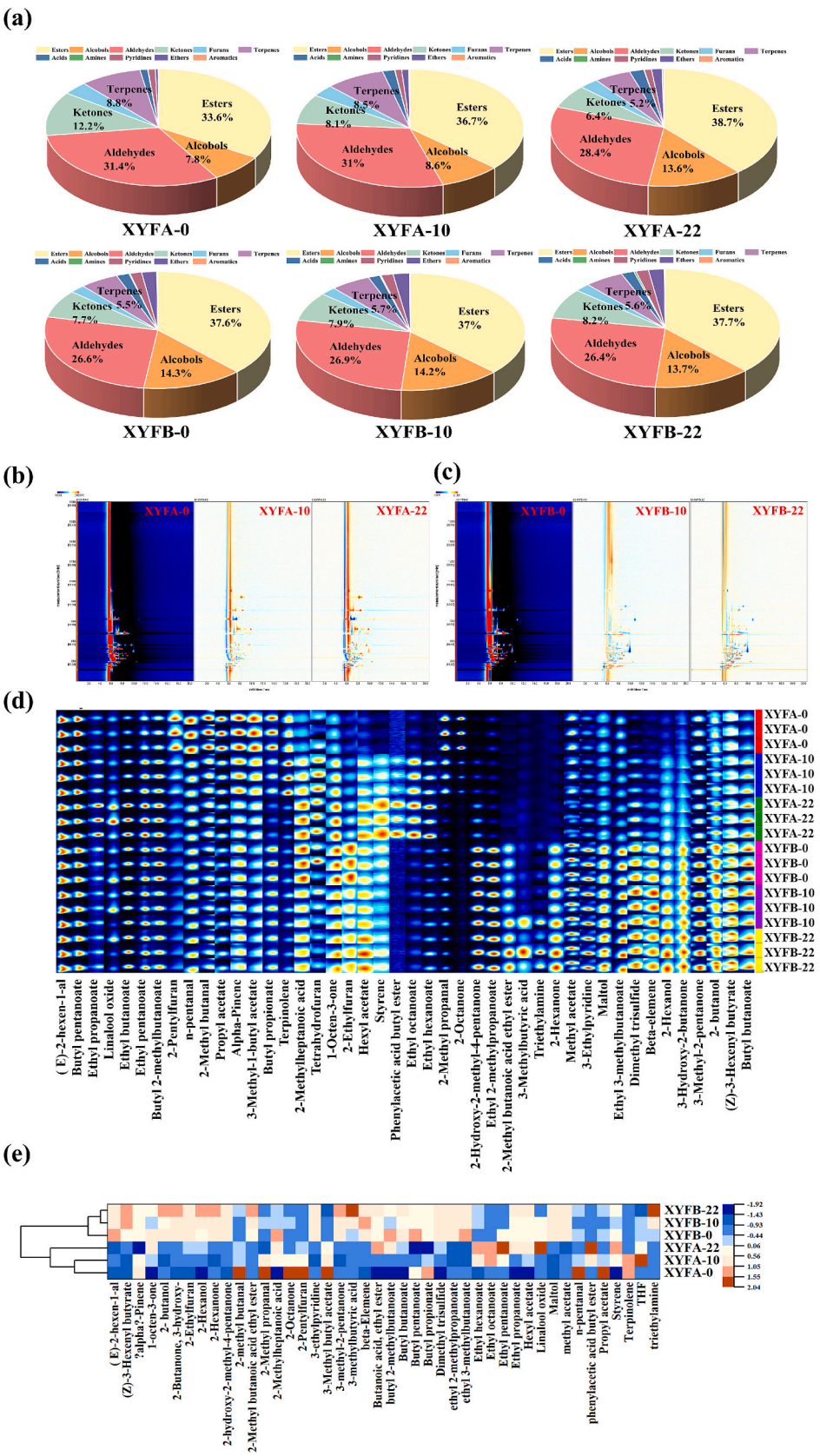

Fig. 4. Analysis of volatile compounds of Xiangyang fresh Huangjiu during storage by GC-IMS. (a) Types and Proportions of volatile compounds. (b) The difference comparison topographic plots of volatile compounds during the storage of XYFA Huangjiu. (c) The difference comparison topographic plots of volatile compounds during the storage of XYFB Huangjiu. (d) Fingerprint of volatile compounds. (e) Volatile compound concentrations and clustering results.

fingerprint was further constructed based on the 44 ion peaks corresponding to volatile compounds, as shown in Fig. 4 (d). The shading and size of the dots correspond to the content of volatile compounds, with darker shades and larger areas signifying a higher concentration. For XYFA Huangjiu, there were predominantly seven volatile substances with relatively high concentrations in fresh Huangjiu, which gradually decreased during storage, including 2-pentylfuran, n-pentanal, 2-methyl butanal, propyl acetate, alpha-Pinene, 3-methyl butyl acetate and butyl propionate. Conversely, there were eleven volatile substances that were initially present in relatively low concentrations in fresh Huangjiu, but increased to relatively high concentrations after storage, including 2-methylheptanoic acid, tetrahydrofuran, 1-octen-3-one, hexyl acetate, styrene, phenylacetic acid butyl ester, ethyl octanoate, ethyl hexanoate, butyl butanoate, ethyl pentanoate and dimethyl trisulfide. For XYFB Huangjiu, the volatile substances exhibited relative stability during storage. There were four volatile compounds that showed a gradual decrease in the concentration, including 2-Methylheptanoic acid, tetrahydrofuran, 1-octen-3-one, 2-Ethylfuran, which were increased in the storage of XYFA Huangjiu. Besides , the concentrations of another five volatile compounds increased during storage, including hexyl acetate, 2-methyl butanoic acid ethyl ester, 3-methylbutyric acid, triethylamine, and 2-hexanone. The clustering heatmap based on the normalized relative content of the volatile compounds were further constructed in Fig. 4 (e) to further analyze the difference in the volatile

Table 2
ROAV values of volatile compounds in Xiangyang Fresh Huangjiu.

No.	Compound	Aroma threshold value (μg/L)	ROAV					
			XYFA-0	XYFA-10	XYFA-22	XYFB-0	XYFB-10	XYFB-22
1	1-octen-3-one	0.03[a]	1.00E+02	1.00E+02	1.00E+02	1.00E+02	1.00E+02	1.00E+02
2	Alpha-Pinene	6[b]	2.00E+00	1.43E+00	1.15E+00	1.21E+00	1.39E+00	1.36E+00
3	2-Methyl butanal	0.35[c]	1.88E+01	1.10E+01	9.54E+00	8.27E+00	9.82E+00	1.03E+01
4	2-Pentylfuran	4.8[d]	2.58E+00	1.41E+00	1.02E+00	7.87E-01	9.36E-01	8.92E-01
5	Dimethyl trisulfide	0.4[e]	4.54E+00	7.90E+00	1.06E+01	1.68E+01	2.03E+01	1.92E+01
6	(E)-2-hexen-1-al	600[d]	1.86E-01	1.39E-01	1.20E-01	1.25E-01	1.41E-01	1.38E-01
7	2-Octanone	50[d]	7.38E-01	2.40E-01	9.88E-02	1.30E-01	1.66E-01	1.54E-01
8	3-Methyl butyl acetate	30[f]	5.10E-01	3.11E-01	2.38E-01	1.81E-01	2.01E-01	1.87E-01
9	Ethyl butanoate	82[e]	1.27E-01	1.14E-01	1.61E-01	1.32E-01	1.49E-01	1.57E-01
10	Ethyl 3-methylbutanoate	6.9[g]	4.64E-01	2.97E-01	2.48E-01	4.51E-01	4.83E-01	4.71E-01
11	Ethyl octanoate	13[e]	1.26E-01	3.15E-01	3.04E-01	8.66E-02	1.14E-01	1.17E-01
12	Ethyl pentanoate	27[e]	2.00E-01	1.81E-01	2.00E-01	1.50E-01	1.50E-01	1.49E-01
13	n-pentanal	12[c]	4.24E-01	2.18E-01	1.78E-01	1.81E-01	2.03E-01	2.03E-01
14	(Z)-3-Hexenyl butyrate	–	–	–	–	–	–	–
15	2- Butanol	3300[d]	4.83E-03	3.28E-03	3.16E-03	4.02E-03	4.34E-03	4.71E-03
16	3-Hydroxy-2-butanone	259[d]	2.01E-02	2.00E-02	1.84E-02	2.57E-02	2.90E-02	3.10E-02
17	2-Ethylfuran	–	–	–	–	–	–	–
18	2-Hexanol	6700[d]	8.48E-05	1.22E-04	1.33E-04	1.36E-04	1.82E-04	2.03E-04
19	2-Hexanone	930[d]	4.91E-04	3.39E-04	3.57E-04	1.39E-03	1.66E-03	1.97E-03
20	2-Hydroxy-2-methyl-4-pentanone	500[d]	3.44E-03	8.02E-03	1.31E-02	3.45E-02	3.97E-02	3.80E-02
21	2-Methyl butanoic acid Ethyl ester	18[h]	1.07E-02	7.66E-03	8.82E-03	3.97E-02	5.26E-02	6.48E-02
22	2-Methyl propanal	1300[i]	1.11E-02	6.45E-03	1.52E-03	2.44E-03	3.50E-03	2.72E-03
23	2-Methylheptanoic acid	–	–	–	–	–	–	–
24	3-Ethylpyridine	–	–	–	–	–	–	–
25	3-Methyl-2-pentanone	–	–	–	–	–	–	–
26	3-Methylbutyric acid	1050[j]	4.40E-04	3.07E-04	2.96E-04	3.87E-04	1.08E-03	1.61E-03
27	Beta-Elemene	–	–	–	–	–	–	–
28	Butyl 2-methylbutanoate	–	–	–	–	–	–	–
29	Butyl butanoate	14066[k]	6.02E-04	6.55E-04	6.64E-04	8.05E-04	8.22E-04	8.45E-04
30	Butyl pentanoate	–	–	–	–	–	–	–
31	Butyl propionate	–	–	–	–	–	–	–
32	Ethyl 2-methylpropanoate	58[g]	5.05E-02	3.10E-02	2.82E-02	1.49E-01	1.67E-01	1.75E-01
33	Ethyl hexanoate	55[h]	2.20E-02	6.37E-02	6.98E-02	3.01E-02	3.99E-02	4.53E-02
34	Ethyl propanoate	19019[g]	6.72E-04	5.68E-04	5.75E-04	5.24E-04	6.00E-04	6.06E-04
35	Hexyl acetate	5560[i]	1.59E-04	2.25E-04	2.35E-04	2.04E-04	2.43E-04	2.84E-04
36	Linalool oxide	3000[l]	5.31E-03	3.87E-03	6.71E-03	4.53E-03	4.95E-03	4.03E-03
37	Maltol	60000[d]	9.54E-05	6.96E-05	6.54E-05	9.24E-05	1.02E-04	1.03E-04
38	Methyl acetate	–	–	–	–	–	–	–
39	Phenylacetic acid butyl ester	–	–	–	–	–	–	–
40	Propyl acetate	–	–	–	–	–	–	–
41	Styrene	3100[d]	7.71E-05	1.80E-04	2.34E-04	1.61E-04	1.43E-04	1.77E-04
42	Terpinolene	–	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
43	THF	–	–	–	–	–	–	–
44	Triethylamine	2000[d]	1.13E-04	7.21E-05	7.78E-05	1.83E-04	3.12E-04	4.84E-04

[a] Odor thresholds from reference (L. Wang et al., 2020).
[b] Odor thresholds from reference (Pino & Mesa, 2006).
[c] Odor thresholds from reference (Yu et al., 2019).
[d] Odor thresholds from reference Van (Gemert 2011).
[e] Odor thresholds from reference (H. Li et al., 2019).
[f] Odor thresholds from reference (Liu & Sun, 2018).
[g] Odor thresholds from reference (W. Gao et al., 2014).
[h] Odor thresholds from reference (Fan et al., 2015).
[i] Odor thresholds from reference (X. Wang et al., 2014).
[j] Odor thresholds from reference (X. Sun et al., 2022).
[k] Odor thresholds from reference (J. Sun et al., 2018).
[l] Odor thresholds from reference (Zhao et al., 2017).

compounds between the two types of Huangjiu during storage. The clustering analysis was conducted based on the Euclidean distance metric. It's obvious that the volatile compounds of XYFA Huangjiu after 0 and 10 days of storage were relatively similar, whereas XYFB Huangjiu stored for 10 and 22 days exhibited more closely aligned flavor characteristics, distinct from the fresh one. Besides, the concentrations of volatile compounds in XYFA Huangjiu is higher than that observed in XYFB Huangjiu during storage. The above results were consistent with those obtained from the electronic nose analysis. Moreover, the results provided a more explicit illustration of the flavor evolution during the storage of XYFA and XYFB Huangjiu, which was consistent with the results in Fig. 4 (d).

In order to ascertain the typical aroma components of the two types of Huangjiu, ROAVs of the 44 volatile compounds were calculated, and the results are presented in Table 2. Five volatile flavor compounds with ROAV >1 were identified in the two types of Huangjiu, including 1-octen-3-one with strong mushroom, earthy, and musty flavor (Zhang et al., 2024), 2-methyl butanal with a grassy and fruity aroma (Marrufo-Curtido, de-la-Fuente-Blanco, Saenz-Navajas, Ferreira, Bueno, & Escudero, 2021; L. Wang et al., 2023), dimethyl trisulfide with an onion-like note (Davis & Qian, 2019), 2-pentylfuran with a bean and fruity scent (Lu et al., 2017), and alpha-pinene with a refreshing pine aroma (X. Sun et al., 2021), which were considered as key aroma compounds. Among them, 1-octen-3-one had the highest ROAV of 100, making it the most prominent flavor compound. Besides, it exhibited an ROAV greater than 1 in both Shaoxing Huangjiu (S. Chen et al., 2019) and Jiujiang Fenggang Huangjiu (Zhang et al., 2024), indicating that 1-octen-3-one is commonly present in these alcoholic beverages and plays a significant role in the overall aroma profile. It might be due to its relatively low odor threshold, which is regarded as the lowest concentration of an aroma compound that can be perceived in a sample. A low threshold of a compound indicates that the compound is more easily detected and make a significant contribution to the sample's flavor under the condition of the same concentration compared to other compounds in the mixed system (Zhai et al., 2024; J. Zhang et al., 2025). Moreover, eight aroma compounds with 0.1 ≤ ROAV <1 were considered as modifying flavor compounds in the two types of Huangjiu, including (E)-2-hexen-1-al with green notes (http://www.flavornet.org), 2-octanone with fruity notes (Mao et al., 2023), 3-methyl butyl acetate with a fruity note (S. Chen et al., 2019), butanoic acid ethyl ester with a fruity note (Hao et al., 2024), ethyl 3-methylbutanoate with fruity and sweet notes (Zhang et al., 2024), ethyl octanoate with a fruity note (Zhu et al., 2023), ethyl pentanoate with a fruity note (Munoz-Gonzalez et al., 2019), and n-pentanal with an almond-like note (Zhou et al., 2024), which contribute to the enhancement and supplementation of the flavor profiles, accentuating the sensory characteristics of specific aromatic components. As can be seen in Fig. 4(d) and (e), during the storage of XYFA Huangjiu, the concentrations of key aroma compounds of 1-octen-3-one and dimethyl trisulfide gradually increased, while the concentrations of the other three key aroma compounds of alpha-pinene, 2-methyl butanal and 2-pentylfuran gradually decreased. Concurrently, modifying flavor compounds of 2-octanone, 3-methyl butyl acetate, ethyl 3-methylbutanoate, and n-pentanal showed decreasing trends, along with a rise in concentrations of ethyl butanoate, ethyl octanoate, and ethyl pentanoate. Compared to XYFA Huangjiu, most of the key aroma compounds and modifying flavor compounds in XYFB Huangjiu remained relatively stable during storage. Except that the modifying flavor compound of ethyl octanoate also increased, key aroma compounds of 1-octen-3-one decreased, while 2-methyl butanal increased, and the modifying flavor compound of ethyl pentanoate decreased, exhibiting an opposite trend to the changes observed in XYFA Huangjiu. Although key aroma compounds and modifying flavor compounds were shared between the two types of Huangjiu, their evolutionary trends during storage were markedly distinct. It's also worth noted that key aroma compounds of dimethyl trisulfide and 2-methyl butanal and the modifying flavor compound of n-pentanal possess pronounced pungent characteristics, which might affect the overall flavor profile of Huangjiu. Thus, appropriate storage of XYFA Huangjiu will conducive to the reduction of its pungent odors, whereas XYFB Huangjiu should be optimally consumed when freshly produced.

3.4. Correlative analysis of microbial communities, sensory characteristics, and flavor compounds during the storage of Xiangyang fresh Huangjiu

A heat map of the Spearman's correlation coefficients for dominant microbial genera, sensory characteristics, and important flavor compounds in the two typs of Huangjiu during storage is provided in Fig. 5. As shown in the heat map, there was a very high correlation between sensory characteristics reflected by electronic nose sensors and 21 dominant microbial genera, and the majority of sensory characteristics exhibited a negative correlation with microbial genera. Especially short-chain alkanes, ketones, alcohols, etc. reflected by electronic nose sensors S7, S8, S10, S11, S12, S13, S16, S21, S22, S24, S25, S26 and S27 had a significant negative correlation with *Streptococcus*, *Enterobacter*, *Acinetobacter*, *Saccharomycopsis*, and *Millerozyma*, the abundance of which in XYFA gradually decreased, leading to the increase of the sensor response values indicated by the analyses in sections 3.1 and 3.2. Besides, the abundance of these microbial genera in XYFB Huangjiu exhibited relative stability, resulting in minimal changes in the sensor response values. Concurrently, a positive correlation was observed between the majority of the same sensory characteristics and microbial genera of *Leuconostoc*, *Lacticaseibacillus*, *Lactococcus*, *Lactobacillus*, *Clavispora*, *Candida* and *Pichia*. Especially some volatile organic compounds, abnormal odor and sterols reflected by sensors S15 and S27 exhibited a highly significant positive correlation with *Leuconostoc* , and a significant positive correlation was observed between short-chain alkanes reflected by sensor S7 and *Lacticaseibacillus*. In summary, the combined action of microbial genera determined the sensory characteristics reflected by specific electronic nose sensors. Given that the same substance may be detected by different sensors, further investigation into the role of microbial genera in flavor formation of Xiangyang fresh Huangjiu was conducted using the conducted using volatile flavor compounds obtained by GC-IMS.

For sensory characteristics reflected by electronic tongue sensors, the sourness demonstrated a relatively high positive correlation with *Saccharomyces*, which constituted the predominant fungal genera in the two types of Huangjiu, significantly contributing to the pronounced sourness in in the two types of Huangjiu. The bitterness was significantly negatively correlated with *Levilactobacillus*, while it was significantly positively correlated with *Lacticaseibacillus* and *Millerozyma*. Astringency showed a significant negative correlation with *Acetobacter*, and aftertaste-A was significantly negatively associated with *Leuconostoc* and *Clavispor*, but positively correlated with *Levilactobacillus*. Umami exhibited a highly significant negative correlation with *Lactiplantibacillus* and a relatively high positive correlation with *Lacticaseibacillus*, *Acetobacter*, *Lactococcus*, *Clavispora*, and *Candida*. The results further confirmed the opposing effects of microorganisms on the umami, astringency, and bitterness of Huangjiu, further validating that umami can offset the bitterness and astringency in Huangjiu, thereby contributing to a more balanced and harmonious taste. Thus, *Leuconostoc*, *Lacticaseibacillus*, *Lactiplantibacillus*, *Levilactobacillus*, *Acetobacter, Saccharomyces, Clavispora, Saccharomycopsis* played a pivotal role in shaping the taste of the two types of Huangjiu.

For volatile flavor compounds, 1-octen-3-one with strong mushroom, earthy and musty flavors, the most significant contributors to the flavor profile of the two types of Huangjiu, was found to be significantly negatively correlated with *Gluconobacterium*, which was ubiquitously distributed in floral tissues, garden soils, fruit matrices, bee microbiota and wine. While the flavor-modifying compound of 2-Octanone showed a significant positive correlation with *Gluconobacterium*. Besides, the key flavor compounds of 2-methyl butanal and 2-Pentylfuran had a highly

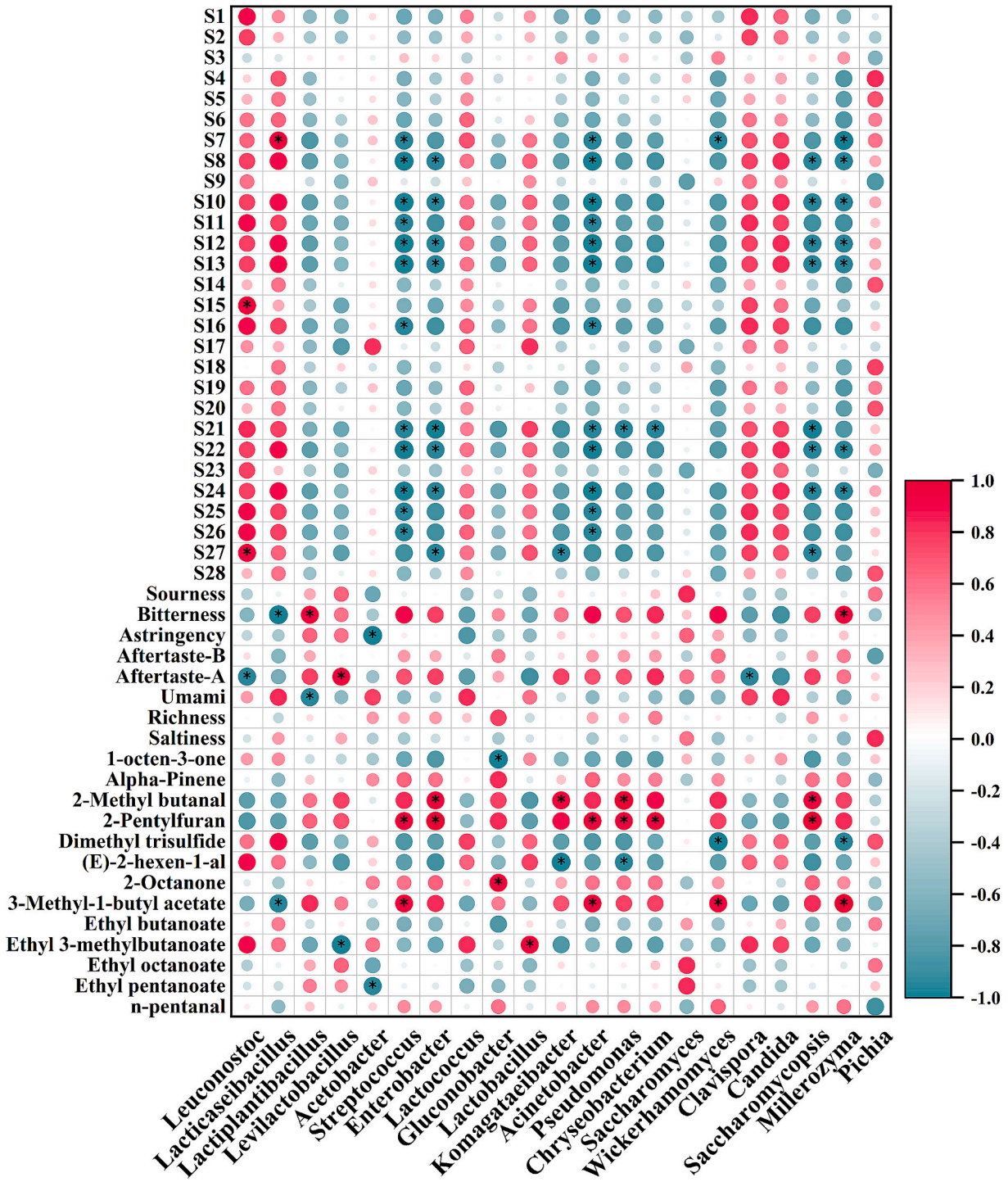

Fig. 5. A heat map of the Spearman's correlation coefficients for dominant microbial communities, sensory characteristics, and critical flavor compounds in Xiangyang fresh Huangjiu. (*) represented the abundance of microbial communities were significantly correlated with sensory characteristics and flavor compounds at $P < 0.001$. The color bar represented R-value of Spearman's correlation.

positive correlation with *Streptococcus*, *Enterobacter*, *Komagataeibacter*, *Acinetobacter*, *Pseudomonas*, *Chryseobacterium*, *Saccharomycopsis*. The key flavor compounds of Dimethyl trisulfide exhibited a highly significant negative correlation with *Wickerhamomyces* and *Millerozyma*. The flavor-modifying compound of (E)-2-hexen-1-al were significantly negatively correlated with *Komagataeibacter* and *Pseudomonas*. Besides, Ester substances including 3-Methyl butyl acetate, Ethyl 3-methylbutanoate and ethyl pentanoate, played a crucial role in the development of the distinctive flavor profile of Xiangyang Huangjiu, were highly correlated with microbial genera. Among them, 3-Methyl butyl acetate exhibited a significantly positive correlation with *Streptococcus, Acinetobacter, Wickerhamomyces* and *Millerozyma,* and was significantly negatively correlated with *Lacticaseibacillus*. Ethyl 3-methylbutanoate was observed to have a highly significant positive association with *Lactobacillus* and a highly significant negative association with *Levilactobacillus*, and ethyl pentanoate displayed a highly significant negative correlation with *Achromobacter*. Consequently, different microbial genera exerted direct influences on the specific flavor profiles in

Xiangyang Fresh Huangjiu, with the collective action of these microorganisms contributing to its rich and characteristic aroma. It's noteworthy that the increase of *Wickerhamomyces* and *Millerozyma*, coupled with the decrease of *Enterobacter*, *Komagataeibacter*, *Pseudomonas*, and *Saccharomycopsis*, contributes to the reduction of the pungent volatile compounds of dimethyl trisulfide and 2-methyl butanal.

4. Conclusions

In this study, the microbial community, sensory characteristics and volatile flavor compounds during the storage of two types of Xiangyang fresh Huangjiu were investigated, and a correlative analysis was conducted between the microbial community and sensory characteristics as well as volatile flavor compounds. 14 dominant bacterial genera, including *Leuconostoc*, *Lacticaseibacillus*, *Lactiplantibacillus*, *Levilactobacillus*, *Acetobacter*, *Streptococcus*, *Enterobacter*, *Lactococcus*, *Gluconobacter*, *Lactobacillus*, *Komagataeibacter*, *Acinetobacter*, *Pseudomonas*, *Chryseobacterium* and 7 dominant fungal genera, including *Saccharomyces*, *Wickerhamomyces*, *Clavispora*, *Candida*, *Saccharomycopsis*, *Millerozyma*, and *Pichia* were indentified in both XYFA and XYFB Huangjiu. The above microbial genera directly influenced the sensory characteristics and volatile flavor compounds of both types of Xiangyang fresh Huangjiu during storage. Compared to XYFA Huangjiu, the relative stability of the microbial genera during the storage of XYFB Huangjiu determined its relatively consistent flavor and taste profile. Additionally, the pronounced sourness in both types of Huangjiu during storage was strongly positively associated with *Saccharomyces*. The predominant flavor profile of 1-octen-3-one was significantly negatively correlated with *Gluconobacterium*, and the increase of *Wickerhamomyces* and *Millerozyma*, along with the decline of *Enterobacter*, *Komagataeibacter*, *Pseudomonas*, and *Saccharomycopsis*, contributed to the reduction of the pungent volatile compounds of dimethyl trisulfide and 2-methyl butanal.The results confirmed the crucial role of microbial genera in the production of flavor and taste in two types of Xiangyang fresh Huangjiu, and provide a useful reference for quality enhancement, storage and consumption in the standardized industrial production of Xiangyang fresh Huangjiu.

CRediT authorship contribution statement

Xianhao Ding: Writing – review & editing, Investigation, Data curation. **Yonghui Yu:** Writing – review & editing. **Wenjing Li:** Writing – review & editing. **Huang Dai:** Writing – review & editing. **Jiahua Wang:** Writing – review & editing, Conceptualization. **Fuwei Pi:** Writing – review & editing. **Mengjie Zhu:** Resources. **Xue Wang:** Resources. **Xiaodan Liu:** Writing – original draft, Investigation, Funding acquisition, Conceptualization. **Dun Wang:** Writing – review & editing, Resources.

Declaration of competing interest

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

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Data availability

Data will be made available on request.

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