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Effects of storage time on flavor characteristics of bran-free fermented Baijiu by using electronic sensory, descriptive sensory analysis, GC × GC–MS, and ICP-MS

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A R T I C L E I N F O

Chemical compounds:
Ethyl Acetate (PubChem CID: 8857), Ethyl Lactate (PubChem CID: 7344), Isoamyl Acetate (PubChem CID: 31276), Ethyl Oleate (PubChem CID: 5363269), Isoamyl Alcohol (PubChem CID: 31260), Acetic Acid (PubChem CID: 176), Acetaldehyde (PubChem CID: 177), Furfural (PubChem CID: 7362).

Keywords:
Bran-free fermented Baijiu
Sensory analysis
Electronic sensory analysis
Flavor characteristics
Metal ions

A B S T R A C T

By examining and analyzing bran-free fermented Baijiu (BFB) with varying storage periods (0–20 years), it was observed that the overall concentration of volatile compounds initially increases and subsequently decreases over time. Furthermore, BFB exhibited more kinds of long chain esters, higher concentration of acetals, and reduced furfural content. The process of cellaring can enhance the aged, sweet, and fruity aroma of BFB. 16 flavor compounds, including 1,1-diethoxyethane, ethyl dodecanoate, and ethyl hexadecanoate, can be used as markers for vintage BFB, and electronic sensory technology was capable of discerning BFB in different years. The results of redundancy analysis (RDA) showed a positive correlation between metals and aldehydes, esters, and ketones, while indicating a negative correlation with acids and alcohols. Al, Fe, and Ca underwent the most significant changes during storage period, and they were positively correlated with differential substances, such as benzaldehyde, vanillin, ethyl isovalerate, and ethyl palmitate ($P < 0.01$).

1. Introduction

Baijiu, a traditional distilled liquor deeply rooted in Chinese culture, holds an esteemed position as one of the world's 6 major distilled liquor. Within our nation's thriving Baijiu industry, Qingxiangxing Baijiu (QXB) stands out as the vanguard with its extensive historical legacy and widespread availability (Zheng & Han, 2016). The Sichuan-style Qingxiangxing Baijiu (SQB) is a renowned QXB originating from southwest China, distinguished by its soft and sweet taste, clean aftertaste, with ethyl acetate serving as the predominant aroma compound. The brewing process of traditional SQB is mainly divided into five steps, including soaking, grain steaming, Xiaoqu inoculation, fermentation, and distillation. Some fiber auxiliary materials such as bran husk, rice husk, and sorghum residue are typically added in the production of traditional SQB (Zheng & Han, 2016) (Fig. S1 B). These auxiliary materials can provide excellent conditions for adhesion and micro-oxygen environments that promote the growth and reproduction of microorganisms during the Baijiu brewing process (Zheng & Han, 2016). However, on one hand, the presence of these crude shell substances may contribute to an undesirable flavor profile known as “bran taste” in the Baijiu. On the other hand, it is important to note that harmful compounds such as furfural and methanol can be generated during the fermentation process involving bran shells (Sun et al., 2022). To mitigate the production of these undesirable substances, we have implemented a novel bran-free fermentation system, resulting in the generation of a Baijiu termed bran-free fermented Baijiu (BFB) (Fig. S1 A).

Like other Baijiu, BFB also requires a period of cellaring to enhance the sensory quality (3–5 years), resulting in a smoother and more

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mellow taste. The aging process can eliminate unpleasant flavor compounds such as sulfides, olefins, methanol, and free ammonia while forming aromatic compounds through hydrolysis, oxidation, association, and esterification (Jia et al., 2023; Wang et al., 2023). Currently, research on cellaring Baijiu primarily employs headspace solid-phase microextraction (HS-SPME), liquid-liquid extraction (LLE) combined with gas chromatography–mass spectrometry (GC–MS), gas chromatography-ion mobility spectrometry (GC-IMS), gas chromatography-olfactometry-mass spectrometry (GC-O-MS), gas chromatography-flame ionization detector (GC-FID), and other technologies to analyze aroma compounds through aroma extraction dilution analysis, aroma recombination, and omission experiments (Jia et al., 2023; Sun et al., 2022; Wang et al., 2023). For example, previous studies on fresh and aged Xiaoqu Baijiu using sensory omics methods such as GC–MS, aroma extraction and dilution analysis, aroma recombination and omission experiments have found that the quantity and intensity of many aroma compounds in aged Xiaoqu Baijiu are higher than those in fresh Xiaoqu Baijiu. Additionally, 3-methylbutane, dimethyl trithioether, ethyl acetate and ethyl isovalerate are also considered important aroma substances in aged Xiaoqu Baijiu (Sun et al., 2022). The total concentration of flavor components was found to decrease slowly during storage by means of HS-SPME/LLE-GC–MS and GC-FID (Huang et al., 2022). The four principal components, ethyl butyrate, ethyl acetate, ethyl caproate, and ethyl lactate, gradually decreased while acetic acid, caproic acid, and lactic acid increased significantly (Huang et al., 2022). However, the composition of flavor compounds in BFB remains unknown, as well as its distinctive characteristics. Additionally, the evolution of flavor in BFB over time and efficient methods for distinguishing BFB years remain unresolved issues. To solve these problems, in addition to employing conventional methods for analyzing aroma compounds of BFB in different years, this study also endeavored to incorporate electronic sensory technology for the discrimination of BFB across various years. The intelligent sensory technology, such as the tongue and electronic nose, is proposed as a research idea to simulate human senses using intelligent instruments. It combines the stability and accuracy of instruments with the overall evaluation ability of humans, while also offering advantages in terms of speed, efficiency, and objectivity (Xu et al., 2022). Currently, intelligent sensory technology has been widely applied in various scientific research fields such as biomedicine, environmental monitoring, and food science. Its practicality has been verified in the food and alcohol beverage industry. For instance, some researchers utilize electronic tongue combined with chemometrics technology to rapidly classify Nongxiangxing Baijiu or employ an electronic nose to identify different quality grades of Jiangxiangxing Baijiu (Ao et al., 2022). However, few studies have applied it to differentiate between QXB of different years. Moreover, as the container gradually releases metal ions into the Baijiu during storage (Wei et al., 2023), these ions have the potential to affect the overall flavor and taste profile. So it is imperative to investigate the impact of metallic elements on key flavors in BFB.

Therefore, the main purpose of this study is to (1) determine whether electronic sensory technology has the potential ability to identify BFB from different years; (2) Study the effect of storage time on BFB aroma compounds and senses, and identify the age-markers; (3) to investigate the release of metal ions during storage and its impact on BFB flavor.

2. Materials and methods

2.1. Baijiu samples

Baijiu samples aged between 0 and 20 years (0, 0.5, 1, 5, 10, 15, and 20 years) were used in this study and were matured in pottery jars. Each year, three clay pots are selected for sampling, and then they were mixed together. The samples were stored in pottery jars at a temperature of 4 °C. All samples were tested within 5 days of being sampled.

2.2. Reagents and chemicals

Sodium chloride (NaCl, >99.5%) and nitric acid (HNO_3, >99.9%) were purchased from the Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Internal standards (2-octanol, >99.5%) were from Aladdin Reagent Co., Ltd. (Shanghai, China). A C_6–C_{30} n-alkane mixture (Sigma-Aldrich, Shanghai, China) was used to determine the linear retention indices (RIs). Mg, Al, Na, Li, K, Ca, V, Cr, Mn, Be, Sr, Fe, Co, In, Ni, Cu, Zn, Ga, Ce, Bi, As, Se, Ag, Rh, Hg, Cd, Ba, and Pb were purchased from Gangyannake Testing Technology Co., Ltd. (Beijing, China).

2.3. Sensory analysis

The previous references were used for sensory quantitative description analysis (Huang et al., 2022; Wang et al., 2023). The aroma profiling was conducted by a panel of ten assessors, comprising an equal number of male and female participants. The assessors will sniff and memorize the aroma reference substances twice a week, spending 50 min each time. In the end, the assessors must achieve an accuracy rate of 80% or higher in blind tasting in order to pass the test. Meanwhile, one sample is selected each week as a representative for the assessors to describe the aroma they perceive, and the results are discussed and shared. After approximately one year of training, the assessors will have mastered the method for sensory evaluation of Baijiu. Eight odor descriptors associated with the sensory characteristics of BFB were chosen: grain, fruity, sweet, aged aroma, alcoholic, acidic, grassy, and floral. The panelists then assessed the strength of each descriptor for each sample on a scale ranging from 0 (indicating very weak) to 9 (indicating very strong). The samples were subjected to three consecutive sniffs, with a 10-min time interval between each sniff. The results obtained from ten evaluators were averaged and subsequently visualized as a radial plot.

2.4. Electronic sensory analysis

2.4.1. Electronic tongue analysis

Following the methodology proposed by Eszter et al. (2023), each sample was subjected to three parallel measurements. Before the e-tongue analysis, all Baijiu samples were diluted fivefold with distilled water to attain the optimal concentration for enhancing the sensitivity of the e-tongue sensors. The e-tongue signal was measured at equilibrium on six sensors, with fixed sensor signal acquisition times of 120 s for sample tests and 20 s for the cleaning solutions (distilled water). The final 10s of the sensor signals, which represent stabilized and optimal sensitivity across different sensors, were exported for statistical analysis using R-project.

2.4.2. Electronic nose analysis

The PEN3 portable electronic nose (Win Muster Airsense Analytics Inc., Schwerin, Germany), equipped with a set of ten metal oxide gas sensors, was employed in this study. The detailed descriptions of the features for each sensor can be found in Table S1. Prior to electronic nose analysis, a 15 mL Baijiu sample was placed in a 60 mL injection sample bottle specifically designed for electronic nose analysis and allowed to equilibrate at room temperature for 15 min, ensuring complete accumulation of the volatile components present in the sample. The procedure for analysis was as follows (Xu et al., 2022): the injection needle and air tonic needle were inserted at the same time. After sampling for 60 s, the injection needle was first removed, followed by the air tonic needle. Then, the system was cleaned (cleaning time lasted for 300 s) and standardized before each sample was determined three times in parallel.

2.5. Two-dimensional gas chromatography coupled to mass spectrometry (GC × GC–MS) analysis

A 5 mL sample of Baijiu was placed in a 20-mL headspace bottle. A

1.5 g of sodium chloride solid was added to the headspace vial to promote saturation of the sample solution. Then, it was mixed with 20 μL of an internal standard solution (2-octanol dissolved in ethanol, 0.822 mg/L). GC × GC–MS analysis was performed using an Shimadzu QP2020NX (Shimadzu Enterprise Management Co., Ltd.; Shanghai; China). The one-dimensional column was polar DB-WAX(30 m × 0.25 mm × 0.25 μm), and the two-dimensional column was medium polar DB-17 ms(1.2 m × 0.18 mm × 0.18 μm). Sample pretreatment: The sample was analyzed using headspace solid-phase microextraction technology. The sample was preheated and balanced for 15 min at 60 °C, and then a three-phase S2 solid-phase microextraction probe is inserted (DVB/C-WR/PDMS, 1 cm, Guangzhou ZhiDa Laboratory Technology Co., Ltd., InLab-4FS0–007). Headspace extraction was carried out at 60 °C for 40 min, and then inserted into the gas chromatograph inlet (inlet temperature 250 °C) for 3 min thermal analysis. The column carrier gas was high-quality helium (≥99.999%) flowing at a rate of 1 mL/min. The temperature program is set as follows: The initial temperature is 40 °C and it is maintained for 5 min. Then, it is heated at a rate of 5 °C per minute to reach 230 °C and maintained for 9 min. The evaporation chamber temperature remains at 250 °C, and no fractionation is performed. The mass spectra were obtained in electron ionization mode using an ionization energy of 70 eV and a scan range from m/z 50 to 550 amu. The temperature of the ion source was set at 230 °C. The solution of C_6–C_{30} n-alkanes was analyzed using the same chromatographic conditions to calculate linear retention index (LRIs) based on retention times. By comparing the mass spectra of the compounds with the standard mass spectra in the NIST17 library, if there is a match of >80% in masses, the compound can be tentatively identified. The identification and quantification procedures for volatile compounds can be referenced from the previously reported method (Wang et al., 2023).

2.6. Inductively coupled plasma-mass spectrometry (ICP-MS) analysis

The samples were tested according to the method described by Wei et al. (2023). Concentrate the 25 mL sample to 1 mL using a 65-degree water bath, and transfer it to a 25 mL volumetric flask. The process should be repeated with the 1% nitric acid solution for approximately three to five cycles, with the washing liquid being transferred to the volumetric flask at each stage. Finally, the solution should be diluted to 25 mL using a 1% nitric acid solution, and a blank test should be performed. The inductively coupled plasma mass spectrometer (PerkinElmer ICPMS NexION 350×, PerkinElmer, America) was employed for the detection of metal ions. The RF power was 1.55 kW; the sampling depth was 8 mm; the plasma gas was 15 L/min; the auxiliary, carrier, and compensating/dilution gases were all at 1 L/min; the temperature of the atomization chamber was 2 °C; and the peristaltic pump speed was 0.1 rps (revolutions per second).

2.7. Statistical analysis

The analysis of variance (ANOVA) was used to test the significance of assay ($P < 0.05$) with software SPSS 25.0. Origin 2019 and GraphPad Prism (v8.0.1) software were utilized for data processing. Principal compounds analysis (PCA) of volatile flavors and the redundancy analysis (RDA) of volatile flavor and metal ions were performed with the online tools (https://www.omicstudio.cn/index). A significant heat map was generated based on Spearman correlation analysis for the flavor substances exhibiting significant differences in metal content and Variable Importance in Projection (VIP) > 1.

3. Results and discussion

3.1. Electronic sensory analysis of BFB samples

In this study, a 10-sensor electronic nose was used for comprehensive flavor evaluation of samples (Fig. S1). The W5S, W1S, and W2S sensors exhibit noticeable responses to BFB samples, whereas other sensors demonstrate a lower intensity of response (Fig. 1 A). The response of the W5S, W1S, and W2S sensors exhibited the highest values, indicating a higher concentration of aromatic compounds such as nitrogen oxides, inorganic sulfides, and ethanol. This is attributed to the presence of volatile substances, such as methanol and sulfur-containing compounds, in newly produced Baijiu. Simultaneously, the equilibrium between these compounds has not yet attained a state of stability, leading to suboptimal sensory attributes including taste and aroma in nascent Baijiu (Jia et al., 2023). Following storage, the concentration of these substances in BFF exhibited a decline, accompanied by a diminished response value. This observation suggests that Baijiu storage can expedite the volatilization and reaction processes of these compounds, thereby improving the sensory attributes of the Baijiu. The electronic tongue sensor array was employed to detect and analyze the sour, bitter, sweet, umami, and saline taste qualities of BFB with varying storage years. Results showed that Baijiu elicited the highest response value from the sour sensor followed by sweet and bitter (Fig. 1 B).

In order to assess the ability of electronic senses in recognizing BFB with varying storage ages, Principal Component Analysis (PCA) was employed to analyze data obtained from samples collected by electronic nose and electronic tongue (Fig. 1 D and E). The samples from different years exhibit distinct discrimination, with PC1 and PC2 accounting for 95.68% and 86.97% of the total variance, respectively, indicating that these principal components effectively capture the information conveyed by the original multiple indicators. In summary, the characteristic indexes obtained through electronic sensory analysis have the potential to quickly identify BFB from different years.

3.2. Sensory analysis

Sensory analysis showed significant differences in sweet, alcoholic, aged aroma, and grassy notes between young and aged BFB ($P < 0.05$) (Fig. 1 C). The grassy and alcoholic notes were prominent in young BFB, whereas cellaring significantly reduced the alcohol and grassy flavors in BFB, resulting in a high sweet and aged aroma, characteristics. Cellaring can enhance the overall flavor profile of BFB. Interestingly, the sensory group and the electronic tongue exhibited divergent trends in their evaluations of the sweet and sour taste profiles of the samples. The sensory panel perceived BFB as exhibiting a higher degree of sweetness, whereas the data obtained from the electronic tongue indicated a slightly higher level of sourness in BFB. The electronic tongue mimics the taste system, while the sensory team evaluates the sample by smell. The substances responsible for taste characteristics are typically non-volatile compounds at room temperature, which interact with taste receptors on the tongue to elicit specific tastes (Wang et al., 2024). Studies have shown that in the taste system, sensitivity to sour tastes is higher than to sweet tastes (Dong et al., 2024). The compounds responsible for olfaction are typically volatile in nature. These compounds enter the nasal cavity and interact with odor receptors, resulting in the perception of smell, which exhibits a sensitivity approximately 10,000 times greater than that of taste (Ellender, 2022). The Baijiu is characterized by a rich array of volatile compounds, which not only contribute to its inherent sweetness but also exhibit a synergistic effect that amplifies the perception of sweetness. For instance, isoamyl acetate, 2-phenylethyl acetate, and ethyl benzoate all contribute to the sweet taste of Baijiu. The omission of ethyl hexanoate resulted in the greatest reduction in the perception of sweetness in Baijiu, followed by ethyl caproate and ethyl 3-methylbutyrate (Sun et al., 2021). The addition of benzene acetate to Nongxiangxing Baijiu results in an enhanced level of sweetness (Chen et al., 2023). In addition, the concentration of non-volatile sweet substances in Baijiu is much lower than the threshold for perceiving sweetness. The volatile components contain a large amount of ester compounds, which may enhance the response of the sweetness receptor to ethanol or activate olfactory receptors expressed in sweet taste cells (Sun et al., 2021). Therefore, the sweetness of volatile components is

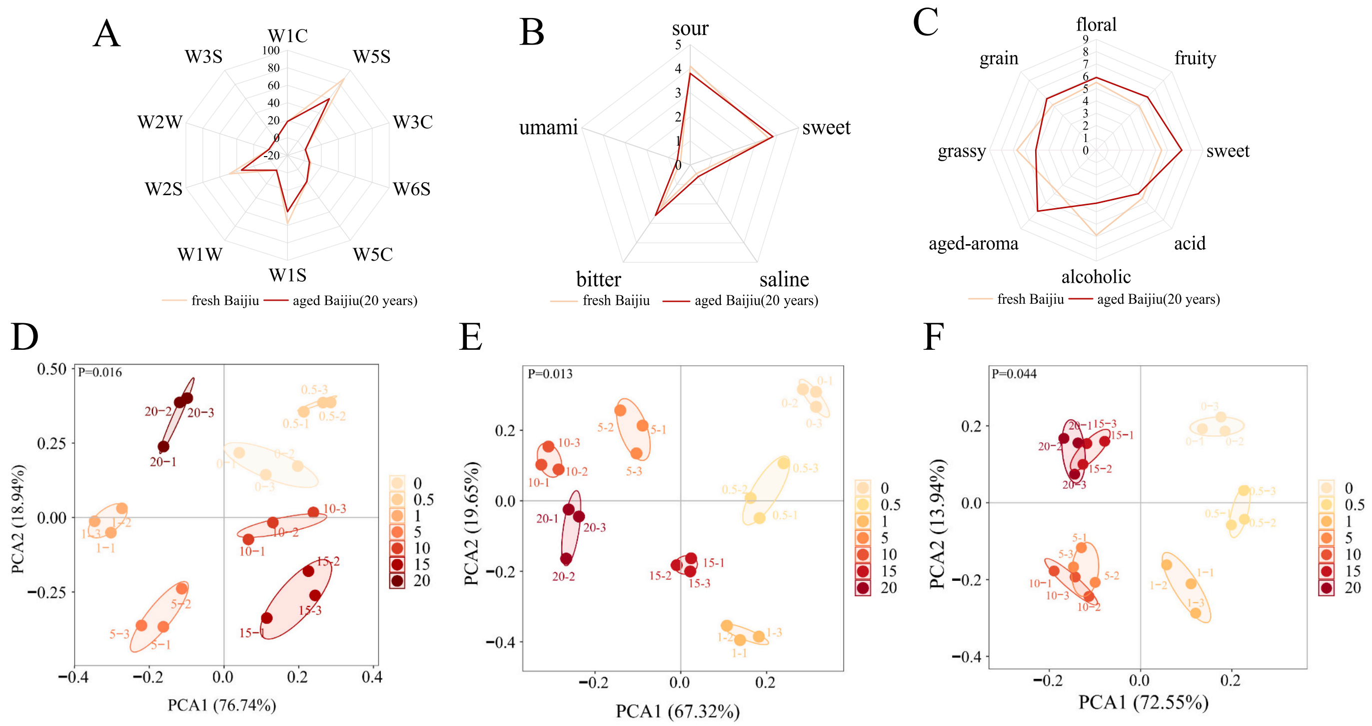

Fig. 1. Electronic nose radar map (A), electronic tongue radar map (B), and sensory radar map (C) of fresh (orange solid line) and aged (red solid line) BFB samples. The PCA analysis results of electronic nose (D), electronic tongue (E), and BFB samples based on all flavor substances (F). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

significantly higher than that of non-volatile substances (Sun et al., 2021). This may be the reason for the difference between electronic tongue and sensory evaluation, but there is no doubt that cellaring can enhance the Baijiu's sweetness and reduce its acidity. The volatile compounds in BFB from different years were further investigated using GC × GC–MS to elucidate the factors contributing to sensory variations.

3.3. Identification of aroma compounds in BFB samples from different years

The chemical constituents in Baijiu undergo intricate physicochemical transformations, encompassing extraction, adsorption, oxidation, esterification, and hydrolysis under specific conditions. These processes culminate in the emergence of distinctive aromatic characteristics (Sun et al., 2022). Revealing the underlying causes for the sensory enhancement of Baijiu after aging can be further achieved through an analysis of the alterations in aroma compounds during storage. A total of 93 volatile flavor compounds were identified in all samples, comprising 37 esters, 26 alcohols, 5 acids, 9 aldehydes and ketones, and 11 alkanes (Table 1). During storage, the total contents of these substances exhibited an initial increase followed by a subsequent decrease. The PCA was performed on all 93 volatile flavor substances to enhance our comprehension of the objective clustering of samples with varying ages (Fig. 1 F). Notably, the composition of BFB exhibited significant alterations within the 0–5 year. The samples stored for 5 and 10 years exhibited similar substance composition, whereas the samples aged 15 and 20 years displayed comparable compound profiles. The aged Baijiu (20 years) significantly deviated from its younger counterparts.

The esters, as is commonly observed in most Baijiu products, play a crucial role as the primary source of aroma components in Baijiu. Their remarkably low aroma threshold contributes significantly to the fruity, sweet, and intricately complex aroma profile exhibited by this traditional Chinese Baijiu (Hong et al., 2023). The esters primarily originate from the microbial esterification biochemical reaction during the fermentation process, while a portion of the esters are predominantly derived from the organic esterification reaction occurring during distillation or aging processes (Fan & Qian, 2006). Esters significantly contribute to the flavor of Baijiu by being one of the key aroma components produced during the storage stage in BFB. For example ethyl hexanoate, ethyl octanoate, ethyl butanoate, ethyl decanoate, ethyl dodecanoate, and ethyl acetate all have a high concentration in the stored procedure. Ethyl acetate, ethyl caproate, ethyl lactate, and ethyl butyrate are the four most important esters in Baijiu, accounting for 64.86–79.42% of the content of ester compounds during the BFB storage stage (Fig. 2 C). The concentration of the majority of ethyl esters in BFB demonstrated a substantial elevation. The concentration of ethyl acetate, the characteristic flavor compound of QXB, significantly increases from 344.70 mg/L to 986.59 mg/L during the process of cellaring (Fig. 2 B). The concentrations of ethyl caproate, ethyl laurate, and ethyl caprate exhibited a significant increase. Ethyl ester compounds serve as the primary source of fruit flavor in Baijiu, and numerous studies have established a positive correlation between fruit flavor and sweet aroma. Notably, ethyl caproate, ethyl 3-methylbutanoate, and ethyl isovalerate make significant contributions to the sweetness of Baijiu (Sun et al., 2021). The increase in ester compounds during cellaring, therefore, enhanced the fruity flavor and sweetness of BFB. In contrast to other QXB studies (Sun et al., 2022), BFB exhibited higher concentrations and more types of long-chain esters (LCES) (>10 carbons), including ethyl oleate, ethyl decanoate, ethyl undecanoate, and ethyl myristate, etc. (Fig. 2 B). The concentration of LCEs exhibited a significant increase during the cellaring process, with the total content of LCEs accounting for 11.84–20.04% of all ester compounds (Fig. 2 A). Baijiu, a distilled liquor primarily composed of ethanol and water, exhibits well-documented colloidal properties akin to other liquors (Morishima et al., 2019). The hydrolysis of the resulting LCEs is also slower because of the greater steric hindrance from the long fatty acid chain. In addition, the alkane chains of the fatty acids should form more stable micelles, which could help protect the LCEs from hydrolysis, by excluding

Table 1
Quantitative data of aroma compounds in bran-free fermented Baijiu.

No.	Compound	RI[a]	Concentration (mg/L)						
			0	0.5	1	5	10	15	20
1	Ethyl Acetate	910	344.72 ± 33.71	559.16 ± 44.73	791.52 ± 71.24	954.67 ± 49.15	1011.93 ± 46.31	980.80 ± 51.58	996.63 ± 54.81
2	Ethyl Butanoate	1033	2.41 ± 0.36	13.45 ± 1.08	25.81 ± 2.32	16.05 ± 1.16	6.39 ± 1.17	5.82 ± 0.73	5.24 ± 0.29
3	Ethyl Lactate	1351	68.55 ± 10.28	64.85 ± 5.19	97.77 ± 8.82	77.92 ± 6.78	59.61 ± 5.55	37.54 ± 3.83	34.47 ± 2.91
4	Ethyl Hexanoate	1238	3.06 ± 0.46	15.71 ± 1.26	21.95 ± 1.98	27.76 ± 1.28	36.38 ± 2.95	33.76 ± 2.74	35.92 ± 2.98
5	Ethyl 4-methylpentanoate	1192	1.91 ± 0.29	1.28 ± 0.11	15.72 ± 1.41	0	0	0	0
6	Isoamyl Acetate	1115	26.58 ± 2.39	38.13 ± 3.05	49.52 ± 3.46	40.46 ± 3.14	45.78 ± 3.19	42.31 ± 3.93	46.39 ± 3.55
7	Ethyl Hexadec-9-enoate	2215	0	0	0	0	0	0	1.01 ± 0.06
8	Ethyl Caprylate	1416	10.89 ± 1.63	17.21 ± 1.38	14.34 ± 1.29	9.89 ± 1.21	7.12 ± 1.19	8.21 ± 0.18	7.89 ± 0.43
9	Ethyl 2-hydroxy-4-methyl Valerate	1217	2.11 ± 0.32	2.22 ± 0.18	2.11 ± 0.19	1.54 ± 0.02	4.81 ± 0.43	2.52 ± 0.16	7.34 ± 0.45
10	Lactic Acid Isoamyl Ester	1227	2.65 ± 0.42	2.67 ± 0.21	2.53 ± 0.23	1.84 ± 0.09	5.78 ± 0.55	3.03 ± 0.97	8.81 ± 0.48
11	Diethyl Succinate	1219	8.36 ± 1.25	15.93 ± 1.27	22.52 ± 2.03	13.54 ± 2.14	16.53 ± 2.43	13.18 ± 1.29	18.27 ± 1.77
12	Ethyl Caprate	1615	27.31 ± 4.11	30.69 ± 2.46	30.69 ± 2.76	36.33 ± 2.36	42.28 ± 3.11	51.79 ± 4.14	67.89 ± 5.73
13	Phenethyl Acetate	1415	25.14 ± 3.77	91.69 ± 7.34	78.63 ± 7.07	54.11 ± 4.54	45.18 ± 3.17	42.43 ± 3.93	35.23 ± 2.94
14	Ethyl Laurate	2230	19.31 ± 2.93	22.69 ± 1.82	11.36 ± 1.02	19.18 ± 1.19	34.24 ± 2.89	21.43 ± 2.47	29.86 ± 1.64
15	Ethyl Tetradecanoate	2045	8.44 ± 1.27	12.75 ± 1.02	3.26 ± 0.29	3.19 ± 0.23	7.07 ± 0.18	1.98 ± 0.24	3.47 ± 0.19
16	(z)-Ethyl Pentadec-9-enoate	1946	0.39 ± 0.06	0.55 ± 0.04	0	0	0	0	0
17	Ethyl Palmitate	2247	29.48 ± 4.42	34.49 ± 2.76	38.79 ± 3.49	51.38 ± 3.51	66.45 ± 5.73	94.41 ± 9.08	110.07 ± 10.05
18	Elaidic Acid Ethyl Ester	2445	4.46 ± 0.67	0	0	0	0	0	0.47 ± 0.03
19	Pentyl Acetate	1169	2.03 ± 0.31	2.57 ± 0.21	17.53 ± 1.58	34.29 ± 3.34	18.01 ± 1.47	18.99 ± 2.42	8.35 ± 1.46
20	Isoamyl Decanoate	2438	2.57 ± 0.39	2.03 ± 0.16	0.93 ± 0.08	0	0	0	0
21	Ethyl Phenyl Lactate	1565	0.06 ± 0.01	0	0	0	0	0	0
22	Diisobutyl Adipate	2235	0.15 ± 0.02	0	0	0	0	0	0
23	Ethyl Oleate	2476	9.67 ± 1.45	22.84 ± 1.83	12.72 ± 1.14	10.48 ± 1.10	8.65 ± 0.22	2.88 ± 0.06	4.77 ± 0.26
24	Ethyl Linoleate	2549	6.81 ± 1.02	12.13 ± 0.97	10.58 ± 0.95	8.24 ± 0.08	7.79 ± 0.24	6.81 ± 0.15	7.13 ± 0.39
25	Benzylcarbinyl Caproate	1950	0.46 ± 0.07	0	0	0	0	0	0
26	Isobutyl Acetate	1149	4.59 ± 0.69	2.73 ± 0.24	1.43 ± 0.13	0.46 ± 0.09	0	0	0
27	Octyl Acetate	1274	6.81 ± 1.02	10.48 ± 0.84	12.84 ± 1.16	13.48 ± 1.13	14.42 ± 1.37	12.43 ± 1.27	11.92 ± 1.66
28	Octyl Formate	1175	0	6.63 ± 0.53	0	0	0	0	0
29	3-Methylbutyl Octanoate	1855	0	1.19 ± 0.09	0	0	0	0	0
30	Ethyl Nonadecanoate	2649	0	0.99 ± 0.08	0	0	0	0.62 ± 0.04	4.33 ± 0.24
31	Ethyl Stearate	2541	0	0	0	1.13 ± 0.17	1.16 ± 0.13	0	0
32	Ethyl Behenate	2939	0	0	0	0	0.86 ± 0.02	0	0
33	Ethyl Isovalerate	1252	0	0	0	0	1.49 ± 0.14	1.92 ± 0.14	3.81 ± 0.21
34	Ethyl Valerate	1245	0	0	0	0	0	2.15 ± 0.15	0
35	Ethyl Heptanoate	1354	0	0	0	0	0	3.63 ± 0.08	0.49 ± 0.03
36	Ethyl Benzoate	1664	0	0	0	0	0	0	1.13 ± 0.06
37	Ethyl Phenylacetate	1765	0	0	0	0	0	0	2.44 ± 0.13
38	Isobutanol	1163	60.54 ± 6.08	109.44 ± 8.75	53.91 ± 4.85	81.13 ± 7.81	118.45 ± 10.08	58.26 ± 4.28	27.74 ± 1.53
39	Butanol	1158	1.81 ± 0.27	4.61 ± 0.37	5.79 ± 0.52	4.77 ± 0.15	3.15 ± 0.18	5.47 ± 0.12	2.61 ± 0.14
40	Isoamyl Alcohol	891	16.33 ± 2.44	13.88 ± 1.11	14.37 ± 1.29	10.43 ± 1.14	7.67 ± 0.23	4.68 ± 0.19	2.23 ± 0.12
41	1-Hexanol	1358	0.24 ± 0.04	2.75 ± 0.22	3.02 ± 0.27	1.95 ± 0.12	1.77 ± 0.07	0.34 ± 0.04	0.97 ± 0.05
42	2,3-Butanediol	1155	3.32 ± 0.93	16.95 ± 1.36	20.43 ± 1.84	9.56 ± 1.11	11.90 ± 1.01	5.21 ± 0.31	7.51 ± 0.41
43	3-Methyl-1-butanol	1206	80.57 ± 12.09	108.86 ± 8.71	117.36 ± 10.56	169.62 ± 11.79	186.95 ± 14.86	90.73 ± 6.92	82.24 ± 6.52
44	β-Phenethyl Alcohol	1561	38.37 ± 5.76	51.84 ± 4.15	55.89 ± 5.03	80.77 ± 7.81	89.02 ± 7.31	43.23 ± 3.95	39.16 ± 3.15
45	Methanethiol	645	3.47 ± 0.07	2.87 ± 0.07	1.88 ± 0.10	1.07 ± 0.07	0.68 ± 0.02	0.66 ± 0.01	0.57 ± 0.04
46	(s)-(+)-1,2-Propanediol	723	8.22 ± 1.23	5.96 ± 0.48	3.92 ± 0.35	3.05 ± 0.03	3.01 ± 0.18	2.71 ± 0.26	2.31 ± 0.13
47	1-Propanol	1031	283.18 ± 42.48	349.86 ± 27.99	446.07 ± 40.15	332.98 ± 33.33	336.48 ± 28.75	376.07 ± 28.27	320.41 ± 17.62
48	(−)-Isolongifolol	2061	0.66 ± 0.11	0	0	0	0	0	0
49	3-Methyl-1,5-pentanediol	1357	0.88 ± 0.13	0.62 ± 0.05	0	0	0	0	0
50	Paclitaxel	2678	0.21 ± 0.03	0.38 ± 0.03	0.91 ± 0.08	1.51 ± 0.12	1.77 ± 0.15	0	0
51	1-Docosanol	2677	1.91 ± 0.29	0	0	0	0	0	0
52	Dl-3-methyl-2-butanol	1273	0.88 ± 0.13	0	0	0	0	0	0
53	2-Phenyl-1-propanol	1382	0.21 ± 0.03	0.34 ± 0.03	0.42 ± 0.04	0.99 ± 0.09	1.66 ± 0.14	1.94 ± 0.14	1.44 ± 0.18
54	Benzyl Alcohol	1173	1.04 ± 0.16	0.83 ± 0.07	0	0	0	0	0
55	Neopentyl Alcohol	1244	0.83 ± 0.12	2.35 ± 0.19	3.34 ± 0.33	5.17 ± 0.45	7.15 ± 0.69	6.89 ± 0.55	9.51 ± 0.72
56	Heptaethylene Glycol	1854	24.78 ± 2.22	20.74 ± 1.51	18.82 ± 1.87	15.01 ± 2.25	12.74 ± 1.33	5.19 ± 0.31	5.01 ± 0.49
57	Hexaethyleneglycol	2673	1.35 ± 0.20	0.85 ± 0.17	0.81 ± 0.11	0.41 ± 0.05	0.31 ± 0.01	0	0
58	Oct-1-en-3-ol	1258	0.03 ± 0.01	0.03 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.11 ± 0.01	0.06 ± 0.01	0.05 ± 0.01
59	Octaethylene Glycol	2031	1.35 ± 0.21	0.85 ± 0.07	0.81 ± 0.07	0.41 ± 0.06	0.31 ± 0.03	0	0
60	Diethylene Glycol	1148	3.82 ± 0.57	1.54 ± 0.12	0.61 ± 0.06	0	0	0	0
61	2-Hexadecanol	2057	2.82 ± 0.42	3.58 ± 0.29	4.82 ± 0.43	1.31 ± 0.21	0.99 ± 0.03	0.61 ± 0.01	1.05 ± 0.16
62	Furfuryl Alcohol	1239	1.32 ± 0.34	0.84 ± 0.12	0.42 ± 0.05	0	0	0	0
63	2-Furanmethanol	1635	1.05 ± 0.16	0.46 ± 0.04	0	0	0	0	0
64	Acetic Acid	1428	799.24 ± 99.89	709.28 ± 56.74	646.29 ± 58.17	589.61 ± 25.9	567.96 ± 24.77	493.26 ± 20.85	519.98 ± 28.6
65	Pentanoic Acid	1712	0.91 ± 0.14	2.58 ± 0.21	3.67 ± 0.33	5.69 ± 0.46	7.86 ± 0.69	7.57 ± 0.77	10.47 ± 0.88
66	Butanoic acid	1600	6.31 ± 0.94	4.88 ± 0.39	4.01 ± 0.36	3.76 ± 0.34	3.07 ± 0.28	2.68 ± 0.16	2.23 ± 0.32

(*continued on next page*)

Table 1 (*continued*)

No.	Compound	RI[a]	Concentration (mg/L)						
			0	0.5	1	5	10	15	20
67	Propionic Acid	1495	10.93 ± 1.64	12.87 ± 1.03	14.17 ± 1.28	18.53 ± 1.19	19.64 ± 1.51	18.45 ± 1.41	19.32 ± 1.06
68	3-Methylbutanoic Acid	1647	2.34 ± 0.35	4.34 ± 0.35	5.41 ± 0.49	2.37 ± 0.12	3.41 ± 0.29	3.31 ± 0.17	3.85 ± 0.21
69	1,1-Diethoxyethane	1441	60.75 ± 9.11	71.27 ± 5.73	89.53 ± 8.06	81.48 ± 7.81	94.71 ± 7.46	79.34 ± 6.75	85.46 ± 7.71
70	1,1-Diethoxy-2-methylbutane	1315	0.51 ± 0.07	0.68 ± 0.05	0	0	1.29 ± 0.11	1.56 ± 0.13	7.85 ± 0.63
71	Tetradecane	1845	2.71 ± 0.81	7.54 ± 0.62	14.73 ± 1.33	13.61 ± 1.14	9.55 ± 1.25	4.69 ± 0.41	2.23 ± 0.12
72	Pentadecane	1951	2.44 ± 0.77	0	0	0	0	0	0
73	9-Methylideneheptadecane	2218	0.71 ± 0.11	6.68 ± 0.53	3.76 ± 0.34	1.49 ± 0.11	3.83 ± 0.14	1.83 ± 0.04	0
74	1,1-Diethoxymethane	1622	64.84 ± 9.73	69.55 ± 5.56	75.16 ± 6.76	81.33 ± 6.81	76.96 ± 6.71	78.33 ± 6.72	77.36 ± 4.25
75	1,1-Diethoxyhexane	1754	0.55 ± 0.08	0.75 ± 0.06	0.79 ± 0.07	1.01 ± 0.01	1.96 ± 0.15	1.42 ± 0.13	0.98 ± 0.05
76	Dodecane	1677	12.88 ± 1.93	11.19 ± 0.89	7.44 ± 0.67	6.17 ± 0.46	2.21 ± 0.16	1.72 ± 0.14	3.53 ± 0.19
77	10-Methylnonadecane	2531	0	13.43 ± 1.07	0	0	0	0	0
78	Tridecane	1882	0	0	8.49 ± 0.76	0	0	0	6.39 ± 0.35
79	1,1-Diethoxy-3-methylbutane	2207	11.79 ± 1.77	12.65 ± 1.01	13.67 ± 1.23	14.79 ± 1.15	13.99 ± 1.36	14.24 ± 1.31	14.07 ± 1.77
80	Vanillin	1553	0.45 ± 0.04	0.69 ± 0.03	0.98 ± 0.04	1.35 ± 0.08	1.78 ± 0.09	1.97 ± 0.09	1.86 ± 0.07
81	Acetaldehyde	1004	94.39 ± 14.16	116.62 ± 12.33	128.69 ± 11.58	110.99 ± 11.11	112.16 ± 11.92	125.36 ± 11.76	116.82 ± 12.42
82	Furfural	1462	1.15 ± 0.17	0.91 ± 0.07	0.73 ± 0.07	0.47 ± 0.09	0.34 ± 0.02	0.16 ± 0.03	0.08 ± 0.01
83	Benzaldehyde	1491	0.49 ± 0.09	0.77 ± 0.06	1.50 ± 0.13	2.29 ± 0.12	4.16 ± 0.51	4.99 ± 0.51	6.01 ± 0.63
84	Hexanal	1067	0.25 ± 0.04	0.39 ± 0.03	0.82 ± 0.07	0.65 ± 0.02	0.57 ± 0.01	0.66 ± 0.03	0.58 ± 0.03
85	Phenylacetaldehyde	1621	1.05 ± 0.26	0.81 ± 0.07	0.67 ± 0.06	0.43 ± 0.03	0.31 ± 0.01	0.14 ± 0.02	0.07 ± 0.01
86	2-Methylbutanal	1347	4.94 ± 0.94	5.74 ± 0.46	5.14 ± 0.46	4.67 ± 0.35	4.26 ± 0.41	3.12 ± 0.37	4.05 ± 0.22
87	Acetoin	1221	0.82 ± 0.12	1.27 ± 0.22	1.64 ± 0.15	2.52 ± 0.23	4.52 ± 0.52	4.69 ± 0.51	4.97 ± 0.47
88	2,3-Butanedione	1254	0.67 ± 0.12	0.57 ± 0.05	0.49 ± 0.04	0.47 ± 0.03	0.43 ± 0.05	0.31 ± 0.04	0.31 ± 0.05
89	2-Phenylphenol	1931	0.45 ± 0.09	0.21 ± 0.01	0.16 ± 0.01	0.07 ± 0.01	0.01 ± 0.01	0	0
90	1-Propoxypropan-2-ol	1426	0.83 ± 0.12	0	0	0	0	0	0
91	2,6-Di-tert-butylhydroquinone	1822	0.53 ± 0.04	0.78 ± 0.06	0.96 ± 0.08	1.21 ± 0.08	1.56 ± 0.09	1.94 ± 0.09	1.72 ± 0.09
92	4-Methylphenol	2069	0.73 ± 0.07	0.70 ± 0.06	0.86 ± 0.08	0.65 ± 0.05	0.53 ± 0.09	0.90 ± 0.08	0.62 ± 0.06
93	Tetraethylene Glycol Monododecyl Ether	2551	0.81 ± 0.13	1.68 ± 0.23	1.16 ± 0.34	0.91 ± 0.13	1.03 ± 0.14	0.83 ± 0.04	0

[a] Retention index (RI).

water. So compared to shorter fatty acid esters, the LCES are more prone to forming stable clusters (Zhang et al., 2021). As a result, the concentration of LCEs increases during Baijiu storage process. Previous studies have revealed that the intricate production process and protracted production cycle of Jiangxiangxing Baijiu lead to a heightened detection of long-chain substances in comparison to other varieties of Baijiu (Zhou et al., 2023). The presence of numerous long-chain substances gives Baijiu a thick and dense body, resulting in clear and transparent Baijiu. It also enhances the sense of hierarchy when drinking, creating the distinctive characteristics of high-quality Baijiu known as "hanging cup" and "empty cup remaining fragrance" (Song et al., 2021). According to the current QXB standard (GB/T 10781.2–2022), the retention time of empty cup aroma serves as a pivotal criterion for evaluating the quality grade of QXB, with higher-grade QXB exhibiting prolonged duration of empty cup aroma. This finding further substantiates the superior efficacy of bran-free fermentation technology in the production of premium grades of QXB.

The boiling point of alcohols in Baijiu is comparatively lower than that of other components, thus facilitating its role as a "carrier" for the volatilization process of other volatile components (Tian et al., 2023). The taste function of alcohols is very important in Baijiu, it is the skeleton of Baijiu taste. Alcohols are mainly shown as stimulating and strong, but sometimes they give the Baijiu a certain bitter taste (Dong et al., 2024). Among them, 1-propanol, isobutanol, and isoamyl alcohol play a role in enhancing the ester aroma, intensifying the flavor and duration. These components are essential for the aroma and taste of Baijiu (Qin et al., 2023). Their concentration increased after storage, which results in a longer aftertaste after cellaring (Fig. 2 B). During the cellaring process, the levels of certain undesirable alcohols such as furfuryl alcohol (1.32–0 mg/L; bitter), methanethiol (3.47–0.57 mg/L; smelly), and heptaethylene glycol (24.78–5.01 mg/L; leather smell) were reduced, which was one of the reasons why cellaring enhances the flavor profile of Baijiu (Fig. 2 B). Moreover, it has been discovered that the —S— functional group present in methanethiol imparts a pungent flavor, which contributes spiciness and stimulation to Baijiu (Zhang et al., 2021). As the storage time increased, the concentration of the —S— functional group decreased (Table 1), resulting in a softer BFB experience.

Acidity is the key factor that determines the taste of Baijiu. The optimal acidity level not only serves as a buffering role but also facilitates the gradual formation of aromatic esters during Baijiu maturation (Zhu et al., 2020). Previous studies have shown that acetic acid, lactic acid, caprylic acid, and pelanoic acid can act as buffers in Baijiu. Additionally, excessive acidity will diminish the sweetness of the Baijiu (Chen et al., 2023). A total of 5 acids were detected in this study, which imparted a cheesy and sour taste. During the process of cellaring, there was a decreasing trend observed in the concentration of acids (Fig. 2 A and B). This could potentially account for the observed decrease in acidity of BFB following the cellaring (Fig. 1 C).

The levels of fruity acetal (137.92–177.86 mg/L) and grassy acetaldehyde (94.39–116.82 mg/L) also increased as the storage time increased (Fig. 2 B). The presence of acetaldehyde in Baijiu can contribute to the development of a mature flavor profile, and the accumulation of acetaldehyde during aging process can intensify the overall aging characteristics in Baijiu (Sun et al., 2022). The presence of acetal in Chinese Baijiu serves as a crucial indicator for the maturation process and is widely recognized as the primary component that enhances the Baijiu's smoothness and aromatic profile (Zhang et al., 2021). Acetal exhibits a distinctive aroma and dry taste. When combined with propanol, it imparts a refreshing and bitter flavor to QXB. In Chinese renowned Baijiu, the acetal content tends to increase with higher quality levels. Some researchers posit that acetal plays a role in reducing acidity and harmonizing the aroma of Baijiu (Hao et al., 2022). Increasing the acetal content during storage enhanced the aromatic and soft BFB flavor. As the storage time increased, both 1,1-diethoxyethane (60.75–85.46 mg/L) and 1,1-Diethoxymethane (64.84–77.36 mg/L) showed a significant increase in their concentration. The content of furfural in BFB was found to be extremely low (Table 1). The main sources of furfural are bran, sorghum husk, and wheat straw combined with fermented grains. These fermentation materials contain abundant lignocellulose,

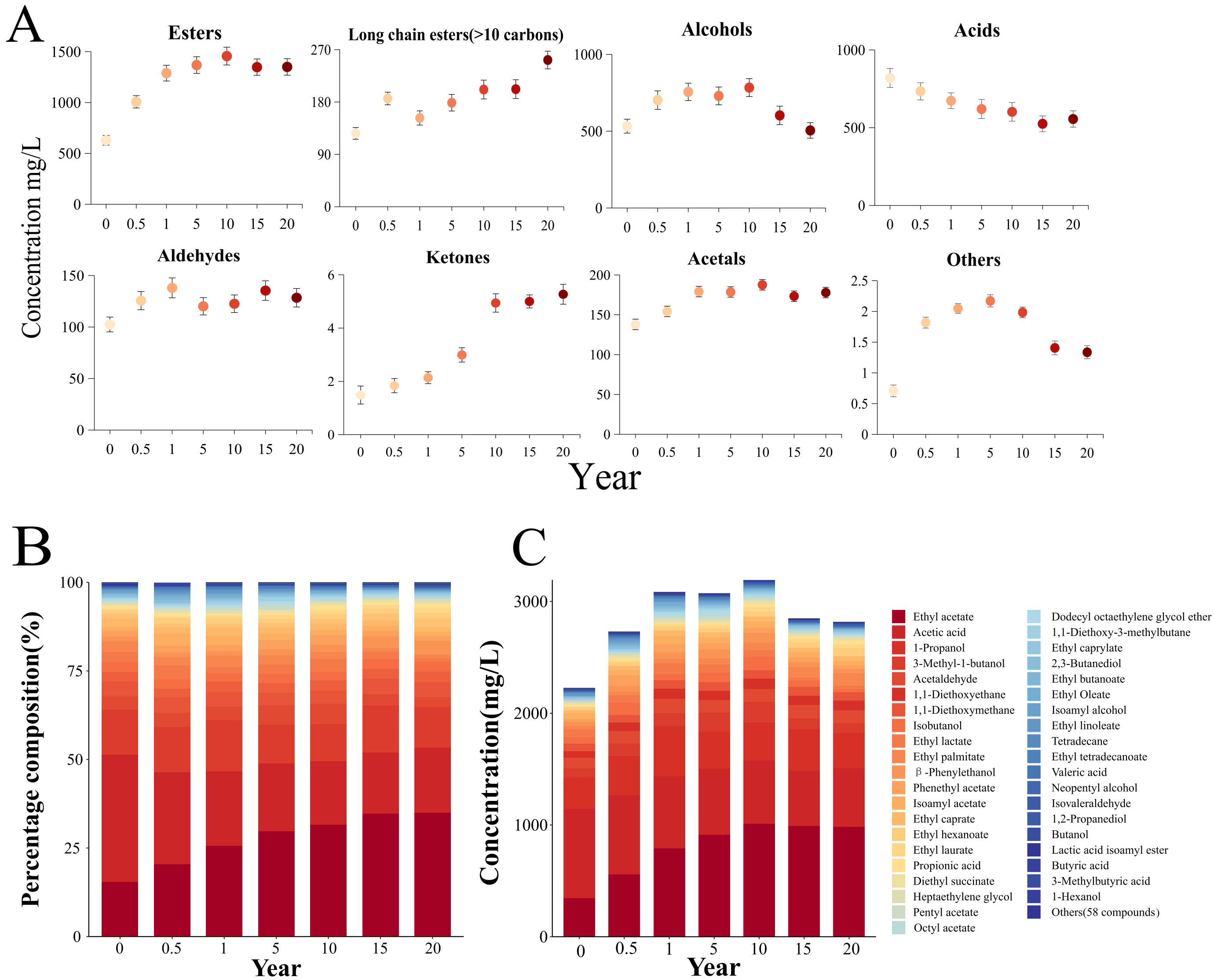

Fig. 2. The total content of each category (A). The percentage composition (B) and cumulative total content (C) of all compounds detected in samples with varied ages.

characterized by a high molecular weight, an irregular structure, and inherent recalcitrance to degradation (Wang et al., 2023). During the fermentation process, lignocellulose serves as a significant substrate for yeast to produce ethanol, which is subsequently metabolized into pentoses (xylose and arabinose) and hexoses (glucose, mannose, and galactose) (Myrto et al., 2019). Furfural and hydroxymethylfurfural are products of pentose and hexose degradation, while the metabolic mechanism of furfural in *Saccharomyces cerevisiae* leads to the production of carcinogens such as furanol and methanol. These compounds also contribute to the bitter, spicy, and complex flavor profile of Baijiu (Sun et al., 2022). Due to the implementation of bran-free fermentation technology in BFB, the production process no longer requires auxiliary materials such as bran husk and rice husk, resulting in a significant reduction in furfural content within Baijiu (Table 1). The furfural content in BFB is halved compared to the conventional production process of QXB (Wang et al., 2023). The presence of vanillin, a main aromatic compound, is significantly enhanced during the maturation process of various wines and exhibits a positive correlation with wine aging (Wang et al., 2023). The vanillin content (0.45–1.86 mg/L) exhibits an increase during the BFB storage process (Table 1). Vanillin not only imparts a sweet and vanilla aroma to Baijiu, but also exhibits significant free radical scavenging activity, rendering it a beneficial constituent in Baijiu (Veena et al., 2023).

3.4. Selection of age-markers based on multivariate analysis of Baijiu volatile profiles

As the PCA analysis based on all flavor substances failed to effectively differentiate samples from each year, we proceeded with a screening process to identify age markers. Orthogonal partial least squares-discriminant analysis (OPLS-DA) and Spearman correlation analysis were used to identify the age markers. As a supervised multivariate statistical analysis, OPLS-DA mitigates the impact of uncontrolled variables on data by incorporating predetermined classification variables to extract additional information from the dataset and quantify the extent of dissimilarity between treatment groups (Zhang et al., 2021). The aforementioned analysis tool is suitable for the classification of data featuring multiple linear and noisy variables. The data of volatile flavor compounds were used to construct a Supervised OPLS-DA model, which involved calculating the VIP score for each compound. Spearman's correlation analysis, a non-parametric test for evaluating the relationship between two variables without assuming any specific data distribution, was employed to investigate the associations between maturation time and volatile compounds. A total of 28 substances with

VIP > 1 and 37 substances with $P < 0.05$ were identified. The Venn analysis revealed that 16 compounds fulfilled both screening conditions, including 12 esters, 3 acetals, and 1 acid (Fig. 3 A and D). The PCA analysis was conducted on a set of 16 age markers to evaluate their discriminatory potential among samples of varying ages, resulting in a significantly enhanced separation efficacy compared to the entire dataset. They can be used as potential markers to distinguish different storage time BFB (Fig. 3 B). It was worth noting that among the 16 identified age-markers, 7 of them belong to long-chain esters (LCEs), namely ethyl tetradecanoate, ethyl nonadecanoate, ethyl hexadecanoate, ethyl dodecanoate, ethyl linoleate, ethyl decanoate, and ethyl oleate. These compounds possess carbon atom chains with lengths of 16, 19, 16, 12,18,12 and 18 respectively (Fig. 3 D). These results indicated that LCEs is a very important characteristic substance in BFB. Cluster analysis of the concentration changes during storage revealed that the 16 substances could be categorized into four distinct groups. For instance, ethyl acetate exhibited an initial increase in concentration followed by a subsequent decrease, whereas ethyl hexadecanoate demonstrated an elevation in concentration after storage (Fig. 3 C).

3.5. Analysis of the metal concentration change and its correlation with flavor substances during storage process

The intake of essential trace metals is necessary for human health, but excessive intake can cause side effects. The metal contents detected in the stored samples in this study were significantly lower than the international legal limits and below the recommended daily allowable intake (ADI) (Iwegbue, 2010; Soylak et al., 2006). The samples were subjected to ICP-MS analysis, revealing the detection of a total of 29 metals. The cumulative concentration of metals exhibits an increasing trend over the storage period. Ca, Mg, K, Na, and Fe emerge as the predominant metals in Baijiu (Fig. 4 A), displaying higher levels, which aligns with previous investigations (Huang et al., 2020). There are two primary sources of metal content in fresh Baijiu: one arises from the migration of metals from the fermented grains into the wine during distillation, while the other stems from their dissolution originating from the distillation equipment (Huang et al., 2020; Zou et al., 2018). However, during the distillation process of Baijiu, the majority of metals predominantly remain sequestered within the fermented grains, with only a minor fraction translocating into the body of the Baijiu. In addition, the distillation time was short, resulting in a limited amount of metal content dissolved from the equipment. These factors contribute to the low metal content in fresh Baijiu. Baijiu is typically stored in pottery jars or stainless steel tanks. Compared with a stainless steel tank, the pottery jars are crafted from fired clay, primarily composed of SiO_2 and Al_2O_3, constituting 52.6% and 45.4%, respectively. Additionally, they contain trace amounts of Fe_2O_3, CaO, CuO, NiO, TiO_2, and other impurities comprising approximately 3% (Schleicher et al., 2008). During storage, the metallic oxides can gradually dissolve into the Baijiu, leading to elevated concentrations of metals such as Ca, Al, Fe, and Cu in the stored Baijiu. The firing process of pottery jars leads to the destruction of certain compounds in clay at high temperatures, resulting in the formation of microporous structures that not only enhance physical and redox reactions of Baijiu, but also facilitate adsorption of specific substances present within it (Li et al., 2021). The concentrations of Se, Hg, In, Ag, Rh and other elements decreased after being stored in BFB, possibly due to adsorption by the pottery (Fig. 4 A).

The metal in liquor has a dual effect. An appropriate amount and type of metal are beneficial for regulating the flavor of Baijiu and human health. Ca, Fe, Mg, and Cu released from pottery jars into Baijiu improve its soft, mellow, and harmonious mouthfeel (Iwegbue, 2010). For example, the sulfur compounds in Baijiu can form odorless complexes with Cu or other metals, thereby reducing the off-flavors caused by sulfur compounds and creating a more pleasant and harmonious fragrance of Baijiu (Ernesto & Vicente, 2014; Franco-Luesma & Ferreira, 2014). Furthermore, metals such as Cu and Fe can act as catalysts for the oxidation, esterification, and condensation reactions of Baijiu. The increase in ester and acetal concentrations helps to reduce the stimulating sensation of Baijiu, enhancing its floral and fruity aroma, resulting in a fragrant and mellow taste (Huang et al., 2020). Some metal ions can

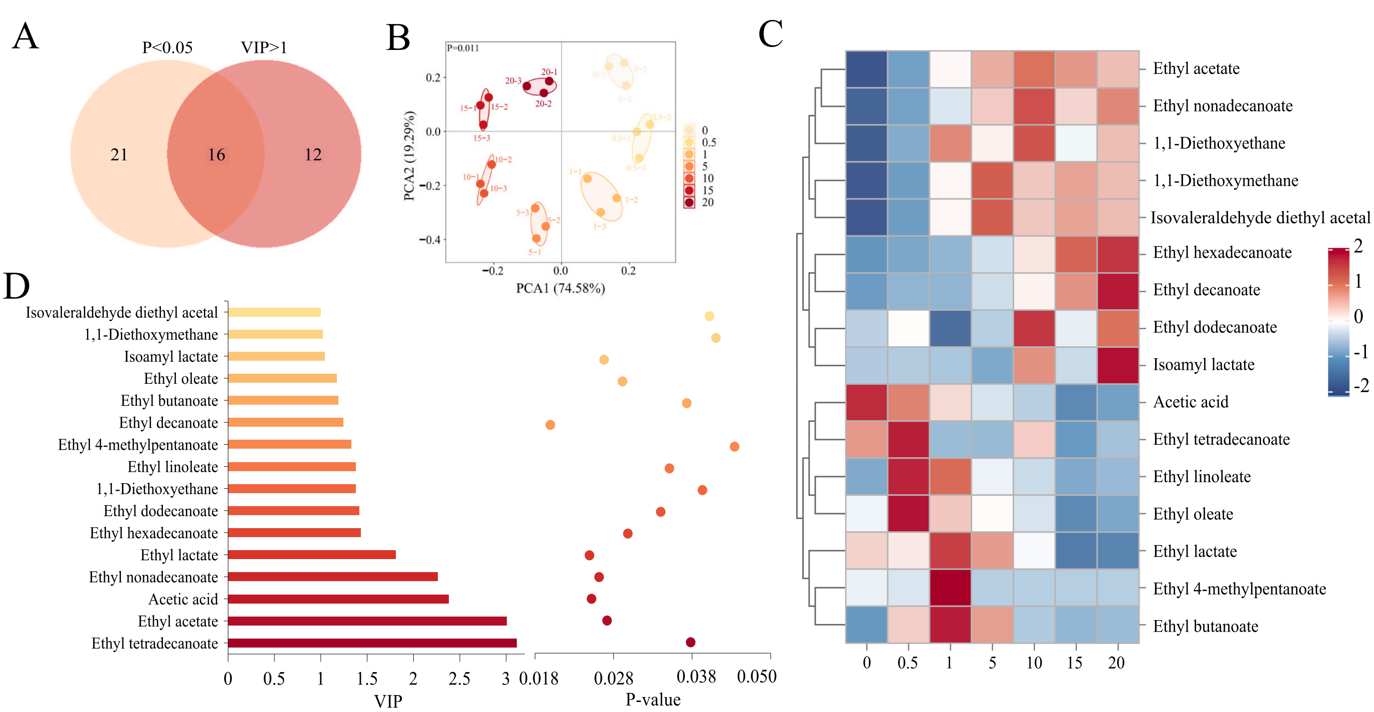

Fig. 3. Venn plot of key characteristic flavor substances showing overlap of the key characteristic substances with VIP > 1 and the Spearman's coefficient ($P < 0.05$) (A). PCA analysis results of Baijiu samples based on 16 potential markers (B). PCA analysis results of Baijiu samples based on 16 potential markers (C).The VIP and P values for 16 compounds (D).

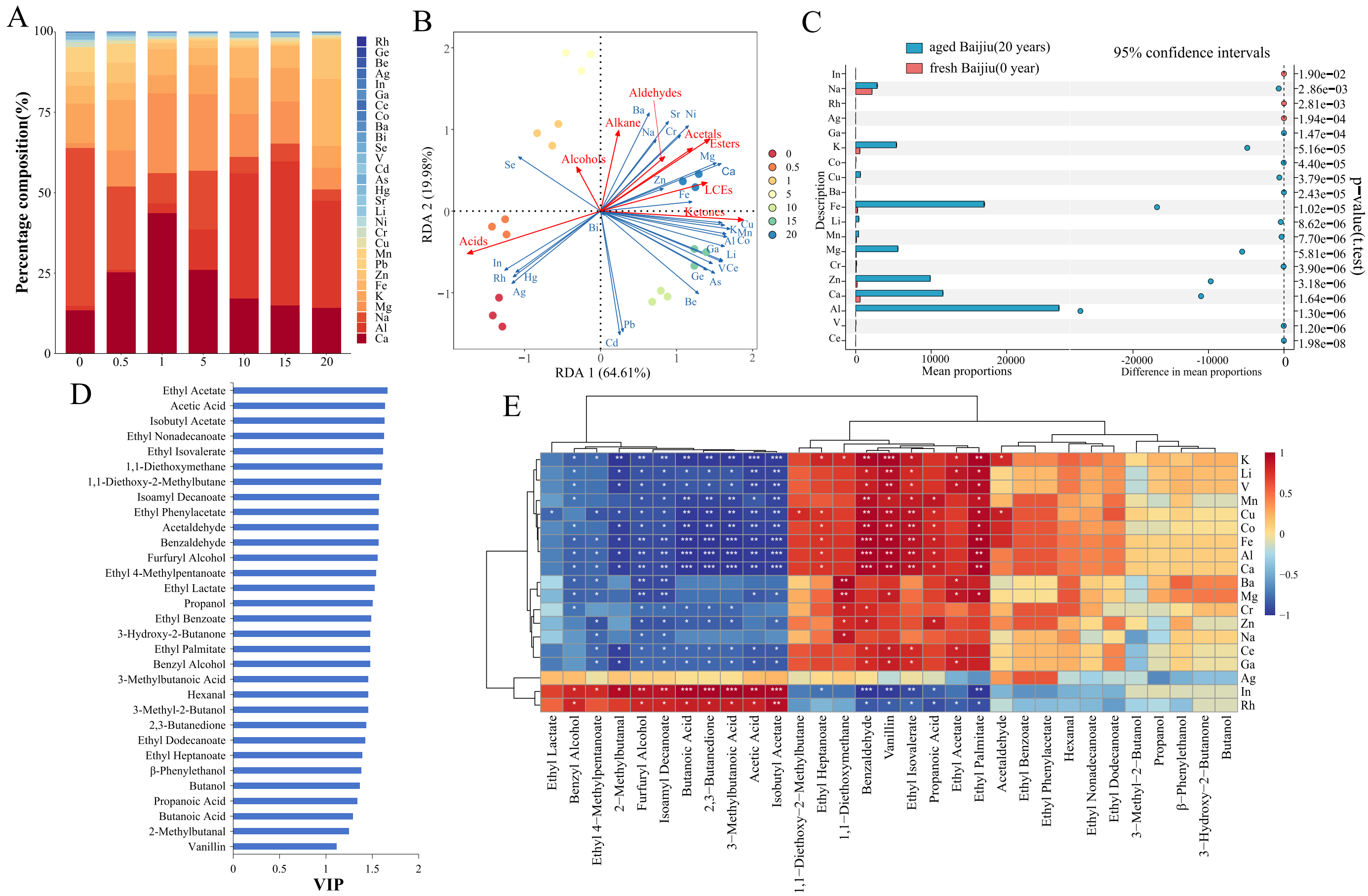

Fig. 4. The percentage composition of metal ions detected in samples with varied storage times (A). The redundancy analysis (RDA) of all the detected metal elements and flavor substances (B). The STAMP analysis between new and aged BFB (C). Different compounds of 0 and 20 year BFB (D). Spearman correlation analysis between these 19 different metals and 31 differential compound (VIP > 1) in old and new BFB (E).

form colloidal particles or complexes with the acids, thereby reducing the sensory stimulation of alcoholic beverages. The presence of a low level of Cu enhances the taste and aroma of brandy (Bonic et al., 2013). K, Ca, Cu, Zn, Fe, and Mg are essential metals in the human body (Wei et al., 2023). However, elevated levels of metals in alcoholic beverages can have a detrimental impact on their sensory attributes and flavor profiles, as well as pose potential risks to human health (Soylak et al., 2006). Excessive Cu results in the formation of minute quantities of volatile and unpleasant compounds containing sulfur (S), while also acting as a catalyst for the production of ethyl carbamate, a known carcinogen (Penteado & Masini, 2009; Pohl, 2007). Therefore, further investigation into the relationship between metal concentration and Baijiu flavor is crucial for elucidating the potential impact of metal concentration on Baijiu quality. The redundancy analysis (RDA) includes all the detected metal elements and flavor substances (Fig. 4 B). RDA analysis is primarily used to explore the relationship between a multi-variable response data set and one or more sets of explanatory variables. Its working principle involves performing a multivariate regression on the response and explanatory variables, and then finding the linear combinations of the response variable that maximize the correlation with the explanatory variable. The combinations are sorted in order to generate a series of ordering axes, each of which exhibits a correlation with the explanatory variable. The RDA analysis revealed a positive correlation between most metal elements and aldehydes, esters, and ketones, while indicating a negative correlation with acids and alcohols. Previous studies have demonstrated that the hydroxyl groups of alcohols and carboxyl groups of acids can form colloidal particles or complexes with metal cations during fermentation, thereby mitigating the sensory impact on alcoholic beverages (Huang et, al., 2022). Therefore, most metals exhibit a negative correlation with acids and alcohols. However, Ni, Ti, and Cu have catalytic effects on esterification and aldol condensation, resulting in a positive correlation with ester and acetal compounds (Bonic et al., 2013). Furthermore, by conducting statistical analysis of taxonomic and functional profiles (STAMP analysis), it is possible to identify 19 metal elements that exhibit significant differences between new and aged Baijiu, including Al, Fe, Ca, Zn, and Mg, etc. (Fig. 4 C). The compounds with a difference of 0 and 20 years were screened based on their VIP values (Fig. 4 D). Through Spearman correlation analysis, heat maps were drawn to illustrate the relationship between these 19 different metals and 31 differential compound (VIP > 1) in old and new Baijiu (Fig. 4 E). The results revealed a strong and significant positive correlation between In and butanoic acid, 2,3 − butanedione, 3 − methyl−2 − butanol, and isobutyl acetate ($P < 0.001$). Ethyl 4-methylpentanoate exhibited a negative correlation with Na, Ba, Mg, etc. ($P < 0.05$). Except for In, Ag, and Rh, the other metals displayed a negative correlation with 2-methylbutanal, 3 − methylbutanoic acid, 2,3-butanedione, butanoic acid, and acetic acid. Furthermore, the introduction of K^+ and Fe^{3+} ions has been observed to significantly reduce the volatility of acetic acid and butanoic acid (Huang et al., 2020). Previous studies have found that K promotes decarboxylation, and Fe^{3+} react with carboxyl groups (Wang et al., 2018). And the acidic compounds contain carboxyl groups, which may be one of the reasons why K^+ and Fe^{3+} reduce the volatility of acetic acid, butyric acid, and 3 − methylbutanoic acid. In this study, K and Fe also exhibited a significant negative correlation with acetic acid and butanoic acid ($P < 0.01$), consistent with previous studies. Cu, Co, Fe, Al, and Ca displayed a positive correlation with benzaldehyde, vanillin, ethyl isovalerate, propanoic acid, ethyl heptanoate, and ethyl palmitate ($P < 0.05$). Cu exhibited a significant positive correlation with benzaldehyde, vanillin, and acetaldehyde ($P < 0.01$). Previous studies have found that Cu can catalyze the formation of carbonyl compounds from alkanes, and aldehydes are a type of carbonyl compound. Under the catalysis of Cu, the concentration of aldehyde compounds increased, and the content of aldehyde compounds volatilized from Baijiu increased (Li et al., 2014).

4. Conclusion

This study presents the first comprehensive analysis of flavor characteristics, volatile compound concentrations, and metal content in BFB during storage. Through the detection by an e-nose and e-tongue, it was found that storage can accelerate the volatilization and reaction of nitrogen oxides, inorganic sulfides, and ethanol substances in these newly produced wines, thereby enhancing the taste and aroma of the Baijiu. Electronic sensory technology has the potential to quickly differentiate between new Baijiu and aged ones. As the aging process was prolonged, there was a decrease in the concentration of compounds such as furfuryl alcohol (bitter), methanethiol (smelly), and heptaethylene glycol (leather smell) in BFB, while the concentration of many esters and acetals increased. This may be why cellar aging can enhance the sensory attributes of BFB, especially its sweetness, aging, and fruit flavor. The storage also enhances the assortment of long-chain esters and augments the concentration of acetal in BFB, thereby imparting a velvety and refined texture to the BFB. Furthermore, the furfural content of BFB was low due to the implementation of bran-free fermentation technology. A total of 16 age-markers, such as 1,1-diethoxyethane, ethyl dodecanoate, and ethyl hexadecanoate, were found in volatile compounds. These markers could significantly differentiate BFB samples at different maturity stages. During the storage process, the total metal concentration increased, and most metal elements were positively correlated with aldehydes, esters, and ketones while being negatively correlated with acids and alcohols. Al, Fe, and Ca underwent the most significant changes during the 20-year storage period. Correlation analysis showed significant positive correlations between them and benzaldehyde, vanillin, ethyl isovalerate, and ethyl palmitate ($P < 0.01$). Additionally, there were significant negative correlations with butanoic acid, 2,3 − butanedione, 3 − methylbutanoic acid, and isobutyl acetate ($P < 0.001$).

In general, this study investigated the effects of storage time on BFB flavor and sensory characteristics. These findings can provide a theoretical foundation for enhancing the quality of BFB and are highly significant for promoting its development.

Ethical statement

The appropriate protocols were utilized to protect the rights and privacy of all participants during the execution of the research and were approved by the Ethics Committee of Xihua University. In this study, participation was not mandatory, and the research requirements and risks were fully disclosed. All participants provided verbal consent and had the option to withdraw from the study at any time.

CRediT authorship contribution statement

Qiao Huang: Writing – review & editing, Writing – original draft, Methodology, Data curation. **Ying Liu:** Validation, Investigation, Conceptualization. **Lei Tian:** Software, Formal analysis. **Fuqiang Xiong:** Visualization, Software, Methodology. **Zongjun He:** Visualization, Software, Methodology. **Yanhui Zhao:** Visualization, Software, Methodology. **Shuangquan Xiang:** Visualization, Resources, Investigation. **Xianping Qiu:** Visualization, Resources, Investigation. **Jianshen Yu:** Visualization, Resources, Investigation. **Tongwei Guan:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101667.

References

Ao, L., Guo, K., Dai, X., Dong, W., Sun, X., Sun, B., … Zheng, F. (2022). Quick classification of strong-aroma types of base Baijiu using potentiometric and voltammetric electronic tongue combined with chemometric techniques. *Frontiers in Nutrition, 9*, Article 977929. https://doi.org/10.3389/fnut.2022.977929

Bonic, M., Tešević, V., Nikicevic, N., Cvejic, J., Milosavljević, S. M., Vajs, V., … Jovanic, S. (2013). The contents of heavy metals in Serbian old plum brandies. *Journal of the Serbian Chemical Society, 78*, 933–945. https://doi.org/10.2298/JSC121106016B

Chen, P. P., Feng, X. X., Zhu, Y. W., Wu, J. H., Li, H. H., Jiang, S., … Liu, Y. (2023). Sweetness science of Baijiu: Unraveling flavor compounds, perception and analytical techniques. *Food Chemistry Advances, 3*, Article 100463. https://doi.org/10.1016/j.focha.2023.100463

Dong, W., Dai, X. R., Jia, Y. T., Ye, S. T., Shen, C. H., Liu, M., … Deng, B. (2024). Association between Baijiu chemistry and taste change: Constituents, sensory properties, and analytical approaches. *Food Chemistry, 437*, Article 137826. https://doi.org/10.1016/j.foodchem.2023.137826

Ellender, G. (2022). Biomedical gastronomy in the interventions of smell and taste disorders in 'altered eating'. *Clinical Nutrition Open Science, 46*, 29–34. https://doi.org/10.1016/j.nutos.2022.11.003

Ernesto, F. L., & Vicente, F. (2014). Quantitative analysis of free and bonded forms of volatile sulfur compouds in wine. Basic methodologies and evidences showing the existence of reversible cation-complexed forms. *Journal of Chromatography A, 1359*, 8–15. https://doi.org/10.1016/j.chroma.2014.07.011

Eszter, F. N., John, Z. Z., Lajos, S., Zoltan, K., & Miklós, P. (2023). Impacts of β-cyclodextrin bead polymer (BBP) treatment on the quality of red and white wines: Color, polyphenol content, and electronic tongue analysis. *LWT - Food Science and Technology, 176*, Article 114567. https://doi.org/10.1016/j.lwt.2023.114567

Fan, W., & Qian, M. C. (2006). Characterization of aroma compounds of Chinese "Wuliangye" and "Jiannanchun" liquors by aroma extract dilution analysis. *Journal of Agricultural and Food Chemistry, 54*(7), 2695–2704. https://doi.org/10.1021/jf052635t

Franco-Luesma, E., & Ferreira, V. (2014). Quantitative analysis of free and bonded forms of volatile sulfur compouds in wine. Basic methodologies and evidences showing the existence of reversible cation-complexed forms. *Journal of Chromatography A, 1359*, 8–15. https://doi.org/10.1016/j.chroma.2014.07.011

Hao, H. Y., Yan, R. Y., Miao, Z. J., Wang, B. W., Sun, J. Y., & Sun, B. G. (2022). Volatile organic compounds mediated endogenous microbial interactions in Chinese *Baijiu* fermentation. *International Journal of Food Microbiology, 383*, Article 109955. https://doi.org/10.1016/j.ijfoodmicro.2022.109955

Hong, J. X., Huang, H., Zhao, D. R., Sun, J. Y., Huang, M. Q., Sun, X. T., & Sun, B. G. (2023). Investigation on the key factors associated with flavor quality in northern strong aroma type of Baijiu by flavor matrix. *Food Chemistry, 426*, Article 136576. https://doi.org/10.1016/j.foodchem.2023.136576

Huang, Z. J., Zeng, Y. H., Liu, W. H., Wang, S. T., Shen, C. H., & Shi, B. (2020). Effects of metals released in strong-flavor baijiu on the evolution of aroma compounds during storage. *Food Science & Nutrition, 8*, 4. https://doi.org/10.1002/fsn3.1475

Huang, Z. J., Zeng, Y. H., Sun, Q. Y., Zhang, W. H., Wang, S. T., Shen, C. H., & Shi, B. (2022). Insights into the mechanism of flavor compound changes in strong flavor *Baijiu* during storage by using the density functional theory and molecular dynamics simulation. *Food Chemistry, 373*, Article 131522. https://doi.org/10.1016/j.foodchem.2021.131522

Iwegbue, C. M. A. (2010). Composition and daily intakes of some trace metals from canned beers in Nigeria. *Journal of the Institute of Brewing, 116*(3), 312–315. https://doi.org/10.1002/j.2050-0416.2010.tb00436.x

Jia, W., Ma, R., Hu, L. B., & Mo, H. Z. (2023). Synergy of physicochemical reactions occurred during aging for harmonizing and improving flavor. *Food Chemistry: X, 17*, Article 100554. https://doi.org/10.1016/j.fochx.2022.100554

Li, H. Q., Neumann, H., Beller, M., & Wu, X. F. (2014). Aryl Formate as bifunctional reagent: Applications in palladium-catalyzed carbonylative coupling reactions using in situ generated CO. *Angewandte Chemie International Edition, 53*, 3183–3186. https://doi.org/10.1002/anie.201311198

Li, M., Fan, W. L., & Xu, Y. (2021). Volatile compounds sorption during the aging of Chinese liquor (baijiu) using pottery powder. *Food Chemistry, 345*, Article 128705. https://doi.org/10.1016/j.foodchem.2020.128705

Morishima, K., Nakamura, N., Matsui, K., Tanaka, Y., Masunaga, H., Mori, S., … Shibayama, M. (2019). Formation of clusters in whiskies during the maturation process. *Journal of Food Science, 84, 1*, 59–64. https://doi.org/10.1111/1750-3841.14398

Myrto, P., Roel, J. T., Kleijwegt, J. S., & Maria, F. N. (2019). Furfural production by continuous reactive extraction in a millireactor under the Taylor flow regime. *Industrial & Engineering Chemistry Research, 58*(35), 16106–1611510.

Penteado, J., & Masini, J. (2009). Multivariate analysis for the classification differentiation of Brazilian sugarcane spirits by analysis of organic and inorganic compounds. *Analytical Letters, 42*, 2747–2757. https://doi.org/10.1080/00032710903060727

Pohl, P. (2007). What do metals tell us about wine? *TrAC Trends in Analytical Chemistry, 26*(9), 941–949. https://doi.org/10.1016/j.trac.2007.07.005

Qin, D., Lv, S., Shen, Y., Shi, J., Jiang, Y., Cheng, W., … Sun, B. (2023). Decoding the key compounds responsible for the empty cup aroma of soy sauce aroma type baijiu. *Food Chemistry*. https://doi.org/10.1016/j.foodchem.2023.137466

Schleicher, L. S., Miller, J. W., Watkins-Kenney, S. C., CarnesMcNaughton, L. F., & Ramsing, M. U. (2008). Nondestructive chemical characterization of ceramic sherds from Shipwreck 31CR314 and Brunswick Town, North Carolina. *Journal of Archaeological Science, 35*(10), 2824–2838. https://doi.org/10.1016/j.jas.2008.05.012

Song, X., Zhu, L., Geng, X., Li, Q., Zheng, F., Zhao, Q., … Sun, B. (2021). Analysis, occurrence, and potential sensory significance of tropical fruit aroma thiols, 3-mercaptohexanol and 4-methyl-4-mercapto-2-pentanone, in Chinese Baijiu. *Food Chemistry, 363*, Article 130232.

Soylak, M., Colak, H., Turkoglu, O., & Dogan, M. (2006). Trace metal content of snacks and appetizers consumed in Turkey. *Bulletin of Environmental Contamination and Toxicology, 76*, 3. https://doi.org/10.1007/s00128-006-0940-z

Sun, X. Z., Qian, Q. Q., Xiong, Y. Q., Xie, Q. Q., Yue, X. X., Liu, J. H., … Q.. (2022). Characterization of the key aroma compounds in aged Chinese Xiaoqu Baijiu by means of the sensomics approach. *Food Chemistry, 384*. https://doi.org/10.1016/j.foodchem.2022.132452

Sun, Y. L., Ma, Y., Chen, S., Xu, Y., & Tang, K. (2021). Exploring the mystery of the sweetness of Baijiu by sensory evaluation, compositional analysis and multivariate data analysis. *Foods, 10*, 2843. https://doi.org/10.3390/foods10112843

Tian, W. X., Liu, X. F., Zheng, J., Ma, Y., Luo, H. B., Luo, X. G., … D.Q.. (2023). Array-based sensing and discrimination of segmented Baijiu using organic molecules-regulated PEI@Ag NPs@Ln as fluorescent probes. *Food Chemistry, 417*, Article 135888. https://doi.org/10.1016/j.foodchem.2023.135888

Veena, P., Nilesh, R., Aparna, A., Vibhav, G., & Abhishek, D. T. (2023). Valorization of lignocellulosic waste (coconut coir) for bio-vanillin production having antioxidant and anticancer activity against human breast cancer cells (MCF-7). *Industrial Crops and Products, 205*, Article 117502. https://doi.org/10.1016/j.indcrop.2023.117502

Wang, L. L., Wu, L., Xiang, D. H., Huang, H., Han, Y., Zhen, P., … Xu, Y. (2023). Characterization of key aroma compounds in aged *Qingxiangxing Baijiu* by comparative aroma extract dilution analysis, quantitative measurements, aroma recombination, and omission studies. *Food Chemistry, 419*, Article 136027. https://doi.org/10.1016/j.foodchem.2023.136027

Wang, P. H., Ye, X., Liu, J., Xiao, Y., Tan, M., Deng, Y., … Han, X. (2024). Recent advancements in the taste transduction mechanism, identification, and characterization of taste components. *Food Chemistry, 433*, Article 137282. https://doi.org/10.1016/j.foodchem.2023.137282

Wang, S. Q., Li, Z. H., Bai, X. Y., Yi, W. M., & Fu, P. (2018). Influence of inherent hierarchical porous char with alkali and alkaline earth metallic species on lignin pyrolysis. *Bioresource Technology, 268*, 323–331. https://doi.org/10.1016/j.biortech.2018.07.117

Wei, L. L., Hu, J. F., Pan, C. K., Cheng, P. Y., Zhang, J., Xi, D. Z., … Hu, F. (2023). Effects of different storage containers on the flavor characteristics of Jiangxiangxing baijiu. *Food Research International, 172*, Article 113196. https://doi.org/10.1016/j.foodres.2023.113196

Xu, J. N., Guo, L., Wang, T. R., Ma, M., Wang, B. Y., Wei, X. Y., & Fan, M. T. (2022). Effect of inorganic and organic nitrogen supplementation on volatile components and aroma profile of cider. *Food Research International, 161*, Article 111765. https://doi.org/10.1016/j.foodres.2022.111765

Zhang, X. J., Meng, L. J., Lu, Z. M., Chai, L. J., Wang, S. T., Shi, J. S., … Xu, Z. H. (2021). Identification of age-markers based on profiling of Baijiu volatiles over a two-year maturation period: Case study of Lu-flavor Baijiu. *LWT- Food Science and Technology, 141*, Article 110913. https://doi.org/10.1016/j.lwt.2021.110913

Zheng, X. W., & Han, B. Z. (2016). *Baijiu*, Chinese liquor: History, classification and manufacture. *Journal of Ethnic Foods, 3*(1), 19–25. https://doi.org/10.1016/j.jef.2016.03.001

Zhou, Z. H., Liu, Z. H., Wen, S. Y., Ouyang, G. W., Shen, Y. X., Yang, Q., … Xu, Y. (2023). Rare short- and medium-chain fatty acid-producing anaerobes from raw soil play vital roles in formation of diverse flavour compounds of Jiangxiangxing Baijiu. *Food Microbiology, 112*, Article 104247. https://doi.org/10.1016/j.fm.2023.104247

Zhu, L., Wang, X. L., Song, X. B., Zheng, F. P., Li, H. H., Chen, F., … Zhang, F. Y. (2020). Evolution of the key odorants and aroma profiles in traditional Laowuzeng baijiu during its one-year ageing. *Food Chemistry, 310*, Article 125898. https://doi.org/10.1016/j.foodchem.2019.125898

Zou, W., Zhao, C., & Luo, H. (2018). Diversity and function of microbial community in Chinese strong-flavor Baijiu ecosystem: A review. *Frontiers in Microbiology, 9*, 671. https://doi.org/10.3389/fmicb.2018.00671