Food Chemistry: X 26 (2025) 102331

Contents lists available at ScienceDirect

Food Chemistry: X

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

Identification of key differential compounds in different quality grades of base liquor during the production of Jiang-flavored baijiu

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ARTICLE INFO

Keywords:
Base liquor
Jiang-flavored baijiu
Fermentation rounds
Quality grades
Sensory evaluation
GC-FID
K-means clustering

ABSTRACT

The production process of Jiang-flavored Baijiu is highly elaborate, and the flavor characteristics and quality of the base liquor (BL) exert a substantial impact on the quality of the final product. A total of 130 typical samples from three quality grades within seven fermentation rounds were examined. Based on sensory evaluation, gas chromatography-flame ionization detection (GC-FID) technology was employed to detect 59 principal volatile compounds within the samples. Subsequently, K-means clustering, partial least squares discriminant analysis, and odor activity values were combined to analyze the compound variation trends and key differentiating compounds. The results demonstrated that in the seven fermentation rounds, 10 key differentiating compounds significantly affect the BL quality; in each round, 6 compounds markedly influence the quality level. Among them, the contents of acetic acid, ethyl acetate, lactic acid, and acetaldehyde were correlated with the BL quality grade and have the potential to act as quality indicators.

Differential compounds

Ethyl acetate (PubChem CID: 8857)
Ethyl lactate (PubChem CID: 7344)
N-propanol (PubChem CID: 1031)
N-butanol (PubChem CID: 263)
Isobutanol (PubChem CID: 6560)
3-Methylbutanol (PubChem CID: 31260)
Acetic acid (PubChem CID: 176)
Acetaldehyde (PubChem CID: 177)
Acetal (PubChem CID: 7765)
3-Hydroxy-2-butanone (PubChem CID: 179).

1. Introduction

Chinese baijiu boasts a long historical background and a rich variety of types. Among them, Jiang-flavored Baijiu has acquired an important position in the Chinese baijiu market due to its distinctive flavor and traditional brewing process. In 2023, the production capacity of Jiang-flavored Baijiu reached 750,000 kiloliters, with an increase of 7.1 %, and achieved sales revenue of 23 billion yuan, with a growth of 9.5 % (Yang, 2024). Its influence in both domestic and international markets is constantly on the rise.

Jiang-flavored Baijiu possesses an intense sauce aroma, and its production process includes eight rounds of fermentation operations to obtain seven batches of base liquor (BL), followed by at least three years of storage and blending to produce the finished product (Duan et al., 2022). In accordance with the sequence of BL collection, BL from the 1st to the 7th fermentation round (FR) are acquired. Each FR of BL is classified into diverse quality grades in accordance with its sensory quality. During the production process, the JiuPei (fermented grains) undergoes multiple fermentations and distillations, leading to significant changes in its chemical composition, resulting in differences in the flavor profiles of BL from different FRs.

The BL of the 1st and 2nd FRs exhibit high alcohol content and low ester content, presenting an astringent and spicy taste, and featuring prominent sour aroma and grain-like aroma; The BL of the 3rd to 5th FRs hold a balanced content of diverse compounds, being characterized by a

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https://doi.org/10.1016/j.fochx.2025.102331
Received 20 November 2024; Received in revised form 15 February 2025; Accepted 25 February 2025
Available online 27 February 2025

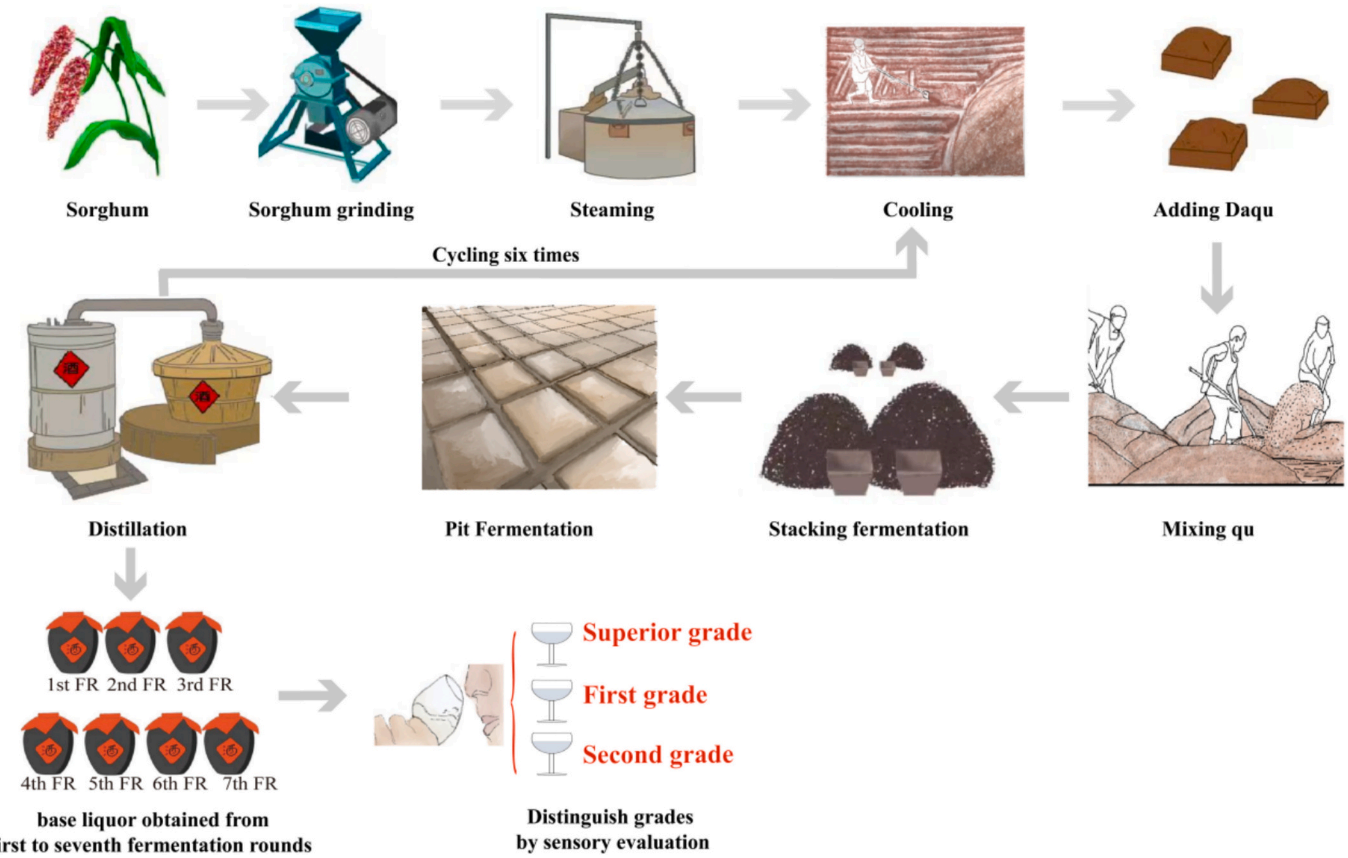

Fig. 1. The production process of Jiang-flavored base liquor. The base liquor obtained from the 1st to 7th fermentation rounds were categorized into superior, first, and second grades by sensory evaluation. FR1-FR7 represent the base liquor from the 1st to 7th fermentation rounds; 'sup', '1st', and '2nd' correspond to the superior, first, and second quality grades of base liquor, respectively.

rich and mellow taste as well as harmonious sauce aroma, and represent the highest quality among all BL. In contrast, the BL of the 6th to 7th FRs have a high content of alcohol and esters and a low acid content, along with prominent roasted and baked aroma (Liu et al., 2023). In accordance with the local recommended standard "Technical Specifications for Quality Evaluation of Base liquor of Jiang-flavored Baijiu" (DB52/T 1767–2023), the quality of BL of each FR is classified hierarchically as superior, first, and second quality grades (sup, 1st and 2nd quality grades), in descending order. The BL being of higher quality, the contents of esters, alcohols, aldehydes, ketones, and aromatic hydrocarbons are richer, and the aromas of sauce, flower, and fruit are more obvious and harmonious (Wu et al., 2023). Therefore, analyzing the differential compounds of BL across various FRs in the production process that show significant changes helps to have a deeper understanding of the chemical foundations underlying the sensory differences among BL. Meanwhile, the BL is an important part in the blending of finished liquor, and its quality directly affects the rate of high-quality finished liquor.

The detection of volatile compounds is mainly carried out by the combination of chromatographic separation technology and various detectors. Among them, the gas chromatography-flame ionization detection (GC-FID) technique has the advantages of high accuracy and effective detection of volatile compounds, and therefore has a wide range of applications in the flavor analysis of foods (Li et al., 2024). For instance, GC-FID was employed to detect adulteration of eucalyptus oil in Moroccan rosemary essential oil (El Mrabet et al., 2024) and to identify bioactive compounds in methanol leaf extracts (Nkwocha et al., 2024). Similarly, GC-FID was utilized to analyze the compositional differences between traditionally handcrafted and mechanized Baijiu. The analysis revealed that handcrafted Baijiu contains higher levels of esters and alcohols, whereas mechanized Baijiu exhibits greater concentrations of acids and aldehydes (Wu et al., 2024). Meanwhile, statistical modeling based on chemical composition is an important way to explore the chemical basis and key chemical compounds underlying the sensory quality differences among different Baijiu samples. Gong et al. analyzed Jiang-flavored Baijiu from different production areas in the Chishui River Basin via Partial Least Squares Discriminant Analysis (PLS-DA), and the results showed that butyric acid and ethyl hexanoate were the key compounds causing the aroma differences of Baijiu in different regions (Gong et al., 2023). Huang et al., upon the detection of Jiang-flavor Baijiu from different origins like Guizhou and Sichuan using GC-FID, further identified regional markers through Principal Component Analysis (PCA) and Odor Activity Value (OAV value) (Huang et al., 2023). More, Wu et al. employed GC–MS to detect the BL of the 3rd to 5th FRs of Jiang-flavored Baijiu and explored the compositional distinctions of BL of different quality grades (Wu et al., 2023). The above research offered a reliable approach for the smooth conduct of this study, establishing a good basis for further analyzing the quality grade disparities among BL of 7 FRs in the production process of Jiang-flavored Baijiu.

Consequently, a differential analysis of the composition in different quality grades of Jiang-flavored BL is necessary. Different quality grades of BL obtained from 7 FRs were collected in this study. First, the flavor profiles of samples were evaluated by sensory analysis. Second, volatile compounds were detected by GC-FID, and the trend of the compounds was visualized by K-means clustering. Finally, multivariate statistical analyses integration with OAV identified the key differential compounds of different quality grades of BL in the production process, establishing a basis for in-depth understanding of baijiu production processes and providing a reference for quality control of production.

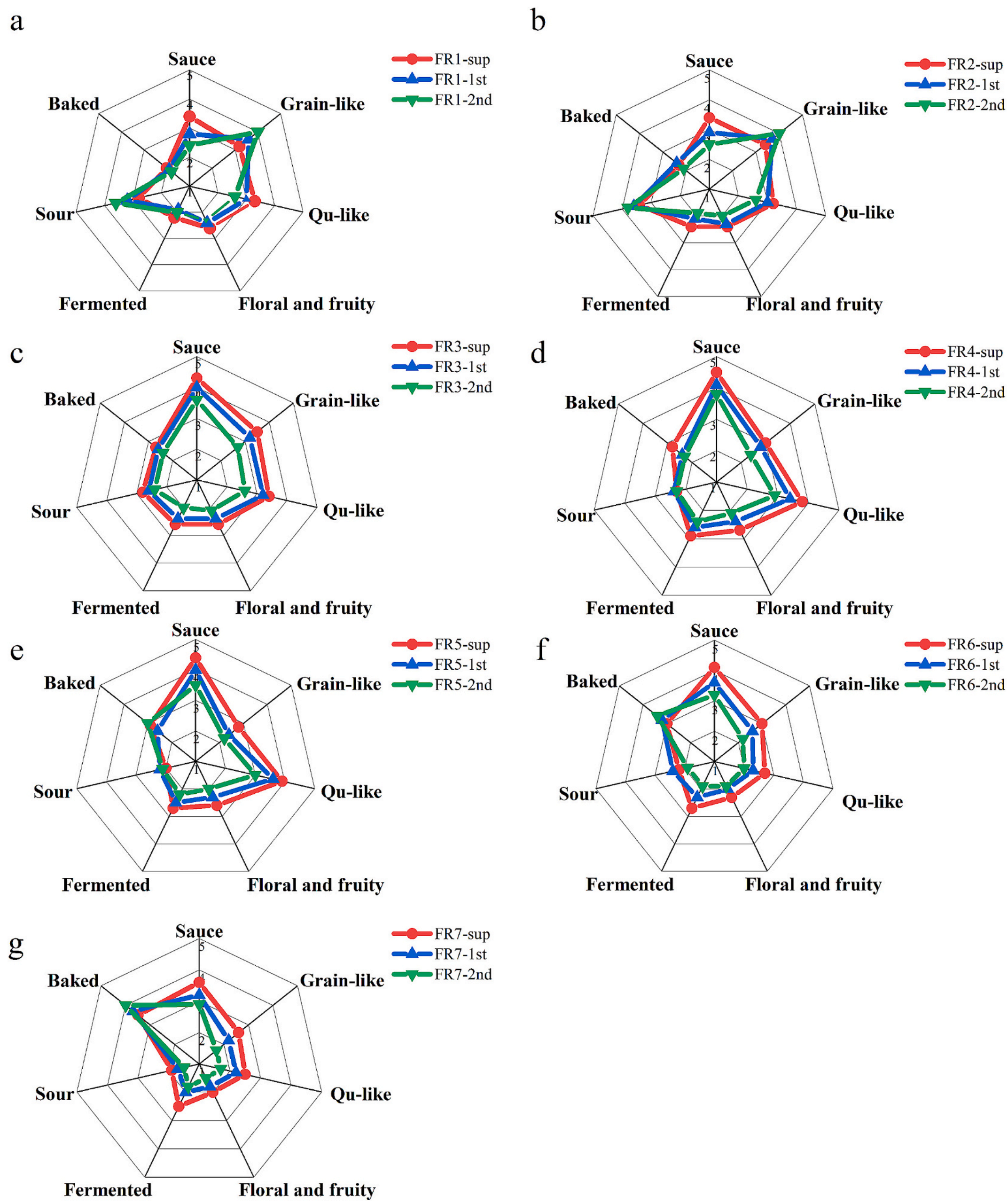

Fig. 2. Sensory analysis of different quality grades of base liquor. Radar map of sensory indexes of base liquor coming from the 1st (a), 2nd (b), 3rd (c), 4th (d), 5th (e), 6th (f), 7th (g) fermentation rounds. FR1-FR7 represent the base liquor from the 1st to 7th fermentation rounds; 'sup', '1st', and '2nd' correspond to the superior, first, and second quality grades of base liquor, respectively.

2. Materials and Methods

2.1. Chemicals and reagents

Anhydrous ethanol of chromatographic grade (purity ≥99.97 %) was obtained from Tianjin Kemio Chemical Reagent Co., Ltd. (Tianjin, China). The standard chemicals used in this study were also of chromatographic grade (purity ≥98 %). These included tert-amyl alcohol (Internal standard, IS1), n-amyl acetate (IS2), 2-ethylbutyric acid (IS3), acetaldehyde, ethyl acetate, n-propanol, 3-hydroxy-2-butanone, acetal, n-butanol, propyl acetate and others. All these chemicals were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). The C7-C30 alkane standard solution (chromatographic grade; purity ≥99.97 %) was sourced from Sigma Aldrich (Beijing, China).

2.2. Samples collection

The 130 samples were collected from the Renhuai production area, which is one of the key regions for Jiang-flavored Baijiu production in Guizhou Province, China (N 27°33′30″-28°10′19″ and E105°59′49″-106°35′50″). BL samples were obtained from the 1st to the 7th distillation of JiuPei (fermented grains) at the same distillery in 2022 (Fig. 1). The collected samples were securely sealed and stored under controlled conditions at approximately 20 °C in a dark environment until utilized for analysis.

2.3. Sensory evaluation

The sensory evaluation team consisted of ten professionally qualified and experienced individuals, aged between 30 and 50 years. After being fully briefed on the research objectives and methodologies, all participants provided informed consent to participate in this study and agreed to the recording and use of their data. The study was conducted in strict adherence to ethical guidelines, ensuring that no ethical issues arose during the experimental process. Following the national standard GB/T 33404–2016 "Guidelines for Sensory Evaluation of liquor" and the group standard T/GZRHJX 001–2019 "Renhuai Daqu Jiang-Flavored Base liquor of the First to Seventh Fermentation Round," different BL were graded based on their aroma and taste characteristics. The aroma indexes primarily included sauce aroma, grain-like aroma, Qu-like aroma, fermented aroma, sour aroma, flower and fruit aroma, and burnt aroma. A five-point scale was employed for scoring with higher scores indicating more pronounced sensory characteristics. The average value was utilized to generate a sensory radar map for visualization analysis.

2.4. Volatile compounds analysis

2.4.1. Preparation of mixed internal standard solution

The mixed internal standard solution (IS1, 80.9 mg/L; IS2, 90.3 mg/L; IS3, 95.6 mg/L) was accurately prepared by adding 1 mL of the standard product to a 100 mL volumetric flask. Subsequently, the flask was filled with a 60 % ethanol solution up to the calibration mark, tightly sealed, and thoroughly mixed for future use.

2.4.2. Detection of volatile compounds by GC-FID

10 μL of the mixed internal standard solution and 990 μL of BL were transferred into a 2 mL injection vial, which was then sealed and agitated. Volatile compounds were detected using GC-FID (Agilent 7890 A, Agilent Technologies Inc., Palo Alto, CA, US) equipped with a DB-FATWAX Ultra Inert GC column (30 m × 0.25 mm × 0.25 μm). A 1 μL of sample volume was injected into the separation mode (30:1 *v*/v), with helium (purity ≥99.999 %) employed as the carrier gas at a constant flow rate of 1.0 mL/min. The flow rates for hydrogen and air were set at 30 mL/min and 300 mL/min respectively. The temperature settings for the injection port and FID detector were maintained at 235 °C and 240 °C respectively throughout the analysis process. The temperature program used in GC-FID consisted of an initial hold at 30 °C for 2 min, followed by a ramping rate of 3.0 °C/min until reaching 180 °C and finally increasing at 15 °C /min to 210 °C for a further 10 min.

2.4.3. Qualitative and quantitative analysis of volatile compounds

The volatile compounds were qualitatively analyzed by comparing the retention times of their peaks in the samples with those of known standards under identical chromatographic conditions. For quantitative analysis, stock solutions of each standard compound were prepared by dissolving them in a 60 % (*v*/*v*) ethanol solution and subsequently serially diluted to five distinct concentration levels. The resulting gradient solutions (990 μL) were then spiked with 10 μL of internal standard solution prior to GC-FID analysis. The standard curve for each compound was constructed by plotting the ratio of the standard concentration to the internal standard concentration on the x-axis against the corresponding peak area ratio on the y-axis. In accordance with China's national recommended standards (GB/T 5009.1–2003), the repeatability of the method was assessed by calculating the relative standard deviations (RSDs) from three replicate measurements for each compound. The limit of detection (LOD) was established as three times the standard deviation of the blank values, and the limit of quantification (LOQ) as ten times the standard deviation of the blank values. Furthermore, recovery rates were evaluated through spiking experiments by adding known quantities of standard compounds to the samples and subsequently analyzing them. The results pertaining to repeatability, LOD, LOQ, and recovery for each compound are summarized in Table S1 of the supplementary materials.

2.5. OAV calculation

The OAV of a volatile compound was determined by dividing its concentration (C) by the odor threshold (OT). Generally, compounds with an OAV ≥ 1 are considered significant contributors to the aroma of the sample (Gong et al., 2023; Mu et al., 2023).

2.6. Statistical analysis

The radar plots, histograms, and line graphs were generated using OriginPro 9.0 (OriginLab Corporation, Northampton, MA, USA). Standard score (z-score) processing of the data were performed using R 4.2.1 software (Auckland University, Auckland, New Zealand), and K-means clustering were generated using OriginPro 9.0 (OriginLab Corporation, Northampton, MA, USA). Variable importance projection (VIP) values were calculated using SIMCA 17.0 (Umetricus Inc., Sweden). Compounds with VIP values >1 were identified as differential compounds. The Venn diagram was created using the Omicshare cloud platform (https://www.omicshare. com/). One-way analysis of variance (ANOVA) followed by Duncan's test for mean comparison was conducted to assess differences at a significance level of $p < 0.05$ using SPSS 22.0 software (SPSS Inc., Chicago, IL).

3. Result and discussion

3.1. Sensory evaluation

By sensory evaluation, the sensory profiles of different quality grades of BL derived from different FRs are presented in Fig. 2. The aroma profiles of BL from the 7 FRs fall into three distinct categories. The BL obtained from the 1st and 2nd FRs exhibit a more pronounced grain-like aroma and sour aroma (Fig. 2a-b). As depicted in Fig. 2c-e, the aroma characteristics of the BL from the 3rd, 4th, and 5th FRs are harmonious and free of off-flavors, particularly showcasing notable sauce aroma and Qu-like aroma. In contrast to other FRs, the BL produced from the 6th and 7th FRs display a prominent baked aroma and roasted aroma while presenting less obvious Qu-like aroma, grain-like aroma, and sour aroma (Fig. 2f-g). Evidently, as the number of FRs increases along with high-temperature distillation, there is a gradual decrease in the intensity of grain-like aromas in the obtained BL samples accompanied by an increase in the prominence of baked aroma. Furthermore, within each FR, higher-quality grade BL demonstrates stronger sauce aroma. In contrast to the relationship observed between grain-like aromas and quality grade for samples from the 1st and 2nd FRs; roasted aroma also appears stronger in low-grade BL samples during the 6th and 7th FRs, indicating that an excessive presence of either outstanding grain-like or roasted aromas is not conducive to overall aromatic coordination, leading to a diminished quality of BL.

The fluctuations in the sensory profile of BL during production reflected changes in the composition and concentration of compounds presented in the fermented grain, influenced by microbial metabolism and biochemical reactions. As a primary raw material for production, sorghum is rich not only in branched starch but also in polyphenolic compounds such as tannins, which supply essential carbohydrates and aroma precursors necessary for fermentation. The degradation products of these polyphenolic compounds, including butyraldehyde and vanillin, impart a grain-like aroma and fruity notes to BL (Zhao et al., 2024). The addition of Daqu with rich flavor compounds gives BL its Qu-like aroma. The sour aroma observed in BL primarily originates from volatile organic acids like acetic acid, propionic acid, butyric acid, and hexanoic acid (Zhao et al., 2018). The fermented aroma presented by BL should be related to the accumulation of esters and acids that were newly produced during the fermentation process (Duan et al., 2022). Consequently, it is essential to investigate the chemical foundations underlying the variations in sensory quality of BL.

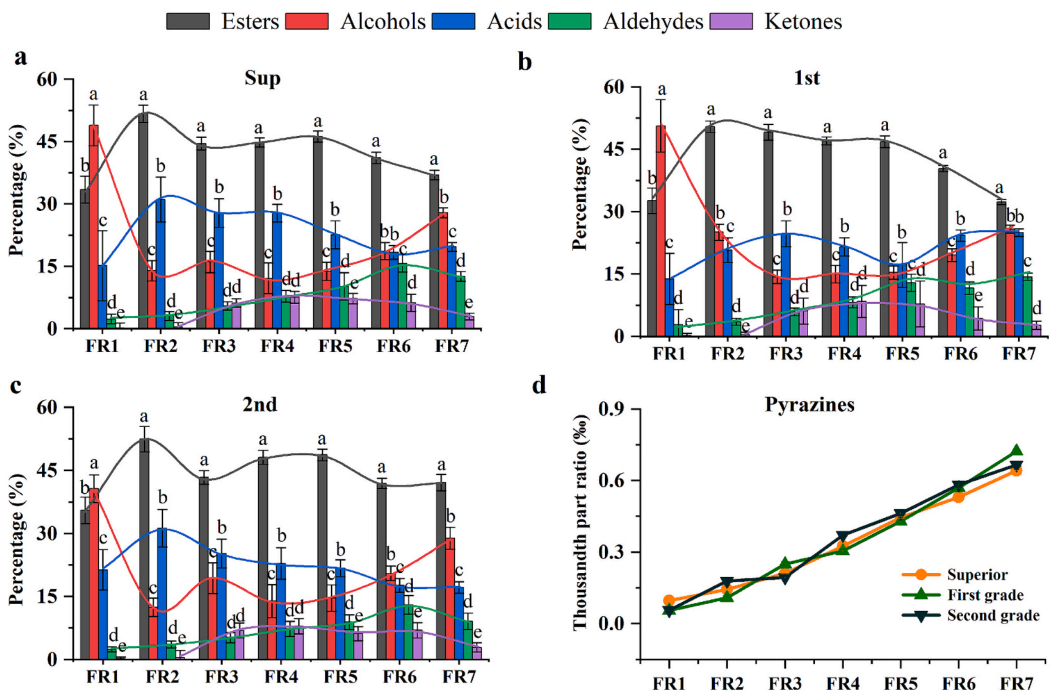

Fig. 3. The percentages of volatile compounds in the superior (a), first (b), and second (c) quality grades BL from the 1st to the 7th FRs are illustrated in bar charts; the percentage of pyrazine compounds is depicted in a line graph (d).
FR1-FR7 represent the base liquor from the 1st to 7th fermentation rounds; 'sup', '1st', and '2nd' correspond to the superior-grade, first-grade, and second-grade quality base liquor, respectively. Significance: a, b, c, d, e: there were significant differences ($p < 0.05$) in the contents of compounds with different letters. The average contents with 'a' were the highest and 'b', 'c', 'd', 'e' descended sequentially.

3.2. Volatile compounds profile of the samples

Owing to the distinctive production process of Jiang-flavored Baijiu, variations in starch and reducing sugar content, as well as fermentation degree from seven FRs, result in diverse compositions and concentrations of volatile compounds within BL, consequently leading to differing sensory profiles. A total of 59 volatile compounds were quantified by GC-FID, including 22 esters, 15 alcohols, 10 acids, 8 aldehydes, 2 ketones and 2 pyrazines. The fluctuations in the volatile compounds content of BL samples across different FRs are depicted in Fig. 3. It is apparent that the trend of compound variation remains consistent throughout BL production, regardless of the differences in quality grades. Notably, alcohols constitute the highest percentage during the 1st FR, while esters and acids become predominant from the 7nd FR onward. The concentration of carbonyl compounds gradually increases with fermentation progression before declining at the seventh FR. In contrast, pyrazines exhibit a continuous increase throughout the entire production process.

The composition and proportion of various compounds in BL play a crucial role in achieving a well-balanced flavor profile. Moderate levels of higher alcohol in liquor contributed pleasant floral and fruity aromas, while higher concentrations of alcohol resulted in an unpleasant odor, such as the "alcoholic" and "nail polish" notes associated with elevated levels of 3-methylbutanol (Wang et al., 2015). The highest quality BL was obtained from the 3rd to 5th FRs, characterized by lower alcohol proportions and higher ester content. This suggested that a higher proportion of esters combined with a lower proportion of alcohols contributed to superior BL quality. The accumulation of acids was closely linked to carbohydrate metabolism by microorganisms. Different types of acids, particularly low molecular weight ones, made the distinct contributions to liquor flavor profiles(Miao et al., 2022). For instance, acetic acid imparted a unique sour taste while caproic acid added a subtle "sweat odor". Furthermore, aldehydes and ketones were significant contributors to Baijiu's flavor profile (Wu et al., 2023). Furfural provided an almond-like aroma whereas 2-methylbutyral contributed cocoa and malt flavors (Niu et al., 2020). According to relevant studies, there were currently two primary pathways for the production of pyrazines in Baijiu. During Qu-making and fermentation stages, pyrazines were predominantly generated through microbial metabolism, whereas during the distillation, they were primarily formed via the Maillard reaction (Shi et al., 2022). Wu et al. found that during the production process of Jiang-flavored Baijiu, the relative abundance of the dominant genera such as Bacillus in the fermented grains gradually increased with the progression of fermentation rounds (Wu et al., 2023). Bacillus possesses the good capability to synthesize pyrazine compounds, which correlates with the observed increase in pyrazine content in the base liquor during the later stages of Jiang-flavored Baijiu production. In addition, the repeated distillation processes during production can also induce the Maillard reaction. As the number of fermentation rounds increases, the accumulation of Maillard reaction products also rises, which might a primary factor contributing to the increased generation of pyrazine compounds (Yan et al., 2020). Given that pyrazine compounds were recognized as significant contributors to the burnt and roasted aromas in Jiang-flavored Baijiu (Zhao et al., 2019), it follows that during the later stages of production (the 6th–7th FRs), an increase in pyrazine content correlated with pronounced characteristics of burnt and roasted flavors in the BL.

3.3. Identification of key differential compounds between seven fermentation rounds of base liquor

Building upon the analysis of various compounds in BL, the identification of key differential compounds responsible for alterations in sensory characteristics was essential for elucidating the mechanisms governing component changes during the fermentation process. Therefore, a comprehensive analysis of the trends and differences in how the BL compound varies in each FR was essential. *Z*-score standardization proved suitable for data with varying magnitudes to ensure

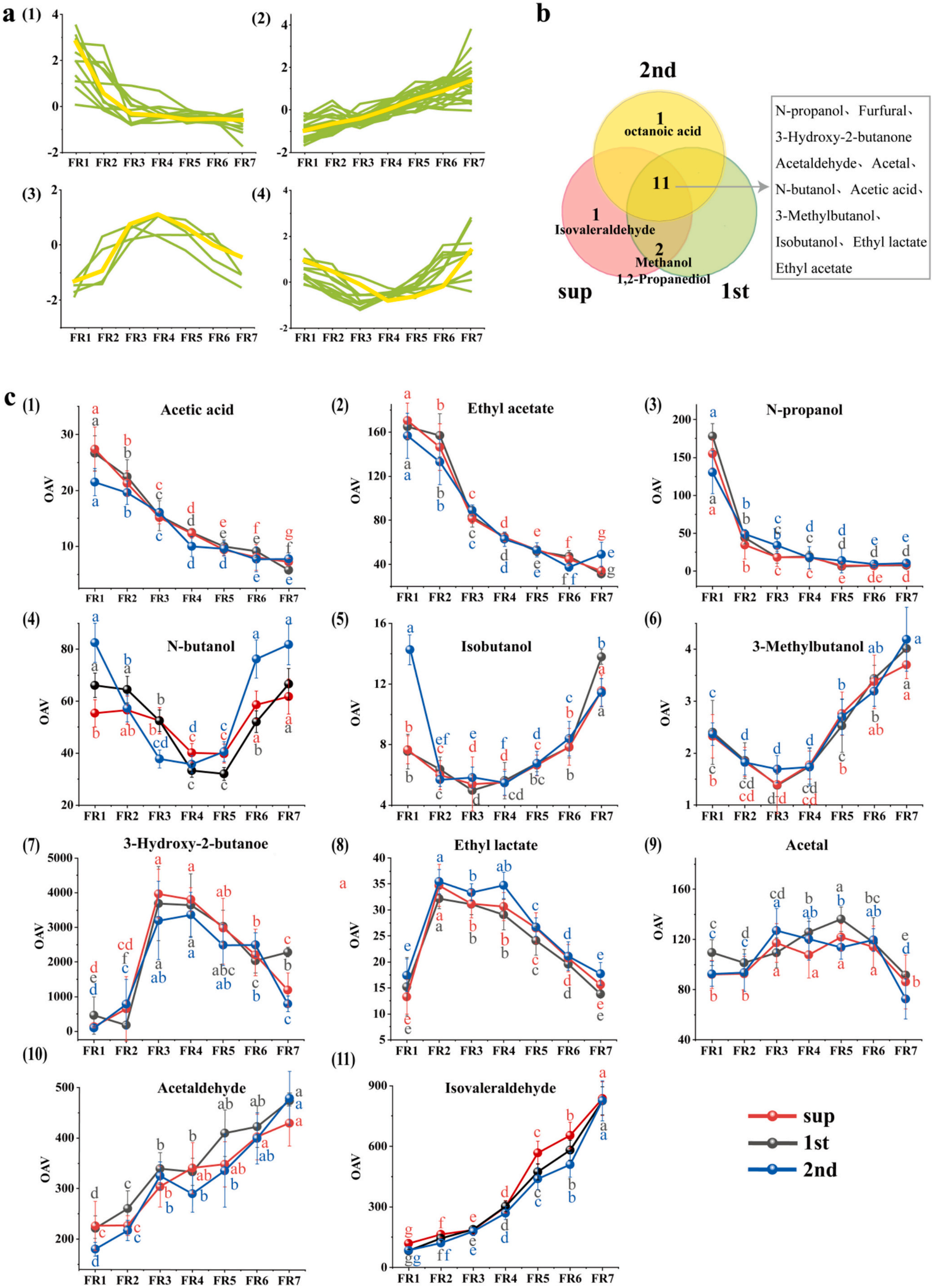

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Fig. 4. K-means clustering graph of dynamic change trend of volatile compounds concentration with the increase of fermentation rounds (a), Venn diagram of differential compounds (b) and line graphs of differential compounds with OAV value >1 (c) of BL from FR1-FR7.
In the K-means clustering chart, the green and yellow lines represent the trends of individual compounds and overall compounds, respectively.
FR1-FR7 represent the base liquor from the 1st to 7th fermentation rounds; 'sup', '1st', and '2nd' correspond to the superior-grade, first-grade, and second-grade quality base liquor, respectively. Significance: a, b, c, d, e, f, g: there were significant differences ($p < 0.05$) in the contents of compounds with different letters. The average contents with 'a' were the highest and 'b', 'c', 'd', 'e', 'f', 'g' descended sequentially. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

comparability in analyses. K-means clustering effectively assesses trends and patterns in compound content (Zhou et al., 2022), similarly illustrating that variations in three amino acids during tea production correspond with changes in tea color (Chen et al., 2024). Therefore, z-score normalization was applied to the data, and the k-means clustering method was employed to assess the trends of volatile compounds in BL across various FRs, aiming to elucidate the relationship between changes in volatile compounds and the production process. The change trend of volatile compounds of BL from 1st to 7th FRs could be classified into four clusters, as shown in Fig. 4. Subclass 1 demonstrates a decreasing trend (Fig. 4a-1) with seven compounds: propyl acetate, ethyl acetate, 2-butanol, n-propanol, 2-pentanol, acetic acid and propionic acid. Subclass 2 displays an increasing trend (Fig. 4a-2) with twenty-two compounds including but not limited to: 2,3,5-trimethylpyrazine, furfural, benzaldehyde and 2,3,5,6-tetramethylpyrazine. Subclass 3 exhibits an initial increase followed by a decrease in trend (Fig. 4a-3), comprising four compounds namely ethyl lactate, acetal, 3-hydroxy-2-butanone and isovaleric acid. Subclass 4 shows a decreasing followed by an increasing trend (Fig. 4a-4), consisting of five compounds namely isobutanol, 2-methylbutanol, 3-methylbutanol, isoamyl acetate, and valeric acid.

To identify the compounds that changed significantly and contributed to the differences observed in samples from the 7 FRs. In this study, BL of 7 FRs were analyzed by PLS-DA (Fig. S1), and differential compounds were screened according to VIP > 1.0, $p < 0.05$ (Sun et al., 2024). The selected differential compounds were listed in Table S2 of supplementary materials. The results showed that there were 15, 14 and 13 differential compounds in BL of superior, first and second quality grades respectively, among which 11 were co-existing as shown in Fig. 4b. In order to evaluate the contribution of different compounds to the aroma of BL, the OAV of each differential compound calculated and listed in Table S3. Among the 11 co-existing differential compounds, 10 exhibited an OAV greater than 1, comprising 2 esters (ethyl acetate and ethyl lactate), 4 alcohols (n-propanol, n-butanol, isobutanol, and 3-methylbutanol), 1 acid (acetic acid), 2 aldehydes (acetaldehyde and acetal), and 1 ketone (3-hydroxy-2-butanone).

Following the analysis of differences among 7 FRs of BL, it was revealed that each quality grade of BL contained 10 common differential compounds with an OAV > 1. It was demonstrated that these compounds played a significant role in aroma differences among BL of all quality grades during the production process. To further investigate grade-specific distinctions, an OAV analysis was conducted on the differential compounds unique to each quality grade of BL. As illustrated in Fig. 4b, isovaleraldehyde and octanoic acid were identified as unique differential compounds for the superior and second quality grades BL, respectively, while methanol and 1,2-propanediol were found to be common differential compounds in both the superior and first quality grades of BL. Notably, isovaleraldehyde emerged as the unique differential compound with an OAV > 1 specific to the superior-grade BL (Table S3), highlighting its critical role in shaping the superior-grade BL's distinct aroma profile.

During the production process, the composition within JiuPei undergoes continuous transformations due to microbial activity, resulting in variations in the compounds of the BL and ultimately leading to differences in sensory profile of BL. The compounds of BL across different quality grades exhibited similar trends in their changes during production. There were 10 co-existing differentiated compounds with OAV > 1 that were considered to be key factors that could effectively explain the changes in the sensory characteristics of different FRs. Among these compounds, acetic acid, ethyl acetate, and n-propanol exhibited elevated OAV values during the1st and 2nd FRs, followed by a continuous decline. This trend aligned with the pronounced sour and grain-like aroma characteristics observed in the BL from these FRs, a finding corroborated by the report of Ding et al. (Ding et al., 2024). In Jiang-flavored Baijiu, the acid content was significantly higher than that of other flavored baijiu (Song et al., 2017). Acetic acid, a precursor for important esters like ethyl acetate, can be produced by yeasts and lactic acid bacteria (Wu et al., 2023). In the stacked fermentation of Jiang-flavored Baijiu, *Saccharomyces cerevisiae* and Lactobacillus spp. were identified as the dominant genera closely associated with the production of lactic and acetic acids (Li et al., 2020). n-Propanol contributes fresh fruity and mellow flavors, with its highest OAV (154.33 ± 19.6) observed in the 1st FR. N-propanol exhibited the highest OAV in the BL of 1st FR, characterized by a fresh floral and fruity aroma profile. Its strong volatility could enhance the permeability of acidic compounds, thereby accentuating the sour aroma of BL (Wei et al., 2020). N-butanol (Fig. 4c-4), isobutanol (Fig. 4c-5) and 3-methylbutanol (Fig. 4c-6),with fruit and mellow characteristics (Wang et al., 2024), showed a decreasing and then increasing trend during the production process. Alcohols in baijiu might be mainly produced via the amino acid Ehrlich pathway (Niu et al., 2022). Similarly, in the study by Wei et al., it was found that the genes involved in isobutanol biosynthesis by *Saccharomyces cerevisiae* and *Pichia kudriavzevii* in the fermented mash had the lowest expression in the 4th FR (Wei et al., 2024). Conversely, the OAV values of 3-hydroxy-2-butanone (Fig. 4c-7), ethyl lactate (Fig. 4c-8), and acetal (Fig. 4c-9) exhibited an up-and-down trend throughout the process. It was important to highlight that 3-hydroxy-2-butanone, characterized by butter and grass aromas, exhibited an OAV reaching up to 3000. This compound was synthesized through the dehydrogenation and reduction of acetoacetic acid derived from amino acid and carbohydrate metabolism (Maina et al., 2022). Ethyl lactate imparts fruity, herbaceous, and sweet flavors to baijiu, which is formed by the esterification reaction between lactic acid and ethanol and is an important flavor (Wang et al., 2020). Acetal is synthesized through the condensation reaction between acetaldehyde and ethanol, serving as a crucial flavor compound in baijiu due to its ability to enhance both the complexity and perceptibility of aroma (Arias-Pérez et al., 2021). Corresponding, acetaldehyde showed an increasing trend and had a high OAV (456.75 ± 22.11) at the 7th FR (Fig. 4c-11), which might be accumulated by the degradation of acetal or the oxidation of ethanol. Additionally, isovaleraldehyde as the unique differentiated compounds found in the superior quality grade BL (Fig. 4c-11), exhibited characteristic nutty, malt, and toasted aromas (Zhang et al., 2021). Isovaleraldehyde primarily arises either from the deamination of branched-chain amino acids (such as valine) or from the degradation of unsaturated fatty acids (Chan & Reineccius, 2005). In summary, the variations in key differential compounds throughout the production process were intricately linked to the flavor transformations of the BL.

3.4. Identification of key differential compounds between superior, first and second quality grades BL

Owing to the variations in microbial metabolism within the JiuPei, the composition and concentration of chemical components in the BL from the same FRs exhibit differences, thereby impacting its quality. The relationship between compounds and quality grades within identical

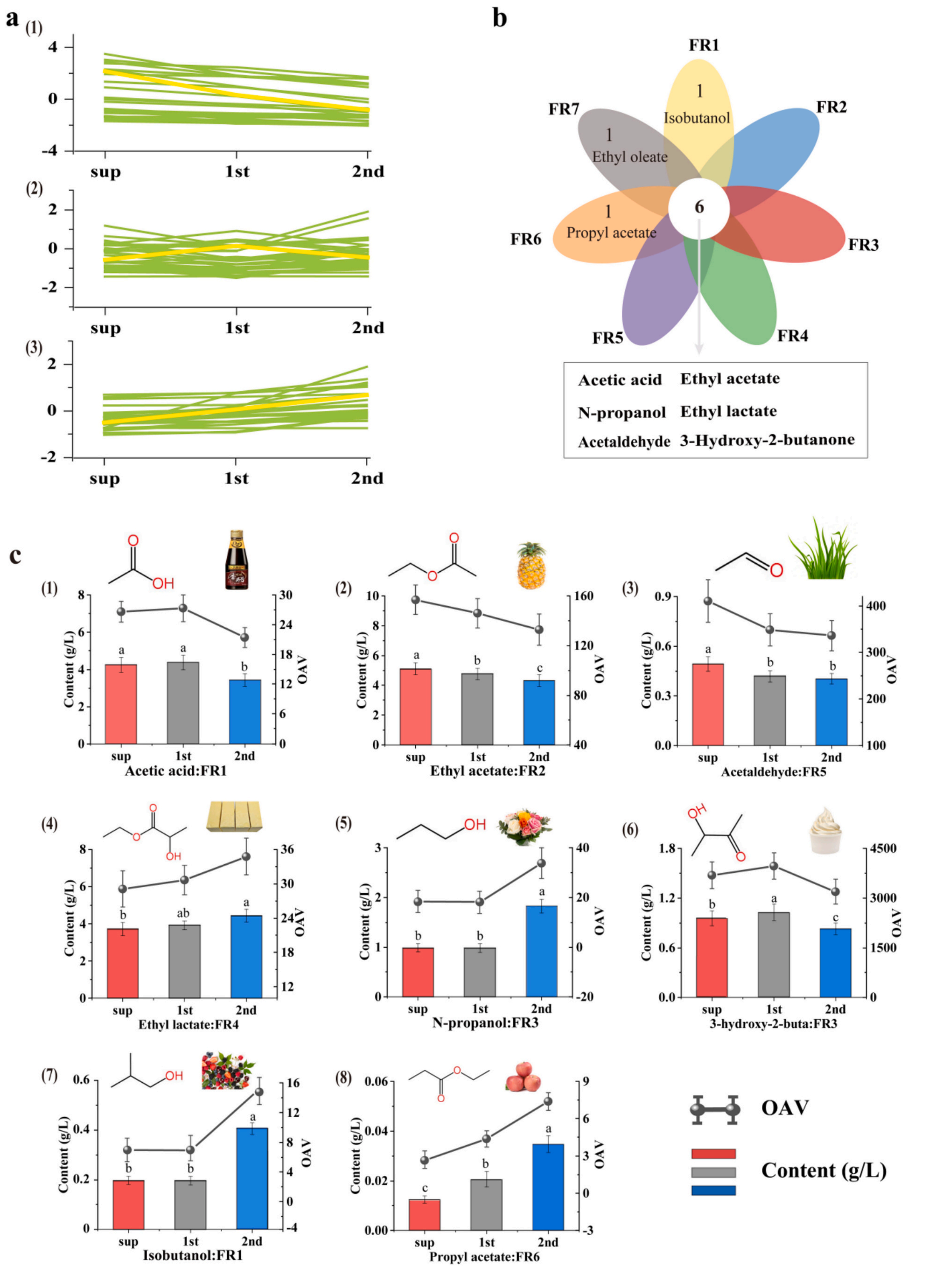

Fig. 5. Relationship between volatile compounds and the quality grades of base liquor in different fermentation rounds: cluster graph of dynamic change trend of volatiles (a), Venn diagram of differential compounds (b), two-coordinate plots of differential compounds with OAV > 1 (c). FR1-FR7 represent the base liquor from the 1st to 7th fermentation rounds; 'sup', '1st', and '2nd' correspond to the superior, first, and second quality grades of base liquor, respectively.

FRs was visualized using K-means clustering (Fig. 5a), resulting in three primary subclusters. Subcluster 1 displays a decreasing trend with four compounds: acetic acid, ethyl acetate, isobutyraldehyde, and hexanal (Fig. 5a-1). Subcluster 2 reveals an irregular trend with ten compounds including but not limited to hydroxy-2-butanone and ethyl oleate (Fig. 5a-2). Subcluster 3 demonstrates an increasing trend with four compounds: ethyl lactate, ethyl laurate, ethyl stearate, and phenethyl acetate (Fig. 5a-3). Through PLS-DA and OAV analysis (Table S4, S5), 6 co-existing differential compounds—acetic acid, ethyl acetate, n-propanol, 3-hydroxy-2-butanone, ethyl lactate, and acetaldehyde—were recognized as critical indicators of quality grade differences that coexist across all seven FRs. Additionally, isobutanol and propyl acetate were identified as unique compounds present in the 1st and 6th FRs respectively; these have significant impacts on quality grading due to their OAV > 1. Ultimately, based on the performance of each differentiating compound across FRs, a specific FR was selected for visualization of its differentiated compound content along with OAV as illustrated in Fig. 5c.

The concentrations and aroma contributions of compounds cause quality variations in BL. Among the 6 co-existing differential compounds, the concentrations of acetic acid (Fig. 5c-1), ethyl acetate (Fig. 5c-2), and formaldehyde (Fig. 5c-3) significantly declined ($p < 0.05$) with decreasing quality. Acetic acid is a crucial flavor compound in baijiu, with a strong sour aroma. At certain concentrations, it enhances BL's complexity and depth and is a precursor to important esters like ethyl acetate and ethyl lactate. Ethyl acetate, with fruity and pineapple notes, is highly present in Jiang-flavored baijiu, giving pure sweetness to BL (Xu et al., 2022). Ethanal is the main contributor to the "grassy aroma" in baijiu, masking bad odors from other aldehydes and enhancing quality (Morakul et al., 2010). So, increasing the concentrations of these three compounds within a range can improve BL quality. But excessive amounts can cause flavor imbalance, like high levels of lactic ethyl ester and n-propanol in lower grades. Moderate lactic ethyl ester gives a creamy and sweet aroma, but too much can cause bad fermented odors (Wei et al., 2020). n-Propanol has floral traits (Niu, Zhu, & Xiao, 2020) and is a high-content alcohol in baijiu. But too much can cause off-flavors, so its content needs control. Finally, the creamy and sweet aroma of 3-hydroxy-2-butanone enriches the flavor (OAV: 3614.56 ± 319.48), but its relationship with BL's quality grade is uncertain (Fig. 5c-6), maybe due to interactions with other substances like lactic acid affecting its aroma release (Wang et al., 2024).

Additionally, isobutanol (Fig. 5c-7) and propyl acetate (Fig. 5c-8) were identified as distinctive differential compounds in the 1st and 6th FRs, respectively, with their concentrations exhibiting an inverse correlation to quality grade. While both isobutanol and propyl acetate are aromatic compounds found in baijiu, elevated levels can detract from the typical flavor profile of BL within a given FR. For instance, high concentrations of isobutanol might impart an unpleasant pungent aroma (Gong et al., 2023), overshadowing the characteristicsour and grain aromas associated with the BL of the 1st FR. Conversely, although propyl acetate provides a fresh aroma reminiscent of apples or pears, it conflicts with the pronounced charred and roasted notes present in the BL of the 6th FR, potentially affecting quality assessments. In summary, most compounds that significantly influence BL quality demonstrate a degree of universality throughout baijiu production processes, thereby offering substantial support for understanding the chemical basis underlying variations in quality grades.

4. Conclusion

This study elucidated the flavor profile and key different compounds of various quality grades of BL during the production of Jiang-flavored Baijiu. Each FR exhibited a distinct aroma profile: the 1st and 2nd FRs presented notable grain-like and sour aromas, while the 3rd, 4th, and 5th FRs were characterized by prominent sauce, fruity, and floral notes; conversely, the 6th and 7th FRs displayed strong burnt aromas. Significant differences in aroma intensity were observed across different grades of BL: high-quality samples exhibited a markedly pronounced sauce aroma, whereas grain-like and burnt aromas were comparatively subdued. Subsequently, concentrations of 59 major volatile aroma compounds in BL were quantified using GC-FID employing an internal standard method. By analyzing changes in component profiles from different FRs, trends among these compounds throughout the production process were visualized through K-means clustering. Utilizing PLS-DA modeling alongside OAV analysis further facilitated identification of key compounds that demonstrated significant variations contributing substantially to flavor differences between FRs during production. Among the three grades of BL studied, 10 common compounds consistently exerted a significant influence on flavor across varying FRs. Furthermore, based on compositional changes within each quality grade BL across different FRs, relationships between specific compounds and quality grades within the same FR were visualized through K-means clustering. Additionally, compounds with significant impacts on quality discrepancies in each respective FR were identified. Ultimately, 6 common compounds present in BL from diverse fermentation rounds consistently affected its quality grading. The identification of these key differentiating compounds offers valuable chemical insights for an extensive exploration into both production processes and quality variations inherent to Jiang-flavored BL while also laying a foundation for understanding microbial metabolism's impact on BL compounds as well as optimizing production methodologies to enhance product quality.

Ethical statement

Ethical review and approval were waived for this study, because experimental samples used in the study are consumed in daily life.

Informed consent

Informed consent was obtained from all individual participants included in the study.

CRediT authorship contribution statement

Xueli Yang: Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation. **Renyuan Chen:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization. **Junhai Wu:** Writing – review & editing, Visualization, Software, Data curation. **Fangqiang Yu:** Writing – review & editing, Project administration, Funding acquisition. **Fengmei Liao:** Writing – review & editing, Software. **Xiaobo Li:** Writing – review & editing. **Ye Wang:** Writing – review & editing. **Cheng Zhao:** Writing – review & editing. **Hanren Zhang:** Supervision. **Xinying Wu:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, 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.

Acknowledgments

This research was funded by Guizhou Province Science and Technology Foundation (NO. QKHZC-[2022]019).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2025.102331.

Data availability

Data will be made available on request.

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