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Aging-induced evolution in rice-flavored Baijiu: analysis of non-volatile organic compounds, volatile organic compounds multispectral characteristics, and viscosity

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Abstract

Aging plays a key role in improving the sensory quality of Chinese Baijiu, yet the molecular evolution of rice-flavored Baijiu (RFB) remains insufficiently explored. This study systematically explored the aging-induced evolution of non-volatile and volatile flavor compounds, spectral features, and viscosity in RFB aged for 0-15 years. 855 non-volatile organic compounds (N-VOCs) were identified, among which 118 key differential compounds were screened, mainly including phosphatidylcholines, long-chain fatty acids, amino acid derivatives, and benzenoids. Aging promoted the accumulation of lipid and lipid-like molecules, especially phosphatidylcholines, while most long-chain fatty acids exhibited an overall decreasing trend. A total of 180 volatile organic compounds (VOCs) were identified, and 34 key aroma compounds were selected, primarily esters, higher alcohols, and organic acids. Early aging enhanced ester formation, whereas prolonged storage led to a decline in low-molecular-weight esters and higher alcohols. Spectroscopic and viscosity results revealed a clear inflection point at 6 years. Hydrogen bonding and ethanol-water clusters formation strengthened before this stage, leading to maximum viscosity. After 6 years, hydrogen networks weakened and clusters dissociated, which differs from strong- and sauce-flavor Baijiu. These findings provides a systematic understanding of the aging evolution of RFB and offers scientific guidance for improving its quality during the aging process.

Keywords: Rice-flavored Baijiu; Volatile organic compounds; Metabolomics; Multispectral characteristics; Viscosity.

Abbreviation: RFB: rice-flavored Baijiu; VOCs, Volatile organic compounds; N-VOCs, Non-volatile organic compounds; Y0, 0-year aged sample; Y3, 3-year aged sample; Y6, 6-year aged sample; Y9, 9-year aged sample; Y15, 15-year aged sample; LC-MS, liquid chromatography-mass spectrometry; GC-MS, Gas chromatography-mass spectrometry.

1. Introduction

Baijiu is one of the oldest and most widely consumed distilled spirits in China. Over centuries of development, it has evolved into a unique brewing system characterized by solid-state fermentation and driven by diverse natural microbial communities^{1,2}. Owing to pronounced differences in fermentation substrates, microbial consortia, and distillation techniques, Chinese Baijiu has developed a highly diverse quality system with distinct flavor profiles³. Baijiu is typically classified into twelve aroma types, among which strong-aroma, sauce-aroma, light-aroma, and rice-aroma types are considered the four most representative categories⁴. Modern analytical techniques have revealed that Baijiu contains hundreds of N-VOCs and VOCs, including esters, alcohols, acids, aldehydes, phenols, lipids, and various heterocyclic substances. In conclusion, these components contribute to the multilayered aroma and taste characteristics of Baijiu.

Among the various aroma types, RFB is highly favored by consumers for its elegant, mild, and clean sensory profile. It is produced using rice as the primary raw material and fermented with Xiaoqu starters⁵. The production process typically includes solid-state cultivation to promote microbial saccharification, followed by liquid fermentation and distillation. RFB is characterized by a distinct rice-derived aroma and a soft, slightly sweet aftertaste⁶. Due to its relatively short fermentation cycle, the variety and concentration of flavor compounds generated are lower than other Baijiu types. Consequently, its chemical composition is less complex, featuring predominantly light ester notes, subtle honey-like aromas, and a smooth mouthfeel. Previous studies have identified ethyl lactate, ethyl acetate, and β -phenylethanol as key aroma-active compounds that collectively define the characteristic flavor of RFB

7.

Freshly distilled Baijiu typically exhibits pungent, irritating, and unpleasant raw aromas^{8,9}. These undesirable characteristics are associated with the presence of high concentrations of small molecular aldehydes and sulfur-containing compounds, as well as the unstable structural state of the ethanol-water system immediately after distillation^{10,11}. Therefore, aging is an indispensable process in Baijiu production, as it helps eliminate these off-flavors and enhances the harmony and overall quality of the liquor. During storage, Baijiu undergoes slow and complex physical and chemical transformations, including esterification, hydrolysis, redox reactions, and condensation¹². These reactions alter the composition of VOCs and influence the evolution of N-VOCs such as long-chain fatty acids, lipids, amino acids, and carbohydrates¹³. Collectively, these changes regulate the integration of aroma and taste, ultimately improving the smoothness, balance, and sensory depth of the Baijiu¹⁴.

Previous studies have demonstrated that different Baijiu systems exhibit pronounced wine body and flavor changes during aging. For example, Zhang et al.¹⁵ reported that irritating aldehydes and sulfur-containing compounds gradually decreased during storage in waxy wheat Baijiu, whereas the content of esters increased markedly, accompanied by the continuous accumulation of amino acids and their derivatives. These transformations enhanced the aroma harmony and improved the mellow taste of the liquor. The research by Liu et al.¹⁶ further revealed that Baijiu aging is not merely a series of simple chemical reactions; it also involves the evolution of intermolecular self-assembly behaviors. Jiang et al.¹⁷ found that in strong-flavor Baijiu, hydrogen-bond-mediated self-assembly of amphiphilic molecules occurs during aging. Ethanol, water, and flavor compounds initially form small colloidal particles, which subsequently undergo Ostwald ripening to merge and grow, thereby affecting the smoothness and flavor persistence of the liquor. Moreover, the hydrogen-bonding network within the ethanol-water system is continuously reconstructed during aging, leading to the formation of multiscale ethanol-water

clusters that modulate viscosity and mouthfeel characteristics. Similar effects have been observed in other fermented alcoholic beverages. Cao et al.¹⁸ reported that the hydrogen-bond interactions between ethanol and water in Huangjiu strengthened during aging, resulting in increased viscosity and a smoother, rounder taste.

Although the effects of aging on strong-flavor and sauce-flavor Baijiu have been well characterized, studies on the aging behavior of RFB remain extremely limited. The unique raw materials and fermentation system of RFB suggest that it may undergo distinct wine body and flavor profile during storage, highlighting the need for systematic investigation. In this context, this study integrates multiple analytical techniques. These include LC-MS, GC-MS, FT-IR, Raman spectroscopy, UV spectroscopy, and 3D fluorescence spectroscopy, together with viscosity measurements. The aim is to systematically characterize changes in chemical composition, the evolution of the hydrogen-bond network, and the formation of ethanol-water clusters in RFB during aging. In addition, the relationships between these structural transformations and liquor viscosity were analyzed. Overall, this work provides a comprehensive theoretical framework for understanding the aging mechanism of RFB and offers scientific guidance for improving its quality during storage.

2. Material and methods

2.1. Samples

RFB samples aged for 0, 3, 6, 9, and 15 years were selected for this study and were designated as Y0 (0-year-aged sample), Y3 (3-year-aged sample), Y6 (6-year-aged sample), Y9 (9-year-aged sample), and Y15 (15-year-aged sample), respectively. All samples were obtained from Guilin Xiangshan Distillery Co., Ltd. (Guangxi Province, China) and were produced using traditional manufacturing processes. The alcohol content of all samples was 53 vol%.

2.2. UPLC-MS analysis

Chromatographic analysis was carried out on a Shimadzu Nexera X2 UHPLC system (Shimadzu, Japan) fitted with a C18 reversed-phase column (2.1 × 100 mm,

1.6 μm ; Waters, Milford, MA, USA), following the procedure described by Wu et al.¹⁴ with minor modifications. The autosampler temperature was consistently maintained at 4 °C. Samples (4 μL) were injected at a column temperature of 40 °C, and separation was achieved at a flow rate of 300 $\mu\text{L min}^{-1}$. The mobile phase consisted of 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B). The gradient program was applied as follows: 2% B from 0 to 1 min; a linear increase to 48% B from 1 to 5 min; a further rise to 80% B between 5 and 7 min; an increase to 100% B from 7 to 11 min; isocratic elution at 100% B for 2 min; a rapid return to 2% B within 0.01 min; and re-equilibration at 2% B until 15 min.

Mass spectrometric detection was performed across an m/z range of 60-1200 Da for full MS scans and 25-1200 Da for product ion scans. The accumulation times were set to 0.08 s per spectrum for TOF MS and 0.03 s per spectrum for MS/MS acquisition. Information-dependent acquisition was conducted in high-sensitivity mode, allowing the selection of up to 15 precursor ions per cycle. Collision-induced dissociation was carried out at a collision energy of 30 V with a ± 15 V energy spread. In positive ionization mode, the ion source parameters were configured as follows: source temperature, 550 °C; nebulizer gas (GAS1), 50; auxiliary gas (GAS2), 50; curtain gas, 50; and ion spray voltage, 5500 V. For negative ionization mode, the source temperature was set to 500 °C, with GAS1, GAS2, and curtain gas all maintained at 50, and the ion spray voltage adjusted to -4500 V.

2.3. GC-MS analysis

Volatile compounds were extracted using a modified protocol adapted from Zhang et al.¹⁹. Briefly, a Baijiu sample was transferred into a 15 mL centrifuge tube, and its ethanol concentration was adjusted to 10% (v/v) by adding saturated sodium chloride (NaCl) solution. Subsequently, 5.0 mL of the diluted sample was placed into a 20 mL headspace vial, followed by the addition of 10 μL of an internal standard solution (n-hexane, 10 mg/L). The sealed vial was equilibrated at 50 °C for 10 min. A solid-phase microextraction (SPME) fiber, preconditioned at 270 °C for 10 min, was

exposed to the headspace and allowed to extract volatiles for 20 min at 50 °C. After extraction, the fiber was immediately inserted into the GC injector, where thermal desorption was carried out at 250 °C for 5 min. The SPME fiber was reconditioned at 270 °C for 10 min prior to the next analysis. For retention index (RI) calibration, 10 µL of an n-alkane standard mixture (C7-C40) was analyzed under identical incubation, extraction, and injection conditions.

GC-MS analysis was performed on an Agilent 8890 gas chromatograph coupled with a 7000D mass spectrometer, equipped with a DB-5MS capillary column (30 m × 0.25 mm × 0.25 µm). High-purity helium (99.999%) was used as the carrier gas at a constant flow rate of 1.0 mL/min, and the injector temperature was maintained at 250 °C. The oven temperature program was as follows: 40 °C for 3 min, increased to 100 °C at 10 °C/min, then to 180 °C at 7 °C/min, and finally to 280 °C at 25 °C/min with a 5 min hold. The transfer line, quadrupole, and ion source temperatures were set at 280, 150, and 230 °C, respectively. Mass spectrometric detection was conducted using electron impact ionization at 70 eV in selected ion monitoring (SIM) mode.

2.4. FT-IR analysis

The SFB sample was analyzed using a FT-IR spectrometer (Nicolet 6700, Thermo Fisher, USA) according to a study by Zheng et al.²⁰ with minor modification. Briefly, an absorbance spectrum in the 4000-400 cm⁻¹ region was obtained by 32 scans with a nominal resolution of 4 cm⁻¹.

2.5. Raman spectra

A Raman spectrometer (Jobin Yvon T64000, Horiba) equipped with an argon laser source of 532 nm was used. The base-line corrections and normalization were applied to the raw Raman spectra as indicated by utilizing Origin 2020.

2.6. UV spectroscopy analysis

UV absorption spectra were collected using a TU-1901 spectrophotometer (Pgeneral, Beijing, China) over a wavelength range of 200-350 nm with a spectral resolution of 1 nm. Absolute ethanol was used as the background reference. Each liquor sample was analyzed in triplicate to ensure measurement reproducibility.

2.7. Three-dimensional fluorescence spectra analysis

The fluorescence spectra were performed using an FL970 fluorescence spectrophotometer (Techcomp Ltd., China). The scanning speed was set to 30,000 nm·min⁻¹, with both excitation and emission slit widths adjusted to 5 nm. Excitation spectra were collected over a wavelength range of 200-450 nm, while emission spectra were recorded from 200 to 600 nm, using a step size of 5 nm. All measurements were conducted in triplicate for each liquor sample to ensure analytical reproducibility.

2.8. Viscosity

Viscosity measurements were conducted using an Ubbelohde viscometer (SGE, Ltd., Australia) with a capillary diameter of 0.3-0.4 mm. RFB samples were equilibrated at 25 °C for 20 min in a thermostatic water jacket before measurement. The viscosity was determined based on flow time recorded with a stopwatch (±0.01 s) and the corresponding sample density. Each sample was measured in triplicate, and the deviation between parallel flow times was maintained below 0.1 s. Viscosity values were calculated according to Eq. (1):

$$\eta = K \times \rho \times t \quad (1)$$

η is the viscosity of the liquid; K is the viscometer constant; ρ is the density of the liquid; t is the liquid flow time.

2.9. Data analysis

Statistical differences were evaluated using one-way analysis of variance (ANOVA) and Pearson correlation analysis implemented in SPSS 27.0 software (SPSS Inc., Chicago, IL), with the significance level defined at $P < 0.05$. Multivariate statistical analyses were carried out using SIMCA 14.1 (Umetrics, Umea, Sweden), while data visualization and figure processing were performed with Origin 2020 software (Microcal, Northampton, MA, USA).

3. Results and discussion

3.1. Analysis of N-VOCs in RFB across different storage years

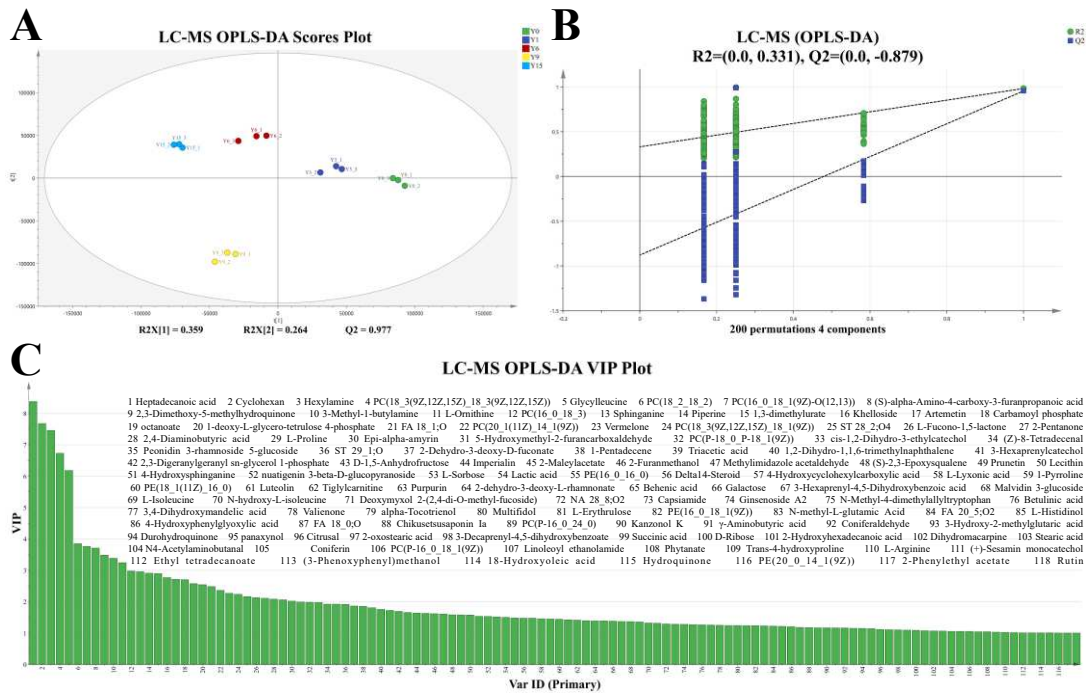


Fig. 1. LC-MS analysis of all aged RFB samples. OPLS-DA score plot (A), model cross-validation results (B), and VIP value plot (C) of naturally aged RFB.

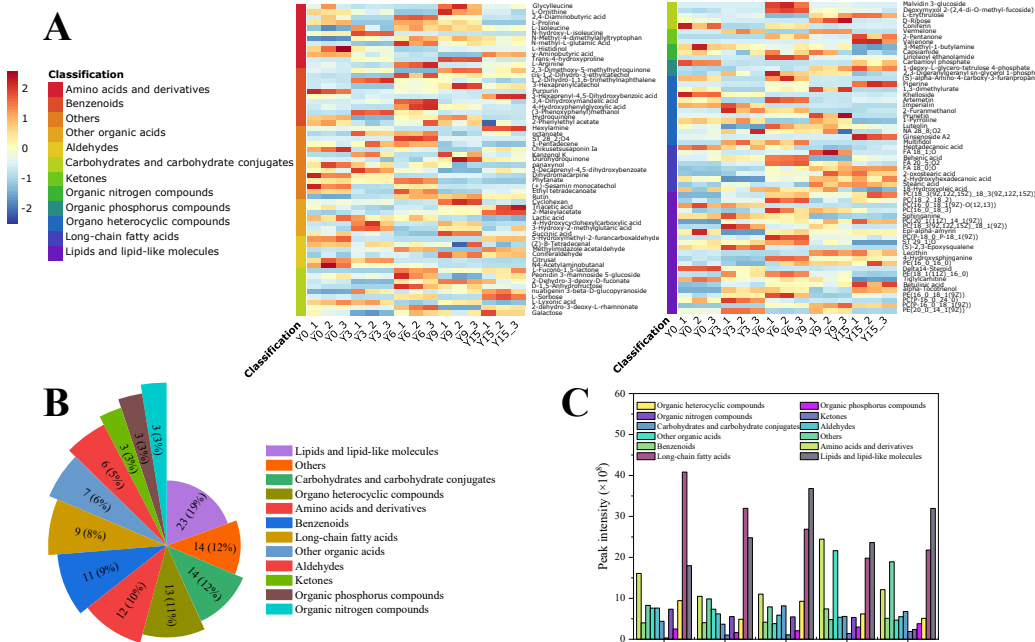


Fig. 2. Heat map (A), proportion (B), and compositional content changes (C) of key N-VOCs (VIP > 1) in RFB aged for different periods (0-15 years).

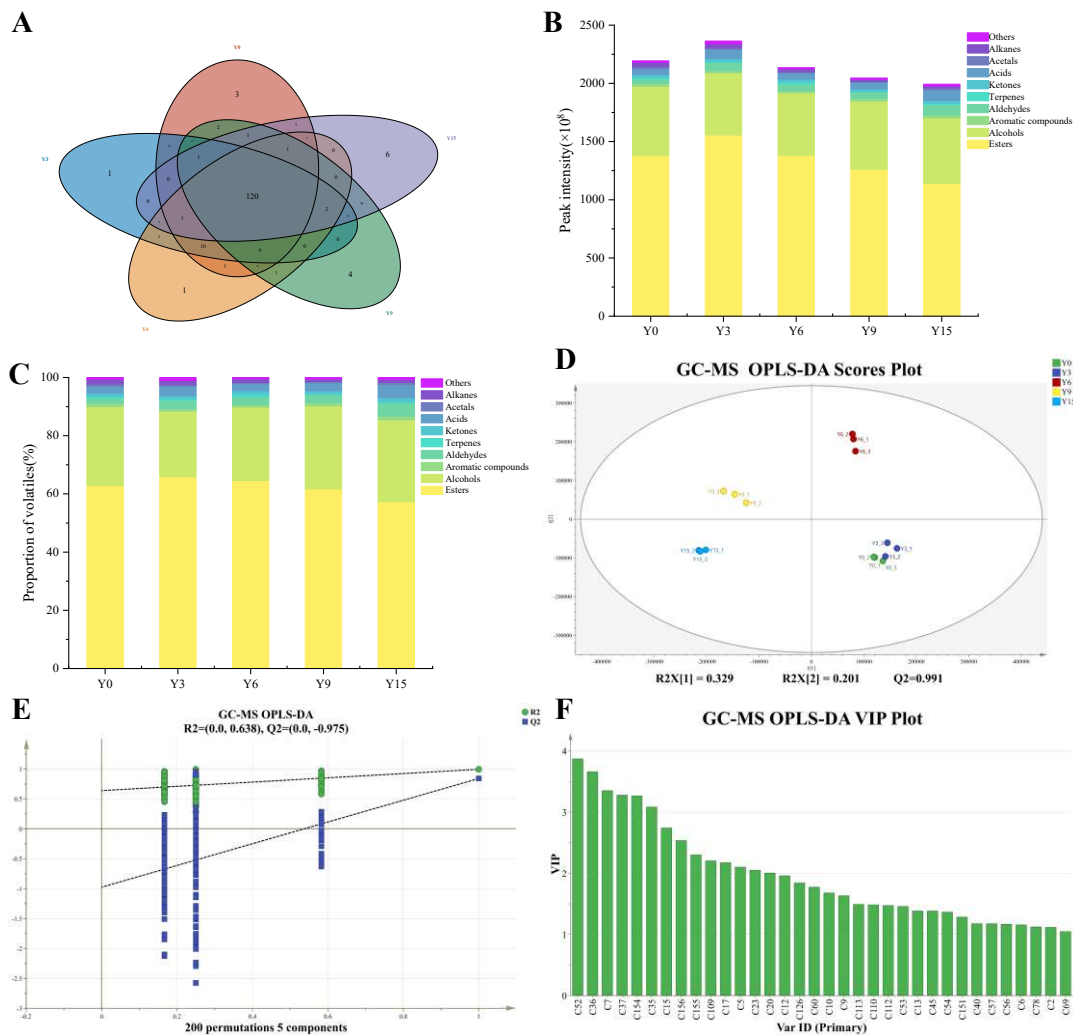
Untargeted metabolomics based on LC-MS was employed to systematically analyze N-VOCs in RFB samples aged for 0, 3, 6, 9, and 15 years from the same

distillery, and a total of 855 differential metabolites were identified (Table S1). The N-VOCs of all RFB samples mainly comprised amino acids and their derivatives, carbohydrates and their conjugates, lipids and lipid-like molecules, and organic acids. To further investigate the effect of aging on the N-VOCs of RFB, orthogonal partial least squares-discriminant analysis (OPLS-DA) was performed to assess the differences among the stored samples (Fig. 1A). The model parameters exhibited high explanatory power ($R^2X = 0.993$, $R^2Y = 0.997$) and strong predictive ability ($Q^2 = 0.977$), confirming the reliability of the model²¹. Specifically, the sample scores of RFB aged for 0 and 3 years were located in the fourth and first quadrants, respectively; those aged for 6 and 9 years were distributed in the second quadrant; while the scores of Y15 clustered in the third quadrant. Notably, all sample scores were clearly distinguishable, indicating that RFB samples of different aging years exhibited significant differences in N-VOCs.

To further explore these differences, a permutation test ($n = 200$) was performed to screen for characteristic differential metabolites between samples and evaluate whether the discriminant model was overfitted. The results showed that the R^2 and Q^2 values were 0.331 and -0.879 (Fig. 1B), respectively, confirming that the OPLS-DA model was free from overfitting²². Key differential N-VOCs were screened based on the Variable Importance in Projection (VIP) values ($VIP > 1$) from the established OPLS-DA model combined with a statistical threshold of $P < 0.05$. Metabolites with $VIP > 1$ were defined as significantly differential metabolites, and a total of 118 such metabolites were identified (Fig. 1C). Hierarchical cluster analysis (HCA) was performed on the relative content distribution of key differential N-VOCs in RFB across different aging years and the results were visualized (Fig. 2A). As shown in Fig. 2B, all differential metabolites were classified into 12 categories: amino acids and derivatives (12), benzenoids (11), other organic acids (7), aldehydes (6), carbohydrates and carbohydrate conjugates (14), ketones (3), organic nitrogen compounds (3), organic phosphorus compounds (3), organic heterocyclic compounds (13), long-chain fatty acids (9), lipids and lipid-like molecules (23), and others (14).

As illustrated in Fig. 2C, lipids and lipid-like molecules, together with long-chain fatty acids, accounted for the major proportion of total differential compounds. Řezanka et al.²³ reported that the cell membrane of *Saccharomyces cerevisiae* contains abundant PCs enriched with long chain fatty acids. The high levels of PCs and long-chain fatty acids detected in RFB are therefore primarily derived from yeast membrane components that are released into the liquor during cell lysis²⁴. A significant trend observed was the progressive accumulation of lipids and lipid-like molecules during the aging process. Specifically, several phosphatidylcholines (PCs) were identified as key differential markers, including PC(18_3(9Z,12Z,15Z)_18_3(9Z,12Z,15Z)) (VIP = 6.74), PC(18_2_18_2) (VIP = 3.86), PC(20_1(11Z)_14_1(9Z)) (VIP = 2.37), PC(18_3(9Z,12Z,15Z)_18_1(9Z)) (VIP = 2.24), and PC(P-18_0_P-18_1(9Z)) (VIP = 1.98). In contrast, the overall relative content of differential long-chain fatty acids (LCFAs) exhibited a decreasing trend, despite a slight increase noted in the Y15. However, this general trend was not uniform across all identified LCFAs. For instance, certain species, such as FA 20_5;O2 (VIP = 1.23), FA 18_0;O (VIP = 1.23), 2-oxostearic acid (VIP = 1.12), and stearic acid (VIP = 1.12), showed moderate accumulation during aging. Conversely, heptadecanoic acid, which presented the highest VIP score (8.39) and was a major identified LCFA, underwent a significant reduction in relative content throughout the aging process. The total relative content of differential ketones demonstrated a significant increase during the aging process; however, vermelone (VIP = 2.27), a specific ketone, exhibited a significant reduction in relative content post-aging. Notably, the Y9 exhibited the maximum relative content of total differential amino acids, with glycyllucine (VIP = 6.19) showing the most prominent change. Consistent with this observation, the total differential benzenoids and other organic acids also peaked in relative content in the Y9.

3.2. Analysis of VOCs in RFB across different storage years



C52 ethyl caprate C36 ethyl caprylate C7 isoamyl acetate C37 ethyl isobutyrate C154 isobutyl alcohol C35 isobutyl acetate C15 ethyl 2-hydroxy-4-methylvalerate
 C156 isoamyl alcohol C155 2-phenylethanol C109 acetic acid C17 dodecanoic acid, ethyl ester C5 ethyl isovalerate C23 ethyl nonanoate C20 isoamyl caprylate
 C12 Ethyl acetate C126 furfural C60 ethyl myristate C10 ethyl caproate C9 butanedioic acid, diethyl ester C113 nonanal C110 octanoic acid C112 Propionic acid
 C53 isobutyl octanoate C13 ethyl lactate C45 Pentanoic acid, 2,2,4-trimethyl-3-carboxyisopropyl, isobutyl ester C54 ethyl 4-decenoate, (4e)- C151 n-propanol
 C40 isoamyl decanoate C57 ethyl palmitate C56 ethyl isopentyl succinate C6 isopentyl lactate C78 2-pentanone, 4-methyl-1,1,1,3,3-d5- C2 n-propyl acetate C69 n-tetradecane

Fig. 3. GC-MS analysis of all aged RFB samples. (A) Venn analysis of the number of VOCs. (B) Proportion of relative content of VOCs. (C) Cumulative total relative content of VOCs. Multivariate statistical analysis of GC-MS results. (D) OPLS-DA score plot. (E) Validation plot of 200 permutation tests for OPLS-DA. (F) Key differential compounds with VIP>1.

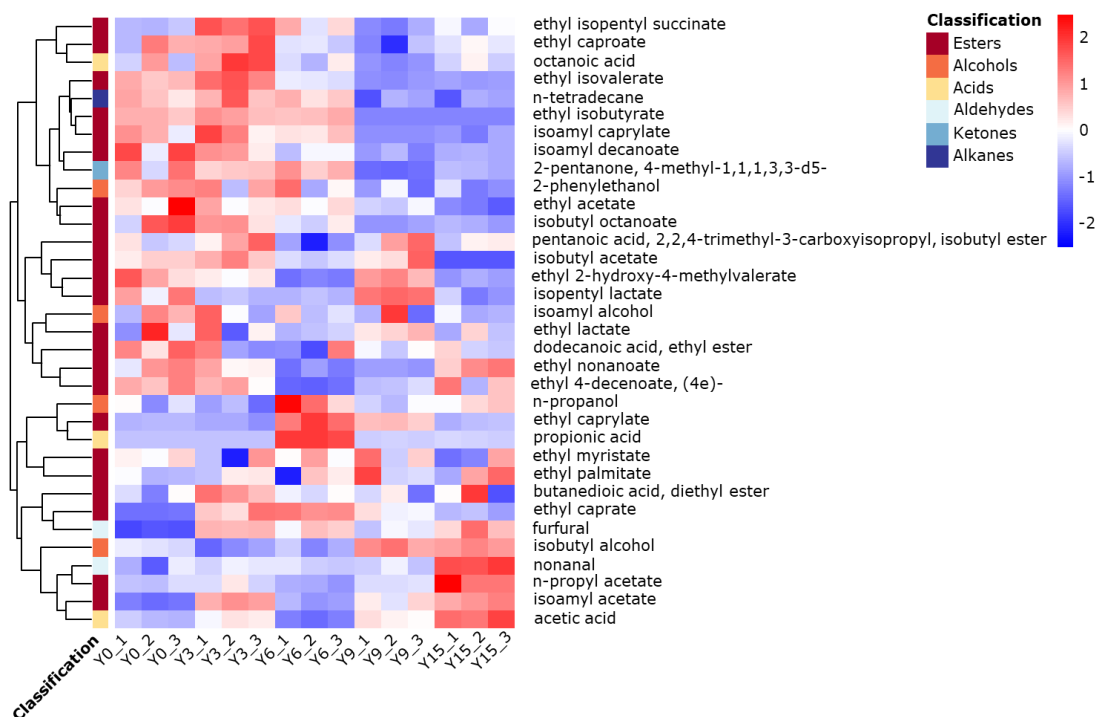


Fig. 4. Heat map of key VOCs in RFB aged for different years (0-15 years).

Baijiu is a chemically complex alcoholic beverage characterized by a wide variety of VOCs, including esters, acids, alcohols, ketones, and aldehydes. Among these constituents, esters, alcohols, and acids are recognized as the predominant VOCs in the majority of Chinese Baijiu²⁵. In contrast to other Baijiu types, such as strong-flavor Baijiu and sauce-flavor Baijiu, RFB is characterized by a shorter fermentation cycle and consequently contains a lower complexity of flavor components⁴. A total of 180 VOCs were identified across all RFB samples spanning 5 different aging years using headspace solid-phase microextraction coupled with GC-MS. These compounds were classified into various chemical categories, comprising 65 esters, 22 alcohols, 21 aromatic compounds, 15 aldehydes, 14 terpenes, 12 ketones, 12 alkanes, 5 acetals, 4 acids, and 10 other compounds (detailed information is provided in Table S2. Notably, esters and alcohols constituted the most diverse categories and exhibited the highest relative content among the VOCs. Analysis of the compound distribution via the Venn diagram (Fig. 3A) showed that RFB aged for 0, 3, 6, 9, and 15 years contained 155, 149, 143, 148, and 143 VOCs, respectively. Overall, the number of identified VOCs demonstrated a minor declining trend with increasing

aging time. Specifically, 120 compounds were consistently detected across all samples, while the 0, 3, 6, 9, and 15-year-aged samples contained 3, 1, 1, 4, and 6 unique compounds, respectively. The stacked bar chart of relative content and the percentage stacked bar chart for each class of VOCs are presented by Fig. 3B and Fig. 3C, respectively. Compared with the Y0, the total relative content of VOCs was found to be significantly higher in Y3, a level in which a declining trend was subsequently demonstrated with further aging. Esters, which are the major VOCs, are considered the primary contributors to the characteristic "elegant honey-like aroma" of RFB, and pleasant fruity and floral notes are imparted to the liquor ²⁶. The proportions of volatile esters in Y0, Y3, Y6, Y9, and Y15 were recorded as 62.85%, 65.82%, 64.60%, 61.70%, and 57.26%, respectively. Mechanistically, the higher ester content observed in Y3 is attributed to the continuous esterification occurring between alcohols and organic acids (e.g., acetic acid, propionic acid, caprylic acid, etc.) during the early aging stage (0-3 years) ¹¹. Subsequently, as aging progresses, the observed decrease in the content of volatile esters is induced by the gradual volatilization of low-boiling-point small-molecule esters through the micropores of the storage container ²⁷.

The VOCs of RFB from different aging years were effectively differentiated by OPLS-DA (Fig. 3D). For the OPLS-DA model parameters, the R^2X and R^2Y values were 0.863 and 0.991, indicating high indicated high explanatory power, while the Q^2 value of 0.930 demonstrated strong predictive ability of the model ²¹. As shown in Fig. 3D, RFB aged for 0 and 3 years were distributed in the fourth quadrant, with no chemical distinction observed between the two periods. This suggests that 3 years of aging has a limited influence on the RFB flavor profile. In contrast, samples aged for 6, 9, and 15 years were clearly separated, distributed in the first, second, and third quadrants, respectively, indicating the RFB flavor profile undergoes significant chemical transformation with prolonged aging. In general, significant changes to the RFB flavor profile are induced when aging exceeds three years. To further explore these differences, a permutation test ($n = 200$) was conducted to screen for characteristic differential markers between Baijiu samples (Fig. 3E). The results

showed that the R^2 and Q^2 values were 0.638 and -0.975, respectively, confirming that the OPLS-DA model was free from overfitting²². Additionally, key differential aroma compounds between samples were screened based on the VIP values ($VIP > 1$) from the established OPLS-DA model combined with a statistical threshold of $P < 0.05$ ²¹. A total of 34 key differential aroma substances were identified among the five samples (Fig. 3F), mainly including esters, alcohols, and acids. Hierarchical cluster analysis was performed based on the relative content of key differential flavor substances in RFB of different aging years (Fig. 4).

A total of 23 esters were identified in the analysis, most of which belong to the ethyl ester group. During the aging of Baijiu, the changes in esters are mainly influenced by the combined effects of hydrolysis, volatilization, and re-esterification¹². Ethyl isobutyrate was found to be below the limit of detection in both the Y9 and Y15. Similarly, isobutyl acetate was not detected in the Y15. This is primarily attributed to the tendency of low molecular weight esters to volatilize during long-term storage²⁸. Crucially, ethyl acetate ($VIP = 1.96$) and ethyl lactate ($VIP = 1.39$) are recognized as ubiquitous and high-concentration compounds in RFB, contributing significantly to the overall flavor profile⁵. However, the relative content of low-molecular-weight esters decreases with aging due to their inherent high volatility. Conversely, long-chain fatty acids have been reported to inhibit the volatilization of VOCs through molecular interactions²⁹. LC-MS results confirmed that the total long-chain fatty acids showed a slight decreasing trend during the aging, resulting in a weakened binding capacity for low-molecular-weight esters. Notably, ethyl decanoate ($VIP = 3.87$) and ethyl caprylate ($VIP = 3.67$) were the two VOCs with the highest VIP values, both exhibiting the highest relative content in the Y6.

The identified alcohols, including isobutanol, isoamyl alcohol, β -phenylethanol, and n-propanol, all are classified as higher alcohols. An appropriate level of higher alcohols is known to enhance the mellow taste, fullness, and harmony of the winebody. Among these, isoamyl alcohol ($VIP = 2.5$) and β -phenylethanol ($VIP = 2.30$), as characteristic flavor compounds of RFB, showed a decreasing trend during

aging. The identified alcohols, including isobutanol, isoamyl alcohol, β -phenylethanol, and n-propanol, were all classified as higher alcohols. An appropriate concentration of these higher alcohols contributes to the mellow mouthfeel, fullness, and overall harmony of the wine body ³⁰. Among these compounds, isoamyl alcohol (VIP = 2.50) and β -phenylethanol (VIP = 2.30), which are characteristic aroma-active components of rice-flavor Baijiu, exhibited a gradual decline during aging. Three differential organic acids were detected, namely acetic acid (VIP = 2.21), propionic acid (VIP = 1.48), and caprylic acid (VIP = 1.49). Aging exerted the most significant influence on acetic acid, which continued to accumulate throughout the aging period. The differential aldehydes included furfural (VIP = 1.85) and nonanal (VIP = 1.50), both of which showed higher relative content compared with the unaged RFB. In addition, other differential VOCs such as 2-pentanone, 4-methyl-1,1,3,3-d5- (VIP = 1.13) and n-tetradecane (VIP = 1.05) were also identified, although no consistent aging-related pattern was observed for these compounds.

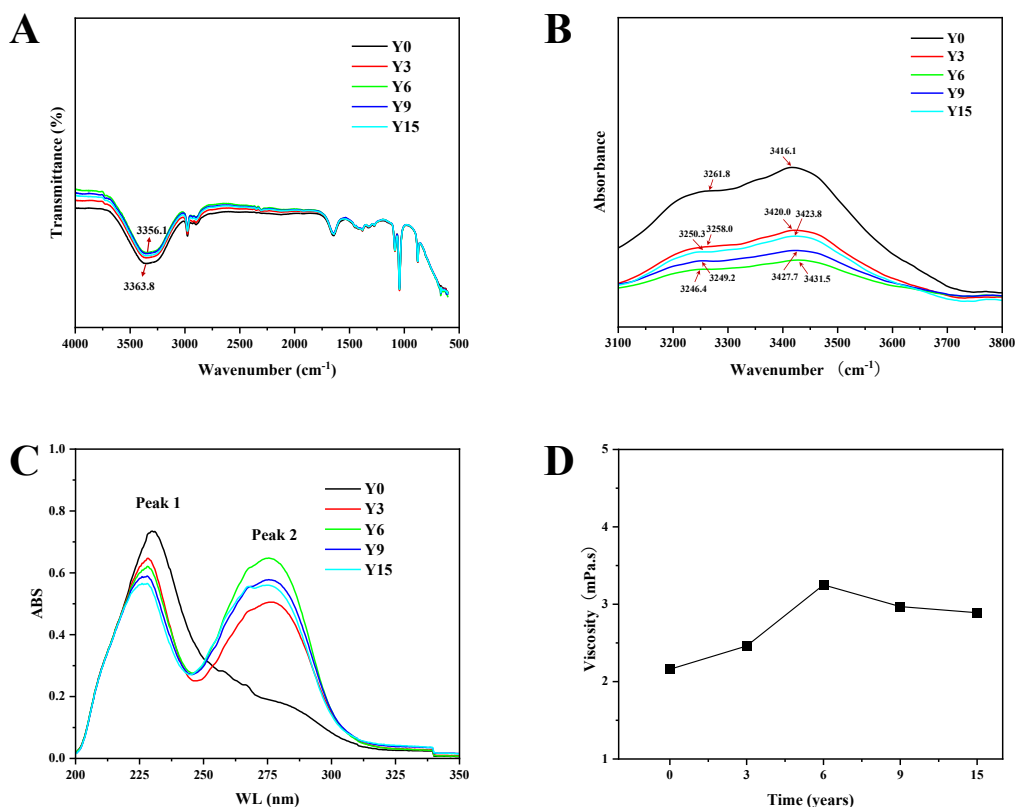


Fig. 5. FT-IR spectra (A), Raman spectra (B), UV spectra (C), and viscosity (D) of RFB aged for 0-15 years.

The FT-IR spectra of RFB with different storage durations are shown in Fig. 5A. The absorption peaks at 2980 cm^{-1} and 2890 cm^{-1} correspond to the stretching vibrations of the CH_3 and CH_2 groups, respectively, while the peak at 1046 cm^{-1} is attributed to the C-OH stretching vibration²⁰. For RFB with different aging years, the positions of the stretching vibration peaks of CH_3 , CH_2 , and C-OH groups exhibited no substantial changes. Because of the high ethanol content in the Baijiu, the FT-IR spectra of RFB with different storage durations showed strong similarity. The spectral region between 3500 and 3200 cm^{-1} corresponds to the O-H stretching vibration of water and ethanol in RFB. Previous studies have shown that the symmetric O-H stretching peak of free water molecules typically appears above 3800 cm^{-1} ³¹. However, hydrogen bonds readily form between water and ethanol molecules, reducing the number of free water and ethanol molecules³². This effect is reflected in the IR spectra as a shift of the O-H stretching peak toward lower wavenumbers. When

the storage duration reached 6 years, the O-H stretching vibration peak shifted from 3363.8 cm^{-1} to 3352.3 cm^{-1} , and with further aging to 15 years, the peak shifted back to 3356.8 cm^{-1} . These observations indicate that hydrogen bonding interactions in the RFB matrix strengthened during aging from 3 to 6 years but weakened thereafter. A study by Jiang et al.³³ reported that hydrogen bonding interactions in strong-flavor and sauce-flavor Baijiu continuously increased as the storage duration from 1 to 17 years. In contrast, this study showed that hydrogen bonding interactions in RFB increased only within the first 6 years of aging. This difference may be related to variations in aroma compounds produced during the aging of different Baijiu types, which influence the strength of hydrogen bonding between ethanol and water molecules. For example, Qiao et al.³⁴ found that the strength of ethanol-water hydrogen bonding in Fenjiu, a light-flavor Baijiu, was inversely correlated with total ester content. In addition, Nose et al.³⁵ demonstrated that acids and phenolic compounds can promote proton exchange between water and ethanol, thereby enhancing the ethanol-water molecular network.

3.4. Raman spectra

Raman spectroscopy has been used to investigate hydrogen-bonding changes during the aging of Baijiu, and the hydrogen-bonding interactions between ethanol and water molecules cause the Raman peak positions to shift toward lower wavenumbers³². For hydrogen-bonding analysis, the Raman spectral region from 3100 to 3600 cm^{-1} was extracted. The O-H stretching band can be divided into three distinct components, each corresponding to a different type of O-H stretching vibration. The peak between 3200 and 3300 cm^{-1} represents the symmetric O-H stretching vibration, which is an indicator of strong hydrogen bonding and is associated with four-coordinated hydrogen-bonded water (4-HB H_2O). The peak between 3400 and 3500 cm^{-1} corresponds to the asymmetric O-H stretching vibration, which reflects two-coordinated hydrogen-bonded water (2-HB H_2O). In this configuration, the two hydrogen atoms of a water molecule form strong and weak hydrogen bonds with neighboring water molecules. The peak in the range of 3600 to

3700 cm^{-1} , which represents the free O-H vibration, is associated with weak hydrogen bonding interactions in free water ³⁶. As shown in Fig. 5B, the symmetric O-H stretching vibration of RFB exhibited a blue shift from 3261.8 to 3246.4 cm^{-1} during aging from 0 to 6 years, followed by a red shift from 6 to 15 years, with the peak position increasing from 3246.4 to 3250.3 cm^{-1} . These results indicate that the Y6 contained the highest number of ethanol-water molecular clusters. In contrast, the asymmetric O-H stretching vibration showed an opposite trend, suggesting a transformation between 2-HB H_2O and 4-HB H_2O in the aging process. Due to the weak hydrogen bonding interactions between free water molecules ³⁷, no obvious peaks corresponding to the free O-H vibration mode were observed in the Raman spectra of RFB samples across different aging years.

3.5. UV spectroscopies

RFB contains a wide range of organic compounds, including alcohols, acids, esters, and phenols. These molecules generally contain chromophoric groups such as carbonyl, carboxyl, and aldehyde groups, which exhibit characteristic absorption in the UV region. Upon exposure to external radiation, electrons in these organic molecules undergo four types of electronic transitions: $\sigma \rightarrow \sigma^*$, $n \rightarrow \sigma^*$, $\pi \rightarrow \pi^*$, and $n \rightarrow \pi^*$ ³⁸. The original UV absorption spectra of RFB with different aging years in the range of 200 to 350 nm and corresponding spectral characteristics are shown in Fig. 5C and Table 1, respectively. Two characteristic absorption peaks were observed in all samples. Peak 1 appeared between 200 and 250 nm, whereas Peak 2 was located between 250 and 300 nm. Peak 1 primarily arises from $n \rightarrow \sigma^*$ transitions between non-bonding orbitals and antibonding σ^* orbitals, which commonly occur in saturated hydrocarbon derivatives containing heteroatoms such as nitrogen, sulfur, and oxygen. This peak is mainly associated with VOCs including alcohols and esters. Peak 2 is attributed to $\pi \rightarrow \pi^*$ transitions in unsaturated organic molecules containing functional groups such as C=O and C $\equiv$ C ³⁹. With increasing aging duration, Peak 1 showed a gradual decrease in intensity accompanied by a blue shift, indicating that aging reduces the relative content of saturated molecules in RFB. GC-MS results further

revealed that isobutanol, β -phenylethanol, ethyl lactate, and ethyl acetate, which are key VOCs in RFB and contain lone-pair electrons capable of undergoing $n \rightarrow \sigma^*$ transitions, decreased in concentration during aging⁴⁰. Consequently, the reduction in compound content caused by aging directly results in a decreased absorption intensity of Peak 1. Compared with unaged Baijiu, aged Baijiu exhibited a significantly higher intensity of Peak 2. This observation confirms that a large number of conjugated unsaturated molecules are formed during the aging process, thereby enhancing the absorption intensity of $\pi \rightarrow \pi^*$ transitions. The content of aldehydes (typical conjugated unsaturated molecules) increased significantly after aging, with the most notable increases observed for furfural and nonanal. Therefore, the UV absorption spectra indicated that RFB undergoes a structural evolution during aging, characterized by a decrease in saturated structures and a concomitant increase in unsaturated structures.

3.6. Three-way fluorescence spectrum

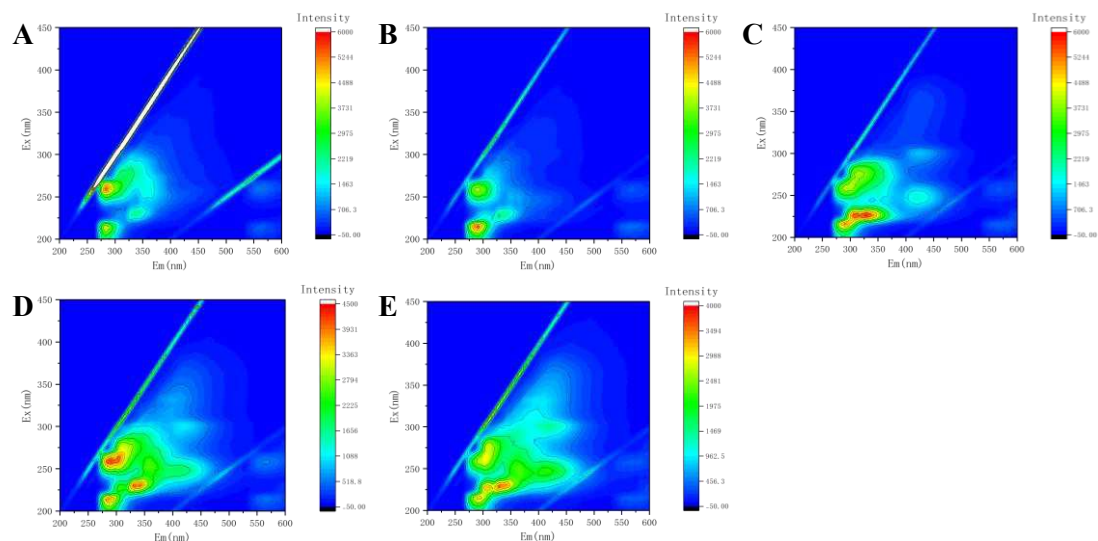


Fig. 6. Three-dimensional fluorescence spectra of RFB across different storage years. (A): 0 years, (B): 3 years, (C): 6 years; (D): 9 years, (E): 15 years.

Table 1. Fluorescence characteristics of RFB across different storage years.

Samples	Peak location Ex/Em (nm)					Fluorescence intensity (a.u.)				
	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5
Y0	214.0/284.0	230.0/334.0	258.0/284.0	-	-	4184	2148	5355	-	-
Y3	214.0/290.0	226.0/330.0	258.0/290.0	-	-	5427	2221	3977	-	-
Y6	216.0/290.0	226.0/326.0	262.0/302.0	300.0/428.0	248.0/420.0	5173	5755	3780	1163	1750
Y9	214.0/286.0	230.0/334.0	262.0/304.0	300.0/416.0	246.0/406.0	3858	3992	4181	1405	1893
Y15	214.0/290.0	228.0/330.0	260.0/304.0	300.0/416.0	248.0/408.0	3350	3596	2968	1551	2169

Fig. 6 illustrates the variation in fluorescence spectral profiles and fluorescence intensity across RFB samples aged for different durations. Baijiu contains a diverse array of fluorescent compounds, including alcohols, esters, acids, and aldehydes. Among these, alcohols, esters, and acids are recognized as the predominant flavor-active components. All RFB samples shared three common fluorescence peaks. These peaks, designated as Peak 1, Peak 2, and Peak 3, were identified by their respective Excitation/Emission (Ex/Em) wavelengths as follows: Peak 1 (214.0-216.0/284.0-290.0), Peak 2 (226.0-230.0/326.0-334.0), and Peak 3 (258.0-262.0/284.0-304.0). Ethanol and water are the predominant components of Baijiu, together accounting for more than 98 vol% of the total composition. Neither ethanol nor water molecules exhibit intrinsic fluorescence, the observed fluorescence originates from supramolecular cluster structures formed through hydrogen-bonding interactions between them ⁴¹. The cross-peak at 225/335 nm is generally regarded as a characteristic signal of the ethanol-water clusters ³⁴, corresponding to Peak 2 in the Fig. 6. Compared with Y0, the fluorescence intensity of Peak 2 increased from 2148 a.u. to 5755 a.u. in Y6 and then gradually decreased to 3596 a.u. in Y15. This suggests that the formation of water-ethanol cluster structures reached its maximum at 6 years of aging. Other peaks were likely associated with flavor constituents in RFB. The fluorescence intensity of Peak 1 increased to a maximum of 5427 a.u. in Y3 and subsequently declined, which aligns with the variation trend of volatile ester compounds revealed by the GC-MS analysis. Long-chain fatty acids are the major components of non-volatile acids in RFB. As the storage time increased, the fluorescence intensity of Peak 3 showed a decreasing trend, which was consistent

with the variation pattern of long-chain fatty acids. Therefore, Peak 3 is likely associated with non-volatile acid components. In Y6, the number of fluorescence peaks in RFB increased to five and remained constant at five in Y9 and Y15. This trend reflects the continuous formation of conjugated structures, indicating that the chemical composition and molecular architecture of the system became progressively more complex, which is characteristic of aging. This evolution is likely associated with the increased presence of aldehydes, aromatic compounds, and related substances. These findings are consistent with the results obtained from the ultraviolet absorption spectra.

3.7. Viscosity

Viscosity is a key property related to the characteristics of the wine body, as it affects the perceived thickness of mouthfeel^{42,43}. In this study, an Ubbelohde viscometer was employed to evaluate the viscosity changes of RFB across different aging years, and the results are presented in Fig. 5B. Compared with the unaged Baijiu (Y0), the viscosity increased to 2.46 and 3.25 mPa·s in Y3 and Y6, respectively, followed by a slight decrease to 3.07 and 2.99 mPa·s in Y9 and Y15. This trend is consistent with the hydrogen-bonding interactions observed in the FT-IR analysis and aligns with previous findings that the macroscopic viscosity of Baijiu is positively correlated with the total microscopic hydrogen bonding in the system¹⁸. Results from Raman spectroscopy and 3D fluorescence spectroscopy further indicated that early-stage aging (0-3 years) promotes the formation of water-ethanol clusters, with the maximum number of these clusters observed in the Y6. These findings suggest that the formation of water-ethanol clusters during aging plays a dominant role in regulating the viscosity of RFB. A study by Jiang et al.³³ reported that in strong-flavor and sauce-flavor Baijiu, the self-assembly of amphiphilic clusters formed through interactions between the hydrophobic moieties of flavor compounds and water-ethanol clusters gradually intensifies as storage time increases. Consistent with this finding, our previous GC-MS and LC-MS results showed that Y6 contained the highest levels of ethyl decanoate, ethyl caprylate, and total differential lipids and

lipid-like molecules, which play key roles in promoting amphiphilic cluster formation. However, with continued aging, the number of water-ethanol clusters decreased in Y9 and Y15. Compared with other Baijiu types, RFB contains a relatively simple composition of flavor substances, and these compounds decline markedly during long-term aging. As a result, the originally formed amphiphilic clusters gradually dissociate, leading to reduced viscosity in Y9 and Y15.

4. Conclusions

This study provides a comprehensive, multi-technique characterization of the chemical composition, structural evolution, and physicochemical properties of RFB across different aging years. LC-MS metabolomics revealed that aging significantly reshapes the N-VOCs of RFB, particularly through the accumulation of lipids and lipid-like molecules and the gradual decline of most long-chain fatty acids. Volatile analysis demonstrated that although esterification increases ester levels during early aging, long-term storage promotes volatilization and hydrolysis, resulting in a gradual decrease in total ester content. Higher alcohols and aldehydes exhibited distinct aging-dependent variation patterns, collectively contributing to the transformation of aroma attributes. During the first 6 years of storage, spectroscopic and viscosity characteristics demonstrated an enhancement of hydrogen-bonding interactions in RFB, which promoted the formation of ethanol-water clusters. However, prolonged aging weakened hydrogen bonding and led to dissociation of amphiphilic clusters, reducing overall viscosity. Compared with other Baijiu types, RFB exhibits a simpler chemical matrix and a more pronounced decline in flavor compounds during long-term storage, which explains the weakening of molecular assemblies in later years. Overall, the results clarify the key chemical and structural pathways governing RFB aging and provide a theoretical basis for optimizing storage strategies, flavor regulation, and product quality improvement in RFB.

Declaration of Interest Statement

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.

CRedit authorship contribution statement

Yongqiang Gong: Data curation, Writing-Original draft preparation. Ting Xie: Formal analysis, Investigation. Hongyu Mou: Visualization, Supervision. Wenjing Xiang: Software. Chengzhi Min: Validation. Tao Yang: Writing- Reviewing and Editing.

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