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Flavor compounds with high odor activity values (OAV > 1) dominate the aroma of aged Chinese rice wine (Huangjiu) by molecular association

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ABSTRACT

Aging is an essential operation to perfect the flavor quality of Hungjiu. In this study, formation mechanism of flavor compounds responsible for the characteristic flavor of aged Huangjiu was investigated. The contents of umami and bitter free amino acids (FAA) increased with the storage period prolonged, while that of sweet FAA showed downward trend. Gas chromatograph-mass spectrometry and principal component analysis indicated that the volatile flavor compounds with OAV exceed 1, especially middle-chain fatty-acid-ethyl-esters and aromatic compounds, dominated the characteristic flavor of aged Huangjiu. Low field-NMR was firstly applied to characterize the molecular association between water and dissolved flavor compounds in aged Huangjiu. The results showed that basic amino acids contributed greatly to the flavor formation of aged Huangjiu via molecular association. In addition, the molecular association significantly promoted the accumulation of flavor compounds with OAV > 1, especially ethyl esters.

1. Introduction

Huangjiu (Chinese rice wine), which has a history of more than 5000 years, is one of the most popular alcoholic beverages in east Asia for its unique flavor and subtle taste (Yu, Zhao, Li, Tian, & Ma, 2015). The manufacturing process of Huangjiu generally involves pretreatment of raw materials, preparation of staters, fermentation, sterilization, and aging (Yang et al., 2020). Fermentation respectively produces ethanol and flavor compounds from the sugar and protein (or other components), however, aging which improves and perfects the properties of Huangjiu makes the final product more pleasurable (Jiao, Xu, & Jin, 2017; Zhu, Xu, Ramaswamy, Yang, & Yu, 2016). Aging is a crucial procedure to produce high-quality Huangjiu due to fresh Huangjiu often taste astringent, flavorless, or bitter. Generally speaking, older wines possess better flavor and taste, as well as a higher market price, compared to young wines (Granato, Katayama, & de Castro, 2011). The process of wine aging usually takes a long time, and both consumers and winemakers are desired to produce high-quality Huangjiu in a short ageing period. Therefore, understanding the formation mechanism of flavor compounds responsible for the aroma in aged wine is of great significance to improve the quality of aged Huangjiu and shorten the aging time.

Throughout the aging process, a series of physical and chemical reaction occur, which tend to improve the organoleptic properties of Huangjiu (Tao, García, & Sun, 2014). The aged Huangjiu is abundant in many chemical components, including volatile flavor compounds, free amino acids (FAA), organic acids, etc. Among which, volatile flavor compounds are one of the most important to the sensory characteristic of

Abbreviations: FAA, Free amino acids; OAV, Odor activity value; SCFAEE, Short-chain fatty acid ethyl esters; MCFAEE, Medium-chain fatty acids ethyl esters; LCFAEE, Long-chain fatty acids ethyl esters.

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Huangjiu along with FAA (Wang, Chen, & Zhou, 2020; Shen, Ying, Li, Zheng, & Zhuge, 2011). Amino acid profiles were considered useful in the classification of wines according to grape variety, origin and vintage (Soufleros, Bouloumpasi, Tsarchopoulos, & Biliaderis, 2003). Shen et al. also reported that the FAA profiles could be used for the classification of Huangjiu with different ages (Shen et al., 2011). Currently, more than 900 kinds of volatile flavor compounds have been detected in Huangjiu (Chen, Xu, & Qian, 2018). It is well-accepted that not all the volatile flavor compounds are involved in the final aroma perception of Huangjiu. Thus, the key volatile flavor compounds and potential "aging-marker" of Huangjiu were investigated by researchers. Chen et al. found that sotolon, vanillin, 3-methylbutanal, and benzaldehyde played key roles in the overall aroma of aged Huangjiu (Guyuelongshan) (Chen, Wang, Qian, Li, & Xu, 2019). Wang et al. identified potential aging markers using metabolomic approach and multivariable statistics (Wang et al., 2020). Those markers might be taking account into the discrimination of Huangjiu age. However, changes of those key flavor compounds and formation mechanism of the aging-markers over aging are still unknown.

Most of the volatile flavor compounds in Huangjiu were presented in trace amount, while ethanol and water accounted for approximately 95% of Huangjiu. Thus, the molecular association between water and ethanol greatly affects the concentration and synthesis of dissolved flavor compounds. As the molecular association and chemical reactions of flavor compounds usually occurs slowly during Huangjiu aging, many physical methods have been proposed to shorten the aging time by strengthen the association between biomolecules (Zhang, Zeng, Lin, Sun, & Cai, 2013; Zhu et al., 2016). Although these aging methods can improve the flavor quality of Huangjiu, they cannot control the flavor characteristic. It has been reported that alcohols, esters, sugar, and acids exhibited a promoting effect on alcohol-water hydrogen-bonding during the aging process of Huangjiu, while aldehydes were exception (Cao et al., 2018). However, until now, to the best of our knowledge, effect of molecular association between water and dissolved compounds on the flavor formation of Huangjiu still remained to be elucidated. Low-field nuclear magnetic resonance (LF-NMR) has been suggested as an excellent noninvasive tool for food quality control and material property measurements (Ezeanaka, Nsor-Atindana, & Zhang, 2019). As the most important parameter of LF-NMR, the spin–spin relaxation (T_2) can provide many information about the relaxation time. According to the distribution and mobility of water and hydrogen protons, protons attached to proteins, carbohydrates and lipids can be distinguished (Blümich, Casanova, & Appelt, 2009). In LF-NMR analysis, the signals of water populations are typically analyzed in the frequency domain, and peak amplitudes are proportional to the amounts of stable hydrogen protons, which could be influenced by the molecular association between water and hydrophilic compounds (Nikolskaya & Hiltunen, 2020). Therefore, the relaxation properties of LF-NMR might be used to characterize the molecular association between macromolecules. In current work, the flavor compounds responsible for the aroma and taste of Huangjiu were analyzed based on gas chromatograph-mass spectrometry (GC–MS) analysis, electronic nose (E-nose) and electronic tongue (E-tongue). Raman spectroscopy and LF-NMR were employed to further characterize the molecular association between water and flavor compounds of Huangjiu during the aging process. This work will provide a substantial basis for the control of flavor formation during the aging process of Huangjiu, the shortening of aging time and the improvement of flavor quality in aged Huangjiu or other alcoholic beverages.

2. Materials and methods

2.1. Materials

Huangjiu samples aged for 1, 2, 3, 4, 5, 6, and 7 years were obtained from Shanghai Jinfeng Wine Co., Ltd (Shanghai, China). Each vintage wine was sampled for three replicates, sealed in sample vial, and stored at −20 °C until analysis. All reagents and standards were purchased at Sigma Aldrich (Shanghai, China). *n*-Alkane (C_7-C_{40}; Supelco, Bellefonte, PA, USA) standards were used to determine the retention index.

2.2. Determination of FAA content

The profiles of FAA in Huangjiu were obtained using an L-8900 automatic amino acid analyzer (Hitachi High-Technologies Corporation, Tokyo, Japan) according to the method described by Yang et al. (2019). To eliminate the interference of large peptides, the filtered Huangjiu sample was precipitated with equal volume of sulfosalicylic acid for 2 h at 4 °C and centrifuged at 12,000×g for 20 min. Then, a 10-μL aliquot of the supernatant was injected into the amino acid analyzer. The contents of FAA were expressed as mg/L Huangjiu sample referring to a standard of amino acid mixture.

2.3. E-nose and E-tongue analysis

A SuperNose electronic nose (Isenso Group Cooperation, New York, USA) equipped with an array of 14 sensors and a Smart Nose intelligent identification software system was used for the aroma classification of Huangjiu with different ages. Huangjiu sample (20 mL) was sealed into 250-mL glass vials and maintained at 25 °C for 1 h prior to the analysis. Then 10 mL of the gas compounds was absorbed from the headspace through the sensor array with clean air (as the carrier gas) at a flow rate of 1.0 L/min for 60 s, and during this time the sensor signals were recorded. The cleaning flow of sensor was 6 L/min and the automatic zero adjustment time was 10 s. Five biological replicates were prepared for each sample.

The taste fingerprint of different Huangjiu samples were characterized by a SuperTongue electronic nose setup (Isenso Group Cooperation, New York, USA) which equipped with MLAPS (Multi-frequency large amplitude pulse scanner) and a multivariate mathematical statistics system. The taste analyzer was composed of six working electrodes including gold, palladium, silver, platinum, titanium, tungsten, and an Ag/AgCl electrode as the reference electrode. Twenty-five mL of wine sample was used for the E-tongue analysis. The measurement was performed at room temperature, and the detection time of each Huangjiu sample was 180 s (30 s for each working electrode). Between the measurements, the device was rinsed with ultrapure water for 10 s to reach stable state. Each sample was analyzed for 5 replicates.

2.4. Raman analysis

Raman spectroscopy measurement was performed using a LabRAM HR Evolution system (HORIBA Co., Paris, France) equipped with a cooled charge-coupled device detector, a 785 nm laser source and motorized microscope at constant temperature (25 °C) and humidity (60%). Each wine sample without dilution was analyzed for triple measurements, with the laser power set to 100 mW and the spectral wavelength ranged from 200 to 4000 cm^{-1} at a resolution of 0.8 cm^{-1}. The LabSpec 6 software provided by HORIBA Co. (Paris, France) was used for the spectral data acquisition and the spectrum was collected as the mean spectrum.

2.5. LF-NMR analysis

An LF-NMR analyzer (PQ001, Niumag Co., Shanghai, China) combined with a multiexponential fitting analysis (*T*-invfit) program was employed for the NMR measurement. Transverse relaxation was measured using the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence. In prior to the detection, 3 mL of wine sample filled into the LF-NMR tube (15 mm of diameter) was equilibrated to 32 °C. The strength of magnetic field was 0.50 ± 0.08 T, and the proton resonance frequency was 22 MHz. The data was collected from 6000 echoes over four scans and waited for 2000 ms between scans. The time between 90° and 180°

pulses was 250 μs. The relaxation measurement was performed at constant operating temperature of 32 °C. T_{2W} (the single component relaxation time) was used to reflect the overall relation distribution of detected sample. Distribution of transverse relaxation time (T_2) was obtained by the multi-exponential fitting, and each relaxation peak represented a certain hydrogen proton population. Among which, transverse relaxation time (T_{2i}), the signal amplitude (A_{2i}), and the percentage relative contribution (RC_{2i}) and could be obtained (Li et al., 2015).

2.6. Analysis of volatile flavor compounds

The analyzed sample was diluted to a final concentration of 6 vol% ethanol, and then added into headspace glass vial with 10 μL internal standard of 4-methyl-2-pentanol (250 μg/mL in absolute ethanol). The volatile compounds were extracted for 30 min at 50 °C by a DVB/CAR/PDMS fiber and desorbed at the GC injection port at 250 °C for 7 min for GC–MS analysis. A Thermo Trace 1300 gas chromatograph (Thermo Fisher Inc., USA) equipped with an Thermo ISQ mass spectrometry was used for the analysis of volatile flavor compounds. A DB-WAX column (60 m × 0.25 mm × 0.25 μm, Agilent Technologies, USA) was used for the separations. The oven temperature was initially programmed at 40 °C for 3 min, raised to 210 °C at 6 °C/min, and finally raised at 8 °C/min to 230 °C for 15 min. Helium was delivered at a flow rate of 1 mL/min as the carrier gas. Data acquisition was in the full scan mode (ionization energy = 70 eV). The compounds were tentatively identified by matching the mass spectra with the NIST and Wiley mass spectral databases. Positive identification was carried out by comparing mass spectra of the authentic standards. The relative contents of volatile compounds were calculated according to the internal standard method.

2.7. Statistic analysis

All data were expressed as means ± standard deviations (SD). One-way ANOVA and Duncan's test were used to analyze the significant differences between data using SPSS 19.0 software (SPSS Inc., Chicago, USA). The E-nose and E-tongue responses of Huangjiu were analyzed by principal component analysis (PCA), which were conducted using the software equipped with the correspondingly analyzer (Isenso Group Cooperation, New York, USA). PCA for the data of volatile flavor compounds were performed by SPSS software was performed to investigate the relationship between the Huangjiu wine different wine ages and the volatile flavor compounds. The discrimination index (DI) was obtained after conducting PCA. The DI which can be between 0 and 100 is determined by calculating the ratio between the sum of areas of sample groups and the area of the whole PCA map. Partial least squares regression analysis (PLSR) was used to explore the relationship between volatile compounds and E-nose, as well as FAA and E-tongue through UNSCRAMBLER ver. 9.8 (CAMO ASA, Oslo, Norway). TBtools was used to visualize the differentiation of free amino acids in different Huangjiu samples (Chen et al., 2020). Spearman's correlation coefficient indicated the correlation among volatile flavor compounds, free amino acids and LF-NMR parameter was calculated and visualized by using OriginPro 2021 software (Origin Lab Inc., Hampton, MS, USA).

3. Results and discussion

3.1. Quantification and changes of FAA in Huangjiu during aging

FAA contributes significantly to the flavor of Huangjiu during aging, as it not only provides nitrogen resources that are used in biochemical reactions but also directly contribute to the flavor (Shen et al., 2011; Xie et al., 2016). The concentration of FAA could be used to evaluate the quality of Huangjiu, as FAA profiles varied with the varieties and origins in different foods and beverages (Shen et al., 2011). The sum of FAA content in different Huangjiu ranged from 3460.88 to 5176.99 mg/L, which was consistent with the result of Shen et al. (2010). The total content of FAA decreased during the first 3-years aging, and then showed an upward trend during the next 4-years storage. The decreased trend could be attributed to amino-carbonyl reactions, while the increased trend might be due to hydrolyzation of peptides and/or proteins by microorganisms (Moreno-Arribas, Pueyo, Polo, & Martín-Álvarez, 1998).

A total of 18 FAA was quantitatively detected in Huangjiu during aging. Ala and Arg with average amounts above 600 mg/L were the most abundant amino acids in the aged Huangjiu, this result was in accordance with those reported by Shen et al. (2010) and Wei (2007). However, in case of Hong Qu Huangjiu, Tian et al. (2016) reported that Glu were the most abundant FAA. Generally, the saccharification starter of Hongqu Huangjiu is mainly made from *Monascus*, while that of current Huangjiuis mainly made from *Aspergillus* spp. The key fungi in the two types of Huangjiu is different, therefore, it could be inferred that the composition of microorganisms played important role in the production of FAA. The contents of Orn, GABA, Cys, His, and Met in the average range of 25.65–99.75 mg/L only accounted for lower than 10% of the total content of FAA, however, all of those FAA exhibited significant difference between Huangjiu aged for 1 year and 7 years ($P < 0.05$). The contents of Lys, Met, Ala and Glu experienced significant increase ($P < 0.001$) after 7-years storage, whereas Ser suffered significant decrease ($P < 0.001$). The contents of Leu and Ile exhibited little difference between Huangjiu of 1-year old and 7-years old (Fig. 1a).

FAA may taste sweet, bitter, fresh, astringent, or umami, which bring Huangjiu a rich taste and enable the wine to be harmonious. Thus, the variety and profile of FAA have significant influence on the taste of Huangjiu. According to the category of Zhang et al. (2018), Asp and Glu belong to the umami taste, Ala, Gly, Ser, and Thr taste sweet, while Arg, His, Ile, Leu, Met, Phe, and Val taste bitter. Overall, with the wine age increased, an upward trend was observed in the relative contents of umami and bitter FAA (Fig. 1b). Although only Glu and Asp contributed to the umami taste, relative contents of the two FAA exhibited opposite trends with the increase of wine age. It is suggested that Glu contributed greater to the umami taste of Huangjiu than Asp. Interestingly, in case of sweet FAA, a downward trend was observed with the aging time, although Ala concentration (577.50–726.18 mg/L) always higher than the sum of Thr, Ser, and Gly (485.36–576.64 mg/L). The Ala might have a higher threshold than the other three FAA in Huangjiu. (Fig. 1a & 1b).

3.2. Changes of volatile flavor compounds in Huangjiu with different wine ages

The typical flavor of Huangjiu is strongly influenced by the volatile flavor compounds (Chen et al., 2019). The contents of volatile flavor compounds in different Huangjiu samples were shown in Table 1. A total of 53 compounds were detected, of those, 45 compounds were positively identified by the comparison of mass spectral database with the authentic standards, and the other 8 compounds were tentatively identified through the comparison with database and literature. These compounds included 13 alcohols, 25 esters, 6 aldehydes, 3 acids, 3 ketones, and 2 phenols (Table 1). Alcohols and esters, which correspondingly accounted for 24.53% and 47.17% of the total number of volatile flavor compounds, were the predominant compounds of aged Huangjiu. With the increase of storage period, the concentration of alcohols gradually deceased (48.51–42.94 mg/L), which could be ascribed to the volatilization, oxidation and esterification reactions (Tian et al., 2016). In terms of esters, an increase tendency (24.22–33.24 mg/L) was observed and chemical esterification of fatty acids interacting with ethanol might be responsible for the increase.

Among the detected alcohols, the most abundant compounds were 2-methyl-1-propanol, 3-methyl-1-butanol, and 2-phenylethyl alcohol. As higher alcohols, excessive content of the 3 alcohols not only spoiled the flavor of Huangjiu, but also damages human health. It could be noted that the contents of the 3 alcohols significantly decreased with the

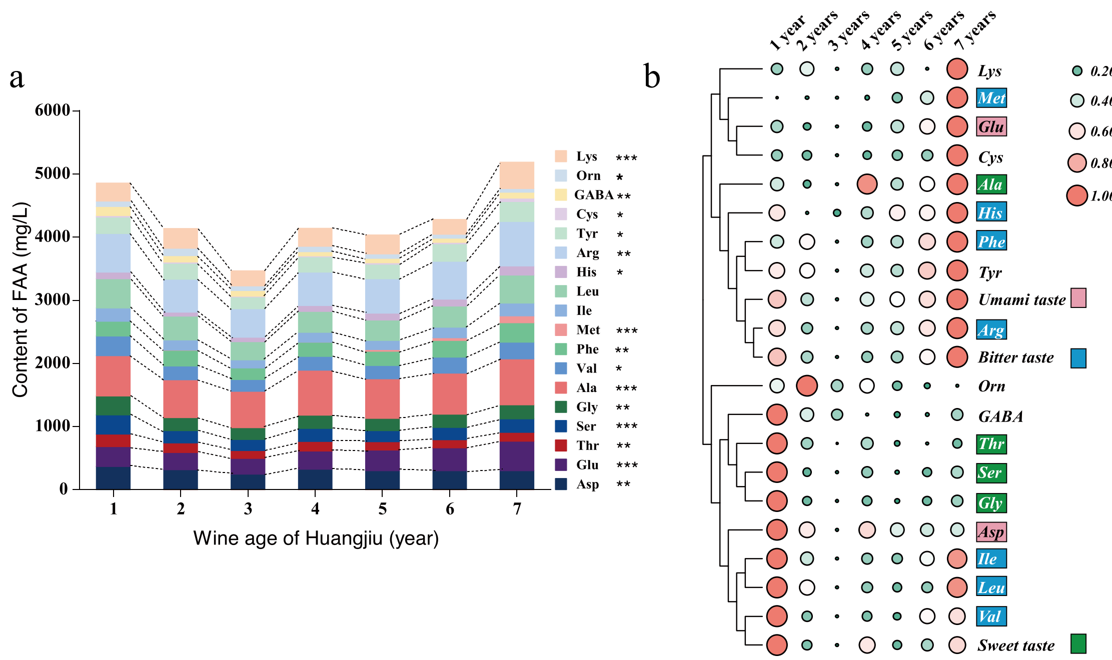

Fig. 1. Changes of FAA profiles in Huangjiu with different ages (a), and differentiation of individual amino acid among Huangjiu aged for different years (visualized by red-white-light green heatmap). All quantified concentrations of FAA were normalized to 0–1 scores for each Huangjiu sample. Clustering was conducted based on Pearson correlation for FAA content. The circle bar denoted the relative content of each individual FAA. The redder (the bigger) of the circle, the higher content of the FAA, and the greener (the smaller) of the circle, the lower content of the FAA. In the color panels for the FAA classification, green, blue, and pink indicated sweet taste, bitter taste, and umami taste, respectively. Statistical differences between Huangjiu aged for 1 year and 7 years were assessed, with (*) (**) and (***) representing the significant difference under $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

storage period. Among which, only 2-phenylethyl alcohol exhibited an OAV (calculated as ration between compound concentration and odor threshold) higher than 1. It has been widely accepted that the flavor compound with OAV > 1 could significantly contribute its flavor notes to the overall aroma of wine (Noguerol-Pato, González-Álvarez, González-Barreiro, Cancho-Grande, & Simal-Gándara, 2012). Thus, 2-phenylethyl alcohol contributed greatly to the final alcohol-aroma of Huangjiu during aging. In addition, 1-octen-3-ol and 2-nonanol with OAVs > 1 also had important effect to the wine aroma. The detection of 1-octen-3-one and 2-nonanone indicated that the two alcohols were oxidized into ketones during Huangjiu storage.

As for esters, ethyl esters constituted the largest group of esters. Based on the chain length, the ethyl esters could be classified into short-chain fatty acid ethyl esters (SCFAEE), medium-chain fatty acid ethyl esters (MCFAEE) and long-chain fatty acid ethyl esters (LCFAEE). The SCFAEE and MCFAEE, which has fairly low odor thresholds (high OAVs), greatly contributed fruity and flower notes to the aroma of Huangjiu, while the LCFAEE might bring aroma of ether or wax into the wine (Table 1). Except for ethyl nonanoate and ethyl dodecanoate, OAVs of the other MCFAEE were all higher than 1. As for SCFAEE and LCFAEE, only ethyl butanoate and ethyl hexadecanoate were found with OAVs > 1. This suggested that MCFAEE had the greatest impact on the aroma of aged Huangjiu. It could be noted that the OAV of 2-phenethyl acetate was lower than its threshold in Huangjiu aged for 1 and 2 years, but exceed 1 since the 3rd year. The longer the aging time, the greater the contribution of 2-phenethyl acetate to the overall aroma, indicating that it was an important component of the aging aroma of Huangjiu. Isoamyl acetate, as the only acetate ester, exhibited the highest OAV in all the detected compounds. In addition, the aging process significantly promoted the accumulation of isoamyl acetate.

The flavors belonged to aromatic compounds (with the structure of benzene rings), except for benzyl alcohol and 4-methyl benzaldehyde, were quantified with OAVs > 1 and contributed flowery, fruity and almond notes to the aroma of Huangjiu. The contents of aromatic compounds slowly increased during the first 4-years aging, and then a substantial rise was observed. In other words, long aging time was needed to promote the accumulation of aromatic compounds in Huangjiu. Although only 7 aromatic compounds were identified, their total concentration was significantly higher than that of esters, suggesting the importance of aromatic compounds on the overall aroma of Huangjiu during aging. Previous studies on the flavor of alcoholic beverages mainly focused on the changes of alcohol, esters and aldehydes (Tian et al., 2016; Wang et al., 2020). However, influence of aromatic compounds on the wine flavor has been largely ignored, and more attention should be paid to study the role of aromatic compounds on the aroma of alcoholic beverages in future research.

As the key flavor compounds of Huangjiu, the contents of higher alcohols decreased with the storage period, while MCFAEE, 2-phenethyl acetate, isoamyl acetate and aromatic compounds conversely exhibited a rising trend.

3.3. Differentiation of the flavor characteristic during Huangjiu aging

PCA was applied to analyze the differentiation of flavor characteristic in Huangjiu with different wine ages, using the concentrations of volatile flavor compounds as analytical variables (Fig. 2). The first and second principal components (PC1 and PC2) correspondingly explained 54.78% and 11.36% of the total variance, which reflected 66.14% of the flavor profile in Huangjiu during aging (Fig. 2a). The loadings of ketones, acids, and phenols were all highly located on the negative axis of PC2. Most esters were highly related with the positive axis of PC1, except for ethyl nonanoate, ethyl dodecanoate, ethyl tetradecanoate, ethyl (9E)-9-octadecenoate, ethyl oleate, and ethyl 2-hydroxy-3-phenylpropanoate, which OAVs were lower than 1. This phenomenon suggested that the contribution of esters to the Huangjiu flavor was different from that of ketones, acids, and phenols during aging. The low numbers and concentrations of ketones, acids and phenols might be responsible for this result.

The distribution of alcohols was highly influenced by 2-methyl-1-propanol, while aldehydes was closely related to furfural,

Table 1
Changes of the volatile flavor compounds in Huangjiu with wine ages ranged from 1 year to 7 years (n = 3).

Code	Compounds [a]	Identity[b]	KI [c]	Aroma [d]	Threshold (mg/L) [e]	Relative content (mg/L)						
						1 year	2 years	3 years	4 years	5 years	6 years	7 years
Alcohols												
al1	2-Methyl-1-propanol	MS, RI	1102	Fusel, alcohol	40	2.25 ± 0.05	2.27 ± 0.09	2.07 ± 0.17	1.73 ± 0.12	2.18 ± 0.06	1.84 ± 0.03	1.57 ± 0.05
al2	1-Butanol	MS, RI	1156	Medicinal	150	0.11 ± 0.008	0.08 ± 0.004	0.05 ± 0.003	0.06 ± 0.002	n. d	n. d	n. d
al3	3-Methyl-1-butanol	MS, RI	1218	Whiskey, malt	30	17.59 ± 0.88	18.57 ± 0.72	18.81 ± 0.54	17.60 ± 0.71	17.28 ± 0.69	16.83 ± 0.43	15.31 ± 0.97
al4	1-Pentanol	MS, RI	1259	Fusel	80	0.06 ± 0.003	0.07 ± 0.003	0.11 ± 0.005	0.02 ± 0.001	0.08 ± 0.001	0.09 ± 0.004	0.05 ± 0.002
al5	2-Heptanol	MS, RI	1341	Fruity, musty	0.20	0.13 ± 0.007	0.18 ± 0.005	0.23 ± 0.004	0.28 ± 0.01	0.18 ± 0.008	n. d	n. d
al6	1-Hexanol	MS, RI	1353	Green, grass	8	0.48 ± 0.015	0.36 ± 0.02	0.63 ± 0.03	0.49 ± 0.01	0.68 ± 0.05	0.76 ± 0.02	0.58 ± 0.01
al7 *	1-Octen-3-ol *	MS, RI	1448	n. f	0.001	0.27 ± 0.008	0.19 ± 0.005	0.13 ± 0.003	0.31 ± 0.01	0.48 ± 0.008	0.34 ± 0.01	0.45 ± 0.009
al8 *	2-Nonanol *	MS, RI	1512	n. f	0.058	0.29 ± 0.011	0.53 ± 0.03	0.72 ± 0.06	0.42 ± 0.02	0.40 ± 0.01	0.78 ± 0.04	0.55 ± 0.03
al9	3-Methylthiopropanol	MS, RI	1716	Meat	1.0	0.11 ± 0.002	0.14 ± 0.004	0.16 ± 0.006	0.08 ± 0.003	0.11 ± 0.005	0.09 ± 0.002	0.10 ± 0.004
al10	Benzyl alcohol	MS, RI	1888	Sweet, flower	200	0.43 ± 0.01	0.52 ± 0.03	0.48 ± 0.02	0.55 ± 0.01	1.40 ± 0.06	0.87 ± 0.04	1.39 ± 0.15
al11 *	2-Phenylethyl alcohol *	MS, RI	1920	Flowery, pollen	14	26.54 ± 0.76	26.94 ± 0.62	25.17 ± 0.71	25.25 ± 0.53	24.03 ± 0.77	23.52 ± 0.82	22.76 ± 0.64
al12	1-Decanol	MS, RI	1769	Flowery	0.4	0.08 ± 0.002	0.05 ± 0.003	0.02 ± 0.001	0.02 ± 0.001	0.05 ± 0.003	n. d	n. d
al13	Cedrol	MS, RIL	2132	Woody	n. f	0.17 ± 0.003	0.13 ± 0.005	0.16 ± 0.001	0.25 ± 0.006	0.14 ± 0.004	0.16 ± 0.002	0.18 ± 0.006
Total						**48.51**	**50.03**	**48.74**	**47.06**	**47.01**	**45.28**	**42.94**
Esters												
Short-chain fatty acids ethyl ester												
et1	Ethyl acetate	MS, RI	895	Fruity, sweet	7.50	2.67 ± 0.07	2.12 ± 0.11	2.84 ± 0.17	2.66 ± 0.05	2.69 ± 0.09	2.75 ± 0.13	2.78 ± 0.19
et2 *	Ethyl butanoate *	MS, RI	1052	Fruity	0.02	0.52 ± 0.018	0.61 ± 0.03	0.63 ± 0.009	0.69 ± 0.01	0.81 ± 0.008	0.81 ± 0.005	1.07 ± 0.06
MCFAEE												
et3 *	Ethyl hexanoate *	MS, RI	1240	Apple, peach	0.014	0.60 ± 0.02	0.73 ± 0.01	0.71 ± 0.008	0.88 ± 0.03	1.23 ± 0.07	1.19 ± 0.18	1.91 ± 0.25
et4 *	Ethyl heptanoate *	MS, RI	1335	Berry, plum	0.002	0.14 ± 0.009	0.27 ± 0.01	0.34 ± 0.008	0.28 ± 0.02	0.19 ± 0.006	0.37 ± 0.01	0.95 ± 0.05
et5 *	Ethyl octanoate *	MS, RI	1440	Pineapple, pear, floral	0.005	1.28 ± 0.06	1.41 ± 0.05	1.22 ± 0.03	1.34 ± 0.07	1.52 ± 0.04	1.87 ± 0.08	1.91 ± 0.13
et6	Ethyl nonanoate	MS, RI	1562	Fruity, coconut	0.20	0.13 ± 0.005	0.15 ± 0.002	0.10 ± 0.008	0.18 ± 0.006	0.12 ± 0.007	0.08 ± 0.005	0.11 ± 0.006
et7 *	Ethyl decanoate *	MS, RI	1652	Fruity, fatty	0.20	0.25 ± 0.03	0.29 ± 0.01	0.16 ± 0.05	0.20 ± 0.009	0.38 ± 0.02	0.32 ± 0.02	0.27 ± 0.03
et8	Ethyl dodecanoate	MS, RI	1830	Rose, honey	1.50	0.64 ± 0.06	0.38 ± 0.05	0.20 ± 0.02	0.10 ± 0.004	n. d	n. d	n. d
LCFAEE												
et9	Ethyl tetradecanoate	MS, RI	2035	Ether	2	1.66 ± 0.21	1.86 ± 0.13	1.85 ± 0.19	2.48 ± 0.10	1.94 ± 0.23	1.40 ± 0.09	1.47 ± 0.12
et10 *	Ethyl hexadecanoate *	MS, RI	2260	Wax	1.50	10.49 ± 0.46	8.74 ± 0.61	8.87 ± 0.68	9.43 ± 0.73	9.88 ± 0.53	10.46 ± 0.77	10.08 ± 0.62
et11	Ethyl stearate	MS, RI	2458	Wax	n. f	0.03 ± 0.001	0.04 ± 0.002	0.02 ± 0.001	0.07 ± 0.002	0.06 ± 0.001	0.06 ± 0.002	0.05 ± 0.003
et12	Ethyl (9E)-9-octadecenoate	MS, RIL	2485	Flowery, fruity	n. f	0.94 ± 0.16	0.58 ± 0.06	0.48 ± 0.03	0.36 ± 0.008	0.44 ± 0.01	0.26 ± 0.004	0.35 ± 0.01
et13	Ethyl oleate	MS, RI	2493	Flowery	n. f	0.19 ± 0.006	0.18 ± 0.003	0.14 ± 0.004	0.13 ± 0.003	0.14 ± 0.002	0.12 ± 0.003	0.15 ± 0.005
et14	Ethyl linoleate	MS, RI	2536	n. f	n. f	1.15 ± 0.15	0.74 ± 0.11	0.87 ± 0.08	1.27 ± 0.11	1.31 ± 0.19	1.71 ± 0.10	0.86 ± 0.13
Acetate ester												
et15 *	Isoamyl acetate *	MS, RI	1135	Banana	0.003	0.79 ± 0.035	1.53 ± 0.09	1.90 ± 0.14	1.81 ± 0.22	1.95 ± 0.17	1.78 ± 0.11	2.16 ± 0.18
Other ester												
et16	Ethyl L-lactate	MS, RI	1355	Lactic, raspberry	154.64	0.94 ± 0.04	1.73 ± 0.06	1.28 ± 0.19	2.31 ± 0.15	2.33 ± 0.26	2.89 ± 0.34	3.49 ± 0.39
et17	Ethyl 2-hydroxy-4-methylvalerate	MS, RIL	1581	n. f	n. f	0.11 ± 0.004	0.16 ± 0.008	0.22 ± 0.01	0.22 ± 0.007	0.47 ± 0.005	0.28 ± 0.02	0.56 ± 0.06
et18 *	Ethyl benzoate *	MS, RI	1670	Camomile, fruity	0.58	0.88 ± 0.06	0.81 ± 0.03	1.07 ± 0.08	0.88 ± 0.02	1.92 ± 0.12	2.04 ± 0.17	2.65 ± 0.14
et19	Diethyl succinate	MS, RI	1696	Fruity	200	0.41 ± 0.05	0.53 ± 0.12	0.77 ± 0.09	0.65 ± 0.07	0.75 ± 0.11	0.73 ± 0.04	0.84 ± 0.15
et20		MS, RI	1811	Caramel	n. f					n. d	n. d	n. d

(continued on next page)

Table 1 (*continued*)

Code	Compounds [a]	Identity[b]	KI [c]	Aroma [d]	Threshold (mg/L) [e]	Relative content (mg/L)						
						1 year	2 years	3 years	4 years	5 years	6 years	7 years
	Ethyl 4-hydroxybutanoate					0.11 ± 0.003	0.07 ± 0.004	0.03 ± 0.002	0.02 ± 0.001			
et21 *	2-Phenethyl acetate *	MS, RI	1830	Floral	0.25	0.19 ± 0.001	0.18 ± 0.006	0.31 ± 0.004	0.3 ± 0.007	0.33 ± 0.01	0.50 ± 0.01	0.68 ± 0.03
et22	Ethyl 3-phenylpropionate	MS, RIL	1937	Fruity, honey	0.07	n. d	n. d	n. d	n. d	0.05 ± 0.002	0.08 ± 0.003	0.09 ± 0.002
et23 *	γ-Nonalactone *	MS, RIL	2028	Coconut	0.03	0.05 ± 0.001	0.04 ± 0.002	0.04 ± 0.002	0.03 ± 0.001	0.26 ± 0.006	0.12 ± 0.002	0.14 ± 0.001
et24	Ethyl 2-hydroxy-3-phenylpropanoate	MS, RIL	2296	n. f	n. f	n. d	n. d	0.02 ± 0.001	0.04 ± 0.002	0.25 ± 0.008	0.12 ± 0.005	0.67 ± 0.01
et25	Ethyl 9-hexadecenoate	MS, RI	2270	n. f	n. f	0.05 ± 0.002	0.06 ± 0.003	0.03 ± 0.001	0.04 ± 0.002	n. d	n. d	n. d
Total						**24.22**	**23.21**	**24.10**	**26.37**	**29.02**	**29.94**	**33.24**
Aldehydes												
ad1	2-Pentenal	MS, RI	1091	Potato	n. f	n. d	n. d	n. d	n. d	0.09 ± 0.004	0.04 ± 0.002	0.13 ± 0.002
ad2 *	Nonanal *	MS, RI	1385	Pungent	0.001	0.40 ± 0.03	0.33 ± 0.01	0.30 ± 0.05	0.21 ± 0.02	0.09 ± 0.003	0.07 ± 0.005	0.10 ± 0.002
ad3	Furfural	MS, RI	1472	Bread, almond	14.10	0.62 ± 0.01	0.94 ± 0.05	1.83 ± 0.08	2.09 ± 0.12	1.69 ± 0.07	1.98 ± 0.26	1.97 ± 0.15
ad4 *	Benzaldehyde *	MS, RI	1526	Almond	2	3.28 ± 0.19	4.74 ± 0.22	4.51 ± 0.15	5.23 ± 0.31	5.49 ± 0.26	6.23 ± 0.24	6.63 ± 0.47
ad5 *	Benzeneacetaldehyde *	MS, RI	1640	Honey	0.004	0.22 ± 0.008	0.23 ± 0.005	0.37 ± 0.02	0.12 ± 0.006	0.61 ± 0.04	0.31 ± 0.009	0.28 ± 0.02
ad6	4-Methyl benzaldehyde	MS, RI	1648	Cherry	n. f	0.11 ± 0.004	0.17 ± 0.005	0.14 ± 0.003	0.23 ± 0.006	0.15 ± 0.004	0.32 ± 0.007	0.35 ± 0.01
Total						**4.63**	**6.41**	**7.15**	**7.88**	**8.12**	**8.95**	**9.46**
Aromatic copmpounds												
al10	Benzyl alcohol	MS, RI	1888	Flowery	200	0.43 ± 0.01	0.52 ± 0.03	0.48 ± 0.02	0.55 ± 0.01	1.40 ± 0.06	0.87 ± 0.04	1.39 ± 0.15
al11 *	2-Phenylethyl alcohol *	MS, RI	1920	Flowery, pollen	14	26.54 ± 0.76	26.94 ± 0.62	25.17 ± 0.71	25.25 ± 0.53	24.03 ± 0.77	23.52 ± 0.82	22.76 ± 0.64
et18 *	Ethyl benzoate *	MS, RI	1670	Camomile, fruity	0.58	0.88 ± 0.06	0.81 ± 0.03	1.07 ± 0.08	0.88 ± 0.02	1.92 ± 0.12	2.04 ± 0.17	2.65 ± 0.14
et21 *	2-Phenethyl acetate *	MS, RI	1830	Floral	0.25	0.19 ± 0.001	0.18 ± 0.006	0.31 ± 0.004	0.3 ± 0.007	0.33 ± 0.01	0.50 ± 0.01	0.68 ± 0.03
ad4 *	Benzaldehyde *	MS, RI	1526	Almond	2	3.28 ± 0.19	4.74 ± 0.22	4.51 ± 0.15	5.23 ± 0.31	5.49 ± 0.26	6.23 ± 0.24	6.63 ± 0.47
ad5 *	Benzeneacetaldehyde *	MS, RI	1640	Honey	0.004	0.22 ± 0.008	0.23 ± 0.005	0.37 ± 0.02	0.12 ± 0.006	0.61 ± 0.04	0.31 ± 0.009	0.28 ± 0.02
ad6	4-Methyl benzaldehyde	MS, RI	1648	Cherry	n. f	0.11 ± 0.004	0.17 ± 0.005	0.14 ± 0.003	0.23 ± 0.006	0.15 ± 0.004	0.32 ± 0.007	0.35 ± 0.01
Total						**31.65**	**33.59**	**32.05**	**32.56**	**33.93**	**33.81**	**34.74**
Acids												
ac1	Acetic acid	MS, RI	1435	Vinegar	200	1.93 ± 0.13	1.67 ± 0.15	1.75 ± 0.11	1.70 ± 0.12	1.43 ± 0.08	1.80 ± 0.10	1.64 ± 0.15
ac2 *	Hexanoic acid *	MS, RI	1859	Cheese, acidic	0.42	1.02 ± 0.11	0.73 ± 0.13	0.89 ± 0.08	0.74 ± 0.10	0.92 ± 0.05	0.83 ± 0.09	0.69 ± 0.007
ac3 *	Octanoic acid *	MS, RI	2067	Sweat,	0.50	0.80 ± 0.06	0.72 ± 0.05	0.61 ± 0.08	0.52 ± 0.04	0.48 ± 0.06	0.34 ± 0.03	0.33 ± 0.04
Total						**3.75**	**3.12**	**3.25**	**2.96**	**2.83**	**2.97**	**2.66**
Ketones												
kt1	1-octen-3-one	MS, RIL	1311	Mushroom	n. f	0.06 ± 0.003	0.08 ± 0.002	0.04 ± 0.002	0.05 ± 0.003	0.08 ± 0.002	0.03 ± 0.001	0.07 ± 0.002
kt2	2-Octanone	MS, RI	1324	Apple peel	1.10	0.17 ± 0.005	0.26 ± 0.01	0.14 ± 0.002	0.22 ± 0.006	0.19 ± 0.004	0.18 ± 0.009	0.11 ± 0.003
kt3 *	2-Nonanone *	MS, RI	1401	Fruity	0.041	0.08 ± 0.003	0.08 ± 0.005	0.05 ± 0.006	0.07 ± 0.002	0.02 ± 0.001	0.06 ± 0.003	0.04 ± 0.001
Total						**0.31**	**0.42**	**0.23**	**0.34**	**0.29**	**0.27**	**0.22**
Phenols												
ph1	2-Methoxy-phenol	MS, RI	1881	Perfume	n. f	0.03 ± 0.002	0.06 ± 0.003	0.03 ± 0.001	0.02 ± 0.002	0.03 ± 0.002	0.02 ± 0.003	0.04 ± 0.002
ph2	2-Methoxy-4-vinylphenol	MS, RIL	2223	Buckwheat	n. f	0.08 ± 0.002	0.05 ± 0.001	0.03 ± 0.003	n. d	n. d	n. d	n. d
Total						**0.11**	**0.11**	**0.06**	**0.02**	**0.03**	**0.02**	**0.04**
Total compounds						**81.53**	**83.30**	**83.53**	**84.63**	**87.30**	**87.43**	**88.56**

a Volatile flavor compounds detected in the aged *Huangjiu*.
b Method of identification: MS, compounds were identified by MS spectra. RI, compounds were identified by comparison to pure standard. RIL, compounds were identified by comparison with RI from http://webbook.nist.gov/.
c KI is the retention index calculated by the Kovats method.
d Aroma descriptor was derived from the web pages: (http://www.leffingwell.com) (http://flavornet.org/flavornet.html) and (http://www.odour.org.uk/).
e Odor threshold from the literature: Yu et al., 2020; Rahayu et al., 2017; Wang et al., 2020.
"n. d": not detected; "n. f": not found; MCFA: medium-chain fatty acids; LCFA: long-chain fatty acids.
The marker of "*" represented the compounds with OAVs > 1.

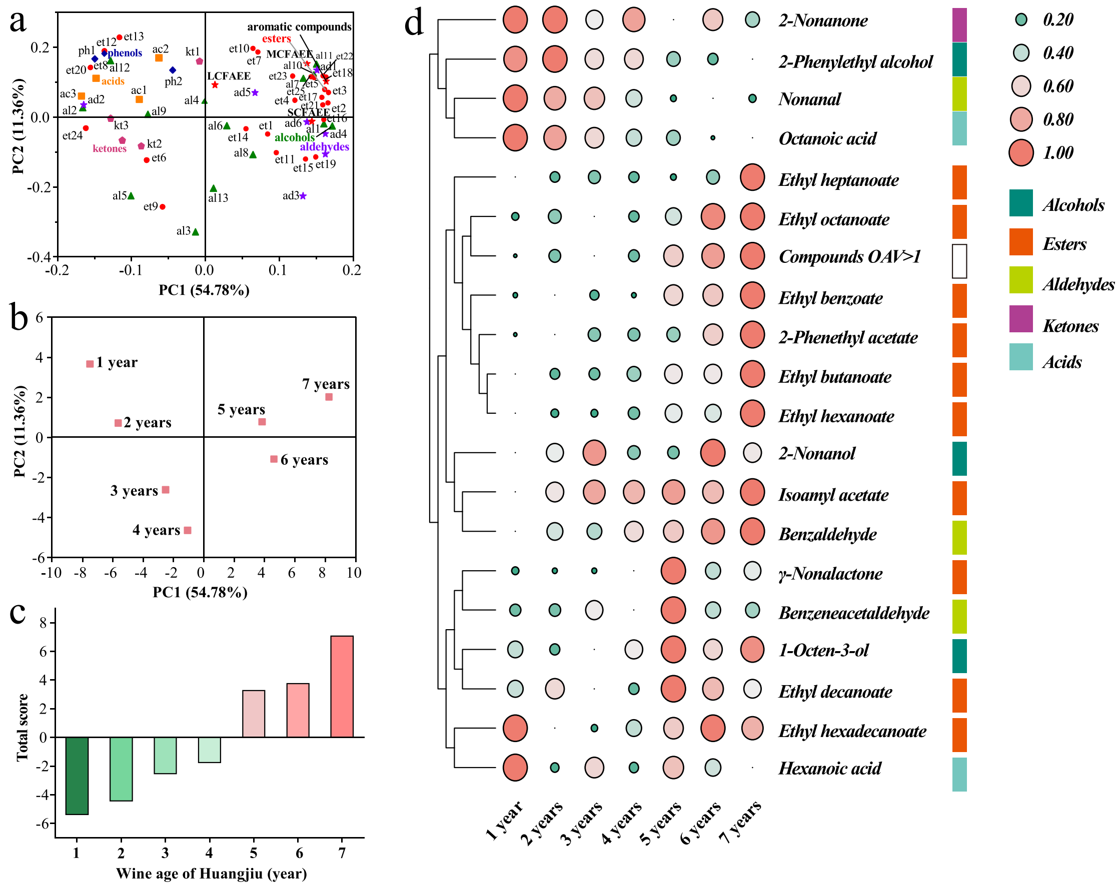

Fig. 2. Flavor profiles of Huangjiu with different ages analyzed by multivariate statistical analysis. (a) Loading plot for the volatile flavor compounds of Huangjiu aged for 1–7 years based on PCA analysis; Score plot (b) and total score (c) of Huangjiu with wine age ranged from 1 year to 7 years based on PCA analysis; (d) Difference of individual volatile flavor compound with OAV > 1 in Huangjiu correspondingly aged for 1 year, 2 years, 3 years, 4 years, 5 years, 6 years, and 7 years. The content of compound with OAV > 1 was normalized to 0–1 scores for each sample. The circle bar denoted the relative content of each individual volatile flavor compound. The redder (the bigger) of the circle, the higher content of the compound, and the greener (the smaller) of the circle, the lower of the content.

benzaldehyde, 4-methyl benzaldehyde, and esters was highly associated with ethyl butanoate, ethyl hexanoate, ethyl (9E)-9-octadecenoate, ethyl benzoate, 2-phenethyl acetate, and ethyl 3-phenylpropionate. The SCFAEE and MCFAEE exhibited higher contribution to the flavor characteristic of Huangjiu than LCFAEE, as LCFAEE was lowly positioned on the positive side of PC1. The abundant aromatic compounds had a strong effect on the positive side of PC1, indicating the importance of aromatic compounds on Huangjiu flavor during aging.

As shown in Fig. 2b, Huangjiu with different ages could be well-defined into four separate group. The Huangjiu aged for 1 year and 2 years could be classified in Group 1, which was characterized by 2-methoxy-phenol, ethyl dodecanoate, ethyl (9E)-9-octadecenoate, ethyl oleate, ethyl 4-hydroxybutanoate, 3-methylthiopropanol, 1-decanol and acetic acid. Except for acetic acid, other compounds were all existed in trace amount. Acids and phenols flavor were highlighted in Group 1. The 3-years old Huangjiu exhibited similar flavor characteristic to the 4-years old Huangjiu (Group 2), which flavor was influenced by 3-methyl-1-butanol, 2-heptanol, ethyl nonanoate, ethyl tetradecanoate, and 2-octanone. Group 3, that formed by wine aged for 5 and 6 years, exhibited similar absolute levels on PC1 and PC2 sides. The flavor characteristic of Huangjiu in Group 3 was mainly affected by the presence of benzaldehyde, benzeneacetaldehyde, 4-methyl benzaldehyde, ethyl acetate, ethyl heptanoate, ethyl stearate, isoamyl acetate, ethyl L-lactate, diethyl succinate and 2-methyl-1-propanol. The aromatic aldehydes and esters with high concentrations or OAVs were the typical flavors of Huangjiu in Group 3. Group 4 was made up of Huangjiu aged for 7 years, which was found with high values in PC1 axis. Esters, especially MCFAEE, 1-octen-3-ol, 2-pentenal and aromatic compounds played predominant role in the flavor characteristic of 7-years old Huangjiu. Notably, most of the typical flavors in Huangjiu aged for 7 years were presented with high OAVs (exceed 1), especially MCFAEE and aromatic compounds. The total score of Huangjiu increased with the storage time, and the 7-years old Huangjiu scored the highest (Fig. 2c), suggesting the best flavor quality. Although complex interaction taken place between the flavor compounds during the aging process, it was speculated that the compounds with OAVs > 1 dominated the characteristic flavor of Huangjiu with the storage period, and this might be the key origin of aging aroma in Huangjiu. As shown in Fig. 2d, the relative contents of compounds with OAV > 1 increased with the storage period and peaked in wine aged for 7 years, which confirmed the above speculation. Thus, it could be concluded that the aging process of Huangjiu was a process of highlighting the flavor compounds with high OAVs (exceed 1). These compounds should be regarded as key monitoring points for improving the flavor quality of aged Huangjiu and developing novel aging technology in future work.

3.4. Discrimination of aged Huangjiu based on E-nose and E-tongue

E-nose and e-tongue technologies, which respectively simulate the olfactory and gustatory of human to rapidly assess food qualities via “olfaction” and “taste”, have been widely applied for the discrimination of wines according to vintage year, geographical origin and variety (Buratti, Malegori, Benedetti, Oliveri, & Giovanelli, 2018; Wang, Zhu, Zhang, & Wei, 2019; Geană, Ciucure, & Apetrei, 2020).

The typical E-nose responses to the different Huangjiu were presented in Fig. S1. The intensity of the sensor response depended not only on the composition of the odor compounds, but also on their concentrations. Response profiles of the 14 sensors exhibited similar trend on the radar fingerprint, while the signal intensities were different. The signal intensities of sensors improved with the aging of Huangjiu, and this was in accordance with the results of volatile flavor compounds (Table 1), and the sensory evaluation that Huangjiu aged for 7 years exhibited the best sensory perception (data not shown). The results were further analyzed via PCA to highlight the discrimination of aged Huangjiu. The cumulative contribution rate of two principal components (PC1 and PC2) reached 100% and the DI was 91.24% indicating that the aroma of Huangjiu with different ages could be effectively distinguished (Fig. 3a). The PC1 contributed 99.94% to the total variability and exhibited high correlation with Huangjiu aged for 6 and 7 years. The distance between loadings of the variables represented the characteristic difference among different Huangjiu samples. It showed that Huangjiu with different ages were classified into seven groups. However, a certain overlapped area was observed between Huangjiu aged for 6 and 7 years, suggesting a similar aroma characteristic between the two wines.

As not all the sensors respond optimally to the flavor compounds of Huangjiu, the combination of E-nose sensors was optimized based on DI. The optimal sensor responses were used to analyze the relationship between volatile flavor compounds and E-nose response by establishing PLSR model (Fig. 3b). The model explained 64% of the variance in X (flavor compounds) and 86% of that in Y (E-nose response). All the 10 sensors showed good relation with the flavor compounds, as it located in the big ellipse which indicated 100% of the explained variance. The E-nose sensors correlated well with esters and aldehydes, indicating that the different flavor characteristic of aged Huangjiu detected with E-nose might be caused by the concentration differences in esters and aldehydes. Combining with the results of GC–MS (Table 1), the E-nose sensors might be sensitive to MCFAEE, 2-phenethyl acetate, isoamyl acetate, furfural, benzaldehyde, and 4-methylbenzaldehyde.

As for E-tongue analysis, the DI was 85.33%, which was lower than the value resulted by E-nose analysis. The PC1 (76.92%) and PC2 (9.13%) explained 86.05% of the total variances, suggesting that they could represent the major taste information of the Huangjiu samples (Fig. 3c). The Huangjiu with different ages were clustered into seven groups. With the increase of wine age, a good separation was observed and the loadings of Huangjiu samples gradually shifted to the right on PC1 axis. Among which, the taste of Huangjiu aged for 2 and 3 years were close to 1-year old wine, while the wine aged for 4, 5 and 6 years showed close distance. The loadings of Huangjiu aged for 7 years were far away from the other wines, demonstrating an obvious different taste was occurred between the Huangjiu aged for 7 years and other Huangjiu. Interestingly, although similar aroma characteristic was observed in Huangjiu aged for 6 and 7 years, the taste of the two were completely different.

In E-tongue analysis, a large data set was obtained by the 6 working electrodes in the experiment (composed of three frequencies: 1 Hz, 10 Hz, and 100 Hz). However, most of the data were unusable and thus the response of optimized electrodes and frequencies that could unambiguously classify Huangjiu was selected as the ideal object for the analysis. The relationship between E-tongue response data and the FAA profiles was shown in Fig. 3d. The X-matrix which explained 87% E-nose sensors was designed as FAA measurements, and Y-matrix which explained 90% E-nose sensors was set as response of the optimal E-tongue sensors. The S3 and S4 sensors covaried well with Met, which taste bitter. S6 sensor correlated with Cys, Phe, and His, which also presented bitterness. In addition, Glu with umani taste was highly associated with the S1 sensor. The contents of above FAA in Huangjiu were all improved with the prolonged aging period, suggesting that the E-tongue could be used to distinguish the tastes of bitterness and umani in Huangjiu with different ages.

3.5. Raman analysis

As alcoholic beverage, Huangjiu contains a large number of biological molecules. Among which, ethanol and water represent about 96% of the total number of molecules in the wine (Martin et al., 2015). Unlike some spectroscopy techniques that affected by the water molecules, the Raman peak of water molecule rarely overlap with the peaks of other molecules (Jin et al., 2016). Thus, the Raman spectroscopy was used to probe the molecular structures of Huangjiu with different ages (Fig. S2).

The Raman shift peaks of Huangjiu were observed at approximately 450, 880, 1045, 1085, 1455, 2885, 2940 and 2980 cm^{-1}, which was similar with the results reported by Wu et al. (2015). The peaks of ethanol and water dominated the spectra, as they were the major compounds in Huangjiu. Therefore, a subtraction of the two signals would allow the observation of differences that appeared among samples, which given by the dissolved minor compounds such as acids, alcohols, esters and aldehydes, some of them having a contribution on wine discrimination (Magdas, Guyon, Feher, & Pinzaru, 2018). In terms of ethanol, the distinctive peaks were observed at around 880, 1045 and 1085 cm^{-1}, which mainly caused by the C—C stretching, C—O stretching and —CH_3 rocking vibrations, respectively (Wu et al., 2016). In addition, the most characteristic band of ethanol is generally attributed to C—C stretching. The intensity of peak observed around 880 cm^{-1} tended to become strong with the storage period. The bands appeared around 450 and 1455 cm^{-1} were probably related to the H—C—H and O—C—C bending of sugars, respectively (Vasko, Blackwell, & Koenig, 1972). The intensities of both bands (correspondingly related to ethanol and sugars) progressively increase with aging time. For the peaks shifted between 2800 and 3000 cm^{-1}, they were assigned to symmetric and asymmetric stretching of -CH_X bonds which were presumably originated from ethanol (Dos Santos et al., 2018). The intensities of the bands around 2885, 2940 and 2980 cm^{-1} seemed to be strong after the wine aged for 3 years. Compared with the Raman spectra of Huangjiu during fermentation (Wu et al., 2015), the Raman shift peaks appeared around 520, 1124, 1280, and 1640 cm^{-1} were not monitored during aging, indicating significant changes were occurred in molecular structures of flavor compounds during aging. The changes might be ascribed to the physical and chemical reactions of flavor compounds, such as volatilization of heterogeneous volatile compounds, oxiadation, esterification between alcohols and fatty acids, and hydrolysis reactions (Tao et al., 2014).

Raman spectroscopy has been used to discriminate wine according to multiple components, variety, and wine flavors (Magdas et al., 2018; Martin et al., 2015; Leong et al., 2021). However, based on the above results, although Raman spectroscopy could identify some differences, it was not sensitive enough in the age identification of Huangjiu.

3.6. The LF-NMR behavior of Huangjiu with different wine ages

In LF-NMR analysis, the signal intensities of water populations are proportional to the quantity of stable hydrogen protons, which affected by the molecular association between water and dissolved flavor compounds (Nikolskaya & Hiltunen, 2020). The single component relaxation time (T_{2W}) of Huangjiu decreased significantly with the aging period, except for the wine aged for 3 and 4 years (Fig. 4a). With the increase of aging time, the molecular association between water and macromolecules in Huangjiu sample was strengthened, which limited the migration of hydrogen protons in the structure, and thus resulted in a decrease of T_{2W}. The increased concentrations of dissolved flavor compounds in Huangjiu might be responsible for the results (Fig. 4a & Table 1).

Generally, multiexponential relaxation behavior of food matrixes could be ascribed to different water populations that exist in heterogeneous microstructures and protons biopolymer (Li et al., 2015). To clearly analyzed the states of different hydrogen protons in Huangjiu during aging, the multi-component relaxation patterns of different wine

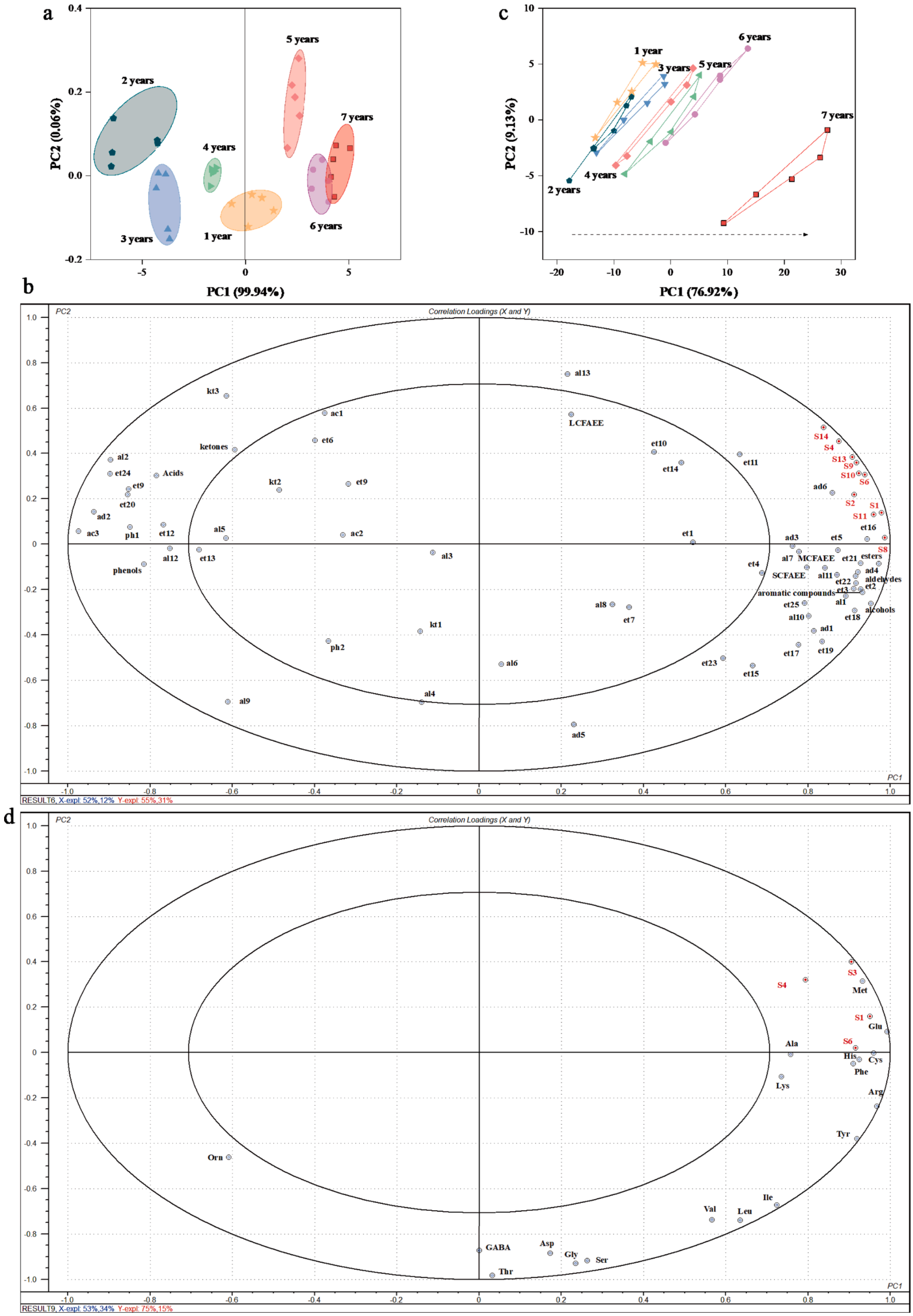

Fig. 3. Multivariate statistical analysis of E-nose and E-tongue dataset in Huangjiu with different ages (ranged from 1 year to 7 years). (a) PCA plot of different Huangjiu samples based on original data for E-nose; (b) correlation between E-nose sensor responses and volatile compounds in Huangjiu with different ages; (c) PCA plot of different Huangjiu samples based on E-tongue data; (d) correlation between E-tongue sensor responses and free amino acids in Huangjiu with different ages.

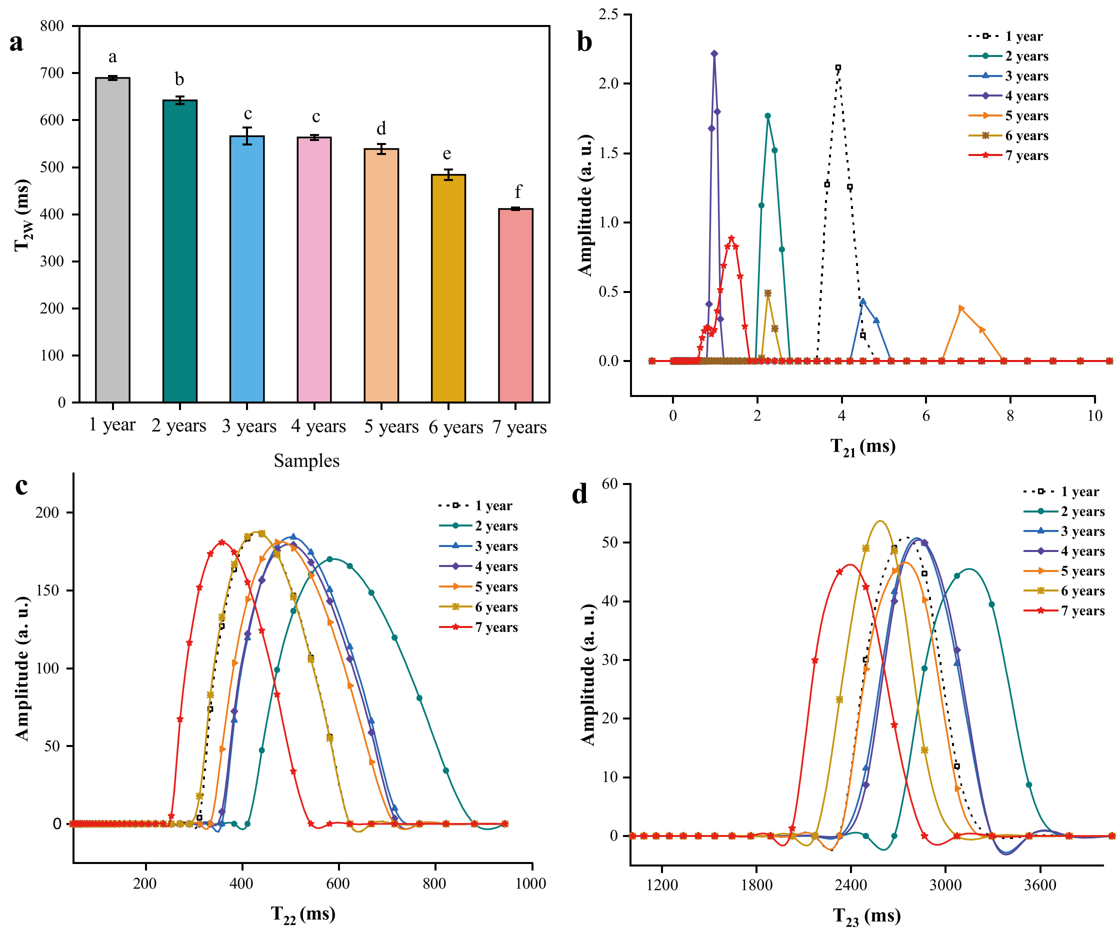

Fig. 4. Transverse relaxation properties of Huangjiu samples aged for different time. a: Single component relaxation time (T_{2W}) spectra of different Huangjiu samples; T_2 relaxation time curves of different Huangjiu samples distributed in 0–10 ms (b), 200–1000 ms (c), and 1800–4000 ms (d).

samples were shown in Fig. 4b, c & d. In all wine samples, three water populations that represented bound water, semi-bound water, free water and distributed in T_{21} (0–10 ms), T_{22} (200–1000 ms), T_{23} (1800–4000 ms) were detected, respectively. Obvious difference was observed in the relaxation time and amplitude of T_{21} peak in different Huangjiu samples (Fig. 4b). With the increase of Huangjiu age, the amplitude of T_{22} peak exhibited small fluctuation, while the relaxation time shifted to the right with the wine age ranged from 1 year to 5 years, and then shifted to the left with wine age higher than 5 years (Fig. 4c). A similar change was observed in the T_{23} peak (Fig. 4d). In other words, the LF-NMR analysis sensitively reflect the differences of different hydrogen states resulted by the age of Huangjiu. The LF-NMR analysis is expected to be a novel approach in the quick discrimination of Huangjiu age.

3.7. Molecular association between water and FAA or volatile flavor compounds during Huangjiu aging

Generally, the hydrogen bonding and chemical reactions simultaneously occurred during the aging of alcoholic beverage (Tao et al., 2014). Both hydrogen bonding and chemical reactions involve molecular association, therefore, the protons which attached to hydrophilic compounds could be distinguished by the relaxation properties, and then used to characterize the molecular association between macromolecules. In LF-NMR analysis, the signals of water populations are generated by the hydrogen protons dissolved in Huangjiu. The molecular association between water and heterogeneous flavor compounds during Huangjiu aging affect the motion state of hydrogen protons. Although water and ethanol are the main signal sources of hydrogen proton, other dissolved flavor compounds (containing hydrogen proton) in Huangjiu also contribute to the amounts of stable hydrogen protons. In other words, the changes of relaxation properties detected with LF-NMR could be affected by the molecular association between water and dissolved flavor compounds. Therefore, the relaxation behavior of LF-NMR was used to characterize the molecular association between water and other dissolved compounds in Huangjiu.

The area of T_2 relaxation peak (A_{2i}) and the percentage of the peak area (RC_{2i}) were closely related to the number of hydrogen protons in the sample under a certain state (Aursand, Erikson, & Veliyulin, 2010). The RC_{22} (ranged from 89.46% to 91.05%) and RC_{23} (ranged from 8.91% to 10.00%) accounted for above 99% of the T_2 relaxation peak area (Table S1). The predominant T_{22} peak explained the most of LF-NMR behavior in aged Huangjiu. Thus, T_{22} relaxation peak (A_{22}) was used to evaluate the molecular association between water and FAA or volatile flavor compounds using Spearman's correlation analysis (Fig. 5). The A_{22} exhibited significant correlation with Glu, Arg, His, and Orn (Fig. 5a). The Glu which significantly positive correlated to A_{22} ($p < 0.05$) belonged to acidic amino acids, while Arg and His positively associated with the peak were basic amino acids. In addition, Orn which also belonged to basic amino acids was correlated negatively with A_{22}. The above results indicated that basic amino acids exhibited stronger molecular association than acidic amino acids in aged Huangjiu. As FAA not only have distinct taste, but also could be used as precursors of flavor compounds (Shen et al., 2010; Tian et al., 2016). Consequently, basic amino acids contributed greatly to the flavor formation of Huangjiu during aging via molecular association.

Although the detected volatile flavor compounds were polar

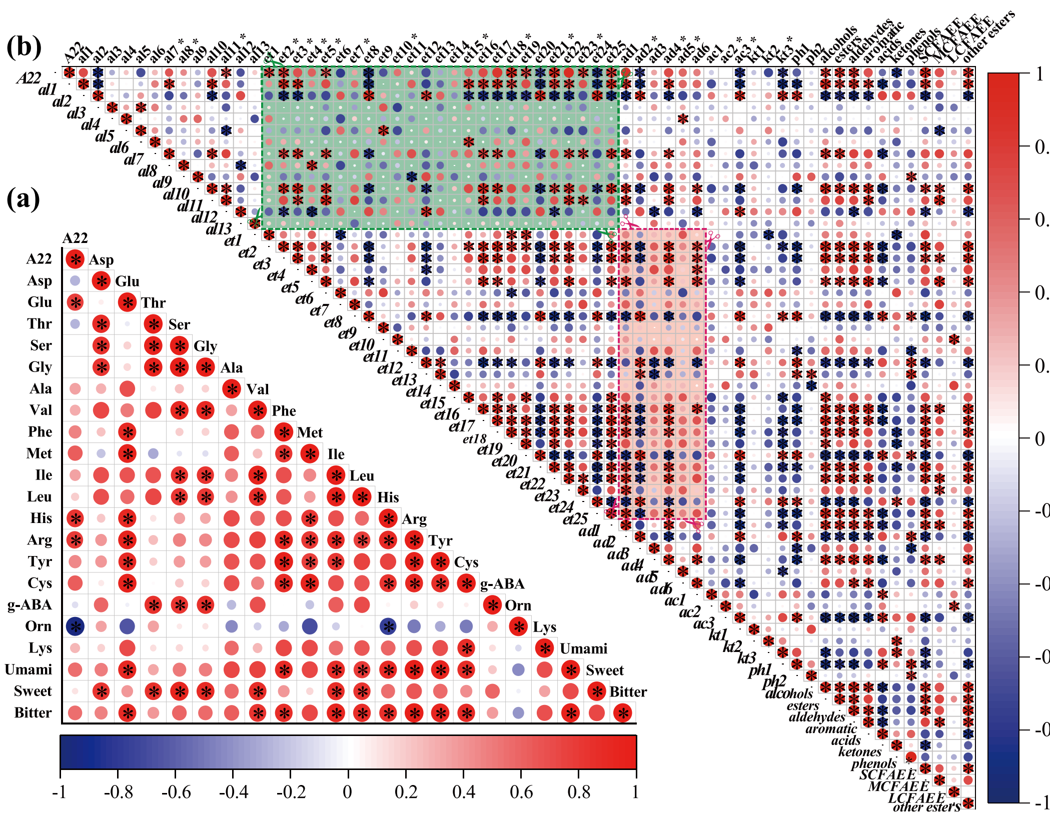

Fig. 5. Spearman's correlation analysis between the concentrations of FAA and LF-NMR behavior (a), as well as correlation between the concentrations of volatile flavor compounds and LF-NMR behavior (b). The large circle indicated a strong correlation, whereas small circle indicated a weak correlation. The color bar denoted R-value of Spearman correlation, with 1 representing a perfect positive correlation (dark red) and −1 representing a perfect negative correlation (dark blue). The "*" in the circle indicated the concentrations of flavor compounds were significantly correlated with the LF-NMR behavior ($p < 0.05$), and the "*" in the upper right of compounds name represented the compounds presented with OAV > 1. The volatile flavor compounds corresponding to each ID could be found in Table 1.

compounds, only alcohols, aldehydes, esters, and ketones showed significant correlation with A_{22} (Fig. 5b). 2-Phenylethyl alcohol, ethyl acetate, ethyl butanoate, ethyl octanoate, ethyl benzoate, diethyl succinate, 2-phenethyl acetate, γ-nonalactone, and ethyl 9-hexadecenoate showed significantly positive correlation with A_{22}, while 1-butanol, 2-nonanol, ethyl 4-hydroxybutanoate, ethyl 2-hydroxy-3-phenylpropanoate, nonanal, octanoic acid, and 2-nonanone exhibited significantly negative association with the A_{22} ($p < 0.05$). Notably, compounds with OAVs exceed 1 accounted for 70.59% of those related compounds, suggesting that the molecular association between water and these compounds significantly promoted the accumulation of flavor compounds with OAV > 1. Among these compounds, ethyl esters exhibited the strongest molecular association, indicating that esters were involved in the most physicochemical changes during Huangjiu aging. Moreover, significant interaction was observed among alcohols, esters and aldehydes. In alcohols, 2-methyl-1-propanol, 1-butanol, 1-octen-3-ol, benzyl alcohol, and 2-phenylethyl alcohol showed significant effect on esters (green box of Fig. 5b). In case of aldehydes, 2-pentenal, nonanal, and benzaldehyde had significant effect on esters (pink box of Fig. 5b). Accordingly, the interaction between different compounds must be considered in studies related to the aroma recombination of Huangjiu.

4. Conclusions

Generally, the process of wine aging governs the flavor quality and economic value of Huangjiu. Thus, the formation mechanism of flavor compounds responsible for the characteristic flavor of aged Huangjiu was investigated in current study. With the storage time prolonged, the contents of FAA with umami and bitter taste increased while the sweet FAA decreased. As the key flavor compounds of Huangjiu, the contents of higher alcohols decreased with the storage period, while MCFAEE, 2-phenethyl acetate, isoamyl acetate and aromatic compounds conversely exhibited a rising trend. Furthermore, we found that the aging of Huangjiu was a process to highlight the flavor compounds with high OAVs (exceed 1). The LF-NMR analysis was firstly applied to discriminate wine age and characterize the molecular association between water and dissolved flavor compounds in aged Huangjiu. The results showed that molecular associations between water and basic amino acids contributed greatly to the flavor formation during the aging process. In addition, the molecular association significantly promoted the accumulation of flavor compounds with OAV > 1 (especially ethyl esters). Thus, the physical methods which strengthen the molecular association of basic amino acids and volatile flavor compounds with OAV > 1 could be applied to shorten the aging time in future studies. This study not only broadens the application field of LF-NMR, but also may provide a substantial basis for the control of flavor formation during the aging process of Huangjiu or other alcoholic beverages. In future work, the molecular interaction between flavor compounds responsible for the flavor characteristic of Huangjiu need to be further explored.

CRediT authorship contribution statement

Yijin Yang: Conceptualization, Investigation, Software, Writing – original draft. **Lianzhong Ai:** Conceptualization, Project administration, Investigation, Writing – review & editing. **Zhiyong Mu:** Methodology, Project administration, Writing – review & editing. **Haodong Liu:** Visualization, Software, Writing – review & editing. **Xin Yan:**

Visualization, Software, Investigation, Writing – review & editing. **Li Ni:** Resources, Methodology, Visualization, Writing – review & editing. **Hui Zhang:** Resources, Visualization, Writing – review & editing. **Yongjun Xia:** Supervision, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

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

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Appendix A. Supplementary data

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