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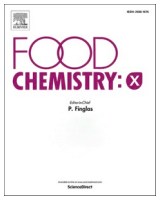

Unraveling *Shengmuxiang* in Jiang-flavor base baijiu using a combination of metabolomics and sensomics strategy

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ABSTRACT

Shengmuxiang (SMX), an important aroma in Jiang-flavor base baijiu, significantly influences the quality of the product. This study employed untargeted metabolomics combined with sensomics to explore the key compounds responsible for SMX. Results indicated that SMX samples had higher intensities of green and woody-like odors compare to control samples. A total of 87 aroma compounds were identified by headspace solid phase micro-extraction combined with gas chromatography–mass spectrometry technology. Based on the variable projection importance, PCA and OPLS-DA were employed to identify 22 potential marker compounds. Quantitative results combined with hierarchical cluster and OAV analysis revealed that 9 aroma compounds (OAV > 1) had high concentrations in SMX samples. Aroma recombination and omission experiments further indicated that acetaldehyde and acetal were the key compounds responsible for the characteristic aroma of SMX in Jiang-flavor base baijiu. These findings provide valuable insights into the distinct aroma profile of SMX and offer a basis for quality control of Jiang-flavor base baijiu.

1. Introduction

Chinese baijiu, one of the six major distilled spirits in the world, has attracted considerable attention both in China and worldwide because of its unique flavor and long history (Liu et al., 2023; Wang et al., 2022). Owing to the differences in brewing materials, processes and environment, baijiu can be divided into 12 flavor types (including jiang-flavor, strong-flavor, light-flavor, rice-flavor, te-flavor, sesame-flavor, laobaigan-flavor, chi-flavor, feng-flavor, jian-flavor, fuyu-flavor, and dong-flavor) according to its aroma and flavor characteristics (Wang, 2022; Zheng & Han, 2016). Jiang-flavor baijiu, strong-flavor baijiu, light-flavor baijiu, and rice-flavor baijiu are the four basic flavor types of Chinese baijiu, and each type has a special smell and taste (Duan et al., 2022; Wang, Tang, et al., 2024). Among them, Jiang-flavor baijiu is deeply loved by consumers according to its outstanding sauce flavor, elegant and delicate, mellow body, long aftertaste and the empty cup with lasting aroma.

As the most representative traditional fermented food in China, Jiang-flavor baijiu has the longest production cycle and the most complex production process. The production of Jiang-flavor baijiu involves a complex series of steps, including two feedings, nine steamings, eight fermentations, and seven distillations. After each distillation round, the resulting product is Jiang-flavor rounded-base baijiu (Liu et al., 2023). The conventional multi-strain mixed and open fermentation process utilized in the production of Jiang-flavor baijiu imparts variety to the base baijiu's style. However, the flavor of base baijiu can easily be impacted by external factors, such as the environment and operating conditions, and may result in the formation of special aromas in the base baijiu (Wu et al., 2021). According to the sensory performance, these special aromas in base baijiu can be divided into positive aromas and off-odors. Among them, the common positive aromas are flower aroma, fruit aroma, green aroma, honey aroma and so on. However, the main off-odors include pickle-like off-odor (Wang et al., 2020), earthy-musty odor (Du et al., 2011), and mud-like off-flavor (Dong et al., 2018). The special aromas reported in Jiang-flavor baijiu mainly include retronasal burnt aroma, empty cup aroma, qu aroma, muddy smell, and pickle-like odor (Duan et al., 2024; Gong et al., 2024; Qin et al., 2024; Zhao et al., 2019). Shengmuxiang (SMX), also one of the important special aromas in Jiang-flavor base baijiu, shows fresh and pleasant grass aroma characteristics at low intensity, but when the intensity is too high, it will show a dull taste similar to fresh wood, which seriously affects the coordination of liquor aroma and taste. Numerous studies have focused on identifying potent odorants that contribute to the flavor of various distilled liquors, which "wood" was one of the keywords with the

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citation bursts (Qiao et al., 2023). However, the characteristics of SMX in Jiang-flavor base baijiu are not clear, and there are no relevant research reports on this special aroma. Therefore, the study of the key compounds that produce the special aroma of SMX in Jiang-flavor base baijiu is crucial for improving product quality.

So far, most reports related to the key aroma compounds in baijiu mainly adopt molecular sensory science strategy (also known as sensomics), which focuses on identifying compounds with important aroma contributions in baijiu through gas chromatography-olfactometry, aroma extraction-dilution analysis, odor activity value (OAV), aroma reconstruction and omission etc. (Duan et al., 2024; Li et al., 2019; Lin et al., 2024; Wang et al., 2020). For example, Wang et al. (2020) successfully identified that the odorants causing a pickle-like off-odor in Jiang-flavor baijiu were volatile sulfur compounds with high concentrations by adopting the molecular sensory science. Although molecular sensory science plays an important role in the identification of key aroma compounds in baijiu, the complexity of flavor composition, the huge amount of data on flavor substances, and the interactions between aroma compounds in baijiu make it difficult to comprehensively analyze the composite aroma. Therefore, it is urgent to introduce other techniques to analyze the flavor composition in baijiu. Metabonomics, as an important branch of omics strategies, is widely used in the field of food research (Hew et al., 2024; Li et al., 2020; Utpott et al., 2022). Especially, metabonomics has found important applications in the identification of liquor quality, the traceability of origins, and the analysis of characteristic flavors (Song et al., 2020; Wang, Zhang, et al., 2024; Wang et al., 2021; Yang et al., 2021). Song et al. (2020) used non-targeted and targeted metabolomics strategies to distinguish strong-flavor baijiu of different origins. Twenty-nine potential important markers were identified through principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA), then 24 compounds were specifically quantified, and it was found that these markers had good discriminatory ability for different origins of baijiu. It can be seen that metabolomics strategy has great advantages in the study of different substances between different categories of samples, which provides a new way for the study of baijiu flavor. Firstly, the non-targeted metabolomics technology was used to screen out the important markers to distinguish different categories of samples, and then the identified markers were validated by using the idea of sensomics. The combination of the two provided a more efficient way for the characterization of special aromas in baijiu.

In this study, the typical SMX samples and control samples were screened by sensory. A series analytical methods were used to analyze the composition of flavor compounds in these baijiu samples, and the different marker compounds between typical SMX and control samples were comprehensively analyzed based on non-targeted metabolomics techniques, such as PCA and orthogonal partial least-squares discrimination analysis (OPLS-DA). Afterwards, the quantitative ratio relationships of the important marker compounds and their OAVs were compared. Finally, the flavor contribution of important marker compounds was analyzed by using sensomics methods such as aroma reconstruction and omission, and the key aroma compounds cause of the special aroma of SMX in Jiang-flavor base baijiu were analyzed successfully. The results of this study enhance the understanding of special aroma in Jiang-flavor baijiu, and provide a new idea for the analysis of key aroma compounds of special aroma in baijiu.

2. Materials and methods

2.1. Baijiu samples

A total of 33 Jiang-flavor base baijiu samples manufactured in Kweichow Moutai Co., Ltd. in 2019 were used in this study. The samples were divided into two groups based on the intensity of SMX odor by professional sensory evaluation team, which consisted of four nationally certified Chinese Baijiu tasters. There were 20 typical SMX samples (high intensity of SMX odor) and 13 control samples (none or very weak) in this study. All the samples were stored at 4 °C before analysis.

2.2. Chemicals

All chemical standards were chromatographic grade with at least 97 % purity. Acetaldehyde, acetal, isoamyl alcohol, ethyl 2-hydroxyhexanoate, isobutanol, isoamyl acetate, ethyl propionate, 2-pentanol, 2-methylpropanal, propionaldehyde, phenylethyl acetate, ethyl lactate, propanol, 2-butanone, furfural, 1,1-diethoxy-3-methylbutane, 3-methylbutanal, ethyl valerate, 2-butanol, diethyl succinate, ethyl heptane, ethyl acetate, 2-methyl-2-butanol, and citronellol (internal standard, IS) were purchased from Sigma-Aldrich (Shanghai, China). Anhydrous ethanol (high performance liquid chromatography, HPLC grade) was purchased from Anaqua Chemicals Supply (America). Pentane (HPLC grade), diethyl ether (analytical grade) and sodium chloride (analytical grade) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China).

2.3. Sensory analysis

A 10-person sensory evaluation team with rich experience in baijiu tasting from the Flavor Research Laboratory of the Technical Center in Kweichow Moutai Co., Ltd. was used in this study. Firstly, the sensory evaluation of typical SMX baijiu samples was carried out, and the sensory descriptors of typical SMX baijiu samples were collected by each reviewer. Among them, eight descriptors with higher frequency were selected, which were floral, fruit, green, sweet, woody-like, acidic, roasted and herbal, and they were used as eight sensory dimensions for evaluating baijiu samples. Then, the intensity of each sensory dimension was scored respectively. The lowest intensity was rated as 0, the highest intensity as 3, and every 0.5 was a gradient (Qin et al., 2024). The average score of all sensory evaluators was calculated and the sensory contour map was drawn.

2.4. Isolation and identification of volatile aroma compounds

2.4.1. Headspace solid phase microextraction (HS-SPME)

A method from the literature reported by Fan et al. (2024) with several modifications was used to extract the volatile compounds in baijiu samples. The baijiu samples were diluted with ultrapure water to a final concentration of 5 vol% ethanol. Then 10 mL diluted baijiu sample was accurately taken into 20 mL screw-capped headspace bottle and saturated with 3 g NaCl. Three microliters of citronellol with concentration of 238.4 mg/L was added to the above solution as IS. An automatic headspace sampling system (MultiPurpose Sample MPS 2 with a SPME adaptor, from Gerstel Inc., Mülheim, Ruhr, Germany) with a 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber (2 cm, Supelco Inc., Bellefonte, PA, U.S.A.) was used to extract the volatile compounds. The extraction conditions were as follows: the sample was equilibrated at 60 °C for 5 min and then extracted for 30 min under stirring (250 rpm). The fiber was then introduced into the gas chromatograph injection port at 250 °C for 5 min of desorption.

2.4.2. Gas chromatography-mass spectrometry (GC–MS)

An Agilent 7890A gas chromatograph (Agilent Technologies, Folsom, CA, U.S.A.) with a 5975C mass selective detector was employed for this analysis. The volatile compounds were separated using a DB-FFAP column (30 m × 0.25 mm, 0.25 μm, Agilent Technologies, Santa Clara, USA). The carrier gas was helium (99.999 %), whose flow rate was 1.6 mL/min. The injection mode was splitless. The oven temperature was initially set at 40 °C and held for 1 min, ramped to 80 °C at 10 °C/min, ramped to 140 °C at 3 °C/min, and finally ramped to 220 °C at 20 °C/min and held for 20 min. The MS was operated in electronic ionization (EI) mode at 70 eV. The temperatures of the ion source,

transmission line, and quadruple were 230 °C, 250 °C, and 150 °C, respectively. The mass range of *m/z* was from 35 to 350 in full scan mode. The processing of raw data was carried out in a sequential manner using Agilent Chem Station. The identification of flavor compounds was achieved by comparing their mass spectra with the information available on NIST 17 databases. In addition, for compounds with authentic standards, the retention time of the authentic standard was analyzed under the same conditions and confirmed by comparison with the retention time of the analyte.

2.5. Quantitative analysis of the marker compounds

2.5.1. DI combined with GC-FID analysis

The compounds with high concentrations (see supporting information Table S1) were quantified using direct injection (DI) with gas chromatography-flame ionization detection (GC-FID) (Agilent 7890B; Agilent Technologies, USA), referring to the method previous reported by Li et al. (2023) with several modifications. Each sample (1.0 μL) was separated on a DB-WAX UI capillary column (30 m × 0.25 mm, 0.25 μm, Agilent Technologies, USA). The temperature of the oven was initially set at 40 °C and held for 4 min, ramped to 100 °C at 5 °C/min, then increased to 200 °C at 10 °C/min and held for 18 min. The carrier gas was H_2 with 99.999 % purity and had a constant flow rate of 1.0 mL/min. The inlet temperature was set at 250 °C, the detector temperature was set at 300 °C, and the split ratio was set at 30:1.

2.5.2. LLME combined with GC–MS analysis

Liquid-liquid microextraction (LLME) combined with GC–MS was used to quantify the trace compounds in baijiu based on the method used in a previous study (Liu et al., 2023) with some modifications. Firstly, one milliliter of baijiu sample was diluted with 9.6 mL of ultrapure water, then saturated with NaCl, then 2 μL of citronellol (11727 mg/L, IS) and 1 mL of extractant (diethyl ether: pentane = 1:1, v/v) were added, followed by shaking for 1 min. After static layering, the upper organic phase was collected and transferred to an injection vial (2 mL) for GC–MS analysis. The injection volume was 1.0 μL, and the inlet temperature was 230 °C. Other instrument conditions were consistent with those described in 2.4.2.

2.5.3. HS combined with GC–MS analysis

The highly volatile compounds with low boiling points in baijiu samples were quantified by static headspace (HS) coupled with GC–MS. The baijiu samples (0.5 mL) were diluted with ultrapure water (1.5 mL), then saturated with NaCl. Three microliters of 2-methyl-2-butanol (1860 mg/L) was added to the above solutions as IS. An Agilent G1888 headspace sampler-7890A gas chromatograph (Agilent Technologies, Folsom, CA, U.S.A.) with a 5975C mass selective detector was employed for this analysis. The volatile compounds were separated using a DB-FFAP column (30 m × 0.25 mm, 0.25 μm, Agilent Technologies, Santa Clara, USA). The carrier gas was helium (99.999 %), whose flow rate was 0.8 mL/min. The inlet temperature was 230 °C, and the split ratio was set at 5:1. The oven temperature was initially set at 40 °C and held for 1 min, ramped to 42.5 °C at 0.5 °C/min, and finally ramped to 220 °C at 35 °C/min and held for 5 min. The MS conditions were consistent with those described in 2.4.2.

2.6. OAV analysis

The OAVs of the aroma compounds in baijiu were calculated based on their concentrations in baijiu and their corresponding thresholds in the alcohol solution. In general, odorants with OAVs ≥1 are considered to be important compounds for the overall aroma profile of baijiu (Zhu et al., 2020). The odor detection thresholds in this study were based on those of previous studies (Li et al., 2023; Liu & Sun, 2018; Wang, Tang, et al., 2024; Wang et al., 2014; Wang, Hao, et al., 2023). The matrix for the measurement of the olfactory threshold of the compounds are indicated in Table 1.

2.7. Aroma recombination and omission tests

Aroma recombination: a total of 9 compounds (OAV > 1) with high content in SMX samples were mixed into 20 mL of control sample at the corresponding concentrations in typical SMX samples. After vigorous shaking and equilibration for 20–30 min at room temperature, the recombination sample was evaluated by trained sensory panel upon eight sensory dimensions using the same sensory evaluation method in the sensory analysis section. The aroma profile of recombination sample was drawn by using the average score of all sensory evaluators.

Omission experiments: The omission experiments were conducted to clarify the contribution of certain compounds to the SMX characteristics by performing a triangular test according to the method ISO 4120:2004. A total of 7 aroma omission models were prepared by omitting aroma component of the recombination model based on the category and aroma characteristics of the 9 aroma compounds. Then, two recombination models and one omission model in 3 separate glasses were arranged in a random three-digit code, and were presented simultaneously for evaluation. The evaluators were asked to identify the sample with significant sensory differences from the other two recombination samples, and the results were subjected to statistical analysis of variance.

2.8. Statistical analysis

PCA was completed using origin2021 software. OPLS-DA, heatmap analysis and hierarchical cluster analysis (HCA) were analyzed using MetaboAnalyst 5.0 (http://www.metaboanalyst.ca/). The significant differences of diverse groups models were defined as $p < 0.05$ using SPSS 20.0.

3. Results and discussion

3.1. Sensory analysis of SMX baijiu

To initially determine the differences in the overall aromas of typical SMX baijiu samples and control samples, aroma profile analyses were carried out according to the method described in “sensory analysis” section based on the research idea of sensomics (Duan et al., 2024). From the eight aroma dimensions of floral, fruit, green, acidic, roasted, sweet, woody-like and herbal, an aroma feature contour map was constructed to analyze the sensory differences between the typical SMX and control samples. As shown in Fig. 1, both samples exhibited similar intensities for floral, fruity, sweet, acidic, roasted and herbal attributes. However, the intensities of green and woody-like odors were significantly different between the typical SMX baijiu samples and the control samples (green: $p < 0.05$, woody-like: $p < 0.001$). The intensities of green and woody-like odors in typical SMX baijiu samples were significantly higher than those in the control samples.

3.2. Identification of marker compounds

To further analyze the differences in flavor composition between the SMX and control samples, HS-SPME/GC–MS was used to analyze the flavor compounds in 20 SMX and 13 control samples. A total of 87 flavor compounds were identified (see Table S1 for detailed information), including 41 esters, 13 alcohols, 15 aldehydes and acetals, 6 acids, 7 ketones, 3 heterocyclic compounds and 2 other compounds.

PCA is a commonly used unsupervised learning method. It uses orthogonal transformation to transform the observation data represented by linear correlation variables into a few data represented by linear independent variables (Zhao et al., 2024). According to the semi-quantitative results, PCA was performed to reveal the distribution and differences between the SMX and control samples. As shown in Fig. S1, the first principal component (PC1) accounted for 32.5 % of the

Table 1
Average concentrations and OAVs of potential marker compounds in SMX baijiu samples and control samples.

No.	Compounds	VIP	Odor description[a]	Odor thresholds (μg/L) [b]	Average concentrations [c](mg/L)		OAVs [d]		Significance [j]
					SMX	control	SMX	control	
1	acetaldehyde	1.74	fruity, grassy	1200[e]	1767.58 ± 471.34	444.07 ± 65.75	1472.98	370.06	***
2	acetal	1.70	grassy, fruity	2090[f]	1586.39 ± 470.97	385.02 ± 68.89	759.04	184.22	***
3	3-methyl-1-butanol	1.67	fusel oil	179191[f]	443.53 ± 44.14	368.42 ± 46.91	2.48	2.06	***
4	ethyl lactate	1.64	fruity	128084[f]	2027.55 ± 296.46	2965.45 ± 514.05	15.83	23.15	***
5	isoamyl acetate	1.64	fruity, banana	94[f]	2.43 ± 0.46	1.24 ± 0.38	25.85	13.19	***
6	2-methyl-1-propanol	1.63	fruity, apple	40000[h]	293.25 ± 35.52	244.11 ± 31.75	7.33	6.10	***
7	ethyl propionate	1.62	fruity	19019[f]	55.39 ± 16.47	16.52 ± 5.24	2.91	<1	***
8	2-pentanol	1.61	alcoholic, fruity	194000[g]	3.25 ± 0.45	2.14 ± 0.47	<1	<1	***
9	ethyl 2-hydroxycaproate	1.58	floral	51400[g]	6.59 ± 1.20	12.26 ± 1.52	<1	<1	***
10	trimethylpyrazine	1.53	roasted, potato, must	730[f]	1.36 ± 0.23	1.36 ± 0.23	1.86	1.86	–
11	isobutyraldehyde	1.52	floral, grassy	1300[g]	7.66 ± 1.61	5.85 ± 1.39	5.89	4.50	*
12	phenylethyl acetate	1.52	honey, rose	909[h]	0.54 ± 0.14	0.23 ± 0.03	<1	<1	***
13	ethyl isovalerate	1.52	fruity	6.89[f]	4.50 ± 0.83	7.58 ± 3.37	653.12	1100.15	**
14	propanol	1.46	alcoholic	53953[f]	3497.11 ± 1571.72	1324.12 ± 650.67	64.82	24.54	***
15	propionaldehyde	1.45	pungent, ethereal	1505[f]	17.15 ± 8.93	2.42 ± 0.42	11.40	1.61	***
16	2-butanone	1.42	solvent, camphor	35400[f]	5.16 ± 3.06	3.81 ± 0.60	<1	<1	–
17	ethyl acetate	1.39	fruity, sweety	32552[f]	2113.10 ± 402.31	2718.86 ± 617.83	64.91	83.52	**
18	ethyl phenylacetate	1.38	fruity, sweety	407[f]	3.70 ± 0.49	5.02 ± 0.27	9.09	12.33	***
19	furfural	1.35	sweety, almond	44000[g]	116.77 ± 21.87	142.62 ± 49.07	2.65	3.24	–
20	isovaleraldehyde	1.33	fruity, peach	17[h]	32.64 ± 4.39	31.39 ± 5.48	1920.00	1846.47	–
21	1,1-diethoxy-3-methylbutane	1.32	fruity	3000[i]	16.05 ± 3.22	15.25 ± 2.33	5.35	5.08	–
22	2-heptanone	1.25	fruit, coconut	140[h]	0.38 ± 0.06	0.30 ± 0.06	2.71	2.36	*

[a] Odor descriptions were obtained from the literatures and the results of smelling the diluted chemical standards by sensory personnel.
[b] The threshold was the minimum concentration of perception through the sense of smell. All odor thresholds were obtained from literatures and determined in 46 % (*v*/v) ethanol/water solution.
[c] Compounds were quantitated by multiple quantitative methods.
[d] OAVs were calculated by dividing the concentration by the odor threshold value of aroma compounds.
[e] Odor threshold previously reported in ref. (Wang, Wu, et al., 2023).
[f] Odor threshold previously reported in ref. (Li et al., 2023).
[g] Odor threshold previously reported in ref. (Wang et al., 2014).
[h] Odor threshold previously reported in ref. (Liu & Sun, 2018).
[i] Odor threshold previously reported in ref. (Wang, Hao, et al., 2023).
[j] Significance was indicated at *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and "-" $p > 0.05$.

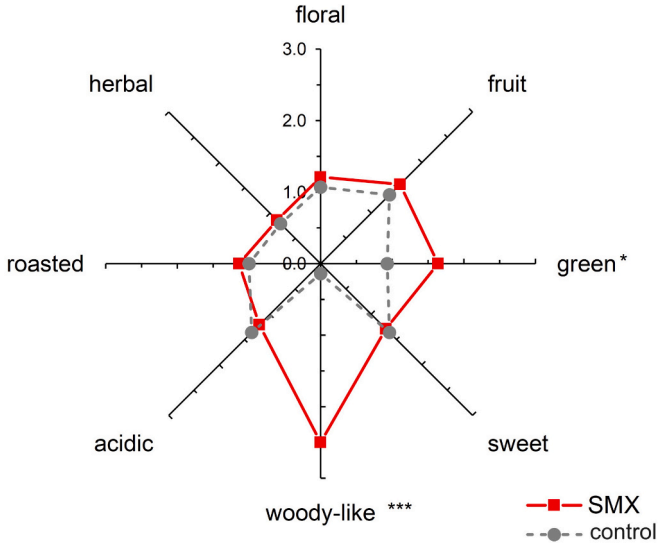

Fig. 1. Aroma profiles of typical SMX baijiu and control samples. The scores of selected descriptors were evaluated by 10 panelists on average. Significance was indicated at * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$

variance, the second principal component (PC2) accounted for 15.5 % of the variance. The first three components (i.e., PC1, PC2 and PC3) together explained 55.8 % of the variance. The SMX baijiu and control samples were clearly separated in PCA score plots. The SMX baijiu samples were almost located in the region with positive scores, whereas the cluster of control samples was well defined by negative PC1 scores. The PCA results indicated a significant difference in the volatile compound profiles between the SMX baijiu samples and the control samples.

PCA can generally reflect the differences between groups of samples and the degree of variation between samples in the group, but it is lacking in the mining of characteristic compounds. OPLS-DA, as a supervised model algorithm, can better distinguish samples between groups using partial least squares regression to establish the relationship between metabolites and sample categories, which is more conducive to finding differences between groups and distinguishing characteristic variables of each group (He et al., 2021). Therefore, it is necessary to further excavate and analyze the obtained data through OPLS-DA to identify the characteristic variables that distinguish the SMX baijiu and control samples.

As shown in Fig. 2A, the SMX baijiu and control samples were clearly distinguished using OPLS-DA model. The R^2Y and Q^2Y represent the degree of interpretation of the data and the predictive ability of the model under the random Y variable model, respectively. The R^2Y and Q^2Y are closer to 1, indicating that more information is available to explain the two categorizations, and the model is more predictable. The results showed that the cumulative explanatory ability parameters R^2Y was 0.911, and the predictive ability parameter Q^2Y was 0.893, indicating that the model was stable and reliable, and the predictive ability was strong, which could be used to distinguish SMX baijiu samples from control samples.

When the number of samples in the OPLS-DA model is much lower than the variable, it is prone to overfitting. In order to evaluate the model performance, 200 permutation tests were performed. As shown in Fig. 2B, all the R^2 and Q^2Y values on the left were lower than the original R^2 and Q^2Y values on the right, and the intercept of the Q^2Y regression line was −0.493, which was lower than 0.05, indicating that the models did not overfit the data. The permutation test results showed that the

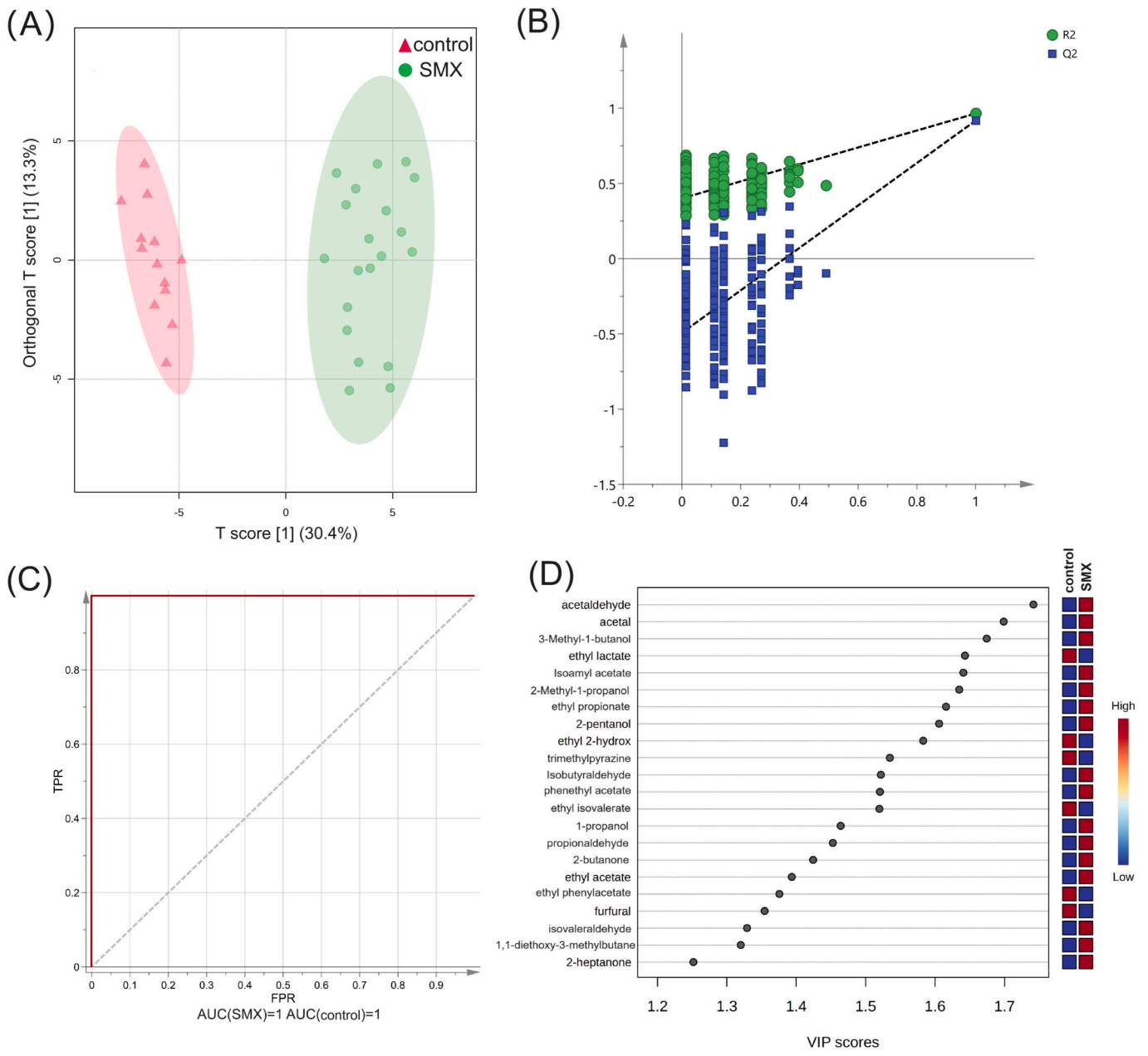

Fig. 2. (A) OPLS-DA plots of the SMX baijiu and control samples. (B) Performance of the permutation test. (C) ROC plots for judging the classification of the SMX baijiu and control samples. (D) Variable importance in projection values of the 22 marker compounds (VIP > 1.2).

initial model was better than the random permutation model, indicating that the model was effective and reliable. In addition, the area under the curve (AUC) in Fig. 2C is 1, indicating that the receiver operating characteristic (ROC) analysis results are good and the model has excellent discriminant ability.

Variable importance in projection (VIP) describes the contribution of each variable to model classification (Wang, Hao, et al., 2024). A factor with VIP value greater than 1.2 in the model was used as the key differential substance to distinguish the SMX baijiu samples from the control samples. As shown in Fig. 2D and Table 1, 22 volatile compounds were identified as potential marker compounds, including 8 esters (ethyl acetate, ethyl propionate, isoamyl acetate, ethyl isovalerate, ethyl lactate, ethyl 2-hydroxycaproate, phenylethyl acetate, and ethyl phenylacetate), 4 alcohols (propanol, 2-methyl-1-propanol, 3-methyl-1-butanol, and 2-pentanol), 5 aldehydes (acetaldehyde, propionaldehyde, isobutyraldehyde, isovaleraldehyde, and furfural), 2 ketones (2-butanone and 2-heptanone), 2 acetals (acetal and 1,1-diethoxy-3-methylbutane) and 1 pyrazine compound (trimethylpyrazine). Among these, acetaldehyde had the highest variable importance in the projection values (1.74), followed by acetal (1.70).

3.3. Quantitative, OAV, heatmap and HCA of marker compounds

To further analyze the differences among the marker compounds in the SMX baijiu and control samples, the identified 22 marker compounds were quantitated using DI/GC-FID, HS/GC–MS and LLME/GC–MS based on a combination of external and internal methods. The calibration curves and corresponding quantitative analysis methods for each compound are listed in Table S2. All marker compounds had good analytical linearities of the standards, with R^2 values of the calibration curves better than 0.99, indicating that the quantitative method was reliable. As shown in Table 1, the quantitation results showed that 15 marker compounds in the SMX baijiu samples had higher concentrations than those in the control samples. Especially, the contents of

acetaldehyde, propionaldehyde, acetal, propanol, 3-methyl-1-butanol, 2-methyl-1-propanol, 2-pentanol, ethyl propionate, isoamyl acetate, and phenylethyl acetate in the SMX samples were significantly ($p < 0.001$) higher than that in the control samples.

To elucidate the associations among the quantitated marker compounds, the concentrations of quantitated compounds in the SMX baijiu and control samples were used to prepare a heatmap, and the color (from light to dark) indicated the relative intensity change from low to high. The differences between the marker compounds that distinguished the SMX and control samples are shown in Fig. 3. HCA clearly separated the quantitative markers into three clusters. Cluster I consisted of the samples originating from the SMX with significantly higher mean concentrations than control samples, which included propanol, propionaldehyde, ethyl propionate, 2-pentanol, acetaldehyde, acetal, isoamyl acetate, phenylethyl acetate, 2-heptanone, 2-methyl-1-propanol, 3-methyl-1-butanol. These 11 compounds at higher levels may be important contributing substances to the SMX characteristic in Jiang-flavor base baijiu. Among these marker compounds, the average contents of acetaldehyde, acetal, propionaldehyde, ethyl propionate and propanol in the SMX samples were significantly higher (3.9-, 4.1-, 6.8-, 3.2- and 2.7-fold change, respectively) than in control samples. Related studies have reported that aldehydes, represented by acetaldehyde, and higher alcohols, represented by isobutanol, are important factors that constitute the characteristics of green taste defects in beer and wine(Arias-Pérez et al., 2021; Cui et al., 2019). The analysis results indicate that the concentrations of these compounds were higher in the SMX samples, consistent with the more pronounced green aroma of the SMX samples in sensory analysis. Cluster II consists of 5 esters, including ethyl acetate, ethyl lactate, ethyl isovalerate, ethyl 2-hydroxycaproate and ethyl phenylacetate. The average contents of these compounds in the control samples were significantly higher than that in SMX samples. Cluster III consists of 6 chemical markers, namely isobutyraldehyde, 2-butanone, isovaleraldehyde, 1,1-diethoxy-3-methylbutane, furfural, and trimethylpyrazine. There was no uniform pattern in the quantitative data for these compounds between the SMX baijiu and control samples, indicating that the compounds in cluster III have no contribution to the aroma of SMX.

It is known that the contribution to baijiu flavor depends not only on the concentration of an odorant but also on its odor threshold. The OAV refers to the ratio of the concentration of a volatile aroma substance to its olfactory threshold, which is an important technical means to measure the contribution of volatile substances and determine the effective aroma components. In general, compounds with OAVs greater than 1 are considered to contribute to the aroma. As shown in Table 1, there are 18 compounds with OAVs greater than 1 among the 22 potential markers in SMX samples. According to Heatmap and HCA results, the 11 compounds in Cluster I were found in high levels in the SMX samples, and are most likely the key compounds that produce SMX characteristic in Jiang-flavor base baijiu. Further analysis of the OAVs of these 11 compounds revealed that the OAVs of propanol, propionaldehyde, ethyl

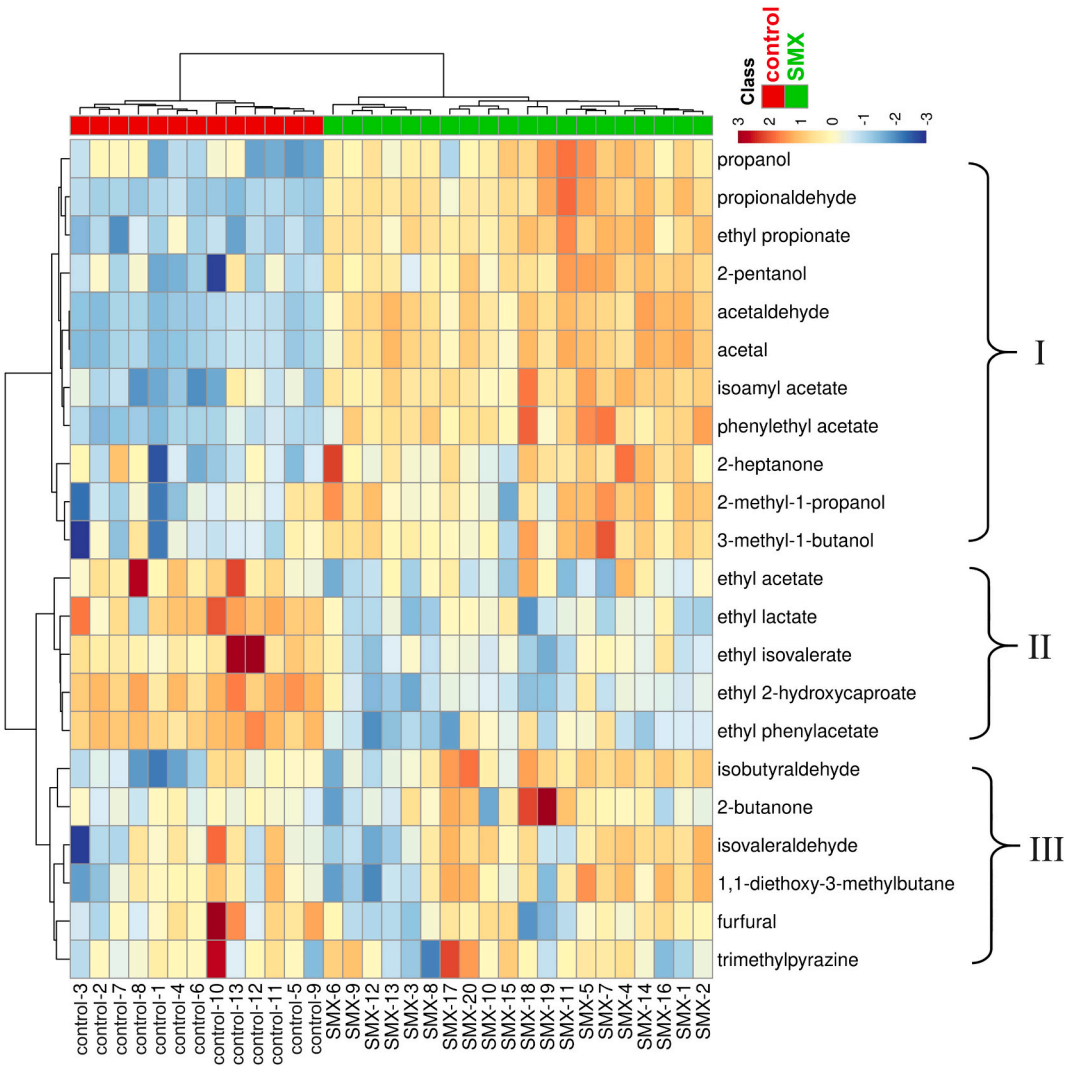

Fig. 3. Heatmap and HCA of the 22 marker compounds in SMX and control samples.

propionate, acetaldehyde, acetal, isoamyl acetate, 2-heptanone, 2-methyl-1-propanol, and 3-methyl-1-butanol were greater than 1. This result indicates that the above 9 compounds may have an important contribution to the formation of the SMX aroma in Jiang-flavor base baijiu.

3.4. Sensory validation

3.4.1. Aroma recombination

Based on the quantitative results and OAVs analysis of the different compounds in the SMX and control samples, it was preliminarily concluded that acetaldehyde, propionaldehyde, acetal, propanol, 2-methyl-1-propanol, 3-methyl-1-butanol, ethyl propionate, isoamyl acetate, and 2-heptanone may strongly contribute to the aroma characteristics of SMX. To verify the above results, aroma recombination was performed to verify that odorants with high OAVs contribute to the aroma of SMX. The above nine compounds were further added to the control sample at the corresponding concentrations of typical SMX sample, and the sensory characteristics were analyzed. As shown in Fig. 4, a comparison of the aroma profiles between the recombinant and typical SMX sample showed that the aroma profile of SMX could be basically simulated, and the woody-like and green aromas were enhanced compare to the control sample. However, the intensities of typical woody-like and green aromas in the recombinant sample still did not reach those in typical SMX sample, which may be related to the higher content of ester compounds in the added matrix (i.e., control sample). Relevant research also shows that esters in baijiu play an important role in aroma perception interactions, and significantly affect the aroma characteristics of baijiu (Niu et al., 2020). The overall aroma profile of the reconstitution sample was highly similar to the typical SMX sample, indicating that the aroma characteristic of SMX in Jiang-flavor base baijiu was successfully simulated by adding the above 9 aroma compounds.

3.4.2. Omission experiments

To further confirm the aroma contribution of the above nine identified compounds to the SMX aroma, seven omission models were prepared, in which either a single component or a group of components with similar chemical structure aroma descriptions were omitted from the recombinant model (Gao et al., 2014; Wang, Wu, et al., 2023). These models were then compared with recombination model (as described in Section 3.4.1) using the triangle test. As shown in Table 2, the study revealed that all aldehydes and acetals significantly influenced the overall aroma attribute of SMX ($p \leq 0.001$) (omission model 1). Furthermore, the effects of acetaldehyde, acetal and propionaldehyde on the SMX aroma were investigated respectively. The absence of acetaldehyde (omission model 1–1) had a considerable impact on the overall aroma profiles of SMX ($p \leq 0.01$). Similarly, removing acetal (omission model 1–2) from the fully reconstituted model resulted in analogous aromatic characteristics ($p \leq 0.01$). However, when propylaldehyde was missed (omission models 1–3), no significant differences were observed between the missing and reconstructed models. In omission models 2 and 3, there was no significant difference ($p \geq 0.05$) in the omission model when all alcohols (propanol, 2-methyl-1-propanol, 3-methyl-1-butanol) and all esters (ethyl propionate and isoamyl acetate) were missing. The strength of the SMX aroma in omission models 2 and 3 showed no significant changes, indicating that propanol, 2-methyl-1-propanol, 3-methyl-1-butanol, ethyl propionate, and isoamyl acetate had no effect on the SMX aroma in Jiang-flavor base baijiu. When 2-heptanone was missing (omission models 4), there was almost no effect on the reconstructed model. In conclusion, the above results indicated that acetaldehyde and acetal were important and contributed substantially to the SMX aroma in Jiang-flavor base baijiu. These two substances both exhibit grassy and green apple-like aroma characteristics, but at high concentrations, they possess a strong stimulating effect, which can cause discomfort and severely affect the aroma and taste of baijiu. Therefore, it is crucial to control the content of acetaldehyde and acetal in baijiu within an appropriate range.

Table 2
Aroma omission experiments from the reconsitution model.

No.	Odorants omitted from the complete recombinant	n[a]	Significance[b]
1	all aldehydes and acetals	10	***
1–1	acetaldehyde	8	**
1–2	acetal	8	**
1–3	propionaldehyde	4	ns
2	All alcohols	4	ns
3	All esters	2	ns
4	2-heptanone	1	ns

[a] Number indicates a significant aroma difference among the three cups via the triangle test based on 10 panelists.
[b] **: highly significant ($p \leq 0.01$); ***: very highly significant ($p \leq 0.001$); and ns: not significant ($p \geq 0.05$).

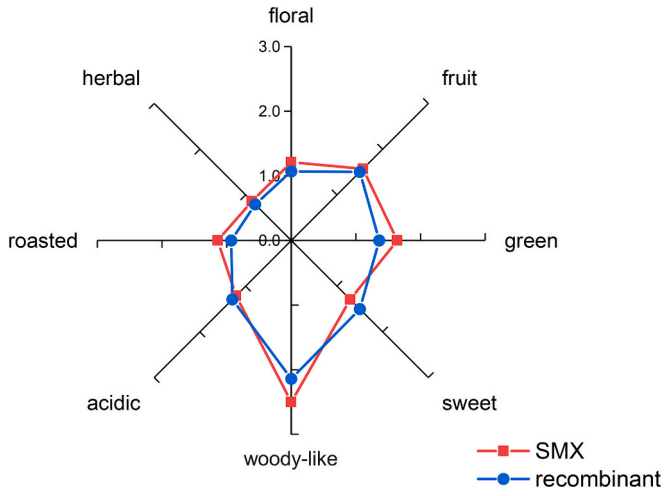

Fig. 4. Aroma profiles of typical SMX baijiu and the recombinant.

4. Conclusions

In this study, a combined strategy of metabolomics and sensomics was used to successfully analyze the key aroma compounds that produce the SMX characteristic in Jiang-flavor base baijiu. Firstly, sensory analysis of SMX baijiu samples revealed significantly higher intensities of green and woody-like odors compared to control samples. Secondly, HS-SPME/GC–MS analysis identified 87 flavor compounds, and PCA showed clear separation between SMX and control samples based on their volatile compound profiles. A total of 22 potential marker compounds were identified using OPLS-DA based on VIP > 1.2. Thirdly, the potential marker compounds were quantified using a variety of methods. HCA and OAV results indicated propanol, propionaldehyde, ethyl propionate, acetaldehyde, acetal, isoamyl acetate, 2-heptanone, 2-methyl-1-propanol, and 3-methyl-1-butanol may strongly contribute to the aroma characteristics of SMX. Finally, through aroma recombination and omission experiments, it was further confirmed that acetaldehyde and acetal were the key factors causing the SMX characteristic in Jiang-flavor base baijiu. This study systematically analyzed the main contributing substances with SMX characteristic in Jiang-flavor base baijiu by combining metabonomics and sensomics, providing a new idea for the analysis of special aroma in baijiu. Moreover, these findings significantly contribute to the quality control of Jiang-flavor base baijiu and the creation of new products with targeted sensory profiles.

CRediT authorship contribution statement

Yang Xu: Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation. **Lizhang Yang:** Writing – review & editing, Validation. **Yubo Yang:** Writing – review & editing. **Fan Yang:** Writing – review & editing, Supervision.

Declaration of competing interest

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

Data availability

The data that has been used is confidential.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101851.

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