Evaluation of flavor substances of rice bran kvass based on electronic nose and gas chromatography–mass spectrometry

Xiaochen Yu, Wenjuan Zhang, Liying Xin, Su Xu [*], Jianjun Cheng [*]

College of Food Science, Northeast Agricultural University, Harbin, Heilongjiang 150030, China

ARTICLE INFO

ABSTRACT

In this paper, the electronic nose (E-nose) and headspace-solid phase microextraction (HS-SPME) combined with gas chromatography–mass spectrometry (GC–MS) were used to analyze the volatiles of rice bran kvass (RBK) with the reference of Qiulin kvass (QLK). Meanwhile, the flavor amino acids of RBK before and after fermentation were determined. The results showed that the kinds of kvass remained consistent in terms of the overall category of volatiles while there were differences in content between them ($p < 0.05$). A total of 35 volatile compounds, mainly including esters, alcohols, phenols, aldehydes, and acids, were identified by GC–MS in the two kinds of kvass. In addition, the total essential amino acid content and the total sweet amino acid content of RBK increased significantly ($p < 0.05$) after fermentation. RBK contains both the main flavor of kvass and its own unique characteristics, making it a new member of the Kvass family.

Introduction

Rice bran stands as a significant by-product in rice processing. In 2021, China's rice yield reached 210 million tons, generating over 14 million tons of rice bran through processing (Yu, 2022). Rice bran comprises about 10 % of the total mass of rice grain (Patra et al., 2023), and harbors 64 % of the rice grain's nutrients and over 90 % of the body's essential elements. It encompasses not only rice bran protein (11–17 %), lipid (15–20 %), carbohydrates (34–62 %) and dietary fiber (20–51 %) and other nutrients, but also contains phenolic acids, tocopherols, polysaccharides, inositol and γ-glutamine and other biologically active substances that have antioxidant and immunity-enhancing properties (Liu, Strappe, Zhou, & Blanchard, 2019; Punia, Kumar, Siroha, & Purewal, 2021). At present in China, rice bran is mainly used for making rice bran oil and directly mixed into livestock feed as raw materials, and a small amount is used in food processing to prepare health care cookies, pasta, noodles and so on (Gul, Yousuf, Singh, Singh, & Wani, 2015). In fact, the rich nutrition in the rice bran is not fully utilized, the value of the rice bran has not been enhanced, which leads to waste of rice bran resources. In view of the large quantity and low utilization rate of rice bran as a by-product of rice in China, it is urgent to develop a high value-added product using rice bran as raw material.

Kvass has been hailed as a healthy drink (Li, Gao, et al., 2023). Traditional Kvass was a drink made from dried bread fermented by yeast and lactic acid bacteria. Kvass boasts a low alcohol content and, a mellow aroma derived from natural fermentation, and tastes somewhere between beer and soda. It contains natural organic acids, amino acids, and carbohydrates, and has the effects of appetizing and strengthening the spleen, antioxidant, and eliminating fatigue (Wang et al., 2022; Zapata Flores, Bùi, Selberg, Herodes, & Leito, 2023). At the same time, kvass has a regulatory effect on intestinal flora, so it is regarded as a functional food by some people (Ai, 2014). As consumers' demands for product quality and flavor continue to improve, the types of kvass tend to be diversified, such as raspberry kvass (Han, Li, Sun, Wang, & Xiao, 2018) and vegetable kvass (Yawei, 2022). But so far, there have been no reports of rice bran kvass. Rice bran was a by-product with high nutritional and application value, and could provide a good material basis for the growth and metabolism of lactic acid bacteria and yeast. it makes the possibility for the development of a new type of kvass, rice bran kvass.

At present, electronic sensory instruments (E-nose) combined with GC–MS techniques are mainly used for the study of different volatile compounds. The E-nose is an electronic system that simulates the human olfactory sense and uses the response value of the gas sensor array to identify odors. It offers advantages such as a short response time, fast detection, a wide measurement range, good repeatability, and the ability to detect the gases that cannot be smelled by the human nose (Yu, Wang, Zhang, Yu, & Yao, 2008). However, the E-nose lacks the capability to identify specific volatile flavor substances (Singh & Gaur, 2023). HS-

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SPME-GC–MS has been applied to the determination of volatile constituents in food. It possesses the advantages like high sensitivity, strong immunity to interference, and the ability to accurately analyze volatile gases qualitatively and quantitatively (Wang, Wang, Deng, Cai, & Chen, 2019). The combination of E-nose and GC–MS gives full play to the advantages of the instruments. This integration not only avoids the limitation of individual analytical technique that fails to detect some crucial compounds, but also provides more comprehensive and reliable scientific information (Rong et al., 2023) and detects volatile compounds more accurately. In recent years, the use of GC–MS combined with E-nose technology has been widely applied to the study of volatile flavor substances in diverse varieties, qualities, and storage conditions of food, such as Jiuquhongmei tea (Peng et al., 2023), Millet Huangjiu (Ye, Wang, Zhan, Tian, & Liu, 2022), meats (Yin et al., 2021), fruits (Wang, Wang, et al., 2023; Wang et al., 2023), and sugarcane juice (Wang et al., 2019), etc. These studies have well demonstrated that the E-nose coupled with GC–MS has good correlation and complementarity in identifying the differences in the flavors of food products, and combined with the statistical analyses, it can distinguish the aroma profiles of the different substances and the specific aroma compounds.

In this study, with QLK as a reference, the volatile flavor components of RBK were analyzed using E-nose and GC–MS techniques to screen out key aroma substances and conduct correlation research. The effect of flavor amino acids on the flavor of kvass was analyzed by measuring the changes of free amino acids in RBK between before and after fermentation. The research in this paper can provide fundamental data for the development and production of new type RBK. It is of great significance for developing functional rice bran drinks, extending the rice bran processing industry chain and increasing the added value of rice bran products.

Materials and methods

2.1. Materials

The strains *Lactobacillus bulgaricus* and *Saccharomyces cerevisiae* (Y-06) were isolated and preserved in the grain engineering laboratory of College of Food Sciences, Northeast Agricultural University, Harbin, China. Rice bran was purchased from Heilongjiang Yihua Rice Co., Ltd; α-amylase, glucoamylase, and glucose (Zhejiang Tianhe Food Co., Ltd); sulfosalicylic acid (Tianjin Tianli Co., Ltd).

QLK was purchased from the market. QLK was a kind of fermented beverage made from Russian Dalieba bread fermented by lactic acid bacteria (*Lactobacillus acidophilus*) and yeast (*Saccharomyces cerevisiae*).

2.2. Preparation of RBK

RBK was prepared according to the process of Fig. 1. The operational essentials were as follows:

(1) Baked rice bran: the rice bran was sieved at 40 mesh and placed in an oven to be baked at 175 ± 1 °C for 5 min.
(2) Gelatinization: The baked rice bran was gelatinized in a water bath at 90 °C for 10–15 min in accordance with a ratio of 1:8 (baked rice bran: water).
(3) Liquefaction: adding α-amylase at 80 U/g and enzymatic hydrolysis in a water bath at 65 °C for 1.5 h.
(4) Saccharification: adding glucoamylase at 40 U/g, enzyme hydrolysis in a water bath at 60 °C for 1 h.
(5) Sterilization: adding 3 % glucose to the solution obtained by filtration after saccharification, mixing well, and then carrying out autoclave sterilization.
(6) Lactobacillus fermentation: the saccharified broth cooled to room temperature after sterilization was inoculated with *Lactobacillus bulgaricus* at 5 %, and then cultured in an incubator at 37 °C for 20 h.
(7) Yeast fermentation: inoculation with 5 % *Saccharomyces cerevisiae*, and then culture in an incubator at 30 °C for 20 h.
(8) Yeast autolysis: the yeast fermentation broth was kept at 55 °C for 18 h.
(9) Sterilization: the fermentation broth was sterilized after the culture was completed, and then was taken out and added 10 % diatomite for filtration.
(10) Blending: Because the Brix of commercial QLK was determined 1.4, the fermentation broth was diluted 5 times to be consistent with that of QLK after sterilization and filtration, and then 3.5 % sugar was added and CO_2 was filled with to 1.5 MPa.

2.3. E-nose analysis

Referring to the method of Hongmei et al. (2019). The odor profiles of two kinds of kvass were analyzed by the E-nose analysis system (PEN3, Airsense, Schwerin, Germany). The PEN3 E-nose system contains 10 different metal oxide semiconductor sensors consisting of a system of
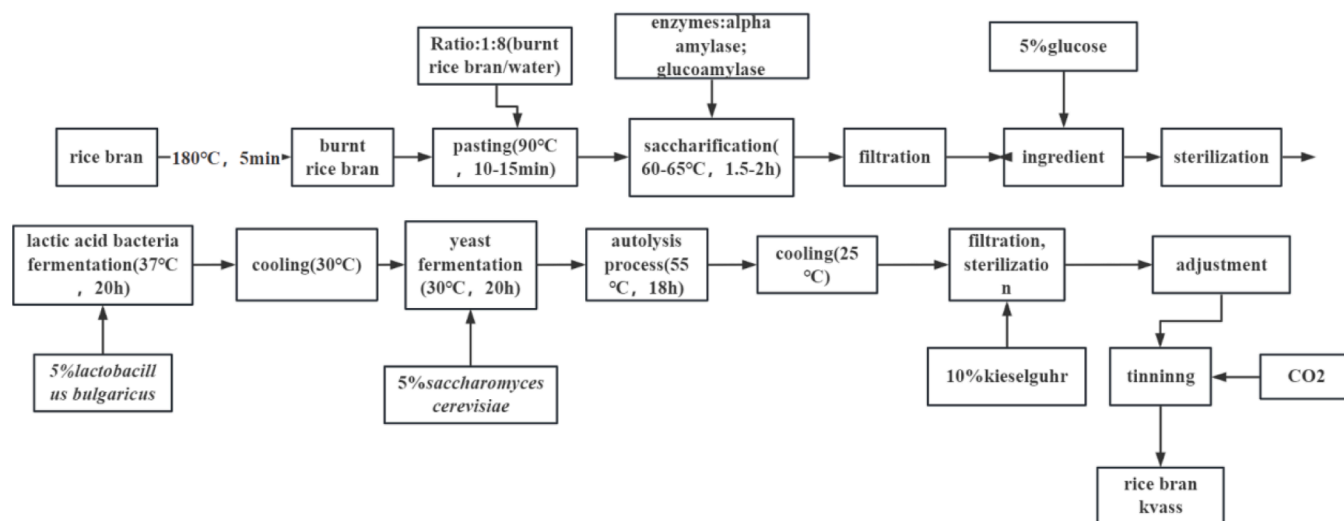

Fig. 1. Flowchart of kvass-making technique using rice bran material.

sensor arrays that provides selectivity for volatile compound classes.

2.3.1. Sample pretreatment

The samples were degassed by ultrasonic wave for 30 min to exclude carbon dioxide. After the degassing was completed, 8 mL of the sample was put into a 20 mL headspace bottle and heated in a water bath for 5 min (70 °C).

2.3.2. Detection conditions

Sampling interval: 1 s, cleaning time: 120 s, zeroing time: 10 s, sample preparation time: 5 s, and determination time: 60 s. The carrier gas and sample flow rates were 150 mL/min and 300 mL/min, respectively. The signal acquisition time was 55 ~ 59 s. Volatile components were characterized using 10 metal oxide semiconductor sensors. The results obtained were subsequently analyzed using the workstation software Winmuster 1.6.2.18 (Airsense, Schwerin, Germany).

2.4. Measurement of volatile compounds

Referring to the method of Hongmei et al. (2019) with slight modification, the volatile compounds were determined by HS-SPME-GC–MS as follows. Accurately measure 8.0 mL of each of the two kvass samples into a 15 mL sample bottle, respectively, and screw the cap tightly.

2.4.1. Headspace solid-phase microextraction conditions

The headspace vials with the sample were equilibrated at 70 °C for 30 min, and an aged extraction head (50/30 μm, DVB/CAR/PDMS, (Supelco, Bellefonte, PA, USA)) was inserted into the headspace portion of the sample vial, and adsorbed for 15 min, then the aged extraction head was desorbed and maintained in the GC injector for 3 min at 250 °C.

2.4.2. Chromatographic conditions

HP-5MS column (30 m × 0.25 mm, 0.25 μm) (Agilent Technologies, Inc. California, USA), carrier gas: helium, inlet temperature 240 °C, injection was performed in a non-split mode, with a carrier gas flow rate of 1.3 mL/min, the temperature increase program: initial temperature 50 °C, hold for 2 min, increase to 200 °C at 5 °C/min, hold for 5 min, increase to 250 °C at 25 °C/min, hold for 5 min.

2.4.3. Mass spectrometry conditions

The ion source temperature was 230 °C, the solvent delay time was 1 min, and the mass scan range was set from 40 to 600 m/z in full scan mode. The unknown compounds were retrieved by the computerized mass spectrometry system NSIT with RTLPEST and results with a match greater than 700 (maximum 1000) and a likelihood greater than 85 % will be reported, and the relative content of each component was calculated using the area normalization method.

2.5. Determination of free amino acids in fermentation broth before and after RBK fermentation

Free amino acids were analyzed based on the method of Fan et al. (2016) with slight modifications. The culture medium before and after the fermentation of RBK were mixed with an equal volume of sulfosalicylic acid solution (10 g/100 mL), and homogenized at high speed for 30 s. The mixture was allowed to stand at 4 °C for 16 h, centrifuged at 4 °C and 8000 r/min for 10 min, and then the supernatant was placed in a refrigerator at 4 °C overnight. The centrifugation was repeated, and filtered, and the supernatant was taken and filtered through a 0.45 μm filter (Yuanye Bio-Tech. Ltd, Shanghai, China). The free amino acid content was measured by the amino acid analyzer LA8080 (Hitachi, Japan). Amino acid analyzer test conditions: 4.6 mm × 60 mm Na$^+$ ion exchange column, buffer flow rate: 0.4 m L/min, ninhydrin flow rate: 0.35 m L/min, column temperature: 57 °C, amino acid standard sample: 10 nmol/L, detection wavelengths: λ_1 = 570 nm, λ_2 = 440 nm, injection

volume: 50 μL. Quantitative analysis on the machine in 50 min program. The content of each free amino acid was reported as mg/100 mL of fermented broth.

2.6. Statistical method

The above experiments were repeated three times. Data were tabulated using Microsoft Excel software (Microsoft Corporation, Seattle, WA, USA) and analyzed for significance of differences using IBM SPSS Statistics 27.0 (SPSS Inc., Chicago, IL, USA) software, $P < 0.05$, significant difference, and $P < 0.01$, highly significant difference. SPME-GC–MS data were processed using MSD Chem Station software (Agilent Technologies, Inc. California, USA), and the unknown volatile compounds were matched to the NIST standard spectral library (NIST (National Institute of Standards and Technology), Gaithersburg, MD, USA). And the GC–MS data were analyzed by SIMCA-P 14.1 (Umetrics, Umea, Sweden) software for orthogonal partial least squares discriminant analysis (OPLS-DA) and plotted on GC–MS data, other plots were produced using Origin 2021 software (Origin Lab Corporation, Northampton, MA, USA).

Results and analysis

3.1. E-nose analysis of RBK and QLK volatiles

The PEN3 E-nose consists of 10 sensors, and a radar map of kvass volatiles was plotted based on the response values of two kinds of kvass across the 10 sensors, as shown in Fig. 2a.The blue and red plots in the figure represent RBK and QLK, respectively, and it could be seen that the shapes of the two plots are similar, but some of the response values were different, indicating that the volatile compound classes of the two were similar in general but the contents were different ($p < 0.05$). As can be seen from Fig. 2a, the response values of two sensors (W1S and W2S) for each of the two kinds of kvass were obvious, that was, short-chain alkanes, as well as alcohols, aldehydes, and ketones, had the highest response values among the two kinds of kvass, which suggested that the substances corresponding to these sensors had an important influence on the formation of the overall flavor of the kvass. In addition, there was higher response values in RBK than in QLK for both types of sensors, which suggested that there was a higher concentration of flavor substance in this category in RBK.

Data visualization combined with data processing allows for a more comprehensive analysis of flavor data. OPLS-DA is a supervised discriminant statistical analysis that screens for difference variables by removing irrelevant differences (Wang, Chen, et al., 2023). In this study, it was mainly used for the identification of the response values of different kinds of kvass to the E-nose probe. As shown in Fig. 2b, the two kinds of kvass were distinguished on the horizontal axis of the score scatterplot, with a fit index (R_x^2) of 0.776 for the independent variable, a fit index (R_y^2) of 0.973 for the dependent variable, and a model prediction index (Q^2) of 0.927. Theoretically, R^2 and Q^2 exceeding 0.5 indicate acceptable fitting results (Yun et al., 2021). The score plot of the OPLS-DA results showed that there was a clear separation of QLK and RBK along component 1, and there were some intragroup differences in both kinds of kvass along component 2. In other words, QLK prepared with dried bread was uniformly distributed in the negative half-axis of the horizontal axis, and RBK with rice bran was uniformly distributed in the positive half-axis of the horizontal axis, which showed there was a good repeatability of the same kvass and there was some difference between the two kinds of kvass. However, the composition and contents of volatile flavor components need to be further analyzed by GC–MS for the kvass qualitatively and quantitatively.

3.2. GC–MS-based analysis of two kinds of kvass volatiles categories

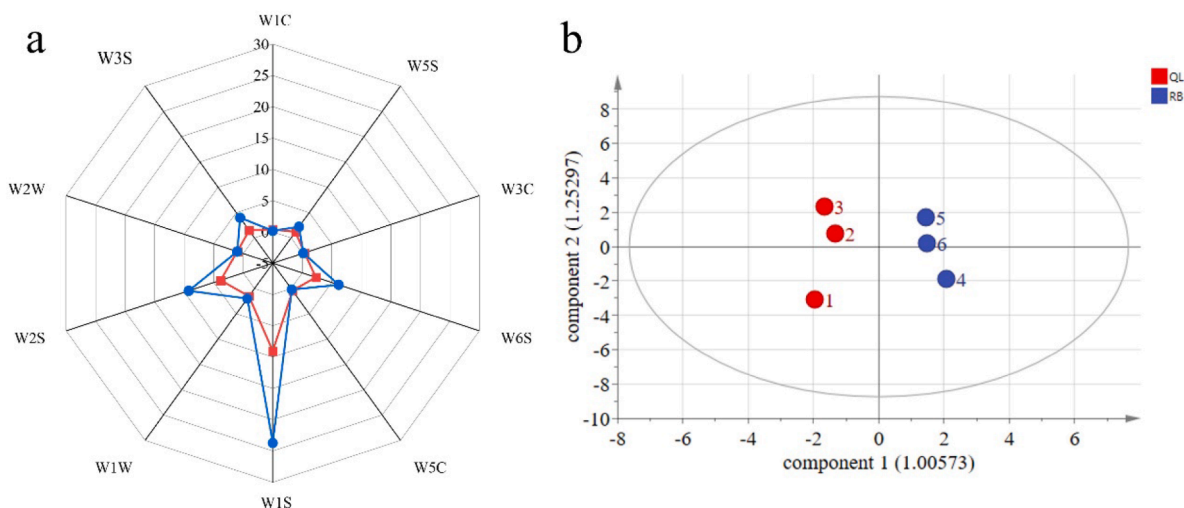GC–MS was used to detect the volatile components in different kvass

Fig. 2. E-nose analysis (a) E-nose radargram (b) OPLA-DA analysis.

samples. As shown in Table 1 and Fig. 3a, a total of 35 volatile compounds from 8 classes were detected. Among them, there were 5 kinds of alcohols, 7 kinds of acids, 4 kinds of aldehydes, 4 kinds of hydrocarbons, 3 kinds of ketones, 3 kinds of phenols, 7 kinds of esters, and 2 kinds of other compounds, of which 22 were QLK and 19 were RBK. The relative content of volatile compounds in the two samples was shown in Fig. 3b. The content of alcohols in QLK was high, accounting for 31.01 % of the relative content, while that in RBK was relatively low, accounting for 3.50 %. Meanwhile, the acid content of QLK accounted for 21.21 % while that of RBK accounted for 9.39 %. It was speculated that in the *Saccharomyces cerevisiae* autolysis process during the preparation process of RBK, the esters were formed by esterification of various fatty acids and alcohols, which resulted in an obvious increase in the ester content of RBK (54.71 %) compared with that of QLK (6.72 %).

Raw materials are processed at high temperatures during baking to undergo a Maillard reaction (Fu, Zhang, Soladoye, & Aluko, 2020), which affects the flavor of the food, and is a means of flavor enhancement during food processing. There are 3 main categories of volatile substances formed by the Maillard reaction: (1) peptide or amino acid degradation products (e.g., aldehydes, ketones), (2) sugar degradation or cleavage products (e.g., furans), and (3) volatiles (e.g., pyrazines) generated by further reaction of peptides and sugar degradation products (Zhang, Yang, Wang, Fan, & Liu, 2021). Ketones and aldehydes are the main compounds produced by the Maillard reaction, in which Strecker degradation of certain peptides or amino acids occurs to generate aldehydes and ketones with low odor thresholds, which contribute significantly to the formation of the characteristic flavor of kvass. In terms of the total amount of aldehydes and ketones, the relative content difference between QLK and RBK was not too great, due to the Maillard reaction that occurred during the baking and preparation of the two kinds of kvass. However, the relative content of ketones in QLK (11.19 %) were high while that in RBK (5.77 %) was low, and the relative content of aldehydes in RBK (9.13 %) were high but that in QLK (0.57 %) was low. It caused on the one hand by the high amount of lipids in rice bran, which could produce aldehydes by lipid oxidation (García-Llatas, Lagarda, Clemente, & Farré, 2006; Zhang et al., 2023; Zhu, Li, Wang, Huang, & Xu, 2023). On the other hand, the process conditions and strains used in the fermentation of the two kinds of kvass were different, and there might be differences in metabolic capacity and product selectivity between the different strains, which could lead to different ketone contents in the two kinds of kvass.

The phenolic content was 7.52 % in QLK and 12.67 % in RBK, respectively. Phenolic compounds were mainly found in the cortical cell wall of rice bran, which were combined with sugars and proteins in the form of ester and glycosidic bonds, whereas the glycosidic bonds bound to the cell wall were destroyed by the saccharification process, so that phenolic compounds were changed from the bound state to the free state, which led to a high content of phenolics in RBK (He et al., 2023). Phenolics have powerful bioactivities and health-promoting effects (Leonard, Zhang, Ying, Adhikari, & Fang, 2021), and the presence of phenolics in both kinds of kvass need be further investigated in depth to provide new ideas for revealing the physiological activities of kvass. Only 6.72 % of esters were present in QLK, whereas the ester content in RBK was as high as 54.71 %. The reason was not only the esterification derived from alcohols and acids the mentioned above, but also related to the microbial autolysis process in the RBK. Microbial autolysis is a process in which the cell itself releases some enzymes to hydrolyze the structure of cell wall and release intracellular substances under certain conditions. A large amount of protease and a small amount of lipase were released after the autolysis of *Lactobacillus bulgaricus* (Jie, Jiaping, Lu, & Shuwen, 2010). After yeast autolysis, protease, nuclease and glucan hydrolase were mainly produced (Xiang, Li, Yi, & Yanglong, 2001). After the rice bran saccharified broth was fermented by *Lactobacillus bulgaricus* and *Saccharomyces cerevisiae*, a large number of cells of *Lactobacillus Bulgaricus* and *Saccharomyces cerevisiae* were accumulated in the fermentation broth. After treatment of the broth at 55 °C, the hydrolases were activated and hydrolyzed the large molecular substances in the rice bran to produce various small molecular substances, including esters, therefore, the content of esters in the RBK were obviously higher than those in QLK.(Li, Qin, Zhong, Hao, & Wu, 2023). Overall, QLK mainly contained the volatile compounds of alcohols, acids, others, ketones and Hydrocarbon, and RBK mainly contained the volatile compounds of esters, phenols, acids, and aldehydes. Tang Hongmei et al. similarly detected that alcohols were important volatile compounds of kvass in analyzing the volatile compounds of QLK (Hongmei et al., 2019). Z.Q. MA et al. studied the volatile compounds of fermented brown rice and similarly found that aldehydes, acids, and esters were the three important types of volatile compounds in fermented brown rice (Ma, Zhai, Zhang, & Tan, 2023).

3.3. GC–MS-based analysis of characteristic flavor substances of two kinds of kvass

To further illustrate the differences between the characteristic flavor substances of the two kinds of kvass, 29 differential aroma substances of the two kinds of kvass were screened according to the criteria of $p < 0.05$ and VIP > 1, and they were standardized and plotted into a clustered heat map. From Fig. 4, it can be observed that the significant differences

Table 1
Table of volatile compound compositions of kvass by GC–MS detection.

No.	Volatile Compounds	Molecular Formula	CAS No.	RT (min)	Relative content (%)		Odor Description
					QLK	RBK	
Alcohols (5)					**31.01**	**3.50**	
1	3-Methyl-1-butanol	$C_5H_{12}O$	123–51-3	3.183	20.70 ± 0.75^a	/	Choking alcohol-like
2	2-Ethyl-1-hexanol	$C_8H_{18}O$	104–76-7	10.147	2.54 ± 0.12^a	/	sweet
3	1-Octanol	C_8H_8O	111–87-5	11.419	0.57 ± 0.21^a	/	Rose-like or lemon-like
4	n-Pentadecanol	$C_{15}H_{32}O$	629–76-5	12.142	/	0.71 ± 0.09^a	/
5	Phenylethyl alcohol	$C_8H_{10}O$	60–12-8	12.701	7.19 ± 0.39^a	2.79 ± 0.11^b	Fruit, honey, lilac, rose, wine
Acids (7)					**21.21**	**9.39**	
6	Sorbic acid	$C_6H_8O_2$	110–44-1	13.505	8.19 ± 0.61^a	/	/
7	7-Hydroxyocta-2,4-dienoic acid	$C_8H_{12}O_3$	/	13.745	7.13 ± 0.20^a	/	/
8	Octanoic acid	$C_{10}H_{20}O_2$	127–07-2	15.135	2.64 ± 0.14^a	/	Cheese, fat
9	n-Decanoic acid	$C_{10}H_{20}O_2$	334–48-5	19.995	2.45 ± 0.26^a	/	Dust, fat, grass
10	Dodecanoic acid	$C_{12}H_{24}O_2$	143–07-7	24.811	0.80 ± 0.17^b	2.12 ± 0.14^a	/
11	Tetradecanoic acid	$C_{14}H_{28}O_2$	544–63-8	29.331	/	2.08 ± 0.14^a	Burnt, Cheese, Harsh, Oil
12	Octadecanoic acid	$C_{18}H_{36}O_2$	57–11-4	38.322	/	5.15 ± 0.77^a	Dairy
Aldehydes (4)					**0.57**	**9.13**	
13	5-Methyl-2-furancarboxaldehyde	$C_6H_6O_2$	620–02-0	8.161	/	3.30 ± 0.21^a	caramel
14	Nonanal	$C_9H_{18}O$	124–19-6	14.514	/	3.12 ± 0.15^a	Rose-orange odor
15	1-Decanal	$C_{10}H_{20}O$	112–31-2	15.417	0.57 ± 0.11^b	1.61 ± 0.28^a	Floral-orange odor
16	5,9,13-Trimethyl-4,8,12-tetradecatrienal	$C_{17}H_{28}O$	67858–78-0	30.839	/	1.09 ± 0.09^a	/
Hydrocarbons (4)					**9.67**	**/**	
17	Propyl-cyclopropane	C_6H_{12}	2415–72-7	5.669	0.80 ± 0.14^a	/	/
18	5-Ethyl-2-methyl-octane	$C_{11}H_{24}$	62016–18-6	10.925	3.91 ± 0.16^a	/	/
19	3,7-Dimethyldecane	$C_{12}H_{26}$	17312–54-8	15.907	3.91 ± 0.22^a	/	/
20	2,6,11-Trimethyl-dodecane	$C_{15}H_{32}$	31295–56-4	17.381	1.05 ± 0.09^a	/	/
Phenols (3)					**7.52**	**12.67**	
21	4-Ethyl-phenol	$C_8H_{10}O$	123–07-09	14.41	/	6.35 ± 0.32^a	smoky
22	Ethyl maltol	$C_7H_8O_3$	490–11-8	15.298	6.42 ± 0.26^a	6.32 ± 0.15^a	caramel
23	2,4-Di-tert-butyl-phenol	$C_{14}H_{22}O$	96–76-4	23.524	1.10 ± 0.18^a	/	/
Ketone (3)					**11.19**	**5.77**	
24	4-Propyl-1,3-cyclohexanedione	$C_9H_{14}O_2$	18456–81-0	13.675	10.39 ± 0.37^a	/	/
25	Dihydro-5-pentyl-2(3 h)-furanone	$C_9H_{16}O_2$	104–61-0	19.683	0.80 ± 0.13^a	/	coconut
26	2-Chloro-1-(2,4-dichlorophenyl)-ethanone	$C_5H_5Cl_3O$	4252–78-2	20.516	/	5.77 ± 0.30^a	/
Ester (7)					**6.72**	**54.71**	
27	Dibutyl phthalate	$C_{16}H_{22}O_4$	84–74-2	8.723	/	1.42 ± 0.11^a	odorless
28	Hexadecanoic acid ethyl ester	$C_{18}H_{36}O_2$	628–97-7	9.237	/	1.73 ± 0.08^a	Waxy
29	2,4-Hexadienoic acid ethyl ester	$C_8H_{16}O_2$	123–66-0	12.21	0.83 ± 0.12^a	/	apple, banana, and pineapple aromas
30	9-Octadecenoic acid (Z)- methyl ester	$C_{19}H_{36}O_2$	112–62-9	12.347	/	1.26 ± 0.08^a	/
31	Diisooctyl phthalate	$C_{24}H_{38}O_4$	27554–26-3	31.381	/	1.49 ± 0.07^a	/
32	l-(+)-Ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$	4218–81-9	33.684	5.89 ± 0.21^b	47.07 ± 0.17^a	/
33	Hexadecanoic acid octyl ester	$C_{24}H_{48}O_2$	16958–85-3	34.417	/	1.74 ± 0.17^a	/
Others (2)					**12.11**	**4.37**	
34	Methoxy-phenyl-oxime	$C_8H_9NO_2$	67160–14-9	7.064	11.62 ± 0.38^a	4.37 ± 0.06^b	/
35	5-(4-Nitrophenoxymethyl)- furane-2-carboxaldehyde	$C_{12}H_9NO_5$	438221–55-7	14.717	0.49 ± 0.17^a	/	/

Note: "/" is not found; there replications were made. RT is retention time (min). Mean values in the same row with different letters are significantly different (p < 0.05). MS, identification based on the NIST14. L.

in the distribution of the sample blocks of volatile compounds of kvass from different raw materials.

There were many kinds of key volatiles in RBK, among which l-(+)-Ascorbic acid 2,6-dihexadecanoate, nonanal, 4-ethyl-phenol, and so on had relatively high content. Jiefang and Zhezhi (2006) found that l-(+)-Ascorbic acid 2,6-dihexadecanoate was the main component (relative content of 6.27 %) in the antitumor medicinal plant Rhodiola rosea in small clusters, and this substance might be a key component in RBK,

and in-depth study of this component may provide new ideas for revealing the health care mechanism of RBK. Li-Yun Chen et al. also detected the substance Nonanal in their study of volatile compounds in bean paste, and the relative contents were all around 3 %. Nonanal is an active flavor compound produced by lipid oxidation, with a strong oil taste and sweet orange odor, which has an important impact on the flavor quality of food (Kuroda, Furusho, Maeba, & Takashio, 2003). 4-ethyl-phenol has a strong woody, rouge, smoky aroma and with a
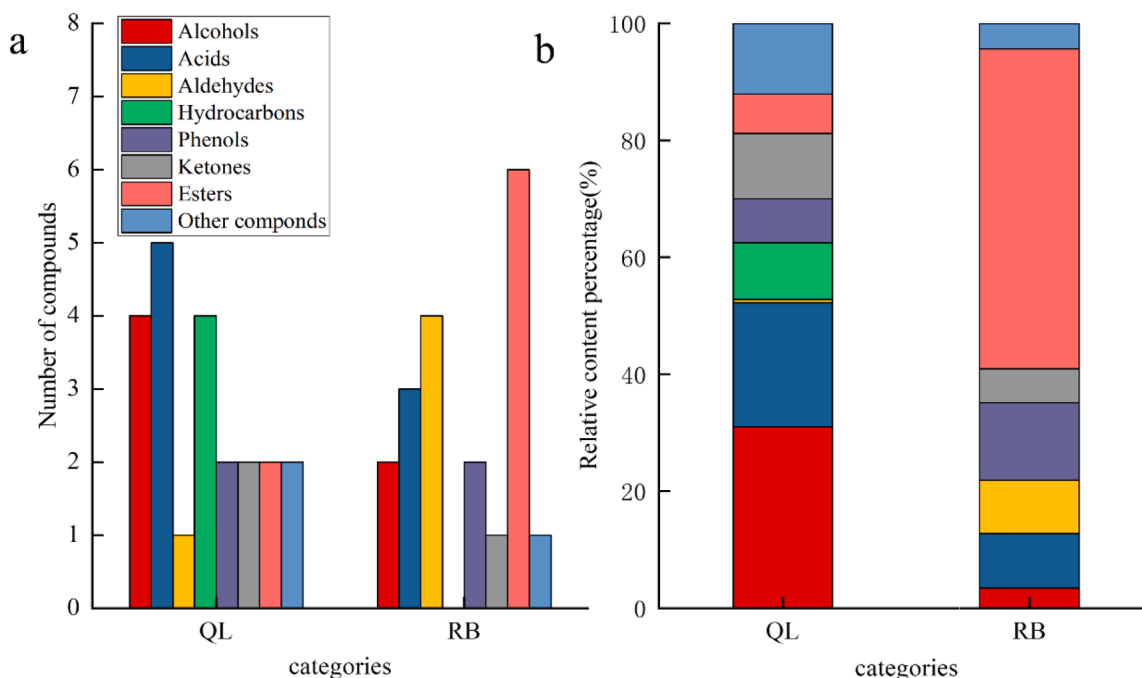
Fig. 3. Volatile compounds identified by HS-SPME/GC–MS about two kinds of kvass. (a) Number of compounds; (b) Relative percentage of content.
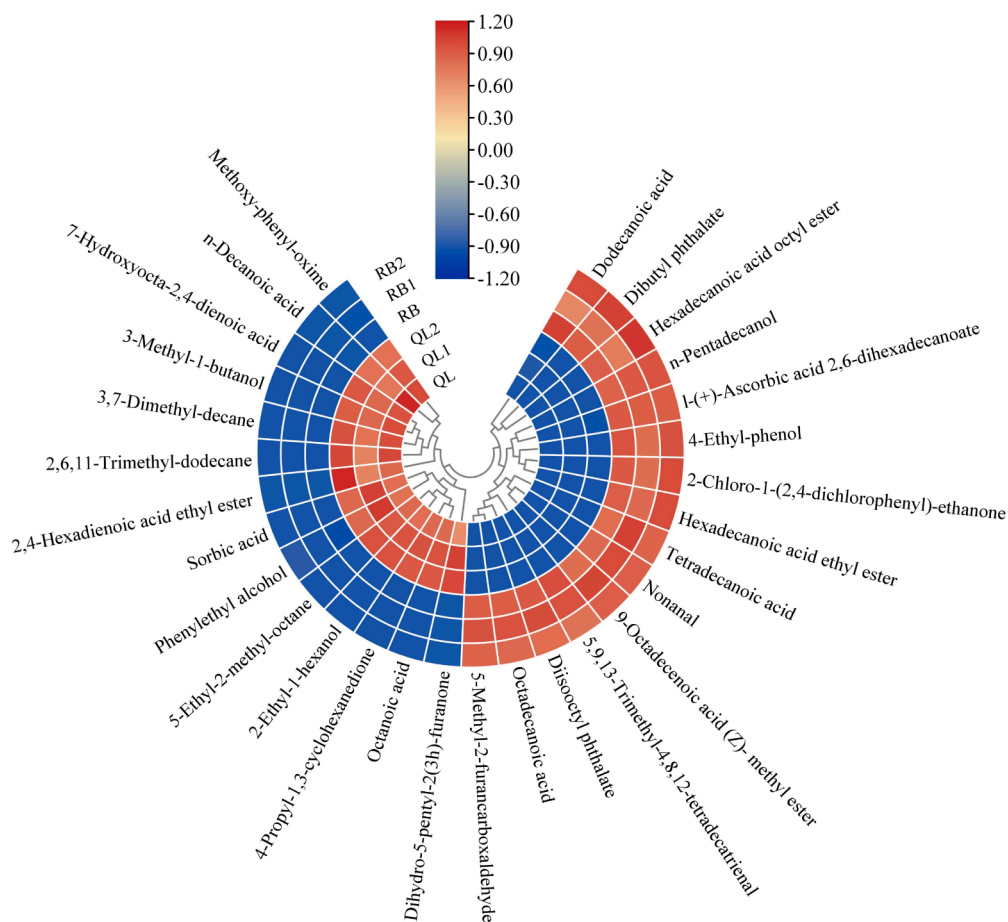

Fig. 4. Thermogram of the differential aroma composition of the two kinds of kvass.

slightly sweet aroma. Tetradecanoic acid has a burnt, cheese, harsh, oil flavor. Octadecanoic acid has dairy flavor. The above volatile compounds gave RBK a kind of fruity and caramel flavor.

The relative content of 3-methyl-1-butanol, phenylethyl alcohol, 2,4-Hexadienoic acid ethyl ester, and octanoic acid were high in the key volatiles of QLK. Hao Yaofei et al. investigated the volatile compounds in fermented Yamalashi rice wine and likewise found phenylethyl alcohol to be the key volatile in rice wine, which has a floral aroma and sweet flavour (Hao et al., 2023). Its source has two pathways: one is from raw materials and the other is from yeast fermentation, i.e., L-phenylalanine is produced by the action of deaminase, decarboxylase, and reductase in the brewer's yeast, which can be esterified with acids during fermentation and maturation (Hongmei et al., 2019), resulting in a more harmonized and balanced QLK aroma (Ebeler & Thorngate, 2009). 3-Methyl-1-butanol in QLK, which is the main component of higher alcohols, has a bitter almond flavor and is the main aroma component of Italian Grappa (Chen et al., 2023) and has also been detected in passion fruit wines (Ye, Zhang, Hao, Lin, & Bao, 2023). Esters usually provide pleasant flavors to food products, and Cynthia Almaguer et al. similarly detected 2,4-hexadienoic acid ethyl ester in barley and rye aroma profiles, which, as a characteristic volatile compound of QLK, contributes to fruit flavors such as apple, banana, and pineapple aromas in QLK (Almaguer, Kollmannsberger, Gastl, & Becker, 2023).The above volatile compounds give QLK its fruity and floral flavor.

3.4. OPLS-DA of volatile compounds in different kinds of kvass

The aroma compounds and relative contents of two kvass samples were analyzed and identified using HS-SPME-GC–MS to study the aroma characteristics of kvass made from different raw materials. The OPLS-DA score plot clearly distinguished the different samples (Fig. 5a), and the two kinds of kvass were well separated from each other with no overlap, which suggested significant differences in volatile compounds among two kinds of kvass. The model was automatically fitted $1 + 1 + 0$. The fit

index for the independent variable (R_x^2) in this analysis was 0.987, the fit index for the dependent variable (R_y^2) was 1, and the model predictive index (Q^2) was 0.999, indicating that the model fit was extremely good (Yun et al., 2021). After 200 replacement tests, as shown in Fig. 5b, R2 = 0.374 and $Q^2 = -0.951$ obtained by the replacement test were both lower than the relevant initial values, and the intercept of the Q^2 regression lines was less than 0, indicating that there was no overfitting in the model and the model validation was effective (Ye et al., 2022). The results could be used for the flavor discrimination analysis of QLK and RBK.

In addition, the VIP values are usually obtained by OPLS-DA methods, which reflect the degree of contribution of each compound to the variables (Chen, Wang, Su, Mu, & Tian, 2023), and the magnitude of the VIP values of the volatile compounds content was ranked and Fig. 5c was drawn, in which, 29 flavor compounds with large contributions were screened and the red areas correspond to the key flavor compounds with large contributions (Xie et al., 2023). Here, we considered that the variables with VIP greater than 1 ($p < 0.05$) had a significant effect on the discrimination of kvass samples (Jiang et al., 2023), among which there were 4 kinds of alcohols, 7 kinds of acids, 3 kinds of aldehydes, 3 kinds of hydrocarbons, 2 kinds of phenols, 3 kinds of ketones, 6 kinds of esters, and 1 kinds of others. The results of OPLS-DA model were also in agreement with those of the heatmap, indicating that the former had good adaptability and predictability.

3.5. Changes in free amino acid before and after RBK fermentation

Amino acids are known as the source of all life. During fermentation, microorganisms can produce proteases that break down large proteins into peptides and amino acids (Aguirre, Garro, & de Gioria, 2008; Christensen, Garcia-Bejar, Heiner Bang-Berthelsen, & Hansen, 2022). As shown in Table 2, a total of 14 free amino acids (6 essential amino acids, 2 semi-essential amino acids, and 6 non-essential amino acids) and γ-aminobutyric acid were detected in the broth before and after
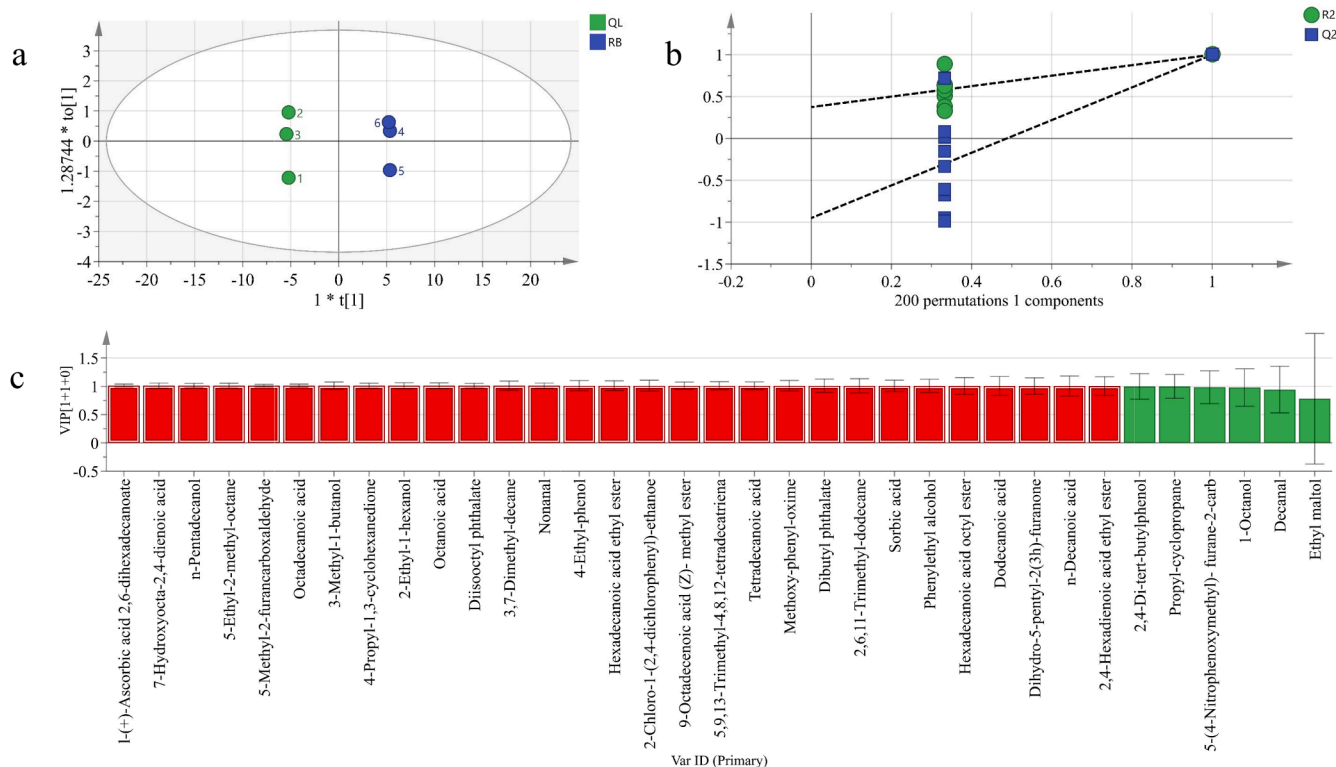

Fig. 5. Analysis of OPLS-DA for volatile compounds of different kinds of kvass. (a) Scores plot, (b) model cross-validation results, (c) VIP predictions.

Table 2
Free amino acid content before and after RBK fermentation.

NO.	Amino acid name	Flavor amino acids	Amino acid content (mg/100 mL)	
			Pre-fermentative	After fermentation
1	Serine	+	6.51 ± 0.28^a	3.72 ± 0.15^b
2	Glutamic	+	48.55 ± 0.02^a	2.07 ± 0.08^b
3	Glutamine	+	5.24 ± 0.19^b	14.94 ± 0.11^a
4	Glycine	+	2.71 ± 0.28^a	28.74 ± 0.18^a
5	Alanine	+	11.81 ± 0.12^a	9.69 ± 0.27^b
6	Cysteine		0.38 ± 0.09^a	/
Total Non-Essential Amino Acids			75.19	59.17
1	Arginine	–	7.71 ± 0.16^a	0.93 ± 0.14^b
2	Histidine	–	1.98 ± 0.20^a	/
Total semi-essential amino acids			9.69	0.93
1	Lysine	–	2.34 ± 0.09^a	1.37 ± 0.04^b
2	Phenylalanine	–	/	1.39 ± 0.12^a
3	Isoleucine	–	/	0.37 ± 0.10^a
4	Leucine	–	/	1.93 ± 0.06^a
5	Valine	+	0.40 ± 0.03^b	1.95 ± 0.04^a
6	Threonine	+	1.05 ± 0.06^b	2.11 ± 0.12^a
Total essential amino acids			3.79	9.12
1	GABA		6.67 ± 0.13^a	3.80 ± 0.04^a
Total free amino acids			88.67	69.22
Total sweet amino acids			27.71	64.47
Total umami Amino Acids			50.89	3.45
Total bitter amino acids			9.69	4.62

Note: Sweet (umami) amino acids are indicated by + and bitter amino acids are indicated by -; "/" in the amino acid content indicates that it is not detected; lowercase letters a, and b indicate the significant difference of the same amino acid before and after fermentation.

fermentation. Compared with the amino acid results of kvass made of bread as an ingredient, which were determined by Zapata Flores et al. (2023), an abundance of glycine in RBK was higher, but proline, tyrosine, and aspartic acid were not detected. The total content of non-essential and semi-essential amino acids in the rice bran broth decreased by fermentation, and the content of essential amino acid increased (Table 2). This change was in agreement with the conclusion that changes in free amino acid content were derived from fermented sweet potato starch processing slurry (Ye, Wang, Zhan, Tian, & Liu, 2023). It has been reported that after fermentation, the content of most free amino acids in honeydew juice was significantly reduced or undetectable (Chong, Zhen, & Tao, 2018), which might be due to the degradation of amino acids by the decarboxylation, deamination and dehydroxylation reactions caused by continuous high temperature during the sterilization process. Moreover, during the fermentation process lactic acid bacteria not only consumed the amino acids to provide nutrients for their growth, but also converted the amino acids into volatile flavor substances (alcohols, aldehydes, acids, and esters in low carbon number), which might reduce the amino acid content (Smid & Kleerebezem, 2014).

Amino acids are important substances for sustaining the life activities of the human body, which not only have a variety of physiological functions, but also most of the amino acids play a role in flavor presentation, with a sweet, bitter, or umami (Gu et al., 2021).The free amino acids can be classified into the following groups according to different taste: umami amino acids (Glutamic acid, Aspartic acid), sweet amino acids (Threonine, Serine, Glycine, Alanine, Proline, Lysine, Glutamine), bitter amino acids (Tyrosine, Tryptophan, Leucine, Phenylalanine, Arginine, Methionine, Histidine, Valine, Isoleucine) and so on (Li, Luo, et al., 2023; Gao et al., 2019). Umami amino acids were considered to be the basic flavor represented by Glu, which was 48.55 mg/100 mL in the rice bran saccharified broth. The content was significantly reduced after fermentation ($p < 0.05$), probably due to the conversion of glutamic acid to glutamine by glutamine synthetase during the fermentation process (Sun et al., 2023). It is worth mentioning that glutamine, slightly sweet, has the function of regulating blood glucose and nutritional metabolism (Cruzat, Rogero, Keane, Curi, &

Newsholme, 2018). The total sweet amino acids were 27.71 mg/100 mL and 64.47 mg/100 mL before and after fermentation, respectively, and it was seen to a significant increase after fermentation. This indicated that during fermentation, *Lactobacillus bulgaricus* and *Saccharomyces cerevisiae* efficiently degraded rice bran protein using their protease system, not only to decompose it into small molecule nitrogen source required for their growth, but also to increase the content of free amino acids for sweetness. The total amount of bitter amino acids in fermented RBK was reduced to 4.62 mg/100 mL, and these bitter amino acids could also be masked by sweet amino acids so that bitter amino acids would not adversely affect the overall flavor of RBK.

Conclusion

In this paper, the volatiles of two kinds of kvass (RBK and QLK) were analyzed by E-nose and GC–MS, and 35 volatile compounds of 8 categories were detected, including alcohols, acids, phenols, esters, aldehydes, ketones, alkanes, and others. The volatile constituents of the two kinds of kvass were similar in terms of compound category, but there were the significant differences in content ($p < 0.05$), and both appeared distinct raw materials characteristics. The ester content of RBK was as high as 54.71 mg/mL, which was the main aroma component, while the alcohol content of QLK was the highest. OPLS-DA results suggested that the volatile components of the two kinds of kvass were well separated from each other, and 29 volatile components were selected as the characteristic volatile components with VIP > 1. After fermentation, the total free amino acids decreased from 88.67 mg/100 mL in the rice bran saccharified broth to 69.22 mg/100 mL in RBK, but the contents of essential amino acids and the sweet amino acids in RBK increased significantly. In conclusion, the flavor of RBK prepared by fermentation was similar to that of QLK, but with the characteristic of caramelized aroma and richness. This paper provides basic data for the development of a new type of RBK and lays a preliminary foundation for further scale development of RBK.

CRediT authorship contribution statement

Xiaochen Yu: Data curation, Investigation, Validation, Writing – original draft. **Wenjuan Zhang:** Data curation, Visualization. **Liying Xin:** Data curation, Investigation, Validation. **Su Xu:** Conceptualization, Methodology, Writing – review & editing. **Jianjun Cheng:** Funding acquisition, 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

I have shared the link to my date at the Attach File step.

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