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ARTICLE

Characterization of Aroma Compounds in Chinese Rice Wine Qu by Solvent-Assisted Flavor Evaporation and Headspace Solid-Phase Microextraction

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The aroma of Chinese rice wine Qu is one of the most important factors that influences the flavor of Chinese rice wine. To better understand the aroma of Qu, aroma compounds in four wheat Qus and two xiao Qus were identified by chromatography−olfactometry (GC-O) after solvent extraction followed by solvent-assisted flavor evaporation (SAFE). A total of 39 aroma compounds were characterized by GC-O. On the basis of aroma intensity, 1-hexanal, ethyl hexanoate, 1-octen-3-ol, and phenylacetaldehyde were found to be the most important aroma compounds in all six Qus. In addition, 3-methylbutanol and 2-phenylethanol also played an important role in the aroma of two xiao Qus. Headspace solid-phase microextraction (HS-SPME) was used for quantifying aroma compounds identified in the Qus. The method enabled limits of detection and quantification of <40.8 and <136.0 μg/L, respectively. Linearity and recovery were satisfied in all cases. Quantitative analysis revealed that volatiles of six Qus had a wide range of concentration. Principal component analysis applied to the data differentiated the six Qus well.

KEYWORDS: Aroma compounds; Chinese rice wine Qu; wheat Qu; xiao Qu; SAFE; GC-O; HS-SPME; quantification; PCA

INTRODUCTION

Chinese rice wine is a popular traditional alcoholic beverage with a long history in China and is typically fermented from rice with Qu and yeast. During Chinese rice wine making, the rice is first cooked with steam and then mixed with Qu and yeast. The mixture is fermented for 20−25 days. After fermentation, the rice wine mash is filtered with a presser, and then the fresh rice wine is heated with steam and aged in sealed pottery jars for at least one year (*1*).

In Chinese rice wine brewing, Qu is a source of microorganisms and crude enzymes, serves as a portion of materials, and provides flavor substances for Chinese rice wine (*1*−*3*). Qu is a molded cereal, which is prepared by natural inoculation of molds, bacteria, and yeasts and their growth on the grains (*2*). During Qu making, the raw materials of Qu are typically milled, mixed with water, and pressed into molds of different sizes. The Qu is then incubated under nonsterile conditions (*1*, *3*). As a result of fermentation, Qu is rich in a wide variety of microorganisms such as *Aspergillus oryzae*, *Rhizopus oryzae*, and *Rhizopus microsporus* (*2*, *3*) and various enzymes including amylases, glucoamylase, proteases, and phosphatase (*3*). Many substances containing amino acids and carbohydrates also accumulate in the Qu during fermentation (*3*). Therefore, as a source of microorganisms and crude enzymes, as well as a portion of brewing materials, Qu is important in the production of Chinese rice wine. On the other hand, Qu also plays a key role in the aroma of Chinese rice wine. It has been reported that when commercial enzymes were used instead of Qu for Chinese rice wine fermentation, the aroma of the fresh rice wine was significantly different from that of traditional Chinese rice wine (*4*). This indicates that the aroma of Chinese rice wine is mainly contributed by the aroma from Qu during Chinese rice wine brewing; without it, the rice wine loses its characteristic aroma and flavor. However, to date, Chinese rice wine's aroma compounds have not yet been characterized, and its aroma is subjectively defined as "the odor of Qu". Up to now, almost all of the studies have focused on enzymes and microorganisms of Qu (*2*, *5*), but the aroma of Qu has not yet been documented, and the aroma of Qu is evaluated only by its simple sensory description with the term "normal or no odor of Qu". It is the aroma compounds of Qu, however, that are the most important factors influencing the aroma of Chinese rice wine (*3*), and for this reason it is important to characterize the aroma compounds of Qu.

Qus can be classified into three categories on the basis of their raw materials: "xiao Qu", "hong Qu", and "wheat Qu". They are made from rice, red rice, and wheat, respectively. Among them, wheat Qu is the most widely used culture in Chinese rice wine, followed by xiao Qu and hong Qu. Because of differences in manufacturing practices, the aroma profiles of various Qus are quite different.

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Using a gas chromatography−olfactometry (GC-O) technique, Takahashi and co-workers (*6*) have identified more than 10 odor-active compounds in Japanese sake koji. The results showed that sake koji aroma was mainly contributed by 1-octen-3-one, 1-octen-3-ol, methional, 2-methyl-2-hepten-6-one, and phenylacetaldehyde. Japanese sake koji is made from steamed rice cultivated with *A. oryzae*. Chinese rice wine Qu, which is made from grains by a spontaneous fermentation containing molds, bacteria, and yeasts, is similar to the sake koji, but is somewhat wider in scope, because it includes many microorganisms. However, the aroma compounds of Chinese rice wine Qu have not yet been investigated.

Many extraction techniques had been used in the analysis of volatiles, such as solid-phase extraction (SPE) (*7*), purge and trap (P&T) (*8*), and simultaneous distillation−extraction (SDE) (*9*). Solvent-assisted flavor evaporation (SAFE) is a good technique for volatile extraction, and it can allow careful isolation of volatile compounds from complex matrices (*10*). High recovery has been achieved even for high-boiling-point compounds. Solid-phase microextraction (SPME) is another good technique for the extraction of volatiles. The main advantages of this method are simplicity, high sensitivity, and small sample volume, and it can also extract a wide range of aroma compounds (*11*−*13*). The SPME method has been employed for many diverse disciplines including the analysis of aroma compounds in mushroom (*14*) and volatiles from apple cider (*12*), orujo spirits (*15*), Chinese liquor (*11*), and Chinese rice wine (*1*).

The objectives of the present work were (1) to identify the aroma compounds in Chinese rice wine Qu by using the SAFE technique and GC-O, (2) to set up a method for quantifying the aroma compounds identified by means of HS-SPME followed by GC-MS, and (3) to investigate potential differences in aroma compounds among six Qus on the basis of the concentrations of aroma compounds.

MATERIALS AND METHODS

Chemicals. Ethanol (special grade reagent) was obtained from Hanbon Science and Technology Co., Ltd. (Jiangsu, China). 1-Propanol (≥99.0%), 2-methylpropanol (99.5%), 3-methylbutanol (99.0%), 1-pentanol (99.0%), 1-hexanol (99.5%), 1-octanol (99.0%), 1-octen-3-ol (98.0%), benzenemethanol (≥99.0%), 2-phenylethanol (≥99.0%), 2-heptanone (98.0%), 2-octanone (98.0%), 1-octen-3-one (50.0 WL% in 1-octen-3-ol), 2-furancarboxaldehyde (furfural) (≥99.0%), 1-hexanal (98.0%), nonanal (95.0%), benzaldehyde (99.0%), phenylacetaldehyde (90.0%), ethyl acetate (≥99.5%), ethyl hexanoate (98.0%), ethyl octanoate (98.0%), ethyl benzoate (99.0%), acetic acid (98.0%), hexanoic acid (99.0%), heptanoic acid (97.0%), octanoic acid (98.0%), nonanoic acid (≥99.0%), guaiacol (2-methoxyphenol) (99.0%), 4-vinylguaiacol (4-vinyl-2-methoxyphenol) (98.0%), *p*-cresol (4-methylphenol) (99.0%), 4-ethylguaiacol (4-ethyl-2-methoxyphenol) (≥98.0%), 2,3-dimethylpyrazine (95.0%), 2,3,5-trimethylpyrazine (99.0%), 2,3,5,6-tetramethylpyrazine (98.0%), γ-nonalactone (98.0%), benzothiazole (96.0%), 2-octanol (96.0%), and 4-(4-methoxyphenyl)-2-butanone (98.0%) were purchased from Sigma-Aldrich (Shanghai, China). Analytical grade sodium chloride, anhydrous sodium sulfate, calcium chloride, diethyl ether, pentane, and absolute ethanol were purchased from China National Pharmaceutical Group Corp. (Shanghai, China). Milli-Q water was obtained from a Milli-Q purification system (Millipore, Bedford, MA).

Chinese Rice Wine Qu Samples. Six samples were gifted by various Chinese rice wine manufacturers: four wheat Qus, including Gunanfeng qiu Qu (GNFQ), Jinfeng sheng Qu (JFSQ), Jiashan sheng Qu (JSSQ), and Wuzhanmao sheng Qu (WZMSQ), and two xiao Qus, including Zhangjiagang gan xing (ZJGGX) and Zhangjiagang tian xing (ZJGTX). All of these Qus were made in 2006.

Identification of Aroma Compounds from Qu. *Ultrasound-Assisted Extraction (UAE).* Qu was ground and stored at 4 °C before analysis. The ground Qu (10 g) was weighed into a 50 mL centrifuge tube with a PTFE-lined screw cap, and 0.1 g of calcium chloride was added to inhibit enzyme activity (*16*). The mixture was soaked with 5 mL of 50% ethanol by volume, and then 15 mL of 50% ethanol was added. The paste was homogenized using a glass stick. The centrifuge tube was sealed with a cap and dipped into an ultrasound cleaning bath (AS2060B, 60 W, China) by the mode of indirect sonication, at the power of 60 W. The temperature of the sonicated bath was 25 ± 3 °C for 30 min. After sonication, the mixture was centrifuged at 7690*g* for 10 min at 4 °C, and the supernatant liquid was then filtered. After two repetitions under the same conditions, the filtrates were combined.

Aroma Extraction and SAFE. The filtrates were diluted with deodorized water (deionized water was boiled for 10 min and then cooled to room temperature) to adjust their alcoholic degree to 10% ethanol by volume and transferred into a separatory funnel. The diluted sample was saturated with NaCl and extracted three times with 50 mL of freshly distilled diethyl ether/pentane (2:1 v/v). All extracts were combined and washed with 30 mL of deodorized water. Volatiles from the organic phase were isolated using the SAFE unit (Glasbläserei Bahr, Manching, Germany) at 40 °C under vacuum (10^{-3} Pa) according to the method proposed by Engel et al. (*10*), and the distillate was dried over anhydrous sodium sulfate overnight and concentrated under a gentle stream of N_2 to 250 μL. The concentrated Qu extract was analyzed by GC-O on a gas chromatography−mass spectrometry (GC-MS) instrument.

GC-MS and GC-O Analysis. Identification of the concentrated Qu extract was carried out using an Agilent 6890 GC−5973 mass selective detector (MSD). The columns were DB-Wax and DB-5 (each 30 m × 0.32 mm i.d., 0.25 μm film thickness; J&W Scientific, Folsom, CA). Helium at a constant flow rate of 2 mL/min was used as carrier gas. Each concentrated sample was injected in splitless mode. Injector and detector temperatures were both kept at 250 °C. The oven temperature was held at 50 °C for 2 min, raised at 10 °C/min to 160 °C, then raised to 230 °C at a rate of 5 °C/min, and held at 230 °C for 35 min. MS was taken at 70 eV, and the ion source temperature was set at 230 °C. Half of the eluate was directed to the MSD, whereas the other half was directed to the sniffing port. Three well-trained panelists performed the GC-O analysis. All panelists were trained on a "flavor language" in several sessions, in which pure reference odorants were evaluated according to the method described by Steinhaus et al. (*17*). Both the retention time and odor qualities were recorded. The perceived aroma intensity was measured by using a 5-point scale ranging from 1 to 5: 1 = very weak, 2 = weak, 3 = moderate, 4 = strong, and 5 = very strong. Each concentrated sample was sniffed twice by each panelist. The aroma intensity values were averaged for all six analyses (three panelists, twice). Unknown compounds were identified by comparison with standard mass spectra in the NIST05a.L database (Agilent Technologies Inc.). Retention indices (RIs) of unknown compounds were calculated by the retention time of a series of alkanes (C_5−C_{30}). Positive identification was carried out by comparing mass spectra, aromas, and RIs of the authentic standards. Tentative identification was performed by comparing aroma or mass spectra only.

Quantitative Analysis of Aroma Compounds. *SPME-GC-MS Analysis.* Quantitative analysis of aroma compounds was carried out using HS-SPME coupled with GC-MS. The ground Qu (10 g) was blended with 0.1 g of calcium chloride and 5 mL of Milli-Q water. The mixture was extracted three times with 15 mL of Milli-Q water by UAE and centrifuged at 7690*g* for 10 min at 4 °C. The conditions of UAE were identical to those of identification of aroma compounds from Qu analysis, described previously. After sonication and centrifugation, the supernatants were combined. The HS-SPME experimental parameters such as fiber coatings extraction temperature and time were evaluated. The number of tentatively identified compounds and the total peak areas were used to assess the effects of these experimental factors on the extraction efficiency of volatile compounds. The optimization of the HS-SPME method of extraction was done with an aliquot of 8 mL of sample of JFSQ. Operating conditions were optimized at different adsorption temperatures (35, 40, 50, and 60 °C) and times (15, 30, 45, and 60 min). Three SPME fiber coatings were tested and used: 100 μm poly(dimethylsiloxane) (PDMS), 75 μm Carboxen/PDMS (CAR/PDMS), and 50/30 μm divinylbenzene/CAR/PDMS (DVB/CAR/PDMS). All fibers were obtained from Supelco (Bellefonte, PA) and were conditioned by keeping them in the GC injector following the manufacturer's instructions before use, and then each fiber was exposed to the headspace of a 25 mL septum-sealed glass vial

containing an 8 mL aliquot of Qu, 15 μL of internal standard (IS) solution (the mixture of 4-(4-methoxyphenyl)-2-butanone (4M2B) and 2-octanol (2OL), 155.52 and 64.55 mg/L, respectively, in ethanol), and 2.5 g of NaCl. The vial was stirred and left to equilibrate for 15 min and extract for 30 min at 50 °C. After extraction, the fiber was introduced into the injection port of the GC-MS system (at 250 °C for 5 min), and the analytes extracted by the fiber were thermally desorbed. The GC-MS conditions were similar to those of GC-MS and GC-O analyse, described previously, except for column and program temperature. The column was a DB-FFAP from J&W Scientific (60 m × 0.25 mm i.d., 0.25 μm film thickness). The oven temperature was held at 50 °C for 2 min, then raised to 230 °C at a rate of 6 °C/min, and held at 230 °C for 15 min.

Calibration of Standard Curves. Synthetic Qu solution was prepared with Milli-Q water, and the pH was adjusted to 6.3 with lactic acid. Each standard compound was accurately weighed and dissolved in absolute ethanol to prepare a standard solution. Then, exact volumes of each standard solution were taken and mixed to give a stock solution, which was dissolved in ethanol at a concentration 3 orders of magnitude higher than typically found in the Qus. These solutions were further diluted to appropriate levels in the synthetic Qu solution to prepare the calibration plots. A total of 8 mL of synthetic Qu solution containing different concentrations of volatile standards, 2.5 g of NaCl, and 15 μL of the mixture of 4-(4-methoxyphenyl)-2-butanone and 2-octanol (IS) was placed in a 25 mL SPME glass vial, which was tightly capped and put in an automatic headspace sampling system. The conditions of HS-SPME and GC-MS were set as described previously. To qualify and quantify the aroma compounds by GC-MS, MS analysis was performed in the selected ion monitoring (SIM) mode using their characteristic m/z values. The selected ion for 4-(4-methoxyphenyl)-2-butanone was m/z 121 and that for 2-octanol was m/z 45. The standard curves for individual compounds were built up by plotting the response ratio of target compound and IS against the concentration ratio. The limits of quantification (LOQs) and detection (LODs) were estimated as the concentration of the analyte of a standard that produced a signal-to-noise ratio of 10 and 3 times, respectively. The linear range experiments provide the necessary information to calculate LOD by extrapolating from the lowest concentration point on the linear calibration curve.

Quantification of Samples and Calculation of Recovery. Quantitative data were acquired by the interpolation of relative peak areas in the calibration curves constructed by the analysis of standard solutions containing known amounts of the analytes. Known amounts of each pure standard were evaluated in the synthetic Qu and JFSQ solutions. For each aroma compound the recovery rates in these samples were determined by the ratio $(C_1 - C_0/C_2) \times 100$, where C_0 is the concentration of the detected amount before addition, C_1 is the concentration of the detected amount after addition, and C_2 is the concentration of added amount. The results were expressed as the mean value of three replicates of Qus.

Statistical Analysis. Statistical analysis was performed using SPSS 13.0 for Windows. Data were analyzed by analysis of variance (ANOVA), and Duncan's multiple-range test was used to compare aroma intensity and contents of the aroma compounds from Qus, respectively. Principal component analysis (PCA) was applied to assess differences in aroma compounds among six Qus.

RESULTS AND DISCUSSION

Identification of Aroma Compounds from Chinese Rice Wine Qu. Aroma compounds were identified using the SAFE technique coupled with GC-O and GC-MS. A total of 39 aroma compounds were detected (**Table 1**). Of them, 35 compounds including 8 carbonyl compounds, 9 alcohols, 4 esters, 5 organic acids, 4 phenols, 1 lactone, 3 pyrazines, and 1 sulfur-containing compound were identified by mass spectra, aroma descriptors, and Kovats indices of a pure standard. Most of them had been detected in Chinese rice wine (*1*).

Table 1 also summarizes the results from the olfactometric study performed in this work. The GC-O data were expressed as the average odor intensity scores given by the panel for each compound. In the GC-O experiment, a total of 33, 32, 22, 24, 28, and 27 odorants were detected in JFSQ, GNFQ, JSSQ, WZMSQ, ZJGGX, and ZJGTX, respectively. On the basis of aroma intensity, 15, 13, 11, 12, 15, and 14 odorants were found to be at least intermediate, with intensity values ranging from 3 to 5 units for JFSQ, GNFQ, JSSQ, WZMSQ, ZJGGX, and ZJGTX, respectively. Among them, 10 odorants with an aroma intensity score of ≥4 were considered to be the potentially significant contributors. These compounds include 1-hexanal, ethyl hexanoate, 1-octen-3-one, 1-octen-3-ol, phenylacetaldehyde, hexanoic acid, 3-methylbutanol, guaiacol, 2-phenylethanol, and 4-vinylguaiacol. Of them, ethyl hexanoate, phenylacetaldehyde, 3-methylbutanol, and 2-phenylethanol, which were also detected in Chinese rice wine (*1*), could contribute to the final aroma of Chinese rice wine. The aroma intensity scores are displayed in **Table 1**. As can be seen, 1-hexanal, ethyl hexanoate, 1-octen-3-ol, and phenylacetaldehyde with strong intensity were the most potent odorants in all of the Qus studied. 4-Vinylguaiacol was also an important odorant in most of the Qus except JSSQ and ZJGTX. In addition, 3-methylbutanol and 2-phenylethanol were significant odorants in the two xiao Qus (ZJGGX, ZJGTX). Takahashi and co-workers reported that phenylacetaldehyde, 1-octen-3-one, and 1-octen-3-ol were the most important odorants in the sake koji (*6*). These three compounds were also detected in the Chinese rice wine Qu. Among them, phenylacetaldehyde and 1-octen-3-ol were also the most important odorants in all of the Qus studied (**Table 1**).

HS-SPME Parameters. A DVB/CAR/PDMS fiber was used to select the optimum extraction temperature and time. Extraction was carried out at 35, 40, 50, and 60 °C for 15, 30, 45, and 60 min. The best results were obtained at 50 °C for 30 min. Once the adsorption temperature and time were fixed, the trapping abilities of three types of fibers (PDMS, CAR/PDMS, and DVB/CAR/PDMS) were compared by the analysis of volatile compounds. The DVB/CAR/PDMS fiber was found to be the most effective for all of the target molecules, whereas the PDMS fiber extracted the fewest compounds. This observation was consistent with the previous results (*11*, *12*); thus, the DVB/CAR/PDMS fiber was selected for the extraction of the aroma compounds in Qu in this study.

Validation of HS-SPME Method. Originally, volatiles from Qu were quantified using the SAFE technique. However, the results showed that not all relative standard deviations (RSDs) for three replicates of samples were satisfied due to its tedious procedure. Then HS-SPME was applied to quantify volatiles of Qu. The results showed that HS-SPME was able to detect all of the volatiles identified by the SAFE technique. The validity of HS-SPME for Qus was checked. As can be seen in **Table 2**, the calibration curves obtained were found to have good linearity within the scope of concentrations studied, with correlation coefficient (R^2) ≥0.99. RSDs for three replicates of samples were generally < 10% except for ethyl benzoate (16.9%). The LODs ranged from 0.03 μg/L for 1-octanol to 40.8 μg/L for ethyl acetate and the LOQs from 0.1 to 136.0 μg/L. The recovery rates were evaluated by the addition of the pure standard to synthetic Qu and JFSQ solutions, respectively (**Table 2**). The range of recoveries of all aroma compounds in the synthetic Qu solution is between 85.6 and 119.8% except for heptanoic acid, octanoic acid, and nonanoic acid, so these three aroma compounds were not discussed in this study, and similar results were obtained in JFSQ.

Analysis of Quantification. Quantitative data of the aroma compounds found in the six Qus are shown in **Table 3**. The data were expressed as the mean value of three replicates of the samples. Thirty-two aroma compounds were detected in the Qus in this study. Of them, a total of 28, 26, 18, 20, 25, and 24 odorants were detected in JFSQ, GNFQ, JSSQ, WZM, ZJGGX,

Table 1. Aroma Compounds from Six Chinese Rice Wine Qus Detected by GC-O on DB-Wax and DB-5 Columns

					aroma intensity ($n = 6$)[d]					
RI_{Wax}	RI_{DB-5}	aroma compound[a]	odor quality[b]	basic of identification[c]	JFSQ	GNFQ	JSSQ	WZMSQ	ZJGGX	ZJGTX
929	606	ethyl acetate	pineapple	MS, aroma, RI	2.7 de	2.5 e	3.0 cd	3.3 bc	3.5 ab	3.7 a
1071		1-propanol	alcoholic, fruity	MS, aroma, RI	ND[e]	0.5 a	0.2 b	0.3 b	ND	ND
1073	797	1-hexanal	apple, green grass	MS, aroma, RI	4.8 a	4.7 a	4.5 ab	4.3 b	4.0 b	4.2 b
1123	618	2-methylpropanol	wine, solvent	MS, aroma, RI	ND	ND	0.2 b	ND	0.3 b	0.7 a
1189	885	2-heptanone	fruity, sweet	MS, aroma, RI	3.3 b	3.7 a	3.8 a	3.5 ab	3.2 b	3.5 ab
1201	771	3-methylbutanol	floral, nail polish	MS, aroma, RI	3.5 c	3.8 bc	3.3 c	3.2 c	4.2 a	4.5 a
1221		unknown	pungent		2.0 b	1.7 c	2.5 a	2.0 b	ND	ND
1229	1012	ethyl hexanoate	fruity, floral, sweet	MS, aroma, RI	4.2 c	4.0 c	4.8 a	4.7 a	4.5 b	4.3 bc
1262		1-pentanol	fruity, balsamic	MS, aroma, RI	2.0 a	2.3 a	1.2 c	1.0 c	1.5 b	1.7 b
1280	990	2-octanone	fruity, green	MS, aroma, RI	3.3 b	2.8 d	3.2 b	3.5 b	3.0 c	3.7 a
1291	983	1-octen-3-one	mushroom	MS, aroma, RI	4.3 a	ND	ND	ND	ND	ND
1344	884	1-hexanol	green, floral	MS, aroma, RI	1.5 bc	1.2 c	0.7 d	0.3 e	1.7 b	2.0 a
1350	920	2,3-dimethylpyrazine	nutty, roasted	MS, aroma, RI	2.0 a	ND	ND	ND	ND	ND
1391	1103	nonanal	green, floral, citrus	MS, aroma, RI	3.2 c	ND	3.5 b	3.8 a	3.3 c	3.7 a
1401	992	2,3,5-trimethylpyrazine	roasted, nutty	MS, aroma, RI	ND	1.8 a	ND	ND	ND	ND
1415	1192	ethyl octanoate	fruity	MS, aroma, RI	3.5 b	3.0 c	3.8 a	3.5 b	3.2 c	3.7 a
1423	980	1-octen-3-ol	mushroom	MS, aroma, RI	4.3 c	4.5 b	4.0 d	4.2 c	4.7 a	4.8 a
1434	653	acetic acid	acidic, vinegar	MS, aroma, RI	2.0 c	2.3 bc	ND	2.5 b	2.8 a	1.7 d
1463	833	2-furancarboxaldehyde	sweet, almond	MS, aroma, RI	1.7 a	ND	ND	ND	ND	ND
1485	1085	2,3,5,6-tetramethylpyrazine	baked	MS, aroma, RI	1.0 a	1.3 a	ND	ND	ND	ND
1502	961	benzaldehyde	fruity, berry	MS, aroma, RI	2.2 d	2.5 c	1.3 e	3.5 a	2.8 b	2.3 d
1548	1070	1-octanol	fruity	MS, aroma, RI	1.7 a	0.7 b	ND	0.8 b	1.5 a	0.5 b
1574		unknown	caramel		3.3 b	1.2 d	2.3 c	2.5 c	3.8 a	3.0 b
1583		unknown	cucumber		2.0 d	3.5 a	2.8 b	2.3 c	2.5 c	3.7 a
1630	1040	phenylacetaldehyde	floral, rose	MS, aroma, RI	5.0 a	4.0 e	4.8 b	4.7 bc	4.3 d	4.5 cd
1643	1180	ethyl benzoate	fruity	MS, aroma, RI	1.2 d	0.5 e	1.8 c	2.0 bc	ND	2.3 a
1839	998	hexanoic acid	sweaty, cheesy	MS, aroma, RI	2.8 c	4.2 a	ND	ND	3.3 b	3.5 b
1848	1091	guaiacol	smoky, spicy	MS, aroma, RI	3.3 b	3.0 b	ND	ND	4.0 a	ND
1861		benzenemethanol	floral	MS, aroma, RI	2.5 a	1.0 d	1.8 bc	1.5 c	1.3 cd	2.2 a
1905	1117	2-phenylethanol	rosy, honey	MS, aroma, RI	3.3 c	3.8 b	3.5 c	ND	4.3 a	4.5 a
1939	1072	heptanoic acid	sweaty	MS, aroma, RI	1.0 b	1.3 a	ND	ND	ND	ND
1986	1226	benzothiazole	rubber	MS, aroma, RI	2.8 a	2.0 d	ND	2.7 a	2.5 bc	2.3 c
2012		unknown	nutty		ND	ND	1.3 c	1.8 a	1.5 bc	1.0 d
2019	1286	4-ethylguaiacol	clove, spicy	MS, aroma, RI	ND	ND	ND	ND	1.7 b	2.0 a
2018	1362	γ-nonalactone	peach, coconut	MS, aroma, RI	3.2 b	3.5 a	ND	ND	2.0 d	2.5 c
2054	1283	octanoic acid	sweaty, cheesy	MS, aroma, RI	2.5 a	1.0 b	ND	ND	ND	ND
2063	1085	*p*-cresol	smoky, animal	MS, aroma, RI	ND	2.0 a	ND	ND	1.5 b	1.0 c
2157	1765	nonanoic acid	fatty	MS, aroma, RI	1.0 b	1.5 a	ND	ND	ND	ND
2183	1313	4-vinylguaiacol	smoky, clove	MS, aroma, RI	4.7 a	4.5 a	ND	4.2 b	4.0 b	ND

[a] Unknown, not identified. [b] Odor quality as perceived at the sniffing port during GC-O. [c] MS, mass spectra (compounds were identified by mass spectra); aroma, compounds were identified by the aroma descriptors; RI, compounds were identified by a comparison to the pure standard. [d] The data correspond to the mean of six times; values followed by the same letter are not significantly different ($p < 0.05$); JFSQ; Jinfeng sheng Qu; GNFQ, Gunanfeng qiu Qu; JSSQ, Jiashan sheng Qu; WZMSQ, Wuzhanmao sheng Qu; ZJGGX, Zhangjiagang gan xing; ZJGTX, Zhangjiagang tian xing. [e] ND, not detected.

and ZJGTX, respectively (**Table 3**). Of these, 13 aroma compounds, which accounted for 40.6% of the total numbers of aroma compounds, including 3-methylbutanol, 1-pentanol, 1-hexanol, 1-octen-3-ol, 2-heptanone, 2-octanone, 1-hexanal, ethyl acetate, ethyl hexanoate, ethyl octanoate, benzaldehyde, phenylacetaldehyde, and benzenemethanol, were detected and identified in all of the Qus. 2,3,5-Trimethylpyrazine was detected only in GNFQ, 1-octen-3-one, 2,3-dimethylpyrazine, 2-furancarboxaldehyde were detected only in JFSQ, and 4-ethylguaiacol was detected only in the xiao Qus.

The different flavor styles of Qu had different amounts of aroma compounds. Among Qus, ZJGGX had the highest concentration of aroma compounds, which is 48775.9 μg/kg, whereas the lowest concentration of aroma compounds was detected in WZMSQ, only 17719.1 μg/kg. The ZJGTX, JFSQ, GNFQ, and JSSQ Qus also had higher total concentrations of aroma compounds, which were 43369.4, 27798.6, 41332.1, and 19681.1 μg/kg, respectively (**Table 3**). These results indicated that concentrations of aroma compounds of xiao Qu (ZJGGX and ZJGTX) were significantly higher than those of wheat Qu.

Alcohols were the major quantitative components of rice wine Qu. As can be seen in **Table 3**, xiao Qu showed by far the highest content of alcohols. Among them, 3-methylbutanol and 2-phenylethanol were markedly the most abundant alcohols. The contents of these two alcohols in xiao Qu (7044.5−9158.9 and 8269.3−10136.8 μg/kg, respectively) were significantly higher than those in wheat Qu (2855.5−4046.8 and 1143.1−2173.8 μg/kg, respectively), whereas 1-octen-3-ol appeared in low contents (172.8−224.9 μg/kg, respectively) in all of the analyzed Qus. However, it is well-known that it is not the higher contents of volatile compounds occurring in a food that contribute to its aroma; only those with concentrations higher than their odor thresholds can contribute to the aroma (*18*). The odor thresholds of 3-methylbutanol, 2-phenylethanol, and 1-octen-3-ol are 300, 1000, and 1 μg/L in water (*19*, *20*), respectively. Thus, this means that 3-methylbutanol, 2-phenylethanol, and 1-octen-3-ol could contribute to the aroma in some Qus. 3-Methylbutanol and 2-phenylethanol, which had higher concentrations in Chinese rice wine (*1*), could also contribute to the flavor of Chinese rice wine. Alcohols commonly come from lipid oxidation. Cramer

Table 2. Calibration Data of Aroma Compound Standards and Their Recovery in Chinese Rice Wine Qu ($n = 3$)

			calibration parameters								synthetic Qu		JFSQ	
no.	compound	m/z[a]	slope	intercept	linear range (μg/L)	n	R^2	IS[b]	LOQ[c] (μg /L)	LOD[d] (μg /L)	recovery (%)	RSD (%)	recovery (%)	RSD (%)
1	ethyl acetate	43	0.0247	−0.4068	151.8−15633	9	0.9974	2OL	136.0	40.8	102.3	8.4	97.0	1.8
2	1-propanol	31	0.0016	0.0430	45.4−14330	9	0.9973	2OL	43.8	13.1	113.2	4.7	106.5	1.9
3	1-hexanal	44	0.0336	0.0419	3.8−785	11	0.9989	2OL	0.4	0.1	100.9	8.7	101.7	5.3
4	2-methylpropanol	43	0.0039	−0.0517	131.5−16828	11	0.9929	2OL	74.0	22.2	98.9	3.7	91.9	8.5
5	2-heptone	43	0.0755	−0.1286	3.8−786	12	0.9977	2OL	2.2	0.7	119.0	8.3	103.3	1.1
6	3-methylbutanol	55	0.0065	−0.0396	97.7−10257	9	0.9954	2OL	1.4	0.4	107.5	4.1	104.2	4.6
7	ethyl hexanoate	88	0.6746	0.3401	1.4−782	10	0.9934	2OL	0.9	0.3	96.7	4.0	92.1	6.8
8	1-pentanol	42	0.0060	−0.0110	32.6−4172	12	0.9969	2OL	9.0	2.7	93.4	10.0	90.7	5.3
9	2-octanone	43	0.4019	−0.3366	1.7−733	12	0.9982	2OL	0.5	0.2	114.1	5.7	102.2	3.9
10	1-octen-3-one	55	0.1240	−0.3748	8.2−839	12	0.9973	2OL	5.8	1.7	109.3	0.6	90.4	0.7
11	1-hexanol	56	0.0282	−0.0088	1.7−9400	12	0.9995	2OL	0.6	0.2	106.4	3.7	98.4	4.1
12	2,3-dimethylpyrazine	67	0.0058	0.0003	6.4−407	8	0.9979	2OL	2.2	0.7	89.3	3.2	86.3	6.4
13	nonanal	57	0.2650	−0.0405	0.8−153	7	0.9998	2OL	0.7	0.2	89.9	9.4	88.2	6.8
14	2,3,5-trimethylpyrazine	42	0.0145	0.0028	23.8−762	6	0.9923	2OL	7.6	2.3	89.1	4.8	84.2	6.4
15	ethyl octanoate	88	1.6938	−2.0349	1.5−573	9	0.9907	2OL	0.9	0.3	92.8	7.6	93.9	7.8
16	1-octen-3-ol	57	0.1862	−0.1282	0.5−397	8	0.9994	2OL	0.1	0.03	119.8	4.8	103.9	5.8
17	acetic acid	43	0.0089	0.0130	46.3−1845	9	0.9929	2OL	25.3	7.6	87.3	9.5	91.4	4.4
18	2,3,5,6-tetramethylpyrazine	54	0.0294	−0.0036	13.1−838	7	0.9999	2OL	4.4	1.3	117.2	1.4	110.3	5.8
19	benzaldehyde	106	0.0916	0.0039	2.6−1344	10	0.9924	2OL	1.4	0.4	98.2	4.9	87.1	6.0
20	1-octanol	56	0.1848	0.0283	0.2−985	12	0.9989	2OL	0.1	0.03	97.2	8.1	91.4	8.6
21	phenylacetaldehyde	91	0.0372	−0.0207	4.1−207	7	0.9998	2OL	0.8	0.2	109.8	3.1	119.3	8.8
22	ethyl benzoate	105	2.2089	−0.1985	1.2−252	8	0.9986	2OL	0.8	0.2	107.8	16.9	92.0	4.2
23	hexanoic acid	60	0.6281	−0.0010	4.9−1118	8	0.9970	4M2B	1.5	0.5	86.3	3.7	90.3	1.4
24	guaiacol	109	0.3614	0.0067	3.1−196	7	0.9967	4M2B	1.3	0.4	85.6	5.5	87.4	7.0
25	benzenemethanol	107	0.0049	−0.0075	31.7−4060	10	0.9987	2OL	15.4	4.6	109.9	3.9	100.3	2.1
26	2-phenylethanol	91	0.1658	0.1040	18.6−19803	10	0.9970	4M2B	6.3	1.9	99.4	7.4	94.3	5.9
27	benzothiazole	135	0.1785	−0.3673	2.8−866	8	0.9956	2OL	0.6	0.2	88.6	3.6	92.5	7.1
28	4-ethylguaiacol	137	1.2196	−0.0769	4.5−143	8	0.9958	4M2B	2.2	0.7	87.7	6.7	91.4	5.3
29	2-furancarboxaldehyde	96	0.0324	−0.1026	7.7−785	12	0.9970	2OL	3.9	1.2	108.7	3.7	104.9	3.8
30	γ-nonalactone	85	0.5883	0.0413	7.4−785	8	0.9951	4M2B	1.1	0.3	91.1	3.9	95.2	9.1
31	*p*-cresol	107	0.2799	0.0197	4.7−602	9	0.9983	4M2B	3.1	0.9	91.4	4.9	95.8	5.9
32	4-vinylguaiacol	150	0.1537	−0.3057	40.9−41739	9	0.9999	4M2B	5.5	1.7	92.7	8.2	95.1	9.4

[a] m/z, quantifying and qualifying ions. [b] IS, internal standard: 2OL, 2-octanol; 4M2B, 4-(4-methoxyphenyl)-2-butanone. [c] LOQ, limit of quantification. [d] LOD, limit of detection.

et al. reported that the lipid content was about 1.70% in wheat varieties, and mostly unsaturated fatty acids, particularly oleic acid and linoleic acids, were about 61% of the total fatty acids (*21*). In storage rice, many volatile compounds may be derived predominantly via lipid oxidation (*22*). Therefore, it is highly probable that lipid oxidation is a major cause of some alcohols in the rice wine Qus.

Acetate esters were the second quantitative components of Qu. Of them, ethyl acetate was markedly the most abundant ester (7743.5−12487.5 μg/kg), being present at levels higher than its odor threshold (5000 μg/L in water) (*23*); thus, it could contribute to aroma in the Qus. Although ethyl hexanoate and ethyl octanoate are present in small amounts (99.9−131.8 and 221.9−237.8 μg/kg, respectively) in the Qus, they are important aroma compounds because their odor thresholds are 1 and 5 μg/L in water (*24*, *25*), respectively. These three esters including ethyl acetate, ethyl hexanoate, and ethyl octanoate, which were also abundant in Chinese rice wine (*1*), could play an important role in the flavor of Chinese rice wine.

Carbonyl compounds including aldehydes and ketones were detected in the Qus. Among them, 1-hexanal, benzaldehyde, phenylacetaldehyde, 2-heptanone, and 2-octanone were detected in all Qus. Most of these five carbonyl compounds except for benzaldehyde were found at concentrations (289.3−515.7, 14.4−290.6, 19.9−64.8, 346.3−402.4, and 178.2−191.2 μg/kg, respectively) above their odor thresholds (5, 350, 6.3, 140, and 50 μg/L in water (*19*, *20*), respectively). Therefore, 1-hexanal, phenylacetaldehyde, 2-heptanone, and 2-octanone were important aroma compounds in the Qus. Of these, phenylacetaldehyde could influence the flavor of Chinese rice wine because it also presents a concentration in Chinese rice wine (*1*). 1-Octen-3-one, present at a level (610.1 μg/kg) higher than its odor threshold (1 μg/L in water) (*26*), was detected only in JFSQ. 2-Heptanone, 2-octanone, and 1-hexanal were derived from lipid β-oxidation (*22*, *27*), whereas benzaldehyde and phenylacetaldehyde came from microbial catabolism amino acid (*28*).

Fatty acids contributed to acidic, cheesy, fatty, and rancid notes. Although five fatty acids were detected in the Qus, only two were quantified by the SPME; the other three fatty acids were not considered because of their poor recoveries.

Some volatile phenolic compounds including guaiacol, *p*-cresol, 4-ethylguaiacol, and 4-vinylguaiacol have been detected in the Qus. Guaiacol, 4-ethylguaiacol, and 4-vinylguaiacol were responsible for clove, spicy, and smoky odors, whereas *p*-cresol contributed medicinal and animal odors. As can been seen in **Table 3**, 4-vinylguaiacol was the most abundant (1063.7−8495.9 μg/kg) in phenols; it is a potential key odorant in most of the Qus except for JSSQ and ZJGTX because of a low odor threshold (3 μg/L in water) (*19*). 4-Ethylguaiacol was detected only in the xiao Qu, and its content was low (33.9−36.0 μg/kg). 4-Vinylguaiacol, guaiacol, and 4-ethylguaiacol came from degradation of ferulic acid, which is abundant in grains (*29*).

Other compounds such as pyrazines, lactones, and sulfur compounds have also been detected in the Qus. Pyrazines, which

Table 3. Concentrations (Micrograms per Kilogram of Dry Weight) of Aroma Compounds in Six Chinese Rice Wine Qus Detected by FFAP Column ($n = 3$)[a]

		JFSQ		GNFQ		JSSQ		WZMSQ		ZJGGX		ZJGTX	
no.	aroma compound	concn[b]	SD[b]	concn	SD	concn	SD	concn	SD	concn	SD	concn	SD
	alcohols												
2	1-propanol	736.5 b	67.4	5216.4 a	471.6	894.3 b	19.0	998.8 b	73.7	ND		ND	
4	2-methylpropanol	<131.5		ND[c]		2837.9 c	39.2	ND		4354.8 a	173.7	4008.5 b	85.9
6	3-methylbutanol	3080.3 e	35.7	3312.1 d	88.6	4046.8 c	26.8	2855.5 f	23.7	9158.9 a	171.8	7044.4 b	307.3
8	1-pentanol	1145.9 c	63.8	2290.3 a	206.7	588.4 d	59.5	544.0 d	16.7	1264.6 bc	47.2	1354.5 b	97.7
11	1-hexanol	2459.7 d	171.7	3478.9 c	310.2	280.1 e	3.2	562.8 e	30.9	5148.1 b	149.2	5597.2 a	423.2
16	1-octen-3-ol	212.3 b	3.6	224.9 a	1.6	200.1 c	4.2	172.8 d	2.1	202.3 c	2.7	221.9 a	4.9
20	1-octanol	39.7 c	0.4	40.1 bc	0.6	ND		35.2 d	0.8	41.6 b	0.8	43.8 a	2.1
25	benzenemethanol	1387.8 a	7.1	459.8 d	14.4	331.1 e	8.2	331.7 e	2.7	622.8 c	17.5	659.2 b	40.9
26	2-phenylethanol	1143.1 e	142.4	2173.8 c	45.4	1405.8 d	226.2	ND		10136.8 a	619.1	8269.3 b	41.1
	subtotal	10205.3 d		17196.3 c		10584.5 d		5500.8 e		30929.9 a		27198.8 b	
	esters												
1	ethyl acetate	11731.6 a	404.5	11532.5 a	314.5	7743.5 b	157.1	8666.6 b	254.7	12040.1 a	1249.3	12487.5 a	733.8
7	ethyl hexanoate	114.8 c	6.8	145.5 a	8.5	99.9 d	4.9	101.6 d	5.0	131.8 b	5.2	129.2 b	4.8
15	ethyl octanoate	237.8 b	0.6	244.5 a	2.9	221.9 c	0.2	223.6 c	0.9	225.2 c	0.4	224.1 c	1.3
22	ethyl benzoate	40.2 a	1.3	27.4 b	0.2	23.2 c	0.9	24.8 c	0.7	ND		19.7 d	0.1
	subtotal	12124.4 a		11949.9 a		8088.5 b		9016.6 b		12397.1 a		12860.5 a	
	carbonyl compounds												
3	1-hexanal	492.9 ab	5.5	515.7 a	33.7	403.7 cd	57.1	349.6 de	20.9	289.3 e	48.3	436.6 bc	18.2
13	nonanal	32.2 b	0.3	ND		32.3 b	0.5	31.7 b	0.2	32.6 b	0.7	35.9 a	0.7
19	benzaldehyde	110.9 b	6.3	69.6 c	2.3	27.7 d	1.3	290.6 a	29.2	14.4 d	0.9	28.1 d	2.6
21	phenylacetaldehyde	63.1 a	4.2	57.2 b	0.9	19.9 d	1.4	52.1 b	4.9	30.6 c	0.6	64.8 a	4.7
5	2-heptanone	362.9 c	0.7	402.4 a	4.7	346.3 e	0.1	355.9 d	3.0	364.5 c	1.8	396.1 b	2.5
9	2-octanone	178.8 c	0.2	186.3 b	1.9	178.2 c	1.8	183.9 b	0.8	185.2 b	2.6	191.2 a	0.7
10	1-octen-3-one	610.1 a	0.1	ND		ND		ND		ND		ND	
29	2-furancarboxaldehyde	647.4 a	37.4	ND		ND		ND		ND		ND	
	subtotal	2498.3 a		1231.2 b		1008.1 d		1263.8 b		916.6 e		1152.7 c	
	acids												
17	acetic acid	624.5 bc	61.6	840.6 b	81.1	ND		458.5 c	87.3	1093.2 a	20.5	746.3 b	57.2
23	hexanoic acid	244.8 c	15.5	616.4 b	79.8	ND		ND		855.2 a	88.9	836.2 a	41.5
	subtotal	869.3 c		1457.0 b				458.5 d		1948.4 a		1582.5 b	
	volatile phenols												
24	guaiacol	29.7 c	1.6	72.6 b	3.8	ND		ND		131.8 a	2.6	ND	
28	4-ethylguaiacol	<4.5		ND		ND		ND		33.8 a	0.4	36.1 a	0.1
31	*p*-cresol	<4.7		61.5 a	0.2	ND		ND		50.8 b	2.3	52.1 b	0.9
32	4-vinylguaiacol	1340.6 c	18.1	8495.9 a	307.1	ND		1063.7 c	52.9	1861.9 b	295.3	ND	
	subtotal	1370.3 c		8630 a				1063 c		2078.3 b		88.2	
	pyrazines												
12	2,3-dimethylpyrazine	131.7 a	10.3	ND		ND		ND		ND		ND	
14	2,3,5-trimethylpyrazine	<23.8		172.8 a	5.9	ND		ND		ND		ND	
18	2,3,5,6-tetramethylpyrazine	37.3 b	1.6	55.3 a	2.7	ND		ND		ND		ND	
	subtotal	169 b		228.1 a									
	lactones												
30	γ-nonalactone	144.3 b	10.9	218.1 a	2.4	<7.39		<7.4		58.3 c	4.2	37.3 d	1.5
	subtotal	114.3 b		218.1 a						58.3 c		37.3 d	
	thiols												
27	benzothiazole	417.7 c	0.3	421.5 b	0.9	ND		415.7 c	0.1	447.3 a	3.0	449.4 a	1.9
	subtotal	417.7 c		421.5 b				415.7 c		447.3 a		449.4 a	
	total	27798.6 c		41332.1 b		19681.1 d		17719.1 d		48775.9 a		43369.4 b	

[a] The data correspond to the mean of triplicates; values followed by the same letter are not significantly different ($p < 0.05$). [b] Concn, mean concentration; SD, standard deviation. [c] ND, not detected.

are mostly formed through the Maillard reaction, were detected only in JFSQ and GNFQ, which had a higher fermentation temperature (50−55 °C). Therefore, the higher temperature would benefit the Maillard reaction, which resulted in the formation of pyrazine compounds in JFSQ and GNFQ. Only one lactone, namely, γ-nonalactone, was detected in most Qus except JSSQ and WZMSQ. Likewise, only one sulfur compound, benzothiazole, was detected in the Qus, and its concentration (415.7−449.4 μg/kg) was above their its threshold (80 μg/L in water) (*19*).

Comparison of Aroma Compounds in Six Chinese Rice Wine Qus Using PCA. The volatiles emanating from six Qus were compared. PCA was applied on the concentrations of the 32 odor-active compounds (**Table 3** and **Figure 1**) to establish differences among the six Qus and to determine which volatiles contributed most to the differences.

As shown in **Figure 1**, biplots revealed that PC 1 and PC 2 explained 39.13 and 30.41% of the total variation. The two xiao Qus (ZJGGX and ZJGTX), which lie on the positive region of PC 1 and the negative region of PC 2, were clearly differentiated from the four wheat Qus (JFSQ, GNFQ, JSSQ, and WZMSQ). The two xiao Qus (ZJGGX and ZJGTX) were also segregated from each other in PC 2 (**Figure 1B**). The compounds, which were strongly correlated with the two xiao Qus, were 2-methylpropanol (**4**), 3-methylbutanol (**6**), 2-phenylethanol (**26**), and

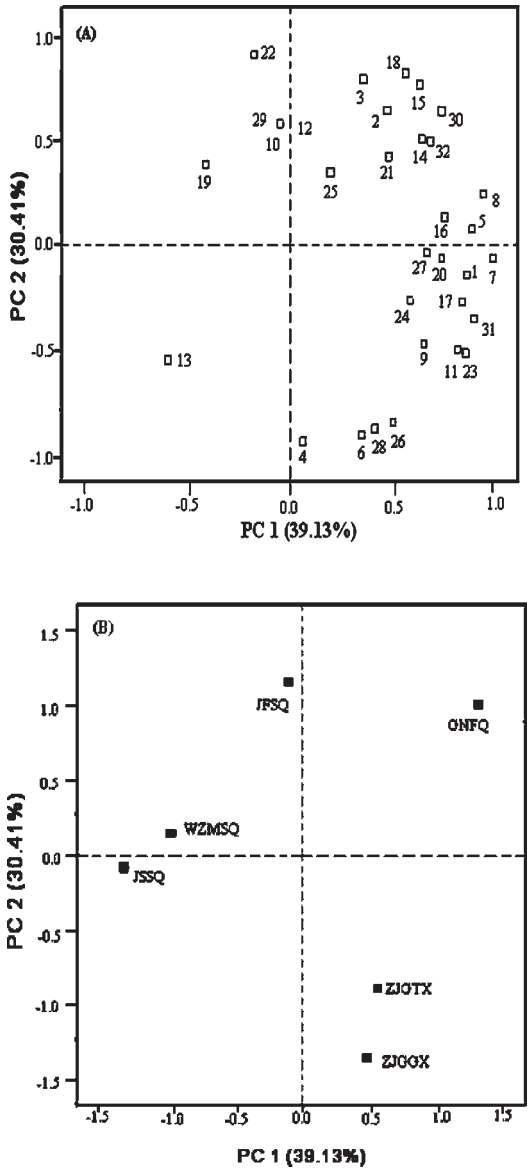

Figure 1. PCA plots from six Chinese rice wine Qus: (**A**) distribution of 32 odorants in six Chinese rice wine Qus (numbers correspond to those in **Table 3**); (**B**) separation of different Chinese rice wine Qus based on the concentrations of 32 odor-active compounds.

4-ethylguaiacol (**28**) (numbers correspond to **Table 3**) (**Figure 1A**). They indicate that these aroma compounds are derived exclusively or predominately from the two xiao Qus. Among the four wheat Qus, GNFQ, which was located on the high positive regions of PC 1 and PC 2, was situated on the positive side of JFSQ, JSSQ, and WZMSQ and clearly isolated from the latter three wheat Qus. 1-Propanol (**2**), 1-hexanal (**3**), 2,3,5-trimethylpyrazine (**14**), ethyl octanoate (**15**), 2,3,5,6-tetramethylpyrazine (**18**), γ-nonalactone (**30**), and 4-vinylguaiacol (**32**) were the variables associated with GNFQ. They are indicative of GNFQ origin. JFSQ located on the positive regions of PC 2 was distinctly separated from another two wheat Qus, namely, JSSQ and WZMSQ, by PC 2 because of their higher levels in 1-octen-3-one (**10**), 2,3-dimethylpyrazine (**12**), 2-furancarboxaldehyde (**29**), ethyl benzoate (**22**), and benzenemethanol (**25**). This suggests that these aroma compounds are responsible for the unique or predominant aroma in JFSQ. WZMSQ was segregated from JSSQ by PC 2 because of high concentrations of benzaldehyde (**19**). Therefore, the main aroma compounds with negative and positive values characterized the aroma difference among the Chinese rice wine Qus.

In summary, 39 aroma compounds were detected by the SAFE technique and GC-O in the Qu. GC-O analysis results showed that important aroma contributors in the Qu were 1-hexanal, ethyl hexanoate, 1-octen-3-ol, and phenylacetaldehyde. HS-SPME-GC-MS was a good method for quantification of the aroma compounds in the Qu. PCA demonstrated that the volatile profile based on the concentrations of aroma compounds enabled us to differentiate the six Qus well.

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