

Aroma Characterization of Chinese Rice Wine by Gas Chromatography–Olfactometry, Chemical Quantitative Analysis, and Aroma Reconstitution

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ABSTRACT: The aroma profile of Chinese rice wine was investigated in this study. The volatile compounds in a traditional Chinese rice wine were extracted using Lichrolut EN and further separated by silica gel normal phase chromatography. Seventy-three aroma-active compounds were identified by gas chromatography–olfactometry (GC-O) and gas chromatography–mass spectrometry (GC-MS). In addition to acids, esters, and alcohols, benzaldehyde, vanillin, geosmin, and γ -nonalactone were identified to be potentially important to Chinese rice wine. The concentration of these aroma-active compounds in the Chinese rice wine was further quantitated by combination of four different methods, including headsapce–gas chromatography, solid phase microextraction–gas chromatography (SPME)-GC-MS, solid-phase extraction–GC-MS, and SPME-GC–pulsed flame photometric detection (PFPD). Quantitative results showed that 34 aroma compounds were at concentrations higher than their corresponding odor thresholds. On the basis of the odor activity values (OAVs), vanillin, dimethyl trisulfide, β -phenylethyl alcohol, guaiacol, geosmin, and benzaldehyde could be responsible for the unique aroma of Chinese rice wine. An aroma reconstitution model prepared by mixing 34 aroma compounds with OAVs > 1 in an odorless Chinese rice wine matrix showed a good similarity to the aroma of the original Chinese rice wine.

KEYWORDS: Chinese rice wine, aroma, normal phase chromatography, GC-O, OAV, aroma reconstitution

INTRODUCTION

Chinese rice wine was a traditional alcoholic beverage in the southern part of China for thousands of years and has regained its popularity in recent decades due to its unique flavor and rich nutrients.^{1,2} The annual production of Chinese rice wine reached 1.5 million metric tons in 2011, with a total value of approximately U.S. \$1.8 billion.³ Chinese rice wine is different from Chinese liquors, which are distilled and have alcohol contents ranging from 36 to 53%. Chinese rice wine is not distilled and has an alcohol content of 8–18%. It is typically fermented from cooked glutinous rice with “wheat Qu” as a saccharifying agent and yeast (*Saccharomyces cerevisiae*) as a fermentation starter.⁴ In the fermentation process, the saccharification of starch and fermentation of sugars are carried out at the same time. After fermentation, the fresh Chinese rice wine is sterilized by thermal treatment with hot water to 85–95 °C for 5 min and matured in a sealed pottery jar at ambient temperature. Typical Chinese rice wines are aged for >3 years before bottling.¹

Chinese rice wines have quite different aroma characters depending on the brand, manufacturing raw materials, and manufacturing process. “Huadiao” is one of the major Chinese rice wine types, produced in Zhejiang province of China. It has aroma characteristics of caramel, herbal, smoke, “yeasty, moldy, and Qu-like aroma”, and honey aroma.⁵ The volatile composition of Chinese rice wine is very complex; more than 100 volatile and semivolatile compounds have been identified.^{4,6} However, the actual odor-contributing compounds are still not well-defined. The aim of this research is to study more

in depth the aroma composition of Chinese rice wine (i) to identify odor-active compounds in Chinese rice wine by normal-phase fractionation and chromatography–olfactometry (GC-O), (ii) to quantitate the aroma compounds in Chinese rice wine by multiple quantitation methods, and (iii) to verify the results by means of aroma reconstitution.

MATERIALS AND METHODS

Chemicals. Chemical standards of the aroma compounds were supplied by Sigma-Aldrich (St. Louis, MO, USA), TCI America (Portland, OR, USA), EKC Inc. (Rosemont, IL, USA), and EMD Chemical Inc. (Gibbstown, NJ, USA), and their purities were >95% in all cases. Methanol (HPLC grade) was from EM Science (Gibbstown, NJ, USA). Dichloromethane (HPLC grade, Burdick & Jackson, Muskegon, MI, USA), pentane (Nanograde, Mallinckrodt Baker, Phillipsburg, NJ, USA), and diethyl ether (EMD Chemical Inc.) were freshly distilled before use. Anhydrous sodium sulfate and sodium chloride (99.9%, ACS certified) were supplied by Mallinckrodt Baker. Pure water was obtained from a Milli-Q purification system (Millipore, Bedford, MA, USA).

Chinese Rice Wine Sample. A Chinese rice wine named “Guyelongshan Huadiao”, was obtained commercially. It was manufactured by Zhejiang Guyelongshan Chinese Wine Co., Ltd. (Shaoxing, China), in 2009. The sample (labeled GYHD, 17% ethanol by volume) belonged to the semidry type Chinese rice wine, which is the most popular type of Chinese rice wine.

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Aroma Extraction. A sample of 300 mL of GYHD was diluted with water at 1:1 ratio (by volume) in a 1 L flask. Two grams of LiChrolut EN resins (CAS Registry No. 165039-45-2; Merck KGaA, Darmstadt, Germany) and a magnetic stir bar were added to the flask. Aroma compounds were extracted for 5 h at room temperature with the magnetic stir bar at a speed of about 800 rpm. After extraction, LiChrolut EN resins were recovered and washed with 30 mL of Mili Q water and then dried by letting the air pass through them (−50 kPa, 20 min). Volatile compounds were eluted with 30 mL of dichloromethane. The aroma extract was washed with 3 × 3 mL of 0.1 M aqueous NaHCO₃ (pH 8.0). The organic phase was dried with anhydrous sodium sulfate and concentrated to 1 mL. This concentrate was labeled “neutral/basic fraction”. The combined aqueous phase was further adjusted to pH 2 with 1.0 M H₂SO₄, saturated with NaCl, and then extracted three times with 2 mL of freshly distilled diethyl ether. The extracts were combined and dried with anhydrous sodium sulfate. After concentration to 200 μL, this concentrate was labeled “acidic fraction”.

Normal Phase Liquid Chromatography. The neutral/basic fraction was further separated with silica gel normal phase liquid chromatography performed on a fast protein liquid chromatography (FPLC) system (Pharmacia, Uppsala, Sweden). A glass column (30 cm × 1.5 cm i.d.) packed with approximately 6.0 g of silica gel (particle size < 0.063 mm and finer than 230 mesh ASTM, Merck) was used for fractionation. The silica column was first washed with 50 mL of methanol and then 50 mL of diethyl ether. After conditioning with 50 mL of pentane, the neutral/basic fraction (1 mL) was loaded on the silica column. Volatile compounds were eluted by a gradient elution with pentane and diethyl ether as the mobile phase at a flow rate at 1 mL/min. Program gradient: phase A, pentane; phase B, diethyl ether; 0–16 min, 100% A; 16–80 min, linear program B until 50%; 80–100 min, linear program B until 100%; 100–120 min, 100% B. An automatic fraction collector was used to collect the eluent for each 8 min. A total of 16 fractions were collected for the whole fractionation. All of those fractions were concentrated to 200 μL and labeled NBF1–NBF16.

GC-O Analysis. GC-O analysis was performed on a Hewlett-Packard 5890 gas chromatograph equipped with a flame ionization detector (FID) and an olfactometer. Samples were separated using a ZB-Wax column (30 m × 0.25 mm i.d., 0.25 μm film thickness, Phenomenex, Torrance, CA, USA) and a DB-5 column (30 m × 0.25 mm i.d., 0.25 μm film thickness, J&W Scientific, Folsom, CA, USA). The column carrier gas was nitrogen at a constant flow rate of 2 mL/min. The column effluent was split 1:1 into FID and olfactometer. Sample (1 μL) was injected into the GC injector at splitless model. The GC injector and detector temperatures were 250 °C. The oven temperature was programmed at 40 °C for a 2 min hold and then to 230 °C at a rate of 4 °C/min, with a 10 min hold at the final temperature. Two well-trained panelists (one female and one male) were selected for GC-O study. The odor intensities were evaluated using 6-point intensity scale from 0 to 5; “0” was none, “3” was moderate, and “5” was extreme. The retention time, intensity value, and odor descriptor were recorded. Each fraction was replicated two times by each panelist. The aroma intensity was the average from both panelists when an aroma was registered.

GC-MS Identification of Aroma Compounds. GC-MS analysis was carried out on an Agilent 6890 GC equipped with an Agilent 5973 mass selective detector (MSD). The samples were analyzed on a ZB-Wax column (30 m × 0.25 mm i.d., 0.25 μm film thickness, Phenomenex) and a DB-5 column (30 m × 0.25 mm i.d., 0.25 μm film thickness, J&W Scientific). The oven and injector temperatures were identical to those of GC-O analysis as described previously. The column carrier gas was helium at a constant flow rate of 2 mL/min. The electron impact energy was 70 eV, and the mass range was from 35 to 350 amu. The ion source and quadrupole temperatures were set at 230 and 150 °C. Mass spectra (MS) of unknown compounds were compared with those in the Wiley 275.L database (Agilent Technologies Inc.).

Aroma Compound Identification. Identification of aroma compounds was based on the following criteria: odor description, mass spectra, and retention indices (RIs) relative to those of pure

reference compounds. Retention indices were determined using a series of standard linear alkanes C₅–C₂₃ under the same chromatographic conditions.

Quantitative Analysis of Aroma Compounds. *Static Headspace–GC-FID (HS-GC-FID).* Acetaldehyde, ethyl acetate, and other highly volatile compounds were quantitated by HS-GC-FID described previously.⁷ One milliliter of wine sample was diluted with 1.0 mL of saturated NaCl solution in a 20 mL autosampler vial and spiked with 10 μL of internal standard (methyl propionate, 12.5 mg/L). Samples were equilibrated at 70 °C for 15 min with shaking at 250 rpm. One milliliter of headspace sample was injected using a heated (70 °C) gastight syringe (2.5 mL) in split mode 10:1. Separation was performed on a ZB-Wax column (30 m × 0.25 mm, 0.25 μm film thickness). Helium was used as the carrier gas at a constant flow rate of 2 mL/min. The oven temperature was initially set at 35 °C for 4 min and then was increased to 150 °C at 10 °C/min and held for 5 min. The injector and detector temperatures were set at 200 and 250 °C separately. All analyses were repeated in triplicate.

Headspace–Solid Phase Microextraction–GC-MS (HS-SPME-GC-MS). An automatic headspace sampling system (CTC HTS PAL, Switzerland) with a 50/30 μm divinylbenzene/carboxen/poly(dimethylsiloxane) (DVB/CAR/PDMS) fiber (2 cm, Supelco, Inc., Bellefonte, PA, USA) was used for extraction of most major compounds (Table 2). An aliquot (2 mL) of wine sample and 8 mL of saturated NaCl solution were put into a 20 mL autosampler vial and spiked with 10 μL of internal standard (3-heptanone, 50 mg/L; 4-octanol, 55 mg/L; octyl propanoate, 60 mg/L) solutions. This sample was equilibrated at 50 °C for 5 min and extracted for 50 min at the same temperature under stirring. After extraction, the fiber was automatically inserted into the injection port of GC (250 °C) for 5 min to desorb the analytes. All analyses were repeated in triplicate. The oven and injector temperatures were identical to GC-O analysis described above on a ZB-Wax column.

A calibration curve was prepared in odorless Chinese rice wine. The odorless wine was prepared by passing 150 mL of GYHD wine through two conditioned Lichrolut EN cartridges (1000 mg) to remove the volatiles. Individual standard compound was accurately weighed and dissolved in absolute ethanol and then mixed and diluted with deodorized wine to obtain a range of concentrations. Ten microliters of internal standards was added to the working solution and then extracted by SPME, as was performed for the sample. The calibration curves were obtained from Chemstation software (Agilent Technologies Inc.) and used for calculation of volatiles in sample.

Solid Phase Extraction–GC-MS (SPE-GC-MS). Acids, phenolics, and minor compounds were quantitated by SPE-GC-MS (Table 2). The wine sample (20 mL) was diluted with Milli-Q water at 1:1 ratio. The diluted sample was spiked with 100 μL of internal standard (2-octanol, 40 mg/L in ethanol) and passed through a Lichrolut EN cartridge (200 mg, Merck KGaA) at a flow rate of 2 mL/min. The SPE cartridge had been previously conditioned with 6 mL of dichloromethane, 6 mL of methanol, and finally 6 mL of Milli-Q water. After the sample had been loaded, the sorbent was dried by letting the air pass through it (−50 kPa, 20 min). Analytes were recovered by elution with 1.8 mL of dichloromethane. The extract was then concentrated to 200 μL and stored at −20 °C until analysis. Each sample was extracted in triplicate. The extract was then analyzed by GC-MS. The oven and injector temperatures were identical to GC-O analysis described above on a ZB-Wax column. The individual standard solution was mixed and diluted with synthetic rice wine [17% (by volume) ethanol–water solution with 5.0 g/L lactic acid, pH 4.0] to obtain a range of concentrations. One hundred microliters of internal standard was added to each working solution and then analyzed by SPE-GC-MS. The calibration curves were obtained from Chemstation software and used for calculation of volatile concentration in the samples.

HS-SPME-GC–Pulsed Flame Photometric Detection (HS-SPME-GC-PFPD). Volatile sulfur compounds were quantitated according to the method described by Fang and Qian.⁸ The analyses were made on a Varian CP-3800 gas chromatograph equipped with a PFPD detector (Varian, Walnut Creek, CA, USA) operating in sulfur mode. Standard calibration curves were obtained by adding increasing amounts of the

Table 1. Aroma Compounds Identified in Huadiao Chinese Rice Wine by GC-O

no.	RI ^a		compound identified	odor descriptors	fraction ^b	identification ^c	odor intensity ^d
	DB-Wax	DB-5					
1	906	nd	ethyl acetate	solvent, fruity	NBF4	RI, odor	4.3
2	925	nd	3-methylbutanal	malty	NBF5	MS, RI, odor	3.2
3	961	nd	ethyl propanoate	sweet, fruity	NBF4	MS, RI, odor	3.1
4	969	nd	ethyl 2-methylpropanoate	fruity	NBF4	MS, RI, odor	2.2
5	1023	nd	1-propanol	alcoholic	NBF7	RI, odor	2.2
6	1043	800	ethyl butanoate	sweet, fruity, pineapple	NBF4	MS, RI, odor	3.8
7	1052	nd	dimethyl disulfide	cabbage	NBF6	MS, RI, odor	2.1
8	1061	860	ethyl 2-methylbutanoate	floral	NBF4	MS, RI, odor	1.6
9	1065	829	hexanal	green, grass	NBF5	MS, RI, odor	1.7
10	1080	867	ethyl 3-methylbutanoate	sweet, fruity	NBF4	MS, RI, odor	2.6
11	1106	752	2-methylpropanol	nail polish	NBF7	MS, RI, odor	3.2
12	1135	878	3-methylbutyl acetate	sweet, banana	NBF4	MS, RI, odor	3.1
13	1148	902	ethyl pentanoate	sweet	NBF4	MS, RI, odor	1.2
14	1158	764	1-butanol	alcoholic	NBF8	MS, RI, odor	1.6
15	1216	nd	2-methylbutanol	alcoholic, nail polish	NBF7	MS, RI, odor	4
16	1243	801	3-methylbutanol	alcoholic, nail polish	NBF7	MS, RI, odor	5
17	1245	996	ethyl hexanoate	fruity, sweet	NBF4	MS, RI, odor	4.2
18	1281	nd	2-methylpyrazine ^e	peanut	NBF12	MS, RI, odor	2.6
19	1287	808	1-pentanol	grassy	NBF7	MS, RI, odor	0.9
20	1341	922	2,6-dimethylpyrazine	cooked rice, nutty	NBF12	MS, RI, odor	3.9
21	1342	nd	ethyl heptanoate	fruity	NBF4	MS, RI, odor	1.4
22	1351	969	dimethyl trisulfide	cabbage	NBF6	MS, RI, odor	3.5
23	1352	836	ethyl lactate	fruity	NBF6	MS, RI, odor	2.6
24	1377	877	1-hexanol	plant, green	NBF8	MS, RI, odor	1.6
25	1404	1002	2,3,5-trimethylpyrazine ^e	peanut	NBF13	MS, RI, odor	2.8
26	1442	1192	ethyl octanoate	fruity	NBF4	MS, RI, odor	2.5
27	1448	977	1-octen-3-ol	mushroom	NBF9	MS, RI, odor	1.8
28	1452	1089	2,3,5,6-tetramethylpyrazine ^e	peanut	NBF11	MS, RI, odor	2.4
29	1466	nd	1-heptanol	plant	NBF8	MS, RI, odor	0.7
30	1468	nd	acetic acid	vinegar	AF	MS, RI, odor	5
31	1483	852	furfural	almond, burnt sugar	NBF7	MS, RI, odor	3.9
32	1543	967	benzaldehyde	almond	NBF6	MS, RI, odor	5
33	1564	675	propanoic acid	acidic	AF	MS, RI, odor	2.1
34	1589	919	5-methylfurfural	burnt sugar	NBF7	MS, RI, odor	2
35	1590	795	2-methylpropanoic acid	acidic, rancid	AF	MS, RI, odor	3.2
36	1634	1037	2-acetyl-5-methylfuran ^e	plant, leaf	NBF7	MS, RI, odor	1.7
37	1641	nd	ethyl 2-furoate	floral	NBF8	MS, RI, odor	2.5
38	nd	865	2-furanmethanol	burnt sugar	NBF9	MS, RI, odor	1.9
39	1650	830	butanoic acid	acidic, cheese	AF	MS, RI, odor	3.6
40	1654	917	γ -butyrolactone ^e	sweet	NBF13	MS, RI, odor	2.1
41	1671	1072	acetophenone	floral	NBF5	MS, RI, odor	1.9
42	1678	1047	phenylacetaldehyde	floral, rose	NBF7	MS, RI, odor	2.5
43	1685	1163	ethyl benzoate	floral	NBF5	MS, RI, odor	1.5
44	1690	859	3-methylbutanoic acid	acidic, smelly	AF	MS, RI, odor	4.9
45	1726	1054	γ -hexalactone ^e	sweet, peach	NBF10	MS, RI, odor	2.2
46	1740	nd	5-methylfurfuryl alcohol ^e	cooked sugar	NBF8	MS, RI, odor	0.8
47	1763	879	pentanoic acid	cheese, acidic	AF	MS, RI, odor	1.7
48	1806	1247	ethyl 2-phenylacetate	rose, honey	NBF5	MS, RI, odor	1.9
49	1830	nd	4-methylpentanoic acid ^e	sweaty	AF	MS, RI, odor	1.7
50	1839	1180	2-phenylethyl acetate	rose, floral	NBF5	MS, RI, odor	1.1
51	1861	1408	geosmin ^e	musty, moldy	NBF4	MS, RI, odor	4.7
52	1871	978	hexanoic acid	cheese, acidic	AF	MS, RI, odor	3.3
53	1885	1094	guaiacol	spicy, clove	NBF11	MS, RI, odor	3.9
54	1900	1037	benzyl alcohol	floral	NBF8	MS, RI, odor	1.2
55	1905	1873	ethyl 3-phenylpropionate	floral	NBF5	MS, RI, odor	0.9
56	1911	1285	2-phenyl-2-butenal	prune, floral	NBF6	MS, RI, odor	2.3
57	1940	1136	β -phenylethyl alcohol	floral, rose	NBF9	MS, RI, odor	4.7
58	1979	nd	heptanoic acid	sweat	AF	MS, RI, odor	1.7
59	2034	975	phenol	phenolic, medicinal	NBF11	MS, RI, odor	2.5

Table 1. continued

no.	RI ^a		compound identified	odor descriptors	fraction ^b	identification ^c	odor intensity ^d
	DB-Wax	DB-5					
60	2056	1358	4-ethylguaiaicol ^e	smoky	NBF11	MS, RI, odor	2.9
61	2059	1369	γ -nonalactone	coconut	NBF10	MS, RI, odor	4.8
62	2062	1054	pantolactone ^e	sweet	NBF13	MS, RI, odor	2.2
63	2069	1281	cinnamaldehyde ^e	cinnamon	NBF6	MS, RI, odor	2.9
64	2118	nd	4-methylphenol	smoky, phenolic	NBF13	MS, RI, odor	1.5
65	2132	1168	octanoic acid	sweat, cheese	AF	MS, RI, odor	2.2
66	2170	1455	ethyl cinnamate ^e	cinnamon, spice	NBF6	MS, RI, odor	2.2
67	2191	1463	γ -decalactone ^e	peach	NBF13	MS, RI, odor	2.8
68	2199	1263	nonanoic acid	sweat	AF	MS, RI, odor	0.7
69	2210	nd	4-ethylphenol	smoky	NBF13	MS, RI, odor	1.3
70	2229	1322	4-vinylguaiaicol	spicy, clove	NBF12	MS, RI, odor	3.4
71	2579	1407	vanillin	sweet, vanilla	NBF13	MS, RI, odor	4.4
72	2618	1558	ethyl vanillate ^e	vanilla	NBF13	MS, RI, odor	2.2
73	2642	1497	acetovanillone ^e	vanilla	NBF13	MS, RI, odor	1.5

^aRI = retention index on different stationary phases; nd = not determined. ^bAF, acidic fraction; NBF, neutral/basic fraction. The numbers mean this fraction has the highest aroma intensity. ^cIdentification based on RI (retention index) or MS (mass spectrometry) or odor description. ^dThe odor intensity values were the average values of four analyses (two panelists, two times). ^eNewly identified aroma compounds in Chinese rice wine.

target compounds to deodorized Chinese rice wine. Concentrations were calculated on the basis of the square root of the peak area ratio of the compound to the internal standard. All analyses were repeated in triplicate.

Aroma Reconstitution Test. The aroma reconstitution test was carried out according to Ferreira et al.⁹ and Poisson et al.¹⁰'s papers with modification. Aroma models were prepared by mixing aroma compounds at their actual concentrations shown in Table 2 in a deodorized Chinese rice wine. Two different aroma models were prepared. One model (model 1) contained those compounds with OAVs ≥ 1.0 ; the second model (model 2) contained all of the aroma compounds quantitated in this study. The overall aroma profiles of the Chinese rice wine aroma and the aroma reconstituted models were evaluated by 10 trained panelists (6 males and 4 females, between 21 and 35 years old). The panel was trained for 1 month (30 min/day) to describe and recognize the odor qualities of about 50 odorants. Before sensory evaluation, two specific 1 h training sessions were carried out in a sensory room at about 20 °C. In the first one, Chinese rice wine sample GYHD was presented and discussed by the panel for the aroma profile. In session two, the sensory panelists were asked to score the strength of alcoholic, caramel-like, fruity, smoky, herb, Qu aroma, and honey note on a seven-point scale from 0 (nondetected) to 3 (very strong); half values were allowed. These seven aroma terms had been selected for the descriptive analysis of Chinese rice wine in a previous study.⁵ Among them, Qu aroma was a specific term to describe the aroma generated from wheat Qu used in Chinese rice wine fermentation.¹¹ These seven aroma terms were defined as the following aromas: 3-methylbutanol for alcoholic note, caramel for caramel-like note, ethyl 3-methylbutanoate for fruity note, 4-ethylguaiaicol for smoky note, "wheat Qu" aroma extract for Qu aroma note, 2-phenylethanol for honey note, and 4-vinylguaiaicol for herb note. After the training period, the aroma profiles of aroma models and Chinese rice wine sample were evaluated by the panel. The samples were poured into a glass cup at 20 °C and presented in coded form. The overall similarity of the Chinese rice wine and aroma models were first investigated by triangular tests. The panelists were then asked to evaluate the difference by using a seven-point scale from 0 (different) to 3 (same). The sensory data were analyzed by one-way analysis of variance (ANOVA) by use of SPSS 15.0 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Odor-Active Compounds Determined Using GC-O.

The aroma extract was first fractionated into acidic and neutral/basic fractions. The acidic fraction was analyzed directly by

GC-O because it has a relatively simple composition. The neutral/basic fraction, however, was too complex to distinguish all of the odors by GC-O analysis. To facilitate GC-O analysis, the neutral/basic fraction was further separated by silica gel chromatography performed on a FPLC system. Because many aroma compounds could be detected by GC-O in more than one fraction, this approach can provide only relative odor intensity results. However, the fractionation process greatly reduced the complexity of aroma composition of each fraction; it was much easier to identify the aroma quality of the compounds, as well as positive MS and GC-O identification. This process enabled us to positively identify 73 odor-active volatiles in Chinese rice wine, 16 more than in a previous study.²

Alcohols, Esters, and Acids. Like most alcoholic beverages, alcohols, esters, and acids were the main aroma-active compounds in Chinese rice wine (Table 1). Most of these aroma compounds are the byproducts of yeast growth and ethanol fermentation. Among the odor-active compounds identified, 3-methylbutanol (**16**), β -phenylethyl alcohol (**57**), and 2-methylbutanol (**15**) were the major alcohols with high aroma intensity. Higher alcohols are formed by decarboxylation and subsequent reduction of keto-acids produced as intermediates of amino acids biosynthesis and catabolism.¹² These higher alcohols were quantitatively the most important volatile compounds produced during yeast fermentation in Chinese rice wine.^{2,13} The concentrations of higher alcohols were greatly affected by the yeast strains and the amount of wheat Qu used in Chinese rice wine fermentation.^{13–15} Esters were the largest group of aroma compounds found in Chinese rice wine. A total of 17 esters were identified in this study, including 7 straight-chain esters, 4 branched-chain esters, and 6 other esters. On the basis of the odor intensities, the potentially important esters were ethyl acetate (**1**), ethyl hexanoate (**17**), ethyl butanoate (**6**), 3-methylbutyl acetate (**12**), and ethyl propanoate (**3**). Due to their low odor threshold and desirable fruity odor, esters play a positive role for the aroma profile of Chinese rice wine. Yeast strains and fermentation processes can greatly influence the formation of esters during Chinese rice wine fermentation.¹³ A total of 11 volatile acids were identified in this study. Among them, acetic acid (**30**), 3-methylbutanoic acid (**44**), butanoic acid (**39**), and hexanoic

acid (52) were the potentially important volatile acids in Chinese rice wine according to their odor intensities.

Phenolics. Besides the alcohols, esters, and acids, volatile phenolics had the highest total aroma intensities in Chinese rice wine. A total of nine phenolic derivatives were detected in GYHD rice wine sample. Ethyl vanillate (72) and acetovanillone (73), which showed vanilla-like aroma, were first identified in Chinese rice wine through the normal phase fractionation–GC-O study. With odor intensity >4, vanillin (71) may be a potentially important aroma compound for Chinese rice wine. Guaiacol (53) and 4-vinylguaiacol (70) had aroma intensity >3 and contributed to the clove, spicy, and smoky odor. Guaiacol and 4-vinylguaiacol could be from wheat Qu, the raw material used in Chinese rice wine fermentation, as these two compounds have been identified in wheat Qu.¹¹ The concentrations of volatile phenolic compounds were significantly higher in Chinese rice wine fermented with wheat Qu than that without wheat Qu.¹⁵

Aldehydes. A total of eight aroma-active aldehydes were identified in this study. Among them, cinnamaldehyde (63) (odor intensity = 2.87) was reported in Chinese rice wine for the first time and contributed to the cinnamon aroma. On the basis of the aroma intensity values, the most potentially important aldehyde was benzaldehyde (32) (odor intensity = 5.0), which imparted bitter almond aroma. It could be formed by the oxidation of the benzyl alcohol or by action of the microorganisms on the aromatic amino acids or from phenyl acetic acid and *p*-hydroxybenzoic acid.^{16,17} Other odor-active aldehydes identified in Chinese rice wine were 3-methylbutanal (2), hexanal (9), phenylacetaldehyde (42), and 2-phenyl-2-butenal (56).

Lactones. In this study, six lactones were identified in Chinese rice wine. γ -Nonalactone (61) had a very high odor intensity (odor intensity = 5) in Chinese rice wine. γ -Nonalactone possesses a “peach”/“coconut” aroma and has been found in high concentration in some Chinese rice wines.⁸ γ -Decalactone (67), which has a “peach” aroma, showed medium aroma intensity. The other lactones appeared at low aroma intensity and may not be important for the aroma of Chinese rice wine.

Sulfur Compounds. Two sulfur compounds, dimethyl disulfide (7) and dimethyl trisulfide (22), were identified in Chinese rice wine sample. Volatile sulfur compounds always appear as “rotten egg”, “onion”, “cooked cabbage”, or “sulfury” off-flavors at high concentration.¹⁸ However, they can also contribute a positive impression to alcoholic beverages at low concentration.^{19,20}

Others. Geosmin (51, *trans*-1,10-dimethyl-*trans*-9-decalol), generally described as an “earthy” and “moldy” aroma, was identified in this study. The aroma intensity of geosmin was 4.5, suggesting it could be a very important aroma contributor in Chinese rice wine. Geosmin is likely from wheat Qu because we have identified geosmin in wheat Qu to have high odor intensity (unpublished data). Geosmin has also been found in Chinese liquors²¹ and is probably from the Daqu used in Chinese liquor manufacturing.²² Daqu is a raw material and used as a saccharifying agent and fermentation starter to initiate fermentations for the production of Chinese liquor.²¹ Four pyrazines were identified in Chinese rice wine. Among them, 2,6-dimethylpyrazine (20) had the highest odor intensity, followed by 2,3,5-trimethylpyrazine (25), 2-methylpyrazine (18), and 2,3,5,6-tetramethylpyrazine (28). Pyrazines, exhibiting the toast, cooked rice, and peanut aroma,

generally formed through the Maillard reaction or microbial metabolism.^{23,24}

Quantitative Analysis of Aroma Compounds. GC-O is a useful tool to locate aroma-active compounds in foods. However, because compounds are ranked by their odor intensity in air, quantitative data are necessary to calculate the odor activity value (OAV) to assess their aroma contribution in the food matrix. Due to the complex chemical character of the aroma-active compounds identified in Chinese rice wine, multiple quantitation approaches were employed in this study, including headspace GC-FID, HS-SPME-GC-MS, SPE-GC-MS, and HS-SPME-GC-PFPD.

A total of 75 compounds were quantitated (Table 2), including alcohols, acids, phenolics, pyrazines, lactones, furans, aldehydes, and sulfur compounds. Among them, acetic acid, ethyl lactate, 3-methylbutanol, acetaldehyde, β -phenylethyl alcohol, 2-methylpropanol, and ethyl acetate were the quantitatively predominating volatile compounds in the Chinese rice wine. The results agreed with previous reports.² Acetaldehyde, methional, diethyl succinate, and 2-acetylfuran, which did not appear in the GC-O list, were quantitated because they have been detected in Chinese rice wine in other reports.⁶

To reveal the aroma contribution of these volatile compounds in Chinese rice wine, the perception thresholds from the literature were used to calculate OAVs. Results shown in the Table 2 confirmed previous GC-O study; most of the aroma compounds with high OAVs also had high aroma intensity. Thirty-four aroma compounds were found to reach concentrations above their aroma thresholds.

Among the quantitated aroma compounds, alcohols, esters, and acids were the most abundant substances. The contributions of these compounds to the Chinese rice wine aroma profile were well discussed previously.^{2,13} In this study, we will focus on the other unique aroma compounds found in Chinese rice wine.

Volatile phenolic compounds, which had high aroma intensity in Chinese rice wine by GC-O, were also confirmed by the quantitative results. Four volatile phenolic compounds including vanillin, guaiacol, 4-vinylguaiacol, and phenol were found with concentrations higher than their thresholds. Among them, vanillin was one of the five aroma compounds with the highest OAVs, suggesting its important aroma contribution to Chinese rice wine. The concentration of vanillin was much higher than in fresh Chinese rice wine (data not showed), revealing it might be generated during the Chinese rice wine aging process. It is quite possible that the vanillin was formed from 4-vinylguaiacol because its concentration was much lower than the level found in fresh Chinese rice wine (6.7 mg/L).²⁵ It is well documented that ferulic acid and 4-vinylguaiacol can be converted to vanillin during food fermentation and storage, and the conversion could be affected by microorganisms, temperature, pH, and oxygen.^{26–28} The wheat Qu used in Chinese rice wine contains high contents of ferulic acid, which can convert to 4-vinylguaiacol and vanillin in Chinese rice wine.²⁵

The aroma contribution of geosmin was confirmed by the quantitative result. Although the concentration of geosmin was at very low level, it also has a very low sensory threshold (10 ng/L in water, 80–90 ng/L in a neutral red wine, and 60–65 ng/L in neutral white wine),²⁹ making it an important aroma contributor to Chinese rice wine. Geosmin has a musty, earthy

Table 2. Quantitative Data Odor Thresholds and OAVs of Aroma Compounds in Huadiao Chinese Rice Wine

compound ^a	mean concn ^b ($\mu\text{g/L}$)	odor threshold ($\mu\text{g/L}$)	OAV ^c	compound ^a	mean concn ^b ($\mu\text{g/L}$)	odor threshold ($\mu\text{g/L}$)	OAV ^c
3-methylbutanoic acid ³	3100 \pm 75	33.4 ^{33d}	93	furfural ²	6250 \pm 310	14100 ³³	0.4
ethyl butanoate ²	1242 \pm 120	20 ³⁴	62	2-methylpropanoic acid ³	897 \pm 28	2300 ³³	0.4
vanillin ³	1276 \pm 10	26 ³⁵	49	ethyl heptanoate ²	82.7 \pm 5.4	220 ³⁷	0.4
ethyl hexanoate ²	392 \pm 11	14 ³³	28	2-furanmethanol ³	655 \pm 55	2000 ⁴¹	0.3
3-methylbutanal ¹	2320 \pm 104	120 ²⁰	19	4-ethylguaiaicol ³	10.3 \pm 0.2	33 ³³	0.3
butanoic acid ³	2790 \pm 140	173 ³³	16	propanoic acid ³	2160 \pm 102	8100 ³⁷	0.3
dimethyl trisulfide ⁴	2.4 \pm 0.3	0.18 ²⁰	13	1-octen-3-ol ²	10.0 \pm 0.1	40 ⁴²	0.2
β -phenylethyl alcohol ²	109310 \pm 9	8500 ³⁵	13	1-propanol ¹	71500 \pm 180	306000 ⁴³	0.2
guaiaicol ³	72 \pm 6	9.5 ³³	7.5	cinnamaldehyde ³	36.1 \pm 0.6	160 ⁴⁴	0.2
geosmin ³	0.36 \pm 0.03	0.05 ³⁶	7.3	ethyl benzoate ²	120 \pm 3	575 ³³	0.2
3-methylbutanol ¹	214200 \pm 20	30000 ³⁴	7.1	4-ethylphenol ³	27.7 \pm 0.6	140 ³³	0.2
ethyl 2-methylpropanoate ²	106 \pm 5	15 ³³	7.1	2-phenylethyl acetate ²	48.8 \pm 1.1	250 ³⁴	0.2
ethyl acetate ¹	83970 \pm 270	12264 ³⁷	6.8	pantolactone ³	304 \pm 53	2000 ⁴⁵	0.2
benzaldehyde ²	5250 \pm 310	990 ²⁰	5.3	pentanoic acid ³	338 \pm 8	3000 ²	0.1
3-methylbutyl acetate ²	145 \pm 4	30 ³⁴	4.8	γ -butyrolactone ³	3790 \pm 460	35000 ³⁹	0.1
ethyl octanoate ²	23.7 \pm 0.7	5 ³³	4.7	diethyl succinate ²	9640 \pm 450	200000 ³⁹	0.05
γ -nonalactone ³	136 \pm 2	30 ³³	4.5	1-hexanol ²	253 \pm 0.7	8000 ³⁴	0.03
acetic acid ²	895000 \pm 28000	200000 ³⁴	4.5	heptanoic acid ³	87.2 \pm 1.5	3000 ²	0.03
phenylacetaldehyde ³	90 \pm 12	25 ²⁰	3.6	4-methylphenol ³	1.9 \pm 1.1	68 ³³	0.03
acetophenone ³	213 \pm 5	65 ³⁸	3.3	dimethyl disulfide ⁴	0.57 \pm 0.13	25 ⁴⁶	0.02
ethyl lactate ²	417000 \pm 16000	154000 ³⁷	2.7	1-butanol ²	3310 \pm 740	150000 ³⁷	0.02
4-vinylguaiaicol ³	102.6 \pm 7.8	40 ³⁴	2.6	2,3,5,6-tetramethylpyrazine ³	151 \pm 1	10000 ⁴⁷	0.02
hexanoic acid ³	1059 \pm 21	420 ³³	2.5	1-heptanol ²	18.0 \pm 0.2	2500 ²	0.01
γ -decalactone ³	220.3 \pm 1.8	88 ³³	2.5	1-pentanol ²	23.8 \pm 1.8	6400 ³⁷	<0.01
ethyl 3-methylbutanoate ²	69.7 \pm 5.6	30 ³³	2.3	5-methylfurfural ³	67.1 \pm 2.5	20000 ³⁷	<0.01
phenol ³	65.3 \pm 0.1	30 ³⁷	2.2	γ -hexalactone ³	22.3 \pm 0.7	13000 ³³	<0.01
2-methylpropanol ¹	85200 \pm 4	40000 ³⁴	2.1	2,3,5-trimethylpyrazine ³	12.0 \pm 0.6	9000 ⁴⁷	<0.01
ethyl cinnamate ³	2.0 \pm 0.3	1.1 ³³	1.8	2-methylpyrazine ³	9.2 \pm 1.0	10500 ⁴⁷	<0.01
methional ⁴	16.3 \pm 0.6	10 ²⁰	1.6	ethyl 2-furoate ³	8.0 \pm 0.4	16000 ³³	<0.01
acetaldehyde ¹	137500 \pm 2	100000 ³⁷	1.4	2-acetylfuran ³	32.2 \pm 0.8	80000 ⁴⁸	<0.01
ethyl 2-methylbutanoate ²	24.6 \pm 1.6	18 ³⁹	1.4	2,6-dimethylpyrazine	19.5 \pm 1.1	54000 ⁴⁷	<0.01
hexanal ²	12.4 \pm 0.6	9.1 ²⁵	1.4	ethyl pentanoate ²	96.4 \pm 2.3	nd ^e	
ethyl propanoate ²	2200 \pm 170	1800 ³⁷	1.2	4-methylpentanoic acid ³	294 \pm 13	nd	
ethyl 2-phenylacetate ²	103.7 \pm 2.1	100 ²⁰	1.0	2-acetyl-5-methylfuran ³	8.0 \pm 0.2	nd	
ethyl vanillate ³	714 \pm 16	990 ⁴⁰	0.7	2-phenyl-2-butenal ²	199 \pm 4	nd	
acetovanillone ³	682 \pm 25	1000 ⁴⁰	0.7	nonanoic acid ³	56.9 \pm 3.9	nd	
benzyl alcohol ²	571 \pm 30	900 ³⁷	0.6	ethyl 3-phenylpropionate ²	53.3 \pm 3.5	nd	
octanoic acid ³	296 \pm 19	500 ³³	0.6				

^aQuantitative method used: ¹static headspace–GC–FID; ²headspace solid phase microextraction–GC–MS; ³solid phase extraction–GC–MS; ⁴headspace solid phase microextraction–GC–pulsed flame photometric detection. ^bMean concentration \pm SD ($n = 3$). ^cOAVs were calculated by dividing the concentration by odor threshold value of the compounds. ^dNumbers following entries indicate the references from which the threshold values have been taken. ^end, threshold value not determined.

odor and is always considered as an off-flavor in water, wine, and juice.

Two lactones (γ -nonalactone and γ -decalactone) were found to have aroma contribution to Chinese rice wine based on their OAVs. γ -Nonalactone (coconut, sweet aroma) was the most important lactone found in Chinese rice wine due to its low threshold (30 $\mu\text{g/L}$).

Concentrations of pyrazines and furans were determined lower than their thresholds, indicating these compounds seem to be more easily detected by GC–O than by the normal olfaction from hydroalcoholic media.

Aroma Reconstitution Test. On the basis of the GC–O results, none of the odorants elicited an odor quality resembling the Chinese rice wine and, thus, it is likely that the overall aroma of Chinese rice wine is a result of the natural composition of the key odorants in appropriate concentrations.

Aroma reconstitution experiments on the basis of the quantitative results were undertaken to mimic the characteristic aroma of the Chinese rice wine. Because the nonvolatile matrix may have a strong influence on the perception of aroma,^{30–32} a deodorized Chinese rice wine was used for the reconstitution experiments. Two different aroma reconstitution models were prepared: a mixture containing all of the aroma compounds in Table 2 (model 2) and a mixture containing only compounds with OAV >1.0 (model 1). The overall aroma similarities of the two reconstitution models with the original Chinese rice wine were judged as 2.6 and 2.5 of 3.0 points. Although the panel was able to discriminate between the Chinese rice wine and these models, their aromas were considered qualitatively very similar to that of the Chinese rice wine. The aroma descriptors of these reconstitution models and Chinese rice wine are shown in Figure 1. The results showed a good similarity for fruity,

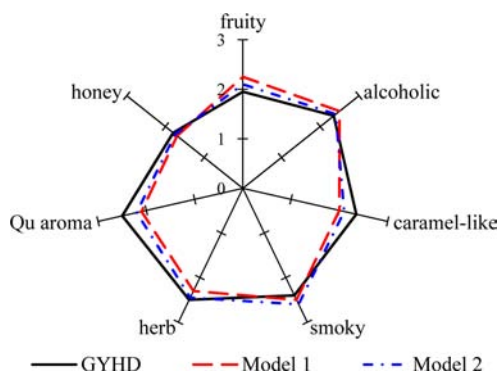


Figure 1. Aroma profiles of Huadiao Chinese rice wine (GYHD) and the aroma reconstituted models (model 1 contained those compounds with OAVs >1.0; model 2 contained all of the aroma compounds quantitated in this study).

alcoholic, smoky aromas, whereas caramel-like and Qu aroma notes were slightly lower in the reconstituted models.

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Notes

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