JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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# Characterization of Odor-Active Compounds in Sweet-type Chinese Rice Wine by Aroma Extract Dilution Analysis with Special Emphasis on Sotolon

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**ABSTRACT:** The aroma characteristics of sweet-type Chinese rice wine were studied by sensory analysis, aroma extract dilution analysis (AEDA), and quantitative analysis. Sensory evaluation demonstrated that a caramel-like note was the most distinctive characteristic for sweet-type Chinese rice wine. AEDA was carried out on the extract of a typical sweet-type Chinese rice wine sample. Thirty-nine odor-active regions were detected in the sample with a flavor dilution (FD) factor ≥8, and 37 of these were further identified. Among them, sotolon and 2- and 3-methylbutanol showed the highest FD factor of 1024, followed by 2-acetyl-1-pyrroline (tentatively identified), dimethyl trisulfide, 2-phenylethanol, and vanillin with a FD factor of 512. Sotolon was identified as a key aroma compound in Chinese rice wine for the first time. AEDA results indicated that sotolon (caramel-like/seasoning-like) was the potentially key contributor to the caramel-like descriptor of sweet-type Chinese rice wine. The concentration of sotolon in Chinese rice wine was further quantitated by Lichrolut-EN solid-phase extraction coupled with microvial insert large volume injection method. The content of sotolon ranged from 35.93 to 526.17 μg/L, which was above its odor threshold (9 μg/L) for all Chinese rice wine samples. The highest concentration of sotolon was found in the sweet-type Chinese rice wine, which highlighted the important aroma role of sotolon for this particular type of Chinese rice wine.

**KEYWORDS:** *Chinese rice wine, sensory evaluation, AEDA, sotolon, large volume injection*

## INTRODUCTION

Chinese rice wine is a very popular traditional fermented alcoholic beverage in China. According to the total sugar content and the fermentation processes, Chinese rice wines can be sorted into four typical varieties: dry (total sugar content ≤15.0 g/L), semidry (total sugar content 15.1−40.0 g/L), semisweet (total sugar content 40.1−100.0 g/L), and sweet (total sugar content ≥100.1 g/L) (Chinese National Standard GB/T 17946−2008).[1] Among them, sweet-type Chinese rice wine is a very special type due to its unique flavor characteristics.[2]

Sweet-type Chinese rice wine is produced following some traditional and specific processes.[2] It is typically fermented from glutinous rice with "wheat Qu" as a saccharifying agent and yeast (*Saccharomyces cerevisiae*) as a fermentation starter.[2] First, Chinese rice wine fermentation mash is prepared by mixing cooked rice, wheat Qu, seed mash (yeast), and water in a ceramic vat.[3] Wheat Qu is a kind of mold culture that spontaneously develops on raw wheat and accumulates amount of protease, amylase, and other enzymes. It was used as a saccharifying agent to degrade the starch in rice into sugar during Chinese rice wine fermentation.[4−6] After about 24 h of saccharification and fermentation at 28−32 °C, the residual sugar and ethanol contents in fermentation mash are approximately 150 g/L and 4% (v/v), respectively. Then the fermentation is stopped by adding natural Chinese rice wine spirits [containing 50% (v/v) ethanol] to obtain a fermentation mash containing approximately 21% (v/v) ethanol. After this, the fermentation mash is transferred to small pottery jars to carry out postfermentation for approximately 5 months. During the postfermentation, enzymes in the fermentation mash will continuously degrade the starch into sugar.[2] At the end of the postfermentation, the residual sugar and ethanol contents are approximately 250 g/L and 19% (v/v), respectively.[7] After fermentation, fresh Chinese rice wine is separated from solids in the fermentation mash by filtration. The fresh Chinese rice wine is then sterilized by thermal treatment (85−95 °C) and maturated in a sealed pottery jar at ambient temperature for more than 3 years before bottling. These specific fermentation and maturation procedures lead to formation of the typical and characteristic aroma of sweet-type Chinese rice wine.

Although it is one of the most popular Chinese rice wines, very few studies have been carried out on sweet-type Chinese rice wine. Shen et al.[8] studied the volatile components of sweet-type Chinese rice wine for the first time in 1986. In this work, volatile compounds in a sweet-type Chinese rice wine were isolated by simultaneous distillation−extraction (SDE) and further identified by gas chromatography−mass spectrometry (GC-MS). A total of 43 volatile compounds were identified, and the majority of them included hydrocarbons, alcohols, aldehydes, ketones, esters, and acetals. This same group of authors also compared the volatile compound differences between aged and unaged sweet-type Chinese rice wine.[9] Wei et al.[10] have recently published a paper studying the effects of ultrahigh pressure treatment on the volatile compositions of sweet-type Chinese rice wine. However, there are no reports carried out on the aroma composition of sweet-type Chinese

**Received:** July 1, 2013
**Revised:** September 4, 2013
**Accepted:** September 12, 2013
**Published:** September 12, 2013

dx.doi.org/10.1021/jf402867m | *J. Agric. Food Chem.* 2013, 61, 9712−9718

rice wine. Volatile compounds identified in sweet-type Chinese rice wine are commonly found in alcoholic beverages, but it is not clear which ones are the most important in characterizing its unique aroma.

Although volatile composition in alcoholic beverages, such as wine, beer, Chinese liquor, etc., are extremely complex, only a few of those volatiles, which are known as key odorants, obviously contribute to the overall aroma.[11,12] So the identification of the key aroma compounds from the complex mixture of volatile components is the most important task in flavor analysis.[13] GC-olfactometry (GC-O) on serial dilutions of the aroma extract, such as aroma extract dilution analysis (AEDA), is one of the most frequently used methods for screening of important aroma compounds in food.[14−16] However, an investigation of the key aroma compounds in Chinese rice wine carried out by AEDA could not be found in the literature.

Therefore, the aims of this research were (i) to characterize the aroma profile of sweet-type Chinese rice wine by sensory evaluation, (ii) to identify the key aroma compounds in sweet-type Chinese rice wine by AEDA, and (iii) to study the quantitative differences in sotolon (one of the key aroma compounds identified in AEDA) between different types of Chinese rice wines through the application of Lichrolut-EN solid-phase extraction coupled with microvial insert large volume injection technique.

## ■ MATERIALS AND METHODS

**Chemicals.** 3-Methylbutanal (97%), benzaldehyde (≥99%), furfural (≥99%), 5-methyl-2-furfural (≥98%), phenylacetaldehyde (≥90%), 2-methylpropanol (99.5%), 3-methylbutanol (99%), 2-phenylethanol (≥99.0%), ethyl acetate (≥99.5%), ethyl 2-methylpropanoate (99%), ethyl butanoate (99%), ethyl 3-methylbutanoate (98.0%), 3-methylbutyl acetate (≥99%), ethyl hexanoate (≥99%), ethyl 2-phenylacetate (≥98%), acetic acid (≥99.7%), 2-methylpropanoic acid (99%), butanoic acid (≥99%), 3-methylbutanoic acid (99%), 2,3-butanedione (97%), 1-octen-3-one (50.0 wt % in 1-octen-3-ol), acetophenone (≥99.0%), dimethyl disulfide (≥99%), dimethyl trisulfide (≥98%), 2,3,5,6-tetramethylpyrazine (≥98%), 2-acetylpyrrole (≥98%), 2-methoxyphenol (guaiacol, 98%), phenol (≥99%), 4-ethylguaiacol (4-ethyl-2-methoxyphenol, ≥98%), 4-vinylguaiacol (4-ethenyl-2-methoxyphenol, ≥98%), vanillin (4-hydroxy-3-methoxybenzaldehyde, 99%), acetovanillone [1-(4-hydroxy-3-methoxyphenyl)-ethanone, ≥98%], γ-nonalactone (≥98%), γ-dodecalactone (≥98.0%), sotolon [3-hydroxy-4,5-dimethylfuran-2(5*H*)-one, ≥98.0%], geosmin (*trans*-1,10-dimethyl-*trans*-9-decalol, 99%, 2 mg/mL in methanol), 4-(4-methoxyphenyl)-2-butanone (≥99.0%), dichloromethane (≥99.8%, HPLC grade), and methanol (≥99.9%, HPLC grade) were obtained from Sigma−Aldrich China Co. (Shanghai, China). Dichloromethane was freshly distilled before use. Lichrolut EN resins and Lichrolut EN solid-phase extraction (SPE) cartridges (200 mg, 3 mL) were supplied by Merck (Darmstadt, Germany). Analytical-grade anhydrous sodium sulfate, sodium chloride, lactic acid, and sodium hydroxide were purchased from China National Pharmaceutical Group Corp. (Shanghai, China). Pure water was obtained from a Milli-Q purification system (Millipore, Bedford, MA).

**Chinese Rice Wine Sample.** Seven bottled Chinese rice wines of four different types were provided as a gift by various Chinese rice manufacturers. Detailed information about these Chinese rice wine samples is shown in Table 2. Among them, YH, SKM, SN, and FG were used for sensory analysis. FG is one of the most typical and famous sweet-type Chinese rice wines and was used for GC-O analysis.

**Chinese Rice Wine Sensory Analysis.** The sensory panel was composed of 26 males and 9 females, 26−63 years of age. All of them were national Chinese rice wine tasters and had long-time experience of Chinese rice wine sensory evaluation. The sensory analysis was carried out according to previous reports in a sensorial analysis room at 20 °C.[17,18] Three specific training sessions were carried out. In the first one, descriptive terms were generated for four types of Chinese rice wines by panelist. In session two, different aroma standards were presented and discussed by the panel. From this session, seven aroma terms were selected for further descriptive analysis. In session three, panelists scored the intensity of each attribute on a seven-point scale from 1 (very weak) to 7 (very strong). Seven aroma terms were defined as the following aroma: 3-methylbutanol for alcoholic note, caramel for caramel-like note, ethyl 3-methylbutanoate for fruity note, 4-ethylguaiacol for smoky note, "wheat Qu" aroma extract for Qu aroma note, 2-phenylethanol for honey note, and 4-vinylguaiacol for herb note. After the training, different types of Chinese rice wine samples were evaluated by the panel. The respective Chinese rice wine samples (20 mL) were poured into a glass cup at 20 °C and presented in coded form. The data processed were an average of the scores from different panelists. The sensory data were analyzed by one-way analysis of variance (ANOVA) by use of SPSS 15.0 (SPSS Inc., Chicago, IL).

**GC-O Analysis.** *Preparation of Aroma Concentrate of FG Chinese Rice Wine.* The aroma compounds in FG Chinese rice wine were extracted by a SPE cartridge (0.8 cm internal diameter, 12 mL internal volume, Sigma−Aldrich, Shanghai, China) packed with 1 g of LiChrolut-EN resins. Before use, the cartridge was conditioned with 10 mL of dichloromethane, 10 mL of methanol, and 10 mL of pure water. A sample of 100 mL of FG Chinese rice wine (to which 15 g of sodium chloride had been previously added) was passed through the conditioned LiChrolut-EN cartridge at a rate not greater than 2 mL/min. After the sample was loaded, the cartridge was washed with 20 mL of pure water, dried by letting the air passing through it (−50 kPa, 20 min), and eluted with 20 mL of dichloromethane. The aroma extract was dried with anhydrous sodium sulfate and slowly concentrated to 2 mL and then to 0.2 mL under a stream of pure $N_2$. This concentrate was labeled as FG and stored at −20 °C before analysis.

*GC-O Analysis.* GC-O analysis was performed on an Agilent 6890 gas chromatograph equipped with an Agilent 5975 mass-selective detector (MSD) and a sniffing port (ODP 2, Gerstel, Germany). Samples were separated using a DB-FFAP column (60 m × 0.25 mm i.d., 0.25 μm film thickness, Agilent, Torrance, CA) and a DB-5 column (30 m × 0.25 mm i.d., 0.25 μm film thickness, Agilent, Torrance, CA). Helium was used as carrier gas at constant flow rate of 2 mL/min. The column effluent was split 1:1 into the MSD and the sniffing port via two deactivated and uncoated fused silica capillaries (0.1 mm i.d.). Sample (1 μL) was injected into the GC injector in splitless mode. The GC injector temperature was 230 °C. The oven temperature was programmed at 40 °C and held for 2 min, and then increased 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 the GC-O study. For each analysis, the sniffing time was 45 min and the capillary, which was connected with the sniffing port, was kept at 250 °C. Analyses were repeated two times by each panelist.

*Aroma Extract Dilution Analysis.* Aroma extract of the FG Chinese rice wine was stepwise diluted with dichloromethane at 1:1 ratio.[19] Each dilution was submitted to GC-O analysis on the DB-FFAP column until no odorant could be detected. The flavor dilution (FD) factor of each compound was determined as the maximum dilution at which the odorant could be perceived. The identification of the odorants was carried out by comparison of their odors, mass spectra, and retention index on both DB-FFAP and DB-5 columns with those of pure reference compounds.

**Quantitation of Sotolon in Chinese Rice Wine.** The concentration of sotolon in Chinese rice wine samples was determined by solid-phase extraction followed by direct microvial insert thermal desorption/GC-MS analysis. This method was developed according to a previously reported method with modification.[20]

*Sotolon Extraction via LiChrolut-EN Solid-Phase Extraction.* Ten microliters of internal standard [4-(4-methoxyphenyl)-2-butanone, boiling point 152 °C, 80 mg/L in ethanol] and 3 g of sodium chloride were first mixed with 20 mL of Chinese rice wine sample. Then the mixture was loaded onto a LiChrolut-EN cartridge (200 mg, 3 mL). The SPE cartridge had been previously conditioned with 6 mL of dichloromethane, 6 mL of methanol, and finally with 6 mL of Milli Q water. After the sample was loaded, the cartridge was washed with 10 mL of water and dried with air. Then, the cartridge was eluted with 5 mL of dichloromethane. The elution was dried with anhydrous sodium sulfate, concentrated to 0.2 mL under a stream of pure $N_2$, and stored at −20 °C until analysis.

*Microvial Insert Large Volume Injection.* Twenty microliters of sample was loaded into a 200 $\mu$L glass microvial insert. Then the insert was transferred into the thermal desorption unit (TDU, Gerstel, Germany) by a multipurpose autosampler (MPS 2, Gerstel, Germany). A programmed temperature vaporizer injector (PTV, CIS4, Gerstel, Germany) with a CIS liner packed with 2 cm of Tenax was used in the system. The initial temperature of the TDU was 35 °C. After the sample was loaded, the TDU was programmed at a rate of 300 °C/min to a final temperature of 230 °C with a 3 min hold. The TDU injection was in splitless mode during thermal desorption, while the CIS4 was in solvent vent mode with a venting flow of 60 mL/min for 4.7 min, at a venting pressure of 157 kPa. After the solvent vent, the PTV was switched to splitless mode. The initial temperature of PTV was kept at 30 °C for 0.2 min and then ramped at a rate of 10 °C/s to a final temperature of 230 °C, with a 5 min hold.

*GC-MS Analysis.* GC-MS analysis was carried out on an Agilent 6890 GC equipped with an Agilent 5973 mass-selective detector (MSD). The separations were carried out on a DB-FFAP column (60 m × 0.25 mm i.d., 0.25 $\mu$m film thickness, Agilent, Torrance, CA). The oven temperature was initially held at 40 °C for 2 min, then raised to 230 °C at 5 °C/min and held for 10 min. The data acquisition was in the selective ion monitoring mode (SIM, ionization energy =70 eV). The ions monitored were $m/z$ 83, 128, and 178. Ions 178 and 83 were used to quantitate 4-(4-methoxyphenyl)-2-butanone and sotolon, respectively. Ion 128 was used to confirm the presence of sotolon.

*Calibration Curve and Method Validation.* Sotolon standard compound was accurately weighed and dissolved in absolute ethanol and then was mixed and diluted with synthetic Chinese rice wine [13% (by volume) ethanol−water solution with 5.0 g/L lactic acid, pH = 4.0] to obtain a range of concentrations. Internal standard (10 $\mu$L) was added to each working solution and then analyzed by SPE/GC-MS. The calibration curve was built up by plotting the response ratio of standard sotolon and internal standard against the concentration ratio. The limits of quantitation (LOQ) and detection (LOD) were estimated as the analyte concentrations of a standard that produced a signal-to-noise ratio of 10 and 3 times, respectively. The reproducibility of the method was determined by means of replicated analysis of a given Chinese rice wine (HD) in four days. Known amounts of sotolon were spiked into YH, SKM, SN, and FG Chinese rice wine, and the spiked samples were analyzed by the methods described above. The recovery of sotolon in different Chinese rice wine matrices was calculated by the ratio $[(C_1 - C_0)/C_2] \times 100$, where $C_0$ is the concentration of determined amount before spiking, $C_1$ is the concentration of determined amount after spiking, and $C_2$ is the concentration of spiked amount. Triplicate analyses were performed for each sample.

## ■ RESULTS AND DISCUSSION

**Chinese Rice Wine Sensory Analysis.** The aroma characteristics of four typical Chinese rice wines were described by a sensory panel of national Chinese rice wine tasters. As shown in Figure 1, alcoholic, caramel-like, fruity, smoky, honey, herb, and Qu aroma-like odor descriptors were chosen. Qu aroma-like was a specific term to describe the aroma generating from wheat Qu used in Chinese rice wine fermentation.[6] Among these odor descriptors, alcoholic was the term with the highest scores, while no statistical difference of this descriptor was found among these four types of Chinese rice wine. This result suggested that alcoholic odor was the generic characteristic of Chinese rice wine. However, scores of the other odor descriptors showed significant variation among the four types of Chinese rice wine. Statistical analysis showed that the caramel-like term was the most discriminative term among these four types of Chinese rice wine. Sweet-type Chinese rice wine (FG) had the highest score for the caramel-like note, followed by semisweet-type Chinese rice wine (SN). The significantly higher score of the caramel-like term for sweet-type Chinese rice wine suggested that it might be the distinctive aroma characteristic of this wine.

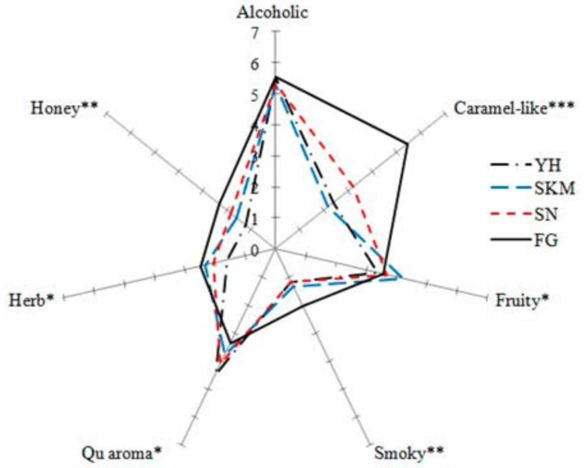

**Figure 1.** Odor profiles of four types of Chinese rice wine. Significance is indicated at $^*p < 0.1$, $^{**}p < 0.05$, and $^{***}p < 0.01$.

**Identification of Key Aroma Compounds in FG Chinese Rice Wine by Aroma Extract Dilution Analysis.** The volatiles were extracted from a sweet-type Chinese rice wine (FG) by SPE with Lichrolut EN resins. The extract showed the typical aroma profile of the FG sample, when it was dripped in a filter paper for sensory evaluation.

GC-O was applied to characterize the aroma compounds in the FG aroma extract, and a total of 46 aroma regions could be detected in the original extract. To evaluate the key aroma compounds in FG Chinese rice wine, AEDA was applied to the aroma extract of FG, and 39 aroma regions were detected with FD factor higher than 8 (Table 1, Figure 2). Among them, 37 aroma regions were further identified by comparison of odor description, retention indices, and mass spectra with those of pure reference compounds.

AEDA and aroma compounds identification results showed that 2- and 3-methylbutanol (10) and sotolon (36) were detected as having the highest FD factor of 1024. Sotolon, exhibiting a caramel-like/seasoning-like note, was identified in Chinese rice wine for the first time. It should be mentioned that the microvial insert large volume injection technique was necessary to get a satisfactory mass spectrum of this odorant for identification. Sotolon is a well-known powerful odorant that contributes to the characteristic aromas of various foods,[21−24] due to its low odor threshold (9 $\mu$g/L in wine)[18] and unique odor note. The highest FD factor of sotolon (caramel-like/seasoning-like) detected in FG rice wine suggested it might contribute to the caramel-like aroma of sweet-type Chinese rice wine. Abhexon [5-ethyl-3-hydroxy-4-methyl-2(5*H*)furanone], the ethyl analogue of sotolon, was always found together with sotolon in yellow wines, Sauterne wines, and gueuze beers.[25−27] However, we could not detect this compound in Chinese rice wine. Compounds 2- and 3-methylbutanol, exhibiting an alcoholic/nail polish-like note, were the major aroma

**Table 1. Most Odor-Active Volatiles (FD ≥ 8) in Sweet-type Chinese Rice Wine FG**

| | RI[a] | | | | | |
|---|---|---|---|---|---|---|
| region[b] | DB-FFAP | HP-5 | odorant | odor description | FD factor | ID basis[c] |
| 1 | 915 | 664 | 2- and 3-methylbutanal | malty | 64 | RI, MS, odor |
| 2 | 957 | nd | ethyl acetate | solvent-like | 128 | RI, MS, odor |
| 3 | 968 | 763 | ethyl 2-methylpropanoate | fruity, sweet | 32 | RI, MS, odor |
| 4 | 996 | nd | 2,3-butanedione | buttery, cream | 256 | RI, MS, odor |
| 5 | 1048 | 810 | ethyl butanoate | fruity, sweet | 32 | RI, MS, odor |
| 6 | 1075 | 863 | ethyl 3-methylbutanoate | fruity | 128 | RI, MS, odor |
| 7 | 1080 | 784 | dimethyl disulfide | rotten cabbage-like | 256 | RI, MS, odor |
| 8 | 1093 | nd | 2-methylpropanol | solvent-like | 8 | RI, MS, odor |
| 9 | 1130 | 873 | 3-methylbutyl acetate | fruity, banana-like | 64 | RI, MS, odor |
| 10 | 1209 | 769 | 2- and 3-methylbutanol | alcoholic, nail polish-like | 1024 | RI, MS, odor |
| 11 | 1238 | 1003 | ethyl hexanoate | fruity, sweet | 16 | RI, MS, odor |
| 12 | 1309 | 985 | 1-octen-3-one | mushroom-like | 16 | RI, MS, odor |
| 13 | 1345 | 931 | 2-acetyl-1-pyrroline[d] | cooked rice, popcorn-like | 512 | RI, odor |
| 14 | 1385 | 979 | dimethyl trisulfide | rotten cabbage-like | 512 | RI, MS, odor |
| 15 | 1438 | nd | unknown | peanuts-like | 128 | odor |
| 16 | 1451 | nd | acetic acid | vinegar-like | 256 | RI, MS, odor |
| 17 | 1476 | 860 | furfural | almond-like | 32 | RI, MS, odor |
| 18 | 1483 | 1093 | 2,3,5,6-tetramethylpyrazine | roasted | 8 | RI, MS, odor |
| 19 | 1541 | 970 | benzaldehyde | bitter almond-like | 256 | RI, MS, odor |
| 20 | 1562 | 791 | 2-methylpropanoic acid | rancid, acidic | 64 | RI, MS, odor |
| 21 | 1590 | 975 | 5-methyl-2-furfural | roasted | 8 | RI, MS, odor |
| 22 | 1600 | nd | unknown | cucumber-like | 16 | odor |
| 23 | 1629 | nd | butanoic acid | rancid | 64 | RI, MS, odor |
| 24 | 1660 | 1053 | phenylacetaldehyde | flowery | 64 | RI, MS, odor |
| 25 | 1667 | 880 | 2- and 3-methylbutanoic acid | rancid, acidic | 128 | RI, MS, odor |
| 26 | 1669 | 1075 | acetophenone | flowery | 8 | RI, MS, odor |
| 27 | 1791 | 1255 | ethyl 2-phenylacetate | sweet | 8 | RI, MS, odor |
| 28 | 1849 | 1412 | geosmin | earthy, moldy | 256 | RI, MS, odor |
| 29 | 1870 | 1099 | guaiacol | smoky, phenolic | 128 | RI, MS, odor |
| 30 | 1921 | 1113 | 2-phenylethanol | flowery, honey-like | 512 | RI, MS, odor |
| 31 | 1985 | nd | 2-acetylpyrrole | nutty | 8 | RI, MS, odor |
| 32 | 2012 | 982 | phenol | phenolic | 16 | RI, MS, odor |
| 33 | 2039 | 1297 | 4-ethylguaiacol | smoky | 64 | RI, MS, odor |
| 34 | 2049 | 1375 | γ-nonalactone | coconut-like, peach-like | 128 | RI, MS, odor |
| 35 | 2208 | 1335 | 4-vinylguaiacol | spicy, clove-like | 64 | RI, MS, odor |
| 36 | 2214 | 1093 | sotolon | caramel-like, seasoning-like | 1024 | RI, MS, odor |
| 37 | 2375 | n.d. | γ-dodecalactone | peach-like | 16 | RI, MS, odor |
| 38 | 2583 | 1392 | vanillin | vanilla-like | 512 | RI, MS, odor |
| 39 | 2676 | nd | acetovanillone | vanilla-like | 8 | RI, MS, odor |

[a] RI = retention index on different stationary phases; nd = not determined. [b] See Figure 2. [c] Identification based on RI (retention index) or MS (mass spectrometry) or odor description. [d] Tentatively identified by comparison of odor description and RI with literature.

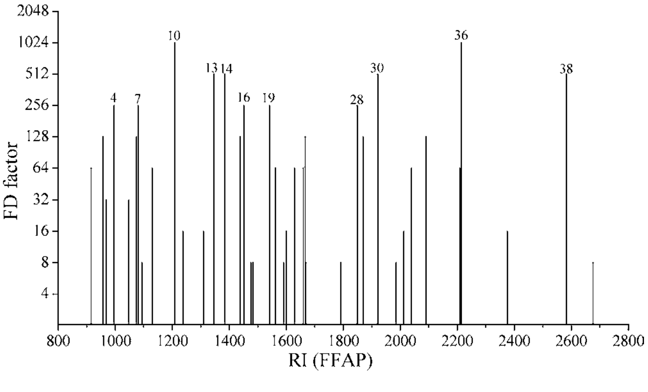

**Figure 2.** Flavor dilution (FD) chromatogram of volatile fractions isolated from sweet-type Chinese rice wine FG.

compounds found in Chinese rice wine previously.[1,28] They are the main byproducts generated by yeast cells during growth and fermentation.[29,30]

Four odor regions detected with FD factor of 512 were regions 13 (cooked rice/popcorn-like), 14 (rotten cabbage-like), 30 (flowery/honey-like), and 38 (vanilla-like). Among them, odor regions 14, 30, and 38 were identified as dimethyl trisulfide, 2-phenylethanol, and vanillin, respectively. However, odor region 13 could not be identified by GC-MS duo to the interferential peak of ethyl lactate. Based on its strong "cooked rice/popcorn-like" odor, 2-acetyl-1-pyrroline was suggested as one possibly responsible for this aromatic region. This hypothesis was supported by the comparison of RIs in both chromatographic columns with literature reported previously.[31,32] However, as there is no commercial reference standard of 2-acetyl-1-pyrroline, we could not confirm this identification. Since this compound has a very low odor

Table 2. Concentrations and Odor Activity Values of Sotolon in Several Bottled Chinese Rice Wines ($n$ = 3)

| code | sample name | sample type | aging period (years) | total sugar (g/L) | ethanol, % (v/v) | sotolon ($\mu$g/L) | OAV[a] |
|---|---|---|---|---|---|---|---|
| YH | Guyuelongshan Yuanhong | dry | 3 | 9.1 | 16.5 | 43.49 ± 2.53 | 5 |
| SKM | Jinfeng Shikumen | semidry | 4 | 32.3 | 12 | 35.93 ± 3.67 | 4 |
| HD | Guyuelongshan Huadiao | semidry | 10 | 36.9 | 15 | 72.31 ± 2.97 | 8 |
| SN | Guyuelongshan Shanniang | semisweet | 3 | 85.8 | 15 | 195.21 ± 11.37 | 22 |
| ZYH | Guyuelongshan Zhuangyuanhong | semisweet | 4 | 65.2 | 13 | 136.37 ± 9.19 | 15 |
| FG | Danyang Fenggang | sweet | 10 | 274.4 | 13 | 526.17 ± 28.83 | 58 |
| XX | Guyuelongshan Xiangxue | sweet | 3 | 184.4 | 19 | 467.35 ± 41.29 | 52 |

[a] Odor activity values (OAV) were calculated by dividing the concentrations by odor threshold value (9 $\mu$g/L) of sotolon determined by Campo et al.[18] The matrix was a 10% water/ethanol mixture containing 5 g/L tartaric acid at pH 3.2.

threshold value (0.053 $\mu$g/L in water),[33] it had been suggested as a key aroma compound in cooked rice.[34,35] Since rice is the main raw material used for Chinese rice wine fermentation, 2-acetyl-1-pyrroline might have originated from the cooked rice during the Chinese rice wine manufacture. Dimethyl trisulfide (14, rotten cabbage-like) was an important aroma compound identified in Chinese rice wine previously. Due to its low odor threshold value (0.18 $\mu$g/L, in sake),[24] dimethyl trisulfide has been quantitated previously with a very high odor activity value in Chinese rice wine.[1] The interaction between dimethyl trisulfide and sotolon has been investigated in aged Japanese sake, which showed that the caramel/burnt odor of sake could be increased by the addition of dimethyl trisulfide.[24]

In addition, 2,3-butanedione (4, buttery/cream-like), dimethyl disulfide (7, rotten cabbage-like), acetic acid (16, vinegar-like), benzaldehyde (19, bitter almond-like), and geosmin (28, earthy/moldy), were also suggested as key contributors to the overall aroma of FG Chinese rice wine due to their high FD factor (256) (Table 1).

**Quantitative Analysis of Sotolon in Chinese Rice Wine.** The AEDA of Chinese rice wine (FG) showed that sotolon had the highest FD factor and might contribute to the caramel-like characteristic of sweet-type Chinese rice wine. Quantitative determination was needed to verify the aroma contribution of sotolon to Chinese rice wine. However, the MS signal response of sotolon was too weak to get satisfying quantitative signal response by normal injection (1 $\mu$L). Large sample size or additional sample cleanup procedures were always needed to get satisfying signal response during analysis of this compound.[23,36] In this study, a sensitive method based on Lichrolut-EN solid-phase extraction coupled with the microvial insert large volume injection technique was developed to quantitate trace amount of sotolon in Chinese rice wine samples. By using this method, the injection volume was increased to 20 $\mu$L and the nonvolatile interferences in the extract could be eliminated, which were beneficial to improving the method sensitivity.

Calibration with seven levels of standards in different concentrations produced a linear response from 3.5 to 916.67 $\mu$g/L (slope = 0.3062, intercept = 0.0359, $R^2$ = 0.993). The limit of quantitation (LOQ, signal-to-noise ratio = 10) of sotolon was 1.77 $\mu$g/L, and the limit of detection (LOD, signal-to-noise ratio = 3) was 0.53 $\mu$g/L. This value was well below the odor threshold value of sotolon (9 $\mu$g/L). In order to examine the accuracy of the method in different Chinese rice wine matrices, a recovery test was performed by spiking four type of Chinese rice wine samples with sotolon standards. The quantitative results showed good recovery ranging from 75.39% to 91.52%. The reproducibility of this method was investigated by analysis of a single Chinese rice wine sample (HD) in four different days. The coefficient of variation was calculated as 7.3%. These results indicated that the method developed in this study was adequate to quantitate the concentration of sotolon in Chinese rice wine.

The developed method was used to quantitate the concentration of sotolon in different Chinese rice wine samples (Table 2). The amount of sotolon in seven Chinese rice wine samples ranged from 35.93 to 526.17 $\mu$g/L. The sotolon content of all samples was above its odor threshold (9 $\mu$g/L), which highlighted the important contribution of sotolon to the overall aroma of Chinese rice wine. However, as sotolon can cause different odor impressions, such as seasoning-like, caramel-like, curry-like, or spicy,[21] it might not be the only aroma compound responsible for the caramel-like note of Chinese rice wine. Comparison of the sotolon contents in different types of Chinese rice wines showed that sweet-type Chinese rice wines had the highest concentrations of sotolon (FG, 526.17 $\mu$g/L, and XX, 467.35 $\mu$g/L), followed by semisweet Chinese rice wines (SN, 195.21 $\mu$g/L, and ZYH, 136.37 $\mu$g/L). Dry and semidry type Chinese rice wines had the lowest contents of sotolon (Table 2). These differences were consistent with the sensory evaluation results that the sweet-type Chinese rice wine (FG) had a much stronger caramel-like note than other types of Chinese rice wines. There are many mechanisms that can possibly explain the formation of sotolon in foodstuffs.[21,37] Among them, the formation of sotolon by aldol condensation between acetaldehyde and 2-ketobutyric acid produced from threonine is well-studied in wines.[37,38] In sweet wines, several reports have suggested that the formation of sotolon might be related to sugar degradation.[39−41] In this study, the highest concentration of sotolon was found in sweet-type Chinese rice wines with the highest total sugar content, indicating that its formation might be related to sugar degradation. However, more studies are needed to explain which mechanisms are responsible for sotolon formation in Chinese rice wine.

In conclusion, the results obtained in this study showed that a caramel-like odor was the distinctive aroma characteristic of sweet-type Chinese rice wine. Sotolon, 2- and 3-methylbutanol, 2-acetyl-1-pyrroline, dimethyl trisulfide, 2-phenylethanol, and vanillin were suggested to be the key odorants in sweet-type Chinese rice wine by aroma extract dilution analysis. Among them, sotolon was identified for the first time in Chinese rice wine and might contribute to the caramel-like note of sweet-type Chinese rice wine. Solid-phase extraction coupled with microvial insert large volume injection technique is a sensitive method to quantitate trace amounts of sotolon in Chinese rice wine. The amounts of sotolon in Chinese rice wine samples were quantitated at levels obviously higher than its odor threshold. Sweet-type Chinese rice wines had significantly higher concentrations of sotolon than other types of Chinese rice wine. This was consistent with the sensory evaluation results carried out for four typical Chinese rice wines. Although further work is needed to investigate the formation mechanism of sotolon in Chinese rice wine, this work highlights the important aroma contribution of sotolon for Chinese rice wine.

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### Funding

This work was supported by grants from the National High Technology Research and Development Program of China (863 Program; 2013AA102108), and 111 Program of Introducing Talents.

### Notes

The authors declare no competing financial interest.

## ACKNOWLEDGMENTS

We thank the panelists from the national Chinese rice wine tasting committee. We particularly thank Professor Michael Qian for helpful suggestions in quantitative analysis.

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