

Characterization of Geosmin as Source of Earthy Odor in Different Aroma Type Chinese Liquors

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S Supporting Information

ABSTRACT: Earthy odor is one of the most frequent and serious causes for the aroma deterioration in Chinese liquor, which causes a dirty and dusty impression. The odor in Chinese liquor is similar to that of rice husk, one kind of auxiliary material widely used as a filler in the distillation process. So it is experientially hypothesized that such odor may derive from rice husk. In this paper, the gas chromatography–olfactometry (GC–O) technique and gas chromatography–mass spectrometry (GC–MS) were used to discover and identify the characteristic odoriferous zone of Chinese liquor marked by earthy odor. Geosmin was found to be responsible for this odor. The levels of the compound in ten bottled liquors and thirty liquors aging for different years belonging to four different aroma types were determined by the optimized headspace–solid phase microextraction–gas chromatography–mass spectrometry (HS–SPME–GC–MS) method. Quantitative analysis of bottled liquor revealed the presence of geosmin in all aroma type liquors with concentrations ranging from 1.10 $\mu\text{g/L}$ to 9.90 $\mu\text{g/L}$, except for strong-aroma type liquor. Meanwhile in the aged liquors belonging to the same aroma type, geosmin was detected with significant concentrations and high odor activity values (OAVs) during different years of aging. However, geosmin was not detected in steamed rice husk nor in nonsteamed rice husk, which suggests that rice husk is not the origin of earthy odor in Chinese liquor, and there may be another origin of it during the brewing process.

KEYWORDS: Chinese liquor, earthy odor, geosmin, rice husk

INTRODUCTION

Chinese liquor is a traditionally indigenous distilled beverage with two thousand years' history and the most popular among alcoholic beverages in China, with a production exceeding 7,000,000 tons in 2010. Compared with other spirits, such as vodka, whisky and brandy, Chinese liquor has higher ethanol content (normally 40–55% by volume). Because of difference in manufacturing practices, the aroma profiles of various Chinese liquors are quite different. Based on aroma characteristics, Chinese liquors are mainly classified into the following categories: light-aroma type, sauce-aroma type, mixed-aroma type, and strong-aroma type. Chinese liquor is typically distilled from fermented sorghum. The sorghum used for liquor fermentation is cooked and then mixed with *Daqu* powder, which is the most widely used as a fermentation starter and substrate complex to initiate fermentations for the production of Chinese liquor culture. *Daqu* is made from wheat or a mixture of wheat, barley, and pea, and is rich in various microorganisms including bacteria, yeast, and fungi. Therefore, in *Daqu* complex enzyme systems are accumulated. Fermentation of Chinese liquor is typically carried out at 28–32 °C under anaerobic conditions in the solid state. After fermentation, a portion of rice husk used as filler is mixed into fermented sorghum to make it fluffy for distillation. The rice husk must be fresh and dry, with no rotten smell. Before being used, rice husk must be presteamed for about 30–40 min to get rid of off-odors and harmful substances. Finally the liquor is distilled out with steam, and aged in a china jar (sealed tightly) for more than 3 years at 15–25 °C.

The quality of Chinese liquor is mainly determined by its aroma and taste. Recent reports on the volatile compositions of

Chinese liquor have revealed its complex and typical characteristics. Zhu et al.¹ have identified 528 components in *Moutai* liquor using comprehensive two-dimensional gas chromatography/time-of-flight mass spectrometry, mainly consisting of esters, acids, alcohols, phenolic compounds, aldehydes, ketones, sulfur-containing compounds and acetals. Fan et al.² have identified and quantified 27 pyrazines in 12 typical commercial Chinese liquors. Most of those compounds contribute positive aroma, such as fruity, sweet, and baked aroma, to Chinese liquor.

Off-odor is one of the most serious problems that the Chinese liquor industry has to face, which has a significantly negative effect on the liquor's aroma and hence leads to economic losses. Up to now, several off-odors, including bran-like, earthy odor, mushroom-like, musty, feculent odor and so on, have been deemed as main sensory defects.^{3,4} Among these defects, earthy odor is most seriously and extensively detrimental to the aromatic quality in Chinese liquors, which, causing the dirty and dusty impression, is similar to a "rice husk-like odor". Thus, it is supposed that such odor may derive from rice husk, and then it is suggested that steaming rice husk for a longer time could decrease earthy odor. Yet it is not effective in actual operation.

Some compounds found in drinking water, as well as in wine, fish, and other food stuffs,^{5–8} have been described as having this odor, such as geosmin (*trans*-1,10-dimethyl-*trans*-9-decalol), 2-methylisoborneol, 2,4,6-trichloroanisole, 2,3,6-trichloroanisole,

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2,3,4-trichloroanisole, 2,3,5,6-tetrachloroanisole, pentachloroanisole and 2,4,6-tribromoanisole.⁹ However, rather little information has been reported about the compounds causing such odors in Chinese liquors. Moreover for the complex aromatic compounds and high alcohol content, it is difficult to discover and analyze the earthy odor in Chinese liquor.

In this study, special attention was drawn to identify the aroma active compound responsible for the earthy odor in Chinese liquor. To investigate the severity and possible origin of the aromatic compound, quantitative analyses were performed in rice husk and aged and bottled liquors of different aroma types.

MATERIALS AND METHODS

Chemicals. Geosmin (99%, 2 mg/mL in methanol) were purchased from Sigma-Aldrich (Shanghai, China). *L*-(-)-Menthol (98.0%, internal standard, IS) was purchased from Acros Organics (New Jersey, USA). Pentane and absolute alcohol were purchased from Tedia (Ohio, USA). Both analytes and IS spiking solutions were prepared in an alcohol solvent. All standard solutions were kept in the freezer at $-20\text{ }^{\circ}\text{C}$ in the dark. Synthetic liquor was a hydroalcoholic solution at 5 vol % of ethanol.

Sodium chloride, anhydrous sodium sulfate, hydrochloric acid, sodium hydroxide, and diethyl ether were purchased from China National Pharmaceutical Group Corporation (Shanghai, China).

Liquor and Rice Husk Samples. All Chinese liquor samples including bottled and aged liquor were supplied by corresponding cooperant distilleries. The studied liquors are *Laobaigan* liquor (LBG), *Xifeng* liquor (XF), *Langjiu* liquor (LJ), *Kouzijiao* liquor (KZJ), *Niulanshan erguotou* liquor (EGT), *Fenjiu* liquor (FJ), *Wuliangye* liquor (WLY), *Gubeichun* liquor (GBC), and *Jiannanchun* liquor (JNC), which are typical representatives of sauce-aroma type, strong-aroma type, mixed-aroma type, light-aroma type, and *Laobaigan* aroma type, respectively. All of these Chinese liquors were sampled in 2007, and stored at $4\text{ }^{\circ}\text{C}$ until analysis. The steamed and unsteamed rice husks were supplied by *Laobaigan* liquor distillery.

Extraction and Fractionation of Aroma in Chinese Liquor.

Aroma Extraction. LBG (300 mL, 67% ethanol by volume) was diluted to 10% ethanol by volume with deodorized water (deionized water was boiled for 5 min, and then cooled to room temperature). The diluted liquor sample was saturated with analytical-grade sodium chloride and extracted 3 times with 100 mL aliquots of pentane:diethyl ether (1:1) in a separatory funnel. All extracts were combined and slowly concentrated to 50 mL under a gentle stream of nitrogen. To facilitate GC–O and GC–MS analysis, the aroma extract of liquor was separated into acidic/water-soluble, neutral, and basic fractions, using a modified method of Fan and Qian.¹⁰ The specific method is shown in Figure S1 in the Supporting Information.

GC–O and Osme Analysis. GC–O analysis was performed on an Agilent 6890N GC coupled to an Agilent 5975 mass selective detector (MSD) and an olfactometer. The column carrier gas was helium, at a constant pressure. Half of the column flow was directed to the MSD, while the other half was directed to a heated sniffing port with a fused silica outlet splitter (Alltech Associates, Inc., Deerfield, IL). The samples were analyzed on a CP-Wax column (60 m length, 0.25 mm i.d., 0.25 μm film thickness, Varian Inc., Palo Alto, CA, USA) and a HP-5 column (60 m length, 0.25 mm i.d., 0.25 μm film thicknesses, Agilent Technology). Each concentrated fraction (1 μL) was injected in splitless mode. The oven temperature was held at $50\text{ }^{\circ}\text{C}$ for 2 min, then raised to $230\text{ }^{\circ}\text{C}$ at a rate of $6\text{ }^{\circ}\text{C}/\text{min}$, and held at $230\text{ }^{\circ}\text{C}$ for 30 min. Injector and detector temperatures were $250\text{ }^{\circ}\text{C}$. The GC–O analysis of each extract was performed by four panelists (2 males and 2 females). They were graduate students and researchers in the Laboratory of Brewing Microbiology and Applied Enzymology at Jiangnan University, whose ages were from 22 to 35. All the panelists were trained with extract of Chinese liquor for 30 h

over a period of 15 days. The panelists responded to the aroma intensity of the stimulus by using a 5-point scale ranging from 0 to 5; “0” was none, “3” was moderate, while “5” was extreme. The retention time, intensity value and aroma descriptor were recorded. Each extract was sniffed in replicate by each panelist. When a volatile compound was detected at least twice, this analyte was determined to be a declared aroma compound. The Osme values for aroma intensity were averaged for the eight analyses (four panelists, two times).

GC–MS Analysis. For the GC–MS analysis, an Agilent 6890N GC coupled to an Agilent 5975 mass selective detector (MSD) was used. The capillary column used was a CP-Wax column (60 m length, 0.25 mm i.d., 0.25 μm film thickness, Varian Inc., Palo Alto, CA, USA) and a HP-5 column (60 m length, 0.25 mm i.d., 0.25 μm film thicknesses, Agilent Technology). The injector temperature was $250\text{ }^{\circ}\text{C}$, and the splitless mode was used. The conditions were as follows: starting temperature $50\text{ }^{\circ}\text{C}$ (holding 2 min), then raised to $230\text{ }^{\circ}\text{C}$ at the rates of $6\text{ }^{\circ}\text{C}/\text{min}$ for HS–SPME and $4\text{ }^{\circ}\text{C}/\text{min}$ for GC–O, and held at $230\text{ }^{\circ}\text{C}$ for 30 min. The column carrier gas was helium with a purity of 99.9995% at a constant flow rate of 2 mL/min. The electron impact energy was 70 eV, and the ion source temperature was set at $230\text{ }^{\circ}\text{C}$. Full-scan acquisition was used in the ranges of masses (30–350 amu).

Identification of Earthy Odor. Mass spectra of unknown compounds were compared with those in the NIST05a.L (Agilent Technologies, Inc.). Then positive identification was achieved by comparing mass spectra, aromas, and retention indices (RIs) of the pure standards on different capillary columns, CP-Wax and HP-5. RIs of unknown compounds were calculated in accordance with a modified Kovats method for temperature programmed chromatography.

Olfactory Perception Threshold Measurement. Corresponding to ASTM E 679-04, the perception threshold of geosmin was determined by a test at seven concentration steps (a step factor of “3” was used) in 46 vol % hydroalcoholic solution (normally ethanol content of Chinese liquor is 40–55% by volume). The solutions were smelled by a 70-person jury. All of them were national liquor tasters and had prior sensory training and experience in Chinese liquor evaluation. The panelist started at the lowest concentration step, which should be two or three concentration steps below the estimated threshold. Each tested sample within the set of three was compared with the other two blank samples (46 vol % hydroalcoholic solution). The blank and test samples were encoded so that there was no visual, audible, tactile, or thermal difference between the samples other than code designators. The panelist indicated which of the three samples was different from the other two. If the panelist could not readily discriminate, a guess had to be made so that all data could be utilized. The series of each panelist’s judgments could be expressed by writing a sequence containing (0) for an incorrect choice or (+) for a correct choice arranged in the order of judgments of ascending concentrations of the added substance. The threshold concentration for the panelist was then the geometric mean of that concentration at which the last miss (0) occurred and the next higher concentration designated by a (+) produced separately by each panelist. Threshold of geosmin was derived by geometrical averaging of all the individual thresholds.

Sensory Verification of Earthy Odor in Chinese Liquor.

A quantity of the good-quality Chinese liquors including LBG, FJ and EGT containing no detectable geosmin were defined as the blank samples. The additions of 5 $\mu\text{g}/\text{L}$ and 50 $\mu\text{g}/\text{L}$ in blank samples were tested respectively. At each concentration step, two blank samples were available to the panelist. The blank and test samples were encoded. The panelists were asked to indicate which of the three samples was different from the other two and try to describe the different odor. The solutions were smelled by a 30-person jury. They were graduate students and researchers in the Laboratory of Brewing Microbiology and Applied Enzymology at Jiangnan University, whose ages were from 22 to 35. All of them had prior sensory training and experience in Chinese liquor evaluation.

Sample Preparation for SPME Extraction. Each liquor sample was diluted with fresh redistilled–deionized water to a final ethanol content of 5 vol % for the extraction of SPME. For rice husk, a 1 g sample was applied to ultrasonic treatment at 40 °C for 30 min after being soaked in distilled water three times (30 mL, 20 mL, 10 mL respectively). The supernatants were combined after centrifugation, and then the volatile of the combined supernatants was extracted by SPME.

HS–SPME Parameter. For the SPME, an automatic headspace sampling system (Multi Purpose Sample MPS 2 with a SPME adapter, from GERSTEL Inc., Baltimore, MD, USA) with a 50/30 μm DVB/CAR/PDMS fiber (2 cm, Supelco Inc., Bellefonte, PA, USA) was used for analyte extraction. A total of 8 mL of diluted sample was transferred to a screw-capped, straight-sided headspace vial with a 15 mL volume and spiked with 10 μL of L-(–)-menthol (internal standard, IS), 4 mg/L in ethanol. The diluted solution was saturated with NaCl. The vial was tightly capped with a Teflon-faced silicone septum. The samples were equilibrated at 60 °C for 5 min and extracted for 45 min at the same temperature under stirring (250 rpm, on for 20 s, off for 0 s). After extraction, the fiber was inserted into the injection port of the GC (250 °C) for 5 min to desorb the analytes. All analyses were made in triplicate.

Calibration of Standard Curve and Quantification of Sample. A calibration curve was derived by plotting the peak area ratios against the concentration ratios of geosmin (m/z 112) to IS (m/z 81). Amounts of geosmin were spiked into synthetic liquor (5 vol % ethanol/water solution), and then it was serially diluted with 5% (by volume) aqueous ethanol solution using a 1:1 dilution to yield concentrations in the range of 2.35×10^{-2} $\mu\text{g/L}$ to 3.00 $\mu\text{g/L}$. The IS spiked to those solutions was at the final concentration of 5 $\mu\text{g/L}$. Geosmin and IS in ethanol solutions were extracted with the SPME fiber and analyzed using GC–MS as described previously.

The liquor samples were diluted to 5 vol % alcohol content before being extracted with SPME fiber. Each sample was tested in triplicate ($n = 3$). The concentration of geosmin in the diluted solution was quantified by comparing the ratio of the peak areas with the calibration curve. Then the quantitative values multiplied the dilution ratio as the concentrations of geosmin in liquors.

The limit of detection (LOD, concentration for signal/noise = 3) and the limit of quantification (LOQ, concentration for signal/noise = 10) were calculated using the Chemstation data analysis software (Agilent Technologies).

A known amount of geosmin was added to the liquor for calculating recovery rate according to the following equation: recovery rate = (detected amount after addition – detected amount before addition) \times 100%/added amount.

RESULTS AND DISCUSSION

Identification of Earthy Odor in Different Extraction Fractions of Chinese Liquor. LBG liquor (bottled, 67% ethanol by volume) was selected as the GC–O sample due to its distinct earthy odor. At first, the aroma compounds of LBG were extracted, and then analyzed by GC–O directly. However, the compounds in the extract were complex, and it was difficult to perform GC–O analysis reliably and detect the earthy odor. So the extract was fractionated into acidic/water-soluble, neutral, and basic fractions as described previously. Then all the fractions were detected and identified by GC–O and GC–MS.

According to the GC–O results, the descriptor of aroma was classified into fruity, green, acidic, floral, herbal, roasted and earthy. Osme values of odorous zones with the same descriptor in each extract fraction were summed to make the Spider plot. The acidic/water-soluble fraction and basic fraction had a relatively simple composition. The number of the odorous zones

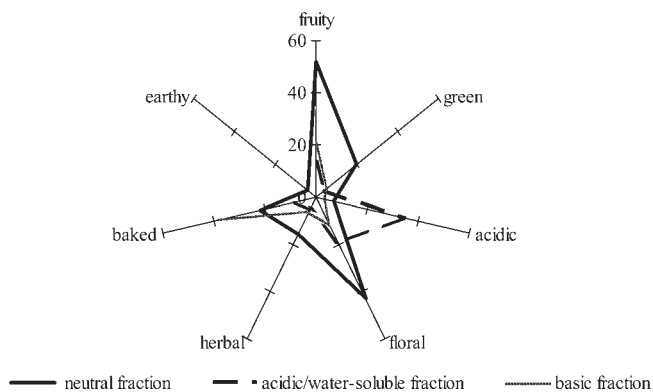


Figure 1. Spider plot of the aroma in neutral, acidic/water-soluble and basic fractions of Chinese liquor extract.

was 21 and 27 respectively. As Figure 1 shows, in the acidic/water-soluble fraction, most of them presented acidic and rancid odor. In the basic fraction fractionation, most of them presented baked and nutty odor. The earthy odor was not detected in the two fractions.

In neutral fraction, there were 67 odorous zones. Most of them contributed floral and fruity odor (see Figure 1). More importantly, one strong odorous zone with earthy odor was found during the retention time of 33–35 min on CP-Wax capillary column by GC–O analysis (see Figure S2 (A) in the Supporting Information). Looking into the chromatogram, the main peak in this zone was ethyl dodecanoate. The aroma was significantly different from the earthy odor. To confirm the actual existence of the compound with earthy odor in the neutral fraction, the same sample was performed on the HP-5 capillary column, with a different polar stationary phase compared to the CP-Wax capillary column. On HP-5 capillary column, the earthy odor appeared during 18.5–19.2 min (see Figure S2 (B) in the Supporting Information). In this zone, there was no interference with high peak. By screening through internal MS reference libraries, at the retention time of 18.7 min a mass spectrum was obtained having ions and abundances similar to those of geosmin (see Figure S3 in the Supporting Information). Back to the total ionic chromatogram performed on a CP-Wax column, we also found the main ion (m/z 112) of geosmin at the retention time of 34.5 min. Then neutral fraction was spiked with 5 $\mu\text{g/L}$ geosmin standard reagent performed on CP-Wax and HP-5 column respectively. Consistent RIs were obtained between the earthy zone and the geosmin standard solution on the two different polar columns. To our knowledge, not many researchers have studied this odor in Chinese liquor.

Verification of Geosmin as the Earthy Odor. To confirm the result we found, thirty panelists were invited to complete a simple sensory experiment as described herein in the Sensory Verification of Earthy Odor in Chinese Liquor section. The additions of 5 $\mu\text{g/L}$ and 50 $\mu\text{g/L}$ were tested respectively. For the distinct earthy odor, all the panelists correctly distinguished the samples spiked with geosmin at the final concentration of 50 $\mu\text{g/L}$. At the final concentration of 5 $\mu\text{g/L}$, 28 of 30 people perceived the spiked liquors, and 25 of 30 people identified the spiked samples for having the earthy off-odor note. These results showed that the intensity of earthy odor was related to the concentration of geosmin in Chinese liquor.

Olfactory Perception Threshold of Geosmin in Solution with High Level Alcohol Content. Geosmin is a well-known

Table 1. Linearity, Limit of Detection (LOD), Limit of Quantification (LOQ), Relative Recovery and Precision of the Quantitative Method Used in Different Aroma Type Liquors and Synthetic Liquor

| sample | R^2 | linear range ($\mu\text{g/L}$) | LOD (ng/L) | LOQ (ng/L) | concn ^a of spiking | | |
|------------------|--------|----------------------------------|------------|------------|-------------------------------|------------------|----------------------|
| | | | | | geosmin ($\mu\text{g/L}$) | rel recovery (%) | RSD ^b (%) |
| LBG | | | | | 0.36 | 82.74 | 7.97 |
| XF | | | | | 0.80 | 110.9 | 4.30 |
| LJ | 0.9997 | 2.34×10^{-2} –3.00 | 6.91 | 22.8 | 0.28 | 96.90 | 6.87 |
| FJ | | | | | 0.12 | 114.1 | 8.11 |
| synthetic liquor | | | | | 0.40 | 96.28 | 7.85 |

^a Concentration. ^b Relative standard deviation (RSD) = standard deviation/mean, $n = 3$.

Table 2. Concentration of Geosmin in Several Bottled Chinese Liquors Determined Using the HS–SPME Method ($n = 3$)

| aroma type | sample | alcohol content (vol %) | geosmin | | |
|-------------------|--------|-------------------------|---|----------------------|------------------|
| | | | mean concn ^a ($\mu\text{g/L}$) | RSD ^b (%) | OAV ^d |
| light-aroma type | LBG | 67 | 9.90 ± 1.18 | 11.9 | 90 |
| | XF | 55 | 8.74 ± 0.61 | 7.01 | 79 |
| | EGT | 50 | 2.55 ± 0.25 | 4.39 | 23 |
| | FJ | 44 | 1.10 ± 0.39 | 6.60 | 10 |
| sauce-aroma type | LJ | 54 | 3.04 ± 0.11 | 8.33 | 28 |
| mixed-aroma type | KZJ | 50 | 4.05 ± 0.07 | 9.67 | 37 |
| strong-aroma type | WLY | 52 | nd ^c | | |
| | GBC | 38 | nd | | |
| | JNC | 52 | nd | | |
| | YH | 46 | nd | | |

^a Mean concentration \pm SD ($n = 3$). ^b Relative standard deviation (RSD) = standard deviation/mean. ^c Not detectable. ^d Odor activity value (OAV) is calculated as the ratio between the mean concentration of geosmin in a sample and the odor threshold value of this substance.

microbial metabolite produced by several species of cyanobacteria, streptomycetes and some fungi,^{11–13} which is responsible for the characteristic smell of moist soil or freshly plowed earth¹⁴ and is mainly found in soil, but also in odor-polluted water, wheat grain, beets, apple juice, cheese, nut, cabbage, fish or wine.^{7,8,15–17} It presents with an undesirable musty or earthy odor even at very low concentration owing to an exceptionally low threshold for human detection (less than 10 ng/L). To study its influence on the aroma of Chinese liquor, we investigated the olfactory perception threshold of geosmin in solution with high level alcohol content according to the mentioned method in Materials and Methods. The olfactory perception threshold of geosmin was 110 ng/L in 46 vol % alcohol solution, which is the typical alcohol content in Chinese liquor. Darriet et al. found it was 10 ng/L in water, 80–90 ng/L in a neutral red wine, and 60–65 ng/L in neutral white wine.⁵ Since matrix effect can be expected for the sensory relevance of the compounds, it is reasonable that high alcohol content in Chinese liquor probably leads to higher odor thresholds than that in water and wine.

Quantification and OAV of Geosmin in Different Aroma Type Chinese Liquors. Generally speaking, HS–SPME is very simple to perform because its coating of polymer on a fused silica fiber combines extraction, concentration, and injection into a single process. Furthermore, this method requires a smaller sample and does not utilize organic solvents. Because of these advantages, we optimized the extraction mode, extraction time, dilution ratio and extraction temperature to develop a quantification method suitable for the analysis of geosmin in Chinese liquor. GC–MS was employed as separation and detection

system. The final extraction procedure was as follows: the samples were diluted to 5 vol % alcohol content, equilibrated at 60 °C for 5 min and extracted by a 50/30 μm DVB/CAR/PDMS fiber for 45 min at the same temperature under stirring. The selected ions (m/z 112 for geosmin, m/z 81 for IS) for quantification were well separated from the other volatile compounds (see Figure S4 in the Supporting Information). As shown in Table 1 and Figure S5 in the Supporting Information, the HS–SPME method yielded an excellent linear response for the calibration curve ($R^2 = 0.9997$) in a wide range from 2.34×10^{-2} to 3.00 $\mu\text{g/L}$. The correlation equation was the following: concentration ratio of geosmin/menthol = $0.2125 \times$ area ratio of geosmin/menthol + 0.0059. The limit of detection (LOD) and limit of quantification (LOQ) of the method were 6.91 ng/L and 22.8 ng/L, which indicated that the method was sensitive enough to detect and quantify geosmin in different aroma type Chinese liquors.

Ten samples of bottled Chinese liquor belonging to light-aroma type, sauce-aroma type, mixed-aroma type and strong-aroma type were analyzed to investigate the concentrations of geosmin using the HS–SPME method. Also odor activity values (OAVs) of geosmin in these liquors were calculated (concentrations and OAVs are listed in Table 2). OAV was calculated as the ratio between the concentration of geosmin in a sample and the odor threshold value of this substance. The value of olfactory perception threshold used for calculating OAVs was 110 ng/L in 46 vol % alcohol solution.

The concentrations of geosmin in different aroma type liquors varied between 1.10 and 9.90 $\mu\text{g/L}$, and the OAVs ranged from 10 to 90, thus indicating that geosmin is important and

Table 3. Concentration of Geosmin in Several Chinese Liquors at Different Vintage ($n = 3$)

| aroma type | sample | vintage ^a | alcohol content (%vol) | geosmin | | |
|------------------|--------|----------------------|------------------------|---|----------------------|------------------|
| | | | | mean concn ^b ($\mu\text{g/L}$) | RSD ^c (%) | OAV ^d |
| light-aroma type | LBG | 1999 | 64 | 4.08 \pm 0.24 | 5.78 | 37 |
| | | 1997 | 66 | 6.30 \pm 0.62 | 9.77 | 57 |
| | | 1995 | 65 | 3.04 \pm 0.04 | 1.31 | 28 |
| | | 1992 | 62 | 4.33 \pm 0.14 | 3.19 | 39 |
| | | 1987 | 65 | 5.16 \pm 0.35 | 6.75 | 47 |
| | | 1982 | 62 | 6.79 \pm 0.78 | 11.5 | 62 |
| | | 1970 | 58 | 8.81 \pm 1.21 | 13.8 | 80 |
| | 1965 | 58 | 6.45 \pm 0.37 | 5.78 | 59 | |
| | XF | 2001 | 45 | 4.15 \pm 0.27 | 6.50 | 38 |
| | | 1992 | 45 | 2.92 \pm 0.34 | 11.6 | 27 |
| 1986 | | 65 | 11.4 \pm 1.56 | 4.96 | 103 | |
| 1979 | | 62 | 20.3 \pm 2.51 | 12.4 | 184 | |
| mixed-aroma type | KZJ | 1957 | 69 | 3.06 \pm 0.30 | 9.84 | 28 |
| | | 2006 | 63 | 1.82 \pm 0.08 | 4.26 | 17 |
| | | 2004 | 65 | 2.62 \pm 0.37 | 14.2 | 24 |
| | | 2002 | 63 | 13.1 \pm 1.42 | 10.9 | 119 |
| | | 1999 | 67 | 5.13 \pm 0.86 | 16.7 | 47 |
| | | 1997 | 66 | 7.12 \pm 0.88 | 12.4 | 65 |
| | | 1992 | 64 | 5.39 \pm 0.30 | 5.51 | 49 |
| | | 1987 | 54 | 4.43 \pm 0.40 | 9.09 | 40 |
| | | 1982 | 68 | 9.29 \pm 0.33 | 3.55 | 84 |
| | | 1977 | 64 | 16.2 \pm 1.10 | 6.81 | 147 |
| sauce-aroma type | LJ | 2006 | 57 | 2.56 \pm 0.07 | 2.66 | 23 |
| | | 2005 | 57 | 2.29 \pm 0.26 | 11.5 | 21 |
| | | 2004 | 55 | 1.29 \pm 0.02 | 1.44 | 12 |
| | | 2003 | 55 | 2.75 \pm 0.04 | 1.36 | 25 |
| | | 2002 | 57 | 2.70 \pm 0.10 | 3.66 | 25 |
| | | 1997 | 56 | 3.77 \pm 0.02 | 0.45 | 34 |
| | | 1987 | 52 | 3.65 \pm 0.09 | 2.43 | 33 |
| | | 1977 | 53 | 3.79 \pm 0.34 | 8.94 | 34 |

^a Vintage, the year when the liquor was produced. ^b Mean concentration \pm SD ($n = 3$). ^c Relative standard deviation (RSD) = standard deviation/mean.

^d Odor activity value (OAV) is calculated as the ratio between the mean concentration of geosmin in a sample and the odor threshold value of this substance.

widespread, corresponding to the frequent presence of earthy odor in Chinese liquors. However, geosmin could not be detected in all the strong-aroma type liquors. Compared with the brewing technology of the other aroma type liquors, the most significant difference is the application of a special fermentor in strong-aroma type liquor making, the inside of which is coated with a layer of weak acidic fermentation mud made of clay, spent grain, bean cake powder and fermentation bacteria (*Clostridium* sp.). Recently, Hoefel et al. found that geosmin can be biodegraded by a flora comprising three Gram-negative bacteria¹⁸ and one Gram-negative bacterium isolated from the biofilm of a sand filter column.¹⁹ Siegmund et al.²⁰ found that available oxygen in apple juice has a significant influence on the *Streptomyces griseus* metabolic process and consequently on the formation rates of the off-odor compounds including geosmin. So during the process of the fermentation of strong-aroma type liquor, there may be some factors related to the special liquor-making technology inhibiting geosmin production or degenerating it.

Before bottling, the aged distillate is adjusted to the designated ethanol concentration and blended to ensure the quality of the finished product. To determine the process (blending, bottling

or fermentation) during which geosmin was introduced, the aged liquors belonging to light-aroma type, sauce-aroma type and mixed-aroma type were examined. The results showed that geosmin was still quantified in all the three aroma type liquors after several years of aging. In the aged liquors the concentrations of geosmin varied between 1.29 and 20.3 $\mu\text{g/L}$, and the OAVs range from 12 to 184 (Table 3). Moreover, geosmin was not detected in all the strong-aroma type liquors aged for different years (data not listed). Those results suggest that geosmin may be introduced during the fermentation process, not during the blending or bottling process. On the other hand, it indicates that the nature of geosmin is stable in acidic solutions (pH is 3.3–3.9 for Chinese liquor and 3.2–3.7 for wines). La Guerche and his co-workers also found that geosmin was relatively stable during alcoholic fermentation and storage, especially at the optimum temperature for aging wine (15 $^{\circ}\text{C}$),²¹ while the results were contrary to Gerber's initial findings, which indicated its rapid degradation in acidified aqueous solutions.¹⁴

Detection of Geosmin in Rice Husk. For a long time, it was experientially hypothesized that such earthy odor in Chinese liquor may derive from rice husk as rice husk has a similar odor. In

order to verify if rice husk is the origin of geosmin responsible for earthy odor in liquor, we set out to study this compound in steamed and nonsteamed rice husks. However, geosmin was not detected in steamed rice husk, nor in nonsteamed rice husk (data not listed). Moreover, rice husk is widely used in the distillation process of all aroma type liquor, including the strong-aroma liquor shown. But geosmin was not detected in all strong-aroma liquors as the previous quantitative results. Based on the above, it suggests that volatile substances of rice husk are not the origin of earthy odor in Chinese liquor, and there may be other origins of it during the brewing process. That is why steaming rice husk for a longer time does not result in the decrease of earthy odor in actual operation.

It has been observed that odors of some compounds including geosmin, described as earthy, musty, or moldy, were generated during storage of cereal grains at elevated moisture contents.²² Geosmin causing earth odor may be the metabolites of microorganism such as *Streptomyces*; and the fermentation process of Chinese liquor is in an open environment. The microbe engaging in the process may be introduced from anywhere connected to grain, water, air, tool, *Daqu* or surrounding earth. However, rice husk is sterilized by steam before being used. So microbe in rice husk will be not introduced in the fermentation process to produce geosmin. Further investigations will be performed to determine the biological origin of the earthy odor in Chinese liquors. Linking between a sensitive analytical method and biological analysis will help to apprehend possible sources of contamination, and consequently enhance the product quality of Chinese liquor.

■ ASSOCIATED CONTENT

S Supporting Information. Flow chart of extract and fractionation of aroma in Chinese liquor, GC–MS total ionic chromatograms of aroma volatile compounds, Comparison of fragmentation ion pattern of the peak with earthy odor extracted from Chinese liquor with geosmin standard spectra, extracted ion chromatograms for L-(–)-menthol and geosmin, and calibration curve for the quantification of geosmin in Chinese liquor by HS–SPME–GC–MS. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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■ ABBREVIATIONS USED

LBG, *Laobaigan* liquor; XF, *Xifeng* liquor; LJ, *Langjiu* liquor; KZJ, *Kouzijiao* liquor; EGT, *Niulanshan erguotou* liquor; FJ,

Fenjiu liquor; WLY, *Wuliangye* liquor; GBC, *Gubeichun* liquor; JNC, *Jiannanchun* liquor; RI, retention indices; GC–O, gas chromatography–olfactometry; GC–MS, gas chromatography–mass spectrometry; HS–SPME, headspace–solid-phase microextraction; OAV, odor activity value.

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