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Determination of 2-Methylisoborneol and Geosmin Produced by *Streptomyces* sp. and *Anabaena* PCC7120

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A new sample preparation and enrichment technique, headspace liquid-phase microextraction (HS-LPME) linked to gas chromatography−mass spectrometry (GC-MS), was developed for the determination of the off-flavor odorants, 2-methylisoborneol and geosmin, produced by *Streptomyces* sp. and *Anabaena* PCC7120. Some of the factors that influence the extraction efficiency of HS-LPME, such as the type of extraction solvent, ionic strength of sample solution, and sample agitation rate, were studied and optimized by a single factor test. Other factors, including extraction temperature, extraction time, microdrop volume, and headspace volume were optimized by orthogonal array design. Extraction of 2-methylisoborneol and geosmin was conducted by exposing 2.5 μL of 1-hexanol for 9 min at 50 °C in the headspace of a 20 mL vial with a 10 mL of sample solution saturated by NaCl and stirred at 800 rpm. The developed protocol demonstrated good repeatability (relative standard deviations (RSDs) < 5%), wide linear ranges (10−5000 ng/L, $r^2 > 0.999$), and low limits of detection (LODs) for 2-methylisoborneol and geosmin (0.05 ng/L for both analytes). Subsequently, the method was successfully applied to extract the analytes in bacterial cultures with high recoveries (from 94% to 98%). Compared with headspace solid-phase microextraction (HS-SPME), HS-LPME demonstrates better linearity, precision, and recovery. Importantly, the sensitivity is about 1 order of magnitude higher than that of most HS-SPME. The results showed that HS-LPME coupled with GC-MS is a simple, convenient, rapid, sensitive, and effective method for the qualitative and quantitative analysis of 2-methylisoborneol and geosmin.

KEYWORDS: 2-Methylisoborneol; geosmin; gas chromatography−mass spectrometry (GC-MS); headspace liquid-phase microextraction (HS-LPME); *Streptomyces* sp.; *Anabaena* PCC7120

INTRODUCTION

Blue-green algae, actinomycetes, and certain fungi are known to produce *trans*-1,10-dimethyl-*trans*-9-decalol (geosmin) and 2-methylisoborneol. These semivolatile compounds have a woody, grassy, rotten, muddy, and earthy and musty odor discernible by human's olfactory system at concentrations as low as 4−10 ng/L for 2-methylisoborneol and geosmin in water (*1*−*3*). Although no known harm to health has been found (*4*), public water supply companies are plagued with consumer complaints once these compounds exceed the threshold of human perception (*3*). Simultaneously, both compounds are found in aquatic products (*5*−*7*), which cause these products to be rejected by consumers. To date, the determination of these target compounds has been challenging, with inaccuracy in detecting them at the level of nanogram grade per liter (ng/L).

Gas chromatography linked to mass spectrometry (GC-MS) combines high sensitivity and efficient separation ability and is the most popular method for the quantification of the earthy and musty odorants. Several conventional approaches have been described for the preconcentration of taste and odor-causing compounds such as liquid−liquid extraction (*8*), closed loop stripping analysis (*2*, *9*), simultaneous distillation extraction (*10*), and purge and trap (*11*). Unfortunately, most of these methods lack sensitivity and require specialized or expensive equipment.

Solid-phase microextraction (SPME) has recently been applied to the pretreatment of off-flavor odorants in water samples, fish tissues, and other samples (*6*, *7*, *12*−*14*). Coupled with GC-MS, the SPME method can detect 2-methylisoborneol and geosmin in water at concentrations less than 10 ng/kg (*14*, *15*). This method was considered to be dependable, convenient, organic-solvent-free, and low-cost. However, the applicability of SPME was occasionally limited by the kind of fibers (*16*). Furthermore the fragility of fibers, limited lifetime, and possibility of sample carry-over made it an unsatisfactory technique for analyzing 2-methylisoborneol and geosmin.

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Stir bar sorptive extraction (SBSE) shows higher extraction efficiency than SPME (*16*−*18*). Nevertheless, it is still time-consuming and inconvenient because 30−120 min stirring is involved, as well as after-extraction desorption with special devices.

In 1996, Jeannot and Cantwell established a new pretreatment technique named liquid-phase microextraction (LPME) or solvent microextraction (*19*). LPME is based on the use of a small amount of organic solvent to extract and concentrate different analytes from moderate amounts of aqueous matrices within a short period (*20*−*24*). Due to the small volumes (1−5 μL) and the minimal exposure of organic solvent used, it is environmental friendly and inexpensive. Furthermore, it is much more efficient because it is easy to find a suitable solvent for one or a group of analytes.

Headspace LPME (HS-LPME), which specializes in extracting volatile analytes, is one type of LPME. In HS-LPME, the analytes can be effectively extracted from the aqueous sample into the single-drop solvent by suspending a microdrop of organic solvent at the tip of a microsyringe needle and placing it into the headspace of a stirred sample solution. After extraction, the microdrop solvent is retracted into the needle and then injected directly into a GC system, so it is quite a fast extraction approach.

The aim of this work is to develop a method with high sensitivity for the determination of 2-methylisoborneol and geosmin in complex bacterial metabolite samples. Because much research focuses on biosynthetic and metabolic pathways of 2-methylisoborneol and gesomin in microorganisms (*25*, *26*), apparently an efficient preconcentration method is requisite. HS-LPME will be adopted in this research. While initial work has been done by Bagheri and Salemi (*27*), they only employed this method to analyze one analyte (geosmin) in water samples, which presents almost no media interference. The optimum conditions will be determined by using both single factor and orthogonal array tests, and also a comparison between HS-LPME and headspace solid-phase microextraction (HS-SPME) will be conducted.

MATERIALS AND METHODS

Materials and Reagents. Standard solutions of 100 μg/mL 2-methylisoborneol and geosmin in methanol were purchased from Supelco Inc. (Bellefonte, PA). Other reagents and solvents were analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

Streptomyces sp. and *Anabaena* PCC7120 were from our own lab.

Instrumentation. A 10-μL syringe for HS-LPME was purchased from Hamilton Co. (Reno, NV). Twenty milliliter headspace vials obtained from Agilent Technologies (Palo Alto, CA) were used for the extraction. A manual fiber holder (no. 57330-U) and a set of 65-μm polydimethylsiloxane (PDMS)/divinylbenzene (DVB) fibers (no. 57310-U) were obtained from Supelco Inc. (Bellefonte, PA). GC-MS was performed with a Finnigan Trace GC Ultra (Thermo Finnigan, Milan, Italy) gas chromatograph equipped with a Finnigan Trace DSQ (Thermo Electron Co., Austin, TX) mass-selective detector. Ultrapure water was produced by a Milli-Q water purification system (Millipore, Bedford, MA).

Analytical Conditions. The separation was conducted on a 30 m × 0.25 mm i.d. × 0.25 μm DB-5 MS capillary column (Agilent Technologies, Palo Alto, CA). The oven temperature was programmed to increase from 50 °C (held for 2 min) to 150 °C (without hold) at 20 °C/min. Helium with a purity of 99.999% was used as carrier gas at a flow rate of 1.0 mL/min. The injector temperature was 250 °C, and all injections were carried out in the splitless mode. The transfer line temperature and ion source temperature were 250 and 200 °C, respectively. The mass spectrometer was operated in the selected ion monitoring (SIM) mode with electron impact (EI) ionization resource (electron energy 70 eV). In SIM mode, four ions were monitored (*m*/*z* 95, 107, and 108 for 2-methylisoborneol; *m*/*z* 112 for geosmin).

Preparation of Standard Solutions. The standard stock solutions of 2-methylisoborneol and geosmin (1−10 μg/mL) were prepared weekly by diluting the standard solution (100 μg/mL) with ultrapure water and stored at 4 °C, and more diluted solutions were prepared daily by diluting the stock solutions.

Extraction Process. A measured volume of a bacterial culture or a diluted standard solution was added to NaCl to a series of concentrations (w/w, %) at 10%, 15%, 20%, 25%, 30%, and saturated concentration and introduced into a 20-mL headspace vial with a 2 cm × 0.5 cm magnetic stirring bar. The vial was then sealed with a polytetrafluoroethylene/silicone septum rounded by an aluminum crimp cap using a manual crimper. The vial was subsequently placed on a homemade magnetic agitator with a temperature controller and preheated for 15 min. The process of HS-LPME is listed as follows: first withdrawing a measured volume (1.5−2.5 μL) of organic solvent in advance and using the microliter syringe to penetrate the septum; then clamping it steadily to fix the needle tip constantly in the headspace of the sample, pressing the plunger, and holding it for a certain time to let the analytes be extracted by the microdrop suspended at the beveled tip; next withdrawing the microdrop into the syringe; and subsequently removing it from the headspace. The extract was finally injected into the GC-MS system. Extraction was performed at the following conditions: stirring rate of 0, 400, 800, and 1200 rpm; extraction temperature of 45, 50, and 55 °C; extraction time of 5, 7, and 9 min; and sample volume of 10, 12, and 14 mL in a 20 mL headspace vial.

The procedure of HS-SPME is somewhat similar to HS-LPME: a 10 mL sample solution was saturated by NaCl and placed into a 20 mL vial with a stirring bar and sealed by a septum. The outer needle of the PDMS/DVB fiber assembly was passed through the septum and the fiber extended into the headspace for extraction. A clamp was used to fix it. The fiber was exposed at 50 °C with a constant rate of 800 rpm for 30 min and then immediately inserted into the GC injection port for desorption.

Bacterial Culture. *Streptomyces* was grown in soybean meal medium. Soybean meal medium was prepared by placing 200 g of soybean meal powder in a vessel with 1000 mL of tap water and boiling for 2 h, then filtering over quantitative filter paper (pore size: 11 μm), and the filtrate was adjusted to 1000 mL with tap water. One loop of *Streptomyces* sp. was used to inoculate one sterilized flask of soybean meal medium (50 mL of medium in a 250-mL Erlenmeyer flask). Then it was incubated at 170 rpm, 28 °C for 48 h in a HQL 150B rotary shaker (Wuhan Scientific Instrument Factory of Chinese Academy of Sciences, Wuhan, China).

Anabaena was cultured in BG11 medium (*28*). *Anabaena* preculture (1 mL) was inoculated to one flask of BG11 (100 mL of medium in a 250 mL Erlenmeyer flask), and the culture was then grown in a HQL 150C rotating shaker (Wuhan Scientific Instrument Factory of Chinese Academy of Sciences, Wuhan, China) with 80−100 microeinsteins of light intensity per square meter per second at 125 rpm, 30 °C, for 96 h.

Biomass Determination. *Streptomyces* sp. cells were separated from the culture by filtration with quantitative filter paper with the same pore size (11 μm) as mentioned above and washed with physiological NaCl solution. Filter papers were dried at 110 °C and then weighed.

Biomass of *Anabaena* was determined spectrophotometrically with the optical density at 750 nm (A_{750}) as described previously (*29*).

RESULTS AND DISCUSSION

Optimization of HS-LPME. *Extraction Solvent.* Selection of extraction solvent was considered in the first place since it is the most important factor affecting HS-LPME. The extraction solvent should satisfy the following three requirements. First, the selected solvent should well dissolve the analytes. In this way, it can ensure a high enrichment and a short extraction time. Second, an appropriate solvent viscosity is required. If the viscosity is too low, the microdrop will fail to suspend at the

Table 1. Assignment of Factors and Levels of the Optimization Experiments Using an OA_9 (3^4) Matrix along with the Range Analysis

trial no.	extraction temp (A) (°C)	extraction time (B) (min)	microdrop vol (C) (μL)	headspace vol[a] (D) (mL)	blank level[b]	peak area of 2-methylisoborneol	peak area of geosmin
1	45	5	1.5	14	1	244603	274445
2	45	7	2.0	12	2	380098	427230
3	45	9	2.5	10	3	427646	481102
4	50	5	2.0	10	1	404342	454076
5	50	7	2.5	14	2	342165	383567
6	50	9	1.5	12	3	345263	388421
7	55	5	2.5	12	1	362823	407087
8	55	7	1.5	10	2	309971	348407
9	55	9	2.0	14	3	324377	364601

	2-methylisoborneol				geosmin			
K	extraction temp	extraction time	microdrop vol	headspace vol	extraction temp	extraction time	microdrop vol	headspace vol
$\sum k_1$	1052347	1011768	899837	911145	1182777	1135608	1011273	1022613
$\sum k_2$	1091770	1032234	1108817	1088184	1226064	1159204	1245907	1222738
$\sum k_3$	997171	1097286	1132634	1141959	1120095	1234124	1271756	1283585
R	94599	85518	232797	230841	105969	98516	260483	260972

[a] The total volume of each vial is 20 mL. [b] The levels are arranged from low to high for every factor; for example, levels 1, 2, and 3 represent 45, 50, and 55 °C for extraction temperature, respectively.

Table 2. ANOVA Table for Experimental Responses in the OA_9 (3^4) Matrix for 2-Methylisoborneol[a]

source	SS	df	MS	F[b]	PC (%)
extraction temp (A)	1.505×10^9	2	7.526×10^8	1.13236	6.41
extraction time (B)	1.329×10^9	2	6.647×10^8	1.00000	5.66
microdrop vol (C)	1.094×10^{10}	2	5.469×10^9	8.22758	46.55
headspace vol (D)	9.723×10^9	2	4.862×10^9	7.31445	41.38
blank	1.329×10^9	2	6.647×10^8		5.66
error	1.329×10^9	2	6.647×10^8		5.66
total	2.350×10^{10}				100

[a] SS = sum of squares; df = degrees of freedom; MS = mean squares; PC = percentage contribution. [b] Critical value is 4.320 ($P < 0.1$).

Table 3. ANOVA Table for Experimental Responses in the OA_9 (3^4) Matrix for Geosmin[a]

source	SS	df	MS	F[b]	PC (%)
extraction temp (A)	1.892×10^9	2	9.462×10^8	1.07288	6.35
extraction time (B)	1.764×10^9	2	8.820×10^8	1.00000	5.92
microdrop vol (C)	1.373×10^{10}	2	6.865×10^9	7.78402	46.05
headspace vol (D)	1.243×10^{10}	2	6.214×10^9	7.04614	41.68
blank	1.764×10^9	2	8.820×10^8		5.92
error	1.764×10^9	2	8.820×10^8		5.92
total	2.982×10^{10}				100

[a] SS = sum of squares; df = degrees of freedom; MS = mean squares; PC = percentage contribution. [b] Critical value is 4.320 ($P < 0.1$).

tip of the needle; whereas if it is too high, the depressing and withdrawing movement will be much more difficult. Furthermore, when it is injected, the solvent will adhere to the inner wall of the capillary column, consequently influencing the separation of the analytes. Final, low volatility is needed, because it helps avoid solvent loss. So, choosing the most suitable extracting solvent is of vital importance. According to the solvent requirements, four organic solvents including 1-butanol, 1-amylalcohol, 1-hexanol, and 1-octanol were investigated. Not only were symmetrical peaks shown but also the highest extraction efficiencies for both 2-methylisoborneol and geosmin were obtained (expressed by peak areas) when the 1-hexanol was applied. Eventually, it was used as the extraction solvent for further studies.

Ionic Strength. The increased ionic strength of the sample solution is expected to decrease the water solubility of the analytes and consequently enhance the extraction efficiency. This is due to the salting-out effect where fewer water molecules are available for dissolving the analyte molecules, preferably forming hydration spheres around the salt ions (*20*). The influence of salt addition on HS-LPME was investigated by adding NaCl to a series of concentration (w/w %) at 10%, 15%, 20%, 25%, 30%, and saturated concentration, and the maximum peak areas of both 2-methylisoborneol and geosmin were achieved when the solution was saturated. Accordingly, saturated NaCl was applied in the experiment.

Sample Agitation. Sample solution agitation with a stirring bar can accelerate the mass transfer in the aqueous phase and induce convection into the headspace and thus shorten the time for achieving a thermodynamic equilibrium. Several different stirring rates, specifically 0, 400, 800, and 1200 rpm, were studied with 10 mL sample solution saturated by NaCl in a 20 mL vial at 50 °C. The results showed that relatively higher peak areas were obtained when a higher speed was employed; however the difference was not so evident when the stirring speed was not less than 800 rpm. Notwithstanding, a higher stirring rate is unpractical since the stability of a microdrop at the tip of the needle could be dramatically affected. Based on the results, a stirring speed of 800 rpm was used in the following experiments.

Table 4. Method Validation for HS-LPME and HS-SPME

method	compound	RSD % ($n = 5$)	linear range (ng/L)	r^2	LOD (ng/L)
HS-LPME	2-methylisoborneol	4.4[a]	10–5000	0.9992	0.05
HS-LPME	geosmin	3.8[a]	10–5000	0.9995	0.05
HS-SPME	2-methylisoborneol	7.2[b]	1–500	0.9987	1.0
HS-SPME	geosmin	6.5[b]	0.5–500	0.9990	0.5

[a] The data were obtained by using a standard solution at 200 ng/L. [b] The data were obtained by using a standard solution at 800 ng/L.

Optimization of Other Factors. Besides the above-mentioned factors, the other four factors including extraction temperature (factor A), extraction time (factor B), microdrop volume (factor

Table 5. Contents and Recoveries of 2-Methylisoborneol and Geosmin in Bacterial Samples Determined by HS-LPME–GC-MS[a] ($n = 3$)

	Streptomyces sp. culture									*Anabaena* PCC7120 culture				
		2 ng/L		4 ng/L		2 μg/L		4 μg/L			1 μg/L		2 μg/L	
analyte	quantity	recovery (%)	RSD (%)	recovery (%)	RSD (%)	recovery (%)	RSD (%)	recovery (%)	RSD (%)	quantity	recovery (%)	RSD (%)	recovery (%)	RSD (%)
2-methylisoborneol	0.77 ng/L	94.7	3.2	95.3	2.9					0.82 μg/L	97.2	3.3	96.1	3.8
geosmin	1.85 μg/L					98.8	2.7	96.4	3.1	*b*				-

[a] These results were obtained under the optimal extraction conditions: exposing 2.5 μL of 1-hexanol at 50 °C for 9 min in the headspace of 20 mL vial with 10 mL of sample solution saturated by NaCl and stirred at 800 rpm. [b] Not detected.

Table 6. Contents and Recoveries of 2-Methylisoborneol and Geosmin in Bacterial Samples Determined by HS-SPME–GC-MS[a] ($n = 3$)

	Streptomyces sp. culture					*Anabaena* PCC7120 culture				
		2 μg/L		4 μg/L			1 μg/L		2 μg/L	
analyte	quantity	recovery (%)	RSD (%)	recovery (%)	RSD (%)	quantity	recovery (%)	RSD (%)	recovery (%)	RSD (%)
2-methylisoborneol	*b*					0.79 μg/L	83.4	8.3	86.5	7.8
geosmin	1.81 μg/L	88.2	6.7	86.3	7.1	*b*				

[a] These results were obtained under the following extraction conditions: exposing 65 μm PDMS/DVB fiber at 50 °C for 30 min in the headspace of 20 mL vial with 10 mL of sample solution saturated by NaCl and stirred at 800 rpm. [b] Not detected.

C), and headspace volume (factor D) can influence the extraction efficiency. In view of the possible interaction among these four factors, orthogonal array design was employed for the optimization of these factors under the conditions of 1-hexanol as the extraction solvent and NaCl saturated test solution with a stirring rate of 800 rpm. The four factors were examined by using a three-level orthogonal array design with an OA_9 (3^4) matrix based on single factor tests (*22*). The chromatographic peak areas of 2-methylisoborneol and geosmin were used to evaluate the extraction efficiency under different experimental conditions.

The effects of headspace and microdrop volume on extraction efficiency can be represented as the following equation (*23*):

$$n_e = K_{es}V_eV_sC_0/(K_{es}V_e + K_{hs}V_h + V_s) \quad (1)$$

where n_e is the amount of analyte in the extraction phase; V_e, V_s, and V_h are the volume of the extraction phase (microdrop), sample, and headspace, respectively; K_{es} and K_{hs} are the extraction phase/sample and headspace/sample distribution coefficients, respectively; and C_0 is the initial concentration of the analyte in the sample. According to the eq 1, an increase in V_s, together with the equivalent decrease in V_h, would improve the extraction efficiency. The statistical results shown in **Table 1** have clearly indicated this relationship. It is unreasonable to choose a smaller headspace volume because the headspace volume should be at least large enough to prevent the direct contact between the microdrop and the aqueous sample. So the headspace of 10 mL was employed for extraction. Theoretically, a larger microdrop volume will definitely impose a positive effect on the extraction of the analytes. As shown in **Table 1**, when the microdrop volume increased from 1.5 to 2.5 μL, the peak areas of the analytes increased. Although a larger microdrop volume increases extraction efficiency, it is impractical to choose too large a volume since it is difficult to suspend a microdrop at the tip of the microsyringe and the chances of losing the drop will increase.

Extraction temperature also plays an important role in the extraction of analytes. In general, the extraction efficiency would increase with the increase of temperature, since temperature has some potential effects on the kinetics and thermodynamics in the sorption process by increasing the mass transfer rates and the partition coefficients of an analyte, accordingly shortening the equilibrium time. At the same time, a higher temperature also leads to a higher vapor pressure of the analyte and consequently increases the analyte concentration in the headspace. However, the analyte absorption by the microdrop is an exothermic process and the amount of analytes absorbed by the microdrop will decrease upon a further increase of the sample temperature (*24*). Moreover, high temperature will lead to the loss of extraction solvent.

For all compounds, mass transfer is a time-dependent process and extraction efficiency correlates with exposure time. However, HS-LPME, which is based on the analyte's partitioning between the aqueous sample and the organic microdrop, is not an exhaustive extraction technique. Consequently, equilibrium could not be reached within a time span that did not cause loss of the microdrop. So, longer sampling period should be avoided. For quantitative analysis, it is not necessary to attain equilibrium if constant conditions are maintained for both analysis of samples and analysis of standard solutions (*23*).

From **Table 1**, we can select the optimum extraction conditions of these four factors according to the $\sum k$ of each level. Therefore the most efficient value of extraction temperature is 50 °C, extraction time is 9 min, microdrop volume is 2.5 μL, and headspace volume is 10 mL.

From the analysis of variance (ANOVA) results in **Tables 2** and **3**, it was observed that microdrop volume and headspace volume are statistically significant at $P < 0.1$, while both extraction temperature and extraction time are not significant ($P > 0.1$). From the percentage contribution (**Tables 2** and **3**), it can be deduced that the most important factor contributing to the extraction efficiency is microdrop volume (about 46% for both analytes), followed by headspace volume (about 41% for both analytes).

Evaluation of Method's Performance. Under the optimal conditions, the performance of this method was investigated, and the results are shown in **Table 4**. Good linearity of response was observed, and the correlation coefficients were higher than 0.999. The limits of detections (LODs) were determined by injecting a low concentration of working standard solution to

produce a signal-to-noise ratio (S/N) of 5, they are both 0.05 ng/L for 2-methylisoborneol and geosmin. The relative standard deviations (RSDs) were less than 5% for both analytes based on the peak areas for five replicates of a standard solution at 200 ng/L.

Application to Real Microorganism Culture Samples. To further demonstrate the feasibility of our method, it was applied to the analyses of 2-methylisoborneol and geosmin in the cultures of *Streptomyces* sp. and *Anabaena* PCC7120 using optimal extraction parameters. The quantities of 2-methylisoborneol and geosmin metabolized by each microbe were calculated by the external standard method. Quantities of 0.77 ng/L 2-methylisoborneol and 1.85 μg/L geosmin were detected from a 10 mL culture containing 0.0982 g of *Streptomyces* sp. cells, while 0.82 μg/L 2-methylisoborneol was detected from a 10 mL *Anabaena* PCC7120 culture ($A_{750} = 0.638$). Results of relative recoveries and RSDs of two microbe cultures are shown in **Table 5**. The data shows that the recoveries ranged from 94.7% to 97.2% for both analytes.

Comparison of HS-LPME Performance with HS-SPME. To further evaluate the performance of HS-LPME, HS-SPME was used as a comparison. There are many commercial SPME fibers, and different fibers have selectivities for certain compounds. For example, PDMS/DVB, DVB/carboxen (CAR)/PDMS and CAR/PDMS were used for the analyses of 2-methylisoborneol and geosmin. Recently, a study showed that the highest extraction efficiency for the two analytes was obtained from 65 μm DVB/CAR/PDMS, followed by 65 μm PDMS/DVB (*30*). However, the latter is the most widely used fiber for the analyses of 2-methylisoborneol and geosmin. So, it was selected to compare the extraction efficiency of HS-SPME with that of HS-LPME. Both extraction techniques exhibited comparable extraction performance in linearity, precision, and recovery. Furthermore, quantity values of the analytes were similar between the two methods (shown in **Tables 5** and **6**). In terms of the sensitivity, that of HS-LPME was much higher than that of HS-SPME. The LODs of the latter were 20 times and 10 times those of the former for 2-methylisoborneol and gesomin, respectively, as shown in **Table 4**.

For good HS-SPME, the LODs for both analytes are in low ng/L levels (*15*, *31*). Up to now, the reported LODs for 2-methylisoborneol and gesomin in several papers were slightly lower than that of our results (0.6 and 0.3 ng/L in ref *32*, 0.59 and 0.48 ng/L in ref *30* and 0.67 and 0.34 ng/L in ref *33*, respectively). The LODs can reach 0.15 ng/L for 2-methylisoborneol and 0.16 ng/L for gesomin using HS-SPME coupled with initial cool programmable temperature vaporizer inlet (*34*); however, it is difficult to perform, since most of the GC systems are equipped with standard split/splitless type inlet. Besides, PDMS coating SBSE usually had lower LODs for 2-methylisoborneol and geosmin (0.33 and 0.15 ng/L reported by Nakamura et al. (*16*) and 1 and 0.5 ng/L described by Benanou et al. (*17*)) than HS-SPME; nevertheless, they were still higher than those of HS-LPME developed by us. Simultaneously, the LOD (0.8 ng/L for geosmin) in another HS-LPME (*27*) was more than 10 times higher than ours. In the experiments, the quantity of 2-methylisoborneol produced by *Streptomyces* sp. could be easily detected by using HS-LPME (0.77 ng/L). It failed to be detected with HS-SPME. Thus, our results indicated that HS-LPME provides higher enrichment of analytes and higher sensitivity. Additionally, the extraction time of HS-LPME is only one-third of that of HS-SPME, and the cost is dramatically lower.

ABBREVIATIONS USED

ANOVA, analysis of variance; CAR, carboxen; DVB, divinylbenzene; HS-LPME, headspace liquid-phase microextraction; HS-SPME, headspace solid-phase microextraction; LODs, limits of detection; LPME, liquid-phase microextraction; PDMS, polydimethylsiloxane; RSDs, relative standard deviations; SBSE, stir bar sorptive extraction; SIM, selected ion monitoring; S/N, signal-to-noise; SPME, solid-phase microextraction.

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