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Head-space gas chromatographic analysis for the volatile flavor compounds from *Ganoderma lucidum* submerged-cultured broth

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Abstract: The volatile flavor from *Ganoderma lucidum* submerged-cultured broth was investigated by head-space gas chromatography-mass spectrometry (GC-MS). More than thirty different volatile flavor compounds from the broth were detected, and twenty-one compounds were identified, which were mainly ketones, alcohols and lactones. Among all the compounds detected in the broth, the compound with its characteristic retention peaks at 22.41min was 4,5-dihydro-3,5-dimethyl-2-furanone, and its content was 64.12% of the total content of all the volatile flavor compounds. In addition, 3-penten-2-one and amyl vinyl carbinol with their characteristic retention peaks at 9.78min and 18.10min, respectively, were the known food spice substances. The results were useful to preliminarily understand the mechanism of light aroma emitted from the submerged-cultured broth of *G. lucidum*.

Key words: higher fungi, submerged fermentation, flavour compounds, gas chromatography-mass spectrometry

顶空气相色谱-质谱联用法分析灵芝发酵物中的挥发性物质

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摘 要：采用顶空-气相色谱-质谱联用法对灵芝发酵物的风味物质进行了定性和定量测定。结果表明，灵芝发酵物中至少含有 31 种风味物质。对其中 21 种主要成分进行了鉴定，这些物质大多是酮类、醇类和内酯类化合物。所有物质中 4,5-二氢-3,5-二甲基-2-呋喃酮的含量最高，达 64.12%。此外，3-戊烯-2-酮和戊基乙烯基原醇是已知的食品香料成分。结果有助于初步理解灵芝发酵物产生清淡香味的原因。

关键词：高等真菌，液体发酵，风味物质，气质联用

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INTRODUCTION

Ganoderma lucidum (Fr.) P. Karst. (Polyporaceae), a medicinal fungus called “Lingzhi” in

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Chinese and “Reishi” in Japanese, has had popularity as a medicine in the Orient for the prevention and treatment of various types of diseases, such as cancer, hepatopathy, arthritis, hypertension, neurasthenia, and chronic hepatitis (Shiao, 2003; Liu & Zhang, 2005). Chemistry studies on *G. lucidum* have shown that pharmaceutically active compounds from fruiting body and mycelium of *G. lucidum* include polysaccharides, triterpenoids, steroids, alkaloids, nucleotides, lactones and fatty acids (Shiao, 2003), among which polysaccharides and triterpenoids are the major source of biological activity and therapeutic use of *G. lucidum* (Wu *et al.*, 2001; Liu & Zhang, 2005; Liu & Wang, 2006).

Currently, submerged fermentation of *G. lucidum* has been viewed as a promising alternative for efficient production of its mycelium as well as intracellular and extracellular valuable metabolites (Yang & Liu, 1998; Tang & Zhong, 2002; Liu & Zhang 2006, 2007). Fermentation broth, the combination of mycelium and fermentation liquid can be used as a convenient product (Yang, 2004). Recent studies have proved that the fermentation broth of *G. lucidum* has the ability of anti-tumor (Yang, 2004), anti-virus and liver protection (Li & Zhang, 2005), and treatment of bronchitis (Wang *et al.*, 2004). However, the data on the volatile flavor of the fermentation broth of *G. lucidum* was scarce. In the present study, the volatile flavor compounds of the fermentation broth of *G. lucidum* were quantitatively detected by the analysis of head-space-GC-MS.

MATERIALS AND METHODS

Main instrumentations

HYG2Ⅱ Rotator (Shanghai Xinrui Auto-Equipments Company).

HS-GC–MS system: 7694E head space autosampler (Hewlett-Packard, Palo Alto, CA, USA). Finnigan gas chromatograph (Trace MS, USA), interfaced with a model 5973 mass detector. The gas chromatograph was equipped with a PEG-20M column (30m long, 0.25mm i.d., 0.25μm film thickness, Trace MS USA). Rubber septa lined with PTFE (150℃ proof) were obtained from Analytical Technology (Milan, Italy).

Preparation of *G. lucidum* broth

Ganoderma lucidum was grown in a 250mL flask containing 80mL medium (see below) at 30℃ for 7d with shaking at 160r/min. This was then inoculated at 10% (v/v) into the same medium, and followed by 7d cultivation. The culture medium contained (g/L): glucose, 35; peptone, 4; KH_2PO_4, 1; $MgSO_4 \cdot 7H_2O$, 0.45; vitamin B1, 0.01.

Analysis of HS-GC-MS

10mL broth was transferred into glass “head space” vials (15mL volume), which were closed with 20mm rubber PTFE lined septa (150℃ proof) and crimped with perforated aluminium seals. After 40min equilibration time on a rotating shaker at 50℃, samples were placed in the auto-sampler where they were heated at 250℃ and shaken for 3min before the head space was withdrawn. The

head space auto-sampler was provided with a 1mL loop kept at 180℃; and the transfer line, heated at 200℃, was connected with the injector of the GC (split 1:20) via an interface heated at 250℃. The oven temperature was kept at 36℃ during the injection (3min) , and then the temperature was increased at 4℃/min up to 120℃ and then to 230℃ at a rate of 10℃/min and kept at 230℃ for 8min. Helium was used as the carrier gas at the constant flow rate of 0.8mL/min. The mass detector was operating in the electron impact (EI+) mode.

Identification of volatile flavor compounds

Volatile flavor compounds were tentatively identified by matching mass spectral data of sample components with those of known compounds in a database (Nist database and Wiley database). The following equations were used to determine their percentage concentration of the identified compounds (w/w).

$$C_i = \frac{A_i}{\sum A_i} \times 100\ \%$$

Where C is concentration of one compound; A is peak area; ΣA is the sum of all peak areas; represents one component.

RESULTS AND DISCUSSION

Head-space gas chromatography was used for the analysis of volatiles and semi-volatile components in solid, liquid and gas samples. The popularity of this technique has grown over recent years and it has now gained worldwide acceptance for analyses of volatile compounds. The PEG-20M column was apparently suitable for separating and identifying the volatile flavor compounds. Fig.1 shows the sharp and non-overlapping total ion peaks (only some of the major peaks are labeled in order to maintain the clarity of the chromatogram). Over thirty different volatile flavor compounds in the submerged-cultured broth were detected. The summarized GC-MS data and the percentage of each individual flavor compound with respect to the total volatile flavors contents were shown in Table 1.

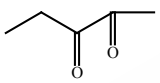
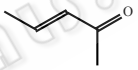
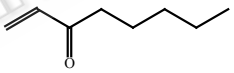
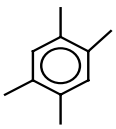
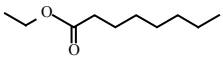
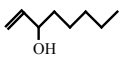
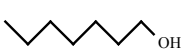
As demonstrated in Table 1, twenty-one kinds of volatile flavor compounds whose match index was more than 800 were identified and their name and chemical structure were listed. There were different types of compounds, including ketones (2,3-pentanedione, hexanal, 3-penten-2-one, 4,5-dihydro-3,5-dimethyl-2-furanone), esters and lactones (ethyl caprylate, 2-methyl-4-hydroxypentanoic acid lactone, 2-methyl-2,4-pentadien-4-olide, 4-hydroxynonanoic acid lactone), alcohols (hexanol, amyl vinyl carbinol, heptanol, octanol, benzenepropanol), aethers (vinyl amyl ketone), acids (octanoic acid), hydroxybenzenes (2.4-di-tert-butylphenol), alkenes (2-ethynyl-bicyclo[4.4.1]undeca-1,3,5,7,9-pentaene), etc.

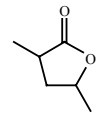
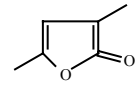
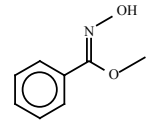
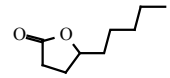
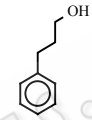
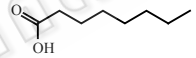
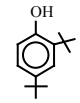
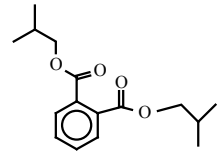
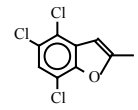
Among the identified compounds, the compound with its characteristic retention peak at 22.41min was 4,5-dihydro-3,5-dimethyl-2-furanone, reaching 64.12% of the total content of all the volatile compounds. It is the first identification of 4,5-dihydro-3,5-dimethyl-2-furanone from *G.*

lucidum. Currently, there is little information on the compound, nevertheless, some of the derivatives of furanone are food spice substance, such as 2,5-dimethyl-3(2H) furanone and 4-hydroxy-5-ethyl-2-methyl-3(2H)-furanone. The submerged-cultured broth of *G. lucidum* has the light aroma, but it is not clear that if the aroma is mainly attributed to the compound 4,5-dihydro-3,5-dimethyl-2-furanone. In addition, 3-penten-2-one and amyl vinyl carbinol with their characteristic retention peaks at 9.78min and 18.10min, respectively, were the known food spice substances (Wen, 2001).

In conclusion, this work firstly reports the volatile flavour compounds from the submerged-cultured broth. The results were useful to preliminarily understand the mechanism of light aroma emitted from the submerged-cultured broth of *G. lucidum*. Further studies were required to identify the detailed flavour of the compound 4,5-dihydro-3,5-dimethyl-2-furanone by sense analysis method.

Table 1 Volatile flavor composition of *G. lucidum* broth

RT[a]	Area	Area %	Chemical structure	Compound name[b]
7.80	5336036.63	0.29		2,3-pentanedione
8.36	5694199.31	0.31		hexanal
9.78	5083235.09	0.28		3-penten-2-one
14.46	7507766.45	0.41		vinyl amyl ketone
15.55	797531.23	0.04		
15.95	53555450.74	2.91		hexanol
16.65	3187147.99	0.17		
17.51	3947606.36	0.21		durene
17.55	6213551.37	0.34		ethyl caprylate
18.10	49654549.33	2.70		amyl vinyl carbinol
18.24	8126190.58	0.44		heptanol
18.97	3889970.23	0.21		

20.42	30612186.79	1.66		octanol
20.54	1496285.28	0.08		
20.73	133504468.39	7.25		3,5-dimethyl-2(3H)-furanone
21.60	5325993.50	0.29		
22.41	1180284069.32	64.12		4,5-dihydro-3,5-dimethyl-2-furanone
23.75	9441443.71	0.51		2-methyl-2,4-pentadien-4-olide
24.08	12216451.54	0.66		methoxy-phenyl oxime
27.62	9808962.45	0.53		4-hydroxynonanoic acid lactone
27.79	4153876.88	0.23		benzenepropanol
27.92	2210807.47	0.12		octanoic acid
29.04	672176.46	0.04		
30.15	1358970.67	0.07		
30.37	27035669.22	1.47		2.4-di-tert-butylphenol
30.76	4231425.67	0.23		2-ethynyl-bicyclo[4.4.1]undeca-1,3,5,7,9-pentaene
30.99	2384304.84	0.13		
32.03	2954745.71	0.16		
32.77	3998943.53	0.22		isobutyl phthalate
33.09	35852930.48	1.95		benzofuran. 4.5.7-trichloro-2-methyl-

Note: [a] Retention time; [b] Data were not given in the table for the compounds whose match index was less than 800.

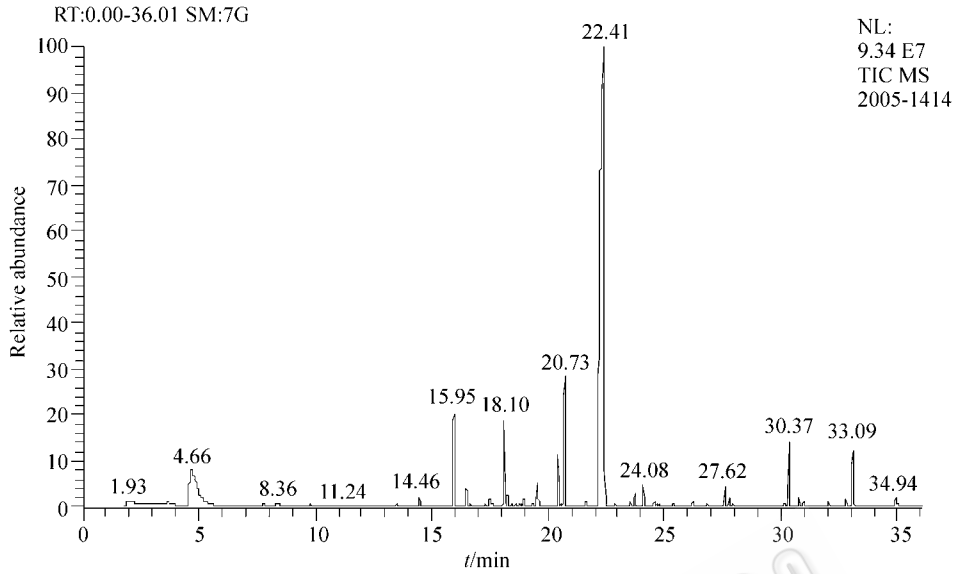


Fig.1 Total ion chromatogram for the volatile flavour in *G. lucidum* broth. Only some of the major peaks are labeled in order to maintain the clarity of the chromatogram.

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