Research Article

Analysis on the Fatty Acids and Volatile Components in *Pleurotus geesteranus* by HS-SPME-GC-MS

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The volatile constituents and fatty acids in *Pleurotus geesteranus* were assayed by headspace solid-phase microextraction coupled with GC-MS. There were 5 volatile compounds in *P. geesteranus* that accounted for 43.43% of the total ion current peak area, and its main compounds were 2-undecanone (13.99%), 3-ethyl-2,5-dimethylpyrazine (12.67%), and l-β-bisabolene (6.79%). Fourteen compounds were identified in the ethanol extract of *P. geesteranus* and 6 fatty acids were identified from the petroleum ether extract, which accounted for 93.72% and 98.48% of the total ion current peak area, the main compounds in the ethanol extract were ethyllinoleate (67.36%) and ethylpalmitate (21.83%), and the main fatty acids in the petroleum ether extract were linoleic acid (78.22%), palmitic acid (10.74%), and oleic acid (8.13%).

1. Introduction

*Pleurotus geesteranus*, which belongs to the family Pleurotaceae, is native to India [1, 2]. *P. geesteranus* contains many types of components including proteins, fat, polysaccharides, vitamins, trace elements, and 8 essential amino acids [3]. The volatile components of edible fungi such as *Morchella esculenta* [4], *P. eryngii* [5], *Tricholoma matsutake* [6], *Boletus edulis* [7], and *Agaricus bisporus* [8], which were detected by solid-phase microextraction (SPME), had been reported, and alcohol compounds are the main chemical components of *Pleurotus* mushrooms. Alcohol and ketone compounds are the key components that affect the flavor of *Pleurotus* mushrooms. Current studies of *P. geesteranus* mainly focus on cultivation techniques [9], preservation [10], and biological characteristics [11]. A number of research studies report that *P. geesteranus* has antioxidant [12], liver protection [13], antitumor [14], hypolipidemia [15], and antibacterial [16] activities. However, the valuable medicinal components present in *P. geesteranus* have not been identified yet. In this study, headspace solid-phase microextraction coupled with the gas chromatography-mass spectrometry (HS-SPME-GC/MS) technique was used to assay the volatile components of *P. geesteranus*.

2. Materials and Methods

2.1. Materials. Dried powder of *P. geesteranus* was provided by Henan Longfeng Industrial Co., Ltd. (Qingfeng, Henan, China) in October 2019.

The GC/MS instrument was an Agilent 7890 gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) equipped with a 5975 mass spectrometer (Agilent Technologies). A solid-phase microextraction (SPME) device (Supelco, USA) and the extraction head were 65 µm polydimethylsiloxane (PDMS-DVB, Supelco, USA). C6–C26 n-alkanes was purchased from Alfa Aesar, Haverhill, USA.
2.2. Extraction. 930 g of *P. geesteranus* powder was taken, and petroleum ether was added to extract 3 times at room temperature for 72 h. After filtration, the filtrate was concentrated by evaporation to obtain the petroleum ether extract with a yield of 1.39%. The residue was extracted with 70% ethanol at room temperature and then filtered. The filtrate was concentrated by evaporation to obtain the ethanol extract with a yield of 5.37%.

2.3. Methyl Esterification. Petroleum ether extract (0.5 g) was added into a 10 mL test tube with petroleum ether/ether (4/3) to 5 mL, and then 4 mL of 0.5 mol/L KOH–CH₃OH solution was added under 70 °C water bath for 10 min. After cooling, distilled water (10 mL) was added to sonicate and centrifuge solutions, and the supernatant was concentrated.

2.4. HS-SPME. A manual SPME device with a fiber precoated with a 65 μm thick layer of polydimethylsiloxane/divinylbenzene (PDMS-DVB) was used for extraction. The dry powder of *P. geesteranus*, ethanol extract, and methylated products was placed in 5 mL vials, and then, the SPME fiber was exposed in the upper space of the sealed vial at 60°C for 30 min. After that, the fiber was withdrawn and directly inserted into the GC-MS inlet (temperature 250°C) for 1 min.

2.5. Determination of Fatty Acids and Volatile Components in *Pleurotus geesteranus*. The fatty acids and volatile constituents were analyzed using the GC/MS instrument. The GC was fitted with a DB-5MS capillary column (30 m × 0.25 mm × 0.25 μm, Agilent Technologies). High-purity helium (99.999%) was used as the carrier gas at a flow rate of 1.0 mL/min. The inlet temperature was 250°C. The temperature program was as follows: the initial column temperature was 50°C, for 2.0 min, then programmed to 120°C at a rate of 8°C/min, and held for 2 min, and programmed finally to 220°C at a rate of 4°C/min and held at 220°C for 5 min. Split injection with a split ratio of 10:1 was used. The MS was operated in the SCAN mode (m/z 30–400) with electron impact ionization at an ionization energy of 80 eV, the ion source temperature was 230°C, the quadrupole temperature was 150°C, transmission line temperature was 280°C, and electron multiplier voltage was 1635 V. According to the previous reports in the literature [17, 18], the Kovats retention index (KI) was calculated by using the retention times of C₆–C₂₆ n-alkanes that were injected under the same chromatographic conditions. The Kovats retention index calculation formula was as follows:

\[
KI = 100n + 100 \times \frac{t_R - t_R^0}{t_{R_{R+1}} - t_R^0},
\]

where \( n \) and \( n + 1 \) are the number of normal alkane carbon atoms before and after the outflow; \( t_R^0 \) and \( t_{R+1}^0 \) are the retention times of the corresponding normal alkane, respectively; and \( t_R \) is the retention time of the unknown substance in the gas chromatography (\( t_R^0 < t_R < t_{R+1}^0 \)).

3. Results and Discussion

According to the above conditions, the components of *P. geesteranus* powder, ethanol extract, and petroleum ether extract were analyzed by GC-MS, and their total ion flow chromatograms were obtained, respectively. The fatty acids and volatile constituents were identified by their mass spectra with the Rt Pest3.L, Nist08.L spectral library, combined with retention index published in the literature [19] and related websites (http://www.vcf-online.nl). Relative percentage amounts of the separated compounds were calculated automatically from peak areas of the total ion chromatograms. Five compounds accounting for 43.43% were identified from the powder of *P. geesteranus*, 14 compounds accounting for 93.72% were identified from ethanol extracts, and 6 fatty acids accounting for 98.46% were identified from petroleum ether extracts after methylation. The specific results are presented in Table 1 and Table 2.

As shown in Table 1 and Figure 1, the main compounds in *P. geesteranus* powder were 2-undecanone (13.99%), 3-ethyl-2,5-dimethylpyrazine (12.67%), 1-β-bisabolene (6.79%), and 2-Phenylcrotonaldehyde (6.08%). Among them, the content of 2-undecanone (13.99%) was the highest. It has been reported that it is not only an important aroma component of *P. geesteranus*, but also has an insect repellent effect [20]. In addition, 3-ethyl-2,5-dimethylpyrazine is a common aroma active substance, and 1-β-bisabolene is mainly used as an edible flavor.

In Table 2 and Figure 2, we could see that the main components in the ethanol extract were ethyl linoleate (67.36%) and ethyl palmitate (21.83%). Compounds in the petroleum ether extract after methylation were linoleate (78.22%), palmitate (10.74%), and elaidate (8.13%) as shown in Table 2 and Figure 3. There were four common compounds: estragole, cis-anethol, elaidate, and linoleate, and the content in the petroleum ether extract was higher than that in the ethanol extract. Among the ethanol extract components, ethyl linoleate had the highest content, and it has a variety of pharmacological effects, such as anti-inflammatory [21], antioxidant [22], and lowering human cholesterol [23]. The relative content of ethyl palmitate in ethanol extracts was also
high, not only as a vasodilator factor for lowering blood pressure [24], but also for preventing nonalcoholic steatohepatitis [25], further having anti-inflammatory [26] and antifibrosis effects [27]. Linoleate was the component with the highest content in petroleum ether extracts and has anti-inflammatory [28], antithrombotic [29], anticancer, and antiatherosclerotic effects [30].

Previous studies have found that the main fatty acid components of edible fungi such as *Lentinus edodes*, *Dictyophora indusiata*, and *Auricularia auricula* are linoleic acid, palmitic acid, and linolenic acid [31,32]. In this study, we also found that linoleic acid is the main fatty acid in *P. geesteranus*. Liu [33] analyzed the volatile components of *P. geesteranus* by the HP-SPME-GC-MS method, 19 compounds were identified, and the major ones are 3-octanol (55.12%), 1-octen-3-ol (20.03%), and 3-octanone (19.22%), which is different from our research.

### 4. Conclusions

The volatile constituents and fatty acids in *P. geesteranus* were assayed by the HS-SPME-GC-MS method. There were 5 volatile constituents in *P. geesteranus*, 14 compounds were

| No. | RT (min) | Compounds name       | Relative content (%) | Similarity | KI     |
|-----|---------|----------------------|----------------------|------------|--------|
|     |         | Ethanol extracts     | Methyalted products  |            |        |
| 1   | 8.621   | γ-Terpinene          | 0.06                 | —          | 86     | 1023   |
| 2   | 11.635  | Terpinen-4-ol        | 0.30                 | —          | 96     | 1168   |
| 3   | 11.981  | Benzoic acid         | 0.23                 | —          | 93     | 1184   |
| 4   | 12.821  | Estragole            | 0.07                 | 0.30       | 98     | 1218   |
| 5   | 15.736  | cis-Anethol          | 0.11                 | 0.60       | 98     | 1322   |
| 6   | 17.169  | Benzene propanoic acid | 0.06              | —          | 92     | 1371   |
| 7   | 20.677  | *trans*-Cinnamic acid | 0.18                | —          | 97     | 1489   |
| 8   | 22.307  | Nicotinamide         | 0.16                 | —          | 95     | 1545   |
| 9   | 28.582  | Pentadecanoate       | —                    | 0.51       | 98     | 1770   |
| 10  | 30.213  | Ethyl pentadecanoate | 1.21                | —          | 99     | 1832   |
| 11  | 31.151  | Palmitate            | —                    | 10.74      | 99     | 1868   |
| 12  | 32.782  | Ethyl palmitate      | 21.83                | —          | 98     | 1933   |
| 13  | 34.956  | Ethyl heptadecanoate | 0.72                | —          | 98     | 2023   |
| 14  | 35.598  | Elaidate             | 0.14                 | 8.13       | 99     | 2050   |
| 15  | 35.845  | Linoleate            | 1.30                 | 78.22      | 99     | 2061   |
| 16  | 37.476  | Ethyl linoleate      | 67.36                | —          | 99     | 2130   |
| Total|         |                      | 93.72                | 98.48      |        |        |

**Table 2: Compounds and relative percentages of ethanol extract and petroleum ether extract.**
identified from the ethanol extract, and 6 fatty acids were identified from the petroleum ether extract. It was found that the main volatile component is 2-undecanone and the main fatty acid is linoleic acid.

Data Availability
The data used to support the findings of this study are included within the article.

Conflicts of Interest
All authors declare that there are no conflicts of interest regarding this study.

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References
[1] F. L. Dai, China Fungi Confluence, Science Press, Beijing, China, 1979.
[2] S.-T. Chang, “The world mushroom industry: Trends and technological development,” International Journal of Medicinal Mushrooms, vol. 8, no. 4, pp. 297–314, 2006.
[3] H. X. Wang and Q. X. Lan, “Research status of Pleurotus Geesteranus in China,” Vegetables, vol. 9, pp. 39–41, 2014.
[4] F. S. Zhang, L. Long, X. R. Yu et al., “Detection and analysis of volatile components in different varieties of morel,” Journal of Sichuan University (Natural Science Edition), vol. 56, no. 05, pp. 963–970, 2019.
[5] C. M. Yin, X. Z. Fan, Z. Fan et al., “Analysis of volatile flavor compounds in different Pleurotus species using HS-SPME-GC-MS,” Food Science, vol. 39, no. 16, pp. 240–246, 2019.
[6] Y. Guo, D. Chen, Y. Dong, H. Ju, C. Wu, and S. Lin, “Characteristic volatile fingerprints and changes of volatile compounds in fresh and dried Tricholoma matsutake singer by HS-GC-IMS and HS-SPME-GC-MS,” Journal of Chromatography B, vol. 1099, pp. 46–55, 2018.
[7] H. Aisala, J. Sola, A. Hopia, K. M. Linderborg, and M. Sandell, “Odor-contributing volatile compounds of wild edible Nordic mushrooms analyzed with HS-SPME-GC-MS and HS-SPME-GC-O/FID,” Food Chemistry, vol. 283, pp. 566–578, 2019.
[8] F. Pei, W. Yang, N. Ma et al., “Effect of the two drying approaches on the volatile profiles of button mushroom (Agaricus bisporus) by headspace GS-MS and electronic nose,” IWT - Food Science and Technology, vol. 72, pp. 343–350, 2016.
[9] B. Q. Liu, D. M. Wang, X. L. Zhuo et al., “Pleurotus geesteranus and its cultivation and management application,” Xianjai Horticulture, vol. 15, pp. 70-71, 2018.
[10] Z. Zhang, X. Zhang, G. Xin et al., “Umami taste and its association with energy status in harvested Pleurotus geesteranus stored at different temperatures,” Food Chemistry, vol. 279, pp. 179–186, 2019.
[11] B. Q. Li, Z. M. Chen, J. Y. Lin et al., “Study on the biological characteristics of Pleurotus geesteranus S3-45 strain,” Edible Fungi of China, vol. 36, no. 02, pp. 13–16, 2017.
[12] X. Song, Q. Shen, M. Liu et al., “Antioxidant and hepatoprotective effects of intracellular mycelium polysaccharides from Pleurotus geesteranus against alcoholic liver diseases,” International Journal of Biological Macromolecules, vol. 114, pp. 979–988, 2018.
[13] X. Song, Z. Liu, J. Zhang et al., “Antioxidative and hepatoprotective effects of enzymatic and acid-hydrolysis of Pleurotus geesteranus mycelium polysaccharides on alcoholic liver diseases,” Carbohydrate Polymers, vol. 201, pp. 75–86, 2018.
[14] M. Zhang, L. Zhu, S. W. Cui, Q. Wang, T. Zhou, and H. Shen, “Fractionation, partial characterization and bioactivity of water-soluble polysaccharides and polysaccharide-protein complexes from Pleurotus geesteranus,” International Journal of Biological Macromolecules, vol. 48, no. 1, pp. 5–12, 2011.
[15] M. Duobin, M. Yuping, G. Lujing, Z. Aijing, Z. Jianqiang, and X. Chunping, “Fermentation characteristics in stirred-tank reactor of exopolysaccharidewith hypolipidemic activity produced by Pleurotus geesteranus 57,” Anais Da Academia Brasileira De Ciencias, vol. 85, no. 4, pp. 1473–1481, 2013.
[16] J. W. Shen, R. R. Wang, C. P. Xu et al., “Study on preparation and bioactivity of sulfated saccharides of Pleurotus geesteranus,” Journal of Henan Agricultural Sciences, vol. 43, no. 07, pp. 102–106, 2014.
[17] Y. B. Zhang and W. Y. Kang, “Volatiles in Potentilla discolor by HS-SPME-GC-MS,” Chemistry of Natural Compounds, vol. 50, no. 6, pp. 1128–1129, 2014.
[18] Y. Niu, G. Hardy, M. Agarwal, L. Hua, and Y. Ren, “Characterization of volatiles Tribolium castaneum (H.) in flour using solid phase microextraction-gas chromatography mass spectrometry (SPME-GCMS),” Food Science and Human Wellness, vol. 5, no. 1, pp. 24–29, 2016.
[19] L. Sun, W. He, G. Xin et al., “Volatole components, total phenolic compounds, and antioxidant capacities of worm-infected Gomphidius rutilus,” Food Science and Human Wellness, vol. 7, no. 2, pp. 148–155, 2018.
[20] D. Bisht and C. S. Chanotiya, “2-undecanone rich leaf essential oil from Zanthoxylum armatum,” Natural Product Communications, vol. 6, no. 1, pp. 111–114, 2011.
[21] G. D. Liu, X. Bing, D. Huang et al., “Ethyl linoleate inhibits inflammatory reaction induced by titanium particles and its mechanism,” Chinese Journal of Tissue Engineering Research, vol. 20, no. 52, pp. 7836–7843, 2016.
[22] T. Masuda, T. Maekawa, K. Hidaka, H. Bando, Y. Takeda, and H. Yamaguchi, “Chemical studies on antioxidant mechanism of curcumin: Analysis of oxidative coupling products from curcumin and linoleate,” Journal of Agricultural and Food Chemistry, vol. 49, no. 5, pp. 2539–2547, 2001.
[23] L. Dan and M. Laposata, “Ethyl palmitate and ethyl oleate are the predominant fatty acid ethyl esters in the blood after ethanol ingestion and their synthesis is differentially influenced by the extracellular concentrations of their corresponding fatty acids,” Alcoholism: Clinical and Experimental Research, vol. 21, no. 2, pp. 286–292, 1997.
[24] Y.-C. Lee, H.-H. Chang, C.-L. Chiang et al., “Role of perivascular adipose tissue-derived methyl palmitate in vascular tone regulation and pathogenesis of hypertension,” Circulation, vol. 124, no. 10, pp. 1160–1171, 2011.
[25] L. Zhang, H.-X. Li, W.-S. Pan et al., “Administration of methyl palmitate prevents non-alcoholic steatohepatitis (NASH) by induction of PPAR-α,” Biomedicine & Pharmacotherapy, vol. 111, pp. 99–108, 2019.
[26] N. M. Saeed, E. El-Demerdash, H. M. Abdel-Rahman, M. M. AlGandaby, F. A. Al-Abbasi, and A. B. Abdel-Naim, “Anti-inflammatory activity of methyl palmitate and ethyl
palmitate in different experimental rat models,” Toxicology and Applied Pharmacology, vol. 264, no. 1, pp. 84–93, 2012.

[27] M. H. Sharawy, D. S. El-Agamy, A. A. Shalaby, and E.-S. M. Ammar, “Protective effects of methyl palmitate against silica-induced pulmonary fibrosis in rats,” International Immunopharmacology, vol. 16, no. 2, pp. 191–198, 2013.

[28] M. Zhao, The Research on the Anti-inflammatory Effect of Linoleic Acid and Methyl Linoleate, Southwest Jiaotong University, Chengdu, China, 2012.

[29] K. Yu, X. Q. Huang, S. L. Peng et al., “Research on plant components inhibiting expression of hIL-1β mRNA,” West China Journal of Pharmaceutical Sciences, vol. 18, no. 6, pp. 420–421, 2003.

[30] O. Berdeaux, L. Voinot, E. Angioni, P. Juanéda, and J. L. Sébédio, “A simple method of preparation of methyl-trans-10, cis-12- and cis-9, trans-11-octadecadienoates from methyl linoleate,” Journal of the American Oil Chemists’ Society, vol. 75, no. 12, pp. 1749–1755, 1998.

[31] T. Y. Li, J. Y. Yang, X. Fan et al., “Determination of 37 fatty acids in fresh edible mushrooms by gas chromatography coupled with mass spectrometry,” Chinese Journal of Health Laboratory Technology, vol. 27, no. 14, pp. 2002–2008, 2017.

[32] Q. Jia, The Research on the Chemical Constituents of the Fungus Cap of Dictyophora indusiata and Analysis of Fatty Acid Composition of 10 Edible Fungi in Xi’an, Shaanxi Normal University, Xi’an, China, 2016.

[33] X. Y. Liu, Optimization of Explosion Puffing Drying Process for Pleurotus Geesteranus at Variable Temperature and Pressure Difference and Analyze of Taste Components and Volatile Components, Shenyang Agricultural University, Shenyang, China, 2018.