Hindawi
Journal of Food Quality
Volume 2020, Article ID 8894066, 5 pages
https://doi.org/10.1155/2020/8894066

Research Article

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

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Received 19 May 2020; Revised 4 June 2020; Accepted 26 August 2020; Published 7 September 2020

Academic Editor: Fatma M. El-Demerdash

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-dimethyl pyrazine (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 ethyl linoleate (67.36%) and ethyl palmitate (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.

Table 1: Compounds and relative percentage of *P. geesteranus* powder.

No.	RT (min)	Compound name	Similarity	Relative content (%)	KI
1	9.955	3-Ethyl-2,5-dimethyl pyrazine	90	12.67	1089
2	14.155	2-Undecanone	94	13.99	1266
3	16.329	2-Phenylcrotonaldehyde	96	6.08	1343
4	19.787	l-β-Bisabolene	95	6.79	1459
5	21.961	Cocal	98	3.91	1533
Total				43.43	

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–CH_3OH 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 C_6–C_{26} *n*-alkanes that were injected under the same chromatographic conditions. The Kovats retention index calculation formula was as follows:

$$\mathrm{KI} = 100n + 100 \times \frac{t_R - t_{Rn}}{t_{Rn+1} - t_{Rn}}, \quad (1)$$

where n and $n+1$ are the number of normal alkane carbon atoms before and after the outflow; t_{Rn} and t_{Rn+1} 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 ($t_{Rn} < t_R < t_{Rn+1}$).

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 Rtlpest3.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 98.48% 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%), l-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 l-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

Table 2: Compounds and relative percentages of ethanol extract and petroleum ether extract.

No.	RT (min)	Compounds name	Relative content (%)		Similarity	KI
			Ethanol extracts	Methylated 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	Benzenepropanoic acid	0.06	—	92	1371
7	20.677	*trans*-Cinnamic acid	0.18	—	97	1489
8	22.307	Niacinamide	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
10	32.782	Ethyl palmitate	21.83	—	98	1933
11	34.956	Ethyl heptadecanoate	0.72	—	98	2023
12	35.598	Elaidate	0.14	8.13	99	2050
13	35.845	Linoleate	1.30	78.22	99	2061
14	37.476	Ethyl linoleate	67.36	—	99	2130
Total			93.72	98.48		

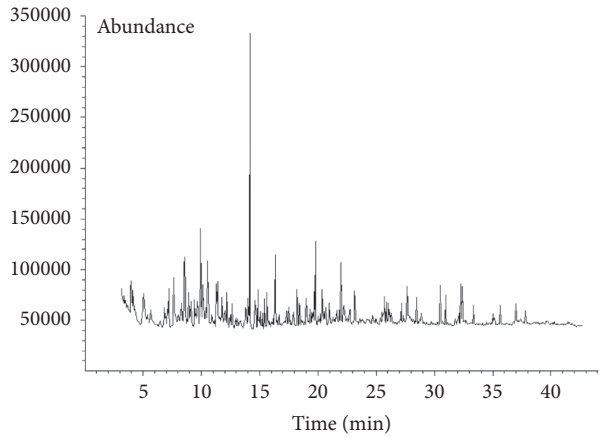

Figure 1: Total ion flow chromatogram of volatile components in *P. geesteranus* powder.

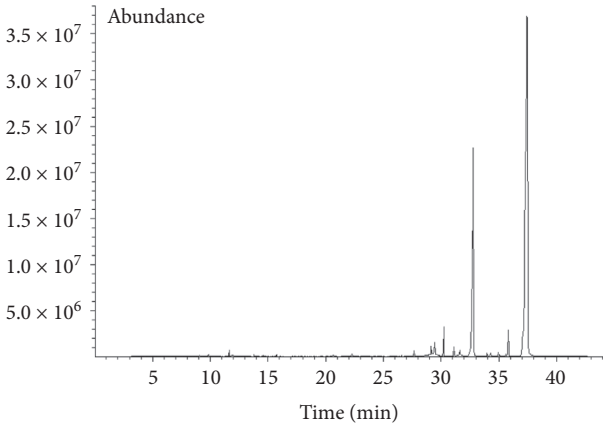

Figure 2: Total ion flow chromatogram of components in ethanol extract.

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

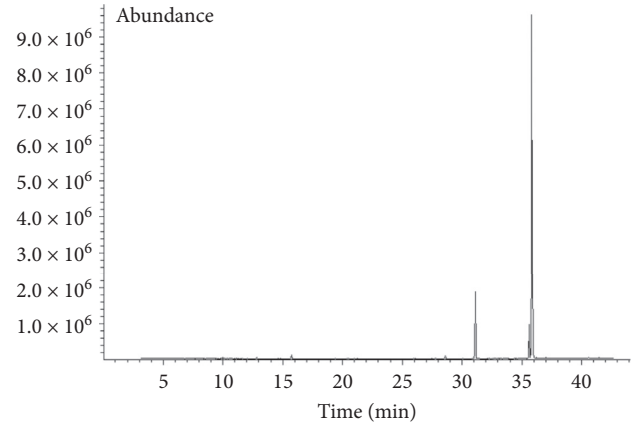

Figure 3: Total ion flow chromatogram of components in petroleum ether extract.

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

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.

Acknowledgments

This work was funded by the National Key R&D Program of China (2018YFD0400200) and Key Project in Science and Technology Agency of Henan Province (202102110283).

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