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Comparison of Agitake (*Pleurotus eryngii* var. *ferulae*) Volatile Components with Characteristic Odors Extracted by Hydrodistillation and Solvent-assisted Flavor Evaporation

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Abstract: The chemical composition of volatile oil from agitake (*Pleurotus eryngii* var. *ferulae*) was established for the first time using gas chromatography (GC) and GC-mass spectrometry. Sixty-seven and 24 components were extracted by hydrodistillation (HD) using diethyl ether (DE) and dichloromethane (DM), respectively; these components accounted for 80.3% and 91.8% of the total oil, respectively. Thirteen and 48 components of were extracted by the solvent-assisted flavor evaporation method (SAFE), using DE and DM, respectively, and identified; these components accounted for 83.5% and 82.0% of the total oil, respectively. Methylsuccinimide and 2,3,7-trimethyl-2-octene were the most characteristic components by SAFE using DM.

Odor evaluation of the volatile oil from agitake was also carried out using GC-olfactometry (GC-O), aroma extraction dilution analysis (AEDA), and the odor activity value (OAV). Sixteen, 8, 5 and 9 aroma-active components were identified using HD (DE and DM) and SAFE (DE and DM), respectively. The main aroma-active components extracted using HD and SAFE were 1-octen-3-ol (mushroom-like) and phenylacetaldehyde (floral), respectively. This study proved that HD and SAFE can be used as complementary extraction techniques for the complete characterization of volatile oil from agitake.

Key words: Agitake (*Pleurotus eryngii* var. *ferulae*), volatile oil, hydrodistillation (HD), solvent-assisted flavor evaporation (SAFE), aroma extraction dilution analysis (AEDA)

1 INTRODUCTION

Mushrooms have been widely consumed since ancient times, not only as food or food-flavoring materials, but also for medicinal or functional purposes, because of their distinctive flavors and textures. Among a range of volatile components, a series of aliphatic components, such as 1-octen-3-ol, 3-octanol, 1-octanol, 1-octen-3-one and 3-octanone have been reported to be the major contributors to the characteristic mushroom flavor.

In particular, an unsaturated alcohol, described as having a "mushroom-like" or "raw mushroom". Its flavor has been found in many mushroom species and, together with its oxidation product, 1-octen-3-one, is considered to be mainly responsible for the characteristic flavor of most edible species of mushroom[1-4]. The genus *Pleurotus* comprises a diverse group of cultivated mushroom species with high nutritional values and significant pharmacological properties. In the past decade, components with medical properties, including antiviral[5], antitumor[6], antibacterial[7], antibiotic[8], anticholesterologenic[9] and immiunostimulatory[10] effects, have been isolated from several *Pleurotus* species[11]. Recently, the mushrooms have been attracting attention as functional foods in Japan. We previously reported such mushroom[12-14].

Pleurotus eryngii var. *ferulae* (in Japanese, "agitake") is an edible mushroom produced by bacteria cultivated in the upper rhizomes of *Ferula assa-foetida* (in Japanese, "agi"), which is a medicinal plant. The mushroom has a

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beautiful shape and is highly flavored. A previous study revealed that an extract liquid submerged culture of agitake has significant antioxidant and antigenotoxicity[15]. An ethanol extract of the fruiting bodies of agitake was reported to show a strong antitumor activity against three human solid carcinomas; a lung carcinoma (A549) and two cervical carcinomas (SiHa and HeLa)[16]. Furthermore, an ethyl acetate fraction of a methanol extract of agitake sporocarps was reported to show considerable human neutrophil elastase (HNE) inhibitory activity[17]. However, so far, there has been describing agitake volatile oil.

In flavor analysis, gas chromatography-olfactometry (GC-O) is the method used most widely for evaluation of odorants. In particular, GC-O, including aroma extraction dilution analysis (AEDA), is a useful method for estimating the contributions of the most odor-active components. AEDA is a useful method for obtaining desirable results on the odor-active components through sniffing analysis. By sniffing analysis of serial dilutions of a volatile oil, the volatile components can be ranked according to odor potency[18]. The odor potency is expressed as the flavor dilution (FD) factor. The FD factor is the ratio of the initial concentration of a component in the initial concentration to the most diluted concentration at which the odor can be detected by GC-O.

The aim of this study is to investigate the characteristic odor components of agitake volatile oil by AEDA and odor activity value (OAV) methods, using hydrodistillation (HD) and solvent-assisted flavor evaporation (SAFE) with two solvents.

2 EXPERIMENTAL

2.1 Materials

Agitake (*P. eryngii* var. *ferulae*) plant material was collected from Takeuchi Nouen (1-2-8 Miyoshi-cho, Nakanoshi, Nagano 383-0025, Japan) in May 2012. Identification of the plant was performed, and a voucher specimen was deposited, at the biotechnology laboratory of Kinki University, Osaka, Japan.

2.2 Isolation of the volatile oil

2.2.1 HD method

The volatile oil from agitake was obtained by HD for 2 h with a Likens-Nickerson-type apparatus using a solvent [diethyl ether (DE) or dichloromethane (DM)]. The yield of oil was 0.068% (DE) and 0.011% (DM). The oils were dried over anhydrous sodium sulfate and stored at 4℃ in a refrigerator prior to analysis.

2.2.2 SAFE

Fresh agitake was frozen in liquid nitrogen. The crushed frozen parts were added to a solvent (DE or DM), and the mixture was stirred and extracted. After standing for 2 d, the residual substances were removed by passing through filter paper. The volatile components were separated from the solvent extracts using SAFE[19]. The filtrate was vacuum distilled using a SAFE apparatus as previously described[20]. After complete introduction of the filtrate into the SAFE system, distillation was carried out for 2 h at 10^{-4} torr. The volatile components were collected in a trap, which was submerged in liquid nitrogen; 0.08% yield (DE) and 0.003% yield (DM) of colorless oils were obtained. The volatile components were stored at 4℃ in a refrigerator prior to analysis.

2.3 GC

Analysis of the oil sample was performed using an Agilent Technologies-6890N gas chromatograph (flame inonization detector) equipped with an HP-5MS (Agilent Technologies) fused-silica capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness). The oven temperature was programmed as follows: initial increase 40–260℃ at 4℃/min, followed by 5 min at 260℃. The carrier gas was He at a flow of 1.8 mL/min; the injector and detector temperatures were 270 and 280℃, respectively. Samples were injected using the split mode, at a split ratio of 1:10, and 1 μL of oil sample was injected.

2.4 Gas chromatography-mass spectrometry (GC-MS)

GC-MS was carried out using an Agilent 6890-5973 instrument. The sample was analyzed using an HP-5MS fused-silica capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm) and a DB-WAX (15 m × 0.25 mm i.d., film thickness 0.25 μm) column. The oven temperature was increased from 40 to 260℃ at a rate of 4℃/min and held at 260℃ for 5 min. The injector and detector temperatures were 270 and 280℃, respectively. The actual temperature in the MS source reached approximately 230℃; the ionization energy was 70 eV and the mass range was 39–450 amu. The oil (6 mg) was diluted with 500 μL of DE, and then, 1 μL of the resulting solution was injected at a 1:10 split ratio.

2.5 GC-O sniffing test

Sniffing tests using GC-O was carried out with an Agilent Technologies 6890N gas chromatograph equipped with an Agilent 5973 MSD mass spectrometer and sniffing port (olfactory detector port, ODP 2, Gerstel, Tokyo, Japan). The GC was equipped with an HP-5MS (30 m × 0.25 mm i.d., 0.25 μm film thickness) column. The sample was injected into the gas chromatograph in splitless mode. The effluent from the capillary column was split 1:1 (v/v) between the MS and the sniffing port. The oven conditions, the carrier gas, flow rate, and ionization mode were the same as those described above for GC-MS.

2.6 AEDA

The highest sample concentration (10 mg/mL) was assigned an FD factor of 1. The volatile oil was diluted stepwise with DE or DM (1:1, v/v), and aliquots of the dilutions (1 μL) were evaluated. The process was stopped when no aromas were detected by assessors. The results were expressed as the FD factor, which is the ratio of the initial concentration of the odorant in the volatile oil to the lowest concentration at which the odor is still detectable by GC-O.

2.7 Identification and quantification of components

The identities of individual components were confirmed by comparison of the MS data with published data[21], and those from our previous studies, and of retention indices (RI) with those of the standards or RIs reported in the literature[22–36]. The RIs were calculated using a series of *n*-alkanes (C8–C27) on two columns of different polarities. Quantitative analysis was performed using an internal standard addition method (alkanes C12 and C19). The volatile oil was diluted 100 times, using DE, to achieve a volume of 1 mL, and then 5 mL of a C12 and C19 mixture solution (1 mg/mL) were added into the diluted oil. The prepared samples were subjected to GC-MS analysis. Quantitative analysis was performed on the basis of calibration curves for pyridine (**4**), 2,3-butanediol (**8**), hexanol (**9**), methional (**13**), benzaldehyde (**16**), 1-octen-3-ol (**18**), 3-octanone (**19**), 2-pentylfuran (**20**), 2-acetylthiazole (**26**), 2-ethylhexanol (**27**), phenylacetaldehyde (**29**), acetophenone (**32**), phenylethyl alcohol (**39**), 2-aminoacetophenone (**54**), and γ-dodecanolactone (**82**) within the concentration range 0.5–1000 μg/mL. The peak area of each component was calculated using the FID response factors. Because of the lack of proper standards, γ-elemene (**62**), γ-muurolene (**66**), β-bisabolene (**68**) and α-copaene (**75**) were quantified using the calibration curves for β-caryophyllene.

3 RESULTS AND DISCUSSIONS

3.1 Volatile components in the agitake.

The volatile oil obtained by HD using DE was yellowish oil and the yield was 0.068% (w/w). A total of 67 components were identified, representing about 80.3% of the total oil (Table 1). The main components of the volatile oil were linoleic acid (**97**; 23.0%) and hexadecanoic acid (**94**; 22.3%). The composition of the oil obtained by HD is summarized in Table 2. The volatile oil consisted mainly of acids (46.6%), followed by esters (24.5%), alcohols (3.4%), and aldehydes (2.0%). On the other hand, a yellowish oil was obtained by HD using DM; the yield was 0.011% (w/w). Twenty-four components were identified, representing about 91.8% of the total oil (Table 1). The main components were 1-octen-3-ol (**18**; 29.1%) and 3-octanone (**19**; 26.1%). The oil consisted mainly of alcohols (35.3%), followed by ketones (31.2%), esters (12.6%), and aldehydes (3.7%).

In contrast, SAFE using DE gave a colorless oil; the yield was 0.08% (w/w). A total of 13 components were identified, representing about 83.5% of the total oil (Table 1). The main components of the volatile oil were 1-octen-3-ol (**18**; 67.5%), octanoic acid (**42**; 9.5%), and 4-hydroxy-2-methoxybenzaldehyde (**61**; 3.9%).

The composition of the oil obtained by SAFE is summarized in Table 2. The volatile oil consisted mainly of alcohols (67.7%), followed by acids (9.5%), aldehydes (3.9%), and ketones (1.6%). When DM was used as the solvent, a colorless oil was obtained in 0.003% (w/w) yield. Forty-eight components were identified, representing about 82.0% of the total oil (Table 1). The main components of the oil were benzoic acid (**52**; 28.2%), 2,3-butanediol (**8**; 14.8%), and 4-methoxybenzaldehyde (**48**; 7.5%). The composition of the oil obtained by SAFE is summarized in Table 2. The volatile oil consisted mainly of acids (30.5%), followed by alcohols (25.2%), aldehydes (10.3%), and esters (4.4%).

Agitake contains characteristic components, such as (*E*)-4-undecene (**28**), 2,3,7-trimethyl-2-octene (**31**), methylsuccinimide (**36**), (*E*)-α-atlantone (**87**), and 2-hexyloxyethanol (**38**). Among these components, 2,3,7-trimethyl-2-octene (**31**) and methylsuccinimide (**36**) are particularly unusual (Fig. 1). It has been reported that 2,3,7-trimethyl-2-octene (**31**) is present in the flowers of *Edgeworthia chrysantha* Lindl[37]. Methylsuccinimide (**36**) is present in the aerial parts of *Brunfelsia grandiflora*[38]. However, this is the first report of these compounds in the *Pleurotus* genus.

3.2 GC-O, AEDA and OAV

The odor-active components of the volatile oil from agitake were also evaluated using GC-O and AEDA. Identification of the components was based on comparisons of their retention times. As shown in Table 3, 20 aroma-active components were detected. The identified components were represented by alcohols [2,3-butanediol (**8**), hexanol (**9**), 1-octen-3-ol (**18**), 2-ethylhexanol (**27**), 1-octanol (**33**), phenylethyl alcohol (**39**)], a nitrogen-containing components [pyridine (**4**)], sulfur-containing components [methional (**13**), 2-acetylthiazole (**26**)], a furan [2-pentylfuran (**20**)], aldehydes [benzaldehyde (**16**), phenylacetaldehyde (**29**)], ketones [3-octanone (**19**), acetophenone (**32**), 2-aminoacetophenone (**54**)], sesquiterpenes [γ-elemene (**62**), γ-muurolene (**66**), β-bisabolene (**68**), α-copaene (**75**)], and a lactone [γ-dodecanolactone (**82**)]. Figures 2 and 3 show a GC-FID chromatogram (above) and AEDA results (below) for a agitake components extracted by HD and SAFE. On the basis of the FD factor, 1-octen-3-ol (**18**; FD = 64, mushroom), methional (**13**; FD = 32, potato), and phenylacetaldehyde (**29**; FD = 32, floral) were the most

Table 1. Chemical components of the volatile oil from agitake (*P. eryngii* var. *ferulae*).

No.	Components	RI[a]		Peak Area (%)[b]				Identification method[c]	Reference source[d]
				HD		SAFE			
		HP-5MS	DB-WAX	DE[e]	DM[f]	DE	DM		
1	3-Hydroxy-2-butanone	751	1312	-	-	-	2.4	RI, MS	TCI
2	(*Z*)-2-Penten-1-ol	753	-	0.2	-	-	-	RI, MS	Wako
3	Isopentyl formate	779	-	1.5	-	-	-	RI, MS	Wako
4	Pyridine	784	1193	tr[g]	-	-	-	RI, MS	Wako
5	Butanoic acid	786	1597	tr	-	-	-	RI, MS	Wako
6	Ethyl butanoate	802	-	tr	-	-	-	RI, MS	Wako
7	Lactonitrile	816	1732	0.2	-	-	-	RI, MS	Aldrich
8	2,3-Butanediol	848	1475	-	-	-	14.8	RI, MS	Wako
9	Hexanol	868	1318	tr	-	-	-	RI, MS	Wako
10	Pentanoic acid	887	1727	tr	-	-	-	RI, MS	Wako
11	Ethyl pentanoate	901	-	tr	-	-	-	RI, MS	Wako
12	2-Butoxyethanol	907	-	-	3.7	-	-	RI, MS	Aldrich
13	Methional	913	1431	0.1	2.5	-	-	RI, MS	Wako
14	2-Butoxyethanol	920	1345	-	-	-	6.1	RI, MS	Wako
15	Dimethyl sulfone	945	1774	-	-	-	1.4	RI, MS	Wako
16	Benzaldehyde	956	1409	0.2	2.1	-	0.2	RI, MS	Wako
17	Hexanoic acid	980	1741	-	-	tr	0.2	RI, MS	Wako
18	1-Octen-3-ol	983	1388	1.8	29.1	67.5	1.5	RI, MS	Wako
19	3-Octanone	988	1272	0.5	26.1	0.6	-	RI, MS	Wako
20	2-Pentylfuran	990	1249	0.2	2.8	-	-	RI, MS	Wako
21	3-Methylthiopropanol	991	1620	-	-	-	0.1	RI, MS	Wako
22	2-Cyclohexen-1-one	997	1424	0.4	-	-	-	RI, MS	Wako
23	Ethyl hexanoate	998	-	tr	-	-	-	RI, MS	Wako
24	Phenol	1004	1861	-	-	0.2	0.6	RI, MS	Wako
25	2-(2-Ethoxyethoxy)-ethanol	1017	-	-	-	-	0.7	RI, MS	Wako
26	2-Acetylthiazole	1020	1596	0.3	-	-	-	RI, MS	Wako
27	2-Ethylhexanol	1034	1425	-	1.1	tr	0.5	RI, MS	Wako
28	(*E*)-4-Undecene	1039	-	-	-	-	0.2	RI, MS	Other
29	Phenylacetaldehyde	1047	1539	0.1	1.6	tr	2.6	RI, MS	Wako
30	4-Hexen-3-one	1055	-	-	4.4	0.6	0.4	RI, MS	Wako
31	2,3,7-Trimethyl-2-octene	1059	-	-	-	-	0.2	RI, MS	Other
32	Acetophenone	1066	1532	tr	0.7	0.4	-	RI, MS	Wako
33	1-Octanol	1072	1493	tr	-	-	tr	RI, MS	Wako
34	Heptanoic acid	1078	1925	tr	-	-	-	RI, MS	Wako
35	Ethyl heptanoate	1095	1332	tr	1.2	-	-	RI, MS	Wako
36	Methylsuccinimide	1107	1792	-	-	-	2.5	RI, MS	Wako
37	Ethyl-2-ethylhexanoate	1108	-	tr	-	-	-	RI, MS	Other
38	2-Hexyloxyethanol	1112	-	-	-	-	0.6	RI, MS	Aldrich
39	Phenylethyl alcohol	1122	1792	tr	-	-	0.3	RI, MS	Wako
40	2-Ethylhexanoic acid	1159	1960	-	-	-	0.9	RI, MS	Wako
41	Ethyl benzoate	1170	1650	0.1	2.0	-	-	RI, MS	Wako
42	Octanoic acid	1182	1948	tr	1.0	9.5	-	RI, MS	Wako
43	Butyldiglycol	1193	-	-	1.3	tr	0.1	RI, MS	Other
44	Ethyl octanoate	1194	1441	tr	-	-	-	RI, MS	Wako
45	Dodecane	1200	1200	-	-	0.5	0.2	RI, MS	Wako
46	4-Allylphenol	1247	-	0.1	0.1	-	-	RI, MS	Other
47	2-(1*E*)-Propenylphenol	1255	-	0.1	tr	-	-	RI, MS	Other
48	4-Methoxy benzaldehyde	1263	1889	-	-	-	7.5	RI, MS	Wako
49	2-Phenyl-2-butenal	1272	1802	0.2	-	-	-	RI, MS	Wako
50	Nonanoic acid	1280	2144	tr	1.1	-	-	RI, MS	Wako
51	Ethyl nonanoate	1294	1521	tr	1.3	-	-	RI, MS	TCI
52	Benzoic acid	1296	2360	-	-	-	28.2	RI, MS	Wako
53	Indolizine	1299	-	tr	-	-	-	RI, MS	TCI
54	2-Aminoacetophenone	1302	2223	tr	-	-	-	RI, MS	Other

Table 1. Continued.

No.	Components	RI[a]		Peak Area (%)[b]				Identification method[c]	Reference source[d]
				HD		SAFE			
		HP-5MS	DB-WAX	DE[e]	DM[f]	DE	DM		
55	Phenylacetic acid	1324	2571	-	-	0.3	0.6	RI, MS	Wako
56	Decanoic acid	1380	2464	tr	-	-	-	RI, MS	Wako
57	3-Methylindole	1389	2459	tr	-	-	-	RI, MS	Wako
58	Ethyl decanoate	1392	1829	tr	-	-	-	RI, MS	Wako
59	Tetradecane	1400	1400	-	-	-	0.3	RI, MS	Wako
60	Acetanilide	1401	-	-	-	-	0.2	RI, MS	Wako
61	4-Hydroxy-2-methoxybenzaldehyde	1416	-	-	-	3.9	0.2	RI, MS	Wako
62	γ-Elemene	1431	1636	tr	-	-	-	RI, MS	Other
63	1,3-Diacetylbenzene	1445	-	-	-	-	0.2	RI, MS	Wako
64	Undecanoic acid	1465	2394	tr	-	-	-	RI, MS	Wako
65	γ-Decalactone	1468	2565	0.1	-	-	-	RI, MS	Wako
66	γ-Muurolene	1476	1679	-	-	-	0.5	RI, MS	Other
67	Ethyl undecanoate	1496	1732	tr	-	-	-	RI, MS	Wako
68	β-Bisabolene	1506	1720	0.2	-	-	1.1	RI, MS	Other
69	δ-Cadinene	1522	1661	-	1.4	-	0.2	RI, MS	Other
70	Dodecanoic acid	1568	2472	tr	-	-	-	RI, MS	Wako
71	Ethyl dodecanoate	1594	1829	tr	4.0	-	-	RI, MS	Wako
72	1,3-Dicyclohexylpropane	1599	-	-	-	-	0.2	RI, MS	Other
73	1-*epi*-Cubenol	1627	2085	0.2	-	-	-	RI, MS	Other
74	β-Cadinene	1641	1769	0.2	-	-	-	RI, MS	Other
75	α-Copaene	1648	1462	tr	-	-	0.1	RI, MS	Other
76	Tridecanoic acid	1664	2603	tr	-	-	-	RI, MS	TCI
77	(*Z*)-6-Dodecene-γ-lactone	1669	2349	7.8	-	-	1.2	RI, MS	Other
78	1,3-Dicyclohexylbutane	1675	-	-	1.8	-	0.2	RI, MS	Other
79	2,2',5,5'-Tetramethylbiphenyl	1679	-	0.3	-	-	-	RI, MS	Other
80	4-Undecanolide	1682	-	-	-	-	0.9	RI, MS	Other
81	Ethyl tridecanoate	1687	1950	tr	-	-	-	RI, MS	Wako
82	γ-Dodecanolactone	1689	2381	4.5	-	-	-	RI, MS	Wako
83	1,2,3-Trimethyl-4-[(*E*)-1-propenyl]naphthalene	1709	2229	0.2	-	-	-	RI, MS	Other
84	(*E,E*)-Farnesol	1721	2291	0.2	-	-	-	RI, MS	Aldrich
85	4-Methylbenzophenone	1756	-	0.8	-	-	0.2	RI, MS	Wako
86	Drimenol	1765	-	0.7	-	-	-	RI, MS	Other
87	(*E*)-α-Atlantone	1774	-	0.3	-	-	-	RI, MS	Other
88	Tetradecanoic acid	1777	2675	0.4	-	-	-	RI, MS	Wako
89	2,4-Diphenyl-4-methyl-1-pentene	1783	-	0.1	-	-	-	RI, MS	Wako
90	Ethyl tetradecanoate	1789	2040	0.2	-	-	0.2	RI, MS	Wako
91	6-Dodecyne	1867	2586	-	-	-	0.3	RI, MS	Wako
92	Pentadecanoic acid	1878	2783	0.9	-	-	-	RI, MS	Wako
93	Ethyl pentadecanoate	1889	-	-	1.1	-	0.3	RI, MS	TCI
94	Hexadecanoic acid	1977	2897	22.3	-	-	-	RI, MS	Wako
95	Ethyl hexadecanoate	1992	2268	5.6	-	-	0.5	RI, MS	Wako
96	(*Z*)-9,17-Octadecadienal	2139	-	-	-	-	tr	RI, MS	Other
97	Linoleic acid	2144	3160	23.0	tr	-	0.6	RI, MS	Wako
98	Ethyl linoleate	2156	2522	6.1	-	-	0.6	RI, MS	TCI
99	Ethyl oleate	2163	2484	-	1.3	-	0.6	RI, MS	Wako
100	Monoethylhexyl phthalate	2541	-	tr	-	-	0.1	RI, MS	Wako
101	Squalene	2819	3058	tr	-	-	0.1	RI, MS	Wako
			Total	80.3	91.8	83.5	82.0		

[a] RI, retention indices determined on HP-5MS and DB-WAX columns, using the homologous series of *n*-alkanes.
[b] Peak area (%) was related to total detected compounds by GC-MS.
[c] Identification methods: RI, retention indice; MS, mass spectrum.
[d] Reference materials were obtained from commercial source: Wako, Wako Pure Chemical Industries Ltd. (Osaka, Japan); TCI, Tokyo Kasei Kogyou Co. Ltd. (Tokyo, Japan); Aldrich, Sigma-Aldrich, St. Louis
[e] DE = diethyl ether
[f] DM = dichloromethane
[g] tr = trace (< 0.1 %)

Table 2. Classification of the components of the volatile oil from agitake (*P. eryngii* var. *ferulae*).

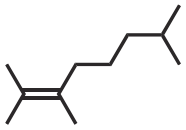
	Peak Area (%)[a]			
	HD		SAFE	
	DE[b]	DM[c]	DE	DM
Hydrocarbon	1.0	1.4	0.5	3.4
Aldehyde	2.0	3.7	3.9	10.3
Alcohol	3.4	35.3	67.7	25.2
Ketone	1.6	31.2	1.6	3.2
Ester	24.5	12.6	-	4.4
Acid	46.6	2.1	9.5	30.5
Nitrogen containing	0.5	-	-	2.6
Sulfur containing	0.1	2.5	-	1.5
Other	0.6	0.1	0.4	0.9

[a] These values were calculated from GC peak area.

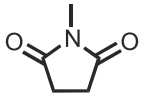
[b] DE = diethyl ether

[c] DM = dichloromethane

intense aroma-active components obtained by HD using DE. Sniffing tests showed that 1-octen-3-ol (**18**) was responsible for the mushroom odor, methional (**13**) produced a potato odor, and phenylacetaldehyde (**29**) produced a floral odor in the oil obtained by HD using DE. When DM was used, 1-octen-3-ol (**18**; FD = 64, mushroom), methional (**13**; FD = 32, potato), 3-octanone (**19**; FD = 32, sweet) and phenylacetaldehyde (**29**; FD = 32, floral) were the most intense aroma-active components. Sniffing tests showed that 1-octen-3-ol (**18**) produced a mushroom odor, methional (**13**) produced a potato odor, 3-octanone (**19**) produced a sweet odor, and phenylacetaldehyde (**29**) produced a floral odor in the oil obtained by HD using DM. The most aroma-active components of SAFE using DE were 1-octen-3-ol (**18**; FD = 64, mushroom), followed by 3-octanone (**19**; FD = 8, sweet) and acetophenone (**32**; FD = 2, sweet). 1-Octen-3-ol (**18**) produced a mushroom odor, and 3-octanone (**19**) and acetophenone produced sweet odors in the SAFE extract. When DM was used as the solvent, the aroma-active components were 1-octen-3-ol (**18**; FD = 64,



2,3,7-Trimethyl-2-octene (**31**)



Methylsuccinimide (**36**)

Fig. 1 The characteristic components of volatile oil from agitake (*P. eryngii* var. *ferulae*).

Table 3. Odor activity components of the volatile oil from agitake (*P. eryngii* var. *ferulae*).

No.	Components	Odor	FD factor[a]				Concentration[b] (ppb)				OT[c] (ppb)	OAV[c]			
			HD		SAFE		HD		SAFE			HD		SAFE	
			DE[d]	DM[e]	DE	DM	DE	DM	DE	DM		DE	DM	DE	DM
4	Pyridine	burnt	2	-	-	-	680	-	-	-	4	170	-	-	-
8	2,3-Butanediol	sweet	-	-	-	32	-	-	-	4425	95	-	-	-	47
9	Hexanol	green	2	-	-	-	680	-	-	-	500	1	-	-	-
13	Methional	potato	32	32	-	-	884	688	-	-	0.2	4420	3438	-	-
16	Benzaldehyde	sweet	1	16	-	2	1224	578	-	69	350	3	2	-	<1
18	1-Octen-3-ol	mushroom	64	64	64	64	12308	8003	135000	438	1	12308	8003	135000	438
19	3-Octanone	sweet	-	32	8	-	-	7178	1200	-	70	-	103	17	-
20	2-Pentylfuran	sweet	8	16	-	-	1224	770	-	-	6	204	128	-	-
26	2-Acetylthiazole	sweet	16	-	-	-	1836	-	-	-	10	184	-	-	-
27	2-Ethylhexanol	green	-	2	1	1	-	303	60	156	N/A[f]	-	N/D[g]	N/D	N/D
29	Phenylacetaldehyde	floral	32	32	1	32	952	440	40	789	4	238	110	10	197
32	Acetophenone	sweet	1	1	2	-	680	193	800	-	65	10	3	12	-
33	1-Octanol	green	2	-	-	-	680	-	-	-	125	5	-	-	-
39	Phenylethyl alcohol	floral	2	-	-	1	680	-	-	96	1000	<1	-	-	<1
54	2-Aminoacetophenone	sweet	1	-	-	-	680	-	-	-	N/A	N/D	-	-	-
62	γ-Elemene	woody	2	-	-	-	680	-	-	-	N/A	N/D	-	-	-
66	γ-Muurolene	woody	-	-	-	2	-	-	-	138	N/A	N/D	-	-	N/D
68	β-Bisabolene	woody	4	-	-	2	1360	-	-	327	N/A	N/D	-	-	N/D
75	α-Copaene	woody, spicy	1	-	-	2	680	-	-	42	N/A	N/D	-	-	N/D
82	γ-Dodecanolactone	woody	4	-	-	-	30736	-	-	-	N/A	N/D	-	-	-

[a] Flavor dilution factor obtained by aroma extract dilution analysis (AEDA) on capillary HP-5MS.

[b] Parts per billion (10^9) or micrograms pf component per kilogram.

[c] Odor activity values were calculated by dividing the concentrations of the component by its recognition threshold.

[d] DE = diethyl ether

[e] DM = dichloromethane

[f] Data not available

[g] Not determined

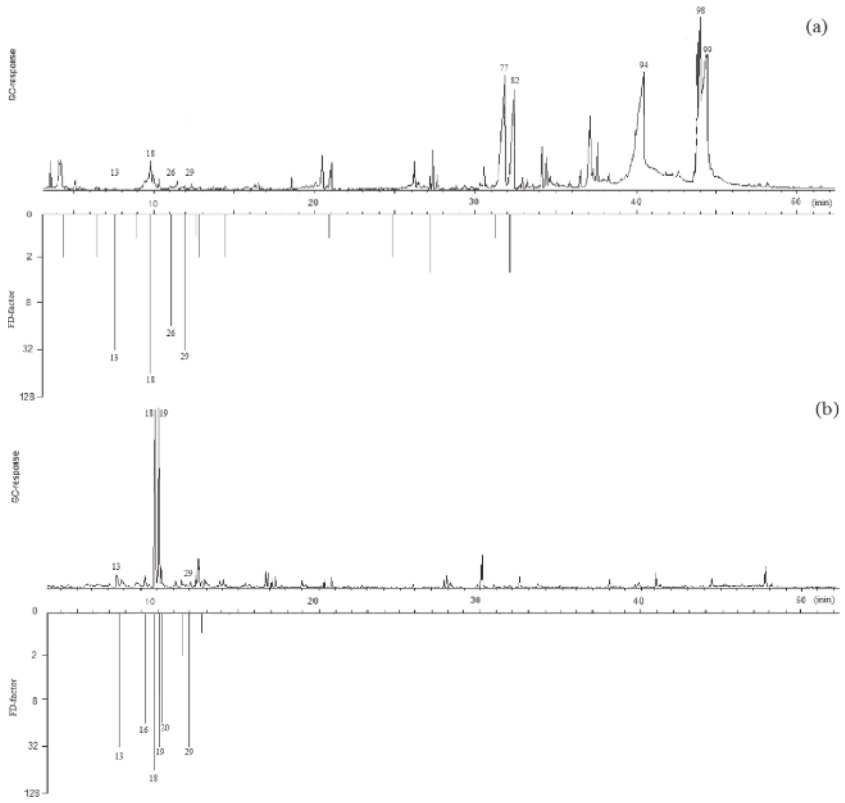


Fig. 2 Gas chromatogram and aromagram (FD factor) of volatile oil from agitake (*P. eryngii* var. *ferulae*) obtained by hydrodistillation (HD); (a) using DE; (b) using DM: **13**, methional; **16**, benzaldehyde; **18**, 1-octen-3-ol; **19**, 3-octanone; **20**, 2-pentylfuran; **26**, 2-acetylthiazole; **29**, phenylacetaldehyde; **77**, (*Z*)-6-dodecene-γ-lactone; **82**, 4-dodecanolide; **94**, hexadecanoic acid; **98**, linoleic acid; **99**, ethyl linoleate.

mushroom), followed by phenylacetaldehyde (**29**; FD = 32, floral) and 2,3-butanediol (**8**; FD = 32, sweet). 1-Octen-3-ol (**18**) produced a mushroom odor, phenylacetaldehyde (**29**) produced a floral odor, and 2,3-butanediol (**8**) produced a sweet odor. These were assumed to contribute strongly to the odor of agitake.

In order to determine the relative contribution of each of the components to the agitake aroma, OAV was used. The OAV was obtained based on the concentration and odor threshold of each component. Because of the unavailability of odor threshold data in the literature, the OAVs of 2-ethylhexanol (**27**), 2-aminoacetophenone (**54**), γ-elemene (**62**), γ-muurolene (**66**), β-bisabolene (**68**), α-copaene (**75**) and γ-dodecanolactone (**82**) were not determined. In the extract obtained by HD using DE, 1-octen-3-ol (**18**) had the highest OAV (12 308), followed by methional (**13**; OAV = 4420), phenylacetaldehyde (**29**; OAV = 238), and 2-pentylfuran (**20**; OAV = 204). When DM was used, 1-octen-3-ol (**18**) had the highest OAV (8003), followed by methional (**13**; OAV = 3438), 2-pentylfuran (**20**; OAV = 128), and phenylacetaldehyde (**29**; OAV = 110). 1-Octen-3-ol (**18**), methional (**13**), and phenylacetaldehyde (**29**) had particularly high FD factors, and were therefore considered to be the main aroma-active components obtained by HD. For SAFE using DE, the three most potent components were 1-octen-3-ol (**18**; OAV = 135 000), 3-octanone (**19**; OAV = 17), and acetophenone (**32**; OAV = 12). When DM was used, the three most potent components were 1-octen-3-ol (**18**; OAV = 438), 2,3-butanediol (**8**; OAV = 47), and phenylacetaldehyde (**29**; OAV = 197). These components showed particularly high FD factors, suggesting that these components make major contributions to the aroma of the SAFE extract. Generally, components with a high FD factor also had high OAVs, which confirms the positive relationship between the FD factor and the OAV[39].

In conclusion, we investigated the characteristic odor components of agitake using sensory evaluation and the concept of OAVs. On the basis of AEDA, OAVs, and sensory evaluations, 1-octen-3-ol (**18**) was found to be the main aroma-active component obtained using two methods. In HD using DE, 1-octen-3-ol (**18**) produced a mushroom odor, methional (**13**) was important in producing a potato odor, and phenylacetaldehyde (**29**) contributed a floral odor. When DM was used, 1-octen-3-ol (**18**) produced a mushroom odor, methional (**13**) was important in producing a potato odor, 3-octanone (**19**) produced a sweet odor, and phenylacetaldehyde (**29**) contributed a floral odor. For the extract obtained by SAFE using DE, 1-octen-3-ol (**18**) pro-

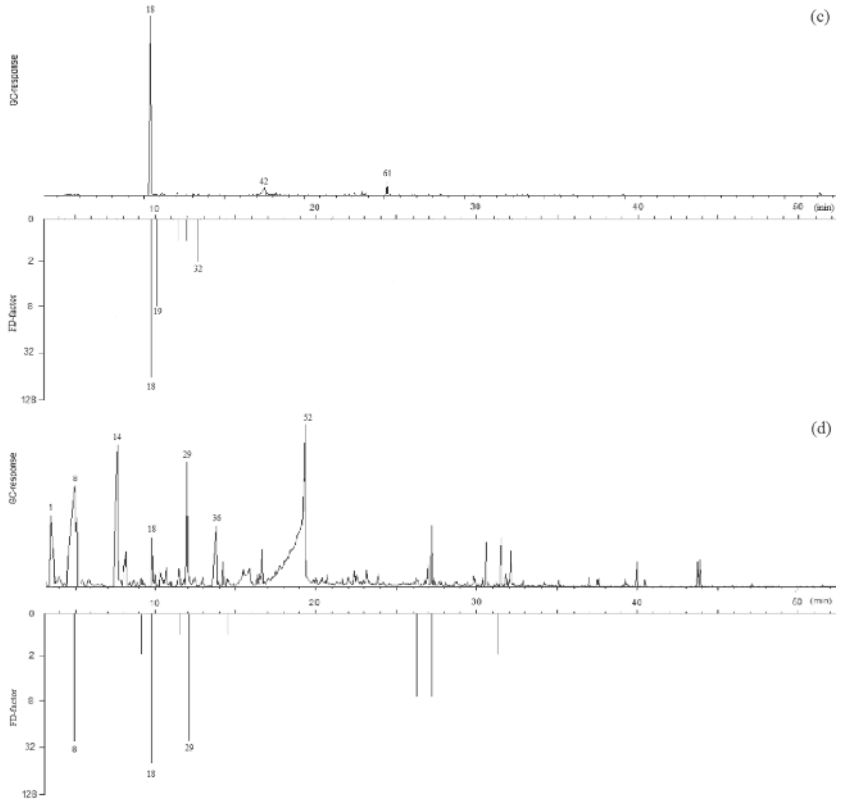


Fig. 3 Gas chromatogram and aromagram (FD factor) of volatile oil from agitake (*P. eryngii* var. *ferulae*) obtained by SAFE; (c) using DE; (d) using DM: **1**, 3-hydroxy-2-butanone; **8**, 2,3-butanediol; **14**, 2-butoxyethanol; **18**, 1-octen-3-ol; **29**, phenylacetaldehyde; **32**, acetophenone; **36**, methylsuccinimide; **42**, octanoic acid; **52**, benzoic acid; **61**, 4-hydroxy-2-methoxybenzaldehyde.

duced a mushroom odor, and 3-octanone (**19**) and acetophenone (**32**) produced sweet odors. When DM was used, 1-octen-3-ol (**18**) produced a mushroom odor, 2,3-butanediol (**8**) contributed a sweet odor, and phenylacetaldehyde (**29**) contributed a floral odor.

The numbers of detected compounds and yields obtained by HD using DM, and SAFE using DE, were poor. It is thought that this was related to the boiling points and densities of the solvents. The most appropriate solvents for detecting large numbers of ingredients are therefore DE for HD and DM for SAFE.

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