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〔Review Article〕

Character impact odorants from mushrooms [*Pleurotus citrinopileatus*, *Pleurotus eryngii* var. *ferulae*, *Lactarius hatsudake*, and *Hericium erinaceus* (Bull.: Fr.) Pers.] used in Japanese traditional food

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ABSTRACT

The composition of volatile oil of Golden oyster mushroom (*Pleurotus citrinopileatus*) were analyzed by capillary gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). Fifty-nine components, representing 95.9% of the total oil were identified. The main components of the oil were fatty acids such as linoleic acid (31.3%), palmitic acid (23.3%), and pentadecylic acid (6.0%). Elsholtzia ketone and curcuphenol were identified in the oil of mushroom for the first time. The result of Gas chromatography-olfactometry (GC-O) and aroma extraction dilution analysis (AEDA) showed that the sulfur and nitrogen-containing components, C8 ketone and C8 aldehyde are responsible for the aroma of *P. citrinopileatus*.

The chemical composition of volatile oil from agitake (*P. eryngii* var. *ferulae*) was established for the first time using GC and GC-MS. 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 in SAFE using DM.

Odor evaluation of the volatile oil from agitake was also carried out using GC-O, AEDA, and the odor activity value (OAV). Sixteen, eight, five and nine 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

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complete characterization of volatile oil from agitake.

The components of the volatile oil from wild mushroom (*Lactarius hatsudake*), used in Japanese traditional food, were analyzed and quantified for the first time by capillary GC and GC-MS. Seventy-six components were separated from the oil and of these 71 components were identified. Odor evaluation of the volatile oil from *L. hatsudake* was also carried out using GC-O and AEDA, from which it was found that hexanol, 4-dehydroviridiflorol, myliol and phenylacetaldehyde seem to contribute to the green, spicy and sweet odor of *L. hatsudake*.

The components of the volatile oil from dried fruiting bodies of *Hericium erinaceus* (Bull.: Fr.) Pers. were analyzed by capillary GC and GC -MS. Seventy-seven components, representing 87.6% of the total oil were identified. The main components of the oil were hexadecenoic acid (26.0%), linoleic acid (13.1%), phenylacetaldehyde (8.9%), and benzaldehyde (2.5%). The oil from *H. erinaceus* was also carried out odor evaluation by GC-O and AEDA. As a result, 2-methyl-3-furanthiol, 2-ethylpyrazine and 2, 6-diethylpyrazine were considered to be principal contributors of *H. erinaceus* odor.

Keywords: volatile oil; *Pleurotus citrinopileatus*; *Pleurotus eryngii* var. *ferulae*; *Lactarius hatsudake*; *Hericium erinaceus*; 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.

Pleurotus citrinopileatus (in Japanese, “tamogi-take”) is an edible mushroom that belongs to the *Pleurotaceae* family and grows on standing and fallen elm trees in Russia, China, Korea, and Japan, where in the latter it is one of the country’s most important mushrooms. It has significant advantages over other mushrooms in terms of rapid fruiting body formation, resulting in a short cultivation period.

Recently, it has been demonstrated that *P. citrinopileatus* has some physiological effects, including antitumor[6], immunoehancement, antihyperglycemia[12,13], antioxidant[14,15], and the reduction of blood sugar level[16]. This mushroom is known as a Japanese traditional food and it is eaten as a tempura because of characteristic aroma with sweet and nut odor.

P. 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 beautiful shape and is highly flavored. 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)[17]. Furthermore, an ethyl acetate fraction of a methanol extract of agitake sporocarps was reported to show considerable human neutrophil elastase (HNE) inhibitory activity[18].

Lactarius hatsudake is an edible, slightly bitter mushroom fungus belonging to the genus *Lactarius* (DC.) Gray, family Russulaceae, which is widely distributed in Japan, Korea and China. *L. hatsudake* is the saprophytic inhabitant of pines such as *Pinus densifl ora* (in Japanese, 'akamatsu'), *Pinus thunbergii* (in Japanese, 'kuromatsu'). This mushroom is called 'hatsutake' in Japan, the name deriving from the fact that it grows earlier than any other type of mushroom. *L. hatsudake* is known as a Japanese traditional food and has been used for a great number of years as a base because of its green, sweet and spicy flavors.

Mushrooms that belong to the genus *Lactarius* (DC.) Gray have the unique property that they are lactescent when their caps are scratched[19]. In the majority of *Lactarius* species, different kinds of sesquiterpenes play important biological roles, being responsible for the pungency and bitterness of the milky juice, and for changes in latex color when exposed to air. The latex of *L. hatsudake* is dark red and later changes color to blue-green. In a previous report, two new azulene pigments, 7-(1-hydroxyl-1-methyl)-4-methylazulene-1-carbaldehyde and 4-methyl-7-(1-methylethyl) azulene-1-carboxylic acid, were isolated from an extract of *L. hatsudake*[20]. Moreover, four ergosterol derivatives [(22*E*, 24*R*)-ergosta-5, 7, 22-dien-3β-ol; 5α, 8α-epidioxy-(22*E*, 24*R*)-ergost α-6,22-dien-3β-ol; 5α, 8 α-epidioxy-(24*S*)-ergosta-6-en-3β-ol; (22*E*, 24*R*)-ergosta-7, 22-dien-3β, 5α, 6β-triol) were isolated from *L. hatsudake*[21].

Hericium erinaceus (Bull.: Fr.) Pers. is an edible mushroom fungus belonging to *Aphyllophorales*, *Hydnaceae* (*Hericiaceae*), *Hericium*, and grows widely in Japan and China. *Hericium erinaceae* is the saprophytic inhabitant on dead trunks of hardwoods such as narra, oak, beech, walnut and among others. This mushroom is called Yamabushitake in Japan because it resembles the ornamental cloth worn by Yamabushi. A great number of studies of the chemical components of *H. erinaceus* have been carried out[22-26]. It has been demonstrated that *H. erinaceus* contains biologically active materials, such as phenol-analogous components (hericenone A-H). The novel phenols (hericenone A and B) were reported that it may have chemotherapeutic effects on cancer[24]. The phenol-analogous components

(hericenone C, D, E, F, G, and H) induce the synthesis of nerve growth factor, might be effective in treating patients suffering from Alzheimer's disease[25,26]. Therefore, *H. erinaceus* has recently become popular as a healthy functional food. Abraham *et al*. reported that H. erinaceus were submerged, cultured, and the volatile components in the culture medium were analyzed[27].

However, there is no detailed report on the volatile oil of *P. citrinopileatus*, *P. eryngii* var. *ferulae*, and *L. hatsudake*. The aim of this study is to determine the components of the volatile oil from three mushrooms.

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[28]. 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.

2 EXPERIMENTAL

2.1 Materials

The fruiting bodies of *P. citrinopileatus* were obtained from Fukushima prefecture in Japan in July 2009 and were air-dried immediately. Agitake (*P. eryngii* var. *ferulae*) plant material was collected from Takeuchi Nouen (1–2–8 Miyoshi-cho, Nakano-shi, Nagano 383–0025, Japan) in May 2012. Plant material of *L. hatsudake* was collected from the Fukushima prefecture, Japan in September 2007. Samples of fruiting bodies of *H. erinaceus* were cultivated in Hokkaido prefecture, Japan in April 2006. 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 Hydrodistillation (HD) method

The volatile oil from dried fruiting bodies of *P. citrinopileatus* (600 g), dried *L. hatsudake* (200 g), or *H. erinaceus* (100 g) were isolated by HD with a Likens-Nickerson-type apparatus. The volatile oil from agitake was obtained by HD for 2 h using a solvent [DE or dichloromethane (DM)]. The oils were dried over anhydrous sodium sulfate and stored at 4°C in a refrigerator prior to analysis.

2.2.2 Solvent-assisted flaror evaporation (SAFE) method

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[29]. The filtrate was vacuum distilled using a SAFE apparatus as previously described[30]. 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°C in a refrigerator prior to analysis.

2.3 Gas chromatography (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 programed as follows: initial increase 40–260°C at 4°C/min, followed by 5 min at 260°C. The carrier gas was He at a flow of 1.8 mL/min; the injector and detector temperatures were 270 and 280°C, 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°C at a rate of 4°C/min and held at 260°C for 5 min. The injector and detector temperatures were 270 and 280°C, respectively. The actual temperature in the MS source reached approximately 230°C; 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 Gas chromatography-olfactometry (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 Aroma extraction dilution analysis (AEDA)

The highest sample concentration (*P. citrinopileatus*.; 8 mg/mL, *P. eryngii* var. *ferulae*; 10 mg/mL,

L. hatsudake; 20 mg/mL, or *H. erinaceus*; 20 mg/mL) was assigned an FD factor of 1. The volatile oil was diluted stepwise with solvent (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[31], and those from our previous studies, and of retention indices (RIs) with those of the standards or RIs reported in the literature[32–46]. 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 3-methylthioprapanal, pyridine, 2, 3-butanediol, hexanol, methional, benzaldehyde, 1-octen-3-ol, 3-octanone, 6-methyl-5-hepten-2-one, 2-pentylfuran, octanal, 2-acetylthiazole, (2*E*)-octen-1-al, 2-ethylhexanol, phenylacetaldehyde, acetophenone, phenylethyl alcohol, nonanal, menthol, furfural, decanal, 2-aminoacetophenone, β-caryophyllene oxide, γ-dodecanolactone, linoleic acid, linolenic acid, and stearic acid 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, 2-ethyl-6-methyl-pyrazine, furfural, γ-elemene, γ-muurolene, β-bisabolene, α-copaene, clovane, α-cedrene epoxide, β-funebrene epoxide, veticadioxide, *cis*-isolongifolanone, or 4-dehydroviridiflorol were quantified using the calibration curves for elsholtzia ketone, β-caryophyllene, or β-caryophyllene oxide.

3 RESULTS AND DISCUSSION

3.1 Constituents of essential oils from mushrooms

The oil of *P. citrinopileatus* was light yellow with nut and fruity odor and was produced in yield of 0.002% (w/w). The components were listed in order in elution in the HP-5MS column. The identified components and peak percentages listed in **Table 1** ***P. c.***.

In total, 59 components were identified, accounting for 95.9% of volatile components. The main components of the oil were fatty acid such as, palmitic acid (23.3%), linoleic acid (31.3%) pentadecylic acid (6.0%) and 2, 3-dimethyl-2-nonen-4-olide (6.0%), followed by phenylacetaldehyde (5.5%). Concerning C8 components, 3-octanone (Peak **12** ***P. c.***) and octanol (Peak **22** ***P. c.***) were only detected in this oil, whereas, other C8 components, 1-octen-3-ol, 2-octen-1-ol, (3*E*)-octen-1-ol, 3-octanol,

Table 1 ***P. c.*.** Components of the volatile oil from *P. citrinopileatus*

No.	HP-5MS	Ri[a] Lit*	DB-WAX	Compounds[b]	Peak area (%)[c]
1 *P. c.*	771	771	1245	Amyl alcohol	0.2
2 *P. c.*	798	800	1068	Hexanal	1.4
3 *P. c.*	835	845	1441	Furfural	0.1
4 *P. c.*	870	864	1131	Hexanol	0.5
5 *P. c.*	893	892	1167	2-Heptanone	0.6
6 *P. c.*	905	901	1164	Heptanal	0.3
7 *P. c.*	909	905	1458	3-Methylthiopropanal	0.1
8 *P. c.*	915	912	-	2, 6-Dimethylpyrazine	0.1
9 *P. c.*	959	960	-	(2*E*)-Heptenal	0.1
10 *P. c.*	963	960	1494	Benzaldehyde	0.6
11 *P. c.*	981	975	1403	5-Methyl-2-cyclohexene-1-one	1.2
12 *P. c.*	983	981	-	3-Octanone	0.4
13 *P. c.*	984	984	1323	6-Methyl-5-hepten-2-one	0.1
14 *P. c.*	988	992	1576	2-Pentylfuran	2.2
15 *P. c.*	1001	1001	1373	2-Ethyl-6-methylpyrazine	0.6
16 *P. c.*	1006	1005	1288	Octanal	0.2
17 *P. c.*	1021	1018	-	2-Acetylthiazole	0.1
18 *P. c.*	1032	1027	1475	2-Ethyl-hexanol	0.2
19 *P. c.*	1040	1038	-	(3*E*)-Octen-2-one	0.2
20 *P. c.*	1047	1044	1383	Phenylacetaldehyde	5.5
21 *P. c.*	1054	1054	-	(2*E*)-Octen-1-al	0.8
22 *P. c.*	1070	1068	1534	Octanol	0.2
23 *P. c.*	1082	1083	-	2-Ethyl-3,6-dimethylpyrazine	0.1
24 *P. c.*	1095	1092	-	2-Nonanone	0.3
25 *P. c.*	1105	1098	1377	Nonanal	0.4
26 *P. c.*	1144	1140	-	3-Nonen-2-one	0.2
27 *P. c.*	1163	1160	1537	(2*E*)-Nonenal	0.5
28 *P. c.*	1175	1172	1410	Nonanol	0.2
29 *P. c.*	1178	1175	1702	Menthol	0.3
30 *P. c.*	1180	1180	-	Elsholtzia ketone[d]	0.4
31 *P. c.*	1205	-	1508	Unknown 1	2.0
32 *P. c.*	1209	1207	1462	Decanal	0.2
33 *P. c.*	1245	1237	-	(3*E*)-Decen-2-one	0.3
34 *P. c.*	1275	1272	-	2-Hexylthiophene	1.5
35 *P. c.*	1282	-	-	Unknown 2	0.4
36 *P. c.*	1286	1287	-	Safrole	0.2
37 *P. c.*	1290	1291	1586	2-Undecanone	0.3
38 *P. c.*	1296	1294	1789	Indole	0.3
39 *P. c.*	1321	1317	1850	(2*E*, 4*E*)-Decadienal	0.2
40 *P. c.*	1323	1319	1584	1-Methoxy-4-propylbenzene	0.4
41 *P. c.*	1368	1366	2099	γ-Nonalactone	0.3
42 *P. c.*	1380	1376	1626	2-Butyl-2-octenal	0.9
43 *P. c.*	1458	1445	1838	Neryl acetone	0.6
44 *P. c.*	1510	1500	-	β-Bisabolene	0.2
45 *P. c.*	1529	1523	1926	2, 3-Dimethyl-2-nonen-4-olide	6.0
46 *P. c.*	1541	1537	-	Dihydroeugenolacetate	0.8
47 *P. c.*	1570	1566	-	(*E*)-Nerolidol	0.6
48 *P. c.*	1571	1574	2080	Tridecanol	0.3
49 *P. c.*	1635	1630	-	Benzophenone	0.3
50 *P. c.*	1650	1649	2150	Torreyol	0.2
51 *P. c.*	1713	1713	-	2, 2'-5, 5'-Tetramethyl-1, 1'-biphenyl	0.4
52 *P. c.*	1718	1718	-	Curcuphenol[d]	0.4
53 *P. c.*	1750	1740	2350	(2*E*, 6*E*)-Farnesal	0.4
54 *P. c.*	1769	1766	-	Myristic acid	0.4
55 *P. c.*	1796	1791	-	2, 4-Diphenyl-4-methyl-1-pentene	0.3
56 *P. c.*	1876	1871	-	Pentadecylic acid	6.0
57 *P. c.*	1930	1942	-	Hexadecanolide	0.7
58 *P. c.*	1983	1983	2604	Palmitic acid	23.3
59 *P. c.*	2035	-	2194	Unknown 3	0.9
60 *P. c.*	2028	2032	2285	(*E*, *E*)-Geranyllinalool	0.6
61 *P. c.*	2087	-	2280	Unknown 4	0.8
62 *P. c.*	2112	2105	2810	Palmito-γ-lactone	0.3
63 *P. c.*	2157	2148	2828	Linoleic acid	31.3
				Total	100

[a] RI = retention indices are determined on HP-5MS and DB-WAX column, using the homologous series of n-alkanes

[b] Compounds are listed in odor of their elution time from a HP-5MS column.

[c] Peak area pecentagee are calculated in GC on HP-5MS column.

[d] Newly identified compounds in the volatile oil of mushroom.

*Literature values.

Unknown 1 ; EI-MS *m/z* (rel. int.): 163 (60), 103 (22), 71 (37), 70 (34), 60 (37), 59 (31), 56 (20), 44 (100).

Unknown 2 ; EI-MS *m/z* (rel. int.): 160 (6), 142(20), 127(27), 99(50), 71(100), 61(60), 55(53).

Unknown 3 ; EI-MS *m/z* (rel. int.): 276 (34), 178 (27), 151 (39), 133 (55), 111 (41), 69 (100), 55 (66).

Unknown 4 ; EI-MS *m/z* (rel. int.): 278 (32), 179 (16), 151 (28), 99 (100), 79 (54), 67 (52), 55 (56), 41 (38).

1-octen-3-one, and 1, 5-octadien-3-one were not identified.

The number of C8 alcohols was little, on the other hand; C8 aldehydes, octanal and (2*E*)-octen-1-al were detected. Furthermore, pyrazine components, 2, 6-diemthylpyrazine and 2-ethyl-6-methylpyrazine also were identified. These pyrazine components are considered to be important flavor components in foods, and may be synthesized by micro-organisms[47], by Maillard reaction[48, 49] and by chemical reactions at room temperature[50]. These C8 aldehydes and pyrazine components would be generated by oxidation or Maillard reaction in the drying process. Regarding the minor components, monoterpenoid, elsholtzia ketone (Peak **30** ***P. c.***) and sesquiterpenoid, curcuphenol (Peak **52** ***P. c.***) showed the peak area percentages of 0.4% each. Elsholtzia ketone has been reported in the volatile oil from *Elsholtzia splendens*[51,52]. Although elsholtzia ketone, which was considered as a generated component from naginata ketone in vivo[53], naginata ketone could not be identified by GC-MS in this study. On the other hand, curcuphenol was isolated and identified from several sources such as the sponges[54–56] and plants[57–59]. Curcuphenol was reported to have antimicrobial activity[54], antifungal activity[56] and antileishmania activity[60], antitumor activity against several human cancer cell lines[61], and antioxidant activity[62]. In the view of bioactive components of volatile oil from *P. citrinopileatus*, curcuphenol is a very important substance. These components were identified in the oil of mushroom for the first time in this study.

The volatile oil of agitake obtained by HD using DE was yellowish oil and the yield was 0.068% (w/w). As shown in **Table 1** ***P. e.***, a gas chromatogram of the volatile oils from agitake was presented. A total of 67 components were identified, representing about 80.3% of the total oil. The main components of the volatile oil were linoleic acid (**97** ***P. e.***; 23.0%) and hexadecanoic acid (**94** ***P. e.***; 22.3%). The volatile oil by HD 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. The main components were 1-octen-3-ol (**18** ***P. e.***; 29.1%) and 3-octanone (**19** ***P. e.***; 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. The main components of the volatile oil were 1-octen-3-ol (**18** ***P. e.***; 67.5%), octanoic acid (**42** ***P. e.***; 9.5%), and 4-hydroxy-2-methoxybenzaldehyde (**61** ***P. e.***; 3.9%).

The volatile oil by SAFE 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. The main components of the oil were benzoic acid (**52** ***P. e.***; 28.2%), 2,3-butanediol (**8** ***P. e.***; 14.8%), and 4-methoxybenzaldehyde (**48** ***P. e.***; 7.5%). The volatile oil by SAFE consisted mainly of acids (30.5%), followed by alcohols (25.2%), aldehydes (10.3%), and esters (4.4%).

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

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

Table 1 ***P. e.*.** Continued

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

[a] RI, retention indices determined on HP-5 and DB-WAX culumns, using the homologous series of *n*-alkanes.

[b] Peak area (%) was related to total detected compounds by GC-MS.

[c] DE = diethyl ether

[d] DM = dichloromethane

[e] tr = trace (< 0.1 %).

Agitake contains characteristic components, such as (*E*)-4-undecene (**28** ***P. e.***), 2,3,7-trimethyl-2-octene (**31** ***P. e.***), methylsuccinimide (**36** ***P. e.***), (*E*)-α-atlantone (**87** ***P. e.***), and 2-hexyloxyethanol (**38** ***P. e.***). Among these components, 2,3,7-trimethyl-2-octene (**31** ***P. e.***) and methylsuccinimide (**36** ***P. e.***) are particularly unusual. It has been reported that 2,3,7-trimethyl-2-octene (**31** ***P. e.***) is present in the flowers of *Edgeworthia chrysantha Lindl*[63]. Methylsuccinimide (**36** ***P. e.***) is present in the aerial parts of *Brunfelsia grandiflora*[64]. However, this is the first report of these components in the *Pleurotus* genus.

L. hatsudake has a delightful aroma, green, sweet and spicy. The volatile oil was obtained by hydrodistillation of *L. hatsudake* and was produced in a yield of 0.003%. The color of the volatile oil was purple and the oil has a green, sweet and spicy odor.

The components were listed in order of their elution from the HP-5MS column. The volatile oil of *L. hatsudake* included 17.6% hydrocarbons, 6.0% alcohols, 6.9% aldehydes, 20.2% ketones, 30.7% fatty acids, 0.3% nitorogen-containing components, 0.8% sulphur-containing components and 6.7% other components. Among these components, 48% of total amount were sesquiterpenes (17.6% hydrocarbons, 16.1% ketones, 9.3% epoxides, 5.0% alcohols).

As shown in **Table 1** ***L. h.***, a gas chromatogram of the volatile oil from *L. hatsudake* was presented in which 76 components were separated and 71 components of these were identified, amounting to 91.8% of the oil. The main constituents were fatty acids, including linoleic acid (11.6%), palmitoleic acid (6.6%), linolenic acid (5.7%) and stearic acid (5.4%); the total content of fatty acids in the oil was 29.2%. As a result, *L. hatsudake* has some characteristic components, such as clovane (**42** ***L. h.***), α-cedrene epoxide (**46** ***L. h.***), β-funebrene epoxide (**47** ***L. h.***), veticanoxide (**48** ***L. h.***), *cis*-isolongifolanone (**49** ***L. h.***), humulene epoxide III (**55** ***L. h.***), chamazulene (**58** ***L. h.***) and 4-dehydroviridiflorol (**59** ***L. h.***). Among these components, 4-dehydroviridiflorol, myliol and veticadinoxide are especially unusual sesquiterpenoids and were identified by comparison of their mass spectra and retention indices with MassFinder 4 Library and published data[65,66]. It has been reported that 4-dehydroviridiflorol and myliol are contained in the liverworts *Mylia taylorii* and *Mylia nuda*[65]. Veticadinoxide was first identified from the essential oil of the hornwort, *Anthoceros caucasicus*[66]. Chamazulene is a blue pigment with an azulene framework, and is contained in camomile; this component is considered to be one of the main contributors of the color of *L. hatsudake*'s oil.

The oil was obtained by hydrodistillation from dried fruiting bodies of *H. erinaceus* in Japan and was produced in a yield of 0.024%. The oil was colorless with a nut-like odor. The components were listed in order of their elution from the HP-5MS and DB-WAX column. A gas chromatogram of the volatile oil from fruiting bodies of *H. erinaceus* was presented in which 111 components were separated. As shown **Table 1** ***H. e.***, 77 components of these were identified, amounting to 87.6% of the oil. They included 12 hydrocarbons, 14 alcohols, 16 aldehydes, 12 ketones, 4 fatty acids, 5 esters, 8 nitorogen-containing components, 3 sulfur-containing components and 3 miscellaneous components. The main

Table 1 *L. h.*. Components of the valatile oil from *L. hatsudake*

No.	Retention Index RI-5[a]	RI-W[b]	Components	Peak area (%)	No.	Retention Index RI-5	RI-W	Components	Peak area (%)
1 *L. h.*	800	1068	Hexanal	0.8	39 *L. h.*	1345		1-Methoxy-4-methyl-bicyclo[2,2,2]octane	0.1
2 *L. h.*	890	1171	2-Heptanone	0.3	40 *L. h.*	1366	2039	γ-Nonalactone	0.4
3 *L. h.*	902	1184	Heptanal	0.2	41 *L. h.*	1376		2-Butyl-2-octenal	0.3
4 *L. h.*	955	1145	(2*Z*)-Heptenal	0.1	42 *L. h.*	1420	1465	Clovane	6.0
5 *L. h.*	960	1641	Benzaldehyde	0.4	43 *L. h.*	1455	1842	Geranyl acetone	0.3
6 *L. h.*	978	1295	1-Octen-3-one	1.6	44 *L. h.*	1473		Unknown 1	1.3
7 *L. h.*	987	1320	6-Methyl-5-hepten-2-one	tr.	45 *L. h.*	1569	2040	(*E*)-Nerolidol	0.2
8 *L. h.*	992	1212	2-Penthyl furan	1.0	46 *L. h.*	1577	1663	α-Cedrene epoxide	4.6
9 *L. h.*	1003	1302	Octanal	0.1	47 *L. h.*	1584	1672	β-Funebrene epoxide	3.2
10 *L. h.*	1029	1475	2-Ethyl hexanol	0.2	48 *L. h.*	1594	1697	Veticadioxide	2.6
11 *L. h.*	1040	1093	3-Octen-2-one	0.3	49 *L. h.*	1606	1680	*cis*-Isolongifolanone	8.8
12 *L. h.*	1046	1644	Phenylacetaldehyde	2.7	50 *L. h.*	1610	2055	Humulene epoxoide II	0.8
13 *L. h.*	1059	990	(2*E*)-Octenal	0.6	51 *L. h.*	1621		Myliol	0.5
14 *L. h.*	1067	1640	Acetophenone	tr.	52 *L. h.*	1630		Benzophenone	0.1
15 *L. h.*	1069		*m*-Tolualdehyde	tr.	53 *L. h.*	1636	1593	5-Cedranone	0.3
16 *L. h.*	1071	1275	Octanal	0.2	54 *L. h.*	1654	1889	Unknown 2	2.5
17 *L. h.*	1092	1398	2-Nonanone	0.2	55 *L. h.*	1659	2060	Humulene epoxide III	6.4
18 *L. h.*	1105	1370	Nonanal	0.5	56 *L. h.*	1678	2131	[d]STK1	1.6
19 *L. h.*	1140	1205	3-Nonen-2-one	0.2	57 *L. h.*	1691	2048	[c]STA 1	1.5
20 *L. h.*	1146	1524	Camphor	0.2	58 *L. h.*	1705	2352	Chamazulene	5.3
21 *L. h.*	1161	1195	(2*E*)-Nonenal	0.7	59 *L. h.*	1718	2056	4-Dehydroviridiflorol	3.2
22 *L. h.*	1172	1652	Nonanol	tr.	60 *L. h.*	1734	1980	α-Cyperone	1.0
23 *L. h.*	1175	1589	Menthol	0.9	61 *L. h.*	1768	2692	Myristic acid	0.2
24 *L. h.*	1187		*p*-Ethyl anisole	0.2	62 *L. h.*	1793	2020	2,4-Diphenyl-4-methyl-1-pentene	0.1
25 *L. h.*	1194	1183	2-Decanone	0.3	63 *L. h.*	1844		Unknown 3	tr.
26 *L. h.*	1199		*trans*-Dehydrocarbone	0.1	64 *L. h.*	1870	2822	Pentadecanoic acid	0.9
27 *L. h.*	1207		Decanal	0.1	65 *L. h.*	1903		2-Heptadecanone	0.1
28 *L. h.*	1220	1510	Isophorone	0.1	66 *L. h.*	1921		Farnesyl acetone	0.1
29 *L. h.*	1242		3-Decen-2-one	0.2	67 *L. h.*	1927		Methyl palmitate	0.1
30 *L. h.*	1267	1867	(2*E*)-Decenal	0.2	68 *L. h.*	1948	2862	Palmitic acid	0.5
31 *L. h.*	1272	1644	2-Hexyl thiophene	0.2	69 *L. h.*	1982	2944	Palmitoleic acid	6.6
32 *L. h.*	1275	1597	2-Phenyl-2-butenal	0.1	70 *L. h.*	2076	2401	1-Acethyl-4,6,8-trimethylazulene	0.8
33 *L. h.*	1280	1476	*S*-Methyl haxanethioate	0.6	71 *L. h.*	2097	2481	Methyl linoate	0.4
34 *L. h.*	1295	1585	2-Undecanone	0.5	72 *L. h.*	2103		Methyl oleate	0.3
35 *L. h.*	1306	1865	Carvacrol	tr.	73 *L. h.*	2130	2115	Methyl stealate	0.4
36 *L. h.*	1318		3,5-Dimethyl-2-propylpyrarzine	0.3	74 *L. h.*	2161	3100	Linoleic acid	11.6
37 *L. h.*	1320	1781	(2*E*, 4*E*)-Decadienal	0.3	75 *L. h.*	2165	3109	Linolenic acid	5.7
38 *L. h.*	1326		Methyl decanoate	0.4	76 *L. h.*	2184		Stearic acid	5.4

[a] RI-5; retention Index on a HP-5MS column.; peak areas were expressed as GC/TIC%.

[b] RI-W; retention Index on a DB-WAX column.; peak areas were expressed as GC/TIC%.

[c] tr.; trace (<0.1%).

[d] STA; sesquiterpene alcohol; [e] STK; sesquiterpene ketone.

Unknown 1; EI-MS *m/z* (rel. int.) 206 (7), 191 (67), 163 (50), 121 (33), 107 (69), 93 (64), 81 (52), 67 (40), 55 (40), 43 (100).

Unknown 2; EI-MS *m/z* (rel. int.) 216 (62), 201 (21), 187 (31), 173 (26), 159 (29), 145 (67), 131 (71), 117 (57), 105 (64), 91 (100), 77 (57), 55 (40), 41 (57).

Unknown 3; EI-MS *m/z* (rel. int.) 228 (100), 212 (21), 199 (38), 185 (69), 169 (36), 157 (36), 141 (48), 128 (60), 115 (62), 91 (40), 85 (57), 71 (76), 57 (93), 43 (90).

STK 1; EI-MS *m/z* (rel. int.) 218 (19), 200 (19), 185 (20), 157 (26), 145 (40), 131 (100), 117 (88), 105 (62), 91 (67), 77 (38), 55 (26), 41 (43).

STA 1; EI-MS *m/z* (rel. int.) 220 (29), 202 (21), 187(40), 159 (), 145 (40), 131 (100), 117 (88), 105 (62), 91 (67), 77 (38), 55 (26), 41 (44).

constituents were fatty acids, which were hexadecanoic acid (26.0%), linoleic acid (13.1%), pentadecylic acid (1.7%) and tetradecanoic acid (0.4%); the total content of fatty acids in the oil was 41.1%. 1-Octen-3-ol, the most important volatile component of the mushroom, was encountered in small amounts.

Table 1 ***H. e.*.** Components of the valatile oil from fruiting bodies of *H. erinaceus*

RI-5[a]	RI-W[b]	Components	Peak area (%)	RI-5	RI-W	Components	Peak area (%)
771	1245	Amyl alcohol	0.2	1225	1919	Benzothiazol	0.3
821	1069	Hexanal	1.0	1233	-	(*E*)-Ocimenone	0.1
823	-	2-Methylpyrazine	tr.[c]	1242	-	Neral	0.2
845	1441	Furfural	0.9	1259	-	Geranial	0.2
862	1191	2-(2-Propenyl)-furan	0.9	1262	-	(2*E*)-Decenal	tr.
874	-	2-Methyl-3-furanthiol	0.1	1275	1901	2-Phenyl-2-butenal	0.5
892	1167	2-Heptanone	0.3	1295	1586	2-Undecanone	1.5
901	1147	Heptanal	0.5	1294	-	Indole	0.2
905	1431	3-Methylthiopropanal	0.6	1317	1789	(*E*, *E*)-2, 4-Decadienal	0.2
910	-	2-Acetylfuran	0.1	1364	1998	γ-Nonalactone	0.3
914	-	2-Ethylpyrazine	0.1	1375	-	2-Butyl-2-Octenel	0.2
925	-	Methyl hexanoate	tr.	1400	1400	Tetradecane	0.3
958	1494	Benzaldehyde	2.5	1454	1838	Neryl acetone	0.3
960	-	5-Methylfurfural	tr.	1476	2006	Unknown 1	1.5
975	1404	5-Methyl-2-cyclohexane-1-one	2.3	1500	1500	Pentadecane	0.3
976	-	1-Octen-3-ol	tr.	1523	2155	2, 3-Dimethyl-2-nonen-4-olide	2.3
981	-	3-Octanone	tr.	1537	-	Dihydroeugenol acetone	0.3
984	1323	6-Methyl-5-heptan-2-one	1.7	1548	1888	α-Calacorene	0.2
989	1219	*p*-Mentha-3-ene	0.8	1566	2030	(*E*)-Nerolidol	1.1
1000	1275	Octanal	0.4	1600	-	Hexadecane	0.5
1012	-	2-Ethyl-3-methylpyrazine	tr.	1638	-	*cis*-Cadin-4-en-7-ol	0.3
1031	1576	2-Acetylpyridine	1.2	1648	-	*epi*-α-Muurolol	0.4
1038	1391	Lavender lactone	0.4	1660	-	α-Cadinol	0.5
1044	1614	Phenylacetaldehyde	8.9	1665	-	1, 2, 3-Trimethyl-(*E*)-4-propenylnaphthalene	2.2
1053	-	Dihydrotogetone	tr.	1699	-	2-Pentadecanone	0.2
1056	1981	*o*-Cresol	2.0	1713	-	2, 2'-5, 5'-Tetramethyl-1, 1'-biphenyl	1.2
1066	-	Acetophenone	0.2	1729	2192	2, 6-Diisopropylnaphthalene	0.3
1072	-	*o*-Toluidine	1.7	1766	-	Tetradecanoic acid	0.4
1085	-	2, 6-Diethylpyrazine	tr.	1791	2328	2, 4-Diphenyl-4-methyl-1-pentene	0.2
1089	1684	*cis*-Linalool oxide (furanoid)	0.3	1826	-	Methyl pentadecyliate	0.1
1096	1541	Linalool	0.3	1871	-	Pentadecylic acid	1.7
1098	-	Nonanal	tr.	1927	-	Methyl hexadecanoate	0.7
1100	1100	Undecane	1.2	1983	2876	Hexadecanoic acid	26.0
1132	-	*cis*-Limonene oxide	0.2	2029	2455	Unknown 2 (diterpenoid)	2.2
1140	-	*trans*-Limonene oxide	0.2	2063	-	epi-13-manool	0.5
1143	-	*cis*-β-Terpineol	tr.	2096	2481	Methyl linoleate	0.8
1160	1517	(2*E*)-Nonenal	0.4	2100	2100	Heneicosane	0.6
1175	1795	Menthol	0.3	2148	3132	Linoleic acid	13.1
1198	-	3-Methoxy-p-cresol	tr.				
1206	-	Decanal	0.1			Total	91.3

[a] RI-5: retention Index on a HP-5MS column.; peak areas were expressed as GC/TIC%.

[b] RI-W: retention Index on a DB-WAX column.; peak areas were expressed as GC/TIC%.

[c] tr.: trace (<0.1%).

Unknown 1; EI-MS *m/z* (rel. int.) 189 (47), 129 (40), 97 (68), 96 (54), 70 (100), 69 (50), 59 (35).

Unknown 2; EI-MS *m/z* (rel. int.) 276 (34), 178 (27), 151 (39), 133 (55), 111 (41), 69 (100), 55 (66), 41 (38).

3.2 GC-O, AEDA and OAV

The volatile oil of *P. citrinopileatus* was subjected to odor evaluation by AEDA and GC-O. **Table 2** ***P. c.*** bears the results. Fifteen components were detected as aroma active components. On the basis of FD factors, 3-methylthiopropanal (FD＝64, nut-like), 2,6-dimethyl pyrazine (FD＝64, nut-like), 6-methyl-5-hepten-2-one (FD＝8, green), 3-octanone (FD＝8, mushroom), (2*E*)-octen-1-al (FD＝8, nut-like) and nonanal (FD＝8, fruity) were most intense aroma-active components among 15 aroma components detected in *P. citrinopileatus* oil by AEDA. Though the contents of 3-methylthiopropanal and 2,

Table 2 *P. c.*. Odor desdription of volatile oil components of *P. citrinopileatus*

No.	Components	HP-5MS	Ri[a] Lit*	DB-WAX	FD factor[b]	Odour description
7 *P. c.*	3-Methylthio-propanal	909	1458	1458	64	nut-like
8 *P. c.*	2,6-Dimethyl-pyrazine	915	-		64	nut-like
10 *P. c.*	Benzlaldehyde	963	1494		4	almond
12 *P. c.*	6-Methyl-5-hepten-2-one	987	-	1323	8	green
13 *P. c.*	3-Octanone	983	1323		8	mushroom, buttery
14 *P. c.*	2-Pentylfuran	988	1576	1576	4	fruity
15 *P. c.*	2-Ethyl-6-methyl-pyrazine	1001	1373	1373	1	nut-like
16 *P. c.*	Octanal	1006	1288	1288	1	fruity
17 *P. c.*	2-Acethylthiazole	1021	-		1	roasted
20 *P. c.*	Phenylacetaldehyde	1047	1388	1383	1	green
21 *P. c.*	(2*E*)-Octen-1-al	1054	-		8	nut-like
25 *P. c.*	Nonanal	1105	1377	1377	8	fruity, soapy
29 *P. c.*	Menthol	1178	1702	1702	2	mentholic
30 *P. c.*	Elsholtzia ketone	1180	-	-	4	green
32 *P. c.*	Decanal	1209	1462	1462	2	green

[a] RI = retention indices on HP-5MS and DB-WAX column.

[b] FD factor : flavour dilution factor in the HP-5MS column, The sample concentration (8 mg/mL) was assigned a FD-factor of 1.

* literature values

6-dimethyl pyrazine were low (**Table 2 *P. c.***), these components had high FD factors. As for other aroma compounds, 2-pentylfuran (FD＝4) and elsholtzia ketone (FD＝4) were also detected by GC-O. The results indicated that the sulfur and nitrogen-containing components with nut-like odor would play the most important role in the aroma of the volatile oil from *P. citrinopileatus*. As further key odorants, nonanal and 2-pentylfuran, which contributed to the sweet aroma of the volatile oil, were identified. As the characteristic aroma of mushroom, 3-octanone was estimated as having a mushroom aroma by the sniffing test; however, C8 alcohols such as octanol were not evaluated as the key aroma component in this study. It is interesting that elsholtzia ketone participates in the aroma of *P. citrinopileatus*.

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

Table 2 ***P. e.***. 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 *P. e.*	Pyridine	burnt	2	-	-	-	680	-	-	-	4	170	-	-	-
8 *P. e.*	2,3-Butanediol	sweet	-	-	-	32	-	-	-	4425	95	-	-	-	47
9 *P. e.*	Hexanol	green	2	-	-	-	680	-	-	-	500	1	-	-	-
13 *P. e.*	Methional	potato	32	32	-	-	884	688	-	-	0.2	4420	3438	-	-
16 *P. e.*	Benzaldehyde	sweet	1	16	-	2	1224	578	-	69	350	3	2	-	<1
18 *P. e.*	1-Octen-3-ol	mushroom	64	64	64	64	12308	8003	1E+05	438	1	12308	8003	1E+05	438
19 *P. e.*	3-Octanone	sweet	-	32	8	-	-	7178	1200	-	70	-	103	17	-
20 *P. e.*	2-Pentylfuran	sweet	8	16	-	-	1224	770	-	-	6	204	128	-	-
26 *P. e.*	2-Acetylthiazole	sweet	16	-	-	-	1836	-	-	-	10	184	-	-	-
27 *P. e.*	2-Ethylhexanol	green	-	2	1	1	-	303	60	156	N/A[f]	-	N/D[g]	N/D	N/D
29 *P. e.*	Phenyl acetaldehyde	floral	32	32	1	32	952	440	40	789	4	238	110	10	197
32 *P. e.*	Acetophenone	sweet	1	1	2	-	680	193	800	-	65	10	3	12	-
33 *P. e.*	1-Octanol	green	2	-	-	-	680	-	-	-	125	5	-	-	-
39 *P. e.*	Phenylethyl alcohol	floral	2	-	-	1	680	-	-	96	1000	<1	-	-	<1
54 *P. e.*	2-Aminoacetophenone	sweet	1	-	-	-	680	-	-	-	N/A	N/D	-	-	-
62 *P. e.*	γ-Elemene	woody	2	-	-	-	680	-	-	-	N/A	N/D	-	-	-
66 *P. e.*	γ-Muurolene	woody	-	-	-	2	-	-	-	138	N/A	N/D	-	-	N/D
68 *P. e.*	β-Bisabolene	woody	4	-	-	2	1360	-	-	327	N/A	N/D	-	-	N/D
75 *P. e.*	α-Copaene	woody, spicy	1	-	-	2	680	-	-	42	N/A	N/D	-	-	N/D
82 *P. e.*	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

[b] DE = diethyl ether, [c] DM = dichloromethane

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

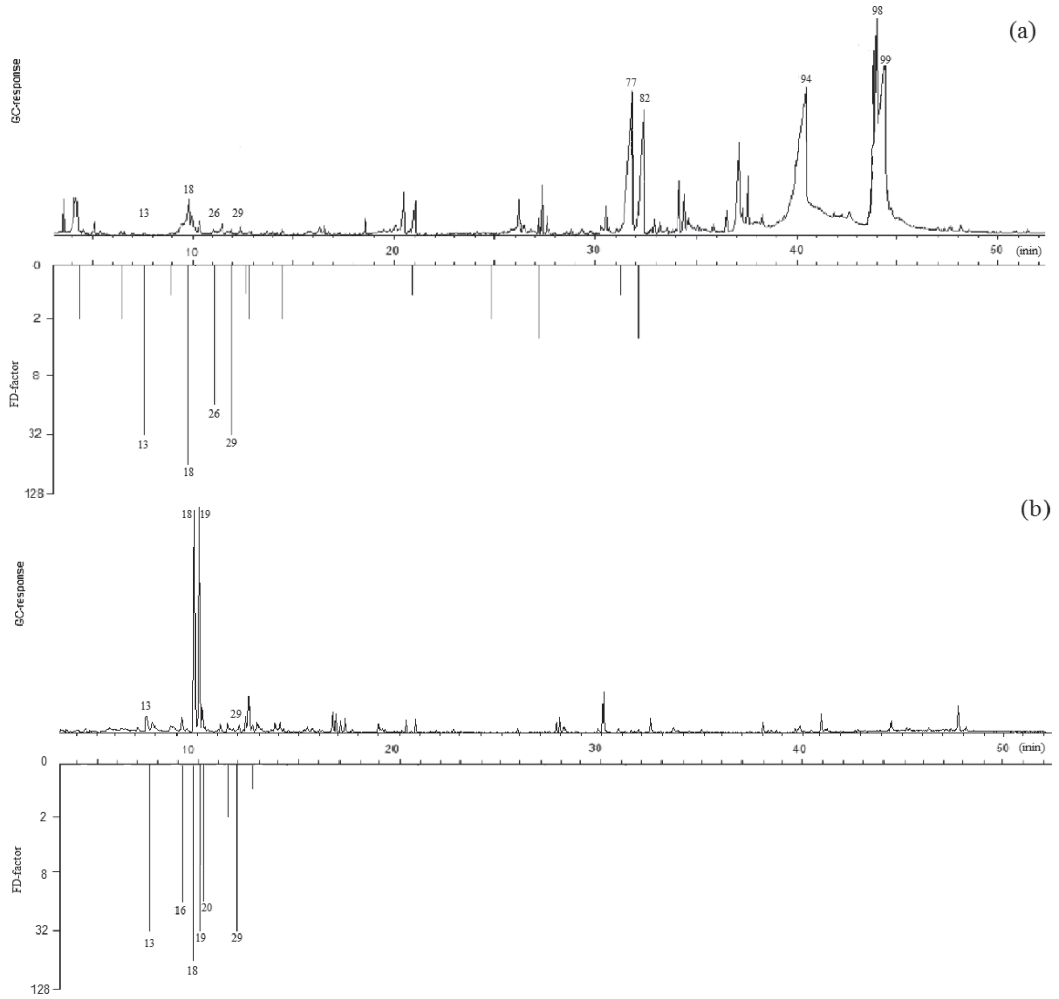

Figure 1 ***P. e.*** **. Gas chromatogram and aromagram (FD factor) of volatile oil from agitake (*P. eryngii* var. *ferulae*) obtained by hydrodistillation; (a) using DE; (b) using DM: 13 *P. e.*, methional; 16 *P. e.*, benzaldehyde; 18 *P. e.*, 1-octen-3-ol; 19 *P. e.*, 3-octanone; 20 *P. e.*, 2-pentylfuran;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

phenylacetaldehyde produced a floral odor, and 2,3-butanediol (**8** ***P. e.***) 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** ***P. e.***), 2-aminoacetophenone (**54** ***P. e.***), γ-elemene (**62** ***P. e.***), γ-muurolene (**66** ***P. e.***), β-bisabolene (**68** ***P. e.***), α-copaene (**75** ***P. e.***) and γ-dodecanolactone (**82** ***P. e.***) were not determined. In the extract obtained

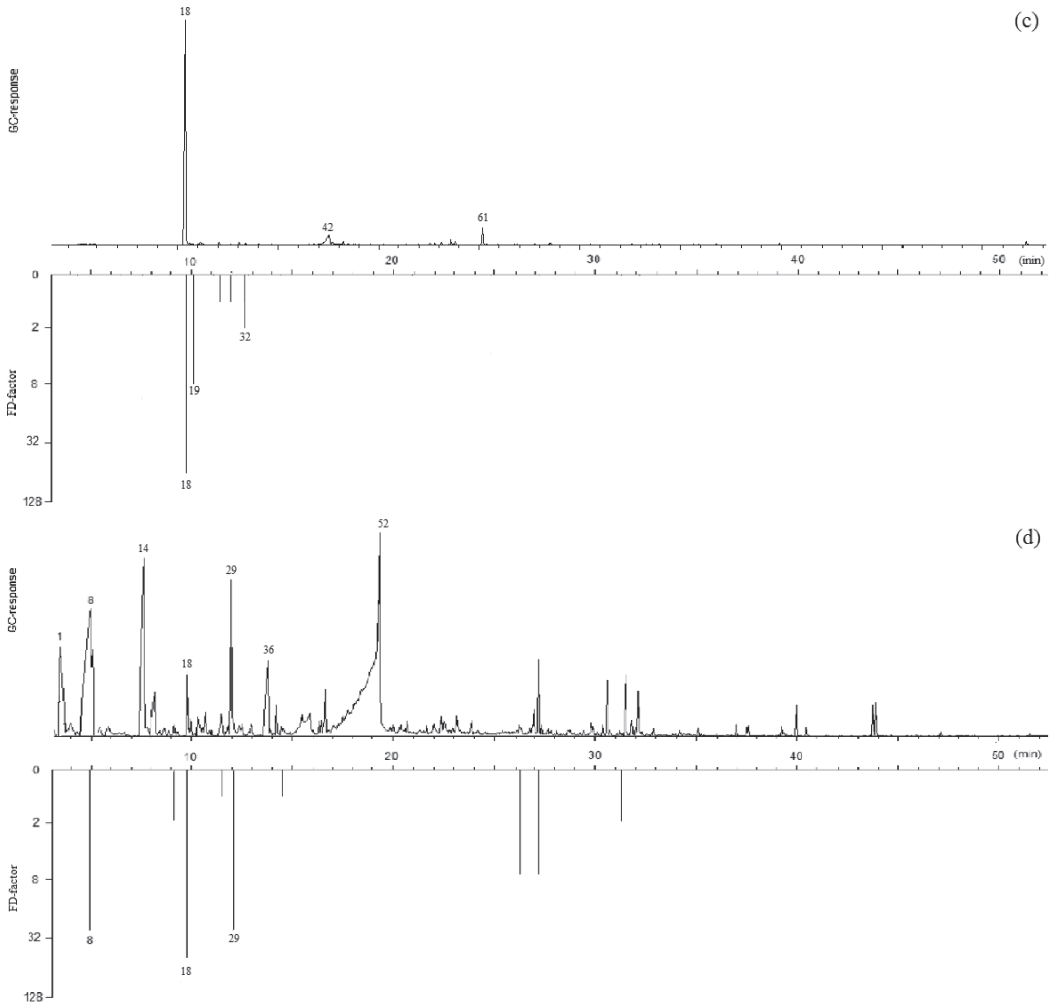

Figure 2 ***P. e.***. **Gas chromatogram and aromagram (FD factor) of volatile oil from agitake (*P. eryngii* var. *ferulae*) obtained by SAFE; (c) using DE; (d) using DM: 1** ***P. e.***, 3-hydroxy-2-butanone; **8** ***P. e.***, 2, 3-butanediol; **14** ***P. e.***, 2-butoxyethanol; **18** ***P. e.***,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

by HD using DE, 1-octen-3-ol (**18** ***P. e.***) had the highest OAV (12308), followed by methional (**13** ***P. e.***; OAV＝4420), phenylacetaldehyde (**29** ***P. e.***; OAV＝238), and 2-pentylfuran (**20** ***P. e.***; OAV＝204). When DM was used, 1-octen-3-ol (**18** ***P. e.***) had the highest OAV (8003), followed by methional (**13** ***P. e.***; OAV＝3438), 2-pentylfuran (**20** ***P. e.***; OAV＝128), and phenylacetaldehyde (**29** ***P. e.***; OAV＝110). 1-Octen-3-ol (**18** ***P. e.***), methional (**13** ***P. e.***), and phenylacetaldehyde (**29** ***P. e.***) 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** ***P. e.***; OAV＝135000),

Table 2 *L. h.*. Odor activity components of the volatile oil from *L. fatsudake*

RI-5[a]	Components	Odor	FD factor[b]
797	Hexanol	green	128
956	1-Octen-3-one	mushroom	2
988	2-Pentylfuran	earthy, green	4
1041	Phenylacetaldehyde	floral	64
1128	3-Nonen-2-one	powdery	4
1143	Camphor	balsamic	8
1155	(2*E*)-Nonanal	fruity, floral	4
1268	*S*-Methyl hexanethioate	mushroom, garlic	8
1306	Carvacrol	spicy	4
1629	Myliol	spicy, herb	32
1714	4-Dehydroviridiflorol	floral, sweet	64

[a] RI-5, retention index on a HP-5 MS column.

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

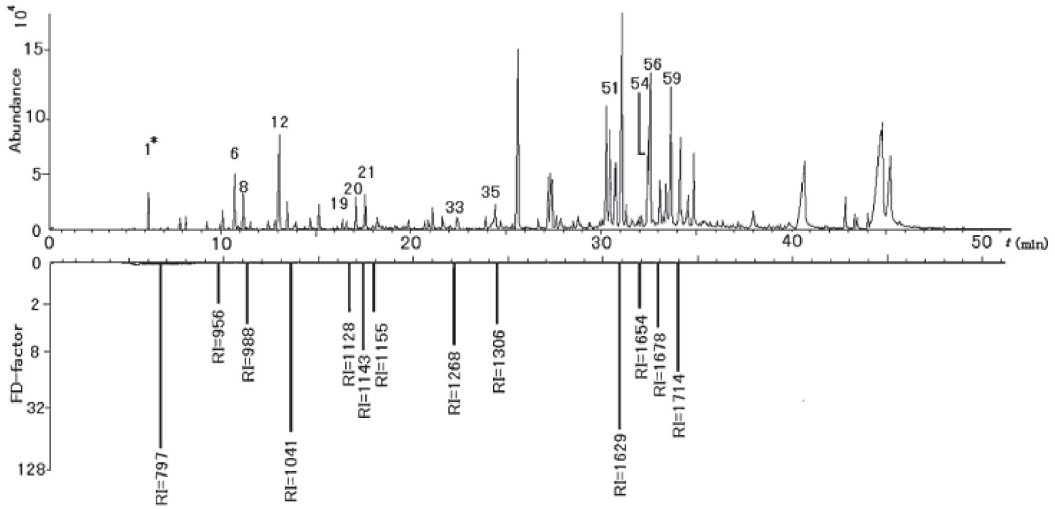

Figure 1 *L. h.*. GC-FID chromatogram (above) and AEDA results (below) for *L. hatsudake*.

[*] Peak number. **1 *L. h.***, hexanal; **6 *L. h.***, 1-octen-3-one; **8 *L. h.***, 2-pentylfuran; **12 *L. h.***, phenylacetaldehyde; **20 *L. h.***, camphor; **21 *L. h.***, (2*E*)-nonenal, **33 *L. h.***, *S*-methyl hexanethioate; **35 *L. h.***, carvacrol; **51 *L. h.***, myliol; **54 *L. h.***, unknown; **56 *L. h.***, sesquiterpene ketone; **59 *L. h.***, 4-dehydroviridiflorol. These results show that the human nose is sensitive to substances that the FID does not detect at the given concentrations.

[*] RI, retention index on a HP-5MS column

3-octanone (**19 *P. e.***; OAV＝17), and acetophenone (**32 *P. e.***; OAV＝12). When DM was used, the three most potent components were 1-octen-3-ol (**18 *P. e.***; OAV＝438), 2, 3-butanediol (**8 *P. e.***; OAV＝47), and phenylacetaldehyde (**29 *P. e.***; 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

Table 2 ***H. e.*** Odor desdription of oil components from fruiting bodies of *H. erinaceus*

RI-5	Aroma components	Odour description	FD factor[b]
823	2-Methylpyrazine	nut-like	8
866	Unkown	sweet	32
874	2-Methyl-3-furanthiol	nut-like	256
905	3-Methylthiopropanal	onion, meat-like	8
914	2-Ethylpyrazine	nut-like	64
925	2-Pentylfuran	fruity, pine-like	8
958	Benzaldehyde	burnt sugar, almond	8
984	6-Methyl-5-heptan-2-one	green	8
1012	2-Ethyl-3-methylpyrazine	nut-like	2
1030	Unkown	nut-like	2
1031	2-Acetylpyridine	popcorn-like	8
1044	Phenylacetaldehyde	green	2
1049	Unkown	almond	8
1053	Dihydrotogetone	almond	8
1072	*o*-Toluidine	nut-like	2
1085	2, 6-Diethylpyrazine	sesame-like	32
1098	Nonanal	fruity, sweet, pine-like	2
1143	*cis*-β-Terpineol	nut-like	2
1175	Menthol	mentholic	2
1476	Unknown 1	bitter	8
1480	Unkown	sweet, bitter	16
1889	Unkown	sweet	8

[a] RI; Retention Indices on HP-5MS column.

[b] FD factor; flavour dilution factor using AEDA method.

relationship between the FD factor and the OAV[67].

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** ***P. e.***) was found to be the main aroma-active component obtained using two methods. In HD using DE, 1-octen-3-ol (**18** ***P. e.***) produced a mushroom odor, methional (**13** ***P. e.***) was important in producing a potato odor, and phenylacetaldehyde (**29** ***P. e.***) contributed a floral odor. When DM was used, 1-octen-3-ol (**18** ***P. e.***) produced a mushroom odor, methional (**13** ***P. e.***) was important in producing a potato odor, 3-octanone (**19** ***P. e.***) produced a sweet odor, and phenylacetaldehyde (**29** ***P. e.***) contributed a floral odor. For the extract obtained by SAFE using DE, 1-octen-3-ol (**18** ***P. e.***) produced a mushroom odor, and 3-octanone (**19** ***P. e.***) and acetophenone produced sweet odors. When DM was used, 1-octen-3-ol produced a mushroom odor, 2, 3-butanediol contributed a sweet odor, and phenylacetaldehyde (**29** ***P. e.***) contributed a floral odor.

The numbers of detected components and yields obained 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.

The odor-active components of the volatile oil from *L. hatsudake* were also examined using odor evaluation by GC-O and AEDA. Identification of components was based on comparison of their retention times. As shown in **Table 2** ***L. h.***, 12 aroma active components were detected as odor-active components and among these 11 components were identified. The identified components were represented by the aldehydes [hexanal, (2*Z*)-heptanal, phenylacetaldehyde], ketones (1-octen-3-one, 3-nonen-2-one, *S*-methyl hexanethioate, camphor) and alcohols (carvacrol, myliol, 4-dehydroviridiflorol). **Figure 1** ***L. h.*** shows a GC-FID chromatogram (above) and AEDA results (below) of *L. hatsudake*. Despite their low levels, hexanal and phenylacetaldehyde possessed high FD factors. They were assumed to contribute strongly to the odor of *L. hatsudake*. In addition, 4-dehydroviridiflorol and myliol are novel components with a high FD factor, which are considered to contribute to *L. hatsudake*'s distinctive odor.

The oil of *H. erinaceus* was also carried out odor evaluation by GC-O and AEDA. The result of the GC-O analysis showing the number of odor detected in the oil listed in **Table 2** ***H. e.***. Of the 22 aroma active components were detected, 16 components were identified. The identified components were represented by the nitrogen-containing components (2-methylpyrazine, 2-ethylpyrazine, 2-ethyl-3-methylpyrazine, 2-acetylpyridine, *o*-toluidine, 2,6-diethylpyrazine), the sulfur-containing components (2-methyl-3-furanthiol, 3-methylthiopropanal), aldehydes (benzaldehyde, phenylacetaldehyde, nonanal), ketones (6-methyl-5-hepten-2-one, dihydrotagetone), alcohols (*cis*-β-terpineol, menthol) and esters (methyl hexanoate). In particular, the sulfur-containing components and nitrogen-containing components possessed high FD factors and most of these components had a nut-like odor, which were the characteristic odor of the oil. Therefore, the sulfur and nitrogen-containing components seemed to play most important role in the characteristic aroma from fruiting bodies of *H. erinaceus*. The profiles of volatile components vary with fresh and dried mushrooms[68,69]. Cho *et al*. reported that the eight carbon components were the main aroma active components in raw *Tricholoma matsutake* (Pine-mushroom) with highest FD factor, followed by 1-octen-3-ol, 1-octen-3-one and 3-octanone[70]. In this paper, the sulfur and nitrogen-containing components in the mushroom volatile seemed to be the key aroma components using GC-O instrument.

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