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Volatiles and key odorants in the pileus and stipe of pine-mushroom (*Tricholoma matsutake* Sing.)

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Abstract

The pileus and stipe of pine-mushroom (*Tricholoma matsutake* Sing.) were compared for differences in their profiles of volatiles and key odorants. We identified 24 and 21 volatile components in the pileus and in the stipe of pine-mushrooms, of which 3-phenyl-2-propenoic acid methyl ester (methyl cinnamate) and 1-octen-3-ol were most abundant, respectively. The C_8 components, such as 1-octen-3-ol, 3-octanol, and (*E*)-2-octen-1-ol, were more prevalent in the stipe than in the pileus. On the other hand, 1-octen-3-one (mushroom-like) was the most potent key odorant in both the pileus and the stipe. The flavor dilution (FD) factors of 1-octen-3-ol (mushroom-like), 3-octanol (mushroom-like/buttery), (*E*)-2-octen-1-ol (mushroom-like), and 3-octanone (mushroom-like/buttery), exhibiting the typical fungal odor note, were higher in the stipe than in the pileus. In contrast, the FD factors of odorants possessing sweet, floral, green, and citrus odor descriptions, such as linalool (sweet and citrus), 2-methyl-butanoic acid ethyl ester (sweet and floral), 2-methyl-3-buten-2-ol (fresh green), α-terpineol (pine-tree-like), and (*E*)-2-decenal (orange-like and fatty), were higher in the pileus than in the stipe.

Keywords: Pine-mushroom (*Tricholoma matsutake* Sing.); Different parts; Volatiles; Key odorant; GC–MS; GC–O; AEDA

1. Introduction

The quality of mushrooms depends on factors such as their aroma, taste, texture, and color, of which the aroma is most critical. The volatiles present in mushrooms have been investigated by many researchers, with nearly 150 different volatile components, representing a variety of chemical classes, identified in various mushroom species (Buchbauer, Jirovetz, Wasicky, & Nikiforov, 1993; Fischer & Grosch, 1987, Lizárraga-Guerra, Guth, & López, 1997; Maga, 1981a, 1981b; Mau, Chyau, Li, & Tseng, 1997; Rapior, Marion, Pélissier, & Bessière, 1997; Venkateshwarlu, Chandravadana, & Tewari, 1999; Wu, Zorn, Krings, & Berger, 2005). Among the diverse volatile components, a series of C_8 aliphatic components, such as 1-octen-3-ol, 2-octen-1-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 (Chen & Wu, 1984; Fischer & Grosch, 1987; Pyysalo & Suihko, 1976). The profiles of volatile components vary with species and varieties and can also be influenced by the cultivating conditions (Mau et al., 1997; Wu et al., 2005). In an earlier study using sensory analysis, Bernard and Simone (1959) found that the various portions of *Agaricus campestris* exhibited significantly different aroma intensities (Bernard & Simone, 1959). Later, Maga (1981) stated that the amounts of desirable mushroom aroma components were higher in the raw stipe (Maga, 1981a). Also, Noël-Suberville and co-workers (1996) reported that the amounts of certain fatty acids and aromatic compounds were higher in the gills rather than in the pileus or the stipe (Noël-Suberville, Cruz, Guinberteau, & Montury, 1996).

The pine-mushroom (*Tricholoma matsutake* Sing.) is one of the most valuable mushroom species worldwide.

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Recently, our research group has performed a series of studies on the aroma characteristics of pine-mushroom (Cho, Choi, & Kim, 2006a; Cho, Kim, Choi, & Kim, 2006b; Cho et al., 2007). The volatile components of pine-mushrooms primarily composed the following C_8 components: 3-octanol, 1-octen-3-ol, 1-octanol, (*E*)-2-octen-1-ol, 3-octanone, 1-octen-3-one, (*E*)-2-octenal, and octanoic acid. Also, 3-phenyl-2-propenoic acid methyl ester (methyl cinnamate) was unusually dominant in pine-mushrooms (Cho et al., 2006a). In addition, we demonstrated that the compositions of volatiles (Cho et al., 2006a) and aroma-active compounds (Cho et al., 2007) in pine-mushrooms differed with their grades.

Although the contents of two major components, 1-octen-3-ol and 3-phenyl-2-propenoic acid methyl ester (methyl cinnamate), were determined in different parts of pine-mushroom (Ohta, 1983), there is still insufficient information on the difference in volatile compositions in each parts of pine-mushroom. In this study, the volatiles and key odorants in different parts of pine-mushroom, the pileus and the stipe, were analyzed and compared using GC–MS and GC–O.

2. Materials and methods

2.1. Materials

We analysed first-grade pine-mushrooms that were cultivated in Inje-eup, Gangwon-do, South Korea, in 2005. The raw pine-mushroom was wrapped in low-density-polyethylene-film and stored at −70 °C until used. The frozen mushroom was thawed at 4 °C for 3 h before use. The mushroom was divided into the pileus and the stipe, which were both sliced using a cutter (Shinomura, Sanjo, Niigata, Japan). The sliced samples were placed into a stainless steel container, frozen in liquid nitrogen, and then ground in a blender (Hanil Electric, Seoul, Korea).

2.2. Chemicals

Dichloromethane (⩾99.9% purity) was obtained from Fisher Scientific (Seoul, Korea). Sodium sulfate and *n*-alkane standards (C_7–C_{22}) were purchased from Sigma–Aldrich (St. Louis, MO). The stock solutions of 33 authentic standard compounds were prepared in dichloromethane. The authentic standards were obtained from various suppliers as follows: Nos. 1–4, 6–8, 11–18, 20, 23–25, 27, i–vi and viii (Sigma–Aldrich); Nos. 5, 9, 10 and vii (Wako Pure Chemical Industries, Osaka, Japan); and No. 22 (Fluka, Buchs, Switzerland).

2.3. Extraction of volatiles

The ground samples (100 g) were extracted with 200 ml of dichloromethane which was re-distilled before use. After 0.1 ml of 100 ppm dodecanoic acid methyl ester (v/v, in dichloromethane) was added as an internal standard, the ground sample which was suspended in dichloromethane was mixed with a magnetical stirrer at 400 rpm for 30 min and then filtered (paper No. 41, Whatman, Maidstone, UK) under a vacuum. Volatile components were then separated from the non-volatiles using high-vacuum sublimation (HVS) at an operating vacuum that was typically below 2×10^{-5} Torr (Cho et al., 2006a). The extract was dehydrated over anhydrous sodium sulfate, evaporated on a Vigreux column (50 cm length × 3 cm i.d.) in a water bath at 45 °C, and then concentrated under a slow stream of nitrogen gas to obtain a final volume of 0.1 ml.

2.4. Fractionation by column chromatography

To identify any odorants not detected by GC–MS, the HVS extracts were subjected to silica gel column chromatography. The mushroom extracts were loaded onto a cooled column (45 cm length × 20 mm i.d.) filled with silica gel (35–70 mesh, 40 Å, Sigma–Aldrich). The concentrated volatile extracts were separated into six fractions using pentane/diethyl ether gradients (F1 extracts = 50/0 ml, F2 extracts = 40/10 ml, F3 extracts = 30/20 ml, F4 extracts = 20/30 ml, F5 extracts = 10/40 ml and F6 extracts = 0/50 ml). Each fraction was further concentrated under a slow stream of nitrogen gas until it could be analysed for the identification of unknowns in GC–MS analysis.

2.5. Gas chromatography–mass spectrometry (GC–MS)

GC–MS analysis was performed using an Agilent 6890N gas chromatography-5975 mass selective detector (GC–MSD) (Agilent Technologies Inc., Palo Alto, CA), equipped with a DB-5 ms column (30 m length × 0.25 mm i.d. × 0.25 mm film thickness, J&W Scientific, Folsom, CA) and HP 5890 series II GC/5972 MSD (Hewlett-Packard, Palo Alto, CA) equipped with a DB-wax column (30 m length × 0.25 mm i.d. × 0.25 mm film thickness). The carrier gas used was helium, at a constant flow rate of 0.8 ml/min. One microliter of mushroom extract was injected into the column using the splitless injection mode. The oven temperature was initially held at 40 °C for 1 min, then raised to 200 °C at a rate of 4 °C/min, and finally held at 200 °C for 10 min. The temperatures of injector and detector were 200 °C and 250 °C, respectively. The mass detector was operated in electron impact mode with an ionization energy of 70 eV, a scanning range of 33–550 a.m.u. and a scan rate of 1.4 scans/s.

2.6. Gas chromatography–olfactometry (GC–O)

GC–O was conducted on an Agilent 6890N GC equipped with a flame ionization detector (FID) and a sniffing port, (Gerstel Inc., Baltimore, MD) using a DB-5 ms column. Effluent from the end of the GC column was split equally between the FID and the sniffing port. The HVS extract was diluted stepwise with dichlorometh-

ane (1:1 by volume). An aliquot (1 μl) was injected into the capillary column. GC conditions were the same as those used for GC–MS. Flavor dilution (FD) factors of the volatile components were determined by aroma extract dilution analysis (AEDA); the FD factor corresponded to the maximum dilution at which each component could be detected (Grosch, 1994). Two experienced sniffers, each with >30 h training on GC–O, participated in AEDA. Then the maximum value of their results was provided as the FD factor of that compound.

2.7. Identification of volatiles and key odorants

Volatile components were positively identified by comparing their mass spectra and RIs with those of the authentic compounds. When standards were not available, compounds were tentatively identified with the aid of the Wiley 275 Imass spectral database (Hewlett-Packard, 1995) or by manual interpretation. All key odorants were positively identified by comparing their mass spectra, linear retention indices (RIs), and aroma properties perceived at the sniffing port with those of authentic standards. The RI of each compound was calculated using *n*-alkanes C_7–C_{22} as external references (van den Dool & Kratz, 1963). The semiquantitative analysis of volatile components was performed by comparing their peak areas to that of the internal standard compound (0.1 ml of 100 ppm dodecanoic acid methyl ester in dichloromethane, v/v) on the GC–MS total ion chromatogram.

3. Results and discussion

3.1. Volatile components in different parts of pine-mushroom

The volatile components in the pileus and the stipe of pine-mushrooms were isolated using HVS and then analyzed by GC–MS. Fig. 1 shows the GC–MS total ion chromatograms for the volatile components and odorants found in the pileus and the stipe of pine-mushrooms.

Table 1 lists the volatile components identified in the pileus and the stipe samples, the relative peak areas, and the retention indices (RIs) on the DB-5 ms column. A total of 24 and 21 volatile components were found in the pileus and the stipe of pine-mushrooms, respectively, including 13 alcohols, 5 carbonyls, 4 acids and esters, 4 terpene hydrocarbons, and 2 miscellaneous components. In particular, (*E*)-linalool oxide (No. 9), benzaldehyde (No. 15), octanoic acid ethyl ester (No. 20), α-pinene (No. 23), camphene (No. 24), and limonene (No. 25) were found only in the pileus, whereas 1-pentanol (No. 2), 2-ethyl-1-haxanol (No. 6), nerolidol (No. 13) and (*E*)-2-octenal (No. 17) were identified only in the stipe. In the pileus, methyl cinnamate (85.053 ± 18.171) (No. 22) was the most abundant component, followed by 1-octen-3-ol (No. 4), (*E*)-linalool oxide (No. 9), (*E*)-2-octen-1-ol (No. 8) and 3-octanol (No. 5). In contrast, 1-octen-3-ol (12.671 ± 2.795) was most dominant in the stipe, followed by methyl cinnamate, (*E*)-2-octen-1-ol, and linalool. As reported previously, the volatile components in pine-mushrooms were primarily

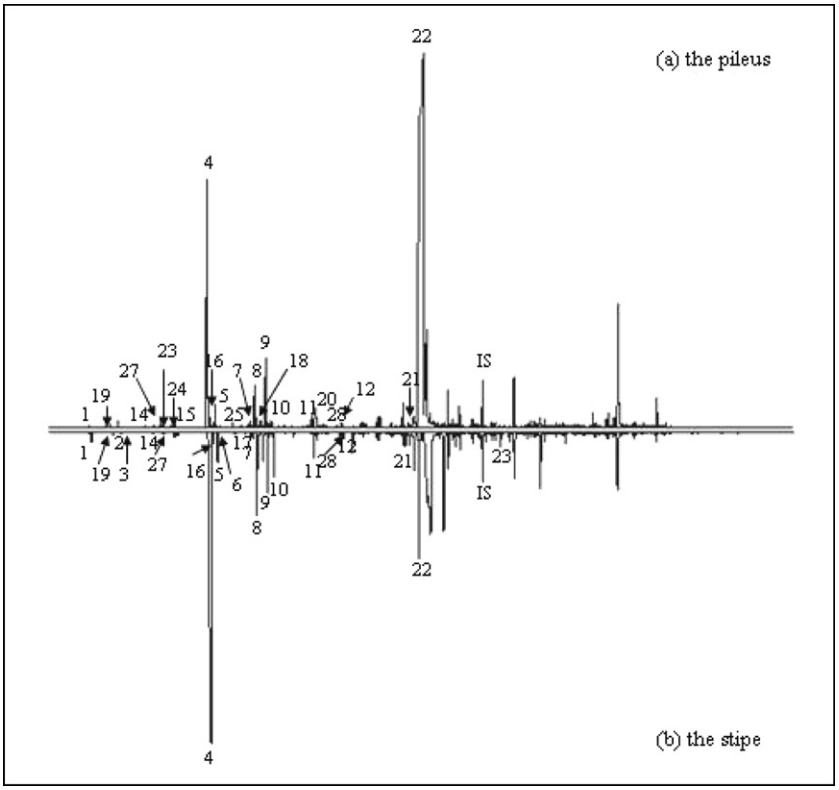

Fig. 1. GC–MS total ion chromatograms for the volatile components identified in the different parts of pine-mushroom.

Table 1
Volatile components identified in the different parts of pine-mushroom by GC–MS

Nos.	Volatile components	RI[a]		Relative peak area (mean ± SD)[b]		ID[c]
		DB-5 ms	DB-wax	Pileus	Stipe	
	Alcohols					
1	3-Penten-2-ol	<700	1170	0.072 ± 0.003	0.072 ± 0.004	A
2	1-Pentanol	764	1252	0.000 ± 0.000	0.040 ± 0.006	A
3	2-Methyl-3-buten-2-ol	776	1037	0.027 ± 0.005	0.004 ± 0.001	A
4	1-Octen-3-ol	983	1458	9.150 ± 0.772	12.700 ± 2.795	A
5	3-Octanol	996	1396	0.321 ± 0.014	0.336 ± 0.070	A
6	2-Ethyl-1-hexanol	1029	1484	0.000 ± 0.000	0.025 ± 0.005	A
7	α-Methyl benzenemethanol	1061	1820	0.099 ± 0.014	0.090 ± 0.013	A
8	(*E*)-2-octen-1-ol	1069	1620	0.525 ± 0.032	1.460 ± 0.251	A
9	(*E*)-linalool oxide (furanoid)	1086	1484	0.947 ± 0.171	0.000 ± 0.000	A
10	Linalool	1100	1547	0.161 ± 0.056	0.470 ± 0.160	A
11	(*Z*)-linalool oxide (pyranoid)	1175	1770	0.229 ± 0.048	0.255 ± 0.026	A
12	Phenylpropyl alcohol	1231	2058	0.105 ± 0.023	0.068 ± 0.002	A
13	Nerolidol	1565	2050	0.000 ± 0.000	0.189 ± 0.007	A
	Carbonyls					
14	2(5H)-Furanone	914	1767	0.070 ± 0.007	0.095 ± 0.011	A
15	Benzaldehyde	960	1530	0.013 ± 0.001	0.000 ± 0.000	A
16	3-Octanone	988	1261	0.127 ± 0.032	0.122 ± 0.026	A
17	(*E*)-2-octenal	1058	1437	0.000 ± 0.000[d]	0.035 ± 0.007	A
18	Nonanal	1071	1390	0.101 ± 0.048	0.000 ± 0.000	A
	Acids and esters					
19	Thiocyanic acid methyl ester	<800	1276	0.013 ± 0.004	0.006 ± 0.001	B
20	Octanoic acid ethyl ester	1178	1440	0.040 ± 0.005	0.000 ± 0.000	A
21	2,4-Furandicarboxylic acid dimethyl ester	1365	2157	0.139 ± 0.031	0.140 ± 0.012	B
22	3-Phenyl-2-propenoic acid methyl ester (methyl cinnamate)	1397	2103	85.050 ± 18.171	1.680 ± 0.356	A
	Terpene hydrocarbons					
23	α-Pinene	933	1026	0.025 ± 0.006	0.000 ± 0.000	A
24	Camphene	948	<1100	0.006 ± 0.001	0.000 ± 0.000	A
25	Limonene	1028	1205	0.090 ± 0.016	0.000 ± 0.000	A
26	Junipene	1412	1590	0.279 ± 0.009	0.048 ± 0.009	B
	Miscellaneous					
27	Dimethyl sulfone	919	1912	0.049 ± 0.007	0.045 ± 0.009	A
28	Benzothiazole	1224	1902	0.024 ± 0.004	0.064 ± 0.017	B

[a] Retention indices were determined using *n*-paraffins C_7–C_{22} as external references.
[b] Average of relative peak areas to that of internal standard ($n = 3$) ± standard deviation.
[c] Volatiles were identified on the basis of the following criteria: A, mass spectrum and retention index were consistent with those of an authentic standard; B, mass spectrum was consistent with that of Wiley 275 Imass spectral database or by manual interpretation (tentative identification).
[d] Not detected.

composed of the following C_8 components: 1-octen-3-ol, 3-octanol, (*E*)-2-octen-1-ol, 1-octanol, 1-octen-3-one, and 3-octanone (Cho et al., 2006a). The contents of all the C_8 components identified, except for 3-octanone, were higher in the stipe than in the pileus in this study. In contrast, Noël-Suberville and co-workers found that the amounts of 1-octen-3-ol, 1-octen-3-one, and 3-octanone were larger in the pileus than in the stipe of fresh blewit (*Lepista nuda*) (Noël-Suberville et al., 1996).

3.2. Key odorants in different parts of pine-mushrooms

In the present study, 15 and 12 key odorants were identified in the pileus and the stipe of pine-mushrooms, respectively (Table 2). Fifteen of the odorants were positively identified by comparing their mass spectra, RIs, and odor descriptions with those of authentic standards. On the other hand, 2-methyl-butanoic acid ethyl ester (No. iii) and methional (No. iv) were identified and confirmed by comparing their RIs and aroma properties perceived at the sniffing port with those of authentic standards. We reported previously that the key odorants of raw pine-mushroom are 1-octen-3-one, 3-octanol, 1-octen-3-ol, (*E*)-2-octen-1-ol, 3-octanone, and 1-octanol, which have typical fungal odor notes including mushroom-like, mushroom-like/buttery and mushroom-like/chemical (Cho et al., 2006b). In addition, 2-methyl-butanoic acid ethyl ester, linalool, methional, hexanal, phenylethyl alcohol, (*E*)-2-decenal, α-terpineol, 2-methyl-3-buten-2-ol, limonene, nonanal, (*E*)-2-octenal, 2-ethyl-1-hexanol, phenylacetaldehyde, 1-hexanol, and

Table 2
Key odorants identified in the different parts of pine-mushroom by GC–O

Nos.	Key odorants	RI[a]		Aroma property[b]	FD factor[c]	
		5 ms	Wax		Pileus	Stipe
3	2-Methyl-3-buten-2-ol	776	1031	Herbaceous	2	1
i	Hexanal	812	1097	Cut grass-like	4	8
ii	1-Hexanol	850	1360	Green and floral	1	0
iii	2-Methyl butanoic acid ethyl ester	893	1073	Sweet and floral	4	1
iv	Methional	903	1480	Boiled potato-like	8	4
15	Benzaldehyde	921	1534	Almond-like	1	0
v	1-Octen-3-one	975	1317	Mushroom-like	32	64
4	1-Octen-3-ol	983	1451	Mushroom-like	8	8
16	3-Octanone	988	1240	Mushroom-like/buttery	4	8
5	3-Octanol	996	1394	Mushroom-like/buttery	4	8
6	2-Ethyl-1-hexanol	1029	1484	Rose-like	0	1
vi	Phenylacetaldehyde	1051	1648	Floral	0	1
8	(*E*)-2-octen-1-ol	1069	1622	Mushroom-like	2	4
19	Nonanal	1071	1390	Fatty and soapy	1	0
10	Linalool	1100	1540	Sweet and citrus	8	4
vii	α-Terpineol	1207	1675	Pine-tree-like	1	0
viii	(*E*)-2-decenal	1234	1630	Orange-like and fatty	1	0

[a] Retention indices were determined using *n*-paraffins C_7–C_{22} as external references.
[b] Aroma properties perceived at the sniffing port.
[c] FD: flavor dilution factor. An FD factor <1 means that the respective compound was not detected during sniffing of the undiluted extract.

octanoic acid ethyl ester could contribute to characteristic odour notes of raw pine-mushrooms (Cho et al., 2006b). In this study, 1-octen-3-one (No. v, mushroom-like) was the most potent key odorant in both the pileus and the stipe. On the other hand, linalool (No. 10, sweet and citrus), methional (No. iv, boiled potato-like), hexanal (No. i, cut grass-like), and 3-octanol (No. 5, mushroom-like/buttery) were major key odorants in the pileus, whereas 3-octanol, hexanal, methional, (*E*)-2-octen-1-ol (No. 8, mushroom-like), and linalool were important in the stipe. A series of C_8 aliphatic compounds, such as 1-octen-3-one, 3-octanol, 1-octen-3-ol, (*E*)-2-octen-1-ol, 3-octanone, have been reported to be the major contributors to the characteristic flavor of diverse mushrooms (Cronin & Ward, 1971; Pyysalo & Suihko, 1976; Fischer & Grosch, 1987; Venkateshwarlu et al., 1999). These C_8 compounds are mainly formed by the oxidation of linoleic or linolenic acids in the presence of enzymes, such as lipoxygenase and hydroperoxide lyase (Wurzenberger & Grosch, 1986; Assaf, Hadar, & Dosoretz, 1997). Although methional, which was reported as one of the major aroma-active compounds in fungal species (Lizárraga-Guerra et al., 1997), could not be identified by GC–MS in this study, it showed a high FD factor, mainly due to its low detection threshold of 0.1–0.2 ng/L in air (Rychlik, Schieberle, & Grosch, 1998) and characteristic odor note.

1-Hexanol (No. ii, green and floral), benzaldehyde (No. 15, almond-like), nonanal (No. 19, fatty and soapy), α-terpineol (No. vii, pine-tree-like), and (*E*)-2-decenal (No. viii, orange-like and fatty) could be perceived only in the pileus, whereas 2-ethyl-1-hexanol (No. 6, rose-like) and phenylacetaldehyde (No. vi, floral) were detected only in the stipe. In addition, the FD factors of 1-octen-3-one, 3-octanol, (*E*)-2-octen-1-ol, and 3-octanone possessing mushroom-like and mushroom-like/buttery odor notes were higher in the stipe than in the pileus. Linalool, 2-methyl-butanoic acid ethyl ester (No. iii, sweet and floral), 2-methyl-3-buten-2-ol (No. 3, fresh green), α-terpineol, and (*E*)-2-decenal, described as sweet, floral, green, and citrus, had higher FD factors in the pileus than in the stipe.

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