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Characterization of Aroma-Active Compounds in Raw and Cooked Pine-Mushrooms (*Tricholoma matsutake* Sing.)

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The characteristic aroma-active compounds in raw and cooked pine-mushrooms (*Tricholoma matsutake* Sing.) were investigated by gas chromatography−olfactometry using aroma extract dilution analysis. 1-Octen-3-one (mushroom-like) was the major aroma-active compound in raw pine-mushrooms; this compound had the highest flavor dilution factor, followed by ethyl 2-methylbutyrate (floral and sweet), linalool (citrus-like), methional (boiled potato-like), 3-octanol (mushroom-like and buttery), 1-octen-3-ol (mushroom-like), (*E*)-2-octen-1-ol (mushroom-like), and 3-octanone (mushroom-like and buttery). By contrast, methional, 2-acetylthiazole (roasted), an unknown compound (chocolate-like), 3-hydroxy-2-butanone (buttery), and phenylacetaldehyde (floral and sweet), which could be formed by diverse thermal reactions during the cooking process, together with C_8 compounds, were identified as the major aroma-active compounds in cooked pine-mushrooms.

KEYWORDS: Pine-mushroom (*Tricholoma matsutake* Sing.); aroma-active compound; gas chromatography-olfactometry; aroma extract dilution analysis

INTRODUCTION

Mushrooms have been widely consumed since ancient times not only as foods or food-flavoring materials but also for medicinal or functional purposes. The characteristic flavor substances of mushrooms can be classified into nonvolatile and volatile components (*1*). The taste of edible mushrooms is primarily attributed to several water-soluble substances, including 5′-nucleotides, free amino acids, and soluble carbohydrates (*2*, *3*). Among the diverse volatile components (*1*, *4*−*12*), a series of aliphatic components, such as 1-octen-3-ol, 2-octen-1-ol, 3-octanol, 1-octanol, 1-octe-3-one, and 3-octanone, have been reported to be the major contributors to the characteristic mushroom flavor (*9*−*12*). In particular, an unsaturated alcohol, 1-octen-3-ol, described as "mushroom-like flavor" and "raw mushroom", 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 (*9*−*12*). The profile of the flavor components varies with species and varieties of mushroom and can also be influenced by cultural conditions (*4*, *5*). In addition, because raw mushrooms contain numerous reactive components, any processing (e.g., drying, canning, or other thermal treatments) normally leads to significant changes in the compositions of diverse components, including volatiles (*4*, *5*, *8*).

Among the various mushroom species, pine-mushroom (*Tricholoma matsutake* Sing.) is the most valuable one throughout the world, exhibiting a characteristic and delicate flavor as well as several biological activities, such as cholesterol lowering, antioxidant, immunomodulating, and antitumor effects in humans (*13*−*15*). In particular, pine-mushrooms that are cultivated in the pine forests of South Korea are highly valued, due to the unique environment and climate of South Korea. Pine-mushrooms are used in a variety of culinary dishes, including stews, soups, and steamed dishes. Even though many methods have been developed to cook pine-mushrooms, they can also be consumed raw, preserving their original taste and aroma. While the effects of origin (*16*), grade (*17*), and thermal processing (*18*) on the volatiles components of pine-mushrooms have been examined, the aroma-active compounds in pine-mushrooms have not been studied previously.

The objective of this study was to determine and compare aroma-active compounds in raw and cooked pine-mushrooms by gas chromatography−olfactometry (GC−O) using aroma extract dilution analysis (AEDA). In AEDA, the assessors evaluated whether or not an aroma could be perceived and described its aroma property. The results were expressed as the flavor dilution (FD) factor that corresponds to the maximum dilution value detected (*19*).

MATERIALS AND METHODS

Materials. We used first-grade pine-mushrooms that were cultivated in Inje-eup, Gangwon-do, South Korea, in 2004. Raw pine-mushrooms were wrapped in low-density polyethylene film before being stored at −70 °C until used. Immediately before use, the frozen mushrooms were thawed at 4 °C for 3 h and then sliced using a cutter (Shinomura, Sanjŏ, Niigata, Japan). The sliced mushrooms were heat-treated at 190 ± 3 °C for 1 min on both sides in a convection broiler (Toastmaster,

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Boonville, MO). The raw or cooked mushrooms were placed into a stainless steel container, frozen in liquid nitrogen, and then ground in a blender (Hanil Electric, Seoul, Korea).

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). Stock solutions of 23 authentic standard compounds were prepared in dichloromethane. Standards 1−9, 11−15, 17−21, and 23 were obtained from Sigma-Aldrich, while standards 10, 16, and 22 were purchased from Wako Pure Chemical Industries (Osaka, Japan).

Extraction of Volatile Components. Samples of the ground mushrooms (100 g) were extracted with 200 mL of dichloromethane that was redistilled before use. The ground sample suspended in dichloromethane was magnetically stirred at 400 rpm for 30 min and then filtered through Whatman no. 41 filter paper (Maidstone, United Kingdom) under a vacuum. Volatile components were then separated from the nonvolatiles using high-vacuum sublimation (HVS) (*17*). The operating vacuum was typically below 2×10^{-5} Torr. 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.

Fractionation by Column Chromatography. To identify odorants not detected by gas chromatography−mass spectrometry (GC−MS), the HVS extracts were subjected to silica gel column chromatography. The mushrooms 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 a pentane/diethyl ether gradient (50/0, 40/10, 30/20, 20/30, 10/40, and 0/50 mL, respectively). Each fraction was concentrated under a slow stream of nitrogen gas to obtain a final volume of 0.1 mL.

GC−MS. GC−MS analysis was performed using a HP 6890N gas chromatography-5973N mass selective detector (GC−MSD) (Hewlett-Packard, Palo Alto, CA) equipped with a DB-wax column (60 m length × 0.25 mm i.d. × 0.25 mm film thickness, J&W Scientific, Folsom, CA) and a HP 5890 series II GC-5972 MSD equipped with a DB-5ms column (30 m length × 0.25 mm i.d. × 0.25 mm film thickness). The carrier gas 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 the injector and detector were 200 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 amu, and a scan rate of 1.4 scans/s.

GC−O. GC−O was conducted on a Varian CP-3800 GC (Varian, Walnut Creek, CA) equipped with a flame ionization detector (FID) and a sniffing port (ODO II, SGE, Ringwood, Australia) using a DB-wax column and a DB-5ms column. Effluent collected from the end of GC column was split equally between the FID and the sniffing port. The HVS extract was diluted stepwise with dichloromethane (1:2 by volume). An aliquot (1 μL) was injected into the capillary column. GC conditions were the same as those used for GC−MS (see above). FD factors of the volatile components were determined by AEDA; the FD factor corresponded to the maximum dilution at which each component could be detected (*19*). Two experienced sniffers, each with >30 h training on GC−O, were used for AEDA. Then, the maximum value of them was provided as the FD factor of that compound.

Identification of Aroma-Active Compounds. For positive identifications, mass spectra, linear retention indices (RIs), and aroma properties of unknowns were compared with those of authentic standards. Tentative identifications were based on matching RIs and aroma properties of unknowns with those in the literature (*20*) or comparing the RIs and aroma properties of unknowns to those of authentic standards. Aroma properties of all authentic standards were determined by GC−O. The RI of each compound was calculated using *n*-alkanes C_7−C_{22} as external references (*21*).

RESULTS AND DISCUSSION

Table 1 lists aroma-active compounds identified in raw and cooked pine-mushrooms, their aroma properties, RI values, and FD factors for each of the two capillary columns of different polarities, such as the DB-wax and DB-5ms columns. An enrichment by column chromatography was necessary for the identification of **8**, **9**, **15**, **19**, **21**, and **22**. Compounds **2** and **14** were only tentatively identified and confirmed by comparing RIs and aroma properties with those of authentic standards using GC−O. One compound (**24**) could not be identified in this study, although it had a very characteristic odor note (chocolate-like) in cooked pine-mushrooms. Most of the aroma-active compounds found on both columns were similar, except for one unidentified compound, which was detected only on the DB-5ms column.

A total of 22 aroma-active compounds were identified in raw pine-mushrooms by GC−O using AEDA. 1-Octen-3-one (**7**) was the most potent aroma-active compound in raw pine-mushrooms with the highest FD factor, followed by ethyl 2-methylbutyrate (**2**), linalool (**16**), and methional (**14**). 1-Octen-3-one, which has a mushroom-like odor, could be formed by the enzymatic degradation of lipids, particularly, unsaturated free fatty acids such as linoleic and linolenic acids (*22*), which had been reported as the impact compound of mushrooms (*9*−*12*) with a detection threshold of 0.03−1.12 ng/L in air (*20*). Ethyl 2-methylbutyrate, which was described as a floral and sweet odor, had a relatively high FD factor of 32 and 64 on the DB-wax and DB-5ms columns, respectively, even though this compound could not be detected by GC−MS. Ethyl 2-methylbutyrate, which had a pleasant odor with a detection threshold of 0.06−0.24 ng/L in air (*20*), was previously identified as one of the aroma-active compounds in apples (*23*) and white wine (*24*). Two other aroma-active compounds, linalool (citrus-like) and methional (boiled potato-like), exhibited a relatively high FD factor on both the DB-wax (FD = 8) and the DB-5ms (FD = 32) column. The odor of linalool has been described as a floral odor in some edible mushroom species (*12*). Methional, which could be formed by enzymatic degradation of methionine (*25*), was reported as one of the major aroma-active compounds in fungus species (*26*). Although methional 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 (*20*). A series of C_8 aliphatic compounds, such as 3-octanol (**10**, mushroom-like and buttery), 1-octen-3-ol (**13**, mushroom-like), (*E*)-2-octen-1-ol (**18**, mushroom-like), 3-octanone (**5**, mushroom-like and buttery), 1-octanol (**17**, chemical and sweet), and (*E*)-2-octenal (**11**, sweet), have been reported to be the major contributors to the characteristic flavor of mushrooms (*9*−*12*). These C_8 compounds were formed by the oxidation of linoleic or linolenic acids in the presence of enzymes such as lipoxygenase and hydroperoxide lyase (*27*−*31*). In this study, 3-octanol and 1-octen-3-ol had relatively high FD factors that ranged from 8 to 24 on both columns in raw pine-mushrooms. By contrast, (*E*)-2-octen-1-ol, 3-octanone, 1-octanol, and (*E*)-2-octenal had relatively low FD factors that ranged from 1 to 4 in raw pine-mushrooms. In addition, hexanal (**3**, cut grass-like), phenylethyl alcohol (**23**, floral and sweet), (*E*)-2-decenal (**19**, orange-like), α-terpineol (**22**, pine tree-like), 2-methyl-3-buten-2-ol (**1**, herbaceous), limonene (**4**, lemon-like), 2-ethyl-1-hexanol (**15**, rose-like), phenylacetaldehyde (**20**, floral and honey-like), 1-hexanol (**8**, green), and ethyl octanoate (**12**, green and sweet), exhibiting relatively low FD factors, were also identified as aroma-active compounds in raw pine-mushrooms. Hexanal (*12*, *26*, *32*), phenylethyl alcohol (*12*, 26), α-terpineol (*9*), phenyl-

Table 1. Aroma-Active Compounds Identified in Raw and Cooked Pine-Mushrooms

	RI[a] on				FD factor[c]				
					raw		cooked		
no.	DB-wax	DB-5ms	aroma-active compounds	aroma property[b]	DB-wax	DB-5ms	DB-wax	DB-5ms	ID[e]
1	1031	<800	2-methyl-3-buten-2-ol	herbaceous	1	2	0[d]	0	MS/RI/odor
2	1073	895	ethyl 2-methylbutyrate	floral and sweet	32	64	16	32	RI/odor
3	1097	807	hexanal	cut grass-like	2	2	1	2	MS/RI/odor
4	1195	1032	limonene	lemon-like	1	2	1	2	MS/RI/odor
5	1240	971	3-octanone	mushroom-like and buttery	2	4	4	8	MS/RI/odor
6	1259	<800	3-hydroxy-2-butanone	buttery	1	1	8	8	MS/RI/odor
7	1317	978	1-octen-3-one	mushroom-like	256	256	64	64	MS/RI/odor
8	1360	865	1-hexanol	green	1	1	1	1	(F5) MS/RI/odor
9	1390	1104	nonanal	fatty and soapy	1	2	0	1	(F3) MS/RI/odor
10	1394	1007	3-octanol	mushroom-like and buttery	8	32	8	32	MS/RI/odor
11	1430	1052	(*E*)-2-octenal	sweet	1	2	0	0	MS/RI/odor
12	1435	1173	ethyl octanoate	green and sweet	1	1	0	0	MS/RI/odor
13	1451	1002	1-octen-3-ol	mushroom-like	8	8	8	8	MS/RI/odor
14	1480	914	methional	boiled potato-like	8	32	64	256	RI/odor
15	1484	1029	2-ethyl-1-hexanol	rose-like	1	2	1	1	(F3-6) MS/RI/odor
16	1540	1105	linalool	citrus-like	8	32	16	64	MS/RI/odor
17	1546	1074	1-octanol	chemical and sweet	2	1	4	4	MS/RI/odor
18	1622	1071	(*E*)-2-octen-1-ol	mushroom-like	2	4	2	4	MS/RI/odor
19	1630	1260	(*E*)-2-decenal	orange-like	2	2	2	2	(F3) MS/RI/odor
20	1648	1043	phenylacetaldehyde	floral and honey-like	1	2	2	4	MS/RI/odor
21	1652	1064	2-acetylthiazole	roasted	0	0	16	32	(F4) MS/RI/odor
22	1675	1198	α-terpineol	pine tree-like	2	2	1	1	(F3-5) MS/RI/odor
23	1810	1060	phenylethyl alcohol	floral and sweet	2	2	2	2	MS/RI/odor
24		1101	unknown	chocolate-like	0	0	0	16	odor

[a] RIs were determined using *n*-paraffins C_7–C_{22} as external references. [b] Aroma properties were perceived at the sniffing port. [c] FD factors by two panelists. [d] Not detected. [e] Aroma-active compounds were identified on the basis of the following criteria: MS/RI/odor, mass spectrum, RI, and aroma properties were consistent with those of authentic standards; RI/odor, RIs and aroma properties were consistent with those of authentic standards; odor, odor was perceived only at sniffing port by two panelists; (Fn) MS/RI/odor, mass spectrum, RI, and aroma properties of compounds were identified by silica gel column chromatography using a pentane:diethyl ether gradient ($n = 1$, 50 mL of pentane; $n = 2$, 40 mL of pentane:10 mL of diethyl ether; $n = 3$, 30 mL of pentane:20 mL of diethyl ether; $n = 4$, 20 mL of pentane:30 mL of diethyl ether; $n = 5$, 10 mL of pentane:40 mL of diethyl ether; and $n = 6$, 50 mL of diethyl ether) were consistent with those of authentic standards.

acetaldehyde (*9*, 26, *32*), and 1-hexanol (*9*, *12*) have been found in other mushroom and fungus species.

By contrast, 23 aroma-active compounds were found in cooked pine-mushrooms by GC−O. Methional was the most potent aroma-active compound in cooked pine-mushrooms with the highest FD factor, followed by 1-octen-3-one, ethyl 2-methyl butyrate, 2-acetylthiazole (*21*), linalool, 3-octanol, and 1-octen-3-ol. In cooked pine-mushrooms, methional could be mainly formed by Strecker degradation of methionine (*33*). 1-Octen-3-one, ethyl 2-methylbutyrate, linalool, 3-octanol, and 1-octen-3-ol were perceived as aroma-active compounds exhibiting relatively high FD factors, in both raw and cooked pine-mushrooms. On the other hand, 2-acetylthiazole and an unknown compound (*24*) were detected only in cooked pine-mushrooms, which suggests that these compounds be formed in response to exposure to heat. 2-Acetylthiazole might be formed in pine-mushrooms by condensation of Maillard intermediates followed by cyclization (*33*). This compound had a relatively high FD factor of 16 and 32 on the DB-wax and DB-5ms column, respectively, and was identified by GC−MS after fractioned by column chromatography and further highly concentrated. Also, 3-hydroxy-2-butanone, 3-octanone, and phenylacetaldehyde, which were formed by the oxidative degradation of unsaturated lipids or by nonenzymatic amino-carbonyl reactions, have been implicated as significant contributors to the flavor of cooked food (*34*, *35*).

These results indicate that major aroma-active compounds of both raw and cooked pine-mushrooms were related to 1-octen-3-one, 3-octanol, 1-octen-3-ol, (*E*)-2-octen-1-ol, 3-octanone, and 1-octanol, which had been described as the fungal odor note. In addition, ethyl 2-methylbutyrate, 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 ethyl octanoate could contribute to the characteristic odor notes of raw pine-mushrooms. By contrast, methional, 2-acetylthiazole, an unknown compound, 3-hydroxy-2-butanone, and phenylacetaldehyde were identified as important aroma-active compounds in cooked pine-mushrooms.

ABBREVIATIONS USED

GC−O, gas chromatography−olfactometry; AEDA, aroma extract dilution analysis; FD, flavor dilution; HVS, high-vacuum sublimation; GC−MS, gas chromatography−mass spectrometry; RI, retention indices.

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