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Characterization of Volatile Components and Odor-active Compounds in the Oil of Edible Mushroom *Boletopsis leucomelas*

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Abstract: The volatile oil from *Boletopsis leucomelas* (Pers.) Fayod was extracted by hydrodistillation with diethylether, and the volatile components of the oil were analyzed by gas chromatography-mass spectrometry. The oil contained 86 components, representing 87.5% of the total oil. The main components of the oil were linoleic acid (15.0%), phenylacetaldehyde (11.2%), and palmitic acid (9.4%). Furthermore, sulfur-containing compounds including 3-thiophenecarboxaldehyde, 2-acetylthiazole, *S*-methyl methanethiosulfonate, and benzothiazole were detected using gas chromatography-pulsed flame photometric detection. The odor components were evaluated by the odor activity value, and aroma extract dilution analysis was performed through gas chromatography-olfactometry analysis. The oil had a mushroom-like, fatty, and burnt odor. The main components contributing to the mushroom-like and fatty odor were hexanal, nonanal, 1-octen-3-ol, and (2*E*)-nonenal, while the burnt odor was due to furfuryl alcohol, benzaldehyde, 5-methyl furfural, 2,3,5-trimethylpyrazine, 2-acethylthiazole, and indole.

Key words: *Boletopsis leucomelas*, GC-O, AEDA, GC-MS/PFPD, edible mushroom

1 INTRODUCTION

Mushrooms have been consumed since ancient times for medicinal and functional purposes, because of their distinctive flavors and textures[1-4]. Among diverse volatile components, a series of aliphatic components including 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 odor of mushrooms. In particular, an unsaturated alcohol contributes to the "mushroom-like odor" and "raw mushroom smell", and has been identified in many mushroom species. Together with its oxidation product, 1-octen-3-one is considered to be responsible for the characteristic odor of most edible mushrooms.

Boletopsis leucomelas (Pers.) Fayod belongs to the genus *Boletopsis* in the family Thelephoraceae. The Japanese name is Kurokawa[5], and it is a traditional Japanese food containing a black fruiting body. *B. leucomelas* contains terphenyl compounds, which exhibit 5-lipoxygenase and kinase domain receptor (KDR) kinase inhibitory abilities and lectin apoptosis-inducing activities[6-11]. In addition, *B. leucomelas* has a characteristic burnt odor. To the best of our knowledge, the burnt odor has not been found in other mushrooms. In fact, this aroma is unique to *B. leucomelas*. The odor components and volatile oil are important factors to be considered in elucidating this characteristic. However, to the best of our knowledge, volatile components of *B. leucomelas* and other Thelephoraceae mushrooms have not been reported.

Gas chromatography-mass spectrometry (GC-MS) and gas chromatography-pulsed flame photometric detection (GC-PFPD) are the most commonly used methods to analyze chemical compounds. In flavor analysis, chromatography-olfactometry (GC-O) is typically used to evaluate odorants. In particular, GC-O in combination with aroma extract dilution analysis (AEDA) is useful for estimating the contribution of odor-active compounds as AEDA utilizes sniffing analysis. By sniffing serial dilutions of a volatile oil (VO), volatile compounds can be ranked according to odor potency[12]. The odor potency is expressed as the flavor dilution factor (FD-factor), which is the ratio of the initial concentration of a compound to the most diluted concentration in which the odor could be detected by GC-O.

The aim of the present work was to investigate the VO from *B. leucomelas*. Herein, the volatile compounds and characteristic odor-active compounds from *B. leucomelas* oil are reported for the first time.

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2 EXPERIMENTAL PROCEDURES

2.1 Plant material

Boletopsis leucomelas (Pers.) Fayod was obtained from Fukushima prefecture, Japan in April 2011. Identification of the mushroom was performed in the biotechnology laboratory at Kinki University in Osaka, Japan.

2.2 Isolation of the VO

The VO from dried *B. leucomelas* (100 g) was isolated by hydrodistillation with a Likens-Nickerson-type apparatus for 2 h in distilled dietylether. After filtration, the oil was treated with sodium sulfate and the solvent was evaporated in vacuo. The yield of the oil was 0.024% (w/w).

2.3 Gas Chromatography (GC)

GC was carried out with an Agilent model 6890 GC-equipped with a flame ionization detector (FID) on a capillary column (HP-5MS; 30 m × 0.25 mm, film thickness 0.25 μm). The oven temperature was programmed from 40 to 260℃ at a rate of 4℃/min and held at 260℃ for 5 min. The injector and detector were 270 and 280℃, respectively. The flow rate of the carrier gas (Helium) was 1.8 mL/min. Peak areas were quantified using a computer integrator.

2.4 Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS analysis was carried out on an Agilent 6890-5973 instrument. The sample was analyzed on a fused-silica capillary column HP-5MS (polydimethylsiloxane, 30 m × 0.25 mm i.d., film thickness 0.25 μm) and DB-WAX (15 m × 0.25 mm i.d., film thickness 0.25 μm). The oven temperature was programmed from 40 to 260℃ at a rate of 4℃/min and held at 260℃ for 5 min. The flow-rate of the carrier gas (Helium) was 1.8 mL/min. The injector and detector temperatures were 270 and 280℃, respectively. The actual temperature in the MS source was approximately 230℃, and the ionization energy was 70 eV. The mass range was 39–450 amu. After 6 mg of the oil was diluted with 500 mL of diethylether, 1 mL of the dilution was injected with a split ratio of 1:10.

2.5 Gas chromatography-Pulsed Flame Photographic Detection (GC-PFPD)

GC-PFPD analysis was carried out using an Agilent 6890-Pulsed Flame Photometric Detector. The sample was analyzed on a fused-silica capillary column HP-5MS (polydimethylsiloxane, 30 m × 0.25 mm i.d., film thickness 0.25 μm). The oven temperature was programmed from 40 to 260℃ at a rate of 4℃/min and held at 260℃ for 5 min. The flow rate of the carrier gas (He) was 1.8 mL/min. The injector and the detector temperatures were 270 and 280℃, respectively.

2.6 Gas chromatography-olfactometry (GC-O)

GC-O analysis was performed on an Agilent-6890-5973N with a capillary column and HP-5MS (polydimethylsiloxane, 30 m × 0.25 mm i.d., film thickness 0.25 μm). The oven temperature was programmed from 40 to 260℃ at a rate of 4℃/min and held at 260℃ for 5 min. The flow-rate of the carrier gas (He) was 1.8 mL/min. The injector and detector temperatures were 270 and 280℃, respectively. The ionization energy was 70 eV. One microliter of oil was injected. At the exit of the capillary column, the effluent was split into channels to the mass detector and the sniffing port at a 1:1 split ratio.

2.7 AEDA

The highest sample concentration (10 mg/mL) was assigned an FD-factor of 1. The volatile oil was diluted stepwise with diethylether (1:1, v/v), and aliquots of the dilutions (1 μL) were evaluated. The process continued until no aroma could be detected by the assessors. The results were expressed as FD-factors, or the ratio of the concentration of the odorant in the initial volatile oil to the concentration in the most diluted volatile oil in which the odor was detected by GC-O[13, 14]. An odorant with a high FD-factor can be considered an important contributor to the characteristic odor.

2.8 Identification and quantification of compounds

Identification of the individual components was based on the comparison of their GC-MS retention indices (RIs) on non-polar and polar columns relative to the retention time of a series of *n*-alkanes (C_5-C_{27}) and authentic compounds [commercial source: Wako Pure Chemical Industries Ltd. (Osaka, Japan), Sigma-Aldrich (St. Louis, Tokyo), Chemical Industry Co. Ltd. (Tokyo, Japan)] or literature data[15]. Computer matching was carried out with commercial mass spectral libraries NIST02, Mass Finder 4, and Aroma Office and compared to literature data. The relative amounts of the individual components were calculated based on GC peak areas of the FID response without using correction factors.

Quantitative analysis of the active aroma components of the oils was performed on the basis of calibration curves for hexanal (**3**), furfural (**4**), and α-copaene (**65**) within the concentration range of 0.5–1000 μg/mL. The weight percent of each compound was calculated using the response factors of the FID.

3 RESULTS AND DISCUSSION

3.1 Chemical components of the volatile oil from *Boletopsis leucomelas*

The hydrodistillation of *B. leucomelas* afforded a yellow oil in a 0.024% (w/w) yield. Eighty-six components were identified in the VO of *B. leucomelas*, representing 87.5% of the total oil (Table 1 and Fig. 1). The main components

Table 1 Compositions of the volatile oil from *Boletopsis leucomelas*.

No.	RI-5[a]	RI-W[b]	Compounds[c]	Peak area (%)[d]	Identification[f]
1	641	1353	acetic acid	tr[e]	MS, RI
2	690	1792	propionic acid	tr	MS, RI
3	802	1088	hexanal	1.8	MS, RI
4	840	1379	furfural	4.2	MS, RI
5	852	1578	furfuryl alcohol	0.2	MS, RI
6	859	1069	ethylbenzene	0.8	MS, RI
7	877	1646	valeric acid	0.1	MS, RI
8	901	1184	heptanal	0.4	MS, RI
9	906		isovaleric acid	0.4	MS, RI
10	909	1420	2-acetylfuran	0.3	MS, RI
11	914	1273	2,5-dimethylpyrazine	1.1	MS, RI
12	931	1035	α-pinene	0.2	MS, RI
13	951	1158	propylbenzene	0.3	MS, RI
14	960	1431	benzaldehyde	0.5	MS, RI
15	966	1560	5-methylfurfural	0.9	MS, RI
16	978	1398	1-octen-3-ol	0.3	MS, RI
17	981	1765	caproic acid	0.1	MS, RI
18	986		6-methyl-5-hepten-2-one	0.3	MS, RI
19	989		2-pentylfuran	0.3	MS, RI
20	997	1539	furfuryl acetate	0.2	MS, RI
21	1007		2,3,5-trimethylpyrazine	0.2	MS, RI
22	1020		3-thiophenecarboxaldehyde	0.3	MS, RI
23	1024		*o*-cymene	0.2	MS, RI
24	1038		2-acetylthiazole	0.2	MS, RI
25	1039	1446	2-ethyl-1-hexanol	2.3	MS, RI
26	1055	1560	phenylacetaldehyde	11.2	MS, RI
27	1059	1398	(2*E*)-octenal	0.3	MS, RI
28	1060	1899	*o*-cresol	0.7	MS, RI
29	1076	1506	octanol	0.4	MS, RI
30	1083		*S*-methyl methanethiosulfonate	0.2	MS, RI
31	1092	1329	2-hexylfuran	0.3	MS, RI
32	1106	1397	nonanal	2.3	MS, RI
33	1143		(*E*)-pinocarveol	0.2	MS, RI
34	1151		2-ethylhexyl acetate	0.6	MS, RI
35	1162	1474	(2*E*)-nonenal	0.4	MS, RI
36	1178	1626	menthol	0.2	MS, RI
37	1181	1478	terpinen-4-ol	0.2	MS, RI
38	1186	1764	*p*-cymen-8-ol	0.1	MS, RI
39	1189		cryptone	0.6	MS, RI
40	1194		2-decanone	0.5	MS, RI

of the VO were linoleic acid (**81**, 15.0%), phenylacetaldehyde (**26**, 11.2%), and palmitic acid (**77**, 9.4%). Generally, sulfur-containing volatiles play an important role among odor active compounds. A PFPD chromatogram confirmed the presence of sulfur-containing compounds (Fig. 1). In particular, four sulfur-containing compounds were identified including 3-thiophenecarboxaldehyde, 2-acethylthiazole, *S*-methyl methanethiosulfonate, and benzothiazole. Notably, 3-thiophenecarboxaldehyde, which was also found in sesame oil[16], was identified for the first time in mushrooms. On the other hand, nitrogen-containing compounds 2,5-dimethylpyrazine, trimethylpyrazine, indole, and *cis*-

Table 1 Continued.

No.	RI-5[a]	RI-W[b]	Compounds[c]	Peak area (%)[d]	Identification[f]
41	1204	1452	decanal	0.4	MS, RI
42	1218	1978	caprylic acid	0.2	MS, RI
43	1243	1611	neral	0.2	MS, RI
44	1245		benzothiazole	0.1	MS, RI
45	1271	1661	geranial	0.2	MS, RI
46	1292		2-undecanone	0.2	MS, RI
47	1301	2289	indole	0.5	MS, RI
48	1334	2202	nonanoic acid	4.1	MS, RI
49	1358	1929	γ-nonalactone	0.3	MS, RI
50	1367		γ-octanoic lactone	0.4	MS, RI
51	1394	2191	decanoic acid	0.6	MS, RI
52	1408	1492	longifolene	0.6	MS, RI
53	1453	1778	(*E*)-geranylacetone	0.3	MS, RI
54	1492	1978	5-methyl-2-phenyl-hexenal	0.3	MS, RI
55	1521	1756	δ-cadinene	0.5	MS, RI
56	1533	1963	(*E*,*Z*)-pseudoionone	0.4	MS, RI
57	1545		α-calacorene	0.3	MS, RI
58	1566	2054	(*E*)-nerolidol	0.3	MS, RI
59	1586	2269	lauric acid	0.6	MS, RI
60	1587	1728	neryl acetate	0.3	MS, RI
61[g]	1595	2390	diethyl phthalate	0.1	MS, RI
62	1602	2032	ledol	0.2	MS, RI
63	1631		1,4-cadinadiene	0.3	MS, RI
64	1636		α-aromadendrene	tr	MS, RI
65	1655	2068	τ-muurolol	0.2	MS, RI
66	1646	2046	α-copaene	0.4	MS, RI
67	1661	1746	valencene	0.4	MS, RI
68	1680		cadalene	0.3	MS, RI
69	1782	2625	myristic acid	1.5	MS, RI
70	1812	1930	tetradecanal	0.3	MS, RI
71[g]	1867	2455	diisobutyl phthalate	0.6	MS, RI
72	1888	2714	pentadecanoic acid	3.6	MS, RI
73	1917		farnesylacetone	0.4	MS, RI
74	1923		methyl palmitate	tr	MS, RI
75	1953	2855	(11*Z*)-hexadecenoic acid	0.3	MS, RI
76	1964	2583	dibutyl phthalate	3.1	MS, RI
77	2002	2831	palmitic acid	9.4	MS, RI
78	2058		palmitoleic acid	tr	MS, RI
79	2074		heptadecanoic acid	0.4	MS, RI
80	2094		methyl octadeca-9,12-dienoate	tr	MS, RI

13-docosenamide (**86**) were also identified. Compound **86** was previously found in the essential oil of *Citrus medica* L[17], and identified in mushrooms for the first time. Category analysis revealed that fatty acids (41.6%) were the most significant components of the VO. Other components detected were hydrocarbons (5.3%), alcohols (5.2%), aldehydes (18.0%), ketones (2.6%), esters (5.3%), furans (6.2%), and nitrogen and sulfur-containing compounds (2.9%).

It was previously reported that only a small amount of furan-containing compounds were found in the VO of other mushrooms (*Pleurotus citrinopileatus*[18] and *Lactarius*

Table 1 Continued.

No.	RI-5[a]	RI-W[b]	Compounds[c]	Peak area (%)[d]	Identification[f]
81	2100		methyl oleate	0.3	MS, RI
82	2184	3097	linoleic acid	15.0	MS, RI
83	2186	3292	linolenic acid	0.7	MS, RI
84	2189	3035	oleic acid	4.1	MS, RI
85	2196	3181	stearic acid	0.8	MS, RI
86	2205		*cis*-13-docosenoamide	0.8	MS, RI
			total	87.5	

a) Retention indices determined on HP-5MS coulmns, using the homologous series of n-alkanes (C_5-C_{27}).
b) Retention indices determined on DB-WAX columns, using the homologous series of n-alkanes (C_5-C_{27}).
c) Compounds are listed in order of their elution time from a HP-5MS column. Presence of compound is indicated by its GC/FID percentage.
d) Total detected compounds by GC-MS.
e) Trace (<0.1%).
f) Identification methods: RI, retention indice MS, mass spectrum.
g) Will be artificial plasticiser.

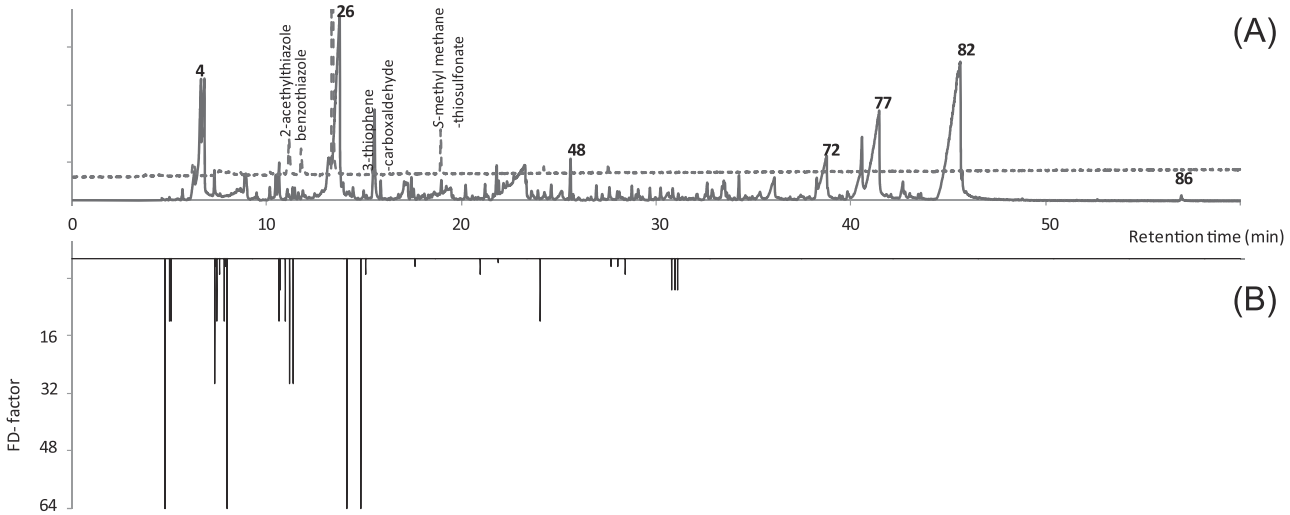

Fig. 1 Chromatograms of VO from *B. leucomelas*: (A) TIC (straight line) and PFPD response (dot line), (B) aromagram (FD-factor). *The peak numbers corresponded to those in **Table 1**.

hatsudake[19]). However, in the VO of *B. leucomelas*, furan-containing compounds such as furfral, furfuryl alcohol, 2-acetylfuran, 5-methylfurfural, 2-pentylfuran, furfuryl acetate, and 2-hexylfuran were identified. Therefore, we hypothesized that furan-containing compounds were characteristic of the *B. leucomelas* oil. Furthermore, (*E*)-geranylacetone and (*E*,*Z*)-pseudoionone (**55**) were also identified in the VO. Importantly, **55** has not been reported in other mushrooms.

3.2 GC-O, AEDA, and odor activity value (OAV) of the VO from *B. leucomelas*

The odor of the VO of *B. leucomelas* was mushroom-like, fatty, and slightly burnt. AEDA was performed through GC-O analysis[20–22]. The flavor components and their odor properties are shown in Fig. 1 and Table 2.

Twenty six compounds were detected by AEDA. Hexanal (fatty), 1-octen-3-ol (mushroom), nonanal (fatty), and (2*E*)-nonenal (fatty) were the most intense odor-active compounds with FD-factors of 64. In addition, heptanal, (2*E*)-octenal, and octanol had FD-factors of 32, and all had fatty odors. Therefore, *B. leucomelas* had a fatty and mushroom odor because of the compounds with high FD-factors (64 or 32).

The characteristic burnt odor of *B. leucomelas* was due to six compounds including furfuryl alcohol (FD = 16, burnt), benzaldehyde (FD = 16, burnt sugar), 5-methyl furfural (FD = 2, burnt sugar), 2,3,5-trimethylpyrazine (FD

Table 2 Odor-active compounds in the volatile oil of *Boletopsis leucomelas*.

No.	RI-5[a]	Compounds	Odor	FD-factor[b]	Concentration (ppb)	OT[c] (ppb)	OAV[d]
3	802	hexanal	fatty	64	4242	10.5	404
4	840	furfural	bread, sweet	16	10080	250	40
5	852	furfuryl alcohol	burnt	16	480	7	69
8	901	heptanal	fatty	32	960	3	320
9	906	isovaleric acid	sweat	2	960	120	8
11	914	2,5-dimethylpyrazine	earthy	16	2730	80	34
12	931	α-pinene	sweet	4	536	190	3
14	960	benzaldehyde	burnt sugar	16	1197	100	12
15	966	5-methylfurfural	almond, burnt sugar	2	2160	500	4
16	978	1-octen-3-ol	mushroom	64	686	1	686
21	1007	2,3,5-trimethylpyrazine	musty, burnt	16	525	23	23
22	1020	3-thiophenecarboxaldehyde	potato	8	720	-	-
24	1038	2-acetylthiazole	burnt	16	480	10	48
27	1059	(2*E*)-octenal	fatty	32	720	3	240
29	1076	octanol	mushroom	32	999	27	37
32	1106	nonanal	fatty	64	5520	5	1104
35	1162	(2*E*)-nonenal	fatty	64	992	0.08	12404
37	1181	terpinen-4-ol	musty	4	455	340	1
41	1204	decanal	fatty	2	935	70	13
47	1301	indole	burnt	4	1175	140	8
49	1358	γ-nonalactone	sweet	1	639	-	-
51	1394	decanoic acid	fatty	16	1510	-	-
55	1526	δ-cadinene	medicine, woody	2	609	60	10
57	1545	α-calacorene	woody	2	813	-	-
58	1566	(*E*)-nerolidol	woody	4	813	260	3
64	1636	α-aromadendrene	woody	8	24	-	-
65	1645	τ-muurolol	spicy	8	521	-	-
66	1652	α-copaene	wood, spicy	8	1040	-	-

a) RI-5:Retention index on HP-5MS column
b) Flavor dilution factor obtained by aroma extract dilution analysis (AEDA) on capillary HP-5MS.
c) Odor threshold
d) Odor-activity values were calculated by dividing the concentrations of the compound by its recognition threshold.
*The peak numbers corresponded to those in **Table 1**.

= 16, burnt), 2-acethylthiazole (FD = 16, burnt), and indole (FD = 4, burnt). On the other hand, 22 other compounds also contributed to the odor (sweet, sweaty, earthy, potato, musty, woody, and spicy).

In the VO, (2*E*)-nonenal had the highest OAV (12,404), followed by nonanal (1104), 1-octen-3-ol (686), hexanal (404), and heptanal (320). Notably, (2*E*)-nonenal, nonanal, 1-octen-3-ol, and hexanal had an FD-factor of 64. Therefore, these compounds were considered to be the main components contributing to the mushroom and fatty odor. In addition, components contributing to the burnt odor were furfuryl alcohol (OAV = 69), 2, 3, 5-trimethylpyrazine (23), and 2-acethylthiazole (48), which were oxygen-, nitrogen- and sulfur-containing compounds. Generally, compounds with high FD-factors also had high OAVs, confirming the positive relationship between FD-factor and OAV[23]. The OAVs of 3-thiophenecarboxaldehyde, decanoic acid, α-aromadendrene, γ-muurolol, and γ-cadinol were not determined owing to the unavailability of odor threshold data in the literature.

4 CONCLUSION

We identified 86 compounds in the VO of *Boletopsis leucomelas* (see Table 1). The furan-containing compounds were characteristic constituents of the oil, and the mushroom, fatty, and burnt odor-active compounds of the oil were detected by AEDA and OAV calculations (see Table 2).

References

1) Frans; Y. Dijkstra. Studies on mushroom flavours 3. Some flavour compounds in fresh, canned and dried edible mushrooms. *Z. Lebensm. Unters. Forsch.* 160 (4), 401-405 (1976).

2) Fang, L. Z.; Shao, H. J.; Yang, W. Q.; Liu, J. K. Two New Azulene Pigments from the Fruiting Bodies of the Basidiomycete *Lactarius hatsudake*. *Helv. Chim. Acta* **89**(7), 1463-1466(2006).
3) Cho, I. H.; Kim, S. Y.; Choi, H. K.; Kim, Y. S. Characterization of Aroma-Active Compounds in Raw and Cooked Pine-Mushrooms(*Tricholoma matsutake* Sing.)*J. Agric. Food Chem.* **54**(17), 6332-6335 (2006).
4) Gao, J. M.; Wang, M.; Liu, L. P.; Wei, G. H.; Zhang, A. L.; Draghici, C.; Konishi, Y. Ergosterol peroxides as phospholipase A2 inhibitors from the fungus *Lactarius hatsudake*. *Phytomed.* **29**(1), 821-824(2007).
5) Imazeki, R.; Otani, Y.; Hongo, T. Fungi of Japan(Nihon no kinoko). Yama-kei Publishers Co. Ltd., Tokyo. p. 445(1988).
6) Takahashi, A.; Kudo, R.; Kusano, G.; Nozoe, S. 5-Lipoxygenase Inhibitors Isolated from the Mushroom *Boletopsis lecmelas*(Pers.)Fayod. *Chem. Pharm. Bull.* **40**(12), 3194-3196(1992).
7) Kaneko, A.; Tsukada, M.; Fukai, M.; Suzuki, T.; Nishio, K.; Miki, K.; Kinoshita, K.; Takahashi, K.; Koyama, K. KDR kinase inhibitor isolated from the mushroom *Boletopsis leucomelas*. *J. Nat. Prod.* **73**(5)1002-1004 (2010).
8) Koyama, Y.; Katsuno, Y.; Miyoshi, N.; Hayakawa, S.; Mita, T.; Muto, H.; Isemura, S.; Aoyagi, Y.; Isemura, M. Apoptosis induction by lectin isolated from the mushroom *Boletopsis leucomelas* in U937 cells. *Biosci. Biotech. Biochem.* **66**(4), 784-789(2002).
9) Koyama, Y.; Suzuki, T.; Kajiya, A.; Isemura, M. Invovement of G2/M cell cycle arrest and the mitochondrial pathway in *Boletopsis leucomelaena*(Pers.)Fayod (Agaricomycetideae)lectin-induced apoptosis of human leukemia U937 cells. *Int. J. Med. Mushrooms* **71** (1&2), 201-212(2005).
10) Koyama, Y.; Suzuki, T.; Odani, S.; Nakamura, S.; Kominami, J.; Hirabayashi, J.; Isemura, M. Carbohydrate specificity of lectins from *Boletopsis leucomelas* and *Aralia cordata*. *Biosci. Biotech. Biochem.* **70**(2), 542-545(2006).
11) Nakamura, S.; Kominami, J.; Kamei, M.; Koyama, Y.; Suzuki, T.; Isemura, M.; Hirabayashi, J. Comparative Analysis by Frontal Affinity Chromatography of Oligosaccharide Specificity of GlcNAc-Binding Lectins, *Griffonia simplicifolia* Lectin-II(GSL-II)and *Boletopsis leucomelas* Lectin(BLL). *J. Biochem.* **140**(2), 285-291(2006).
12) Buettner, A.; Schieberle, P. Characterization of the most odor-active volatiles in fresh, hand squeezed juice of grapefruit(*Citrus paradise* Macfayden). *J. Agric. Food Chem.* **47**(12), 5189-5193(1999).
13) Grosch, W. Evaluation of the key odorants of foods by dilution experiments, aroma models and omission. *Chem. Senses.* **26**(5), 533-545(2001).
14) Bailly, S.; Jerkovis, V.; Marchand, B. J.; Collin, S. Aroma extraction aroma analysis of sauternes wines. Key role of polyfunctional thiols. *J. Agric. Food Chem.* **54** (19), 7227-7234(2006).
15) Adam, R. P. Identification of essential oil components by gas chromatography/quadrupole mass spectroscopy. Allured: Carol Stream, IL(2001).
16) Dong, X. Y.; Li, P. P.; Wei, F.; Jiang, M. I.; Zhao, Y. Z.; Li, G. M.; Chen, H.; Zhao, Y. D. The impact of processing on the profile of volatile compounds in sesame oil. *Eur. J. Lipid Sci. Technol.* **114**(3), 277-286(2012).
17) Bhuiyan, M. N. I.; Begum, J.; Sardar, P. K.; Rahman, M. S. Constituents of peel and leaf essential oils of *Citrus medica* L. *J. Sci. Res.* **1**(2), 387-392(2009).
18) Miyazawa, M.; Dejima, Y.; Takahashi, T.; Matsuda, N.; Ishikawa, R. Characteristic odor components of essential oil from dried fruiting bodies of golden oyster mushroom(*Pleurotus citrinopiloatus*). *J. Essent. Oil Res.* **23**(3), 58-63(2011).
19) Miyazawa, M.; Kawauchi, Y.; Matsuda, N. Character impact odorants from wild mushroom(*Lactarius hatsudake*)used in Japanese traditional food. *Flav. Fragr. J.* **25**(4), 197-201(2010).
20) Schieberle, P.; Grosch, W. Evaluation of the flavor of wheat and rye bread crusts by aroma extract dilution analysis. *Z. Lebensm. Unters. Forsch.* **185**(2), 111-113(1987).
21) Grosch, W. Detection of potent odorants in foods by aroma extract dilution analysis. *Trends Food Sci. Technol*. **4**(3), 68-73(1993).
22) Grosch, W. Determination of potent odourants in food by aroma ectract dilution analysis(AEDA)and calculation of odour actibity balues(OAVs). *Flav. Fragr. J.* **9** (4), 147-158(1994).
23) Pino, J. A.; Mesa, J. Contribution of volatile compounds to mango(*Mangifera indica* L.)aroma. *Flav. Fragr. J.* **21**(2), 207-213(2006).