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Analysis of the Volatile Flavor Compounds Produced during the Growth Stages of the Shiitake Mushrooms (*Lentinus edodes*)

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Abstract

Volatile flavor components, produced during the young (P-1), immature (P-2), mature (P-3) and old (P-4) growth stages, of shiitake mushrooms (*Lentinus edodes*), were extracted by simultaneous steam distillation and extraction (SDE), using a mixture of n-pentane and diethyl ether (1:1, v/v) as the extraction solvent. Analyses of the concentrates, by capillary gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS), led to the identification of 129, 129, 111 and 120 components in the P-1, 2, 3 and 4 stages, respectively. The major volatile compounds were 1-octen-3-ol, 3-octanol, 3-octanone and 4-octen-3-one. Ethanol and ethyl acetate were also detected in large amounts. The characteristic volatile compounds found in shiitake mushrooms, such as dimethyl disulfide, dimethyl trisulfide and 1, 2, 4-thiolane, were at low concentrations in all samples. The amount of 1-octen-3-ol decreased as growth progressed, but concentrations of 3-octanone increased. The amount of 4-octen-3-ol decreased from P-1 to P-3, but was at a high concentrations in P-4. The concentration of 3-octanol gradually increased and reached its highest concentration in P-3, but decreased in P-4. The C8-compounds comprised 70.91, 64.09, 64.29 and 60.01% in the P-1, 2, 3 and 4 stages, respectively, so decreased gradually with growth. The S-compounds were found in the highest concentrations in P-3.

Key words: shiitake mushroom (*Lentinus edodes*), volatile organic compounds

INTRODUCTION

Most mushrooms originally grew wild on hills and moors. There are approximately 15,000 mushroom species known globally, about 2,000 of which are edible. 1,000 of species of mushroom grow wild in Korea, and about 300 are edible (1-5). Shiitake mushrooms are among these edible mushrooms, and have been cultured for many years, but most other mushrooms are wild. More recently, methods for the artificial cultivation of oyster (*Plerotus ostreatus*), button (*Agaricus bisporus*) and hackberry mushrooms (*Collybia velutupes*) have been developed, leading to the expansion of the mushroom industry.

Edible mushrooms are used as foods, or food flavoring materials, due to their unique and subtle flavors, and some mushroom parts are highly valued for their medicinal and functional effects. In particular, the yield of shiitake mushrooms is continuously increasing due to their mass-production from artificial cultivation. Therefore, their applications within the food industry, such as dried shiitake mushrooms (6,7), drinks, and so forth, are being actively investigated.

Numerous investigations on mushrooms have been reported. Mattila et al. (8) reported on the vitamin, mineral element and some phenolic compound contents in button (*Agaricus bispourus*/white and brown), shiitake (*Lentinus edodes*) and oyster mushrooms (*Pleurotus ostreatus*), etc. Mau et al. (9-11) reported on the non-volatile components of several commercial, speciality and medicinal mushrooms. The volatile components that impart flavor in native Korean mushrooms have been investigated for *Agaricus bisporus* (12), *Tricholoma matsutake* (13) and *Pleurotus ostreatus* (14). Research on non-Korean mushrooms includes a report of MacLeod and Panchasara (15) on volatile aromatic components of *Agaricus bisporus*, which were analyzed during cooking and when cooked dried. The volatile components from the mycelium of *Agaricus bisporus* have been analyzed by Grove (16), and those of some edible (Basidiomycetes) and oyster mushrooms (*Pleurotus florida*), in submerged cultures, by Venkateshwarlu et al. (17,18). Investigation of the volatile components of shiitake mushrooms have been reported by both Hong et al. (19) and Ahn et al. (20), who analyzed for the volatiles in fresh and dried, and fresh and cooked mushrooms, with the latter group also evaluating the concentration and changes

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in state caused by the heating of shiitake mushrooms (*Lentinus edodes*). Yang et al. (21) reported on the effects of irradiation and drying on the volatile components of fresh shiitake mushrooms.

The array of volatile flavor components in natural and processed shiitake mushrooms has also been reported (19-21), but the volatile components during their growth stages have not. Therefore, in the present investigation, we were analyzed the volatile flavor compounds produced during the growth stages of the shiitake mushroom, with a comparison of 4 different types of shiitake mushroom.

MATERIALS AND METHODS

Materials

The shiitake mushroom (*Lentinus edodes* Sing.) strain used in these experiments was the Rym-hyeap 1, and were obtained from the Jangheung Pyogo Marketing Corporation on May 10, 2000. Samples of the four different stages, young (P-1), immature (P-2), mature (P-3) and old (P-4) were harvested for analysis at the same time (Fig. 1). The samples were stored -18°C until required for the experiments.

Reagents

All the reagents used in the experiments were purchased from Sigma Co. (USA) and Fisher Scientific (USA). The organic solvents used for the extraction and in the chromatography were redistilled using a wire spiral packed double distilling apparatus (Normschliff Geratebau, Wertheim, Germany) and Milli-Q water that was generated with a water purification system (Millpore Corporation, Bedford, USA).

Extraction of volatile components from shiitake mushroom by SDE

100 g samples were homogenized in a blender (MR 350CA, Braun, Spain), and mixed 1 L of distilled water. The resultant slurry was used for the quantitative analysis, with 1 μL of n-butyl benzene added as an internal standard. The volatiles were extracted for 2 h, with 200 ml of a redistilled n-pentane/diethyl ether (1:1, v/v) mixture, using a SDE (Likens & Nickerson type simultaneous steam distillation and extraction) apparatus (22), as modified by Schultz et al. (23), under atmospheric pressure.

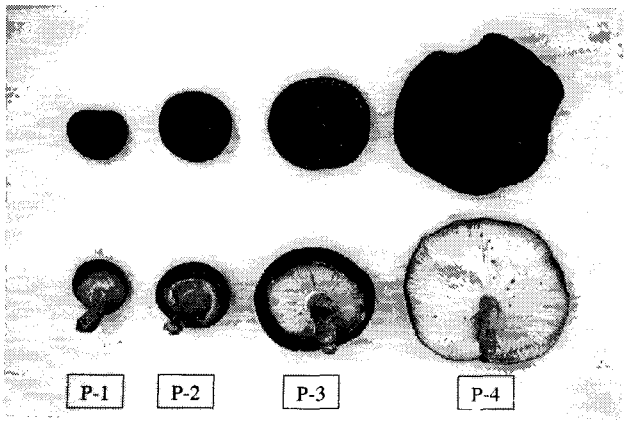

Fig. 1. Growth stage of shiitake mushrooms (P-1: young stage, P-2: immature stage, P-3: mature stage and P-4: old stage).

The extract was dried over anhydrous sodium sulfate, concentrated to approximately 2 mL, using a Vigreux column. The 2 mL sample was transferred to a GC vial, and then further reduced, to a final volume of 0.1 mL, under a stream of nitrogen. The final sample was then used for the GC-FID and GC/MS analyses.

Analysis of volatile compounds by GC-FID

The GC analyses were carried out on an HP 5890 II Plus gas chromatograph, equipped with a flame ionization detector. A DB-WAX capillary column (60 m × 0.2 mm i.d., 0.25 μm film thickness, J&W, USA) was used for the separation. The oven temperature program was as follows: 40°C (isothermal for 3 min) which was ramped to 150°C at 2°C/min, and then to 210°C at 4°C/min. The injector and detector temperatures were 250°C and 300°C, respectively. Helium was used as the carrier gas at a flow rate of 1.0 mL/min, with an injector volume of 1 μL, using a 1:20 split ratio.

Identification of volatile compounds by GC/MS

The GC/MS used for the quantitative analysis was a Shimadzu GC/MS QP-5000 (Kyoto, Japan), in the EI (electron impact) mode. The ionization voltage and ion source temperature were 70 eV and 230°C, respectively. The mass spectrometer scanned from 41 to 450 m/z. The other conditions were the same as those used for the GC analysis. Mass spectra were identified with the aid of our own mass spectral data and those contained within the WILEY 139, NIST 62 and NIST 12 libraries and mass spectral data books (24,25). The compounds were also identified by a comparison of the retention indices to reference data (26,27), retention indices from GC-FID analysis and the laboratory data of the authentic compounds.

RESULTS AND DISCUSSION

Volatile flavor compounds produced during the growth stages of shiitake mushrooms

The volatile flavor compounds were collected, and concentrated to an appropriate volume, using SDE, from the various growth stages of the shiitake mushroom (*Lentinus edodes*). The compounds were detected and

identified by GC and GC/MS. The GC/MS chromatograms are presented in Fig. 2, and the volatile organic components, from the RI of the GC and GC/MS analysis, and their percentage peak areas are shown in Table 1.

A total of 129 compounds were identified and quantified from P-1, including 24 alcohols, 22 ketones, 18 S-compounds, 18 aldehydes, 12 N-compounds, 8 terpenes, 6 esters and 4 acids (Table 1). The relative areas obtained for each functional group, in descending order were: alcohols (76.40%), ketones (6.67%), esters (5.96%), aldehydes (5.49%), S-compounds (1.87%), acids (1.32%), N-compounds (1.07%) and terpenes (0.31%) (Table 2).

Of these compounds, 1-octen-3-ol (62.79%) was the major compound, with ethyl acetate and 3-octanone, at 4.13 and 3.63%, respectively, also detected in large amounts. Ethanol, (*E*)-2-octenal, 4-octen-3-one, acetaldehyde an ethyl formate were the other major components. The percentage of C8-compounds constituted 70.91% of the total, which was in accordance with the C8-compounds being the primary volatiles in many edible

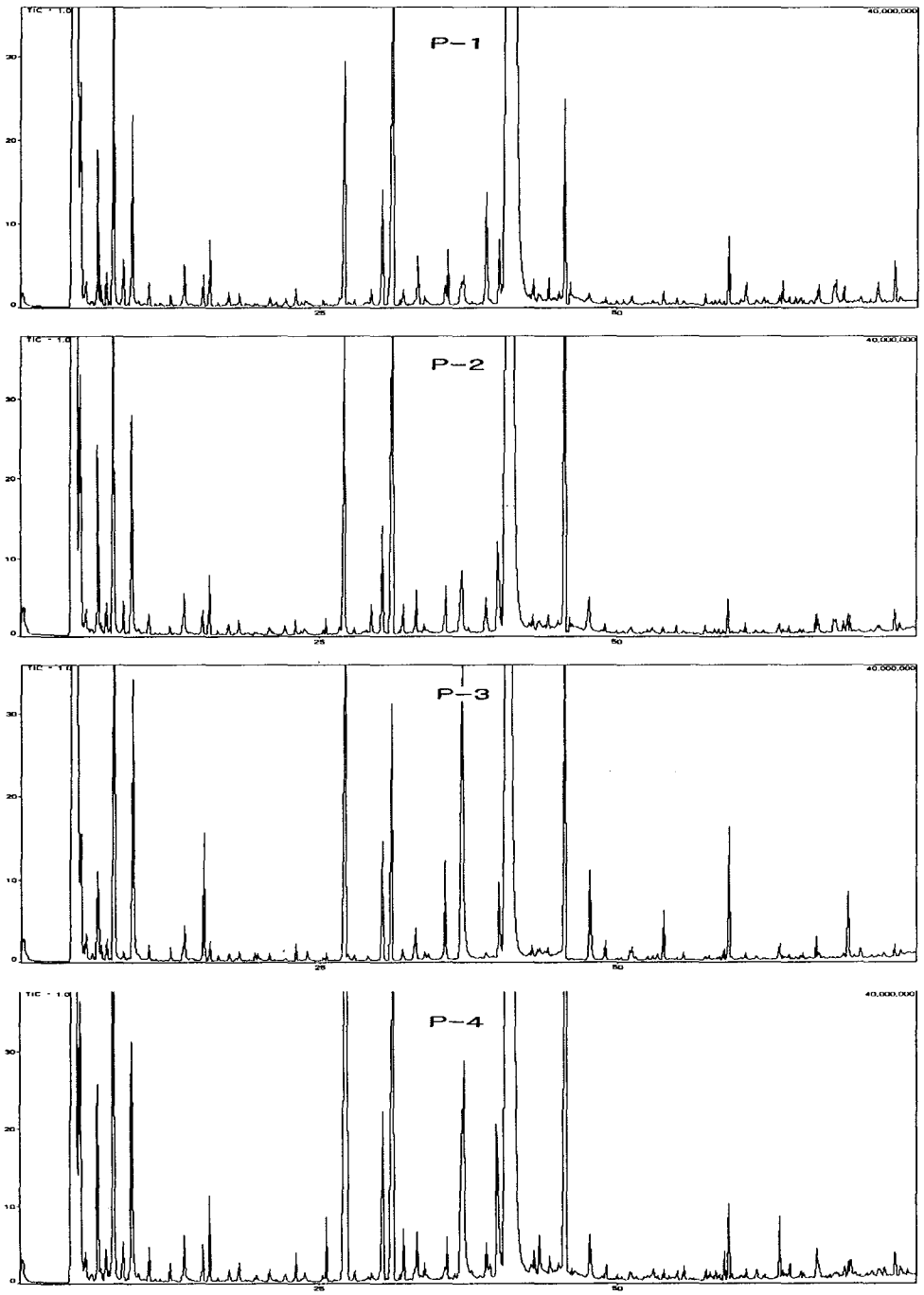

Fig. 2. GC/MS chromatograms of the volatile flavor components in young (P-1), immature (P-2), mature (P-3) and old (P-4) shiitake mushrooms.

Table 1. Volatile flavor components in shiitake mushroom

Peak No.	RT[1]	RI[2]	Compound name	Peak area %			
				P-1[3]	P-2	P-3	P-4
1	5.033	695	Acetaldehyde	1.52	1.39	0.67	2.00
2	5.312	727	Ethylbutyl ether	-	0.02	-	-
3	5.475	744	Dimethyl sulfide	0.13	0.13	0.19	0.18
4	5.924	789	Propanal	0.02	0.02	0.01	0.03
5	6.044	800	Octane	0.02	0.02	-	0.04
6	6.317	815	2-Propanone	0.01	-	0.01	0.01
7	6.466	823	Ethyl formate	1.07	1.52	1.00	1.53
8	6.850	843	2-Propenal	0.02	-	-	-
9	7.199	860	Tetrahydrofuran	0.21	0.28	0.17	0.24
10	7.496	874	Butanal	0.02	0.05	0.02	0.07
11	7.799	888	Ethyl acetate	4.13	5.22	4.30	4.70
12	7.916	893	Diethyl acetal	0.14	-	0.25	-
13	8.079	900	Nonane	0.02	0.03	0.03	-
14	8.486	913	2-Methylbutanal	0.04	0.03	0.01	0.04
15	8.614	917	3-Methylbutanal	0.30	0.25	0.08	0.24
16	9.025	929	3-Methyl-2-butanone	0.02	0.02	0.01	0.02
17	9.341	938	Ethanol	1.79	2.79	5.22	2.44
18	9.888	954	5-Hexen-1-ol	0.04	0.09	-	0.08
19	9.975	956	1,3-Octadiene	0.01	-	0.03	-
20	10.462	969	Acetaldehyde, ethylpropyl acetal	0.01	0.01	0.01	0.01
21	10.756	976	2-Pentanone	0.25	-	0.19	0.31
22	11.017	982	Ethyl propyl sulfide	-	0.01	0.01	0.02
23	11.311	989	2-Methylpentanal	0.01	0.01	0.01	0.01
24	11.689	998	Decane	0.03	0.02	0.01	0.02
25	12.625	1019	α-Pinene	0.01	-	-	-
26	12.800	1022	1-Propanethiol	-	-	0.01	0.01
27	12.900	1024	2,5-Diethyltetrahydrofuran	0.01	0.02	-	-
28	13.042	1027	2-Butanol	0.01	0.01	0.01	-
29	13.703	1041	Propanol	0.39	0.49	0.43	0.45
30	13.958	1046	S-Methyl thioacetate	0.01	0.01	0.02	0.03
31	14.199	1050	2,3-Pentanedione	0.01	0.01	0.04	-
32	14.350	1053	2-Methyl-3-pentanethiol	0.01	0.01	0.02	0.01
33	15.115	1067	Ethyl isopropyl ether	0.02	0.02	0.03	0.02
34	15.277	1070	Dimethyl disulfide	0.23	0.21	1.29	0.24
35	15.672	1077	Diethoxyethane	0.03	0.02	0.01	0.02
36	15.836	1079	Hexanal	0.50	0.53	0.17	0.61
37	16.525	1091	2-Methyl-1-propanol	0.02	0.03	0.06	0.02
38	17.213	1102	2-β-Pinene	0.03	-	0.01	0.01
39	17.430	1106	Propyl isopropyl ether	0.13	0.13	0.10	0.11
40	17.875	1114	Allyl hexanoate	0.02	0.02	-	-
41	17.943	1115	3-Methyl thiophene	-	-	0.01	-
42	18.092	1118	Methylpentyl sulfide	0.01	0.02	0.01	0.01
43	18.310	1121	2-Pentanol	0.11	0.15	0.10	0.14
44	18.862	1131	2-Butylfuran	0.02	0.01	-	0.01
45	19.167	1136	1-Methylpyrrole	0.01	0.01	0.01	0.01
46	19.581	1142	Methylethyl disulfide	0.04	0.02	0.07	0.02
47	19.808	1146	Butanol	0.02	0.01	0.08	0.05
48	20.042	1150	3-Mercaptopropionic acid	-	-	0.01	-
49	20.822	1162	β-Myrcene	0.04	0.06	0.06	0.09
50	21.129	1166	2-Butyltetrahydrofuran	-	0.04	-	-
51	22.050	1180	2-Heptanone	0.02	0.04	0.01	0.03
52	22.228	1182	Heptanal	0.06	0.16	0.01	0.05
53	23.066	1194	α-Limonene	0.15	0.15	0.17	0.23
54	23.650	1202	β-Phellandrene	-	-	0.01	-
55	23.980	1207	3-Methyl-1-butanol	0.06	0.04	0.12	0.01
56	24.232	1211	Thiazole	0.02	0.01	0.01	0.01
57	24.400	1214	(*E*)-2-Hexenal	0.01	-	-	-
58	25.331	1229	2-Pentylfuran	0.05	0.04	0.03	0.05
59	25.597	1233	Ethyl pyruvate	0.02	0.14	0.08	0.39
60	26.317	1243	γ-Terpinene	0.01	0.01	0.01	0.01

Table 1. Continued

Peak No.	RT[1]	RI[2]	Compound name	Peak area %			
				P-1[3]	P-2	P-3	P-4
61	26.704	1249	(*E*)-3,4-Epoxynonane	0.09	0.08	-	-
62	27.119	1255	3-Octanone	3.20	5.27	7.31	8.38
63	27.434	1260	Mercaptoacetic acid	0.02	0.04	0.05	0.04
64	27.614	1262	1,2-Ethanedithiol	0.02	-	0.03	0.01
65	27.800	1265	Methyl pyrazine	-	-	0.01	-
66	27.825	1265	2-Methyl-3-octanone	-	0.01	-	-
67	27.959	1267	ρ -Cymene	0.04	0.07	0.05	0.10
68	29.008	1281	3-Hydorxy-2-butanone	0.02	0.05	-	-
69	29.066	1282	2,4-Dithiapentane	0.02	0.02	0.05	0.03
70	29.380	1286	Octanal	0.17	0.36	0.02	0.08
71	30.317	1298	4-Octen-3-one	1.59	1.53	1.50	2.11
I.S.	31.226	1313	Butyl benzene	-	-	-	-
72	31.748	1321	(*E*)-2-Heptenal	0.04	0.04	0.01	0.04
73	32.033	1325	4-Nonanone	0.21	0.33	0.12	0.45
74	32.442	1331	2-Phenylpropanal	-	0.01	-	0.01
75	33.258	1343	3,7-Diemthyl-3-octanol	0.45	0.47	0.02	0.42
76	33.500	1347	3-Methyl-4-nonanone	-	0.05	-	0.04
77	33.823	1352	1-Hepten-3-ol	0.07	0.09	0.10	0.12
78	34.158	1357	Methylpentyl disulfide	0.03	0.02	0.07	0.03
79	35.523	1376	Dimethyl trisulfide	0.18	0.14	1.14	0.12
80	35.775	1379	3,6-Diemthyl-3-octanol	0.48	0.47	0.01	0.35
81	36.400	1388	2-Ethyl-2-hexenal	0.01	0.02	-	0.04
82	36.934	1395	3-Octanol	0.76	1.64	8.93	5.17
83	39.078	1429	(*E*)-2-Octenal	1.77	0.42	0.09	0.38
84	39.395	1434	5-Decanone	0.06	0.12	-	0.18
85	39.875	1442	2-Methyl-2-heptanol	-	0.01	-	-
86	40.116	1446	Acetic acid	0.89	1.53	1.36	2.38
87	41.568	1469	1-Octen-3-ol	62.79	59.50	44.04	42.48
88	42.695	1486	7-Methyl-4-octanol	0.03	0.07	0.04	0.06
89	42.758	1487	2-Octen-4-one	-	0.01	-	0.04
90	43.419	1496	2-Ethylhexanol	0.09	0.1	0.1	0.4
91	44.30	1510	4-Ethyl-1-octyn-3-ol	0.25	0.17	0.11	0.16
92	44.60	1515	1H-Pyrrole	0.09	0.01	-	0.03
93	45.14	1523	Benzaldehyde	0.23	0.16	0.1	0.18
94	45.977	1536	6-Undecanone	-	-	-	0.02
95	46.091	1538	2,4-Dimethyl-3-heptanol	0.19	0.15	-	0.07
96	46.312	1541	Pantolactone	0.06	0.04	-	-
97	46.758	1548	4-Dodecalactone	-	0.02	-	-
98	47.689	1561	Octanol	0.15	0.63	1.78	0.57
99	48.812	1578	2,2-Dimethylpropanoic acid	0.02	0.02	0.01	0.03
100	49.065	1581	Dimethyl sulfoxide	0.07	0.1	0.24	0.12
101	50.041	1595	2-Undecanone	0.04	0.03	0.03	0.05
102	50.325	1599	3,5-Dimethyl-1,2,4-trithiolane	0.01	0.01	0.04	0.03
103	50.573	1603	3,6-Dimethyl-3-heptanol	0.04	-	-	-
104	51.275	1615	(*E*)-2-Octen-1-ol	0.08	0.09	0.23	0.15
105	51.933	1626	Butanoic acid	-	0.02	-	0.01
106	52.243	1631	6-Dodecanone	-	0.02	-	0.02
107	53.036	1644	2-Acetyl thiazole	0.04	0.06	0.04	0.07
108	53.233	1648	Acetophenone	0.01	0.01	-	0.01
109	53.422	1651	Methyl 1-(methylthio) ethyl disulfide	0.02	0.01	0.06	0.03
110	53.897	1658	Methyl(methylthio) methyl disufide	0.12	0.06	0.58	0.07
111	54.392	1666	Estragole	0.01	0.01	0.02	0.03
112	54.683	1671	Cryptone	0.02	-	-	0.02
113	54.987	1676	6-Tridecanone	-	0.07	-	-
114	55.027	1677	2-Dodecanone	0.05	-	0.02	0.05
115	55.562	1685	γ -Muurolene	0.02	0.05	0.07	0.12
116	57.408	1717	N-Cyclopentylidene methylamine	0.11	0.1	0.03	0.09
117	58.197	1731	6-Tridecanone	0.05	0.07	-	0.06
118	57.672	1722	α -Muurolene	-	-	0.04	0.07
119	58.271	1733	6-Tridecanone	-	-	0.01	-

Table 1. Continued

Peak No.	RT[1]	RI[2]	Compound name	Peak area %			
				P-1[3]	P-2	P-3	P-4
120	58.546	1738	Azulene	0.07	0.07	0.03	0.06
121	58.922	1745	3,5-Dimethylbenzotriazole N-oxide	0.03	0.04	0.09	0.17
122	59.321	1752	1,2,4-Trithiolane	0.71	0.39	1.81	0.64
123	59.633	1758	δ -Cadinene	0.01	0.03	0.04	0.05
124	60.319	1770	7-Methyl-4-octanol	0.06	0.03	-	0.01
125	60.749	1778	2,6-Pyridinediol	0.21	0.11	0.07	0.07
126	61.589	1793	1-Phenyl-1-butanone	0.03	0.03	0.02	0.05
127	61.732	1795	α -Indanone	0.02	-	-	-
128	62.274	1806	(*E*,*E*)-2,4-Decadienal	0.05	0.04	0.01	0.03
129	63.541	1836	Cyclooctanamine	0.08	0.11	0.17	0.46
130	63.812	1842	Hexanoic acid	0.22	0.06	0.05	0.07
131	64.363	1855	N-Acetyl-2-pyrrolidone	0.08	0.09	0.09	0.15
132	64.887	1867	N-(3-Methylbutyl)acetamide	0.05	-	0.02	-
133	65.285	1876	1,3-Di(Isobutoxycarbonyl)-2,4,4-trimethylpentane	0.04	0.03	0.03	0.04
134	65.448	1880	Dipentyl sulfide	-	-	0.06	-
136	65.535	1882	4-Methyl-4-nitropentanol	0.03	0.04	-	-
137	66.822	1913	Phenethyl alcohol	0.18	0.15	0.07	0.09
138	67.492	1932	2-Phenyl-2-butenal	-	0.01	-	-
139	67.925	1945	Tetradecyl glycidyl ether	0.02	-	-	-
140	68.228	1953	Dodecanol	0.27	0.15	-	0.04
141	68.80	1969	1-Undecanethiol	-	-	-	0.01
142	68.907	1972	2-Methyl-1-phenyl-propanol	0.17	0.13	0.04	0.08
143	69.236	1982	S-Methylmethyl thiosulphonate	0.01	0.16	0.69	0.11
144	69.429	1987	1,4-Dihydroxy-p-menth-2-one	0.01	0.12	-	-
145	70.288	2010	Methyl methylsulfinylmethyl sulfide	0.04	0.02	0.11	-
146	70.633	2020	2-Pentadecanone	0.01	-	-	0.01
147	71.09	2032	Propyl tetradecanoate	0.02	0.02	0.01	0.01
148	71.208	2035	(*E*)-Nerolidol	0.01	0.02	0.01	0.02
149	71.713	2048	2-Pyrrolidinone	0.21	0.08	0.03	0.07
150	71.88	2052	Octanoic acid	0.05	0.06	0.02	0.06
151	72.058	2057	2,4-Dimethyl-6-tertbutylphenol	-	0.01	-	-
152	72.157	2060	δ -Cadinol	-	-	0.08	0.07
153	72.674	2073	Tridecanol	-	-	-	0.01
154	73.113	2084	1,5-Di-T-butyl-3,3-dimethylbicyclo [3.1.0]hexan-2-one	0.27	0.16	0.10	0.12
155	73.544	2095	Methyl pentadecanoate	0.08	0.04	-	0.06
156	73.944	2109	2-Hexadecanone	0.02	0.04	-	-
			Total	89.66	85.03	87.36	82.71

[1]Retention time. [2]Retention index. [3]P-1: sampled young stage, P-2: sampled immature stage, P-3: sampled mature stage, P-4: sampled old stage.

Table 2. Relative content of functional groups in shiitake mushroom (Relative peak area %)

Functional group	P-1[1]		P-2		P-3		P-4	
	No.	Area %	No.	Area %	No.	Area %	No.	Area %
Aldehydes	18	5.49	18	4.13	15	1.68	16	4.62
Esters	6	5.96	6	8.17	4	6.17	5	8.09
Alcohols	24	76.40	25	72.14	19	70.4	23	64.53
S-Compounds	18	1.87	18	1.62	23	7.52	20	2.13
Terpenes and derivatives	8	0.31	6	0.38	10	0.57	9	0.80
Ketones	22	6.67	23	9.74	13	10.73	20	14.50
N-Compounds	12	1.07	11	0.77	11	0.65	10	1.37
Acids	4	1.32	4	1.91	4	1.65	5	3.08
Miscellaneous	17	0.91	18	1.14	12	0.62	12	0.89

[1]P-1: sampled young stage, P-2: sampled immature stage, P-3: sampled mature stage, P-4: sampled old stage.

mushrooms (28).

A total of 129 compounds were detected, and identified, at the P-2 stage, including 25 alcohols, which comprised the largest portion, with a relative area of 72.14%. The other volatile flavor compounds consisted of 23 ketones (9.74%), 8 aldehydes (4.13%), 18 S-compounds (4.13%), 11 N-compounds (0.77%), 6 esters (8.17%) and 8 acids (1.91%), all of which are known to influence the flavor composition of the mushrooms. 1-Octen-3-ol (53.5%) was the major compound in P-2. Ethyl acetate and 3-octanone accounted for the largest portions of the esters and ketones, respectively, at 5.22 and 5.27%, respectively (Table 2). The composition of the volatile compounds in P-2 was similar to that of P-1. The percentage of C8-compounds, the primary volatiles in edible mushroom constituted 64.09% in P-2.

A total of 111 compounds were detected in P-3, including 19 alcohols 23 S-compounds, 15 aldehydes, 13 ketones, 11 N-compounds, 4 esters and 4 acids, with relative areas of 70.04, 7.25, 1.68, 10.73, 0.65, 6.17 and 1.65%, respectively (Table 2). The most abundant volatile compound in shiitake mushrooms was 1-octen-3-ol, at 44.04%, with 3-octanol and 3-octanone presenting in large quantities, at 8.93 and 7.31%, respectively (Table 1). The volatile compounds detected in P-3 were also similar to those of P-1, with the C8-compounds accounting for 64.2% of the total.

There were 120 volatile compounds detected in P-4, comprising of 23 alcohols (64.53%), which accounted for the majority of the volatile compounds in P-4. Ketones were the second most abundant volatiles, with 20 kinds comprising a total area of 14.50%. The other volatiles were esters (8.09%), aldehydes (4.62%), acids (3.08%), S-compounds (2.13%), N-compounds (1.37%) and terpenes (0.80%). The amounts of 1-octen-3-ol, 3-octanone and 3-octanol were 42.48, 8.38 and 5.17%, respectively, so were also detected in large amounts. The C8-compounds accounted for 60.01% of the total compounds in P-4, with all other compounds being present at much lower levels.

Comparison of the volatile flavor compounds produced during the growth stages of shiitake mushrooms

Shiitake mushrooms have two types of volatile flavor compounds, the C8- and S-compounds (29,30). The C8-compounds are the major flavor components of the fresh mushrooms, which include 3-octanone, 3-octanol, 1-octen-3-ol, octanol and (*E*)-2-octen-1-ol. The S-compounds, including dimethyl disulfide, dimethyl trisulfide, methyl (methylthio) ethyl disulfide and 1, 2, 4-trithiolane, are important compounds, and are characteristically present in the dried shiitake mushrooms (30-32). Major compounds in the four shiitake mushroom growth stages (young stage; P-1, immature stage; P-2, mature stage; P-3 and old stage; P-4) used in this investigation were: 1-octen-3-ol, 3-octanol, 3-octanone and 4-octen-3-one, which was in accordance with previous studies. Ethanol and ethyl acetate were present in large amounts. The S-compounds, including dimethyl disulfide, dimethyl trisulfide and 1, 2, 4-trithiolane, were present at low concentrations. The S-compounds were also identified as being at lower concentrations in fresh, as opposed to dry, shiitake mushrooms.

As the mushrooms progressed through the growth stages, the major C8-compounds and 3-octanone increased (Fig. 3), but the concentration of the 1-octen-3-ol decreased. This resembled the results obtained by Ku et al. (33), where the major volatile flavor compounds of the pine mushroom, 1-octen-3-ol, decreased as the growth progressed, but the levels of the 3-octanone increased. The amount of 4-octen-3-one decreased from P-1 to P-3, but high concentrations were found in P-4. The amount of the 3-octanol gradually increased with growth stage, with the highest concentrations in the P-3 stage; but decreased in the P-4 stage. The C8-compounds are derived, enzymatically, from unsaturated fatty acids, by the action of lipoxygenase (34). The lipoxygenase catalyzed degradation of fatty acids usually occurs during the late phase of cultivation, due to the increased contact of the enzyme with the substrate, which results from enhanced cell lysis. However, the amount of the C8-comounds, during the growth stage of the shiitake mushrooms used in this study, tended to decrease, from 70.91% during P-1, to 64.09%, 64.29% and 60.01% in P-2, 3 and 4, respectively. Venkateshwarlu et al. (18) investigated the volatile flavor components of oyster mushrooms in submerged cultured, and reported that when the culti-

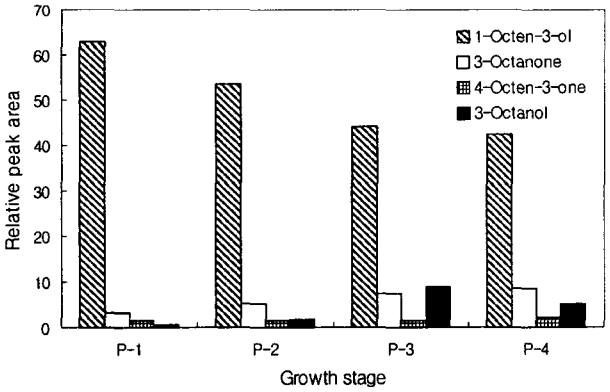

Fig. 3. The changing trend for the relative area % of the major C8 compounds during the growth stages of shiitake mushrooms.

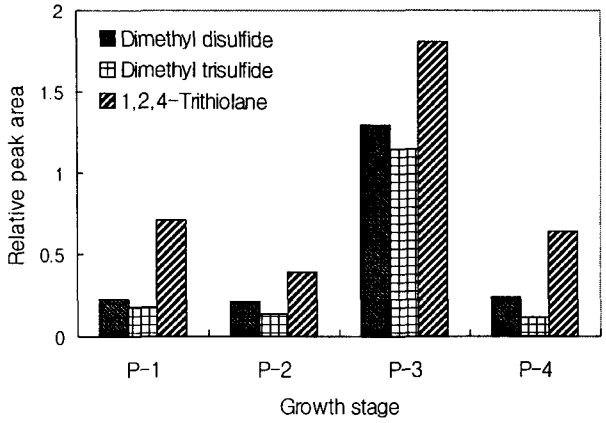

Fig. 4. The changing trend for the relative area % of the S-containing compounds during the growth stages of shiitake mushrooms.

vation period was extended up to 50 days, the synthesis of the C8-compounds was not improved.

The other characteristic flavor compounds in shiitake mushrooms, the S-compounds, were identified at low concentrations, and were also observed to change during the growth stages. Their concentrations were highest during the P-3, as shown in Fig. 4. The amount of the dimethyl disulfide in P-3 was twice as high as the 5.6%, 6.1% and 5.4% of the P-1, P-2 and P-4 stages, respectively. The amounts of dimethyl trisulfide and 1, 2, 4-trithiolane were both twice as high during P-3, than the respective 6.3%, 8.1% and 9.5%, and the 2.5%, 4.6% and 2.8%, produced during the P-1, 2 and 4 stages, respectively. Similarly, the S-compounds showed relatively high concentration in P-3. Therefore, it was adjudged that they affect the flavor components of P-3 in a similar fashion to the C8-compounds. It has been reported that S-compounds, such as dimethyl disulfide or dimethyl trisulfide, originate from lentinic acid, which is a derivative of γ-glutamylcysteine sulfoxide. S-compounds are produced by the lysis of the CH_2-S-bond in lenthionine (1,2,3,5,6-pentathiephane), which is derived, enzymatically, from lentinic acid and peptides, by the action of γ-glutamyltranspeptidase and cysteine sulfoxide lyase. The lenthionine is the characteristic flavor component, which is present in small amounts, and is generally affected by pH and temperature during the rehydration of dried mushrooms (35,36). Therefore, we can assume that the lenthionine, the characteristic flavor component of shiitake mushrooms, is present in high concentrations in P-3, as the S-compounds were identified as being present in high concentrations during P-3.

To conclude, the C8-compounds comprised 70.91, 54.09, 64.29 and 60.01% at the P-1, 2, 3 and 4 stages, respectively, so gradually decreased with the stage of growth. The S-compounds were found in the highest concentrations during the P-3 stage.

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