Volatile Components in Culture Fluid of *Polyporus tuberaster*

Tatsuya Kawabe* and Hideo Morita

Food Research Laboratories, Takara Shuzo Company, Ltd., Seta 3-4-1, Otsu, Shiga 520-21, Japan

Of 117 mushrooms grown in liquid culture and given a sensory evaluation by 10 panelists, *Polyporus tuberaster* K2606 was the most highly rated. This strain had a fruity and floral odor suitable for beverages. Forty-seven compounds from the culture fluid of strain K2606 were identified, 14 of which are reported for the first time as volatile components from mushrooms grown by liquid culture. The most abundant component was benzaldehyde, which accounted for 61% of the total amount of volatile compounds in this strain. Other main components were other aromatic compounds and aliphatic alcohols, of which 3-methyl-1-butanol was the most abundant. By cultivation of strain K2606 in a medium to which L-phenylalanine was added, benzaldehyde and benzyl alcohol were obtained in substantially increased yields.

INTRODUCTION

In the food industry, many kinds of flavors are used, and there is a demand for new and better flavors, especially natural ones. Much work has been done on the development of flavors derived from fermentation products. Yeasts and lactic acid bacteria often produce desirable flavors, and mushrooms can produce a variety of flavors, as well. Mushrooms are usually produced by solid-state fermentation that is time- and labor-consuming. Since submerged fermentation is a fast and controlled method, it has been adopted in many studies attempting to produce mushroom flavor in high yield. The best-known volatile compound of mushrooms is 1-octen-3-ol; its in vivo synthetic pathway has been studied in green beans (de Lumen et al., 1978), mushrooms (Tressl et al., 1982; Chen and Wu, 1984b; Mau et al., 1992), etc. Other middle-chain aliphatic alcohols, aldehydes, and ketones, which are the degradation products of fatty acids, have been found in mushrooms (Chen and Wu, 1984b; Tressl et al., 1982). Lenthionin, which contains sulfur, has been found in *Lentinus edodes* (Wada et al., 1967). A strain of *Polyporus durus* that can produce lactones in high yields (Berger et al., 1986b) and a strain of *Lentinus lepideus* that can produce terpenes (Hanssen, 1982, 1985) have been reported. There are other reports [e.g., Grove (1981), Drawert et al. (1983), Chen and Wu (1984a), Sastry et al. (1980), Berger et al. (1986a, 1987), and Hanssen and Abraham (1987)] and reviews (Hadar and Dosoretz, 1991; Maga, 1981) of volatile compounds of mushrooms grown in liquid culture.

In this study, a number of edible mushrooms were cultured in liquid medium and the odor characteristics of the medium after filtration were then evaluated organoleptically by 10 panelists. The volatile components of the strain given the best score were analyzed by gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS), and the possibility of increasing the amount of the volatile compounds contributing most to the characteristic aroma of that mushroom by the addition of a precursor in the medium was investigated.

MATERIALS AND METHODS

Materials. A total of 117 strains of mushrooms (84 Agaricales, 23 Aphyllophorales, 4 Lycoperdales, 2 Auriculariales, 1 Tremellales, 1 Phallales, 1 Pezizales, and 1 Hymenogastrales) stored at our laboratory were used.

Porous polymer resin Porapak Q (50/80 mesh) was purchased from Millipore Corp. Authentic samples of volatile compounds were obtained from Tokyo Chemical Industry Co., Ltd., Wako Pure Chemical Industries, Ltd., and Sigma Chemical Co.

Table I. Mushrooms Given High Organoleptic Score

species and strain	score	odor
Polyporus tuberaster K2606	3.9	floral, fruity, cinnamon-like
Pleurotus cornucopiae K18	3.8	resembles Japanese pickles
Cryptoporus volvatus K3051	3.7	sweet, floral
Sparassis crispa K1440	3.6	tea-like
Fomes officinalis K2096	3.6	roasted, tea-like
Hericium laciniatum K2682	3.6	mushroom-like, spicy
Hohenbuehelia serotina K2100	3.5	floral, fruity
Mycoleptodonoides aitchisonii K3110	3.5	sweet, floral, ester-like
Pleurotus ostreatus K2	3.5	sweet, floral, cinnamon-like
Cortinarius purpurascens K131	3.5	sweet, fruity

Sensory Evaluation of Cultures. A 100-mL portion of PGY medium (0.2% polypeptone, 2% glucose, 0.2% yeast extract, 0.05% KH_2PO_4, and 0.05% $MgSO_4 \cdot 7H_2O$) in a 500-mL flask with baffles was inoculated with a strain and incubated at 25 °C with shaking (100 rpm) until cell pellets were formed. The cell pellets were removed by filtration, and the odor of the filtrate was evaluated by 10 panelists. Each panelist gave a score of 1 (very bad) to 5 (very good) to the filtrates and commented on their odor characteristics and what kind of foods they might be suitable in, if any. The panelists evaluated 15–20 strains in a single day.

Isolation of Volatile Compounds. *Polyporus tuberaster* K2606, which was given the highest sensory score, was incubated in 100 mL of PGY medium for 11 days, transferred to a 2-L flask with baffles containing 700 mL of PGY medium, and incubated at 25 °C with shaking (100 rpm) for 13 days. A flavor concentrate was prepared from the filtered culture medium by Porapak Q adsorption (Shimoda et al., 1987); 15 mL of Porapak Q in a glass column (1.5 × 9 cm) was washed with 150 mL of ethyl ether first, 60 mL of methyl alcohol next, and 120 mL of water last, and the filtered culture medium of K2606 was passed through the column at the rate of 10 mL/min. Before that process, 25 μL of methyl alcohol containing 0.2% *m*-xylene was added to the filtered culture medium as an internal standard. Then the column was washed with 50 mL of water, and the adsorbed components were eluted with 60 mL of ethyl ether. To remove free acids, 60 mL of 5% $NaHCO_3$ was added to the ethyl ether solution and the solution was mixed for 10 min. The ethyl ether layer was then dried over anhydrous sodium sulfate, concentrated at atmospheric pressure at 38 °C to the volume of 0.1 mL, and analyzed by GC and GC–MS.

Identification of Volatile Compounds. Flavor compounds were identified by comparison of the retention time and mass spectrum with those of authentic samples.

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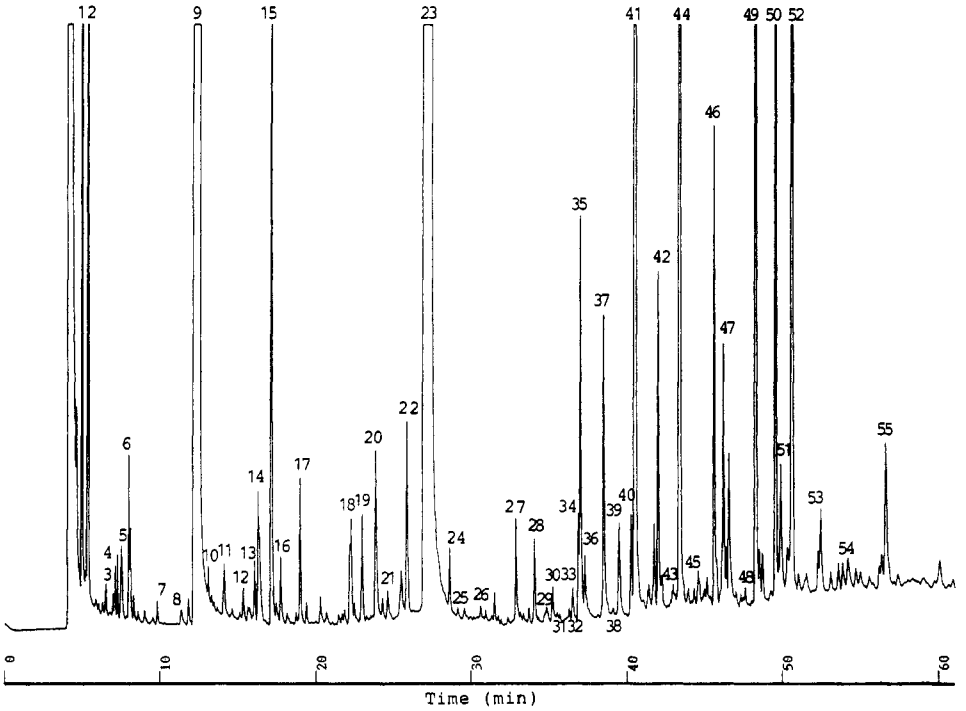

Figure 1. Gas chromatogram of volatile compounds of *P. tuberaster* K2606.

Gas Chromatography. A gas chromatograph (Shimadzu GC-15A) equipped with a fused-silica capillary column (50 m × 0.25 mm, Ulbon HR-20M, Shinwa Chemical Industries Co., Kyoto, Japan) and a flame ionization detector was used. The column temperature was programmed to stay at 60 °C for 8 min and to increase to 190 °C at the rate of 3 °C/min; the temperature of the injector and detector was 230 °C. The pressure of the carrier gas, He, was 1.3 kg/cm^2, the split ratio was 40:1, and the sampling time was 0.1 min. The injection volume of each sample was 1 μL.

Gas Chromatography–Mass Spectrometry. A JEOL JMS-DX302 GC–MS system was used with the same gas chromatographic conditions as described above. During MS, the ionization voltage was 70 eV and the ion source temperature was 150 °C.

RESULTS AND DISCUSSION

Table I shows the mean scores and flavor characteristics of the 10 species given the highest scores. The best score was for *P. tuberaster* K2606, which produced an unusual odor, which was floral and slightly cinnamon-like, not mushroom-like, musty, or rancid, as the odor of many strains was. *P. tuberaster* is an edible mushroom with a round, hollow pileus. It grows in Japan, Europe, and North America. Up to now, no research has been done on its volatile components.

Figure 1 shows a typical gas chromatogram of the volatile compounds of strain K2606, and Table II lists the compounds identified and gives peaks for some unidentified ones. Fourteen of the 47 compounds identified have not been reported as being found in mushrooms before, to the best of our knowledge.

The most abundant component was benzaldehyde, which seems to be important for the flavor characteristics of the culture fluid, to judge from its specific organoleptic property and its abundance; its concentration in the culture fluid was about 8 ppm, which was more than its threshold in water, 1.5 ppm (Yamanishi, 1970). Other aromatic compounds (benzonitrile, acetophenone, benzyl alcohol, 2-phenylethanol, 2-phenylpropanol, and 3-phenylpropanol) that have been found in the culture fluid of many other mushrooms were found. Benzaldehyde and benzonitrile have an almond-like odor, acetophenone has a sweet and slightly pungent odor, benzyl alcohol has a weakly fruity odor, and 2-phenylethanol, 2-phenylpropanol, and 3-phenylpropanol have floral odors. These aromatic compounds may contribute much to the characteristic aroma of strain K2606.

2-Methylbenzaldehyde, 4-methylbenzaldehyde, 2-hydroxybenzaldehyde, 4-ethylbenzaldehyde, 4-ethylacetophenone, 4-isopropylbenzyl alcohol, and 4-methoxyacetophenone, also reported here for the first time as being found in mushrooms, as far as we know, probably contributed to the aroma. Berger et al. (1987) reported that prominent volatile compounds of the culture fluid of *Ischnoderma benzoinum* were benzaldehyde and 4-methoxybenzaldehyde. Both *I. benzoinum* and *P. tuberaster* are wood-destroying mushrooms, but *P. tuberaster* K2606 did not accumulate 4-methoxybenzaldehyde in the liquid medium. It seems that there exist some differences in the varieties or the activity of ligninolytic enzymes among wood-destroying mushrooms.

3-Methyl-1-butanol was the most abundant aliphatic compound found. It accounted for 26% of the total amount of volatile compounds. Other aliphatic saturated alcohols, such as 1-propanol, 2-methyl-1-propanol, 1-butanol, 2-methyl-1-pentanol, 4-methyl-1-pentanol, 1-hexanol, and 1-heptanol, were identified. 1-Octen-3-ol was not found, as expected, because none of the panelists had commented that strain K2606 had a mushroom-like odor. 1-Octen-3-one and 3-octanone were not found, either. Therefore, the activity of lipoxygenase and hydroperoxide lyase producing C_8 compounds from linoleic acid seemed to be weak in this strain.

2-Formylpyrrole, which has been found in the pentane extract of dried *Boletus edulis* (Thomas, 1973), was the only nitrogenous compound identified. 2-Formylpyrrole has been identified in cocoa and bread; it may be produced by nonenzymic browning reactions. By GC and GC–MS analyses, even with mass chromatography at m/z 66 and 95, 2-formylpyrrole was not detected in the concentrate of volatile compounds in PGY medium sterilized in an autoclave and treated with Porapak Q as was done after the culture of strain K2606. This result suggests that

Table II. Volatile Compounds Isolated from the Culture Fluid of Strain K2606

peak[a]	compound	peak area, %	ID by
1	ethyl acetate	0.53	MS, GC
2	3-methylbutanal	0.53	MS, GC
3	2-butanol	+[e]	MS, GC
4	1-propanol	+	MS, GC
5	unknown[d] 88 (100), 58 (54), 43 (21), 70 (15), 55 (13), 57 (12)	0.07	
6	2-methyl-1-propanol	0.09	MS, GC
7	1-butanol	+	MS, GC
8	2-heptanone	+	MS, GC
9	3-methyl-1-butanol	26.18	MS, GC
10	2-pentylfuran	+	MS
11	3-methyl-3-buten-1-ol	+	MS, GC
12	1-octanal	+	MS, GC
13	2-methyl-1-pentanol[b]	+	MS, GC
14	2-ethyl-1-butanol[b]	0.15	MS, GC
15	4-methyl-1-pentanol[b]	0.60	MS, GC
16	3-methyl-1-pentanol	+	MS, GC
17	1-hexanol	0.10	MS, GC
	+ 4-methylstyrene[b]		MS, GC
18	acetic acid	0.12	MS
19	unknown[d] 117 (100), 118 (83), 43 (65), 60 (61), 45 (57), 115 (33), 91 (25)	0.09	
20	1-heptanol	0.16	MS, GC
21	furfural[c]	+	MS, GC
22	unknown[d] 45 (100), 43 (66), 73 (59), 61 (39), 44 (37), 72 (20)	0.15	
23	benzaldehyde	60.88	MS, GC
24	5-methylfurfural	+	MS, GC
25	benzonitrile	+	MS, GC
26	methylbenzoate	+	MS, GC
	+ 2-methylbenzaldehyde[b]		MS, GC
27	4-methylbenzaldehyde[b]	0.09	MS, GC
28	acetophenone	0.07	MS, GC
29	ethyl benzoate	+	MS, GC
30	2-hydroxybenzaldehyde[b]	+	MS, GC
	+ 3-thiophenecarboxaldehyde[b]		MS, GC
31	2-thiophenecarboxaldehyde	+	MS, GC
32	γ-muurolene	+	MS
33	dimethylbenzaldehyde[b]	+	MS
34	unknown	+	
35	unknown[d] 91 (100), 69 (71), 45 (65), 92 (50), 87 (48), 43 (43), 77 (36), 128 (35), 65 (34)	0.36	
36	4-ethylbenzaldehyde[b]	0.07	MS, GC
37	unknown[d] 69 (100), 45 (97), 87 (84), 43 (81), 86 (50), 41 (34), 57 (30)	0.25	
38	2-phenylethyl acetate	+	MS, GC
39	1-phenylethanol	0.10	MS, GC
40	1-phenyl-1,2-propanedione	+	MS, GC
41	unknown[c,d] 65 (100), 92 (99), 51 (98), 79 (94), 77 (91), 91 (89), 66 (88),95 (63), 110 (32)	4.21	
42	benzyl alcohol	0.27	MS, GC
43	4-ethylacetophenone[b]	+	MS, GC
44	2-phenylethanol	2.02	MS, GC
45	2-phenylpropanol	+	MS, GC
46	unknown[c,d] 150 (100), 107 (91), 79 (82), 135 (80), 43 (65), 91 (62), 77 (61), 121 (54)	0.35	
47	2-methylphenol	0.25	MC, GC
	+ unknown[d] 135 (78), 83 (52), 91 (42), 77 (42), 55 (42), 150 (38)		
48	2-formylpyrrole	+	MS, GC
49	3-phenylpropanol	1.20	MS, GC
50	4-methylphenol[b]	0.69	MS, GC
51	4-isopropylbenzyl alcohol[b]	0.12	MS
52	unknown[d] 51 (100), 43 (94), 79 (91), 107 (87), 105 (85),108 (84), 150 (45)	1.49	
53	unknown[d] 167 (100), 182 (93), 165 (34), 152 (23), 183 (15)	0.11	
54	4-methoxyacetophenone[b]	+	MS, GC
55	unknown[d] 182 (100), 167 (92), 45 (37), 73 (34), 152 (21), 183 (14)	0.24	

[a] Number refers to Figure 1. [b] Newly identified in mushroom. [c] From PGY medium. [d] Mass spectra data: *m/z* (relative intensity). [e] Less than 0.05%.

2-formylpyrrole was produced by strain K2606 or by secondary chemical reactions of metabolites of strain K2606 and constituents of the PGY medium. The sulfur compounds 2- and 3-thiophenecarboxaldehyde were identified; the former was first found in the pentane extract of dried *B. edulis* (Thomas, 1973), but the latter was found for the first time in the culture medium of mushrooms. Both compounds have been identified in processed ham and pork (Mussinan and Walradt, 1974). This suggests that they are produced by chemical reactions in which one reactant is an amino acid containing sulfur, but they were not detected by mass chromatography at *m/z* 111 and 112 in PGY medium sterilized in an autoclave and treated as described above. Autoclaved PGY medium contained furfural and the unknown compounds of peaks 42 and 46 in Figure 1.

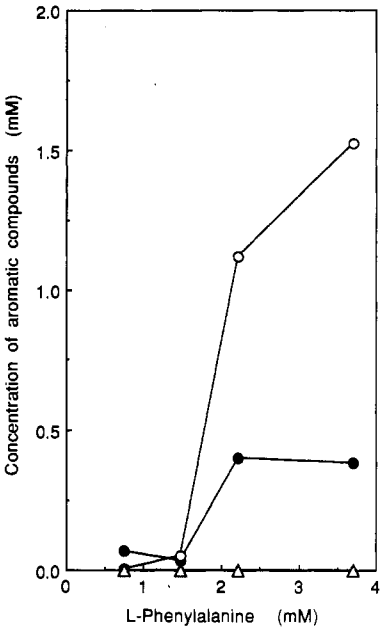

Figure 2. Effects of the L-phenylalanine concentration on the amounts of benzaldehyde (●), benzyl alcohol (○), and 2-phenylethanol (△) produced by *P. tuberaster* K2606 in PGY medium.

The aromatic compounds we detected in the culture medium of strain K2606 were probably produced by its ligninolytic enzymes; *P. tuberaster* is a white-rot fungus that can degrade lignin and lignocellulosic materials. Since PGY medium contains 0.74 mM L-phenylalanine, which is a precursor of 3,4-dimethoxybenzyl alcohol, a secondary metabolite of lignin (Shimada et al., 1981), L-phenylalanine may be a precursor of benzaldehyde, benzyl alcohol, and so on. We cultured strain K2606 in PGY medium to which L-phenylalanine was added to see if the amount of aromatic compounds produced increased. With 1.48 mM L-phenylalanine or less, the amounts of benzaldehyde and benzyl alcohol did not change, but when more was added, the concentrations of both aromatic compounds increased, with the concentration of benzaldehyde leveling off soon (Figure 2). 2-Phenylethanol hardly increased. These results suggest that the ligninolytic enzymes of strain K2606 are more active than the enzymes of the Ehrlich pathway, by which yeasts produce 2-phenylethanol from L-phenylalanine.

Thus, it was possible to obtain considerably more benzaldehyde and benzyl alcohol from *P. tuberaster* K2606. Different media and culture conditions may make it possible to obtain even larger amounts of volatile compounds, especially aromatic ones.

ACKNOWLEDGMENT

We thank Y. Yoshihama and C. Abe for assistance in the cultivation of mushrooms. We also thank C. Latta for help in preparing the manuscript.

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Received for review September 16, 1992. Accepted January 4, 1993.