Effects of pH Value on the Formation of Volatiles of Shiitake (*Lentinus edodes*), an Edible Mushroom

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The formation of volatiles of Shiitake (*Lentinus edodes*), an edible mushroom, is affected greatly by the pH value during blending. The formation of eight-carbon compounds such as 1-octen-3-ol and 2-octen-1-ol is dominant around pH 5.0–5.5 while the formation of sulfurous compounds such as dimethyl disulfide and dimethyl trisulfide is dominant around pH 7.0. There may be two enzymic systems responsible for the formation of eight-carbon and sulfurous compounds.

Shiitake (*Lentinus edodes*) is a kind of edible mushroom highly prized in Japan and China. Due to the difficulties of postharvest storage, the mushroom was traditionally preserved and consumed in dried form. Lenthionine ($C_2H_4S_5$), a cyclic sulfurous compound noted to have the characteristic aroma of the Shiitake mushroom, was first identified in dried Shiitake and then synthesized by chemical methods (Morita and Kobayashi, 1966; Wada et al., 1967). Stability studies of the lenthionine indicated that it was unstable when heated at 100 °C in 10% alcohol solution for 1 h at a pH value greater than 5.0 (Wada et al., 1967). Yasumoto et al. (1976) proposed that lenthionine may come from the enzymic degradation of a nonvolatile precursor—lentinic acid—during blending of Shiitake mushroom. The studies on steam-distilled volatiles of fresh Shiitake mushroom (Kameoka and Higuchi, 1976) indicated the presence of eight-carbon compounds, sulfurous compounds, terpeneous compounds, and some miscellaneous compounds. It is interesting to note that there was no existence of lenthionine in the steam-distilled volatiles of fresh Shiitake mushroom; instead, a cyclic compound, 1,2,4-trithiolane ($C_2H_4S_3$), described to have an "intense garlic ordor" by Gil and MacLeod (1981), was found. That the formation of volatiles in mushroom is influenced by the enzymic activities has been described previously (De Lumen et al., 1978; Tressl et al., 1980, 1982; Wurzenberger and Grosch, 1982, 1983; Chen and Wu, 1983a), but the relationship between pH value and volatile formation has not been discussed yet. In this study, volatiles formed at pH 4.0–9.0 were analyzed so as to investigate the enzymic activities affected by the pH value.

EXPERIMENTAL SECTION

Sample Preparation. Fresh Shiitake mushrooms were picked daily from the local cultivation houses near the Hsin-Chu, Taiwan. A total of 250 g of fresh mushrooms was blended with 750 mL of distilled water for 5 min while the pH of the whole mixture was maintained at a constant value by adding 0.1 N HCl or 0.1 N NaOH solution. The pH value of the mixtures ranged from 4.0 to 9.0 with 0.5 unit/interval. Volatiles of each mixture were extracted for 1 h by using the modified Likens-Nickerson apparatus (Römer and Renner, 1974), in which steam is the heat source; *n*-pentane (95%, E. Merck, glass distilled) was used as the extracting solvent. A fixed amount of *n*-amyl alcohol (99%, E. Merck) was added as the internal standard. Controlled experiments were conducted by using blanched mushrooms (97 °C, 5 min) under the same conditions. The extracted volatiles were injected directly into the gas chromatograph without further concentration to avoid any loss.

Gas Chromatography. Gas chromatography was carried out on a Shimadzu GC-8APF equipped with dual flame ionization detectors and dual glass columns (2 m × 2.6 mm i.d.). The column packing was 10% Carbowax-20M (Varian aerograph) coated on Chromosorb W A/W DMCS (80–100 mesh, Supelco, Inc.). The oven temperature was programmed from 60 to 200 °C at 3 °C/min. The injector and detector temperatures were 250 °C. Carrier gas was nitrogen at a flow rate of 20 mL/min. About 6 μL of extractant was injected for each run. Rention indices were calculated by using normal paraffin (C_8–C_{22}, Alltech Associates) as standards. The reported data were recorded by a Hewlett-Packard 3390A integrator.

Gas Chromatography–Mass Spectrometry. A Hewlett-Packard 5985B gas chromatography–mass spectrometry system was used. Operation parameters were as follows: carrier gas, helium; ionization voltage, 70 eV; electron multiplier voltage, 2200 V; ion source temperature, 200 °C.

RESULTS AND DISCUSSION

Figure 1 shows the typical gas chromatograms of volatiles of Shiitake mushroom formed at pH 5.0 (A) and pH 7.0 (B). *n*-Pentane was used as the extracting solvent; it seems sufficient enough for the extraction purpose of this study. The resulting solvent, which contained the extracted volatiles, was clear and transparent; the quantitation of this study was carried out under such conditions.

Table I shows the amount of each volatile eight-carbon or sulfurous compound of Shiitake mushroom formed from pH 4.0 to pH 9.0. The identification of the volatile compounds was done according to their GC retention indices and mass spectra (Buttery et al., 1976; Kameoka and Higuchi, 1976; Jennings and Shibamoto, 1980; De Brauw et al., 1981; Gil and MacLeod, 1981; Tressl et al., 1982). Other volatiles that are less important and are not shown in Table I included some six-carbon, eight-carbon, ten-carbon, and terpeneous compounds (Chen and Wu, 1983b).

Figure 2 shows the effects of pH values to the formation of eight-carbon and sulfurous compounds. The formation of eight-carbon compounds reaches the highest point at around pH 5.0–5.5; 1-octen-3-ol is the most abundant compound formed. The formation of sulfurous compounds begings at pH 5.5 and reaches the highest point at around pH 7.0; dimethyl disulfide (DMDS) and dimethyl trisulfide (DMTS) are the two most abundant compounds formed.

The control experiments were conducted by using blanched mushrooms (97 °C, 5 min) instead of fresh mushrooms; only a small amount of volatiles (ca. 3%) could be extracted. The result gives the following infor-

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Table I. Effects of pH Value on the Formation of Eight-Carbon and Sulfurous Compounds

peak no.[a]	compd	I_k[b] (CW-20M)	yield,[c] mg/250 g of mushroom at pH										
			4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5	8.0	8.5	9.0
1	3-octanone	1224	0.42	2.63	1.66	2.43	1.72	2.09	3.27	2.70	2.67	3.50	1.95
4	1-octen-3-ol	1427	37.35	43.37	48.86	45.72	43.43	39.79	28.33	24.27	12.69	14.36	1.34
5	1-octanol	1522	0.54	0.56	0.96	0.94	0.62	1.50	1.16	0.99	0.66	0.59	0.10
6	2-octen-1-ol	1577	3.41	4.38	4.40	4.16	3.02	3.27	1.87	1.54	0.93	0.92	–[d]
	total amount of 8-C compounds		41.72	50.94	55.88	53.25	48.79	46.65	34.63	29.50	16.95	19.36	3.39
2	dimethyl disulfide	1044	0.12	2.63	0.76	1.27	1.52	2.10	6.86	6.82	4.85	2.75	0.43
3	dimethyl trisulfide	1325	0.06	1.10	0.95	1.41	2.86	6.26	11.36	6.53	3.53	0.94	0.03
7	1-(methylthio)dimethyl disulfide	1587	0.04	0.11	0.10	0.10	0.05	0.24	0.58	0.39	0.33	0.17	0.36
8	1,2,4-trithiolane	1660	0.05	0.04	0.09	0.17	0.31	0.15	0.39	0.27	0.21	0.22	–[d]
	total amount of sulfurous compounds		0.27	3.88	1.90	2.94	4.74	8.75	19.19	14.01	8.92	4.08	0.81

[a] Number refers to Figure 1. [b] Calculated Kovats' retention indices. [c] Average of four experiments. [d] Does not exist.

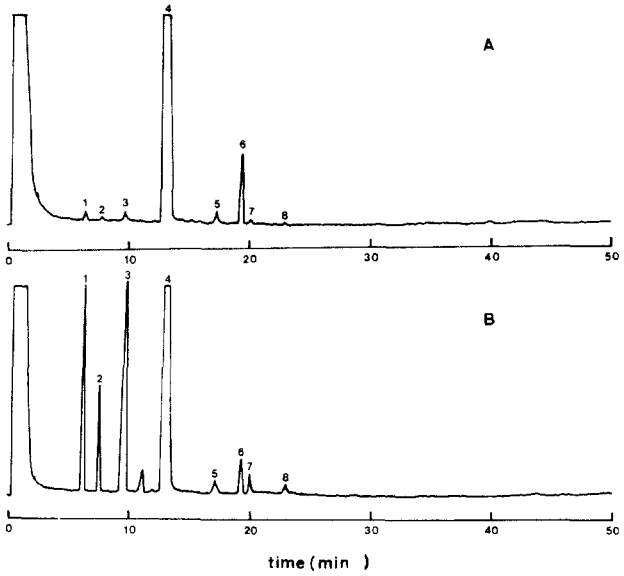

Figure 1. Gas chromatograms of volatiles of Shiitake mushroom formed at pH 5.0 (A) and pH 7.0 (B). Conditions: 2 m × 2.6 mm glass columns packed with 10% Carbowax 20 M coated on Chromosorb W A/W DMCS; carrier gas, nitrogen; flow rate, 20 mL/min; injector and detector temperatures, 250 °C; range, 10^2; attenuation, 8×. Temperatures were as follows: initial temperature, 60 °C; program rate, 3 °C/min; final temperature, 200 °C.

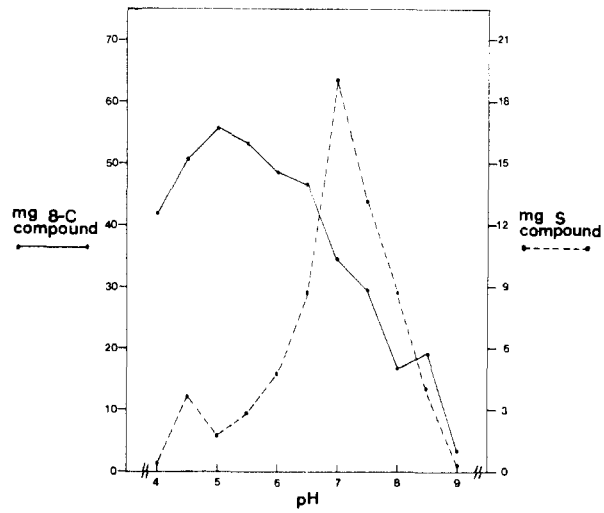

Figure 2. Volatile eight-carbon compounds (—) and sulfurous compounds (- - -) of Shiitake mushroom formed at different pH values.

mation: (a) The small amount of volatiles are due to the enzymic reaction(s) after the mushroom opens its veil; usually, the mushroom is picked at this stage. (b) Enzymes involved in the formation of volatiles were inactivated by the heat treatment; artifacts caused by the thermal degradation can be eliminated (Chen and Wu, 1983b).

In mushroom *Agaricus bisporus* or *Agaricus campestris*, the formation of eight-carbon compounds is known as the result of enzymic reaction(s) using linoleic acid ($C_{18:2}$) as the substrate (De Lumen et al., 1978; Tressl et al., 1980, 1982; Wurzenberger and Grosch, 1982, 1983; Chen and Wu, 1983a); the optimal pH value is about 6.0–7.0. In Shiitake mushroom, the formation of eight-carbon compounds also uses linoleic acid as the substrate of enzymic reaction(s) (Chen and Wu, 1983b), but the optimal pH is around 5.0–5.5.

Lenthionine, the characteristic compound of Shiitake mushroom reported by Morita and Kobayashi (1966) and Wada et al. (1967), cannot be found in this study; this is in accordance with the previous report by Kameoka and Higuchi (1976). DMDS and DMTS, two sulfurous compounds with threshold values of only 12 and 0.01 ppb, respectively (Buttery et al., 1976), were the two most abundant sulfurous compounds found at pH 6.5–7.5. The existence of DMDS and DMTS may originate from the degradation of CH_2–S bonding of lenthionine, since this compound is unstable if the pH value is greater than 5.0 (in 10% alcohol solution, 100 °C, 1 h) as Wada et al. (1967) described. The formation of DMDS and DMTS began at pH 5.5 and reached the highest point at pH 7.0 in this study, indicating that the degradation may be due to the enzymic activity and is affected by the pH value. 1-(Methylthio)dimethyl disulfide and 1,2,4-trithiolane, two sulfurous compounds in smaller amounts as compared with those of DMDS and DMTS, may be formed by the similar mechanism as that of DMDS and DMTS. However, it is also possible that the sulfurous compounds may originate from another sulfurous compound or other sulfurous compounds instead of lenthionine.

Registry No. 3-Octanone, 106-68-3; 1-octen-3-ol, 3391-86-4; 1-octanol, 111-87-5; 2-octen-1-ol, 22104-78-5; dimethyl disulfide, 624-92-0; dimethyl trisulfide, 3658-80-8; 1-(methylthio)dimethyl disulfide, 1618-26-4; 1,2,4-trithiolane, 289-16-7.

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Isolation and Characterization of Methyl Epijasmonate from Lemon (*Citrus limon* Burm.)

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Two epimers of methyl jasmonate, methyl 2-[2(*Z*)-pentenyl]-3-oxocyclopentane-1-acetate, were isolated from lemon peel (*Citrus limon* Burm.). ^{1}H NMR, mass spectra, hydrogenation, ozonolysis, and acid-catalyzed isomerization were used to establish their identity. Gas chromatography indicated the presence of 75 μg of methyl jasmonate isomers in the peel of one lemon with more than 95% of it in the thermodynamically less stable methyl epijasmonate form.

During the study of the pheromones produced by the male oriental fruit moth [*Grapholitha molesta* (Busck.)], a lemon-like odor was recognized in specialized organs called "hairpencils", located at the base of the abdomen. Ethyl cinnamate and methyl jasmonate, and not the isoprenoid compounds commonly associated with lemons, were shown to cause this lemon-like odor (Nishida et al., 1982).

Many volatile compounds have been identified in lemons (Ranganna et al., 1983), some in the juice (Mussinan et al., 1981), but most in the oil expressed from the peel (Shaw, 1979). The major components, limonene, γ-terpinene, β-pinene, β-bisabolene, and citral, arise through isoprenoid biosynthesis. Of these, citral, a mixture of the *E* and *Z* isomers, geranial and neral, contribute much to the characteristic odor of lemon peel oil. This report described an odoriferous lipid metabolite methyl epijasmonate [methyl 3-oxo-2-[2(*Z*)-pentenyl]cyclopentane-1-acetate] (Demole, 1982; Vick and Zimmerman, 1983), which was determined to be a component of lemon peel. The presence of this compound in lemons explains why the hairpencils of the oriental fruit moth seem to have a lemon-like smell. We find the odor of methyl epijasmonate especially strong in very ripe lemons.

EXPERIMENTAL SECTION

Crude Extract of Lemon Peels. Peels from 150 lemons obtained from a local grocer were twice soaked in 2.5 L of acetone and ether (1:1) for 1-week periods. The combined extracts were concentrated at 45 °C in a rotary evaporator to an oily residue, shaken with 350 mL ether, and extracted with 150 mL of saturated NaCl. The ether extract was dried over anhydrous Na_2SO_4 and then concentrated to yield 22.2 g of a yellow oil. Approximately, 17 g of volatile components (limonene, etc.) were removed, by vacuum distillation at 0.02 mmHg and 45 °C, yielding 4.4 g of brown oily residue.

Florisil chromatography of the nonvolatile residue in a column 200 mm × 35 mm i.d. yielded five fractions successively eluted with the following solvents: 400 mL of Skelleysolve B (abbreviated SKB), followed by 400 mL of 5% ether in SKB, 400 mL of 10% ether in SKB, 800 mL of 20% ether in SKB, and then 800 mL of 30% ether in SKB. Evaporation of the solvent from the 30% ether–SKB fraction yielded 640 mg of residue with the characteristic hairpencil lemon-like smell of the Oriental fruit moth. This fraction was chromatographed on a 50 mm × 9 mm i.d. column filled with 30 g of 200-mesh silica gel deactivated with 10% water. Six fractions were obtained by successive elution with the following solvents: 45 mL of SKB, followed by 90 mL of 5% ether in SKB, 150 mL of 10% ether in SKB, 60 mL of 20% ether in SKB, and then 75 mL of 20% ether in SKB. Evaporation of the last 20% ether in SKB fraction yielded 130 mg of a yellowish oil with the characteristic hairpencil lemon-like odor.

Isolation of Methyl Jasmonate Epimers. Portions of the final 20% ether–SKB fraction were separated on a 380 mm × 10 mm i.d. column filled with 5-μm Lichrosorb SI-100 eluted with 20% ether in SKB at 25 kg/cm^2 and 2 mL/min by using an Altex 110 pump. One fraction was collected between 45 and 50 min and another between 51 and 57 min. GLC of each fraction on a 2 m × 4 mm i.d. glass column filled with 100–200-mesh Gas-Chrom Q coated with 3% OV-101 yielded two peaks in both fractions; I at an *n*-paraffin retention index of approximately

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