

Volatile Flavor Compounds Produced by Molds of *Aspergillus*, *Penicillium*, and *Fungi imperfecti*

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Strains of molds *Aspergillus niger*, *A. ochraceus*, *A. oryzae*, *A. parasiticus*, *Penicillium chrysogenum*, *P. citrinum*, *P. funiculosum*, *P. raistrickii*, *P. viridicatum*, *Alternaria*, *Cephalosporium*, and *Fusarium* sp. were grown on sterile coarse wheat meal at 26 to 28 C for 120 h. The volatiles from mature cultures were distilled at low temperature under reduced pressure. The distillates from traps -40 and -78 C were extracted with methylene chloride and subsequently concentrated. All the concentrates thus obtained were analyzed by gas-liquid chromatography, mass spectrometry, chemical reactions of functional groups, and olfactory evaluation. Six components detected in the culture distillates were identified positively: 3-methylbutanol, 3-octanone, 3-octanol, 1-octen-3-ol, 1-octanol, and 2-octen-1-ol. They represented 67 to 97% of all the volatiles occurring in the concentrated distillate. The following 14 components were identified tentatively: octane, isobutyl alcohol, butyl alcohol, butyl acetate, amyl acetate, octyl acetate, pyridine, hexanol, nonanone, dimethylpyrazine, tetramethylpyrazine, benzaldehyde, propylbenzene, and phenethyl alcohol. Among the volatiles produced by molds, 1-octen-3-ol yielding a characteristic fungal odor was found predominant.

In our previous paper (9) a description was presented of different volatiles produced by *Aspergillus flavus*. The purpose of this study was the identification of the volatiles produced by molds of the group *Aspergillus*, *Penicillium*, and *Fungi imperfecti*.

MATERIALS AND METHODS

Microorganisms. All the microorganisms studied were isolated from wheat grain and were maintained on Czapek-Dox agar slants at 3 C until used.

Culture media and growth conditions. The culture medium used was coarse wheat meal sterilized at 1 atm for 45 min. The wheat meal was moistened to 60% water content and inoculated with conidia of pure culture suspended in physiological saline as described previously (9). After 3 to 4 days the culture medium (1 kg) was collected for the isolation step.

Isolation of volatiles for the medium. The isolation of volatiles from the culture medium was carried out by vacuum distillation in an all-glass apparatus (10). The distillation step took 4 h and was done under nitrogen at 5 mm Hg. The temperature of the water bath was 35 C, whereas that of the cold traps in which the distillate was collected ranged from -10 to -80 C. The distillate collected in traps cooled to -40 and -80 C was extracted with CH_2Cl_2 and concentrated to a volume of 100 μl (10).

Gas chromatography. The separation of the volatile substances in the concentrated distillates was carried out with a Willy Giede model GCHF 18.3 gas

chromatograph equipped with a flame ionization detector. The columns were stainless steel (3 m long; inner diameter 3 mm) packed with 15% Carbowax 20 M terminated with terephthalic acid on 80- to 100-mesh-acid-washed, dimethyldichlorosilane (DMCS)-treated Chromosorb W. Nitrogen was used as the carrier gas at a flow rate of 20 ml/min. Samples (2 μl) were applied to the column which was held isothermally at 120 C. The percentage of individual volatiles was calculated from the peak area, the area of all the peaks on the chromatogram serving as the 100% value.

Identification of the predominant volatiles. The volatile compounds occurring in the extracts were separated on a gas-liquid chromatography column and then were identified by coincidence of relative retention times with those of known compounds, by mass spectrometry, chemical modification of the sample, and by olfactory evaluation.

For identification purposes a combined apparatus gas chromatograph-mass spectrometer LKB 9000 was used. The columns were glass (3 m long, inner diameter 2.5 mm) packed with 10% Carbowax 20 M terminated with terephthalic acid on 80- to 100-mesh, acid-washed, DMCS-treated Chromosorb W. Helium, at a flow rate 10 to 20 cm^3/min , served as the carrier gas. The columns were temperature programmed as follows: 5 C/min in the range 70 to 170 C. The ionization chamber was operated at 10^{-6} to 10^{-7} torr. A 70-eV source provided the mass spectra. Spectra were recorded from mass 30 to 250 in 100 s. Components were identified by comparing the mass spectra of the unknown to authentic compounds.

TABLE 1. Volatile compounds occurring in the concentrated distillate from molds

Molds	Identified compounds												Total identified compounds
	3-Methylbutanol		3-Octanone		3-Octanol		1-Octen-3-ol		1-Octanol		2-Octen-1-ol		
	%	1 ^a	%	2	%	3	%	4	%	5	%	6	
<i>A. niger</i>	0.5	GLC, ChR, OE	1.3	GLC, MS, ChR, OE	0.3	GLC	91.5	GLC, MS, ChR, OE			1.5	GLC, OE	95.1
<i>A. ochraceus</i>	0.85	GLC, MS, ChR, OE	1.2	GLC, MS, ChR, OE	1.2	GLC, MS, ChR, OE	83.8	GLC, MS, ChR, OE	1.4	GLC, MS, OE	2.6	GLC, MS, OE	91.0
<i>A. oryzae</i>	15.1	GLC, MS, ChR, OE		GLC, ChR, OE	48	GLC, OE	72.8	GLC, MS, ChR, OE		GLC, OE	1.5	GLC, MS, OE	94.2
<i>A. parasiticus</i>	7.6	GLC, MS, ChR, OE		GLC, ChR, OE	13.6	GLC	35.6	GLC, MS, ChR, OE	0.7	GLC, MS, OE	34.8	GLC, MS, OE	96.2
<i>P. chrysogenum</i>	6.7	GLC, MS, ChR, OE	1.1	GLC, ChR, OE	6.9	GLC	70.5	GLC, MS, ChR, OE	0.7	GLC	11.7	GLC, MS, OE	97.6
<i>P. citrinum</i>	0.7	GLC, MS, ChR, OE	0.3	GLC, ChR, OE	0.2	GLC, ChR	93.1	GLC, MS, ChR, OE	0.1	GLC, MS, OE	1.1	GLC, MS, OE	95.5
<i>P. funiculosus</i>	12.5	GLC, MS, OE	0.2	GLC, ChR, OE			89.0	GLC, MS, ChR, OE			0.9	GLC, MS, OE	92.6
<i>P. raistricki</i>	1.8	GLC, MS, ChR, OE	0.7	GLC, ChR, OE			92.1	GLC, MS, ChR, OE			0.1	GLC, OE	94.7
<i>P. viridicatum</i>	1.4	GLC, MS, ChR, OE	0.2	GLC, ChR, OE	0.5	GLC, MS, OE	63.5	GLC, MS, ChR, OE	5.3	GLC, MS, OE	21.4	GLC, MS, OE	92.4
<i>F. imperfecti</i>	1.1	GLC, ChR	7.0	GLC, MS, ChR, OE			76.4	GLC, MS, ChR, OE			2.3	GLC, MS, OE	86.8
<i>Alternaria</i>	0.4	GLC, ChR	0.5	GLC, MS, ChR, OE	2.0	GLC, MS, ChR, OE	92.4	GLC, MS, ChR, OE			0.7	GLC, MS, OE	96.0
<i>Cephalosporium</i>	3.0	GLC, MS, ChR, OE	0.6	GLC, ChR, OE			82.9	GLC, MS, ChR, OE					86.5

^a Abbreviations: methods of identification: GLC, gas-liquid chromatography; MS, mass spectrometry; ChR, chemical reactions; OE, olfactory evaluation; %, contents of individual components, percent in relation to total volatiles.

The functional groups were identified also in the head space above the culture medium. The method of Hoff and Feit modified in this laboratory was used (11). The method is based on the reactions of functional groups of the volatile compounds with chemical reagents.

The sensory evaluation was performed by a panel of 2 to 3 members by smelling the effluent from the column.

RESULTS AND DISCUSSION

The volatiles identified in the concentrated distillates from various strain cultures are listed in Table 1. 1-Octen-3-ol was found to be the main volatile component produced by all the molds studied. Its contents varied from 36.6 to 93.1% of the total volatiles.

In this respect the most efficient appeared to be such molds as *P. citrinum*, *P. raistrickii*, *Cephalosporium*, *P. funiculosum*, and *A. niger*.

Pure 1-octen-3-ol isolated from the molds by a gas chromatography trapping procedure exhibited a strong fungal resinous odor. In concentrations close to the threshold value its odor resembled that of the mushroom, *Agaricus bisporus*. According to the data presented in Table 1, the quantity of 1-octen-3-ol depends on the mold species and composition of the growth medium. Thus, *A. ochraceus* grown on such media as coarse wheat meal, starch, gluten, and plant oil was stated to produce volatile fractions differing both qualitatively and quantitatively. The contents of 1-octen-3-ol were calculated by measuring the peak area on chromatograms of the concentrated distillates. This strain was found to produce the highest amounts of 1-octen-3-ol when grown on coarse wheat meal (83.8%); lower yields were obtained on gluten (26.9%), starch (9.3%), and only traces on media containing plant oils.

It should be stressed that the uninoculated coarse wheat meal contained only traces of the above mentioned compounds (9).

A research was also carried out in this laboratory on the mushrooms: *Agaricus bisporus* and *Boletus edulis* (14, 15). According to the data obtained, 1-octen-3-ol was the predominant volatile in *Agaricus bisporus* and *B. edulis*—78 and 82.5% of the total volatiles, respectively.

It is noteworthy that this compound occurs in many other foods; its origin has not been established, however (1, 2, 4, 5, 7, 8, 12, 13).

According to the results of mass spectrometry and infrared analysis, 1-octen-3-ol produced by molds and mushrooms is identical (14, 15). Thus, a number of strains can be used to produce foods showing the odor typical of mushrooms. In Table 2, data are presented on the contents of 1-octen-3-ol in the head space above *Agaricus bi-*

TABLE 2. Contents of 1-octen-3-ol in vapors above *Agaricus bisporus* and mycellia of molds grown on different media

Product	1-octen-3-ol (μg of vapors per ml)
Unprocessed mushroom	0.036
Boiled mushroom	0.0009
<i>A. oryzae</i> on wheat	0.0015
<i>A. oryzae</i> on soybean	0.001
<i>Cephalosporium</i> on wheat	0.012
<i>Cephalosporium</i> on soybean	0.018

sporus and above the coarse wheat meal on which molds were cultivated.

For *A. oryzae*, the vapors above the substrate contained 200 to 300 times lower quantities of 1-octen-3-ol as compared with those above the freshly harvested mushrooms, but more than in boiled ones. In the case of *Cephalosporium*, the level of 1-octen-3-ol in vapors was found to be only 2 to 3 times lower than in vapors above the fresh mushrooms.

Certain mold species, for example *A. oryze*, are used in the production of soybean products (3, 6). The mold species studied were found to be capable of producing other volatiles also (Table 1). Among the latter compounds, of special interest is 2-octen-1-ol, which yields a characteristic, unpleasant musty-oil odor. Some mold species, for example *A. flavus* (9) and *A. parasiticus*, produce this compound in large quantities. There are other mold strains, however, which produce 2-octen-1-ol in small amounts.

Besides the compounds presented in Table 1, the following volatiles were identified tentatively by chemical reactions and mass spectrometry: octane, isobutyl alcohol, butyl alcohol, butyl acetate, octyl acetate, pyridine, hexanol, nonanone, dimethylpyrazine, benzaldehyde, propylbenzene, and phenethyl alcohol. Although the above compounds occur in low concentrations, they affect considerably the flavor of foods infected by molds.

The volatiles produced by molds may be used as an index for the detection of food contamination by rapid instrumental methods.

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