# Investigation of Some Volatile Constituents of Mushrooms (*Agaricus bisporus*): Changes which Occur during Heating

Shirley M. Picardi* and Phillip Issenberg[1]

Aroma concentrates characteristic of both "raw" and "cooked" flavors of *Agaricus bisporus* were obtained by simultaneous distillation-extraction in a Likens-Nickerson apparatus, and analyzed by gas chromatography and combined gas chromatography–mass spectrometry. The major volatile constituents of raw mushrooms were 3-octanone, 3-octanol, 1-octen-3-ol, benzaldehyde, octanol, and 2-octen-1-ol. The major volatile constituents of cooked mushrooms were the above six compounds plus 1-octen-3-one. Of these, octanol had not been previously identified as a mushroom component, and 2-octen-1-ol had been only tentatively identified. The changes of these volatile compounds during the course of heating were also investigated. 1-Octen-3-one was formed in detectable amounts only after the mushrooms had been boiled for approximately 15 min, and its greatest production appeared after one-half hour. Odor evaluation investigations were conducted using an effluent splitter attached to a gas chromatographic column.

A report of an investigation of the volatile constituents of the cultivated mushroom *Agaricus bisporus* by Cronin and Ward (1971) summarized past research on mushroom flavor. They obtained a mushroom concentrate by steam distillation in a Likens-Nickerson apparatus (Likens and Nickerson, 1964) and used combined gas chromatography–mass spectrometry to identify its major components. They positively determined the presence of 3-methylbutanal, butanol, 3-methylbutanol, pentanol, 3-octanone, 1-octen-3-one, hexanol, 3-octanol, furfural, 1-octen-3-ol, benzaldehyde, phenylacetaldehyde, $\alpha$-terpineol, and benzyl alcohol. They tentatively identified butyl methyl ether, a monoterpene hydrocarbon, and 2-octen-1-ol. Odor evaluations of the components were carried out as they eluted from a gas chromatographic column. The aroma properties of 1-octen-3-one and 1-octen-3-ol in dilute aqueous solutions were studied, and both of these compounds were considered to have mushroom-like odors.

This research was undertaken to determine the major volatile constituents of *Agaricus bisporus* (both cooked and uncooked) and changes which occur in the composition and flavor as cooking progresses. The techniques of gas chromatography, combined gas chromatography–mass spectrometry, and sensory analysis were employed.

## EXPERIMENTAL SECTION

Fresh mushrooms (*Agaricus bisporus*, "cream" variety) were obtained by air freight from the Kennett Canning Co., Kennett Square, Pa. They arrived unwashed and were stored at 4° until analyzed (24 to 48 hr).

**Isolation of "Raw" Mushroom Aroma Extract.** Mushrooms (1000 g) were washed and blended with 1000 ml of distilled water in a Waring Blendor at low speed for 10 sec. The slurry was transferred to a two-necked 5000-ml round-bottomed flask fitted with a thermometer and a Likens-Nickerson continuous distillation-extraction apparatus, modified slightly by addition of a Dewar-type condenser containing a 23% by weight NaCl–ice mixture (approximately −16°) at the top. Hexane (25 ml), 99 mol% pure (Fischer Scientific Co., Fair Lawn, N. J.), was the extracting solvent, with prewashed carborundum boiling chips added. Coolant fluid circulated through the extractor was maintained at approximately −8° by a constant temperature bath (Ultra-Kryomat TK30, Lauda Instruments, Inc., Westbury, N. Y.). The solvent vapor tube of the extractor was wrapped with 1 in.-wide asbestos tape; the aqueous vapor tube was wrapped with 1 in.-wide fiberglas-insulated electrical heating tape maintained at approximately 60° to 80°. The adapter at the top of the apparatus was connected to a mercury manometer and a vacuum pump. The pressure was approximately 51 to 58 Torr during extraction of the mushroom slurry, which was continued for 3 hr after boiling commenced. During this extraction, the mushrooms were exposed to temperatures no greater than 45.5° to 49°.

The resulting hexane extract (10.5 ml) was diluted to 25 ml with fresh hexane and stored at 4° until it could be concentrated and analyzed. (Concentration took place 2 to 3 days later; analyses took place within 1 to 2 weeks.)

**Isolation of "Cooked" Mushroom Aroma Extract.** The mushroom slurry was prepared as described above, except that no vacuum pump or manometer was employed. Coolant was maintained at −1° to −2°. The heating tape on the aqueous vapor tube of the apparatus was maintained at approximately 105°.

The hexane extract (19.5 ml) was diluted to 25 ml with fresh hexane and stored at 4°.

**Isolation of Mushroom Aroma Fractions Obtained during the Course of Cooking.** The procedure was identical to that employed for isolation of the "cooked" aroma extract, except that extract was withdrawn and fresh solvent was added to the extractor periodically, so the time course of changes in volatile components due to the cooking process could be determined. The following fractions were obtained (note that time "zero" was when the mushroom slurry started to boil): A: preboiling fraction (−1 hr to 0); B: 0 to +15 min; C: +15 to +30 min; D: +30 min to +1 hr; E: +1 to +2 hr; F: +2 to +3 hr.

The fractions, which varied from 22.0 to 25.0 ml in volume, were diluted to 25 ml with fresh hexane and stored at 4°.

**Concentration of Aroma Extracts.** The hexane extracts were concentrated to a volume of approximately 0.12 ml with a stream of prepurified nitrogen. The final stages of concentration were done in 0.3-ml vials with cone-shaped interiors (microvials with "Mininert" Teflon valves on screw caps, Cat. No. 614569, Precision Sampling Corp., Baton Rouge, La.) which had been calibrated using mercury.

**Solvent Blanks.** Blanks were extracted at both reduced and atmospheric pressures using 25 ml of hexane and 2000 ml of distilled water. All conditions were identical to those described above.

**Identification of Components.** Aroma extracts were separated on a 150 m × 0.5 mm i.d. Carbowax 20M open-

Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139.

[1] Present address: Eppley Institute for Research in Cancer, University of Nebraska Medical Center, Omaha, Nebraska 68105.

tubular stainless steel column, prepared as described by Mon (1971), in an Aerograph Series 200 gas chromatograph (Varian Aerograph, Walnut Creek, Calif.) coupled to a Hitachi Perkin-Elmer RMU-7 double-focusing mass spectrometer (Perkin-Elmer Corp., Norwalk, Conn.). Sample size was 0.1 μl. The gc injection port temperature was 215° and the gc column temperature was 110°. Helium flow was 9.0 ml/min.

The effluent from the gc column passed through a stainless steel capillary tube (1.0 m × 0.5 mm i.d.) maintained at 250° and a glass Watson–Biemann-type separator maintained at 200° (Watson and Biemann, 1965) into the ion source. Ion source temperature was 150°, electron current was 100 μA, electron energy was 70 eV, and the accelerating voltage was 2500 V. Source and collector slits were set at 0.5 mm to give a resolving power of approximately 400. Scanning range was $m/e$ 4 to $m/e$ 400 in 5 sec. Total ion current (TIC) chromatograms were recorded from a monitor located between the electrostatic and magnetic analyzers of the mass spectrometer. The gcms system and its characteristics have been described by Issenberg *et al.* (1969).

The spectra thus obtained, together with published mass spectral data (Cornu and Massot, 1966, 1967; Stark and Forss, 1962, 1964; Stenhagen *et al.*, 1969; Von Sydow *et al.*, 1971), were used to postulate identities of seven major components of the extracts. Identification was confirmed by comparison with spectra and retention data of reference compounds obtained commercially or synthesized.

Further gc confirmation of the above identifications based on spectra and retention data was obtained by separation on a 150 m × 0.5 mm i.d. SF 96(50) + 5% Igepal open-tubular stainless steel column, prepared as described by Mon (1971), in an Aerograph Series 1200 gas chromatograph (Varian Aerograph, Walnut Creek, Calif.), equipped with a flame ionization detector (FID). Column temperature was 150° and helium flow was 10.8 ml/min. Injection port temperature was 205° and detector temperature was 230°. Sample size was 0.1 μl.

**Synthesis of 1-Octen-3-one.** The method of Brown and Garg (1961) was used to synthesize 1-octen-3-one from the corresponding alcohol (obtained from Givaudan Corp., Clifton, N. J.). Gcms analysis of the ether extract showed only one component (other than the solvent) which had a mass spectrum like that of 1-octen-3-one (Stark and Forss, 1962).

**Synthesis of 2-Octen-1-ol.** The method of de Gaudemaris and Arnaud (1962) was used to synthesize 2-octen-1-ol from the corresponding trans acid (*trans*-2-octenoic acid, tech, 80%, obtained from Aldrich Chemical Co., Milwaukee, Wis.). Gcms analysis of the ether extract showed two major components in addition to the solvent. The larger of the two had a mass spectrum like that of *trans*-2-octen-1-ol (Von Sydow *et al.*, 1971).

**Odor Evaluation of Components.** The "cooked" mushroom aroma extract (0.5 μl) was separated on a 3 m × 3.2 mm i.d. stainless steel column packed with 1% Carbowax 20M on Chromosorb G (100–120 mesh) in an Aerograph Series 1200 gas chromatograph (Varian Aerograph, Walnut Creek, Calif.) equipped with an FID. An effluent splitter made of 3.2 mm i.d. stainless steel tubing was placed between the column exit and the detector, extending just through the wall of the gc oven. The mass split ratio (170:1 in favor of the collecting arm) was determined by comparison of the peak areas of 3-octanone recorded with the splitter open and closed. The column was maintained at 90° and prepurified nitrogen at 30 ml/min was the carrier gas. Injection port temperature was 215° and detector temperature was 220°. The chromatogram obtained was similar to that obtained in the gcms analysis, and the fact that the peaks eluted in the same order on this packed Carbowax 20M column as on the open-tubular Carbowax 20M column was confirmed by mass spectral analysis. The solvent peak and the seven peaks of interest were collected in a glass U-shaped collector tube (Varian Aerograph, Walnut Creek, Calif.) chilled in liquid nitrogen. The trap was broken into a vial containing 10 ml of distilled water and the odor was compared to that of 0.5 μl of the "cooked" mushroom aroma extract in the same amount of distilled water.

An alternative odor evaluation procedure was also employed; the effluent stream was sniffed and odors were recorded on the chromatogram. Evaluations were based on only one individual's judgment.

**Table I. Seven Gc Peaks Representing the Major Volatile Constituents of Mushroom Flavor**[a]

| Peak designation | Identity | Retention time, min: Carbowax 20M open-tubular column, Isolated | Carbowax 20M open-tubular column, Reference | SF 96(50) open-tubular column, Isolated | SF 96(50) open-tubular column, Reference |
|---|---|---|---|---|---|
| 1 | 3-Octanone | 7.6 | 7.6[b] | 21.0 | 21.0[b] |
| 2 | 1-Octen-3-one | 8.1 | 8.1[c] | 20.6 | 20.6[c] |
| 3 | 3-Octanol | 9.3 | 9.3[d] | 22.3 | 22.3[d] |
| 4 | 1-Octen-3-ol | 10.6 | 10.6[e] | 21.3 | 21.3[e] |
| 5 | Benzaldehyde | 13.6 | 13.6[f] | 21.6 | 21.6[f] |
| 6 | Octanol | 14.5 | 14.5[g] | 29.3 | 29.3[g] |
| 7 | 2-Octen-1-ol | 17.7 | 17.7[h] | 29.6 | 29.6[h] |

[a] See text of article for which compounds are present in raw and cooked mushrooms. [b] Aldrich Chemical Co., Milwaukee, Wis. [c] Synthesized. [d] Aldrich Chemical Co., Milwaukee, Wis. [e] Givaudan Corp., Clifton, N. J. [f] Eastman Organic Chemicals, Rochester, N. Y. [g] Aldrich Chemical Co., Milwaukee, Wis. [h] Synthesized.

## RESULTS AND DISCUSSION

The "raw" mushroom aroma extract had a strong, raw mushroom-like odor. Gcms analysis using the Carbowax 20M open-tubular column showed six major components which were identified as 3-octanone, 3-octanol, 1-octen-3-ol, benzaldehyde, octanol, and 2-octen-1-ol by comparison of their retention times and mass spectra with those of known compounds (see Table I). Gc analysis on the SF 96(50) column confirmed these conclusions.

The "cooked" mushroom aroma extract had a strong, cooked mushroom-like aroma. Gcms analysis using the Carbowax 20M open-tubular column showed seven major peaks. Retention data for these peaks are presented in Table I. With the exception of peak =2 which was detected only in the "cooked" extract, the mass spectra and gc retention data were identical in both the "raw" and "cooked" mushroom extracts. Peak =2 was identified as 1-octen-3-one by comparison of its retention time and mass spectrum with those of the synthesized compound. Gc analysis on the SF 96(50) column confirmed these conclusions.

Gcms analysis of the two solvent blanks on the Carbowax 20M open-tubular column showed that no artifacts were detected which would interfere with the gc and gcms analyses of the mushroom aroma extracts.

The six aroma fractions A through F obtained during cooking had varying odors. Fractions A through C smelled like raw mushroom, D was mushroomy, E smelled like cooked mushroom, while F had a "buttery" overtone in addition to smelling like cooked mushroom. Gcms analyses of the fractions using the Carbowax 20M open-tubular column yielded the chromatograms presented in Figure 1. Extract A contained the same major components as the "raw" mushroom aroma extract. No 1-octen-3-one was detected. Extract B had a similar composition, but the relative amounts of the major components were slightly dif-

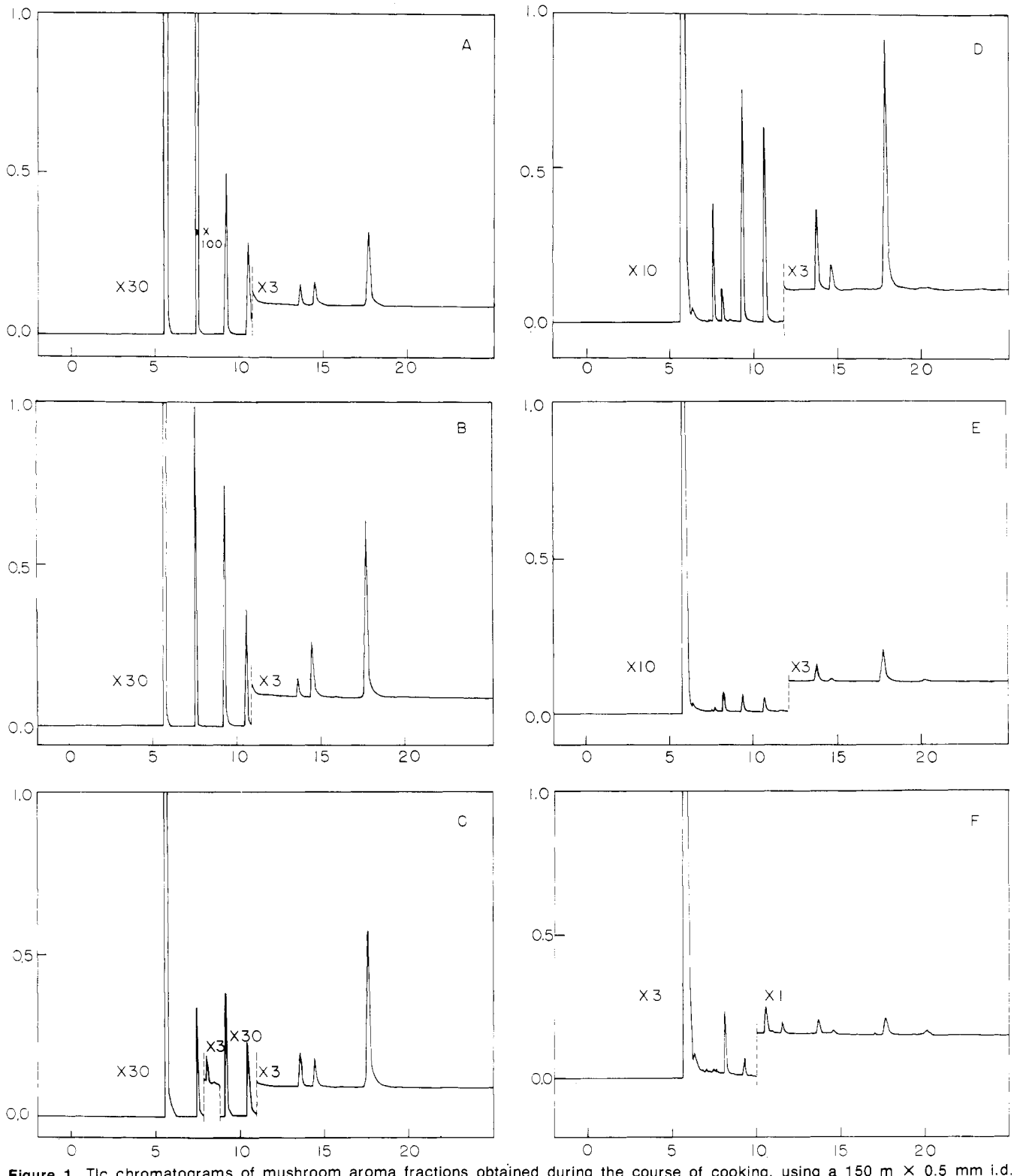


**Figure 1.** Tlc chromatograms of mushroom aroma fractions obtained during the course of cooking, using a 150 m × 0.5 mm i.d. Carbowax 20M open-tubular stainless steel column maintained at 110°. The letters A through F represent the fractions A through F. Abscissa: retention time in minutes; ordinate: detector response in A × $10^{11}$.

ferent than in extract A. Extract C contained the same six components as A and B (in different relative amounts), plus a small amount of 1-octen-3-one. In extract D, 1-octen-3-one had increased at least threefold relative to its amount in C. The other six components were also present, in different relative amounts. Extracts E and F showed very small peaks.

Odor evaluation showed the trapped "cooked" mushroom aroma extract (containing the solvent and peaks =1 through =7) had a qualitatively similar odor to that of the "cooked" mushroom extract made up to the same approximate dilution. Therefore, artifact formation or loss of volatile constituents significant in mushroom aroma under the gc conditions employed was unlikely.

Odor evaluations obtained by sniffing peaks as they eluted from the packed Carbowax 20M gc column during separation of the "cooked" mushroom extract are shown in Figure 2. Many different odors were detected, and some

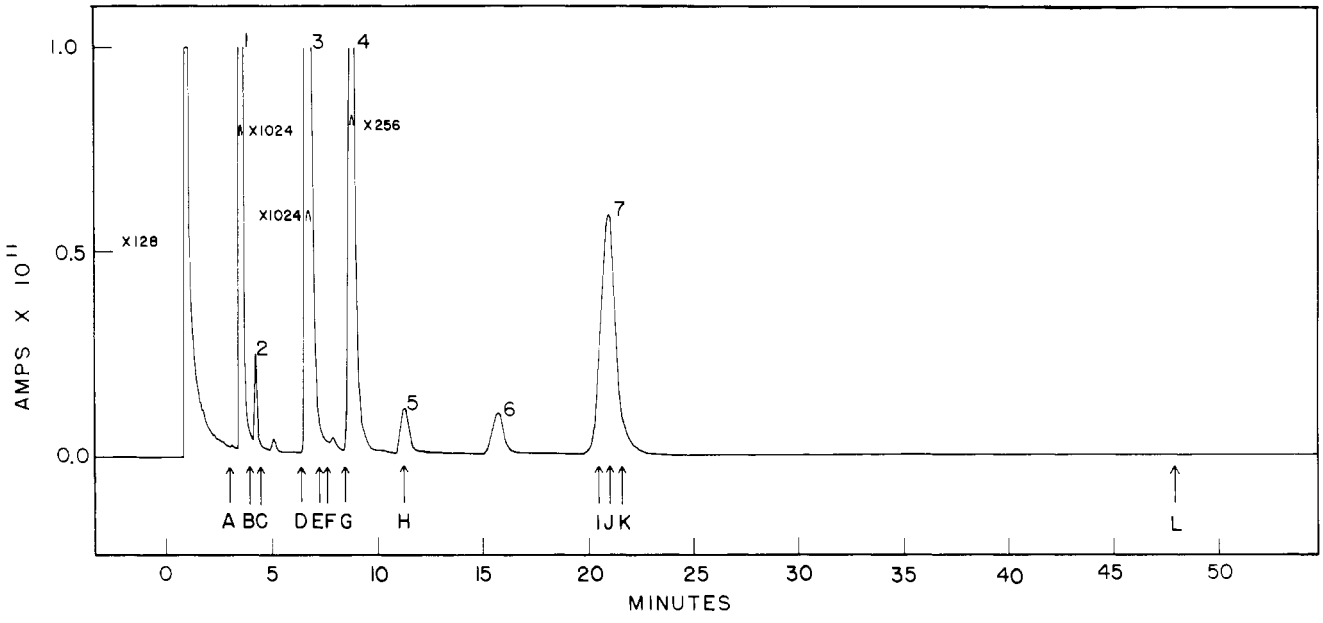


**Figure 2.** FID chromatogram of "cooked" mushroom aroma extract using a 3 m × 3.2 mm i.d. stainless steel column packed with 1% Carbowax 20M on Chromosorb G (100–120 mesh) maintained at 90°. The major gc peaks of the extract are indicated by the numbers 1 through 7. The odor responses are indicated by the letters A through L and correspond to the following observations: A: metallic, mushroomy; B: sweet; C: popcorn-like, buttery; D: strongly metallic; E: dusty; F: faintly musty; G: sweet, slightly mushroomy; H: slightly musty, almond-like; I: urine-like; J: stale urine-like; K: similar to odor response J, but greener; and L: sweet, slightly mushroomy.

coincided with the retention times of the major peaks. A "buttery" odor detected between peaks =2 and =3 may have been formed only after the mushrooms were boiled for 2 hr, because this odor was also the odor of the fraction F obtained in the study of the time course of volatile changes during cooking. A "sweet, slightly mushroomy" odor detected near the end of the chromatogram was not correlated with any observable peak. The detection of more than one odor for a peak (=7, for example) could be due to a changing concentration of that component or the presence of small amounts of powerful odorants which do not appear as large peaks on the chromatogram.

## SUMMARY

These investigations demonstrated that 3-octanone, 3-octanol, 1-octen-3-ol, benzaldehyde, octanol, and 2-octen-1-ol are the major volatile components of raw mushroom flavor, while the above compounds plus 1-octen-3-one are the major volatile components of cooked mushroom flavor. 1-Octen-3-one was formed in detectable amounts only after the mushrooms had been boiled for approximately 15 min, and its greatest production appeared after one-half hour.

The detection of 1-octen-3-one in the "cooked" mushroom extract but not in the "raw" mushroom extract might have been due to either of two possible reasons. Either 1-octen-3-one is not an important contributor to the flavor of raw mushrooms, but is an important "character impact" compound in the flavor of cooked mushrooms, or 1-octen-3-one is present in both raw and cooked mushrooms, but its level in raw mushrooms is below the detection limits of the analytical methods used in this investigation. If the former possibility is true, 1-octen-3-one may play an important role in differentiating raw mushroom flavor from cooked mushroom flavor. If the latter possibility is true, the differences in the concentrations of 1-octen-3-one may be a differentiating factor.

While 1-octen-3-one appears to play a significant role in the transition from raw to cooked mushroom flavor, it is also possible that the changing concentrations of some of the other major volatile constituents may also play a role.

In this investigation, the presence of five of the flavor compounds of *Agaricus bisporus* identified by Cronin and Ward (1971) was confirmed (3-octanone, 1-octen-3-one, 3-octanol, 1-octen-3-ol, and benzaldehyde). The presence of 2-octen-1-ol, which was tentatively identified as a mushroom component, was demonstrated to be present.

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