High-Resolution Gas Chromatographic Profiles of Volatile Organic Compounds Produced by Microorganisms at Refrigerated Temperatures

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Three different strains of bacteria isolated from spoiled, uncooked chicken were grown in pure culture on Trypticase soy agar supplemented with yeast extract. The volatile organic compounds produced by each culture were concentrated on a porous polymer precolumn and analyzed by high-resolution gas chromatographic mass spectrometry. Twenty different compounds were identified. Both qualitative and quantitative differences in the chromatographic profiles from each culture were found.

At refrigerated temperatures, the spoilage of meat, poultry, and fish is ascribed to microbial action (1, 8, 9, 10) and not to tissue autolysis (8, 9, 16). The prevalent spoilage odor is directly related to the volatile chemicals produced by microbial action. The types of microorganisms present during spoilage are responsible for the chemicals produced and hence the spoilage odor. Furthermore, different conditions favor the growth of different types of microorganisms, which thus affect the nature of the spoilage and the spoilage aroma (2).

In general, the specific activities of microorganisms on fresh meat, poultry, and fish at refrigerated temperatures are poorly understood (10). It seems reasonable to expect, however, that determination of the compounds which characterize spoilage aroma, the environmental conditions under which these compounds are produced, and the microorganisms responsible for the specific compounds in the spoilage aroma would help to further elucidate this microbial spoilage pattern. One approach to such a study is the isolation of the different bacterial strains from a spoiled product and then the collection and identification of the volatile metabolites produced by each of these strains grown separately under controlled conditions on several substrate types. These substrate types could include well-defined and carefully prepared agar plates as well as aseptic muscle tissues. With the information gained from these experiments, one should be able to identify the microorganisms responsible for spoilage of refrigerated meat products by identification of the volatile compounds in the spoilage aroma.

The identifications of a number of highly volatile metabolites produced by microorganisms growing on chicken and fish have recently been reported (4, 7, 11). Although the highly volatile compounds are important to the spoilage aroma, the less volatile components may exert an equally important influence as far as spoilage parameters are concerned. This paper reports the first in a series of studies involving the spoilage aroma of uncooked, refrigerated chicken. Three different strains of bacteria that had previously been isolated from spoiled, uncooked chicken (7) were grown in pure culture on Trypticase soy agar, and the compounds produced by each of these strains were identified. A methodology is described for the preconcentration of spoilage aroma volatiles and subsequent identification of these compounds by high-resolution gas chromatographic mass spectrometry.

MATERIALS AND METHODS

Incubation of cultures. Cultures of Pseudomonas putida, Pseudomonas fluorescens, and a Moraxella oxidative genus, previously isolated from spoiled, uncooked chicken (7), were grown separately on prepared TSY agar plates enclosed in oxygen-permeable, medium-density polyethylene bags to aid in moisture control. The cultures were allowed to incubate for 14 days at 2 to 4°C. The TSY agar medium was composed of 4% Trypticase soy agar (BBL) supplemented with 0.5% yeast extract (Difco).

Collection and analysis of volatiles. After 14 days of incubation, 10 to 20 g of TSY agar containing the specific culture was transferred to a water-jacketed headspace sampling bottle. Purified, dry helium was swept over the agar-bacteria sample for 60 min at a rate of 100 ml/min while the temperature of the water-jacketed sample bottle was held at 60°C. The volatile organic compounds were trapped on 2.0 mg of the
porous polymer Tenax GC, which was packed in a 1-
mm-ID precolumn.

After the sample collection, the precolumn was
inserted into the injection port (held at 250°C) of a
Perkin-Elmer model 3920B series gas chromatograph
equipped with a glass capillary column (80 m by 0.28
mm ID), coated with SF-96 methylallicone stationary
phase and purged with helium carrier gas. The chro-
matographic oven was maintained at -50°C for 5 min
during purging to trap the desorbed compounds in the
first few loops of the capillary column. After trapping
for 5 min, the oven temperature was programmed from
-50 to 20°C at a rate of 8°C/min and then from 20 to
190°C at a rate of 4°C/min to record the chromatog-
raphic profiles. Several TSY agar samples were ana-
lized in the same way to serve as control blanks.
Selective detection for nitrogen-containing com-
ounds was accomplished by splitting the column effluent to both a flame ionization detector and a
nitrogen selective thermionic detector (Perkin-Elmer)
and recording both signals simultaneously.

Identification of compounds was accomplished by
combined gas chromatography-mass spectrometry us-
ing a Hewlett-Packard model 5920A gas chromato-
graph-mass spectrometer and 5934A data system. The
glass capillary column used in this system had the
same specifications as the one used in the Perkin-
Elmer 3920B series gas chromatograph described
above. Electron impact ionization with an ionization
energy of 70 eV was used. Spectra were obtained at
the scan rate of 80 atomic mass units/s.

RESULTS

The compounds identified from the three cul-
tures in this study are listed in Table 1. The
peak number assigned to each compound in
Table 1 can be used to locate the peak of that
specific compound in the illustrated chro-
matograms (Fig. 1 and 2). The chromatogram of
the uninoculated TSY agar is shown in Fig. 1B. The
compounds identified in this blank are benzal-
dehyde, 1-undecene, and 4-methyl-2,6-di-tertiar-
ybutylphenol. The latter compound results from
decomposition of the porous polymer in the
injection port. A very polar compound exhibiting
significant tailing was found in the TSY agar
control but not in the chromatograms produced
by the volatiles of the three cultures.

The chromatogram produced by the volatiles
from P. fluorescens is illustrated in Fig. 1A. The
compound indicated as peak 19 was unique to
this culture. Although this compound was not
identified, its mass spectrum is similar to those
for purines and pteridines. It has a molecular
weight of 222 determined by chemical ionization
mass spectrometry. The compound also gave a
strong response from the nitrogen detector.
Since the molecular weight was even numbered,
the molecule will contain an even number of
nitrogen atoms.

Volatiles produced by the Moraxella oxida-
tive culture led to the chromatogram illustrated
in Fig. 2A. Methylisobutyrate (peak 7), n-bu-
tylacetate (peak 11), and dimethylbenzene (peak
13) were found in this culture but not detected
from the volatiles of the other two cultures.

Figure 2B represents the chromatogram pro-
duced from the separation of the volatiles pro-

<table>
<thead>
<tr>
<th>Peak no.</th>
<th>Compound</th>
<th>TSY agar alone</th>
<th>Culture grown on TSY agar</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>P. putida</td>
</tr>
<tr>
<td>1</td>
<td>Acetone</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>2-Butanone</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Methyl propionate</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Methyl butanal</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Methyl thiolacetate</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Methyl isothiocyanate</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Methyl isobutyrate</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Dimethyl disulfide</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Toluene</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>Methyl-2-methyl butyrate</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>n-Butyl acetate</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td>Methyl pent-2-enoate</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>13</td>
<td>Dimethyl benzene</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>14</td>
<td>Benzaldehyde</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>15</td>
<td>Dimethyl trisulfide</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>16</td>
<td>Methyl benzoate</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>17</td>
<td>2-Nonanone</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>18</td>
<td>1-Undecene</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>19</td>
<td>Unidentified</td>
<td></td>
<td>+</td>
</tr>
</tbody>
</table>
| 20      | 4-Methyl-2,6-di-tertiary-
                          |               | +        | +            | +                     |
                          butylphenol    |               |           |               |                       |
produced by *P. putida*. Methylbenzoate (peak 16) was detected in significant amounts from this culture and was not found in the analysis of the volatiles from the other two cultures. Although 1-undecene (peak 18) was found in the volatiles from the uninoculated TSY agar, it increased significantly in concentration in all three bacteria cultures, which suggests that all three are capable of producing this compound.

Figure 3 illustrates the degree of reproducibility of the chromatograms obtained from two additional samplings of the volatiles generated by the *Moraxella* oxidative culture. All three cultures consistently displayed reproducible chromatograms.

**DISCUSSION**

In an earlier study by Freeman et al. (7), a low-temperature, high-vacuum distillation technique was used to collect a total aroma condensate that was then separated into several fractions and subsequently analyzed by combined gas chromatography-mass spectrometry. Although over 20 compounds were identified, the procedures were time consuming, and only the more volatile constituents were effectively analyzed. On the other hand, the precolumn concentration technique using Tenax GC porous polymer as an adsorbent has been shown to be very efficient for sampling and concentrating volatile
organic compounds from the headspace of a number of samples including body fluids (14, 17), drinking water (5, 6), tobacco leaf aroma (13), and air (3, 15). Tenax GC has an extremely high capacity for the retention of organic compounds and little retention for water. Furthermore, sample collection is simplified to one easy step which requires very minimal and inexpensive equipment. This simplification greatly enhances the reproducibility of comparative analyses as long as all samples are treated in an identical fashion. A detailed discussion of the preconcentration step and the significance of the resultant chromatographic profile is given in an earlier study (12). It is sufficient to say here that the chromatographic profile is a result of a number of factors, including the amount of Tenax in the precolumn, the temperature of the precolumn during sampling, the temperature of the sample during sampling, and the total sampling time. If these parameters are kept constant, very precise measurements can be made, as can be seen by comparison of the chromatograms in Fig. 3.

In comparing results of this study with those reported earlier (7), several generalizations can be made. The cryogenic trapping technique included the identification of several highly vola-
Volatiles from Three Microorganisms

tile compounds that were not effectively concentrated by the precolumn technique. On the other hand, in this study, the analyses were faster and more precise, more compounds were identified per sample, and many less volatile organic compounds were effectively concentrated and identified.

More specifically, only dimethyl disulfide was identified from the P. putida culture growing on TSY agar in the earlier study (7), whereas in the current study dimethyl disulfide was identified in addition to a number of other compounds listed in Table 1.

The volatiles identified from P. fluorescens growing on TSY agar in the earlier study were methyl propionate, methyl thiolacetate, dimethyl disulfide, dimethyl sulfide, hydrogen cyanide, and ethyl acetate. Of these compounds, dimethyl sulfide, hydrogen cyanide, and ethyl acetate were not detected in the current study, but again additional compounds were.

From the volatiles produced by the Moraxella oxidative organisms in the earlier study, 2-propanethiol, ethyl acetate, methyl isopropyl sulfide, and an unidentified compound having a molecular weight of 102 were detected. The first three compounds were not detected in the current study. However, methyl isobutyrate, having

Fig. 3. Capillary-column gas chromatograms illustrating the degree of reproducibility of the volatiles produced by a Moraxella oxidative culture.
a molecular weight of 102, was identified.

In summary, a method has been developed which uses a combination of sample preconcentration, high-resolution gas chromatographic separation, and mass spectrometric identification for the analysis of complex mixtures of organic compounds produced by microorganisms during spoilage. The resultant chromatographic profiles are unique to the microorganisms studied. This methodology is presently being used for the study of compounds produced by microorganisms on uncooked chicken muscle.

ACKNOWLEDGMENTS

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LITERATURE CITED