

# Fungal Infections of Fresh-Cut Fruit Can Be Detected by the Gas Chromatography–Mass Spectrometric Identification of Microbial Volatile Organic Compounds

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## ABSTRACT

There is a large and rapidly growing market for fresh-cut fruit. Microbial volatile organic compounds indicate the presence of fungal or bacterial contamination in fruit. In order to determine whether microbial volatile organic compounds can be used to detect contamination before fruit becomes unmarketable, pieces of cantaloupe, apple, pineapple, and orange were inoculated with a variety of fungal species, incubated at 25°C, then sealed in glass vials. The volatiles were extracted by headspace solid-phase microextraction and analyzed by gas chromatography–mass spectrometry. Forty-five compounds were identified that might serve as unique identifiers of fungal contamination. Fungal contamination can be detected as early as 24 h after inoculation.

Fresh-cut fruit is a large and rapidly growing market consisting of fruit that is peeled, sliced, packaged, and refrigerated. It is more convenient than whole fruit and fresher tasting than canned or frozen fruit; however, it spoils faster than the whole or canned product. This disadvantage can result in more than inconvenience and minor monetary loss to the consumer if human pathogens are present (3).

Preservation technologies for retarding spoilage include chemical preservatives (15), modified atmosphere packaging (4), sterilizing agents (16), biocontrol (use of nonpathogenic microorganisms that compete with and prevent the growth of spoilage organisms) (1, 5), and irradiation (8). Preservatives (for example nisin-EDTA (15)) might change the fruit's taste or appearance and would have to be labeled, so they are rarely used. Modified atmosphere (4) packaging has good consumer acceptance and is widely used, as are sterilizing agents such as chlorine and hydrogen peroxide (16). Sterilizing agents need not be labeled because they are not in the product as sold.

Several authors (7, 9, 10) have proposed that microbial volatile organic compounds (MVOCs) can be used to detect and identify species of fungi. Schnürer et al. (14) proposed the use of fungal volatiles as indicators of food and feed spoilage. The purpose of this article is to extend this research to the rapid detection of spoilage organisms in fresh-cut fruit. Ultimately, our goal is to detect fungal or bacterial contamination before the fruit becomes unmarketable. The impact of this is twofold: first as an aid to research into new preservation technologies and second as a useful tool in the processing plant. A plant manager could use results from this technique to determine the source of contamination

or to direct contaminated but edible fruit to a canning process.

## MATERIALS AND METHODS

Cantaloupes (*Cucumis melo* L. var. *cantalupensis*), apples (*Malus domestica* Borkh. var. *gala*), potatoes (var. *Russet*), pineapples (*Ananas comosus* (L.) Mett. var. *gold*), and oranges (*Citrus sinensis* var. *navel*) were obtained from local supermarkets. Fungal cultures were obtained from the collection of the Southern Regional Research Center. The species of fungi used are listed in Table 1. Fungi were chosen from lists of pathogens found in Farr et al. (6). The chosen fungi are representative of postharvest pathogens, not ones that infect the plant or fruit prior to harvest. Chemicals were purchased from J. T. Baker (Phillipsburg, N.J.), Difco Laboratories (Sparks, Md.), Sigma-Aldrich (St. Louis, Mo.), TCI America (Portland, Ore.), and Wako Fine Chemicals (Richmond, Va.). Dr. Jocelyn Millar, University of California, provided sesquiphellandrene and zingiberene. Potato dextrose agar, Czapek yeast autolysate, and yeast extract sucrose were made in the laboratory.

Conidial suspensions were made in an aqueous inoculation medium (12) using 7- to 14-day-old cultures grown on potato dextrose agar slants. Spore concentrations were determined using a hemacytometer (Hausser Scientific, Horsham, Pa.). Counts ranged from  $5.0 \times 10^4$  to  $3.8 \times 10^7$  CFU/ml.

Whole fruits were soaked in a 5% bleach solution for 5 min. The fruit was removed from the bleach solution and placed on the alcohol-sterilized surface of a laminar flow hood (Nuair, Inc., Minneapolis, Minn.). Fruit was sliced, and portions weighing approximately 1 g were removed with a sterile cork borer and placed in sterilized glass vials (10-ml clear vial, beveled crimp; Microliter Analytical Supplies, Inc., Suwanee, Ga.). Agar slants were made in the same sterilized vials using Czapek yeast autolysate and yeast extract sucrose. Slants, fruit, and an empty vial were inoculated with 50  $\mu$ l of inoculation media. Each treatment was replicated three times. Vials were covered with aluminum foil and incubated at room temperature for time periods ranging from 1 to

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TABLE 1. Fruit and fungi used in this research

| Apple<br>( <i>Malus domestica</i> )<br>gala | Cantaloupe<br>( <i>Cucumis melo</i> )<br>cantalupensis | Orange<br>( <i>Citrus sinensis</i> )<br>navel | Pineapple<br>( <i>Ananas comosus</i> )<br>gold |
|---|--|---|--|
| <i>Aspergillus clavatus</i>                 | <i>Alternaria alternate</i>                            | <i>Penicillium brevicompactum</i>             | <i>Fusarium subglutinans</i>                   |
| <i>Cladosporium cladosporioides</i>         | <i>Cladosporium cladosporioides</i>                    | <i>Penicillium italicum</i>                   |  |
| <i>Penicillium expansum</i>                 | <i>Fusarium oxysporum</i>                              | <i>Trichoderma viride</i>                     | <i>Trichoderma viride</i>                      |
|   | <i>Rhizopus oryzae</i>                                 |   |  |
| <i>Rhizopus stolonifer</i>                  | <i>Rhizopus stolonifer</i>                             | <i>Rhizopus stolonifer</i>                    | <i>Rhizopus stolonifer</i>                     |

4 days. After incubation, an internal standard (5  $\mu$ l 10 mg/liter aqueous solution of *cis*-decahydro-1-naphthol [Chemical Abstract Service number: 36159-47-4; Sigma-Aldrich]) was added and the vials were sealed with crimp caps (20-mm alumin large opening with natural Teflon-blue silicon, Microliter Analytical Supplies, Inc.). Volatile compounds were extracted and analyzed using automated solid-phase microextraction-gas chromatography-mass spectrometry.

A Combi-Pal autosampler (Leap Technologies, Carrboro, N.C.) was used for the automated solid-phase microextraction analysis of the samples. Vials were heated to 65°C and agitated at 500 rpm for 15 min. Volatiles were extracted from the headspace onto a 1-cm divinylbenzene/carboxen/polydimethylsiloxane fiber (57329-U, Supelco, Inc. Bellefonte, Pa.) for 15 min. The fiber was desorbed for 1 min in the injection port at 270°C and baked for 2.5 min in a needle heater at 270°C between samples. An Agilent 6890 gas chromatograph (Agilent, Inc., Palo Alto, Calif.) was equipped with a low polarity capillary column (30-m long, 0.25-mm internal diameter, 1.0- $\mu$ m-thick 5%-phenyl-95%-dimethylpolysiloxane phase, ZB-5; Phenomenex, Torrance, Calif.). Helium carrier gas flowed through the column at a constant 40 cm/s except during the 1-min pulsed splitless desorption pe-

riod, when the head pressure was maintained at 172 kPa. Column temperature was held for 1 min at 50°C, then increased to 100°C at 5°C/min, then to 200°C at 10°C/min, then to 270°C at 25°C/min, and held for 3 min. An Agilent 5973 mass selective detector was used for volatile detection and identification. The scan range was from *m/z* 33 to 300, and 5.24 scans were collected per second.

Compounds were initially identified by library match (Wiley Registry of Mass Spectral Data 7th edition with NIST98 Spectra, Palisade Corporation, Newfield, N.Y.). Reference standards and extracts were analyzed under the same conditions to confirm the library identification. The esters (methyl R-[+]-citronellate, methyl S-[-]-citronellate, ethyl R-[+]-citronellate, ethyl S-[-]-citronellate, and methyl 2,4-hexadienoate) were synthesized from R-(+)-citronellic acid, S(-)-citronellic acid, and 2,4-hexadienoic acid, respectively, by acid catalyzed esterification with methyl or ethyl alcohols using a procedure modified from that of Rodríguez-Ruiz et al. (13). One milliliter of each alcohol containing 5% by volume acetyl chloride was mixed with 0.5 ml of hexane and 50 to 150 mg of each organic acid in screw-top glass tubes (100 by 13 mm). The tubes were sealed with Teflon lined caps and heated at 100°C for 10 min. After cooling, 1 ml of water was added and the tube was inverted several times. The hexane layer was re-

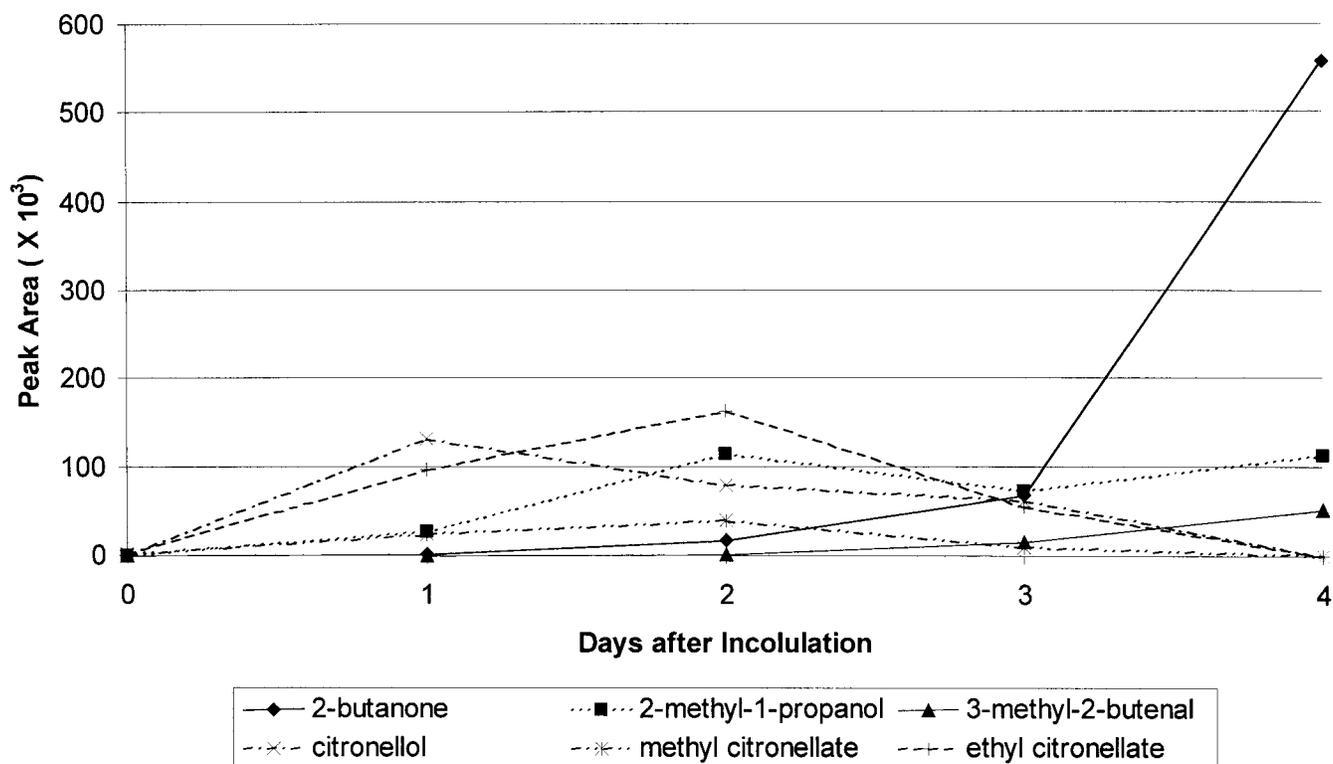


FIGURE 1. Change in relative concentrations of six microbial volatile organic compounds from cantaloupe pieces inoculated with *Rhizopus stolonifer*.

TABLE 2. Microbial volatile organic compounds from fruit inoculated with fungi

| ID <sup>a</sup> | Compound                      | R.T. <sup>b</sup> | CAS no. <sup>c</sup> | Class    | Fruit <sup>d</sup> | Fungus <sup>e</sup> |
|-----------------|-------------------------------|-------------------|----------------------|----------|--------------------|---------------------|
| 1               | 1-Propanol                    | 2.50              | 71-23-8              | Alcohol  | A                  | R.s.                |
| 2               | 2,3-Butanediol                | 2.98              | 513-85-9             | Alcohol  | A, P               | R.s.                |
| 3               | 2,3-Butanedione               | 2.98              | 431-03-8             | Ketone   | P                  | T.v.                |
| 4               | 2-Butanone                    | 3.13              | 78-93-3              | Ketone   | A, C, O, P         | R.s.                |
| 5               | 2-Methyl-propanol             | 3.67              | 78-83-1              | Alcohol  | A, C, O, P         | R.s., Fo., T.v.     |
| 6               | 3-Methyl-butanal              | 4.30              | 590-86-3             | Aldehyde | O                  | Pb.                 |
| 7               | 2-Methyl-butanal              | 4.53              | 96-17-3              | Aldehyde | O                  | Pi.                 |
| 8               | 2,3-Pentanedione              | 5.21              | 600-14-6             | Ketone   | C                  | C.c.                |
| 9               | 3-Hydroxy-2-butanone          | 5.64              | 513-86-0             | Ketone   | A                  | Pe.                 |
| 10              | 2-Methyl-1-butanol            | 6.27              | 137-32-6             | Alcohol  | P                  | Fs.                 |
| 11              | 3-Methyl-butanol              | 6.28              | 123-51-3             | Alcohol  | C                  | Fo., R.o.           |
| 12              | 2,3-Hexanedione               | 7.77              | 3848-24-6            | Ketone   | C                  | C.c.                |
| 13              | 3-Methyl-2-butenal            | 7.83              | 107-86-8             | Aldehyde | C                  | R.s.                |
| 14              | 2-Hexenal                     | 10.03             | 6728-26-3            | Aldehyde | A                  | C.c.                |
| 15              | 4-Methyl-3-penten-1-ol        | 10.25             | 763-89-3             | Terpene  | A, O               | R.s.                |
| 16              | 2-Heptanone                   | 11.28             | 110-43-0             | Ketone   | C                  | Fo.                 |
| 17              | Styrene                       | 11.54             | 100-42-5             | Aromatic | O                  | Pb.                 |
| 18              | Methyl 2,5-hexadienoate       | 13.09             | 5889-42-9            | Ester    | O                  | Pi.                 |
| 19              | (E)-2-Heptenal                | 13.28             | 18829-55-5           | Aldehyde | A                  | Pe.                 |
| 20              | 2-Nonanol                     | 16.48             | 628-99-9             | Alcohol  | P                  | Fs.                 |
| 21              | Benzeneethanol                | 16.98             | 60-12-8              | Aromatic | O                  | T.v.                |
| 22              | Benzoic acid                  | 17.64             | 65-85-0              | Aromatic | A                  | C.c.                |
| 23              | Ethyl benzoate                | 18.01             | 93-89-0              | Ester    | O                  | Pb.                 |
| 24              | $\beta$ -Citronellol          | 18.82             | 106-22-9             | Terpene  | C                  | R.o., R.s.          |
| 25              | 4-(2-Propenyl)-phenol         | 19.26             | 501-92-8             | Alcohol  | P                  | T.v.                |
| 26              | cis-Geraniol                  | 19.27             | 106-24-1             | Terpene  | O                  | Pi.                 |
| 27              | Methyl citronellate           | 19.31             | 2270-60-2            | Terpene  | C, P               | R.s.                |
| 28              | Ethyl 3-(acetyloxy)-hexanoate | 19.45             | 21188-61-4           | Ester    | O                  | T.v.                |
| 29              | Ethyl citronellate            | 20.38             |                      | Terpene  | C, O, P            | R.o., R.s.          |
| 30              | Theaspirane                   | 20.40             | 36431-72-8           | Terpene  | O                  | Pi.                 |
| 31              | $\alpha$ -Cedrene             | 21.68             | 469-61-4             | Terpene  | O                  | Pb.                 |
| 32              | <i>t</i> -Caryophyllene       | 22.12             | 87-44-5              | Terpene  | A                  | C.c.                |
| 33              | $\beta$ -Cedrene              | 22.22             | 546-28-1             | Terpene  | C                  | A.a.                |
| 34              | Thujopsene                    | 22.29             | 470-40-6             | Terpene  | C                  | A.a.                |
| 35              | $\gamma$ -Curcumene           | 22.55             |                      | Terpene  | C, O               | Fo., T.v.           |
| 36              | $\beta$ -Himachalene          | 22.56             | 1461-03-6            | Terpene  | O                  | Pb.                 |
| 37              | $\alpha$ -Murolene            | 22.61             | 31983-22-9           | Terpene  | A                  | A.c.                |
| 38              | Zingiberene                   | 22.63             | 495-60-3             | Terpene  | O, P               | T.v.                |
| 39              | $\beta$ -Bisbolene            | 22.76             | 495-61-4             | Terpene  | A                  | Pe.                 |
| 40              | $\beta$ -Sesquiphellandrene   | 22.93             | 20307-83-9           | Terpene  | O, P               | T.v.                |
| 41              | Longiborneol                  | 23.83             | 465-24-7             | Terpene  | C                  | Fo.                 |
| 42              | $\alpha$ -Elemene             | 23.89             | 5951-67-7            | Terpene  | P                  | T.v.                |
| 43              | Ethyl hexadecanoate           | 26.11             | 112-39-0             | Ester    | P                  | Fs.                 |

<sup>a</sup> ID, identification, used for labels in Figure 2.

<sup>b</sup> R.T., retention time.

<sup>c</sup> CAS no., Chemical Abstract Service number.

<sup>d</sup> A, apple; C, cantaloupe; O, orange; P, pineapple.

<sup>e</sup> A.a., *Alternaria alternata*; A.c., *Aspergillus clavatus*; C.c., *Cladosporium cladosporioides*; Fo., *Fusarium oxysporum*; Fs., *Fusarium subglutinans*; Pb., *Penicillium brevicompactum*; Pe., *Penicillium expansum*; Pi., *Penicillium italicum*; R.o., *Rhizopus oryzae*; R.s., *Rhizopus stolonifer*; T.v., *Trichoderma viride*.

moved. One microliter was added to 6 ml of salt-saturated water in a 10-ml crimp-top vial and analyzed by solid-phase microextraction–gas chromatography–mass spectrometry.

Volatile chemical abundance was measured by recording the area under the peak of a unique ion for each chemical. The abundances were corrected for day-to-day variation in mass spectrometer response by using the *m/z* 136 ion from decahydro-naphthol.

## RESULTS AND DISCUSSION

Total ion chromatograms from fruit analyzed 4 days after inoculation were compared with total ion chromatograms from uninoculated fruit. We found a total of 43 compounds that might serve as indicators of fungal contamination because they were present in the inoculated fruit but not in the uninoculated controls. Chemical classes repre-

TABLE 3. Microbial volatile organic chemicals identified in fruits inoculated with fungi; each chemical is found in inoculated fruit but not in the control

| Apple  | Cantaloupe  | Orange  | Pineapple   |
|--|---|---|---|
| <i>Aspergillus clavatus</i><br>α-Muurolene   | <i>Alternaria alternata</i><br>β-Cedrene <sup>a</sup><br>Thujopsene <sup>a</sup>  | <i>Penicillium brevicompactum</i><br>3-Methyl-butanal <sup>a</sup><br>Styrene <sup>a</sup><br>Ethyl benzoate <sup>a</sup><br>α-Cedrene <sup>a</sup><br>β-Himachalene  | <i>Fusarium subglutinans</i><br>2-Methyl-1-butanol <sup>a</sup><br>2-Nonanol <sup>a</sup><br>Ethyl hexadecanoate <sup>a</sup>   |
| <i>Penicillium expansum</i><br>3-Hydroxy-2-butanone <sup>a</sup><br>3-Methyl-phenol <sup>a</sup><br>β-Bisabolene <sup>b</sup><br>( <i>E</i> )-2-Heptenal <sup>a</sup>                  | <i>Fusarium oxysporum</i><br>2-Methyl-propanol <sup>a</sup><br>3-Methyl-butanol <sup>a</sup><br>2-Heptanone <sup>a</sup><br>γ-Curcumene<br>Longiborneol   | <i>Penicillium italicum</i><br>3-Methyl-butanal <sup>a</sup><br>2-Methyl-butanol <sup>a</sup><br>Methyl 2,5-hexadienoate<br><i>cis</i> -Geraniol <sup>a</sup><br>Theaspirane <sup>a</sup>                     |   |
| <i>Cladosporium cladosporioides</i><br>2-Hexenal <sup>a</sup><br>Benzoic acid <sup>a</sup><br><i>t</i> -Caryophyllene <sup>a</sup>   | <i>Cladosporium cladosporioides</i><br>2,3-Pentanedione <sup>a</sup><br>2,3-Hexanedione <sup>a</sup>  | <i>Trichoderma viride</i><br>2-Methyl-1-propanol <sup>a</sup><br>Benzeneethanol <sup>a</sup><br>Ethyl 3-(acetyloxy)-hexanoate<br>γ-Curcumene<br>Zingiberene <sup>b</sup><br>β-Sesquiphellandrene <sup>b</sup> | <i>Trichoderma viride</i><br>2-Methyl-1-propanol <sup>a</sup><br>2,3-Butanedione <sup>a</sup><br>4-(2-Propenyl)-phenol<br>α-Elementene<br>Zingiberene <sup>b</sup><br>β-Sesquiphellandrene <sup>b</sup> |
|  | <i>Rhizopus oryzae</i><br>3-Methyl-butanol <sup>a</sup><br>Ethyl citronellate <sup>a</sup><br>β-Citronellol <sup>a</sup>  |   |   |
| <i>Rhizopus stolonifer</i><br>2-Butanone <sup>a</sup><br>Ethyl citronellate <sup>a</sup><br>2-Methyl-1-propanol <sup>a</sup><br>1-Propanol <sup>a</sup><br>2,3-Butanediol <sup>a</sup> | <i>Rhizopus stolonifer</i><br>2-Butanone <sup>a</sup><br>Ethyl citronellate <sup>a</sup><br>2-Methyl-1-propanol <sup>a</sup><br>3-Methyl-2-butenal <sup>a</sup><br><br>Methyl citronellate <sup>a</sup><br>β-Citronellol <sup>a</sup> | <i>Rhizopus stolonifer</i><br>2-Butanone <sup>a</sup><br>Ethyl citronellate <sup>a</sup>  | <i>Rhizopus stolonifer</i><br>2-Butanone <sup>a</sup><br>Ethyl citronellate <sup>a</sup><br><br>2,3-Butanediol <sup>a</sup><br>Methyl citronellate <sup>a</sup>   |
| 4-Methyl-3-penten-1-ol <sup>a</sup>  |   | 4-Methyl-3-penten-1-ol <sup>a</sup>   |   |

<sup>a</sup> Confirmed by analysis of authentic compound.

<sup>b</sup> Confirmed by analysis of extract.

sented include alcohols, aldehydes, ketones, aromatics, esters, and terpenes (Table 2). For each fruit, at least one compound is unique to each fungus (Table 3). The best candidates for identification are those that are completely unique to a fungal infection on a particular fruit. Since many fungi exist, more work needs to be done to identify volatile chemicals indicative of a particular fungus.

The best candidates for markers of fungal infection are those produced early in the infection. Of the 43 candidate compounds, all but 11 were detected 1 day after inoculation. The exceptions were benzoic acid (apple–*Cladosporium cladosporioides*); β-bisabolene (apple–*Penicillium expansum*); 2-methyl-propanol (cantaloupe–*Fusarium oxysporum*); ethyl citronellate and β-citronellol (cantaloupe–*Rhizopus oryzae*); 3-methyl-butanol, α-cedrene, and β-himachalene (orange–*Penicillium brevicompactum*); and methyl 2,5-hexadienoate, geraniol, and theaspirane (orange–*Penicillium italicum*). Interestingly, ethyl citronellate appeared in the day 0 or 1 samples of all four fruits inoculated with *Rhizopus stolonifer*, which grew more rapidly than the other fungi.

In order to determine whether volatiles come from the fruit or the fungus, comparisons were made between fruits inoculated with the same fungus. 2-Butanone and ethyl citronellate were found in the headspace of all fruits inoculated with *R. stolonifer*. 2-Butanone is also present in the *R. stolonifer* inoculum and in the headspace over cultures of *R. stolonifer* growing on yeast extract sucrose and Czapek yeast autolysate agar. Acetophenone is also present in both agar cultures, inoculum and zero time cantaloupe samples. It is present in control cantaloupe samples (in concentrations smaller than present in fruit or agar samples), so it does not qualify as a marker of fungal contamination of cantaloupe. This indicates that both 2-butanone and acetophenone are produced by *R. stolonifer* regardless of substrate. Ethyl citronellate is not present in the headspace over agar cultures, but it is produced by *R. stolonifer* on all four fruits studied here. Other MVOCs produced by *R. stolonifer* in more than one of the fruits examined include 2-methyl-1-propanol (in both apple and cantaloupe), methyl citronellate (cantaloupe and pineapple), and 4-methyl-3-pentenol (apple and orange).

TABLE 4. Volatile esters present in cantaloupe

| ID <sup>a</sup> | Compound                      | R.T. <sup>b</sup> | CAS no. <sup>c</sup> |
|-----------------|-------------------------------|-------------------|----------------------|
| 44              | Ethyl acetate                 | 3.45              | 141-78-6             |
| 45              | 2-Methylpropyl acetate        | 7.45              | 110-19-0             |
| 46              | Ethyl butanoate               | 8.27              | 105-54-4             |
| 47              | Butyl acetate                 | 8.71              | 123-86-4             |
| 48              | Ethyl 2-methyl-butanoate      | 9.93              | 7452-79-1            |
| 49              | 2-Methylbutyl acetate         | 10.86             | 624-41-9             |
| 50              | Ethyl hexanoate               | 14.28             | 123-66-0             |
| 51              | <i>cis</i> -3-Hexenyl acetate | 14.46             | 3681-71-8            |
| 52              | Hexyl acetate                 | 14.57             | 142-92-7             |
| 53              | Phenylmethyl acetate          | 17.84             | 140-11-4             |
| 54              | Ethyl <i>cis</i> -4-octenoate | 18.10             | 34495-71-1           |
| 55              | Ethyl octanoate               | 18.22             | 106-32-1             |
| 56              | Octyl acetate                 | 18.46             | 112-14-1             |
| 57              | 2-Phenylethyl acetate         | 19.44             | 103-45-7             |
| 58              | 3-Phenylpropyl acetate        | 21.18             | 122-72-5             |
| 59              | Ethyl decanoate               | 21.28             | 110-38-3             |

<sup>a</sup> ID, identification (continued from Table 3), used for labels in Figure 2.

<sup>b</sup> R.T., retention time.

<sup>c</sup> CAS no., Chemical Abstract Service number.

Two fruits (apple and cantaloupe) were inoculated with *C. cladosporioides*, and two (orange and pineapple) were inoculated with *Trichoderma viride*. Apple and cantaloupe inoculated with *C. cladosporioides* exhibited no inoculation-unique volatiles in common. Both orange and pineapple inoculated with *T. viride* produced 2-methyl-propanol, zingiberene, and  $\beta$ -sesquiphellandrene. These three compounds are not found in *T. viride* inoculation media, but they are found in both agar cultures.

The MVOCs are produced either by the fruit (in response to the presence of the fungus or in response to injury) or by the fungus itself. Fungi produce different volatiles when growing on different substrates. The MVOCs that we chose as unique indicators of fungal infection were not present in uninoculated controls; therefore, they are not produced by the fruit in response to injury alone. It appears from these data that 2-butanone and acetophenone are produced by *R. stolonifer* independent of substrate. Conversely, the *C. cladosporioides*-produced volatiles examined here are substrate dependent. *T. viride* produces three volatiles (2-methyl-1-propanol, zingiberene, and  $\beta$ -sesquiphellandrene) independent of substrate and several other volatiles (butyl 3-hydroxy-butanoate, 2,3-butanedione, benzeneethanol, ethyl 3-[acetyloxy]-hexanoate, 4-[2-propenyl]-phenol,  $\gamma$ -curcumene, and  $\alpha$ -elemene) that are substrate dependent. 2-Hexenal (which is produced in apple inoculated with *C. cladosporioides*) has been reported to inhibit the growth of *Botrytis cineria* on strawberries, blackberries, and grapes (1). Apples may produce this compound as a defense against infection by *C. cladosporioides*.

Because no standard curves were analyzed, absolute quantification is not possible. Semiquantitative data can be used to compare relative trends. There is no consistent pattern to how the amounts of the volatiles present in the headspace change over time. Concentration (as measured by abundance of extracted ions) can increase from day 1 to day 4 or peak at any time and then decrease. A few compounds were found in the day 0 treatment but not in the uninoculated control. This indicates that some volatile production is so rapid that it takes place even in sealed vials in the 2 to 8 h between inoculation and analysis while the

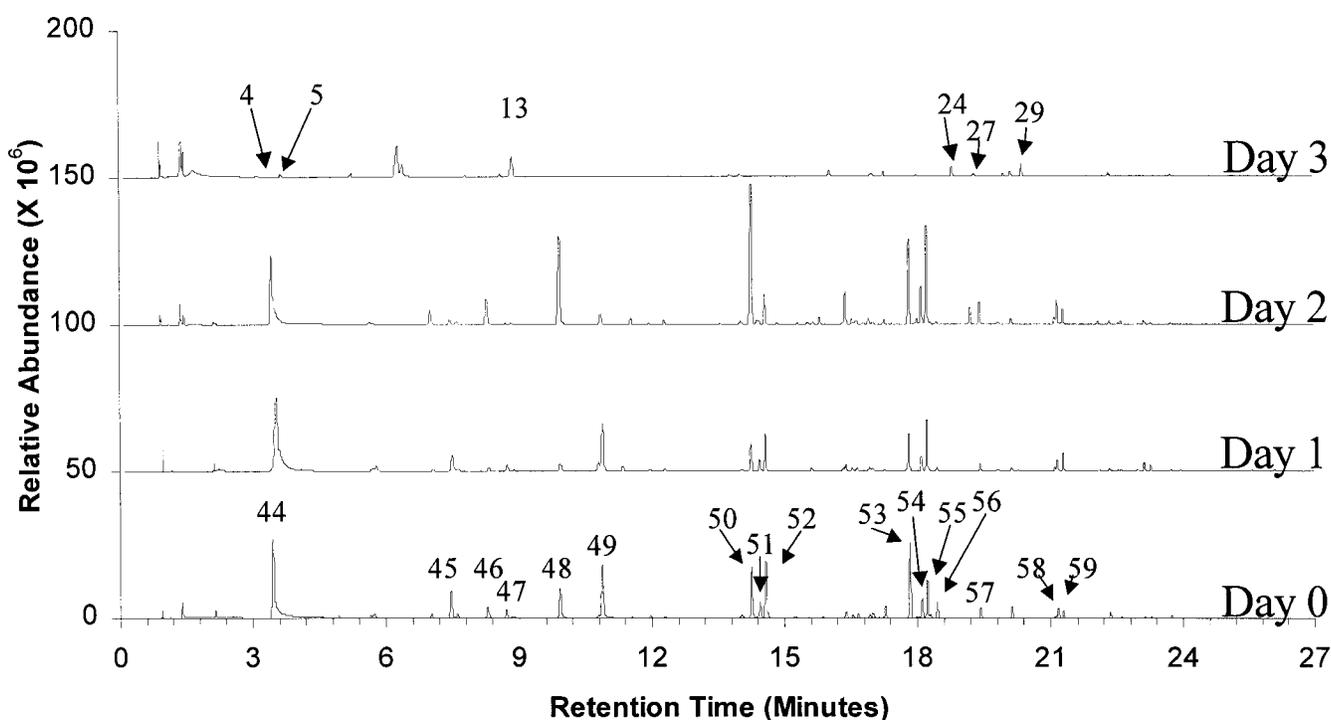


FIGURE 2. Total ion chromatograms from the solid-phase microextraction–gas chromatography–mass spectrometry analysis of the headspace over cantaloupe pieces inoculated with *Rhizopus stolonifer*. Each chromatogram is offset by  $50 \times 10^6$  units for clarity.

sealed vials were sitting on the autosampler at room temperature. Figure 1 illustrates these observations. 2-Butanone rises from day 1 to day 4, while citronellol peaks at day 1 and then decreases until the end. Ethyl and methyl citronellate were both found in small amounts (2,227 and 932 area counts, respectively) in the day 0 samples.

Volatile compounds from the fruit are also detected by this method. These are not MVOCs, but their concentrations are effected by the presence of fungi. They appear to be consumed or broken down by the fungus or by the fruit in response to the fungus. In cantaloupe inoculated with *R. stolonifer*, the compounds listed in Table 4 (previously identified as contributors to cantaloupe flavor (2, 11)) are greatly reduced or entirely absent 3 days after inoculation (Fig. 2). These same compounds did not decrease in the uninoculated fruit during the same time period.

Compounds such as 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol, and 1-octen-3-ol have been reported from many fungal cultures (7, 9). The propanol and butanols are found in several different fruits inoculated by different fungi, but 1-octen-3-ol was not found in any of the inoculated fruits.

Fungal growth on fresh-cut fruit can be detected by this technique. With the limited number of fungi considered here, the species of fungus can be identified by MVOC analysis as long as the fruit is known. Only one fruit of each type was sampled, and only one strain of fungus from each species was used. Before these results can be used in a commercial or regulatory application, a broader investigation using a range of fruit varieties and fungal strains must be performed. Further investigations will also examine naturally occurring fungal infections. The relationship between the number of CFUs and the concentration of MVOCs in the headspace must be determined to ensure identification of contaminated fruit before it is unsafe to eat.

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