Taylor, S. L.; Lieber, E. R.; Leatherwood, M. *J. Food Sci.* **1978**, *43*, 247.
Turner, A. *Food Manuf.* **1960**, *35*, 386.
Wortberg, B.; Woller, R. *Fleischwirtschaft* **1982**, *62*, 1457.
Yamamoto, S.; Itano, H.; Kataoka, H.; Makita, M. *J. Agric. Food Chem.* **1982**, *30*, 435.

Received for review February 21, 1984. Revised manuscript received December 20, 1984. Accepted February 14, 1985. This study was supported by the Deutsche Forschungsgemeinschaft.

Volatile Compounds from *Pencillium sp.* Contributing Musty–Earthy Notes to Brie and Camembert Cheese Flavors

Carol Karahadian, David B. Josephson, and Robert C. Lindsay*

Volatile headspace compounds collected on Tenax GC at room temperature (21 °C) from pure cultures of *Penicillium caseicolum* and *Penicillium camemberti* grown on potato dextrose and Czapek's agar were separated by gas chromatography and identified by mass spectrometry, retention indices (I_E), and odor quality. *P. camemberti* and *P. caseicolum* produced similar volatile compound profiles. Mushroom-like, green plant-like aromas were contributed by 1-octen-3-ol, 1,5-octadien-3-ol, 1,5-octadien-3-one, 3-octanol, and 3-octanone. 2-Methylisoborneol contributed strongly to the overall musty/moldy component of aromas of *Penicillium* cultures. 2-Methoxy-3-isopropylpyrazine was found in aged mold cultures (4–6 weeks) only and caused these cultures to exhibit intense earthy/raw potato aromas.

INTRODUCTION

Surface-ripened cheeses involving extensive mold growth during ripening, especially Brie and Camembert, long have been popular in European countries. Although early production of Camembert cheese was accomplished through the use of *Penicillium camemberti* (Thom and Fisk, 1918), current production of both Camembert and Brie cheeses is largely achieved by employing *Penicillium caseicolum* (Kosikowski, 1982). Very limited information exists concerning the biochemical differentiation of these two species of *Penicillium* (Raper and Thom, 1949), but morpologically *P. caseicolum* exhibits a white mycelial mat while *P. camemberti* yields a light tan to greyish mat because of the production of slightly colored conidia.

European consumers generally prefer extensively aged Brie and Camembert cheeses whose flavors reflect not only primary lactic acid fermentations and mold contributions, but also metabolites of secondary surface ripening by yeasts, *Brevibacterium linens*, and some related coryneforms (Olson, 1969; Greenberg and Ledford, 1978; Law, 1982; Rousseau, 1984). Recent interest in these varieties of cheeses by U.S. consumers, however, has stemmed from an acceptance of the less pronounced but nutty, mushroom-like flavors that are found in modestly aged cheeses. Moinas et al. (1973, 1975) and Dumont et al. (1974b, 1976) have identified a substantial number of volatiles in aging Camembert cheeses of French origin, and both groups of researchers concluded that 1-octen-3-ol was a major characterizing compound in the flavor of younger cheeses. A number of alcohol, ester, and sulfur compound metabolites was believed to characterize extensively aged Camembert flavors.

Critical evaluation of the aroma and flavors of both mold cultures and modestly aged Brie and Camembert cheeses revealed that additional musty–earthy notes were present besides the distinctly raw mushroom-like aroma of 1-octen-3-ol. Information concerning the identity of compounds with earthy–mushroom-like quality that are produced by molds and mushrooms is generally limited to saturated or monounsaturated eight-carbon primary and secondary alcohols and corresponding carbonyl compounds (Cronin and Ward, 1971; Kaminski et al., 1972, 1974; Moinas et al., 1973; Dumont et al., 1974b; Halim et al., 1975; Maga, 1981; Tressel et al., 1982). Some claims have been made that certain alkyl benzenes produced by *Penicillium roqueforti* in Blue and Roquefort cheeses contribute musty aromas (Boyd et al., 1965; Day, 1967; Law, 1982), but these claims are largely unsubstantiated and have not been analytically confirmed. Therefore, the objective of this research was to investigate the volatile compounds produced by cultures of *P. camemberti* and *P. caseicolum*, particularly those capable of contributing musty–earthy notes to the flavors of Brie and Camembert cheeses.

MATERIALS AND METHODS

Cultures of *P. caseicolum* no. 874 and *P. camemberti* no. 877 were obtained from the collection of K. B. Raper (Bacteriology Department, University of Wisconsin, Madison, WI) and cultures of *P. caseicolum* ATCC no. 6986 and *P. camemberti* ATCC no. 6985 were obtained from the American Type Culture Collection (Rockville, MD). One culture of *P. caseicolum* was propagated from an isolate obtained recently from a commercial, domestically produced Brie cheese.

Pure cultures of each *Penicillium sp.* were grown on sterile acidified potato dextrose agar slants or Czapek's agar slants (100 mL, Difco Laboratories, Detroit, MI) held in loosely capped 900-mL glass prescription bottles at 17 ± 1 °C. Headspace volatiles produced by young (5–12 days) and mature (30–45 days) cultures were collected during 3- and 24-h periods by purging the headspace of bottles containing the mold cultures at room temperature (21 °C) with a stream of humidified air at a rate of 240 mL/min. Humidified air was produced by bubbling the air stream through 5 mL of sterile water placed in the

Department of Food Science, University of Wisconsin, Madison, Wisconsin 53706.

0021-8561/85/1433-0339$01.50/0

bottom of each vertically positioned mold culture bottle in the entrainment apparatus, and this water contained 10 μg of 4-decanol as an internal standard (Aldrich Chemical Co., Milwaukee, WI). Volatile compounds were collected onto two tandem Tenax GC traps (80 mg each, 60–80 mesh, product of ENKA N.V., Holland) which were prepared as described by Steinke (1978) and Josephson et al. (1983). Volatile compounds were eluted from individual traps with approximately 1 mL of diethyl ether each, and samples were concentrated to about 15 μL under a slow stream of nitrogen.

Diethyl ether extracts were separated with a Varian 1740 gas chromatograph (Varian Associates, Palo Alto, CA). Odor qualities of eluting compounds (split ratio 100:1 in favor of exit port) were assessed. Separations for odor assessment were achieved with a 3 m × 2 mm id silane-deactivated glass column packed with 7% Carbowax 20M on 80–100 mesh Chromosorb W AW/DMCS that was programed from 50 to 200 °C at 2 °C/min.

Capillary column gas chromatography–mass spectrometry was performed with a Finnigan 4021 GC–MS as reported by Josephson et al. (1983) with a Supelcowax-10 (permanently bonded Carbowax 20M, 60 m × 0.25 mm id) fused silica column (Supelco, Inc., Bellefonte, PA). The column was operated with helium carrier gas (head pressure 10 psi, split; 10 mL/min, sweep; 5 mL/min), and a program rate of 6 °C/min from 50 to 140 °C followed by a rate of 10 °C/min from 140 to 220 °C was employed. Additionally, a Carbowax 20M (30 m × 0.25 mm id) fused silica column (J & W Scientific, Inc., Rancho Cordova, CA) was employed using an isothermal hold at 50 °C for 5 min, then a program rate of 5 °C/min from 50 to 140 °C, and finally a rate of 10 °C/min from 140 to 220 °C.

Compounds were identified by computer matching of experimental mass spectra of compounds with those published in "EPA/NIH Mass Spectral Data Base" (Heller and Milne, 1975, 1980) as well as by manual matching with published mass spectral data. Coincidence of retention indices (I_E; Van den Dool and Kratz, 1963) for unknown and authentic compounds and where possible agreement of aromas of eluting compounds were also employed in assigning identities of compounds.

RESULTS AND DISCUSSION

Profiles of volatile compounds from surface cultures of *P. camemberti* and *P. caseicolum* were qualitatively similar to each other on both potato dextrose agar and Czapek's agar at all stages of growth. The molds were grown on the sterile media to allow segregation of volatile metabolites produced by the molds from those arising from other microflora existing on ripening cheeses. This approach also allowed exclusion of obscuring volatile compounds arising from the action of molds on milk constituents, particularly lipids (Gehrig and Knight, 1963; Hawke, 1966; Kinsella and Hwang, 1976). Analysis of uninoculated control media samples allowed determination of compounds arising from either the media or mold metabolism. Czapek's agar was employed to assure that methoxy alkyl pyrazines were not contributed by the potato ingredient of the potato dextrose agar (Buttery and Ling, 1973).

Raper and Thom (1949) have suggested that *P. caseicolum* may be a colorless mutant of *P. camemberti* because the two molds appear to differ morphologically mainly by the tan to greyish conidia of *P. camemberti* compared to the white conidia for *P. caseicolum*. The similarity in their abilities to produce the volatile compounds observed in this study supports the view that these two species of molds are very closely related. Because of the lack of differentation in qualitative profiles of volatile compounds between

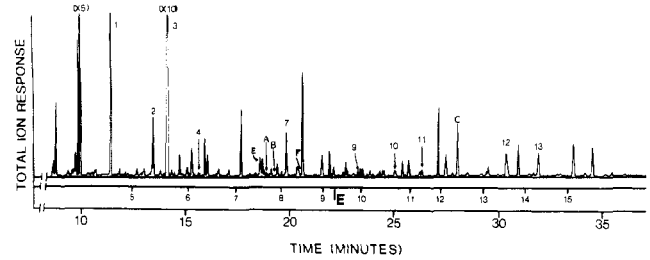

Figure 1. Gas chromatogram of headspace volatiles produced by a young culture of *Penicillium caseicolum* grown on acidified potato dextrose agar and purged for 3 h with a fused silica bonded 60-m Carbowax capillary column. The identities of numbered peaks are listed in Table I.

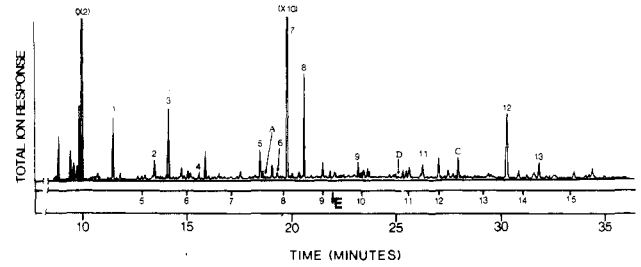

Figure 2. Gas chromatogram of headspace volatiles produced by a mature culture of *Penicillium caseicolum* grown on acidified potato dextrose agar and purged for 3 h with a fused silica bonded 60-m Carbowax 20M capillary column. The identities of numbered peaks are listed in Table I.

strains and species of molds included in this study, data are presented for the 874 strain of *P. caseicolum*, but similar observations were made for each of the other cultures studied.

Typical bonded Carbowax 20M capillary column GC–MS total ion gas chromatograms of headspace volatiles from young and mature cultures of *P. caseicolum* strain 874 are shown in Figures 1 and 2, respectively. Odor assessments of compounds eluting from the packed Carbowax 20M column indicated that compounds with mushroomy, earthy, and heavy green plant-like aromas eluted between I_E 6 and 8.4 and the influential compounds in this region were identified as 1-octen-3-ol, 1,5-octadien-3-ol, 1,5-octadien-3-one, 3-octanol, and 3-octanone (Table I). Of the eight-carbon compounds identified, 1-octen-3-ol would be expected to contribute influential flavor and aroma qualities to cheeses because of its abundance (Table I) and distinct mushroom aroma. 1-Octen-3-ol has long been recognized as an important flavor compound produced by *P. roqueforti* in blue cheese (Shimp and Kinsella, 1977) and has been viewed similarly for an important role in all but extensively and modestly aged Camembert cheese flavors (Dumont et al., 1974b; Moinas et al., 1973; Adda et al., 1982). 1-Octen-3-ol along with 3-octanone, 3-octanol, 1-octanol, and 2-octen-1-ol have also been identified as metabolites of a number of *Aspergillus* and *Penicillium* species associated with moldy grains (Kaminski et al., 1972, 1974) and pure cultures grown on defined media (Halim et al., 1975; Siefert and King, 1982).

The strong, crushed geranium leaf-like aromas of 1,5-octadien-3-ol and 1,5-octadien-3-one can be considered capable of contributing to the overall earthy notes of Brie and Camembert cheeses and probably blend well with the aroma notes of methyl ketones found in these cheeses (Moinas et al., 1973; Dumont et al., 1974b). 1,5-Octadien-3-one was present in only trace quantities as determined by odor assessment of the eluting compound on a Carbowax 20M packed column but exhibits a recognition threshold of 0.001 ppb (Swoboda and Peers, 1977). The

Table I. Volatile Compounds Produced by Cultures of *Penicillium caseicolum* Grown on Defined Media

peak no./ positn[a]	compd	I_E[b]	GC effluent odor quality (packed column)	odor threshold,[c] ppb	rel concn, μg[d] young culture (5 days)	rel concn, μg[d] mature culture (29 days)	means of ID
			Eight-Carbon Compounds				
4	3-octanone	6.26	earthy/ketonic/mushroom-like	50[e]	0.02	0.09	MS, Rt
5,E	3-octanol	7.48		18[f]	g	0.25	MS, Rt
A	1,5-octadien-3-one	7.61	geranium leaves	0.001[h]	tr[i]	tr	Rt, odor
7	1-octen-3-ol	8.06	raw mushrooms	10[e]	0.35	17.40	MS, Rt, odor
8,F	1,5-octadien-3-ol	8.48	earthy/geranium	10[j]	tr	1.03	MS, Rt, odor
			Earthy–Musty Compounds				
6,B	2-methoxy-3-isopropyl-pyrazine	7.90	earthy/nutty/potato-like	0.002[k]	g	0.01	MS, Rt, odor
9	2-methylisoborneol	9.80	musty/earthy	0.10[l]	0.05	0.16	MS, Rt, odor
			Others				
1	2-methyl-1-propanol	4.30			1.60	0.65	MS, Rt
2	2-methyl-2-pentenal	5.30			0.45	0.19	MS, Rt
3	3-methyl-1-butanol	5.64		4.75×10^3[m]	14.08	0.78	MS, Rt
11	naphthalene	11.62			0.06	0.14	MS, Rt
C	damascenone	12.62		10[n]	tr	tr	Rt, odor
13	octanoic acid	14.17			0.03	0.08	MS, Rt

[a] Numbers identify peaks and letters indicate location based on odor assessment from a packed Carbowax 20M column. [b] Retention indices; Van den Dool and Kratz (1963). The column is bonded Carbowax 20M. [c] Threshold concentrations in water. [d] Based on 10 μg of internal standard (4-decanol) in 54 L of headspace. [e] Pyysalo and Suihko (1976). [f] Frazzalari (1978). [g] None detected. [h] Swoboda and Peers (1977). [i] Trace amounts, detected only by retention indices and odor assessment on a packed Carbowax 20M column. [j] Whitfield et al. (1982). [k] Seifert et al. (1970). [l] Medsker et al. (1969). [m] Sheldon et al. (1971). [n] Ohloff (1978).

odor qualities of 3-octanone and 3–octanol (Cronin and Ward; 1971; Pyysalo and Suihko, 1976; MacLeod and Panchasara, 1983) indicate that these compounds likely contribute to the mushroom-like flavor notes encountered in surface mold-ripened cheeses.

Production of eight-carbon compounds via lipoxygenase mediated conversions of linoleic and linolenic acid is well-established for many plants (Tressl et al., 1975, 1981; Lumen et al., 1978; Eskin, 1979; Grosch, 1982) and mushrooms (Tressl et al., 1981, 1982; Hanssen and Klingenberg, 1983). While actual data for the enzymic production of eight-carbon compounds by *Penicillium sp.* have not been reported, it has been generally regarded parallel to that for mushrooms (Tressl et al., 1982). For example, Shimp and Kinsella (1977) have suggested that concentrations of linoleic and linolenic acids in the media could influence amounts of 1-octen-3-ol produced by *P. roqueforti*.

The distinctly musty/earthy compound eluting at I_E 9.45 (packed Carbowax 20M column, non-bonded) from mold cultures was identified as 2-methylisoborneol. This compound was also observed in the analysis of Brie and Camembert cheeses (Karahadian et al., 1985) and, when added to Brie cheese homogenates (25 ppb), provided generally desirable flavor notes to the cheeses. Historically, 2-methylisoborneol has been associated with the musty/muddy odors produced by actinomycetes (Medsker et al., 1969; Gerber, 1969; Rosen et al., 1970), cyanobacteria (Izaguirre et al. 1982), and blue-green algae (Tabachek and Yurkowski, 1976) and, along with geosmin, accounts for musty/muddy flavors found in fish and water systems (Steinke, 1978; Lane, 1981; Persson, 1980a, 1980b, 1983). Interestingly, Whitfield et al. (1983) recently have identified 2-methylisoborneol as the compound responsible for a musty, earthy off-favor occurring in canned champignons (mushrooms) and attributed its presence to naturally contaminated processing water supplies.

2-Methylisoborneol appears to arise through pathways that are related to terpene biosynthesis (Gerber, 1979). Earlier encounters with terpene compounds in Swiss cheese (pinenes and fenchenes; Langler et al., 1967) and Beaufort cheese (sequiterpenes; Dumont and Adda, 1978) have been attributed to the occurrence of these compounds in the cheese milk which arise from forages consumed by cows. Terpenes in Vacherin cheese (Dumont et al., 1974a) have been attributed to migration of the compounds from straps of spruce wood used in hooping the cheese.

An intensely musty, green pea-like, raw potato-like aroma consistently developed in old cultures of the molds and was especially pronounced in cultures exhibiting atrophied mycelia. The distinctive aroma eluted at I_E 7.95 on the packed Carbowax 20M column, and the compound was identified as 2-methoxy-3-isopropylpyrazine. Methoxy alkylpyrazines have been found in a variety of vegetables (Murray and Whitfield; 1975), including bell peppers (Buttery et al., 1969), potatoes (Buttery and Ling, 1973), and green peas (Murray et al., 1970). Flavor defects of microbial origin that have been attributed to 2-methoxy-3-isopropylpyrazine include those occurring in milk cultures of *Pseudomonas taetrolens* (Morgan et al., 1972), lamb caracasses contaminated with *P. taetrolens* (Tompkin and Shaparis, 1972), fish muscle inoculated with *Pseudomonas perolens* (Miller et al., 1973), and in smear-coated cheeses contaminated with *Pseudomonas sp.* (Dumont et al., 1983). Recently, Mottram and Patterson (1984) identified a compound causing musty/sour odors produced by certain Gram-negative bacteria as 2,6-dimethyl-3-methoxypyrazine. In addition, molds have also been shown to metabolically produce aspergillic acid (2-hydroxy-3-isobutyl-6-*sec*-butylpyrazine 1-oxide) and flavacol (2-hydroxy-3,6-diisobutylpyrazine) (Dutcher, 1947). Because of the low threshold for 2-methoxy-3-isopropylpyrazine (0.002 ppb; Seifert et al., 1970), it is probable that low concentrations of this compound contribute to the usual flavors of Brie and Camembert cheeses, especially extensively aged cheeses. Excessive production of 2-methoxy-3-isopropylpyrazine could easily contribute to the defective earthy/mushroomy flavor encountered in French Camembert by Dumont et al. (1974b).

Fungi biosynthesize a variety of flavor compounds (Maga, 1976, 1981; Cronin and Ward, 1971; Kaminski et al., 1972, 1974; Picardi and Issenberg, 1973; Pyysalo, 1976; Collins, 1979; Hanssen and Sprecher, 1981; Tressl et al., 1982) and have been viewed as potential sources of naturally derived flavors (Maga, 1976; Margalith; 1981 Drawert et al., 1983a, 1983b; Kempler, 1983). Of the remaining

volatile compounds listed in Table I, damascenone (2,6,6-trimethyl-1-crotonyl-1,3-cyclohexadiene) seems worthy of mention. This compound has been identified in alcoholic beverages (Strating and Van Eerde, 1973; Masuda and Nishimura, 1980), grapes (Schreier et al., 1976; Acree et al., 1981), fish (Steinke, 1978; Lane, 1981), and a variety of other food products (Ohloff, 1978). Although found in very low concentrations in young mold cutures of both *P. caseicolum* and *P. camemberti* by odor assessment on a packed Carbowax 20M column (I_E 11.68 on nonbonded Carbowax column), this compound could contribute because it exhibits a low detection threshold (10 ppb; Ohloff, 1978). It is likely that damascenone provides desirable flavor-blending effects to Brie and Camembert cheese flavors as we have observed it also in flavor isolates from these cheeses (Karahadian et al., 1985). The addition of damascenone to Brie cheese homogenates (25 ppb) suppressed distinct musty notes and enhanced woody, cedar-like notes in the flavor.

In summary, volatile compounds with earthy–musty notes that are produced by *P. camemberti* and *P. caseicolum* include a group of eight-carbon alcohols and corresponding ketones, 2-methylisoborneol, and 2-methoxy-3-isopropylpyrazine. Further investigations on mechanisms of formation and means for regulating biosynthesis of these compounds should prove useful in the development of manufacturing processes for mold surface-ripened soft cheeses with extended shelf lives and uniform flavor.

ACKNOWLEDGMENT

We thank Dr. K. Raper of the Department of Bacteriology, University of Wisconsin, for providing some of the mold cultures used in this study and for discussions about the taxomony of *Penicillium sp.* The assistance of D. Dube, Wisconsin State Laboratory of Hygiene, in obtaining mass spectra is gratefully acknowledged.

Registry No. 3-Octanone, 106-68-3; 3-octanol, 589-98-0; 1,5-octadien-3-one, 65213-86-7; 1-octen-3-ol, 3391-86-4; 1,5-octadien-3-ol, 83861-74-9; 2-methoxy-3-isopropylpyrazine, 25773-40-4; 2-methylisoborneol, 2371-42-8; 2-methyl-1propanol, 78-83-1; 2-methyl-2-pentenal, 623-36-9; 3-methyl-1-butanol, 123-51-3; naphthalene, 91-20-3; damascenone, 23726-93-4; octanoic acid, 124-07-2.

LITERATURE CITED

Acree, T. E.; Braell, P. Butts, R. M. *J. Agric. Food Chem.* **1981**, *29*, 688.
Adda, J.; Gripon, J. C.; Vassal, L. *Food Chem.* **1982**, *9*, 115.
Boyd, E. N.; Patton, S.; Day, E. A., unpublished data, 1965.
Buttery, R. G.; Ling, L. C. *J. Agric. Food Chem.* **1973**, *21*(4), 745.
Buttery, R. G.; Seifert, R. M.; Guadagni, D. G.; Ling, L. C. *J. Agric. Food Chem.* **1969**, *17*, 1322.
Collins, R. P. "35th Proceedings of the Society for Industrial Microbiology", Houston, TX, 1979; Vol. 20, ppp 239–245.
Cronin, D. A.; Ward, M. K. *J. Sci. Food Agric.* **1971**, *22*, 477.
Day, E. A. In "Symposium on Foods: the Chemistry and Physiology of Flavors"; Schultz, H. W., Day, E. A., Libbey, L. M., Eds.; The Avi Publ. Co.: Wesport, CT, 1967; pp 342–343.
Drawert, F.; Berger, R. G.; Neuhauser, K. *Chem. Mikrobiol. Technol. Lebensm.* **1983a**, *8*, 91.
Drawert, F.; Berger, R. G.; Neuhauser, K. *Eur. J. Appl. Microbiol. Biotechnol.* **1983b**, *18*, 124.
Dumont, J. P.; Adda, J. *J. Agric. Food Chem.* **1978**, *26* (2), 364.
Dumont, J. P.; Mourgues, R.; Adda, J. In "Sensory Quality in Foods and Beverages: Definition, Measurement and Control"; Williams, A. A., Atkin, R. K., Eds.; Ellis Horwood Limited Pub.: Chichester, 1983; pp 424–428.
Dumont, J. P.; Roger, S.; Adda, J. *Lait* **1976**, *56*, (559), 595.
Dumont, J. P.; Roger, S.; Cerf, P.; Adda, J. *Lait* **1974a**, *54* (535), 243.
Dumont, J. P.; Roger, S.; Cerf. P.; Adda, J. *Lait* **1974b**, *54*, (538), 501.
Dutcher, J. D. *J. Biol. Chem.* **1947**, *171*, 321.
Eskin, M. N. A. In "Plant Pigments, Flavors and Textures. The Chemistry and Biochemistry of Selected Compounds"; Academic Press: New York, 1979; pp 94–119.
Frazzalari, F. A., Ed. In "Compilations of Odor and Taste Threshold Value Data"; American Society for Testing and Materials: Philadelphia, PA, 1978.
Gehrig, R. F.; Knight, S. G. *Appl. Microbiol.* **1963**, *11*, 166.
Gerber, N. N. *J. Antibiot.* **1969**, *22*, 508.
Gerber, N. N. *CRC Crit. Rev. Microbiol.* **1979**, *7* (3), 191.
Greenberg, R. L.; Ledford, R. A. *J. Dairy Sci.* **1978**, *61*, 106 (abstract).
Grosch, W. In "Food Flavours; Part A, Introduction"; Morton, I. D., Macleod, A. J., Eds.; Elsevier Scientific Pub. Co.: New York, 1982; pp 325–398.
Halim, A. F.; Narcisco, J. A.; Collins, R. P. *Mycologia* **1975**, *67*, 1158.
Hanssen, H. P.; Klingenberg, A. *Z. Lebensm.*—Unters. *Forsch.* **1983**, *177*, 333.
Hanssen, P. H.; Sprecher, E. In "Flavour '81"; Schreier, P., Ed.; Walter de Gruyter & Co.: Berlin, New York, 1981; pp 547–556.
Hawke, J. C. *J. Dairy Res.* **1966**, *33*, 225.
Heller, S. R.; Milne, G. W. A. "EPA/NIH Mass Spectral Data Base"; U.S. Government Printing Office: Washington D.C., 1975; Vol. 1–4.
Heller, S. R.; Milne, G. W. A. "EPA/NIH Mass Spectral Data Base"; U.S. Government Printing Office: Washington D.C., 1980; Supplement 1.
Izaguirre, G.; Hwang, C. J.; Krasner, S. W.; McGuier, M. J. *Appl. Environ. Microbiol.* **1982**, *43*, 708.
Josephson, D. B.; Lindsay, R. C.; Stuiber, D. A. *J. Agric. Food Chem.* **1983**, *31* (2), 326.
Kaminski, E.; Libbey, L. M.; Stawicki, S.; Wasowicz, E. *Appl. Microbiol.* **1972**, *24* (5), 721.
Kaminski, E.; Stawicki, S.; Wasowicz, E. *Appl. Microbiol.* **1974**, *27* (6), 1001.
Karahadian, C.; Josephson, D. B.; Lindsay, R. C. *J. Dairy Sci.* **1985**, in press.
Kempler, G. M. *Adv. Appl. Microbiol.* **1983**, *29*, 29.
Kinsella, J. E.; Hwang, D. H. *CRC Crit. Rev. Food Sci. Nutr.* **1976**, *8*, 191.
Kosikowski, F. V. In "Cheese and Fermented Milk Foods", 2nd ed.; F. V. Kosikowski and Associates: Brooktondale, NY, 1982; pp 329–333.
Lane, N. A. M.S. dissertation, University of Wisconsin, 1981.
Langler, J. E.; Libbey, L. M.; Day, E. A. *J. Agric. Food Chem.* **1967**, *15* (3), 386.
Law, B. A. *Perfum. Flavor.* **1982**, *7*, 9.
Lumen, B. O.; Stone, E. J.; Kazeniac, S. J.; Forsythe, R. H. *J. Food Sci.* **1978**, *43*, 698.
MacLeod, A. J.; Panchasara, S. D. *Phytochemistry* **1983**, *22* (3), 705.
Maga, J. A. *Chem. Senses Flavour* **1976**, *2*, 255.
Maga, J. A. *J. Agric. Food Chem.* **1981**, *29* (1), 1.
Margalith, P. Z. In "Flavor Microbiology"; Charles Thomas, Pub.: Springfield, IL. 1981; pp 256–278.
Masuda, M.; Nishimura, K. *J. Food Sci.* **1980**, *45*, 396.
Medsker, L. L.; Jenkins, D.; Thomas, J. F.; Koch, C. *Environ. Sci. Technol.* **1969**, *3*, 476.
Miller, A.; Scanlan, R. A.; Lee, J. J.; Libbey, L. M.; Morgan, M. E. *Appl. Microbiol.* **1973**, *25* (2), 257.
Moinas, M.; Groux, M.; Horman, I. *Lait* **1973**, *53* (529), 601.
Moinas, M.; Groux, M.; Horman, I. *Lait* **1975**, *55* (547), 414.
Morgan, M. E.; Libbey, L. M.; Scanlan, R. A. *J. Dairy Sci.* **1972**, *55* (5), 666 (abstract).
Mottram, D. S.; Patterson, R. L. S. *Chem. Ind. (London)* **1984**, (12), 448.
Murray, K. E.; Shipton, J.; Whitfield, F. B. *Chem. Ind. (London)* **1970**, 897.
Murray, K. E.; Whitfield, F. B. *J. Sci. Food. Agric.* **1975**, *26*, 973.
Ohloff, G. *Perfum. Flavor.* **1978**, *3*, 11.
Olson, N. F. "Pfizer Cheese Monographs"; Chas. Pfizer and Co., Inc.: New York, 1969; Vol. IV.
Persson, P.-E. *Water Res.* **1980a**, *14*, 1113.
Persson, P.-E. *Dev. Hydrobiol.* **1980b**, *2*, 203.
Persson, P.-E. *Water Sci. Technol.* **1983**, *15* (6/7), 1.
Picardi, S. M.; Issenberg, P. *J. Agric. Food Chem.* **1973**, *21*, 959.

Pyysalo, H. *Acta Chem. Scand., Ser. B.* **1976**, *B30*, 235.
Pyysalo, H.; Suihko, M. *Lebensm.-Wiss. Technol.* **1976**, *9*, 371.
Raper, D. B.; Thom, C. In "A Manual of the Penicillia"; The Williams & Wilkins Co.: Baltimore, 1949; pp 421-509.
Rosen, A. A.; Mashni, C. I.; Safferman, R. S. *Water Treat. Exam.* **1970**, *19*, 106.
Rousseau, M. *Milchwissenschaft* **1984**, *39* (3), 129.
Schreier, P.; Drawert, F.; Junker, A. *J. Agric. Food Chem.* **1976**, *24* (2), 331.
Seifert, R. M.; Buttery, R. G.; Guadagni, D. G.; Black, D. R.; Harris, J. G. *J. Agric. Food Chem.* **1970**, *18* (2), 246.
Siefert, R. M.; King, D. A. *J. Agric. Food Chem.* **1982**, *30*, 786.
Sheldon, M. R.; Lindsay, R. C.; Libbey, M. L.; Morgan, M. E. *Appl. Microbiol.* **1971**, *22* (3), 263.
Shimp, J. L.; Kinsella, J. E. *J. Agric. Food Chem.* **1977**, *25* (4), 793.
Steinke, J. Ph.D. Dissertation, University of Wisconsin, 1978.
Strating, J.; Van Eerde, P. *J. Inst. Brew.* **1973**, *79*, 414.
Swoboda, P. A. T.; Peers, K. E. *J. Sci. Food Agric.* **1977**, *28*, 1019.
Tabachek, J. L.; Yurkowski, M. *J. Fish. Res. Board Can.* **1976**, *33* (1), 25.
Thom, C.; Fisk, W. W. "The Book of Cheese"; MacMillan Co.: New York, 1918.
Tompkin, R. B.; Shaparis, A. B. *Appl. Microbiol.* **1972**, *24* (6), 1003.
Tressl, R.; Bahri, D.; Engel, K. In "Quality of Selected Fruits and Vegetables in North America"; Teranishi, R., Barrera-Benitez, H., Eds.; American Chemical Society: Washington D.C., 1981; pp 213-232.
Tressl, R.; Bahri, D.; Engel, K. *J. Agric. Food Chem.* **1982**, *30*, 89.
Tressl, R.; Holzer, M.; Apetz, M. "Proceeding of the International Symposium on Aroma Research"; Maarse, M., Groenen, P. J., Eds.; Pudoc Wageningen Pub.: Zeist, The Netherlands, 1975; pp 41-62.
Van den Dool, H.; Kratz, P. D. *J. Chromatogr.* **1963**, *11*, 463.
Whitfield, F. B.; Freeman, D. J.; Last, J. G.; Bannister, P. A.; Kennett, B. H. *Aust. J. Chem.* **1982**, *35*, 373.
Whitfield, F. B.; Tindale, C. R.; Last, J. H. *Chem. Ind. (London)* **1983**, 316.

Received for review October 23, 1984. Accepted February 2, 1985. Research supported by the College of Agriculture and Life Sciences, Department of Food Science, University of Wisconsin—Madison.

Direct Sampling Capillary GLC Analysis of Flavor Volatiles from Ovine Fat

Jun Suzuki and Milton E. Bailey*

A rapid, direct sampling capillary gas chromatographic procedure for the quantitation of volatiles from lamb fat was developed. Following trapping of volatiles by heating 300 mg of fat containing an internal standard (2-methyl-3-octanone), volatiles were eluted in an external sampling device onto a SE-54 coated capillary column, separated, and identified by mass spectrometry. Fifty-two volatile compounds were quantitated by GC. Accuracy and precision were determined on selected compounds. Average recovery of these compounds was 95.4% and the average relative standard deviation was 6.29%.

Although lamb is potentially an excellent source of meat that can be finished on different forages, it has low acceptance in many countries due to undesirable flavor. Studies by Wong et al. (1975) established that 4-methyloctanoic acid contributed to mutton flavor and suggested that nonacidic fractions might also contribute to mutton odor.

Nixon et al. (1979) published information on 93 nonacidic volatiles from cooked mutton identified by GC/MS. Sample preparation involved refluxing minced meat for 3 h, steam distillation, extraction in a continuous ether extraction apparatus (Likens and Nickerson), and removal of acidic compounds with Na_2CO_3.

A similar study was published by Lorenz et al. (1983) on volatile compounds from sheep liver. These investigators identified 108 compounds from 35 kg of lamb liver. Extensive sample fractionation was necessary prior to identification of volatiles by GC/MS.

Simple quantitative chemical methodologies are essential for further studies of the influence of various factors including diet on the flavor volatiles of lamb and similar meat animals.

Clark and Cronin (1975) and Cronin (1982) described direct sampling procedures for trapping volatiles on charcoal packed into a glass capillary tube and eluting onto a capillary GC column by heating in an injection port at 260 °C.

Galt and MacLeod (1984) trapped volatiles from beef in Tenax contained in a 20 cm long × 4 mm id tube cooled in dry ice, then desorbed heating at 250 °C, and flashed than to a packed column with N_2.

The innovative direct GC method of Dupuy et al. (1976) for the analysis of volatiles related to undesirable oil flavor was an important contribution to methodology needed to relate chemical constituents of food products to acceptability. This procedure was improved for packed column GC by the more versatile procedure of Legendre et al. (1979). Both methods are limited by the inefficiency of the packed column for separating complex mixtures of volatile compounds such as those from animal fat.

Recently developed fused silica capillary columns are much more efficient for separating the complex mixture of hydrocarbons, ketones, alcohols, aldehydes, acids, esters, and lactones found in these samples.

In this communication we report on a new, rapid, quantitative direct sampling procedure for analyzing ovine depot fat for volatiles that can be used to study the influence of different factors on flavor.

EXPERIMENTAL SECTION

Lamb Fat Samples. Two fat samples were analyzed in these experiments; one was taken from the loin of a lamb

Department of Food Science and Nutrition, University of Missouri, Columbia, Missouri 65211.

0021-8561/85/1433-0343$01.50/0