Gas Chromatographic and Mass Spectral Identification of Natural Components of the Aroma Fraction of Blue Cheese

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The aroma fraction of blue cheese fat was isolated by centrifugation of intact cheese and molecular distillation of the recovered fat. The aroma fraction was separated by gas chromatography on packed columns containing polar and nonpolar phases and by programmed temperature capillary column gas chromatography. The effluent from the capillary column was admitted directly to the inlet of a rapid-scan mass spectrometer, enabling simultaneous recordings of spectra for each chromatographic peak. By mass spectral and gas chromatography retention data, it was possible to identify the major neutral components in the aroma fraction: methyl ketones, secondary alcohols, and the methyl and ethyl esters of the normal aliphatic acids.

According to the literature (*3*), the characteristic flavor of blue cheese is largely composed of fatty acids (*18*, *20*), methyl ketones (*15*), and secondary alcohols (*9*). In addition to contributing to the flavor *per se*, the fatty acids also function as precursors of methyl ketones (*7*) and secondary alcohols (*9*). Hence, most of the ketones and alcohols isolated thus far contain an odd number of carbon atoms. There have been several reports, however, of methyl ketones with an even number of carbons in the chain—i.e., butanone (*14*) and 2-octanone (*4*).

Most of the above work, as well as that of others, has been of a qualitative nature, and it is difficult, therefore, to evaluate a mixture of compounds for authenticity of blue cheese flavor. In an effort to establish the quantitative relation of the various flavor compounds, recently developed procedures (*10*) were employed for cheese flavor isolation. Gas chromatography of the isolated material gave approximately 100 fractions. Since the ketones, alcohols, and fatty acids combined could account for only about one fifth of the observed fractions, it seemed appropriate to investigate the qualitative nature of the flavor material to determine if there were heretofore unrecognized compounds of possible flavor significance. The findings reported herein deal with the major neutral compounds.

Procedures

The fat from 7 pounds of domestic-type blue cheese was isolated by the centrifugation procedure of Libbey, Bills, and Day (*10*). The yield was approximately 60%, and the aroma of the fat was typical of the intact cheese. The volatiles were isolated from 500 ml. of the fat by means of the molecular distillation apparatus of Libbey *et al.* (*10*), except that trap 1 was eliminated; hence, only two traps were used, a trap packed with 3-mm. glass beads and a safety trap. The molecular still was operated at 45° C. Upon completion of the distillation, portions of the distillate contained in the glass bead trap were transferred to a gas chromatograph by the technique of Libbey *et al.* (*10*) and chromatograms were obtained using polar (20% diethylene glycol succinate on 80- to 100-mesh Celite 545) and nonpolar (20% Apiezon M on 80- to 100-mesh Celite 545) columns operated at 70° C. The columns were stainless steel, $^1/_8$-inch o.d. × 11 feet, and a β-ionization detector was used in the chromatograph. Relative retention times of resulting peaks were calculated and compared with data for authentic compounds.

The remainder of the distillate, contained in the glass bead trap, was recovered in diethyl ether. The excess ether was removed through a fractional distillation column, and the residue was separated by programmed temperature gas chromatography. A Perkin-Elmer Model 800 gas chromatograph equipped with a hydrogen flame detector and containing a stainless steel capillary 300 feet × 0.01-inch i.d. coated with polypropylene glycol was used. The capillary column was operated isothermally at 73° C. for 8 minutes, then temperature programmed at 25° per minute to 174° C., and operated isothermally at 174° C. until the separation was complete (actual column temperature has undetermined lag from this temperature). Retention data were obtained for both the cheese volatiles and authentic compounds under these conditions. The same conditions were employed for gas chromatography analysis in combination with fast-scan mass spectrometry.

The mass spectrometric analyses were conducted as described by McFadden *et al.* (*12*, *13*) and Teranishi *et al.* (*19*), except that the positive ions occurring at *m/e* 43 (*m/e* is the mass to charge ratio) were monitored by gate 2 of the electron multiplier to obtain a concurrent strip chart recording of the gas chromatogram. The other electron multiplier gate was used to scan *m/e* 12 to 250 in 2 or 6 seconds and the spectra were recorded by an oscillographic recorder. The spectra were observed concurrently on an oscilloscope which provides an important means of monitoring the effluent from the capillary column.

Additional gas chromatographic data were obtained by temperature-programming the capillary columns from 30° to 50° C. at 2.5° per minute, then at 25° per minute to 170° C.

Results and Discussion

Figure 1 is a gas chromatogram depicting separation of the blue cheese aroma concentrate. Data supporting identification of the peaks having assigned numbers are presented in Table I. The peaks from the gas chromatogram obtained by monitoring *m/e* 43 in the mass spectrometer gave relative retention values that agreed with those shown in Figure 1. As reported previously (*5*), response differences occur when the two types of detectors are compared, but retention data can be correlated. By rapidly scanning each chromatographic peak as it entered the mass spectrometer, molecular structure assignments were made by interpretation of the fragmentation patterns of the resulting mass spectra. Analysis of the mass spectral data also was augmented by the retention data obtained for both the cheese aroma concentrate and authentic compounds on capillary and packed columns using the aforementioned stationary phases and operating parameters. All retention data are reported relative to ethyl acetate = 1.000. Retention data were not obtained for some of the compounds (Table I). The characteristics of the mass spectra were adequate for either tentative or positive identification, as indicated in the table.

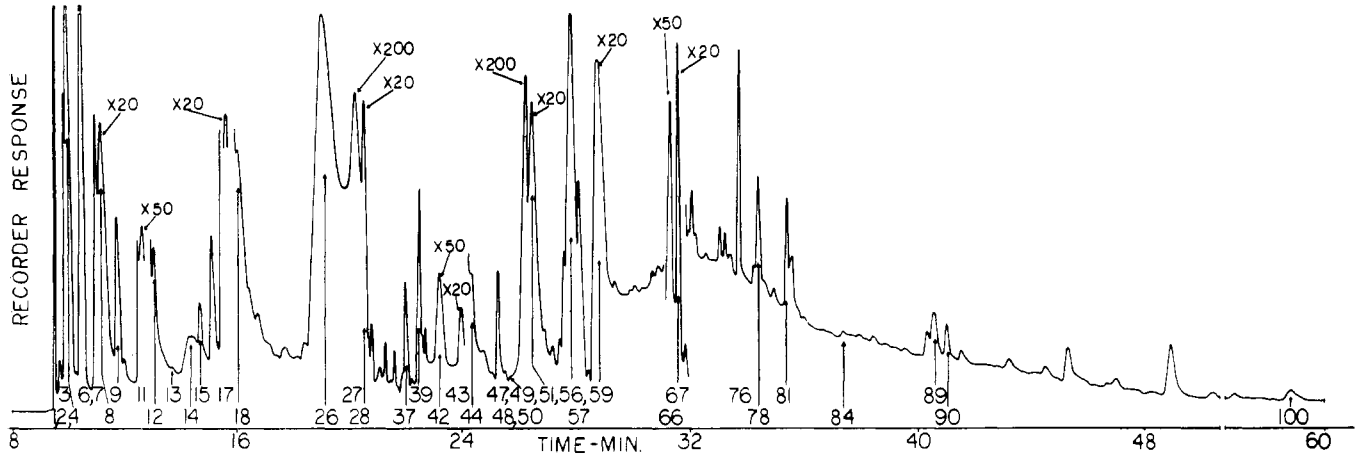

Figure 1. Gas chromatogram of neutral components of blue cheese aroma

Capillary column, 300 feet × 0.01-inch i.d. coated with polypropylene glycol; isothermal at 73° C. for 8 minutes, then temperature programmed at 25° per minute to 174° C.

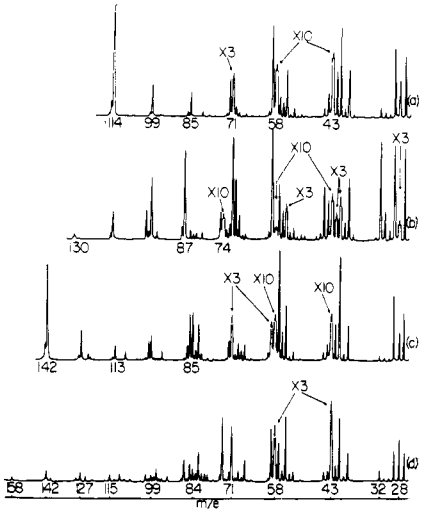

Figure 2. Mass spectral charts for chromatographic fractions

a corresponds to chromatographic peak 27 in Figure 1; b, peak 28; c, peak 50; d, peak 51

To show the type of data obtained by the technique, mass spectra charts *a*, *b*, *c*, and *d* representing the spectra for chromatographic peaks 27, 28, 50, and 51 (Figure 1), respectively, are presented in Figure 2. In mass spectrum *a*, the large ion peaks at *m/e* 43 (OC_2H_3+) and 58 (OC_3H_6+) are indicative of a methyl ketone. The ion fragment at *m/e* 114 is the parent ion for 2-heptanone. Comparison of the remainder of the spectrum, especially ion peaks 99, 85, 71, and 59, with the spectrum in the API tables (*1*) supports this interpretation. Coincidence of gas chromatography retention data for authentic 2-heptanone and chromatographic peak 27, Table I, is sufficient evidence for confirming the identification. Spectrum *b*, which represents chromatographic peak 28 in Figure 1, still contains

Table I. Gas Chromatographic and Mass Spectral Identification of Components of Blue Cheese Aroma

GC Peak No.	Identity	t_R/t_R[a], Blue Cheese	t_R/t_R, Authentics	Confirmed by Packed Col. GC	Mass Spectral Identification	Ref.
1	Ethanal	0.867	0.867	Yes	Positive	(*1*)
2	Diethyl ether	0.888	0.883	Yes	Positive	(*1*)
3	Acetone	0.902	0.917	Yes	Tentative	(*1*)
4	Ethyl formate	0.906	0.924	Yes	Positive	(*1*)
7	Ethyl acetate	1.000	1.000	Yes	Positive	(*1*)
7	2-Propanol	1.004	1.007	Yes	Positive	(*1*)
8	3-Methyl butanal	1.007	...	Yes	Positive	(*8*)
9	Benzene	1.069	...		Positive	(*1*)
11	2-Pentanone	1.159	1.151	Yes	Positive	(*1*)
12	Methyl butanoate	1.198	1.200	Yes	Positive	(*1*)
13	Toluene	1.250	...		Positive	(*1*)
14	2-Pentanol	1.315	1.352	Yes	Positive	(*6*)
15	Dimethylcyclohexane	1.344	...		Tentative	(*1*)
17	Ethyl butanoate	1.414	1.414	Yes	Positive	(*1*)
18	2-Hexanone	1.464	1.467	Yes	Positive	(*16*)
25	2-Methylbutanol	1.678	...		Positive	(*6*)
26	3-Methylbutanol	1.735	1.730		Positive	(*6*)
26	1-Pentanol	1.735	...		Positive	(*6*)
27	2-Heptanone	1.842	1.834	Yes	Positive	(*16*)
28	Methyl hexanoate	1.875	1.879	Yes	Positive	(*17*)
37	Furfural	2.003	2.007	Yes	Tentative	(*1*)
39	Ethyl hexanoate	2.050	2.055	Yes	Positive	(*17*)
42	2-Heptanol	2.108	...	Yes	Positive	(*11*)
43	2-Octanone	2.183	...	Yes	Positive	(*16*)
44	Isopropyl hexanoate	2.217	...		Tentative	(*17*)
47	3-Methylbutyl butanoate	2.300	...		Tentative	(*11*)
48	2-Octanol	2.325	2.310	Yes	Positive	(*8*)
49	Cresyl methyl ether	2.342	...		Tentative	(*1*)
50	2-Nonanone	2.392	2.359	Yes	Positive	(*2*)
51	Methyl octanoate	2.410	2.376	Yes	Positive	(*2*)
56	Ethyl octanoate	2.528	2.510	Yes	Positive	(*2*)
57	2-Decanone	2.560	...	Yes	Positive	(*11*)
59	2-Nonanol	2.617	...	Yes	Positive	(*11*)
60	Pentyl hexanoate	2.621	...	Yes	Tentative	(*11*)
66	2-Undecanone	2.857	2.866	Yes	Positive	(*16*)
67	Methyl decanoate	2.892	2.886	Yes	Positive	(*2*)
76	Ethyl decanoate	3.078	3.103	Yes	Positive	(*2*)
77	2-Phenylethanol	3.128	...		Tentative	(*11*)
78	Ethyl-2-methylnonanoate	3.139	...		Tentative	(*1*)
81	Isopropyl decanoate	3.236	...		Tentative	(*11*)
84	δ-Octalactone	3.414	3.400		Tentative	(*11*)
89	2-Tridecanone	3.707	3.800	Yes	Positive	(*11*)
90	Methyl dodecanoate	3.750	3.880		Positive	(*2*)
100	δ-Decalactone	5.178	4.952		Tentative	(*11*)

[a] t_R/t_R = relative retention time calculated on basis t_R/t_R of ethyl acetate = 1.000. Stainless steel capillary, 300 feet × 0.01-inch i.d. coated with polypropylene glycol; operated at 73° C. for 8 minutes, then at 25° per minute to 174° C.

heptanone as shown by ion fragments at *m/e* 43, 58, 71, 85, 94, and 114. However, the appearance of ions at *m/e* 87 (CH_2-CH_2COOCH_3+) and 74 (the rearrangement ion of methyl esters) points to an ester. The ion peak at 130 is indicative of the parent ion for methyl hexanoate. Examination of the aforementioned peak ratios plus those at *m/e* 101, 99, 75, 59, 55, and 43 to 39 and the gas chromatography retention data for peak 28 in Table I substantiates the identity of the compound.

Chromatographic peaks 50 and 51 in Figure 1 also were chosen for illustration purposes, since they further demonstrate the utility of the method, even though the fractions were not completely separated by the capillary column. Mass spectra *c* and *d* of Figure 2 were taken for chromatographic peaks 50 and 51, respectively. Again in spectrum *c* as in the case of spectrum *a* ion fragments at *m/e* 43 and 58 suggest a methyl ketone and the parent ion at *m/e* 142 indicates it is 2-nonanone. The ratios of ion peaks at *m/e* 127, 100, 99, 85, 82, and 71 plus the chromatographic evidence in Table I confirm this point. The spectrum also shows traces of other compounds—for example, *m/e* 95—and while the residual peaks were inadequate for identification of the compounds, they did not interfere with the interpretation of 2-nonanone. In this case as well as in others, it will be necessary to carry out additional analyses in which chromatographic parameters are varied to allow better separation of low concentration components. The resulting improvement in the quality of the mass spectra for these components will facilitate identification.

Mass spectrum *d*, which represents GC peak 51, still contains a substantial amount of the methyl ketone; however, ion fragments not attributable to the ketone occur in the correct ratios for methyl octanoate. A comparison of spectra *c* and *d* in Figure 2 shows the appearance of an ion fragment at *m/e* 158 (parent ion for methyl octanoate), 127, 115, 101, 87, and 74 (methyl ester rearrangement ion) and changes in the ratios of ion peaks in the 59 to 55 and 43 to 39 regions, all of which may be due to methyl octanoate. The agreement in chromatographic properties of the authentic ester with the chromatographic fraction of the blue cheese aroma supports the mass spectrum interpretation.

In a similar fashion the other compounds listed in Table I were characterized. The composition of several of the chromatographic peaks requires some discussion at this point. Peaks 25 and 26 contained the three isomers of primary pentanol. An estimate of the ratio is 65/25/10 for *n*-pentanol–3-me-1-butanol–2-me-1-butanol. The spectrum for peak 60 is positive for a pentyl hexanoate. At this point, the evidence is not sufficient to assign the specific isomer. If branching exists in the structure, it undoubtedly occurs in the alcohol moiety. The spectrum for 2-me-butyl-*n*-hexanoate does not give a suitable fit with the unknown. The remaining possibilities are being studied.

The mass spectra for a number of the peaks listed in Table I were not strong enough to enable unequivocal identification. In these cases the peak assignments are tentative. Hence, even though the chromatographic data for the authentic δ-lactones agreed with peaks 84 and 100, the mass spectral patterns were only suggestive and further work will be required to confirm the peak assignments.

All of the major compounds in the mixture were characterized and it is notable that these mainly consisted of methyl ketones, secondary alcohols, and esters of the aliphatic acids. The presence of methyl ketones in the flavor has been well substantiated by previous workers (*4*, *14*, *15*) and the secondary alcohols also have been observed (*9*). This is the first work, however, in which a substantial number of esters have been identified. These compounds were not unexpected, in view of the type of reactions predominating during blue cheese ripening.

The origin of some of the other compounds is not easily explained. Diethyl ether was used as a solvent and prior to use it was treated for removal of peroxides, distilled through a fractionating column, and when checked on a gas chromatograph, it gave one peak. Hence, this does not appear to be the logical source for the observed aryl compounds. Although they are not shown in the table, methane and methyl chloride were evident prior to the diethyl ether peak in the analysis. Methyl chloride may have resulted from its use as a fumigant in the cheese-curing rooms.

The sensory properties of the blue cheese aroma concentrate were typical of the intact cheese. This was particularly evident at the time of injection of the sample into the gas chromatograph on observing the effluent from the injection splitter. It was apparent that the concentrate contained free fatty acids, but these did not elute from the capillary column during an analysis. This point was confirmed by injection of authentic compounds. Similar observations were made for other highly polar compounds such as amines. Hence, the chromatogram in Figure 1 does not represent the complete pattern of the blue cheese aroma. These points and the fact that unidentified low-concentration compounds may have flavor-contributing qualities forbid conclusions on the significance of various compounds in the complete blue cheese aroma. In view of the sensory properties of the major components identified here and in other work (*9*, *15*), these, in conjunction with the free fatty acids, obviously are the key aroma components. The minor constituents may play an essential role, however, in giving a flavor that exactly duplicates that of the natural product.

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