AN ABSTRACT OF THE THESIS OF

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Title FLAVOR CHEMISTRY OF SWISS CHEESE

Abstract approved _____
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The unique flavor of high quality Swiss cheese is difficult to reproduce in commercial market cheese. Swiss cheese flavor has never been duplicated or thoroughly understood. New techniques and advances in flavor research have enabled better definition and understanding of food flavors. Therefore, it was desirable to make a detailed investigation of Swiss cheese flavor.

Neutral volatile flavor compounds were isolated from Swiss cheese fat by low-temperature low-pressure distillation. The compounds were separated by temperature programmed gas chromatography. Direct analysis of cheese fat and whole cheese from four domestic and two imported good flavored cheeses by gas entrainment and on-column trapping provided a further means of isolation of volatile flavor compounds in Swiss cheese. Gas chromatography in conjunction with rapid scan mass spectrometry and relative retention time data were used to identify compounds.

Compounds positively identified by the distillation and on-column

trapping techniques were as follows: methanol, ethanol, 1-propanol, 1-butanol, 2-pentanol, trans-2-hexene-1-ol, 2-phenylethanol, acetaldehyde, 2-methyl propanal, 2-methyl butyraldehyde, benzaldehyde, phenylacetaldehyde, acetone, butanone, 2-pentanone, 2-hexanone, 2-heptanone, 2-nonanone, 2-undecanone, 2-tridecanone, 2-pentadecanone, hexane, octane, 1-octene, nonane, 1-nonene, dodecane, pentadecane, toluene, α-pinene, methyl acetate, methyl hexanoate, methyl octanoate, methyl decanoate, ethyl propionate, ethyl butanoate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl dodecanoate, butyl acetate, 3-methyl butyl acetate, γ-valerolactone, γ-dodecalactone, δ-octalactone, δ-decalactone, δ-dodecalactone, dimethyl sulfide, diacetyl, benzothiazole, o-dichlorobenzene, 1, 2, 4-trichlorobenzene, di-isobutyl adipate, and chloroform.

Compounds tentatively identified include an aromatic hydrocarbon, pinane, α-fenchene, ethyl benzene, a di-methyl benzene, methyl benzoate, 2-phenyl-2-methyl butane, 5-methyl-5-ethyl decane, 3-methyl butyl octanoate, 2, 5-dimethyl tetra decane, methyl vinyl ether and 2-methyl propenal.

The concentration of selected volatile compounds identified by the on-column trapping technique were determined by relating their peak heights to known quantities of compound. Average concentrations calculated from the mean values for all the six cheeses and expressed in parts per million were as follows: dimethyl sulfide,

0.107; diacetyl, 0.8; acetaldehyde, 1.4; acetone, 1.6; butanone, 0.3; 2-methyl butyraldehyde, 0.42; 2-pentanone, 0.98; 2-heptanone, 0.45; ethanol, 16.3; 2-butanol, 0.3; 1-propanol, 2.9; 1-butanol, 0.7; methyl hexanoate, 1.5; and ethyl butanoate, 0.6.

Liquid-liquid partition chromatography and gas chromatography were utilized to determine quantitatively the major free fatty acids in the six Swiss cheeses. 2-Methyl butyric acid was detected in all cheeses and varied from 9.0 to 100.0 mg/kg cheese. The other isomeric acid, 3-methyl butyric, was detected in only two cheeses. Formic acid was detected in only one cheese. No n-valeric or 2-methyl propionic acids were detected.

A synthetic Swiss cheese flavor was prepared utilizing the data obtained in this investigation and that available in the literature for free amino acids. A satisfactory reproduction of Swiss cheese flavor could be achieved only if the mixture contained free fatty acids, volatile constituents, and free amino acids and was adjusted to the pH of natural cheese.

FLAVOR CHEMISTRY OF SWISS CHEESE

by

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FLAVOR CHEMISTRY OF SWISS CHEESE

INTRODUCTION

Of the hard cheeses, Swiss is second in production only to Cheddar in the United States. It is considered one of the most difficult cheeses to manufacture. The unique, nut-like, sweet flavor of high quality Swiss cheeses is difficult to find duplicated in most commercial domestic cheeses available.

Recent advances in flavor chemistry techniques have enabled significant progress to be made in defining, duplicating, and establishing the origins of food flavors. Due to its subtle mild flavor, Swiss cheese has been avoided or ignored by most investigators. Significant progress has been made, however during the past decade in determining the quantities of free amino acids present in the ripened cheese. The importance of volatile fatty acids in Swiss cheese flavor has long been recognized, but the identity of all of these acids was not known until recently. Heretofore efforts to reproduce Swiss cheese flavor have not been successful. It was, therefore, desirable to make a detailed investigation of Swiss cheese flavor.

A major objective of this investigation was to isolate and identify the compounds that might contribute to the flavor of a good quality cheese. Quantitative determination of the concentrations of compounds contributing to good flavor was another objective. A final

objective was to utilize the data obtained and that available in the literature to reproduce Swiss flavor and evaluate the importance of the compounds identified on flavor. It is believed that the information reported here will contribute to a more thorough understanding of Swiss cheese flavor and thereby establish further criteria of quality.

REVIEW OF LITERATURE

The subtle flavor of Swiss cheese has eluded food chemists for many years. Recent advances in flavor research techniques have made possible the identification and characterization of the components contributing to cheese flavors (33, 15, 16). A major portion of the flavor compounds arise from the metabolism of the bacteria used in the manufacture and curing of the cheese; however, natural milk enzymes and the milk itself, as well as the conditions of manufacture, contribute flavor compounds to the cheese. Compounds formed during manufacture and curing of cheeses and their contribution to flavors have been reviewed by Harper (20).

Flavor Compounds of Swiss Cheese

Free Fatty Acids

In 1921, Sherman (49) concluded that Bacterium acidi propionici (now designated Propioni bacterium shermanii) was essential for the production of eyes and to ensure proper flavor development in Swiss cheese. Shaw and Sherman (48) reported that this organism produced propionic acid, acetic acid and carbon dioxide from lactose and lactates. These compounds were also produced from succinate, glycerol, and nitrogenous compounds, and to a limited extent from butterfat.

Babel and Hammer (6) found that Swiss-type cheese of characteristic flavor yielded a much higher quantity of volatile acids on steam distillation than cheese of poor flavor. The addition of calcium or sodium propionate to processed Swiss-type cheese lacking in flavor gave the product a sweet flavor. These findings implicated the propionates as significant flavor contributors to Swiss-type cheese.

Krett and Stine (25) found good quality imported and domestic Swiss cheese to contain relatively large amounts of acetic and propionic acids with little or no butyric acid and a small quantity of higher acids. Poor quality cheese varied from good cheese in its content of lower fatty acids. Cheeses with little or no propionic or butyric acids had an unobjectionable flat flavor. Objectionable flavor occurred in cheeses containing a high quantity of butyric acid. Krett et al. (26) later determined the quantity of lower fatty acids in 140 day old cheese by steam distillation. One kilogram of cheese was found to contain 2.9 g of acetic, 6.3 g of propionic, 0.38 g of butyric and 0.50 g of higher acids (calculated as caproic). Hintz et al. (22) also determined the quantity of lower fatty acids in Swiss cheese using the direct chromatographic method developed by Harper (19). Butyric, propionic and higher acids were found in all cheeses investigated and all but one cheese contained valeric acid. A typical flavored cheese contained 3.0 g acetic, 5.0 g propionic, 1.1 g

butyric, 0. 19 g valeric and 24. 2 g of higher acids (calculated as caproic) per kg. There was no relation between the age of the cheese and the content of free fatty acids. Other investigators (29) using the same technique have found comparable values for the acetic, propionic, butyric and combined higher acids.

In attempting to assess the role of propionic acid in the flavor of Swiss cheese Kurtz et al. (29) made an organoleptic evaluation of aqueous solutions of propionic acid and sodium propionate. The solutions were devoid of either the sweet or nutty characteristics of Swiss cheese flavor. This refutes the claim previously put forth by Babel and Hammer (6) that these compounds are responsible for typical Swiss flavor. Propionic acid was found to be a component of the flavor complex; however, other compounds are responsible for both the nutty and sweet characteristics of Swiss cheese flavor.

Recently, Patton (43) detected 2- and 3- methyl butyric acids in Swiss cheese volatiles. Langler and Day (31) have determined quantitatively these and the other major free fatty acids in several good flavored cheeses. None of these investigators detected n-valeric acid. Investigators who have detected this acid (22) used the method of Harper, which would not separate the branched chain five carbon acids from n-valeric acid. Therefore it would appear that the quantity of n-valeric acid reported corresponds to the branched chain five carbon acids.

Production of Propionic and Acetic Acid by Propionibacterium shermanii

The formation of propionate and acetate by the propionibacteria has been extensively investigated (53, p. 684-686). The pathway in Propionibacterium shermanii is known to involve a number of enzymes which have been purified and studied (1). The principal carbohydrate of milk, lactose, is hydroxylyzed in the cheese to yield glucose and galactose. The galactose can be converted by well known reactions (14, p. 185) to glucose which is then utilized in the scheme shown in Figure 1 to yield propionic and acetic acids. In Figure 1, Me-malonyl-CoA is the methyl malonyl ester of coenzyme A and (a) and (b) designate the two possible isomers. Flavoprotein is designated by FP and reduced flavoprotein by FPH_2.

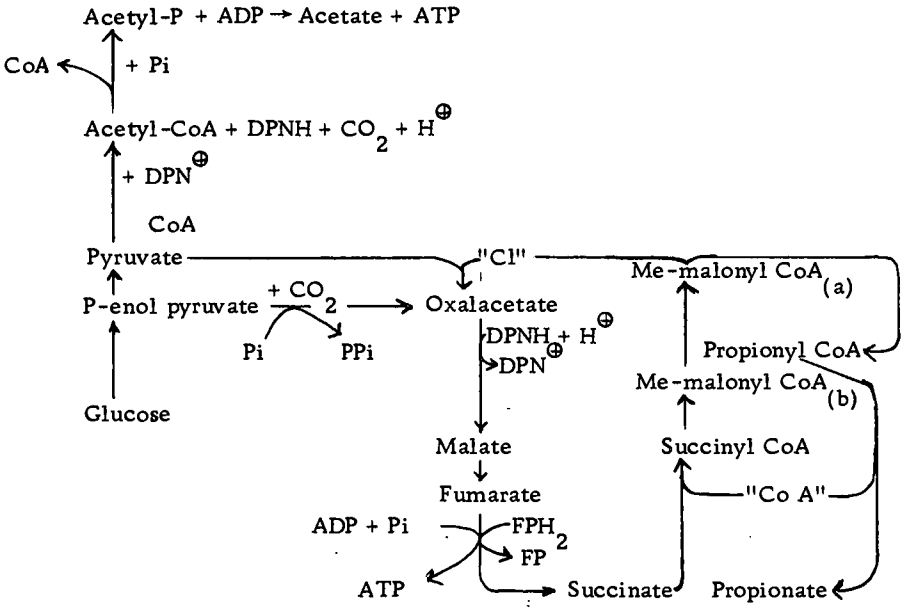

Figure 1. Reactions of propionic acid fermentation and the formation of acetate, propionate and carbon dioxide as described by Allen et al. (1, p. 183).

Amino Acids

Considerable attention has been devoted to the influence upon taste and flavor exerted by amino acids in ripe Swiss cheese. The role of proline in the flavor of Swiss cheese was first recognized by Virtanen et al. (52) who ascribed the sweetness of the cheese to its proline content. Storgårds and Hietaranta (51) reported that glutamic acid, of all the sour tasting amino acids, contributes to the flavor of Swiss cheese. When these investigators added glutamic acid to skim milk in the concentration calculated to be present in ripe cheese, a typical pleasant taste of cheese was noticed. They found that the quantity of basic amino acids, which have been described to have a bitter flavor (41), decreased during storage of the cheese.

Using ion exchange chromatography, Hintz et al. (22) determined quantatively the free amino acids in 13 aged Swiss cheeses. Not all of the amino acids were present in every cheese. A correlation between proline and propionic acid content was found for cheeses of typical flavor. A minimum proline concentration of 2.0 g/kg, accompanied by 5.0 g/kg of propionic acid, was necessary before a cheese possessed a satisfactory flavor. In a comparison of rindless block and wheel-type cured cheeses, Kristoffersen and Slater (28) detected a dramatic difference in the proline content of the cheeses. In the rindless variety proline varied from 0.01 to

2.1 g/kg, whereas in the wheel-type 3.7 to 7.4 g/kg of proline was found.

Carbonyl Compounds

Of the compounds that might contribute to Swiss cheese flavor, the least is known about carbonyl compounds. Bassett and Harper (7) have isolated and identified the following neutral carbonyl compounds from Swiss cheese: acetaldehyde, acetone, 2-pentanone, diacetyl, acetyl-methyl carbinol, a five carbon and a seven carbon aldehyde or ketone. These investigators also found a group of acidic carbonyl compounds. Oxalsuccinic, α-ketoglutaric, oxalacetic, pyruvic and α-acetolactic acids were detected. The concentration of pyruvic acid in a cheese of typical flavor varied from 0.1 to 0.5 g/kg of cheese. Kreula and Virtanen (27) found in addition to the above mentioned acidic carbonyl compounds, glyoxylic and hydroxy pyruvic acids in three and six month old cheese. Cheese that was 12 months old also contained α-ketocaproic. The "tear fluid" contained pyruvic, α-ketoisovaleric and α-ketoglutaric acids. No correlations between flavor and quantities of these compounds have been made.

EXPERIMENTAL

Isolation of Flavor Compounds from Swiss Cheese Fat by Low-Temperature Low-Pressure Distillation

An 80 pound block of domestic rindless Swiss cheese donated by a local plant was analyzed. Neutral volatile flavor compounds were isolated by the distillation technique of Libbey et al. (33). Fat was separated from 8.7 kg of cheese by high speed centrifugation (30,000 × g for 20 minutes). Recovery of fat was 52% of the total fat in the cheese. The fat fraction had a typical Swiss like aroma.

Volatile compounds were removed from 1500 ml of fat by low-temperature low-pressure distillation (33). One glass bead trap was used instead of two. The fat was fed through the still at a rate of three ml/minute while maintaining one to five microns pressure and a temperature of 54° C. A bland flavor that was not reminiscent of Swiss cheese was perceived on organoleptic evaluation of the distilled fat.

Reagent grade ethyl chloride which had been purified by stirring 24 hours at -29° C over sodium carbonate (to remove traces of HCl) and glass distilled, was used to extract the contents of the glass bead trap. At the completion of the cheese distillation the glass bead trap was allowed to warm up to 4° C in the cold room. Three 50 ml washings of the trap at 4° C were sufficient to remove the flavor

compounds. The combined trap washings were dried over anhydrous sodium sulfate at 0° C. The solvent was removed after drying using the apparatus designed by Bills et al. (16). The concentrate was stored at -29° C until subjected to gas liquid chromatography (GLC).

During the course of the analysis with the first concentrate it became evident that a more desirable chromatographic separation and better mass spectral interpretations would be achieved if the concentrate contained no free fatty acids (FFA). This was easily accomplished by means of an alkaline wash. The concentrates obtained on subsequent distillations were each washed at 4° C three times with 100 ml portions of 1% sodium carbonate-saturated sodium chloride aqueous solution. The FFA free ethyl chloride solution of flavor compounds was dried and concentrated as previously described.

Separation and Identification of Isolated Flavor Compounds

Temperature programmed packed column GLC was used to separate compounds in the flavor concentrate. In the case of the FFA containing concentrate it was necessary to employ a base treated pre-column (5, p. 45) placed in front of the regular column to remove the acids. The conditions of analysis are summarized below:

Instrument	Barber Colman model 5000
Detector	Hydrogen flame
Injection	On column

Column	20% Apiezon M on 100/120 mesh acid-alkali washed Celite 545
Column dimensions	12 feet × 1/8 inch OD
Initial temperature	55 ° C for 15 minutes
Temperature program	3 ° C/minute to 200 ° C
Flow rate	35 ml/minute of He
Detector temperature	205 ° C
Attenuation	x300

The instrument was equipped with a 1:1 splitter so that GLC effluent odor could be evaluated. It became evident that the neutral compounds in the concentrate were too dilute due to the high concentration of FFA. A trapping technique (5, p. 49) was used to increase the concentration for mass spectral identification.

The traps used were 2.0 feet × 1/8 OD tubing packed with 20% Apiezon M on 100/120 mesh Celite 545. The GLC effluent emerging from the column was split 20:1, the smallest portion going to the detector, and the sample was collected in a trap cooled in dry ice-methyl cellosolve as described (5, p. 49). Several consecutive trappings of three regions of the GLC effluent from the Apiezon separation were made.

The trapped components were rechromatographed by temperature programming on a polar column in the same instrument. Conditions were adjusted with each trap so that maximum separation and resolution were obtained. A 12 feet × 1/8 inch OD column packed with diethylene glycol succinate (DEGS) on 80/100 mesh acid-alkali washed Celite 545 was used. The collection trap containing flavor

compounds was connected in front of the DEGS column, the oven temperature adjusted, and the carrier gas turned on at a flow rate of 35 ml/min. Retention times of known compounds were obtained under similar conditions by on column injection with the collection trap connected in front of the DEGS column.

Rapid scan mass spectrometry was used in conjunction with GLC to positively identify the major peaks in the chromatograms. An Atlas-MAT CH-4 Nier-type mass spectrometer (nine inch, 60 degree sector, single focusing instrument) was used for all mass spectral analysis. The mass spectra were recorded with a Honeywell Model 1508 Visicorder. The GLC instrument was equipped with a 10:1 splitter so that 10% of the effluent went to the hydrogen flame detector. Of the column effluent going to the mass spectrometer approximately 5% of the flow went through the EC-1 gas inlet valve to the ion source. The remaining 95% of the effluent was directed through a heated tube so that the odor of the components being analyzed could be simultaneously evaluated. The operating conditions used for analysis of DEGS column GLC effluent were:

Filament current	60 μ-amps
Electron voltage	30 eV
Accelerating voltage	3000 V
Analyzer pressure	2×10^{-7} mm Hg
Multiplier voltage	1.60-1.85 KV
Scanning speed	5 sec. to scan m/e 25 to m/e 250

Although good spectra were obtained for several major peaks

from each trapping, it became apparent that a higher concentration of neutral compounds would facilitate spectral interpretation. Hence, flavor concentrates obtained by the distillation procedure were washed with alkali to remove FFA prior to GLC as described previously. The concentrates obtained in this manner were also subjected to temperature programmed GLC. No trapping step was necessary.

One FFA free concentrate was analyzed using the same instrument, temperature program and column conditions as used for the initial separation of the FFA containing concentrate. A base treated pre-column was not used. A 10:1 splitter directed a major portion of the column effluent to the mass spectrometer. The operating conditions used for this mass spectral analysis were as follows:

Filament current	60 μ-amps
Electron voltage	70 eV
Accelerating voltage	3000 V
Analyzer pressure	5×10^{-7} mm Hg
Multiplier voltage	2.2 KV
Scanning speed	4.5 sec. to scan m/e 25 to m/e 250

Previous analysis and retention time data indicated that compounds emerging from the column during the first 40 minutes were of molecular weights less than 200. Wide slit geometry was used to increase sensitivity with no serious loss in resolution.

Another FFA free concentrate was analyzed using the following conditions:

Instrument	F and M model 810
Detector	Hydrogen flame
Injection	On column
Column	20% Apiezon M on 80/100 mesh acid-alkali washed Celite 545
Column dimensions	12 feet × 1/8 inch OD
Initial temperature	60 ° C for 12 minutes
Temperature program	4 ° C/minute to 200 ° C
Flow rate	35 ml/minute of He
Detector temperature	195 ° C
Injector port	205 ° C
Auxiliary line	190 ° C
Range	10
Attenuator	x1
Effluent splitter	1:5

Mass spectral analysis of the column effluent was carried out under the same conditions as with the previous concentrate and wide slit geometry was used for the first 35 minutes.

Determination of Swiss Cheese Volatiles by On-Column Trapping

Six Swiss-type cheeses of typical flavor were examined. Four were domestic rindless Swiss cheese. Three of these domestic cheeses were obtained from local retail outlets. The other cheese was that used in the low-temperature low-pressure distillations. The remaining two were imported Emmenthal cheeses purchased as five pound wedges from a retail delicatessen.

Separation and Identification

The flavor volatiles of the six Swiss cheeses were determined

by the gas-entrainment and on column trapping technique developed by Morgan and Day (38). The GLC columns used for the analysis had a three-inch U-shaped bend near the injection port end. These U-shaped sections were cooled in a methyl cellosolve-dry ice bath during sample purging. The apparatus shown in Figure 2 was used. Nitrogen gas passed through the sample at a rate of eight ml/minute.

Whole cheese samples were prepared as reported by Bills (9, p. 56). Two grams of cheese was gound intimately with five g of anhydrous sodium sulfate in a 25 × 55 mm screw caped vial. Six ml of distilled water was added to the sample. Vigorous shaking suspended the sample in the aqueous phase prior to purging. During the purging the sample vial was immersed in an isothermal 80° C water bath.

Cheese fat that had been prepared as described previously was also analyzed. In these experiments 10 ml (9.2 g) of fat was pipetted into a sample vial containing five g of anhydrous sodium sulfate. The remainder of the purging procedure was the same as used with whole cheese.

The GLC operating conditions were as follows:

Instrument	F and M 810
Detector	Hydrogen flame
Columns	20% Tris 1, 2, 3-(2-cyanoethoxy) propane (TRIS) and 20% Carbowax 600, both on 60/80 mesh acid-alkali washed Celite 545
Column dimensions	Each column 12 feet × 1/8 inch OD
Temperature	Isothermal at 50° C and 80° C

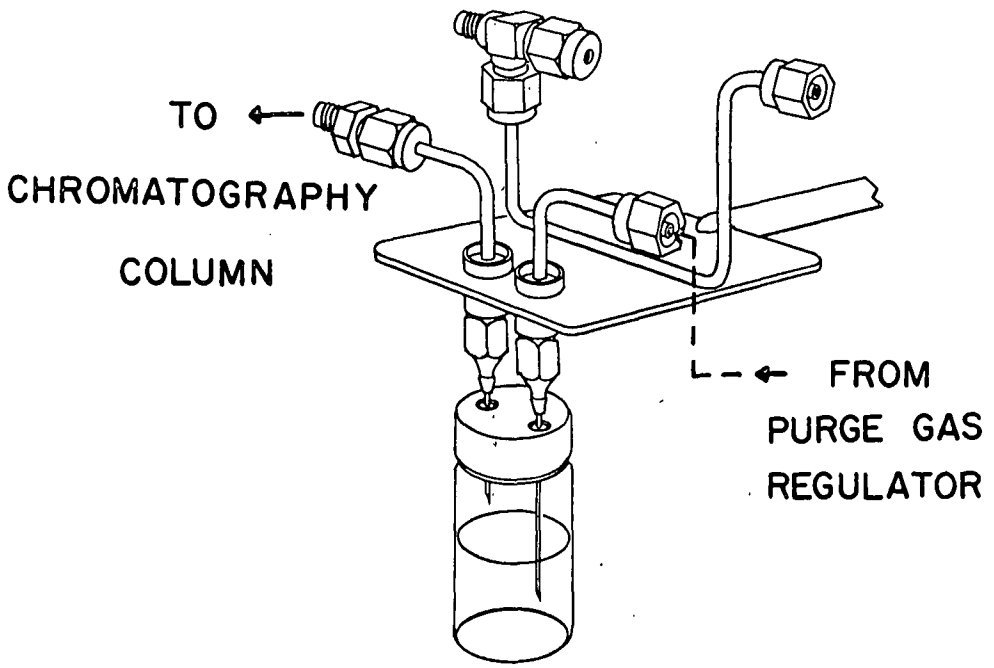

Figure 2. Apparatus used for entrainment and on-column trapping of Swiss cheese volatiles.

Flow rate	30 ml/minute of He
Purge flow rate	8 ml/minute of N_2
Detector temperature	200° C
Injector port	190° C
Range	10
Attenuation	x1
Splitter	Not used

The retention times of known compounds were determined by sample enrichment with knowns. For the whole cheese, knowns were added to the aqueous phase. In the fat samples knowns were added either directly to the fat or dissolved in small quantities of mineral oil.

For confirmation of retention data and positive identification, mass spectral analyses were obtained on entrained volatiles from one of the cheeses analyzed. Two 15 ml (13.8 g) cheese fat samples were each purged for 30 minutes. Trapped volatiles were analyzed isothermally at 50° C and 80° C on the TRIS column. Wide slit geometry was used throughout the mass spectral analysis of the volatiles. The conditions of the mass spectral analysis were the same as those used in the anlaysis on the F and M model 810 of the FFA free concentrate.

Quantitative Analysis

The concentration of several volatile compounds identified by the gas entrainment and on-column trapping technique were determined by relating their peak heights to known quantities of compound. Standard compounds were added in known concentrations to whole

cheese (Dagano variety) or butter fat as described above. Dagano cheese, which has some similarity to Swiss cheese, served as a good carrier for knowns since the GLC pattern of volatiles from this cheese was different than that of Swiss cheese and much less intense. Butter fat served as carrier for knowns used in the determination of the concentration of some volatiles from cheese fat. Volatiles identified in cheese fat were either absent in butterfat or in very low concentration.

Quantitative Determination of the Free Fatty Acids in Swiss Cheese

The same six cheeses used in the analysis of Swiss cheese volatiles by on-column trapping were analyzed. Small pieces, obtained at random from several parts of each cheese, were throughly mixed to ensure that the samples were representative. All subsequent analyses were performed on the mixed samples.

Formic, Acetic, Propionic and Butyric Acids

The liquid-liquid partition column of Wiseman and Irwin (54) was used to quantify these acids. The cap material consisted of 2.5 g of cheese and 4.5 g of silicic acid. Fractions eluted from the column were titrated with standardized-isopropanolic potassium hydroxide (4, p. 18-19).

Higher Acids

The higher acids, including 2- and 3-methyl butyric acids were determined by the method of Bills and Day (10). Since no n-valeric acid was detected in any of the cheeses examined, this acid was used as an internal standard for the determination of 2- and 3- methyl butyric acids. A 500 g sample of cheese was ground in a motar with sufficient 50% sulfuric acid to reduce the pH to 1.9 and 10 ml of a hexane solution containing 20 mg n-valeric, 25 mg enanthic, and 250 mg margaric acids for internal standards was added. The free fatty acids were isolated and converted to their methyl esters as previously reported (10).

The methyl esters of 2- and 3- methyl butyric acid were separated using an Aerograph Model A-100 and a 20 feet $\times \frac{1}{4}$ inch OD aluminum column packed with 20% Carbowax 1540 on 100/120 mesh acid-alkali washed Celite 545 operated at 82° C. Carrier gas flow rate (He) was 80 ml/minute. Detector response is not linear with respect to weight for different esters (11); hence, factors for relating the peak area of the ester of the internal standard (n-valeric acid) to the peak areas of the esters of 2- and 3- methyl butyric acids were determined (11). These factors are shown in Table 1.

TABLE 1. Factors for relating the internal standard to the methyl esters of 2- and 3- methyl butyric acids.

Acid	Number of trials	Average factor[a]	Standard deviation
2-methyl butyric	4	1.363	0.0226
3-methyl butyric	4	0.983	0.0143
n-valeric	4	1.000[b]	----

[a] Corrected for acid purity

[b] Assigned

The total moles of higher fatty acids (longer than butyric) determined by the Wiseman Irvin column (54) were distributed according to their molar ratios, as determined by GLC (10).

Compounding Synthetic Swiss Cheese Flavor

A base for evaluating synthetic flavor was made by mixing pasteurized cream, milk fat, dry curd cottage cheese and salt in a Waring Blender. Flavor compounds were added during the blending directly or dissolved in milk fat or aqueous solutions. In compounding the synthetic mixtures an attempt was made to maintain the proportions of protein, fat, water and salt in the natural cheese i. e. approximately 28%, 30%, 36% and 1.3% respectively.

RESULTS AND DISCUSSION

Identification of Isolated Flavor Compounds from Swiss Cheese

Low-Temperature Low-Pressure Distillation of Cheese Fat

The concentrate obtained from the ethyl chloride extraction of the trap contents from the low-temperature low-pressure distillation of Swiss cheese fat was chromatographed by temperature programming (55° C for 15 minutes then programmed at 3° C/minute to 200° C and held) on an Apiezon column. Figure 3 shows the resulting chromatogram from a six μl injection under these conditions. A base-treated pre-column was employed to remove the FFA from the injected sample. The large quantities of low molecular weight volatile fatty acids in Swiss cheese which were concentrated in the flavor extract, if not removed, interfered with the separation and resolution of components shown in Figure 3. Results with known compounds, on the stale flavor of concentrated milk (5, p. 62-65) and the flavor of irradiated milk fat (24, p. 74), indicate that the base-treated pre-column does not induce artifacts or cause serious decompositions of extract components.

In order to obtain usable mass spectra it was necessary to concentrate the components separated on the Apiezon column. Hence,

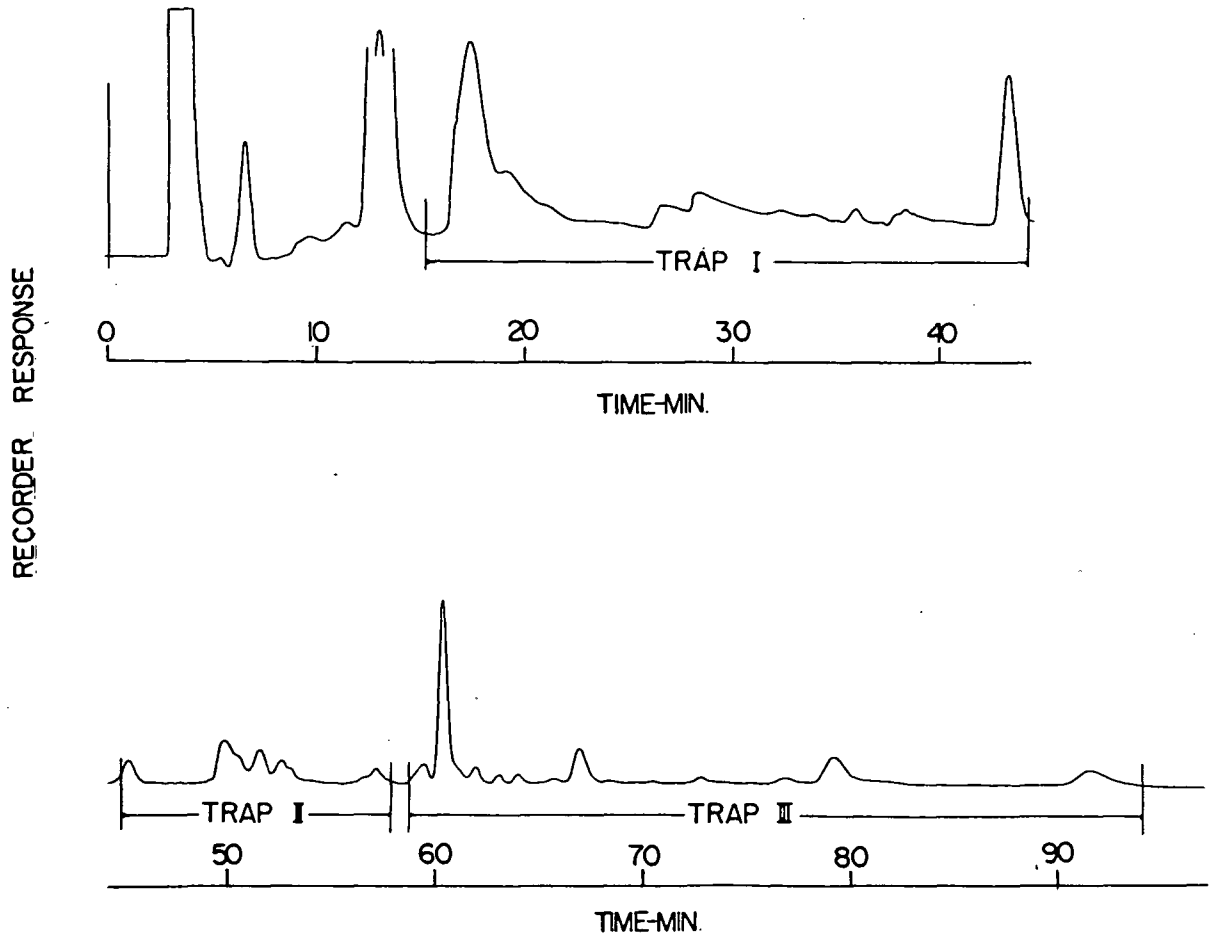

Figure 3. Chromatogram of components in flavor concentrate. Apiezon column, temperature programmed at 3°C/minute from 55°C (15 minutes isothermal) to 200°C. Base-treated (5% by weight) pre-column used to remove FFA.

the trapping technique was used. The three regions as designated in Figure 3 were selected. Odor evaluation of GLC effluent during the temperature programmed run indicated that some components in these three regions had very intense aromas.

The trapped components were rechromatographed on a polar DEGS column in an attempt to attain better separation and resolution of components. Conditions found most suitable for the trapped components on DEGS are given in Table 2.

TABLE 2. Chromatographic conditions for trapped components on DEGS.

Trap no.	Initial temperature	Post injection interval	Temperature program[a]	Number of consecutive trappings[b]
I	50° C	20 min.	2° C/min to 175° C	10
II	60° C	15 min.	3° C/min. to 175° C	10
III	80° C	15 min.	3° C/min. to 175° C	10

[a]Temperature held at 175° C on completion of program.

[b]Trapped from Apiezon column shown in Figure 3.

Chromatograms of the components of traps I, II and III are shown in Figures 4, 5 and 6, respectively. Peak identifications made by comparison of retention times of knowns and unknowns relative to 2-heptanone, ethyl hexanoate, and 2-nonanone for traps I, II and III

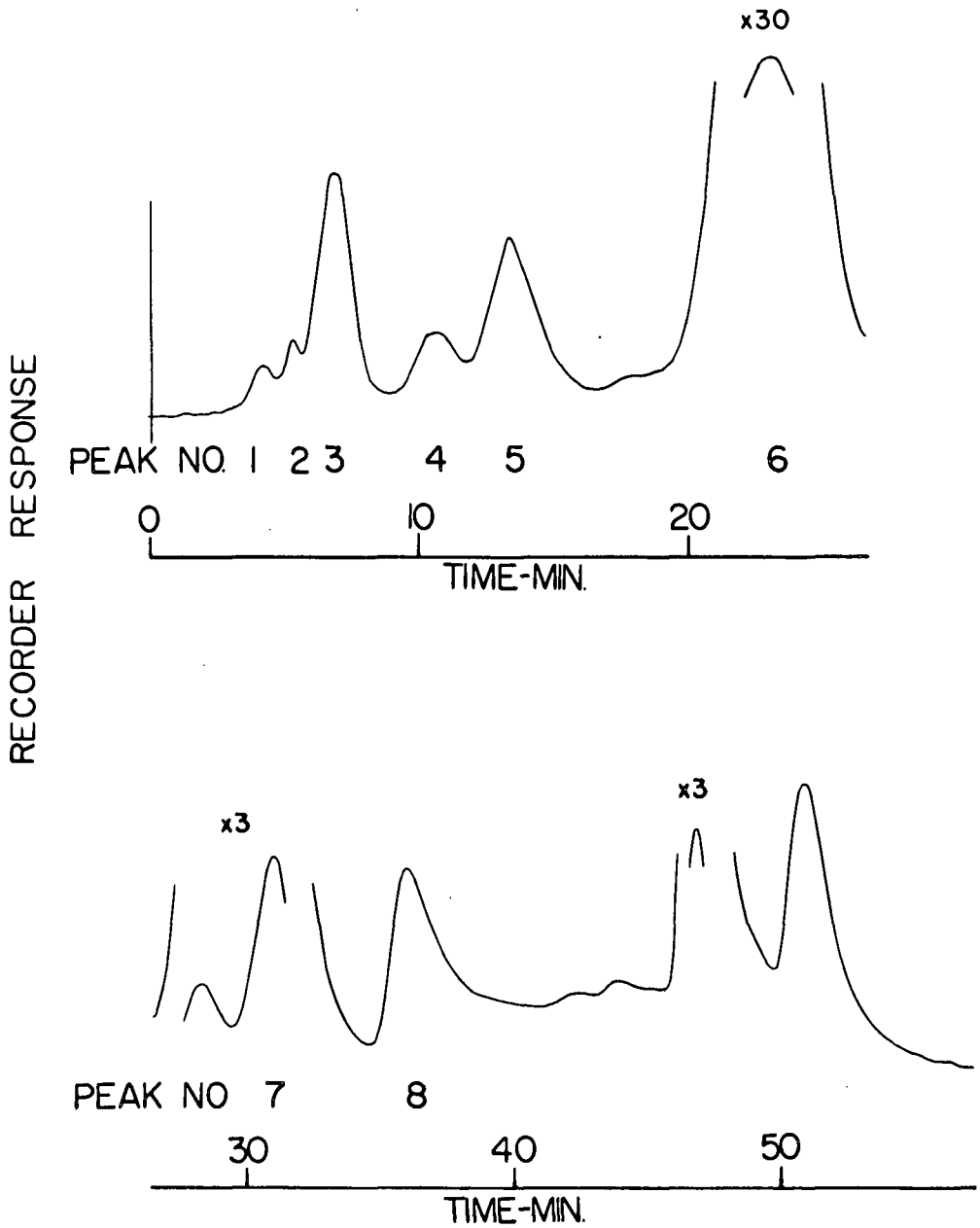

Figure 4. Chromatogram of the components in Trap I on DEGS.

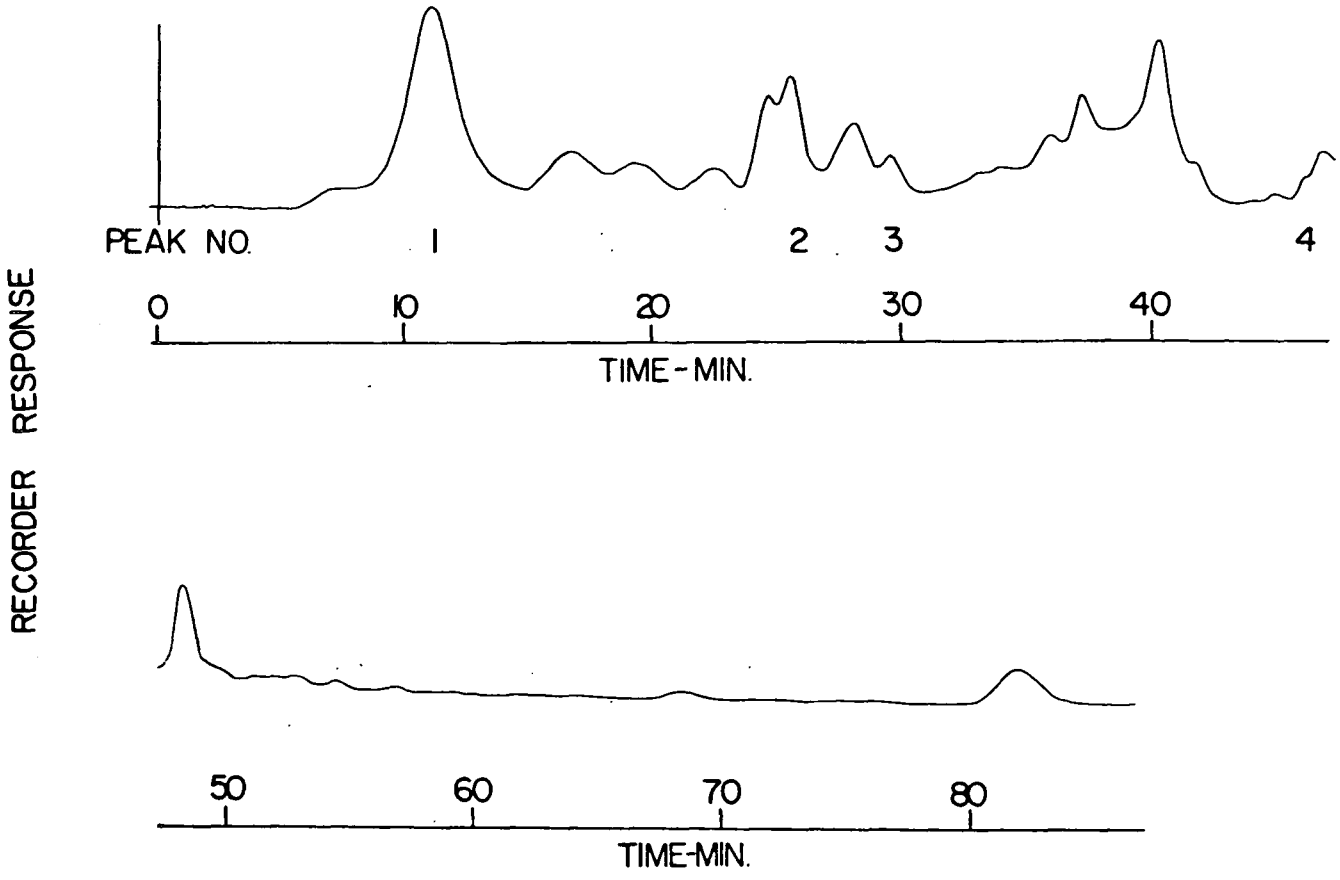

Figure 5. Chromatogram of the components in Trap II on DEGS.

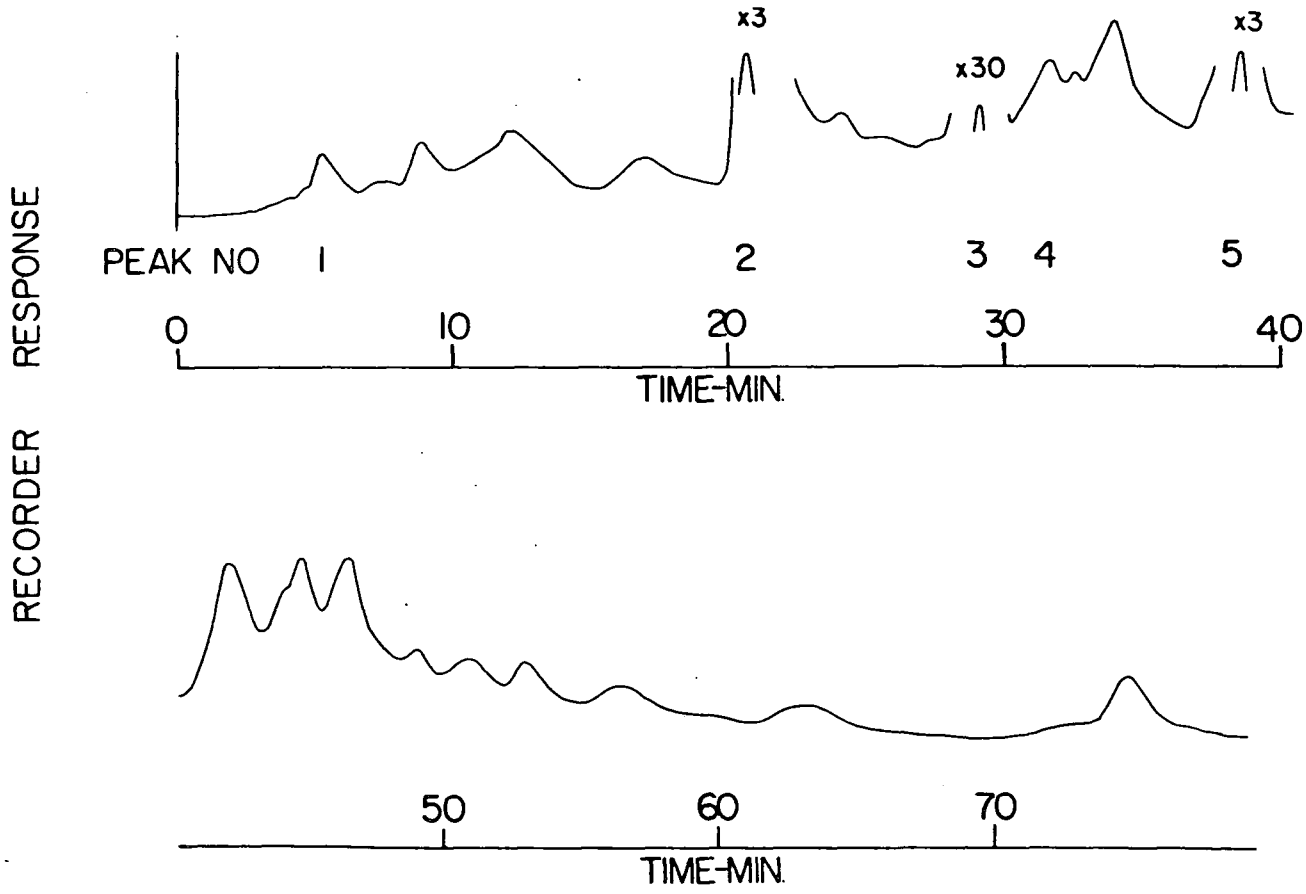

Figure 6. Chromatogram of the components in Trap III on DEGS.

respectively are summarized in Table 3. The cooling bath solvent, 2-methoxyethanol was found in Trap I and III. Mass spectral identifications and the mass spectral references are also indicated in the table.

Figure 7 shows the improved separation and resolution obtained when the concentrate was washed free of FFA prior to GLC analysis on Apiezon. Component concentrations from the two μl injections were sufficient for direct mass spectral identification. Identifications are shown in Table 4. Informal odor evaluations of GLC effluent are shown in the last column of Table 4. Peak assignments were made by comparison of retention times of knowns and unknowns relative to 2-heptanone. One component, peak 25, had a sufficiently strong spectra to make a positive identification although the authentic 1, 2, 4- trichlorobenzene was not available for confirmation of retention time. The parent peak (molecular weight) of the mass spectrum of an aromatic halogen compound is readily discernible (50, p. 17). Due to the presence of ions containing the Cl^{37} isotope, the number of chlorine atoms in a molecule can be determined by the number of alternate peaks beyond the parent peak for the compound. In the case of a tri-chloro-substituted benzene there are isotope contributions at m/e 182 (P+2), m/e 184 (P+4) and m/e 186 (P+6). Of the possible isomers, the 1, 2, 4 isomer has a peak at m/e 74 of much greater intensity than found with the others,

TABLE 3. Gas chromatographic and mass spectral identification of trapped components. See Figures 4, 5, and 6 for the corresponding chromatogram.

	Peak no.	Compound	t_R/t_R Swiss cheese	t_R/t_R Authentic	Mass Spectral Identification	Ref.
Trap I	1	Hexane	0.076	0.072	Positive	(2)
	2	Octane	0.160	0.152	Positive	(2)
	3	Methanol	0.195	0.201	Positive	(18)
	4	2-Pentanone	0.338	0.345	Positive	(47)
	5	Toluene	0.426	0.426	Positive	(2)
	6	Butanol	0.744	0.742	Positive	(18)
	7	2-Heptanone	1.000	1.000	Positive	(47)
	8	2-Methoxyethanol	1.160	1.120	Positive	(2)
Trap II	1	α-Pinene	0.430	0.439	Positive	(46)
	2	Ethyl hexanoate	1 000	1.000	Positive	(3)
	3	Methyl octanoate	1.159	1.209	Positive	(3)
	4	Benzaldehyde	1.816	1.785	Positive	(3)
Trap III	1	Nonane	0.180	0.185	Positive	(2)
	2	2-Methoxyethanol	0.709	0.718	Positive	(2)
	3	2-Nonanone	1.000	1.000	Positive	(3)
	4	o-Dichlorobenzene	1.085	1.097	Positive	(3)
	5	2-Undecanone	1.321	1.370	Positive	(47)

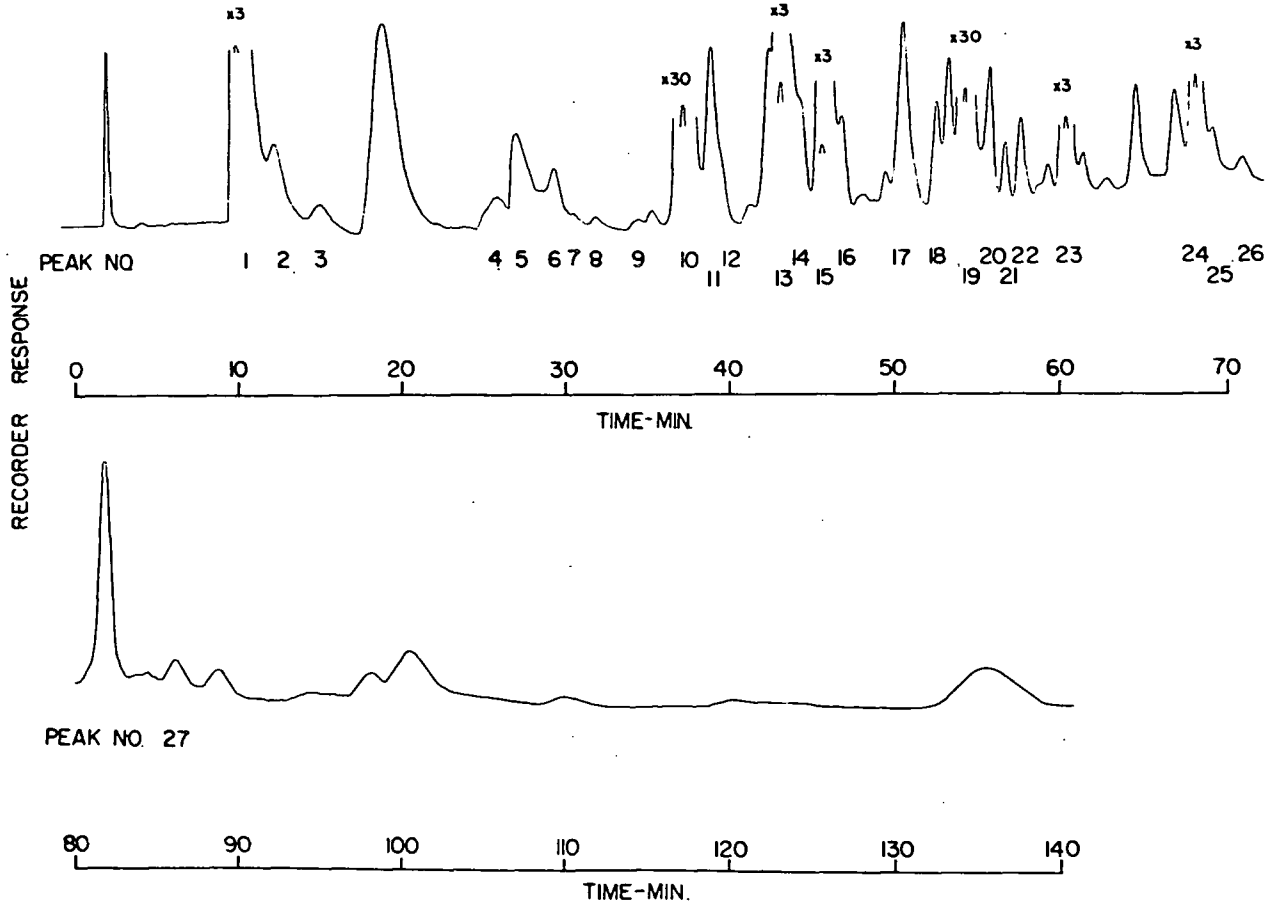

Figure 7. Chromatogram of components in the FFA free flavor concentrate. Apiezon column, temperature programmed at 3° C/minute from 55° C (15 minutes isothermal) to 200° C.

TABLE 4. Gas chromatographic and mass spectral identification of components from the FFA free concentrate. See Figure 7 for the chromatogram.

Peak no.	Compound	t_R/t_R[a] Swiss cheese	t_R/t_R[a] Authentic	Mass Spectral Identification	Ref.	Odor
1	1-Butanol	0.274	0.283	Positive	(18)	
2	2-Pentanone	0.334	0.343	Positive	(47)	Buttery
3	Butanol	0.408	0.389	---		Alcohol
4	Ethyl butanoate	0.696	0.699	Positive	(2)	Ethyl butanoate
5	Trans-2-Hexene-1-ol	0.727	0.723	Positive	(23)	Green
6	2-Hexanol	0.789	0.790	---		Alcohol
7	Toluene	0.821	0.798	Positive	(2)	Estery
8	Nonane	0.857	0.860	---		
9	Cis-3-hexene-1-ol	0.919	0.907	---		Grass like
10	2-Heptanone	1.000	1.000	Positive	(47)	2-Heptanone
11	Heptanal	1.045	1.042	---		Aldehyde
12	Methyl hexanoate	1.063	1.062	Positive	(3)	Estery
13	Aromatic hydrocarbon	1.159	----	Tentative	(2)	Caramel
14	Pinane	1.191	----	Tentative	(45)	Pungent alcohol
15	Ethyl hexanoate	1.226	1.228	Positive	(3)	Ethyl hexanoate
16	Benzaldehyde	1.257	1.265	Positive	(3)	Aldehyde
17	α - Fenchene	1.359	----	Tentative	(46)	Acetophenone- old corn
18	Phenylacetaldehyde	1.411	1.409	----		Phenylacetaldehyde
19	2-Nonanone	1.460	1.449	Positive	(3)	2-Nonanone
20	Methyl octanoate	1.499	1.497	Positive	(3)	Ester
21	Dodecane	1.524	1.532	---		Aromatic
22	2-Phenylethanol	1.549	1.553	----		Sherry wine
23	Ethyl octanoate	1.622	1.623	Positive	(3)	Ester
24	2-Undecanone	1.830	1.859	Positive	(47)	Methyl ketone
25	1, 2, 4-Trichlorobenzene	1.859	----	Positive	(3)	
26	Methyl decanoate	1.907	1.906	Positive	(3)	Green
27	Ethyl decanoate	2.112	2.133	----		Alcohol

[a]Relative retention time with t_R/t_R of 2-heptanone = 1.000.

which simplified identity of peak 25 (Table 4).

Another FFA free concentrate was analyzed under different conditions and the chromatographic separation is shown in Figure 8. A larger injection (eight instead of two μl) than used in the preceding analysis enabled the positive identification of a greater number of components in the concentrate. A summary of identifications made from the GLC separation of Figure 8 are shown in Table 5. The basis of peak assignments was the comparison of the retention times of known and unknown components relative to 2-heptanone. On examination of the mass spectra obtained it appeared that peaks 6, 7, and 29 (see Table 5) were mixtures. The utility of mass spectrometry in flavor chemistry where mixtures of components often are not entirely resolvable has been discussed (45). If mass spectra are taken at several points on a given peak suspected to be a mixture of components, it is sometimes possible to ascertain the identity of components in the mixture by comparison of m/e intensity changes from spectrum to spectrum. This was the case with peak 6. A spectrum taken prior to the attainment of maximum GLC peak height showed a base peak at m/e 57. Also a prominent parent at m/e 86. These data suggested 2-methylbutyraldehyde. Retention time data confirmed the presence of this compound. A spectrum taken after attainment of maximum peak height showed a base peak now at m/e 31. The peak at m/e 57 had dropped considerably in intensity and

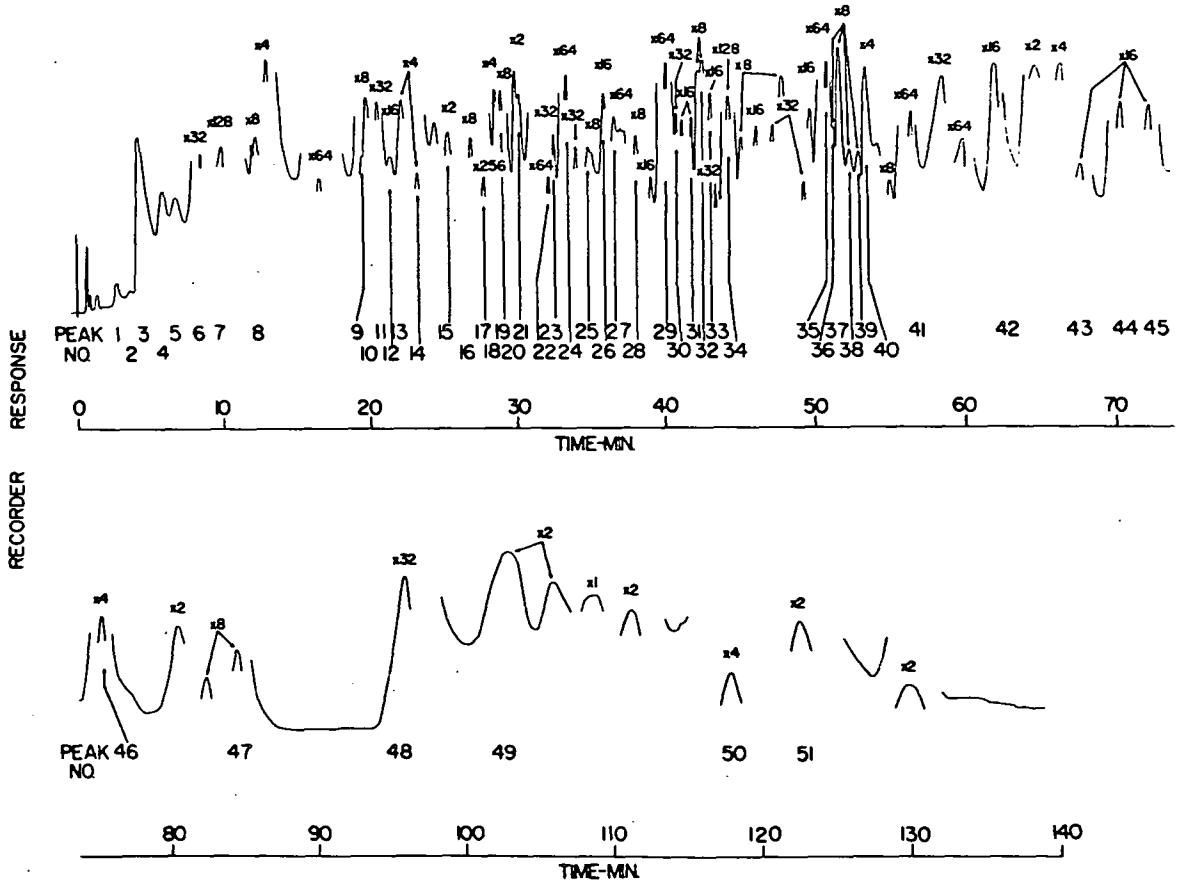

Figure 8. Chromatogram of components in the FFA free flavor concentrate. Apiezon column, temperature programmed at 4° C/minute from 60° C (12 minutes isothermal) to 200° C.

TABLE 5. Gas chromatographic and mass spectral identification of components from the FFA free concentrate. See Figure 8 for the chromatogram.

Peak no.	Compound	t_R/t_R^a Swiss cheese	t_R/t_R^a Authentic	Mass Spectral Identification	Ref.	Odor
1	Methyl acetate	0.098	0.094	Positive	(2)	
2	1-Propanol	0.130	0.123	Positive	(18)	
3	Butanone	0.152	0.160	Positive	(47)	
4	2-Methyl propanal	0.212	0.216	Positive	(3)	
5	Chloroform	0.244	0.253	Positive	(2)	
6	2-Methyl butyraldehyde	0.306	0.291	Positive	(3)	Alcohol, spicy
	1-Butanol	0.306	0.305	Positive	(18)	
7	2-Pentanone	0.359	0.352	Positive	(47)	Estery
	Ethyl propionate	0.359	0.359	Positive	(2)	
8	2-Pentanol	0.443	0.431	Positive	(18)	
9	Ethyl butanoate	0.704	0.684	Positive	(2)	Ethyl ester
10	2-Hexanone	0.717	0.718	Positive	(47)	Methyl ketone
11	Butyl acetate	0.744	0.735	Positive	(2)	Fruity
12	Toluene	0.771	0.787	Positive	(2)	
13	1-Octene	0.803	0.802	Positive	(2)	
14	Nonane	0.838	0.848	Positive	(2)	
15	3-Methylbutyl acetate	0.916	0.924	Positive	(3)	Fruity
16	Trans-2-hexene-1-ol	0.972	0.943	Positive	(23)	Green
17	2-Heptanone	1.000	1.000	Positive	(47)	2-Heptanone
18	Ethyl benzene	1.032	----	Tentative	(2)	Aromatic
19	Methyl hexanoate	1.048	1.065	Positive	(3)	Methyl ester
20	1, 2-; 1, 3-; or 1, 4-Dimethyl benzene	1.083	----	Tentative	(2)	
21	1-Nonene	1.093	1.077	Positive	(2)	
22	γ-Valerolactone	1.162	1.139	Positive	(37)	
23	α-Pinene	1.175	1.225	Positive	(46)	Woody
24	Ethyl hexanoate	1.208	1.207	Positive	(3)	Ethyl hexanoate
25	Benzaldehyde	1.259	1.278	Positive	(3)	Benzaldehyde

TABLE 5. (Continued)

Peak no.	Compound	t_R/t_R[a] Swiss cheese	t_R/t_R[a] Authentic	Mass Spectral Identification	Ref.	Odor
26	Methyl benzoate	1.295	----	Tentative	(2)	Aromatic
27	α-Fenchene	1.325	----	Tentative	(46)	
28	Phenylacetaldehyde	1.376	1.419	Positive	(3)	Flowery
29	2-Nonanone	1.453	1.455	Positive	(3)	2-Nonanone
	o-Dichlorobenzene	1.453	1.470	Positive	(3)	
30	Methyl octanoate	1.484	1.509	Positive	(3)	Estery
31	Dodecane	1.521	1.542	Positive	(2)	Hydrocarbon
32	2-Phenyl-2-methylbutane	1.549	----	Tentative	(2)	Aromatic
33	2-Phenylethanol	1.573	1.579	Positive	(3)	Sherry wine
34	Ethyl octanoate	1.610	1.617	Positive	(3)	Ethyl ester
35	2-Undecanone	1.852	1.854	Positive	(47)	Ketone
36	δ-Octalactone	1.866	1.876	Positive	(37)	Coconut
37	Methyl decanoate	1.880	1.921	Positive	(3)	Estery
38	1,2,4-Trichlorobenzene	1.906	----	Positive	(3)	
39	5-Methyl-5-Ethyl decane	1.927	----	Tentative	(2)	Hydrocarbon
40	Benzothiazole	1.949	1.982	Positive	(2)	Rubbery
41	Ethyl decanoate	2.057	2.049	Positive	(2)	Estery
42	3-Methyl butyl octanoate	2.259	----	Tentative	(3)	
43	2,5-Dimethyl tetradecane	2.467	----	Tentative	(2)	Hydrocarbon
44	2-Tridecanone	2.563	2.553	Positive	(3)	Ketone
45	δ-Decalactone	2.630	2.613	Positive	(37)	Peach, lactone
46	Pentadecane	2.735	2.753	Positive	(2)	Hydrocarbon
47	Ethyl dodecanoate	3.071	2.976	Positive	(3)	Faint ester
48	Di-isobutyl adipate	3.468	----	Positive	(3)	
49	γ-Dodecalactone	3.717	----	Positive	(37)	Spice, lactone
50	2-Pentadecanone	4.265	----	Positive	(3)	Heavy ketone
51	δ-Dodecalactone	4.432	4.379	Positive	(47)	Coconut

[a]Relative retention time with t_R/t_R of 2-heptanone = 1.000.

the parent peak of 2-methyl butyraldehyde at m/e 86 had disappeared. The intensity of the peak at m/e 56 was largely increased. These data were suggestive of 1-butanol which was confirmed by retention time data. Similar techniques and methods of spectra interpretation led to identifications of the components in peaks 7 and 29. The spectra for peaks 38, 48, 49 and 50 were sufficiently strong to make positive identifications.

On-Column Trapping of Swiss Cheese Volatiles

Six typical flavored cheeses were examined by the on-column trapping technique (38). The TRIS column and the Carbowax 600 column, both operated isothermally at 50 and 80° C, were used for analyses. An example of the chromatographic separation obtained by on-column trapping on TRIS of volatiles purged from a sample of Swiss cheese fat is shown in Figure 9. Sample purging time was ten minutes. Peak identifications are indicated in Table 6. Table 6 also shows if the peak identity was confirmed by GLC on the Carbowax 600 column. GLC in conjunction with mass spectrometry was used to positively or tentatively identify some of the volatiles constitutents purged from the cheese. Mass spectral identifications and the mass spectral reference are indicated in the last two columns respectively of Table 6. In order to increase concentration for the mass spectral analysis, two fat samples were each purged 30 minutes

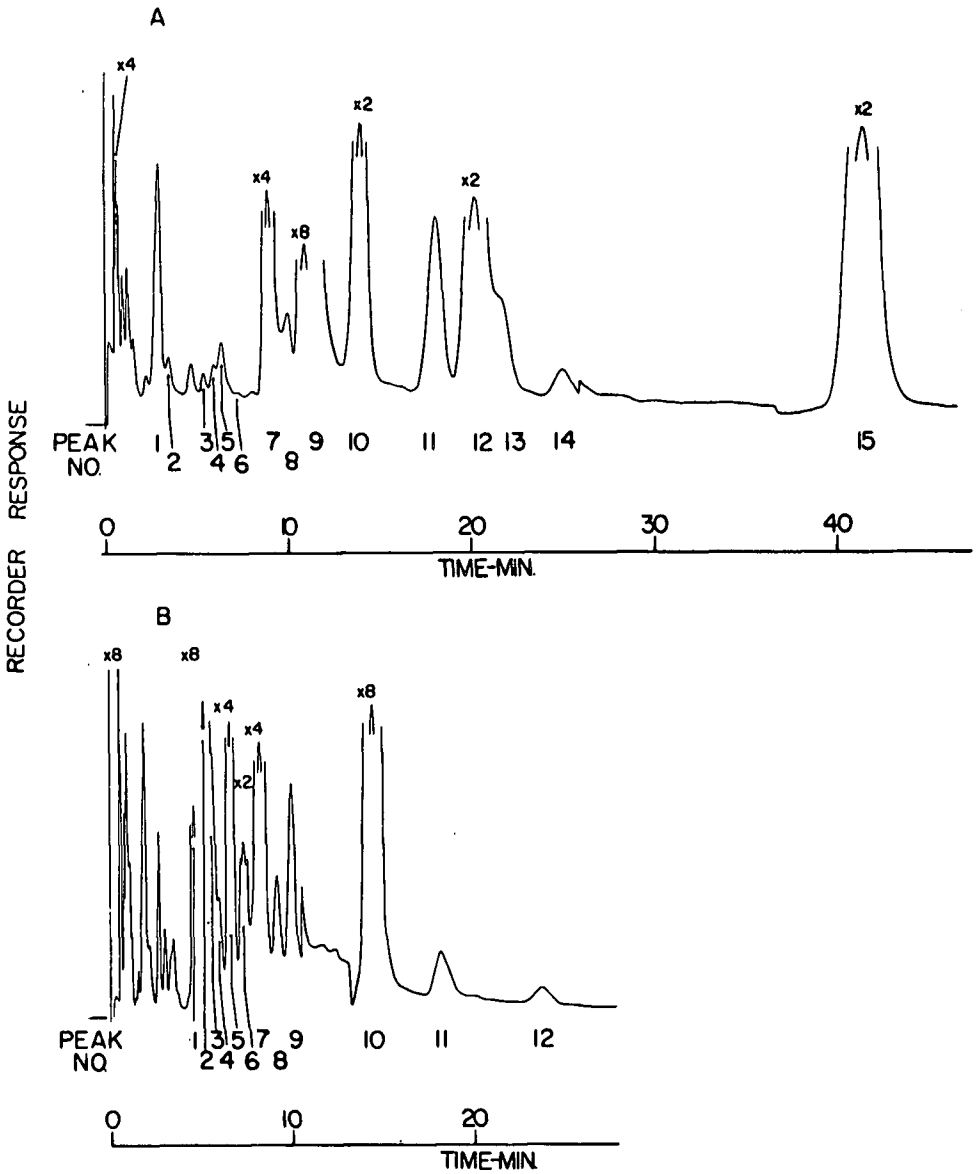

Figure 9. Chromatograms of the volatile components of a Swiss cheese obtained by on-column trapping. TRIS column. Chromatogram A obtained at 50° C and B at 80° C.

TABLE 6. Gas chromatographic and mass spectral identification of the volatile components of a Swiss cheese obtained by on-column trapping. See Figure 9 for the chromatogram.

Peak no.	Compound	t_R/t_R[a] Swiss cheese	t_R/t_R[a] Authentic	Confirmed on Carbowax 600	Mass Spectral Identification	Ref.
Column at 50°C						
1	Dimethyl sulfide	0.262	0.260	yes	Positive	(2)
2	Acetaldehyde	0.316	0.310	yes	Positive	(2)
3	Ethyl formate	0.485	0.498	yes	----	
4	Propanal	0.537	0.525	yes	----	
5	2-Methyl propanal	0.581	0.580	yes	----	
6	Methyl acetate	0.654	0.653	yes	----	
7	Acetone	0.816	0.815	yes	Positive	(47)
8	Butanal	0.882	0.874	yes	----	
	Ethyl acetate	0.882	0.870	yes	----	
	Methyl vinyl ether	0.882	---	---	Tentative	(3)
9	Ethanol	1.000	1.000	yes	Positive	(18)
	2- and 3- Methyl butyraldehyde	1.000	1.010	yes	---	
10	Butanone	1.294	1.280	yes	Positive	(47)
	Ethyl propionate	1.294	1.238	yes	----	
11	2-Butanol	1.639	1.640	yes	----	
	Toluene	1.639	1.645	yes	Positive	(2)
12	1-Propanol	1.827	1.834	yes	Positive	(18)
13	Ethyl butyrate	1.978	1.913	yes	----	
	2-Pentanone	1.978	2.020	yes	----	
14	2-Methyl propenal	2.357	----	----	Tentative	(3)
15	1-Butanol	3.662	3.639	yes	Positive	

TABLE 6. (Continued)

Peak no.	Compound	t_R/t_R^a Swiss cheese	t_R/t_R^a Authentic	Confirmed on Carbowax 600	Mass Spectral Identification	Ref.
Column at 80°C						
1	Acetone	0.888	0.881	yes	Positive	(47)
2	Ethanol	1.000	1.000	yes	Positive	(18)
3	2- and 3- Methyl butyraldehyde	1.083	1.105	yes	----	
4	Ethyl propionate	1.156	1.175	yes	Positive	(2)
5	Butanone	1.287	1.298	yes	Positive	(47)
6	2-Butanol	1.438	1.444	yes	----	
7	1-Propanol	1.603	1.597	yes	Positive	(18)
	Ethyl butanoate	1.603	1.566	yes	----	
	Toluene	1.603	1.616	yes	Positive	(2)
8	2-Pentanone	1.780	1.805	yes	Positive	(47)
9	Diacetyl	1.948	1.936	yes	Positive	(2)
10	1-Butanol	2.809	2.777	yes	Positive	
11	Methyl hexanoate	3.534	3.492	yes	----	
12	2-Heptanone	4.691	4.743	yes	Positive	(47)

[a]Relative retention time with t_R/t_R of Ethanol = 1.000.

onto the TRIS column. This enabled the identification of most major components of Swiss cheese volatiles. Peak 7 of the 80°C chromatogram proved to be a mixture of components. Toluene was identified as one component of this mixture. The parent ion of aromatic compounds is stabilized by the presence of the ring (50, p. 8). Toluene, which is an alkyl-substituted aromatic compound, fragments at the bond beta to the ring giving the base peak of the spectrum at m/e 91. The resulting tropylium ion is resonance-stabilized. This considerably simplifies spectral interpretation. Thus toluene is easily detected even in mixtures such as peak 7. Chromatograms similar to Figure 9 were obtained when whole cheese samples were purged instead of fat, but the peak heights were considerably reduced.

A summary of all positively identified flavor compounds in Swiss cheese is shown in Table 7. This table includes the results from the low-temperature low-pressure distillations of Swiss cheese fat and from the on-column trapping technique for determination of Swiss cheese volatiles.

Origin and Significance of Compounds Identified in Swiss Cheese

A total of 54 compounds representing several chemical classes were identified in Swiss cheese. Previously only acetaldehyde,

TABLE 7. Summary of flavor compounds positively identified in Swiss cheese.

Alcohols
- Methanol
- Ethanol
- 1-Propanol
- 1-Butanol
- 2-Pentanol
- Trans-2-hexene-1-ol
- 2-Phenylethanol

Aldehydes
- Acetaldehyde
- 2-Methyl propanal
- 2-Methyl butyraldehyde
- Benzaldehyde
- Phenylacetaldehyde

Methyl esters
- Methyl acetate
- Methyl hexanoate
- Methyl octanoate
- Methyl decanoate

Ethyl esters
- Ethyl propionate
- Ethyl butanoate
- Ethyl hexanoate
- Ethyl octanoate
- Ethyl decanoate
- Ethyl dodecanoate

Miscellaneous compounds
- Dimethyl sulfide
- Diacetyl
- Benzothiazole
- o-Dichlorobenzene
- 1, 2, 4-Trichlorobenzene
- Di-isobutyl adipate
- Chloroform

Methyl ketones
- Acetone
- Butanone
- 2-Pentanone
- 2-Hexanone
- 2-Heptanone
- 2-Nonanone
- 2-Undecanone
- 2-Tridecanone
- 2-Pentadecanone

Hydrocarbons
- Hexane
- Octane
- 1-Octene
- Nonane
- Nonene
- Dodecane
- Pentadecane
- Toluene
- α-Pinene

Other esters
- Butyl acetate
- 3-Methylbutyl acetate

Lactones
- γ-Valerolactone
- γ-Dodecalactone
- δ-Octalactone
- δ-Decalactone
- δ-Dodecalactone

acetone, 2-pentanone and diacetyl had been positively identified in Swiss cheese (7).

Alcohols

The importance of methanol and ethanol in cheese is their availability for methyl and ethyl ester formation. Ethanol is a common fermentation product; however, the mode of formation of methanol is unknown. 1-Propanol and 1-butanol might be expected to result from the reduction of the corresponding aldehydes. Although molds and yeasts have been shown to reduce 2-pentanone to 2-pentanol (4, p. 76), no evidence exists to demonstrate whether this reaction can be accomplished by the starter organisms used in Swiss cheese manufacture. A possible source of 2-pentanol may be the film yeast commonly used in the cultivation of Swiss cheese starter organisms in milk (17, p. 376). Morgan and Pereira (39) have identified trans-2-hexenal in the grassy aroma constituents of green forages. Reduction of this compound during ruminant metabolism to the corresponding alcohol and subsequent transport to the mammary gland probably accounts for the alcohol's presence in the milk and consequently the cheese manufactured from it. A case might be made for this constituent arising in the cheese from autoxidation of linolenate (34) and subsequent reduction of the resulting hex-2-enal to the alcohol. Many other products of autoxidation would be expected,

however, in such a cheese. These were not found; hence, autoxidation appears an unlikely source of the hex-2-enal which on reduction would yield the alcohol. 2-Phenylethanol has been found in blue cheese (15) and many other foods. Anderson (4, p. 65) found that this compound, when added with esters to a synthetic blue cheese flavor mixture imparted a very desirable character to the overall flavor of the sample. This compound may also be of some significance in Swiss cheese flavor. A probable mode of formation is via degradation of the amino acid phenylalanine.

Methyl Ketones

The presence of an homologous series of methyl ketones in heat treated dairy products has been well substantiated (30). They should therefore be expected to be present in Swiss cheese which is cooked at a high temperature (54° C). Langler and Day (30) have shown that water is required for ketone formation in milk fat. The environment during the cooking of the cheese should thus be ideal for ketone formation. Other sources of methyl ketones such as microbial metabolism cannot be entirely ruled out although the starter organisms used in Swiss cheese manufacture have never been demonstrated to produce these compounds. The relative amounts of some of the ketones will be discussed in a subsequent section.

Lactones

Lactones were found to be normal trace constituents of milk fat by Boldingh and Taylor (13). It is not surprising, therefore that they have been identified in Swiss cheese. The γ-valerolactone is probably not a normal constituent of milk fat and may originate from microbial metabolism. Various micro-organisms have been reported to be capable of converting keto-acids into hydroxy-acids and the corresponding lactone (13).

Aldehydes

Acetaldehyde is a common constituent of fermented dairy products and has been found in Cheddar (16) and Blue cheese (15). The branched chain aldehydes might arise as intermediates in the conversion of amino acids to volatile fatty acids by the lactic acid bacteria (42). Phenylacetaldehyde may be formed by oxidative deamination and decarboxylation of phenylalanine. Benzaldehyde and phenylacetaldehyde were both found in the volatile constituents of grass and corn silage (40) which is yet another source of these compounds in milk and subsequently cheese.

Esters

Esters have been found to exert an influence on cheese

flavors (12). Their presence in Swiss cheese is presumably due to the existence of free fatty acids and alcohols in the cheese. It is still unknown whether the formation of the esters in cheese is accomplished by simple mass action or by enzymatic means.

Hydrocarbons

Aromatic hydrocarbons have been detected in Cheddar (16) and Blue cheese (15) but not alkyl hydrocarbons. The alkyl hydrocarbons identified in Swiss cheese probably arise from the wax or packaging material used for the cheese. They probably contribute little if at all to flavor. It is interesting to note that toluene was detected in entrained Swiss cheese volatiles. Previous work from this laboratory (15, 16) indicated the presence of toluene in concentrates obtained by low-temperature low-pressure distillation. Since toluene was detected in volatiles purged from Swiss cheese, it may be assumed that toluene is not an artifact introduced by distillations and is indeed a normal constituent of the aforementioned cheeses. The terpene, α-pinene, is probably transmitted to the milk and the cheese subsequently made from it by the forage eaten by the bovine.

Miscellaneous Compounds

Dimethyl sulfide and diacetyl have been shown to be important flavor compounds in butter culture (35, p. 203). These compounds

exist in most fermented dairy products. Dimethyl sulfide is a normal constituent of milk and has been found to contribute to its typical flavor (44). Both of these compounds are important contributors to Swiss cheese flavor. Their contribution will be discussed in a subsequent section. Benzothiazole was detected in the sterile concentrated milk by Arnold (5, p. 83). Other investigators using a similar low-temperature low-pressure distillation technique for the isolation of flavor compounds from cheese fat have not identified with this compound. In the data reported here, however, the temperature of the still (54° C) was higher than used in previous studies with Cheddar and Blue cheese (15, 16) and may account for the compound's recovery from Swiss cheese and not the aforementioned cheeses. The chlorinated aromatic compounds might be expected to be solvent contaminants. The purified ethyl chloride used in the extraction procedure was checked for purity by injecting a corresponding amount of concentrated solvent into the chromatograph. The GLC instrument was allowed to complete the same program used in a sample analysis. No contaminants were evident in the ethyl chloride. Another possible source of the chlorinated aromatic compounds might be herbicides used to control plant growth. For example, ruminant metabolism might cause o-dichlorobenzene to be formed from 1-n-butyl-3(3,4-dichloro-phenyl)-1-methylurea (55, p. 157) and 1, 2, 4-trichlorobenzene from 2, 4, 5-trichlorophenoxyacetic acid (55, p. 247). These

chlorinated aromatic compounds probably make no contribution to the flavor of the cheese. The origin of chloroform and di-isobutyl adipate in the cheese is not known.

Determination of the Concentration of Selected Volatile Constituents in Swiss Cheese

After identification of the components of a food that might contribute to flavor, a second objective of flavor chemistry is to determine the quantities of these flavor components. The on-column trapping technique provided an excellent means for the determination of the quantities of some of the volatile constituents in Swiss cheese. Since this technique is essentially a direct analysis of the cheese, distillations and elaborate extractions are avoided.

The peak height of the selected volatile compounds was related to their concentration in cheese by adding known amounts of these compounds to a whole cheese (9, p. 53) or to milk fat carrier. The conditions used for the determinations of the various compounds are outlined in Table 8. The peak heights observed with various concentrations of known compounds added to the carrier system versus the concentration in parts per million (ppm) were plotted and are shown in Figures 10, 11, 12 and 13. Four concentrations were chromatographed for each compound. Concentrations were adjusted so that the peak heights were in the same range as found in the Swiss

TABLE 8. Conditions of volatile analysis.

Curve no.	Compound	Carrier[a]	Column	Temperature °C	Purge Time Min.	Standard Curve Fig. no.
1	Dimethyl sulfide	F	TRIS	50	10	10
2	Diacetyl	F	TRIS	80	10	12
3	Acetaldehyde	WC	TRIS	50	6	11
4	Acetone	WC	TRIS	50	6	11
5	Butanone	WC	TRIS	80	8	12
6	2-Methyl butyraldehyde	F	TRIS	80	8	12
7	2-Pentanone	F	TRIS	80	10	12
8	2-Heptanone	F	TRIS	80	10	12
9[b]	Ethanol	WC	TRIS	50	6	b
10	2-Butanol	WC	TRIS	50	6	11
11	1-Propanol	WC	TRIS	50	6	11
12	1-Butanol	WC	TRIS	50	6	11
13	Methyl hexanoate	WC	TRIS	80	8	12
14	Ethyl butanoate	WC	Carbowax 600	80	8	13

[a]F = milk fat; WC = whole cheese (Dagano variety).

[b]Curve not shown. It was linear throughout the range of occurrence in the samples.

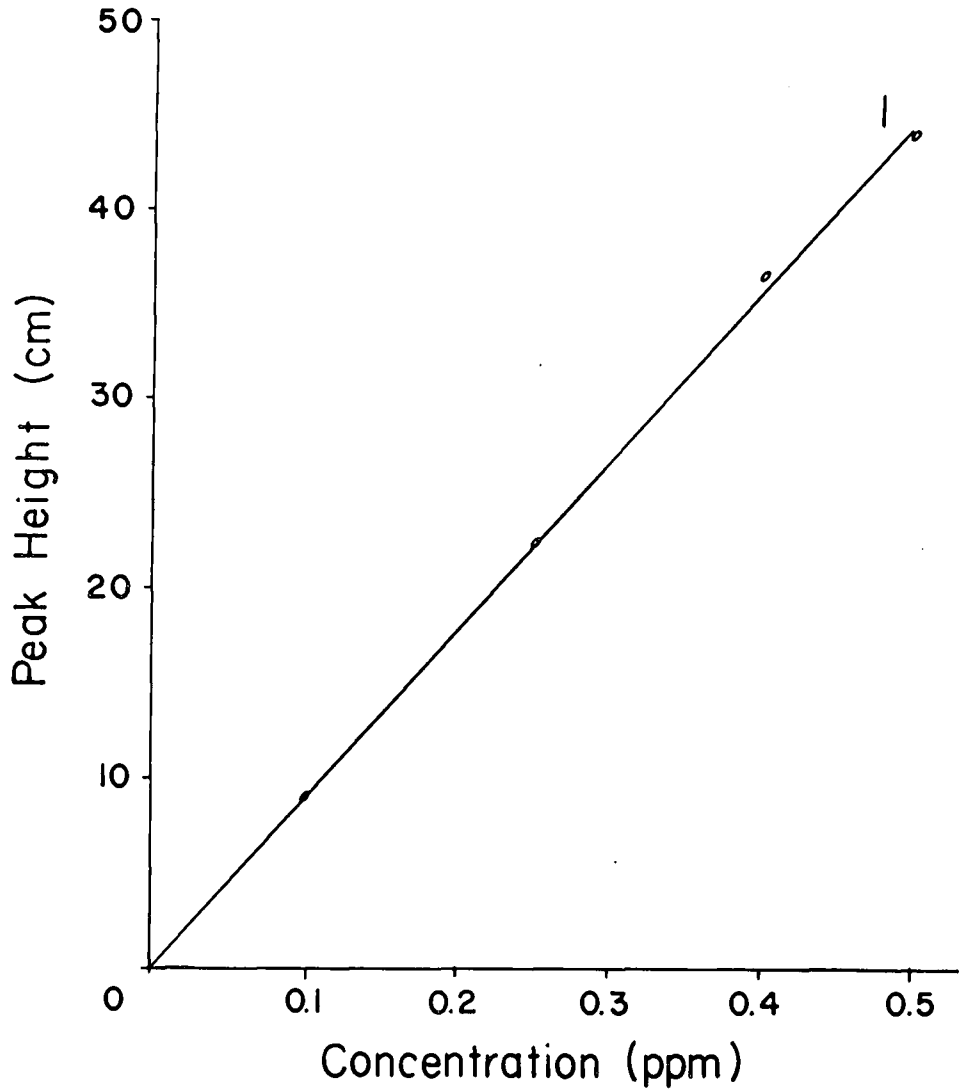

Figure 10. Recorder response with various concentrations of known compound. Curve (1) dimethyl sulfide.

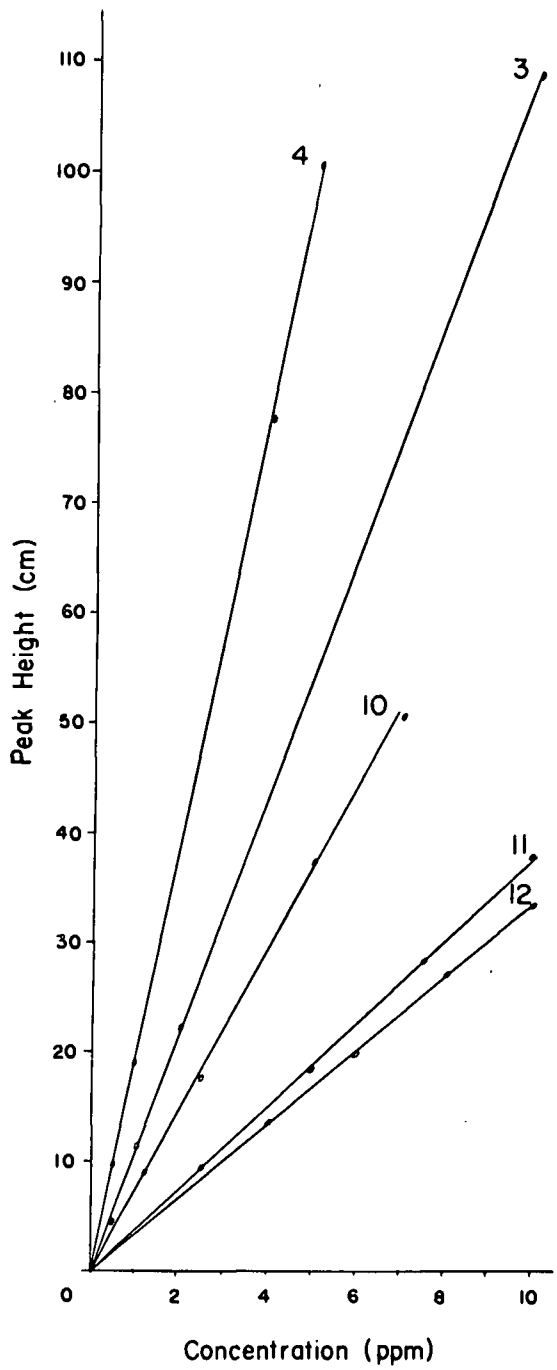

Figure 11. Recorder response with various concentrations of known compound. Curve (4) acetone; (3) acetaldehyde; (10) 2-butanol; (11) 1-propanol; (12) 1-butanol.

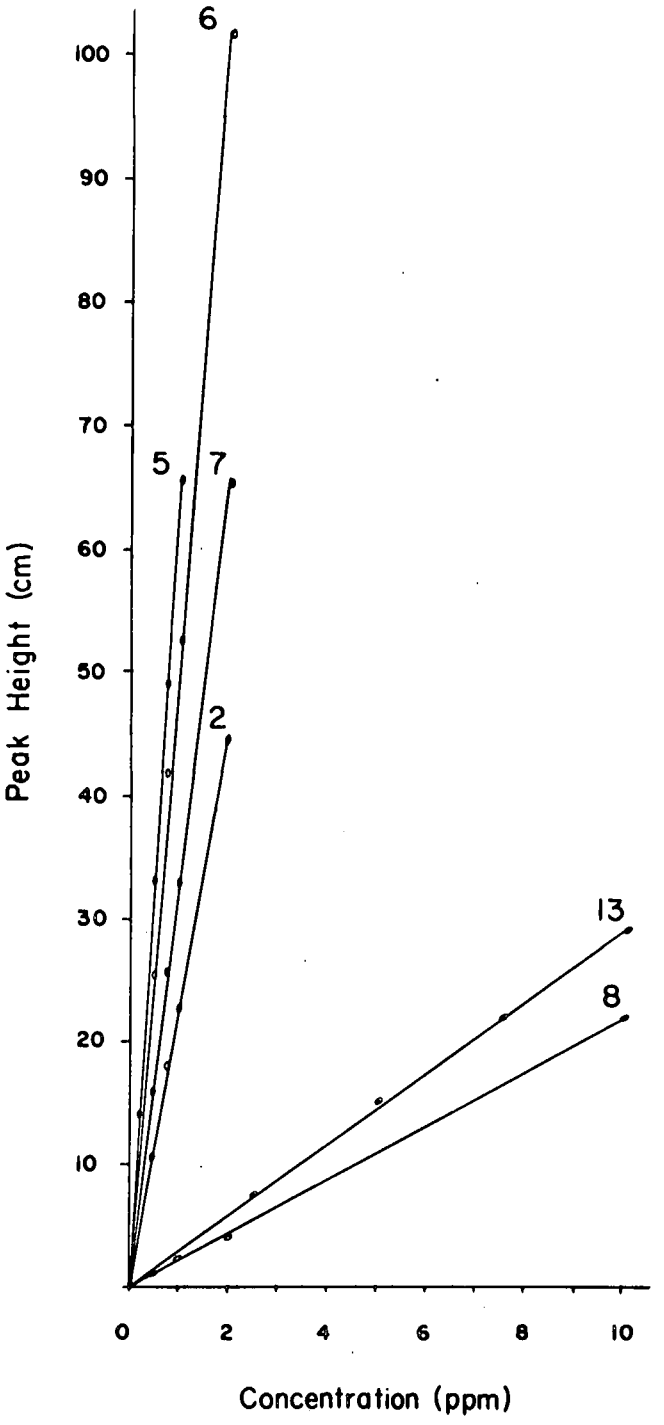

Figure 12. Recorder response with various concentrations of known compound. Curve (5) butanone; (6) 2-methyl butyraldehyde; (7) 2-pentanone; (2) diacetyl; (13) methyl hexanoate; (8) 2-heptanone.

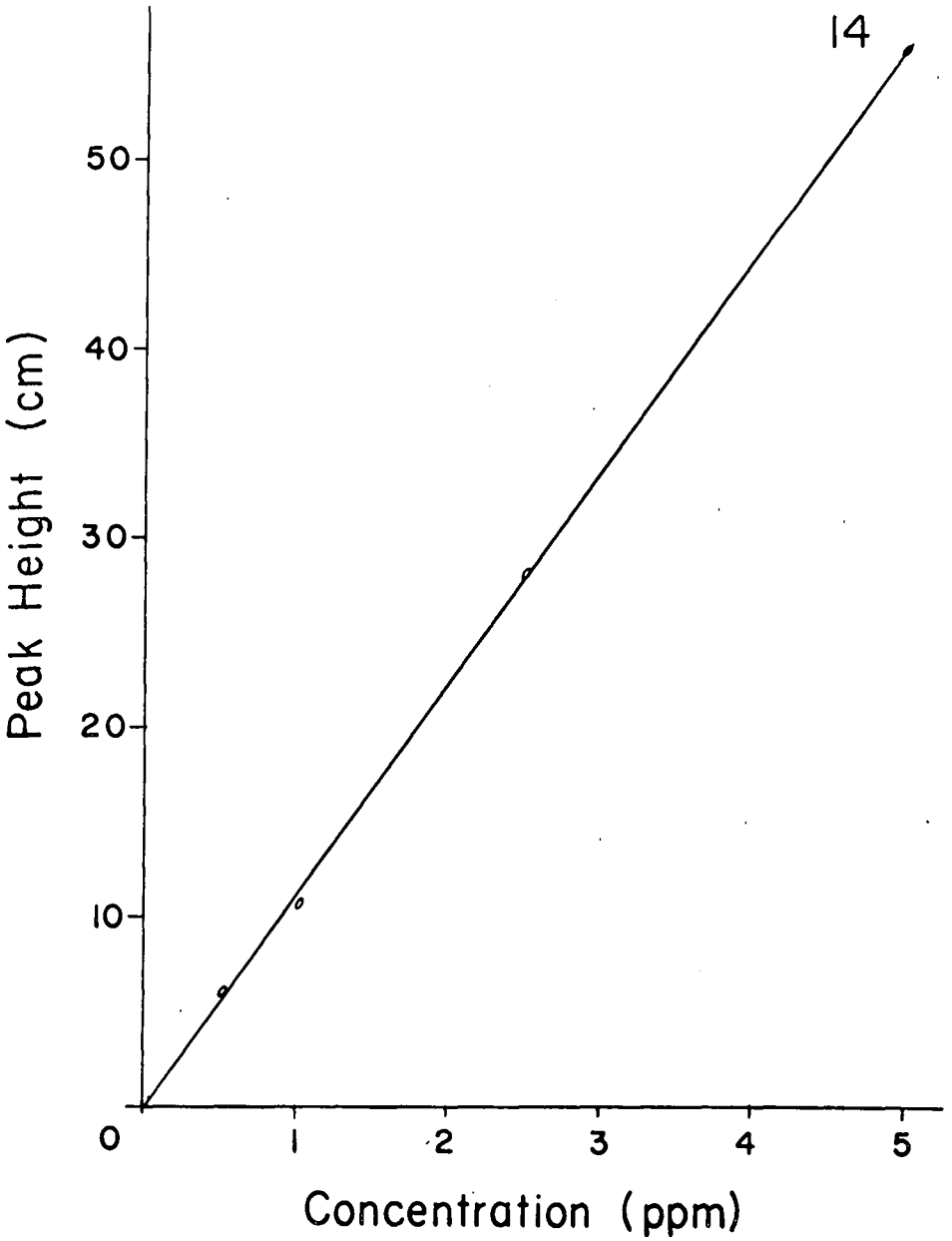

Figure 13. Recorder response with various concentrations of known compound. Curve (14) ethyl hexanoate.

cheese samples. It can be seen from examination of Figures 10, 11, 12 and 13 that recorder response was linear in the range of concentrations used. The curve for ethanol was not plotted although response was linear in the concentration range found in cheese. The concentration of ethanol was calculated from the following relationship: ppm ethanol = peak height (cm) × 0. 14.

The concentrations determined for duplicate analysis of typical flavored cheeses are shown in Table 9. Cheeses A, B, E and F were of the domestic rindless type. Cheese B was analyzed also by low-temperature low-pressure distillation. C and D were imported Emmenthal cheeses. The average concentration of each compound in all the cheeses is shown in the second to last column. The last column shows the average percent deviation of duplicates from their mean.

From Table 9 it is apparent that some components varied greatly while others varied little. Dimethyl sulfide, which ranged from 0. 056 to 0. 183 ppm, is expected to play a significant role in Swiss cheese flavor. Bills (9, p. 72) found dimethyl sulfide in Cheddar cheese, but judging from observed peak heights it probably is present in Cheddar cheese in much lower concentrations. Lindsay (35, p. 161) determined the average flavor threshold of dimethyl sulfide in bland butter oil to be 0. 024 ppm. He also found that very desirable synthetic culture flavors could be obtained if the flavor

TABLE 9. Concentration (ppm) of selected compounds in Swiss cheese volatiles.

Compound	Cheese Sample A	B	C	D	E	F	Average Concentration[b]	Average Percent Deviation[c]
Dimethyl sulfide[a]	0.106	0.056	0.079	0.094	0.123	0.183	0.107	2.2
Diacetyl[a]	0.8	0.2	1.4	1.4	0.6	0.4	0.8	3.8
Acetaldehyde	2.0	0.6	1.1	1.0	1.8	1.8	1.4	2.4
Acetone	0.6	0.6	1.6	3.9	2.1	0.7	1.6	4.4
Butanone	0.7	0.1	0.2	0.6	< 0.1	0.1	0.3	4.5
2-Methyl butyraldehyde[a]	0.06	0.06	0.3	0.2	0.09	0.1	0.42	3.9
2-Pentanone[a]	0.06	0.09	1.36	4.24	0.06	0.09	0.98	1.7
2-Heptanone[a]	0.06	0.15	0.73	1.49	0.12	0.12	0.45	2.0
Ethanol	9.6	3.5	16.7	8.0	35.3	24.9	16.3	4.9
2-Butanol	0.5	0.1	0.7	1.0	0.1	0.1	0.3	3.1
1-Propanol	2.1	0.8	2.7	1.1	4.1	3.5	2.9	1.9
1-Butanol	0.8	1.1	0.4	0.2	1.1	0.6	0.7	4.5
Methyl hexanoate	4.1	0.3	1.7	0.1	1.8	0.8	1.5	3.5
Ethyl butanoate	< 0.1	< 0.1	0.2	0.2	1.0	1.0	0.6	1.2

[a]Calculated from cheese fat.

[b]Calculated from the values determined for each cheese.

[c]Average percent deviation of the duplicates from their mean.

mixture contained 0.025 ppm of dimethyl sulfide. The average concentration of dimethyl sulfide in all the cheeses studied was approximately four times this value. In preliminary laboratory work with Swiss cheese, the whole cheese was sluried in water and lyophilized. When the trap containing the removed aqueous phase of the cheese was allowed to warm up to room temperature a definite dimethyl sulfide odor was evident along with a Swiss like aroma.

Diacetyl plays a significant role in the flavor of cultured dairy products (17, p. 321). Bennett et al. (8) have found its threshold dependent on the medium in which it exists and its pH. Lindsay (35, p. 197) found 2.0 ppm of diacetyl to impart a very desirable flavor to synthetically flavored butter culture. The level of diacetyl found in Swiss cheese is probably well above its threshold and undoubtedly is an important constituent of the flavor.

Harvey (21) found the flavor threshold for acetaldehyde to be 0.4 ppm. Again comparing the value found in Swiss cheese to that in good flavored butter culture (35, p. 178) acetaldehyde would be expected to play a role in the flavor of Swiss cheese.

The carbonyl compounds in the Swiss cheeses examined varied considerably in concentration. It is interesting to note that the imported cheeses, C and D in Table 9, had the highest levels of methyl ketones. This would suggest that these cheeses were probably cooked for a longer period or at a higher temperature than domestic Swiss

cheeses. From the data of Langler and Day (30) it would appear that milk fat contains more than enough precursor to account for the levels of methyl ketones observed in Swiss cheese. The possibility that foreign bovines produce a more keto-genic milk does not seem feasible. The 2-heptanone in cheeses C and D occurs near and slightly above its threshold respectively. Taking into account the syngeristic effect observed for methyl ketone mixtures (30), it is probable that the methyl ketones make some contribution to the flavor of Swiss cheese.

The alcohols determined have no direct influence on the flavor of the cheese but may be an index to the fermentation processes in each cheese. As discussed earlier, the alcohols may also contribute indirectly to flavor by their ability to form esters with fatty acids. It is of interest though that no propyl esters (derived from 1-propanol and a fatty acid) were detected by low-temperature low-pressure distillation or in Swiss cheese volatiles.

Ethyl butanoate and methyl hexanoate, if above threshold concentration in cheese, may play an important role in its flavor. Bills (9, p. 69) found that the addition of 5 ppm each of ethyl butanoate and ethyl hexanoate to Cheddar cheese caused it to have a fruity flavor. None of the Swiss cheeses used in this study had a detectable fruity flavor.

All six of the cheeses used in this investigation were judged

to be of typical good flavor but the two imported Emmenthal cheeses had outstanding flavor. In general the volatile pattern obtained by GLC for these cheeses (C and D, Table 9) was much more intense than those obtained with the domestic cheeses. The higher carbonyl concentration, aluded to previously, might also be the result of the curing process which is quite different from that used with domestic cheese. Other differences in cheese composition should be expected which would contribute to the superior flavor of the Emmenthal cheese.

Quantitative Analysis of the Free Fatty Acids in Swiss Cheese

Table 10 gives the quantities of free fatty acids found in the cheeses analyzed. The same six cheeses were analyzed as used in the on-column trapping technique study of volatiles. Cheeses A, B, E and F were of the domestic rindless variety. C and D were imported Emmenthal cheeses. Cheese B was also analyzed by low-temperature low-pressure distillation.

As shown in Table 10, 2-methyl butyric acid was detected in all cheeses. Although there is considerable variation in the quantity of this acid in the cheeses examined, it may be an essential component of the flavor complex. Samples E and F were judged to have the least 'Swiss like" flavor of the six and also the lowest quantities of this acid. The other isomeric five carbon acid, 3-methyl butyric,

TABLE 10. Free fatty acids in Swiss cheese.[a]

Acid	Mg acid/Kg cheese Cheese Sample A	B	C	D	E	F	Average percent deviation[c]
Formic	314	b	b	b	b	b	6.3
Acetic	3,568	1,835	3,724	3,680	2,539	2,890	1.1
Propionic	2,181	3,868	5,919	4,941	2,899	3,210	0.6
Butyric	86	337	329	89	127	255	1.4
2-Methyl butyric	32	29	100	22	12	9	1.4
3-Methyl butyric	47	b	13	b	b	b	0.8
Caproic	37	225	115	41	70	29	1.0
Caprylic	38	216	94	40	79	39	0.8
Capric	86	190	113	40	98	36	1.7
Lauric	133	310	174	74	157	51	1.8
Myristic	318	966	592	258	523	146	1.3
Palmitic	587	3,091	1,727	721	1,390	444	0.6
Stearic	97	650	629	193	334	126	1.3
Oleic	363	2,026	1,787	481	985	366	1.2
Linoleic	65	272	238	47	143	56	2.0
Linolenic	78	231	175	62	115	42	1.6
pH of cheese	5.8	5.4	5.7	5.7	5.6	5.6	

[a]Average of duplicate analysis.

[b]Not detected in sample.

[c]Average percent deviation of the duplicates from their mean.

was detected only in samples A and C. Since its highest quantity occurred in Sample A, which was judged to have a mild flavor, it would appear that 3-methyl butyric acid is not an essential component of the flavor complex.

Formic acid was detected in only one of the cheeses (Sample A) and probably makes no contribution to the flavor complex. Kurtz et al. (29) reported that the propionic to acetic acid ratios varied in Swiss cheeses between 0.8 and 1.0; however, their values for acetic acid may be high due to contamination with pyruvic acid (32, p. 25). The molar propionic:acetic ratio determined from the six samples reported in Table 10 varied from 0.5 to 1.7 indicating no simple correlations of this ratio to flavor quality.

When the proportions of the free fatty acids of Swiss cheese are compared to those of the esterfied fatty acids of milk fat, the percentages of butyric through linolenic are quite comparable, suggesting that these acids arise from a non-specific hydrolysis of milk fat. Similar results for Cheddar cheese have been reported (10).

Since the branched chain fatty acids are presumably derived from oxidative deamination and decarboxylation of the appropriate amino acids (42), it is interesting that 2-methyl propionic acid (from valine) was not found in any cheese and that 3-methyl butyric acid (from leucine) was found in only two (A and C). This suggests either differences in the specificities of the enzymes involved or alternate

metabolic pathways for these compounds.

Taking into account the pH of the cheeses studied, it would be expected that 65 to 90 percent of the acids reported in Table 10 would exist in the salt form. This would affect the aroma resulting from volatile short-chain acids and the contribution to flavor made by long-chain acids. Unfortunately it is not possible to determine experimentally the ratio of a given acid to its salt in cheese.

Evaluation of Synthetic Swiss Cheese Flavor

The quantitative data obtained for free fatty acids and selected volatiles in addition to that published for free amino acids (22) were utilized to compound a synthetic Swiss cheese mixture. Selected flavor compounds were added to a mixture of cream, milk fat, dry curd cottage cheese and salt prepared so that the approximate proportions of protein, fat, water and salt found in natural cheese were maintained. The initial mixture contained the quantities of free fatty acids found in cheese C from acetic to octanoic inclusive. The higher acids were omitted from the mixture to avoid the soapy flavor which Anderson (4, p. 64) found in compounded synthetic blue cheese flavor. The data of Hintz et al. (22) for free amino acids was used. The average concentrations of the volatiles shown in Table 9 excluding the alcohols were also added to this mixture.

Two standards of comparison were prepared. The fat from

a good flavored cheese was prepared by high speed centrifugation as previously described. This fat was used in place of milk fat in the synthetic mixture. This provided one comparison standard sample. Another sample was prepared in the same manner except the amino acids from the data of Hintz et al. (22) were added. Thus, a standard with and one without amino acids was used in comparison of synthetically compounded mixtures.

On organoleptic evaluation the initial synthetic mixture had a strong vinegar or acid flavor but was somewhat reminiscent of Swiss cheese. A second mixture was prepared in which the acid concentrations were reduced to one-half the value found in cheese. Anderson (4, p. 65) found that the quantities of FFA in Blue cheese also had to be reduced. This reduced the harshness of the acid but the sample still lacked a desirable Swiss flavor. The pH of this sample was found to be approximately 4.5. This value is considerably lower than that found in natural cheese. Therefore, the pH of the second sample was adjusted with two normal alkali to approximately 5.6 and then evaluated. This sample was judged to have typical Swiss like character and resembled the flavor of the natural cheese. The amounts of compounds used in this mixture are shown in Table 11.

A series of synthetic mixtures were prepared to evaluate the effects of FFA, amino acids and selected volatiles on flavor. The standards, described above, were also used in the evaluation. The

TABLE 11. Compounds used in synthetic Swiss cheese flavor (ppm).

Compound	Added to mixture	Found in cheese[a]
Fatty acids		
Acetic	1,862	3,724
Propionic	2,960	5.919
Butyric	165	329
2-Methyl butyric	50	100
3-Methyl butyric	6	13
Caproic	58	115
Caprylic	47	94
Amino acids		
Proline	6,000	--
Glycine	1,600	--
Serine	1,950	--
Threonine	1,950	--
Aspartic	2,500	--
Glutamic	3,000	--
Cysteic	760	--
Tryptophan	2,200	--
Histidine	3,700	--
Lysine	2,200	--
Selected volatiles		
Dimethyl sulfide	0.107	0.107
Diacetyl	0.8	0.8
Acetaldehyde	1.4	1.4
Acetone	1.6	1.6
Butanone	0.3	0.3
2-Methyl butyraldehyde	0.42	0.42
2-Pentanone	0.98	0.98
2-Heptanone	0.45	0.45
Methyl hexanoate	1.5	1.5
Ethyl butanoate	0.6	0.6

[a]Fatty acids, Table 10, sample C; amino acids, Hintz et al. (22); selected volatiles, Table 9, average concentrations.

standard that did not have added amino acids was found to be bland and lacking in the full sweet taste associated with Swiss cheese. The amino acid containing standard (same quantities as shown in Table 11) had a typical Swiss like character and taste although the texture and body were quite unlike the natural cheese. The synthetic mixture containing the quantities of compounds shown in Table 11 and adjusted to pH 5.6 was comparable to the amino acid containing standard. Several of the judges detected the so called nutty characteristic in this synthetic mixture. However, no single component detected in Swiss cheese volatiles had a nutty character.

In order to evaluate the effect of amino acids on flavor, another synthetic sample was prepared containing the quantities of compounds shown in Table 11 excepting the amino acids and adjusted to pH 5.6. This mixture was criticized for lacking fullness of flavor. It did not have the sweet characteristic of natural Swiss cheese. This sample was comparable to the amino acid free standard.

The effect of the selected volatile compounds on flavor was evaluated by preparing a mixture containing only the FFA. The pH of the mixture was adjusted to 5.6 and quantities of FFA shown in Table 11 used. This mixture was completely lacking in Swiss like character and reminiscent of FFA only.

Several general conclusions may be drawn from these evaluations. It is obvious that FFA alone are not responsible for Swiss

cheese flavor. The selected volatiles in combination with the fatty acids do not yield a typical full flavor. A Swiss like flavor could be obtained only when the amino acids, FFA, and selected volatiles were present and at the pH of natural cheese.

Although a mixture was formulated which had a Swiss like flavor, the flavor of a high quality Swiss cheese was not duplicated. This was probably due to several reasons. The greatest problem in formulating a cheese flavor is the duplication of the natural medium. The texture and body of the synthetic medium was unlike natural cheese. Not all compounds that might contribute to the flavor were added. The tear fluid of the eyes of Swiss cheese, for example may play a significant role in flavor. It has been found to contain certain keto acids (27). In the natural cheeses peptides which were absent in the synthetic mixture would also be suspected to contribute to flavor. Finally, the state in which the flavor compounds exist in the natural cheese would influence the flavor perceived.

SUMMARY AND CONCLUSIONS

Low-temperature low-pressure distillation was used to isolate the volatile neutral flavor components from Swiss cheese fat. The isolated compounds were separated by gas liquid chromatography (GLC). Compounds were identified by comparison of retention times with authentic compounds and by mass spectral analysis in conjunction with GLC.

On-column trapping of volatiles from intact cheese and cheese fat provided a direct analysis of cheese volatiles. Identifications were made by comparison of retention times on two packed columns and mass spectral analysis.

The quantities of selected volatiles were determined by the on-column trapping technique. Relative peak heights were related to known concentrations of compound. Six typical flavored cheeses, which included two Emmenthal cheeses, were analyzed. One of the cheeses was that used in the low-temperature low-pressure distillation.

The major free fatty acids (FFA) ranging from acetic to linolenic and including 2-methyl and 3-methyl butyric acids were quantitatively determined in the six cheeses. Liquid-liquid chromatography and GLC were used for the analysis.

A synthetic Swiss cheese mixture was compounded utilizing

the data from the FFA and selected volatile determinations and that published for free amino acids.

The findings of this investigation were as follows:

1. A total of 54 compounds were positively identified in Swiss cheese from the low-temperature low-pressure distillation of cheese fat and entrainment and on-column trapping of volatiles. Alcohols, hydrocarbons, methyl ketones, aldehydes, esters, lactones and several miscellaneous compounds comprised the identified compounds. Heretofore only four of the 54 compounds were positively identified in Swiss cheese. No single component or fraction was found that could be described as the so called nutty flavor. Thus, it appears that this flavor is due to a combination of flavors.

2. The concentration of the selected volatile components varied from cheese to cheese. Some constituents such as the methyl ketones varied considerably while others remained fairly constant. The imported Emmenthal cheeses contained much higher total volatile concentrations and especially higher methyl ketone concentrations. It is suggested that cooking temperature and/or time influences the methyl ketone concentration and the overall flavor of the cheese.

3. The concentration of FFA in the Swiss cheeses was variable. 2-Methyl butyric acid was detected in all cheeses. The other isomeric five carbon acid, 3-methyl butyric, was detected in only

two. The relative proportions of FFA from butyric to linolenic inclusive are quite comparable to those of the esterfied fatty acids of milk fat.

4. An acceptable synthetic Swiss cheese flavor was obtained when FFA, selected volatiles and amino acids were blended with cream, dry curd cottage cheese, milk fat and salt. When amino acids were absent from the mixture, it lacked the typical sweet full flavor associated with high quality cheese. If amino acids and the selected volatiles were omitted from the mixture, its flavor was bland and quite unlike Swiss cheese.

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