Identification and Evaluation of Selected Compounds in Swiss Cheese Flavor

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The major neutral volatiles of Swiss cheese were isolated by low-temperature low-pressure distillation of the cheese fat. Free fatty acids were removed from the trapped volatiles, and the acid-free extract was analyzed by temperature-programmed gas-liquid chromatography (GLC) with rapid-scan mass spectrometry (MS) of the chromatographic effluent. Semiquantitative analysis of the highly volatile constituents of Swiss cheese was performed by gas-entrainment and on-column trapping coupled with GLC and MS. Concentrations of selected compounds were estimated by relating peak heights to those of known quantities of compound. Average concentrations from the mean values for six cheeses, expressed in parts per million were: dimethyl sulfide, 0.11; diacetyl, 0.8; acetaldehyde, 1.4; acetone, 1.6; butanone, 0.3; 2-methylbutyraldehyde, 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. A synthetic Swiss cheese flavor of fair quality was prepared from these data, free fatty acids, and amino acid analyses available in the literature. A qualitative and quantitative investigation was made of the volatile compounds that might contribute to typical Swiss cheese flavor and their importance was evaluated.

In preliminary work the major neutral volatiles of Swiss cheese were obtained by a low-temperature low-pressure distillation of cheese fat (*15*). The trapped volatiles were extracted with ethyl chloride, and partitioning against dilute aqueous Na_2CO_3 was used to remove free fatty acids (FFA). The FFA-free extract was analyzed by temperature-programmed packed column gas-liquid chromatography (GLC) in conjunction with rapid scan mass spectrometry of the GLC effluent. GLC retention data and mass spectral analyses permitted identification of 46 of the 83 GLC peaks observed. Figure 1 indicates the chromatographic separation of the volatiles. Identifications of peaks are presented in Table I. It is doubtful that all of the compounds reported herein contribute significantly to the flavor of Swiss cheese. Further quantitative work was required to determine which compounds exist in concentrations great enough to influence flavor.

The unique flavor of high quality Swiss cheese is difficult to reproduce in commercial market cheese. Swiss cheese flavor has never been synthesized or thoroughly understood. Newer techniques (*16*, *18*) and advances in flavor research have enabled better definition of food flavors (*3*, *6*, *17*). Although the literature contains data on the free fatty acids (*9*, *11*, *12*, *14*, *20*) and amino acids (*9*) of Swiss cheese, little is known concerning the identities and quantities of volatile flavor compounds in Swiss cheese (*4*).

The gas-entrainment and on-column trapping technique developed by Morgan and Day (*19*) provided a method for determining concentrations of volatiles in foods without extraction or distillation. This method was found useful for the semiquantitative analysis of Swiss cheese volatiles.

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Experimental

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 was an 80-pound block donated by a local plant. The remaining two were imported Emmenthal cheeses purchased as 5-pound wedges from a retail delicatessen.

The flavor volatiles of the six cheeses were determined by the gas-entrainment and on-column trapping technique (*19*). Nitrogen gas was passed through the sample at the rate of 8 ml. per minute. Whole cheese samples were prepared as reported by Bills (*6*). During the purging, the sample vial was immersed in an isothermal 80° C. water bath. Cheese fat, prepared by high speed centrifugation (*16*), also was analyzed. In these experiments 10 ml. (9.2 grams) of fat was pipetted into a sample vial containing 5 grams of anhydrous sodium sulfate and purged as described (*19*).

The gas liquid chromatographic (GLC) conditions were as follows: instrument, F and M Model 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; dimensions, each column was 12 feet × 1/8-inch o.d.; temperature, isothermal at 50° and 80° C; flow rate, 30 ml. per minute of He. The retention times of known compounds were determined by sample enrichment. 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.

Mass spectral analyses were obtained on volatiles entrained from one of the cheeses. Two 15-ml. (13.8-gram) cheese fat samples were each purged for 30 minutes. The entrained volatiles were analyzed isothermally at 50° and 80° C. on the Tris column. An Atlas-MATCH-4 mass spectrometer (9-inch, 60-degree sector,

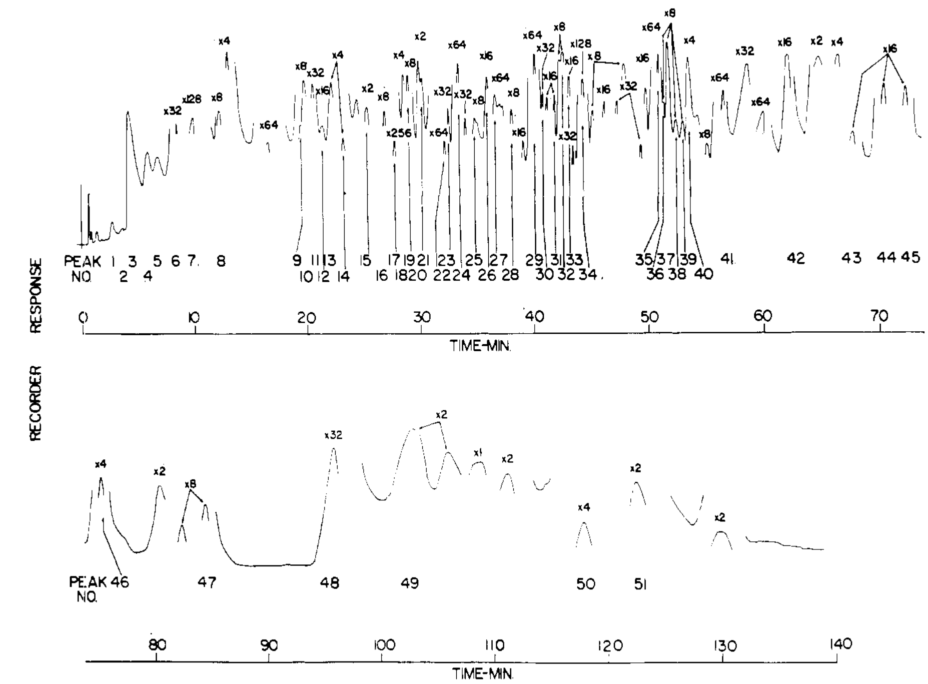

Figure 1. Gas chromatogram of FFA-free volatile concentrate from Swiss cheese

Column packed with 20% Apiezon M on 80- to 100-mesh acid-alkali-washed Celite 545 and operated at 60° C. for 12 minutes then programmed at 4° C./minute to 200° C. and held until completion of analysis

single-focusing instrument) modified for direct connection to GLC effluent was used. Spectra were recorded with a Honeywell Model 1508 Visicorder.

The concentrations of several volatile compounds identified were determined by relating their GLC peak heights to those of known quantities of standard compounds added to whole cheese (Dagano) or butterfat. Dagano cheese, which is similar in some respects to Swiss cheese, served as a good carrier for knowns, since the GLC pattern on volatiles from this cheese was different from that of Swiss cheese and much less intense. Butterfat served as a carrier for knowns in the cheese fat studies. Volatiles identified in cheese fat were either absent or in very low concentration in butterfat.

A base for evaluating synthetic Swiss cheese flavor was made by mixing pasteurized cream, milk fat, dry curd cottage cheese, and salt in a Waring Blendor. Flavor compounds were added in milk fat, water solution, or directly during the blending. In compounding the synthetic mixture, an attempt was made to maintain the proportions of protein, fat, water, and salt found in the natural cheese.

Results and Discussion

An example of the chromatographic separation obtained from a 10-minute purge (on-column trapping) of Swiss cheese fat on the Tris column is shown in Figure 2. Peak identifications were considered positive if the mass spectrum and retention time of the unknown component were equivalent to that of the known compound; supporting data are given in Table II. If the known compound was not available for retention time determination, the identification was considered tentative. Chromatograms similar to Figure 2 were obtained when whole cheese samples were purged instead of fat, but the peak heights were considerably smaller.

Quantitative data were obtained from standard curves prepared from the peak heights of four different concentrations of standard compounds. The concentrations were selected to be near the range found in normal Swiss cheese. Figure 3 demonstrates the linearity of detector response to concentration. Similar results were obtained with the other compounds. The concentrations determined from duplicate analyses of the six cheeses expressed in parts per million are shown in Table III. The precision of duplicate analyses is also shown. Table III shows that some components varied greatly while others varied little. Dimethyl sulfide, which ranged from 0.056 to 0.183 p.p.m., probably plays a significant role in Swiss cheese flavor, since these concentrations are above the flavor threshold (*17*).

Diacetyl plays a significant role in the flavor of cultured dairy products (*17*). Bennett, Liska, and Hempenius (*5*) have found its threshold dependent upon the composition and the pH of the medium. Lindsay (*17*) found 2.0 p.p.m. 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 (*8*) found the flavor threshold for acetaldehyde to be 0.4 p.p.m. Again comparing the value found in Swiss cheese to that in good-flavored butter culture (*17*), acetaldehyde would be expected to play a role in the flavor of Swiss cheese.

The concentrations of the carbonyl compounds in the cheeses examined varied considerably. The imported

Table I. Gas Chromatographic and Mass Spectral Identification of Components from the Concentrate of Volatiles from Swiss Cheese

Peak No.	Compound	t_R/t_R[a] Swiss cheese	t_R/t_R[a] Authentic	Mass[b] Spectral Ident.	Ref.
1	Methyl acetate	0.098	0.094	Yes	*(1)*
2	1-Propanol	0.130	0.123	Yes	*(7)*
3	Butanone	0.152	0.160	Yes	*(22)*
4	2-Methylpropanal	0.212	0.216	Yes	*(2)*
5	Chloroform	0.244	0.253	Yes	*(1)*
6	2-Methylbutyraldehyde	0.306	0.291	Yes	*(2)*
	1-Butanol	0.306	0.305	Yes	*(7)*
7	2-Pentanone	0.359	0.352	Yes	*(22)*
	Ethyl propionate	0.359	0.359	Yes	*(1)*
8	2-Pentanol	0.443	0.431	Yes	*(7)*
9	Ethyl butanoate	0.704	0.684	Yes	*(1)*
10	2-Hexanone	0.717	0.718	Yes	*(22)*
11	Butyl acetate	0.744	0.735	Yes	*(1)*
12	Toluene	0.771	0.787	Yes	*(1)*
13	1-Octene	0.803	0.802	Yes	*(1)*
14	Nonane	0.838	0.848	Yes	*(1)*
15	3-Methylbutyl acetate	0.916	0.924	Yes	*(2)*
16	*trans*-2-Hexene-1-ol	0.972	0.943	Yes	*(10)*
17	2-Heptanone	1.000	1.000	Yes	*(22)*
18	Ethyl benzene	1.032	...	Tent.	*(1)*
19	Methyl hexanoate	1.048	1.065	Yes	*(2)*
20	1,2-; 1,3-; or 1,4-dimethylbenzene	1.083	...	Tent.	*(1)*
21	1-Nonene	1.093	1.077	Yes	*(1)*
22	γ-Valerolactone	1.162	1.139	Yes	*(18)*
23	α-Pinene	1.175	1.225	Yes	*(21)*
24	Ethyl hexanoate	1.208	1.207	Yes	*(2)*
25	Benzaldehyde	1.259	1.278	Yes	*(2)*
26	Methyl benzoate	1.295	...	Tent.	*(1)*
27	α-Fenchene	1.325	...	Tent.	*(21)*
28	Phenylacetaldehyde	1.376	1.419	Yes	*(2)*
29	2-Nonanone	1.453	1.455	Yes	*(2)*
	o-Dichlorobenzene	1.453	1.470	Yes	*(2)*
30	Methyl octanoate	1.484	1.509	Yes	*(2)*
31	Dodecane	1.521	1.542	Yes	*(1)*
32	2-Phenyl-2-methylbutane	1.549	...	Tent.	*(1)*
33	2-Phenylethanol	1.573	1.579	Yes	*(2)*
34	Ethyl octanoate	1.610	1.617	Yes	*(2)*
35	2-Undecanone	1.852	1.854	Yes	*(22)*
36	δ-Octalactone	1.866	1.876	Yes	*(18)*
37	Methyl decanoate	1.880	1.921	Yes	*(2)*
38	1,2,4-Trichlorobenzene	1.906	...	Yes	*(2)*
39	5-Methyl-5-ethyldecane	1.927	...	Tent.	*(1)*
40	Benzothiazole	1.949	1.982	Yes	*(1)*
41	Ethyl decanoate	2.057	2.049	Yes	*(1)*
42	3-Methyl butyl octanoate	2.259	...	Tent.	*(2)*
43	2,5-Dimethyltetradecane	2.467	...	Tent.	*(1)*
44	2-Tridecanone	2.563	2.553	Yes	*(2)*
45	δ-Decalactone	2.630	2.613	Yes	*(18)*
46	Pentadecane	2.735	2.753	Yes	*(1)*
47	Ethyl dodecanoate	3.071	2.976	Yes	*(2)*
48	Diisobutyl adipate	3.468	...	Yes	*(2)*
49	γ-Dodecalactone	3.717	...	Yes	*(18)*
50	2-Pentadecanone	4.265	...	Yes	*(2)*
51	δ-Dodecalactone	4.432	4.379	Yes	*(18)*

[a] Relative retention time with t_R/t_R of 2-heptanone = 1.00.
[b] Tent. = tentative identification; not confirmed by retention time.

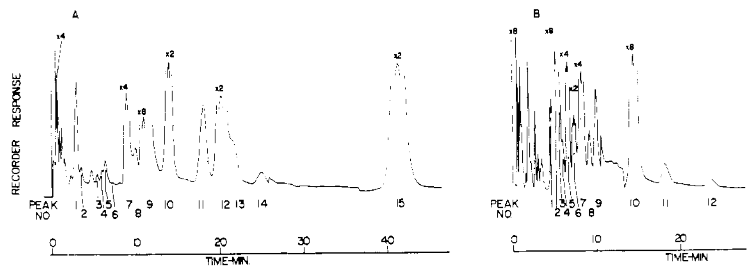

Figure 2. Gas chromatogram of the volatile components of a Swiss cheese obtained by on-column trapping

A obtained at 50° C. *B* at 80° C., both using the Tris column

Table II. Gas Chromatographic and Mass Spectral Identification of Volatile Components of a Swiss Cheese Obtained by On-Column Trapping

Peak No.	Compound	t_R/t_R[a] Swiss cheese	t_R/t_R[a] Authentic	Confirmed on Carbowax 600	Mass Spectral Ident.	Ref.
		Tris column at 50° C.				
1	Dimethyl sulfide	0.262	0.260	Yes	Positive	*(1)*
2	Acetaldehyde	0.316	0.310	Yes	Positive	*(1)*
3	Ethyl formate	0.485	0.498	Yes	...	
4	Propanal	0.537	0.525	Yes	...	
5	2-Methylpropanal	0.581	0.580	Yes	...	
6	Methyl acetate	0.654	0.653	Yes	...	
7	Acetone	0.816	0.815	Yes	Positive	*(22)*
8	Butanal	0.882	0.874	Yes	...	
	Ethyl acetate	0.882	0.870	Yes	...	
	Methyl vinyl ether	0.882	...	...	Tentative	*(2)*
9	Ethanol	1.000	1.000	Yes	Positive	*(7)*
	2- and 3-Methyl butyraldehyde	1.000	1.010	Yes	...	
10	Butanone	1.294	1.280	Yes	Positive	*(22)*
	Ethyl propionate	1.294	1.238	Yes	...	
11	2-Butanol	1.639	1.640	Yes	...	
	Toluene	1.639	1.645	Yes	Positive	*(1)*
12	1-Propanol	1.827	1.834	Yes	Positive	*(7)*
13	Ethyl butanoate	1.978	1.913	Yes	...	
	2-Pentanone	1.978	2.020	Yes	...	
14	2-Methylpropenal	2.357	...	...	Tentative	*(2)*
15	1-Butanol	3.662	3.639	Yes	Positive	*(7)*
		Tris column at 80° C.				
1	Acetone	0.888	0.881	Yes	Positive	*(22)*
2	Ethanol	1.000	1.000	Yes	Positive	*(7)*
3	2- and 3-Methyl butyraldehyde	1.083	1.105	Yes	...	
4	Ethyl propionate	1.156	1.175	Yes	Positive	*(1)*
5	Butanone	1.287	1.298	Yes	Positive	*(22)*
6	2-Butanol	1.438	1.444	Yes	...	
7	1-Propanol	1.603	1.597	Yes	Positive	*(7)*
	Ethyl butanoate	1.603	1.566	Yes	...	
	Toluene	1.603	1.616	Yes	Positive	*(1)*
8	2-Pentanone	1.780	1.805	Yes	Positive	*(22)*
9	Diacetyl	1.948	1.936	Yes	Positive	*(1)*
10	1-Butanol	2.809	2.777	Yes	Positive	*(7)*
11	Methyl hexanoate	3.534	3.492	Yes	...	
12	2-Heptanone	4.691	4.743	Yes	Positive	*(22)*

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

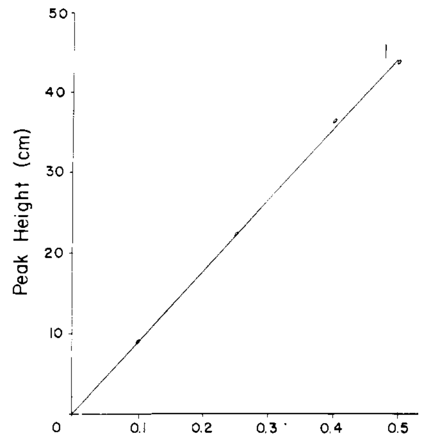

Figure 3. Recorder response with various concentrations of dimethyl sulfide

cheeses, E and F in Table III, had the highest levels of methyl ketones. This would suggest that these cheeses were cooked for a longer period and/or at a higher temperature than domestic Swiss cheese. Langler and Day (*14*) demonstrated that milk fat contains more than enough precursor to account for the levels of methyl ketones observed in Swiss cheese. The 2-heptanone in cheeses E and F occurs near and slightly above its threshold, respectively (*14*). Taking into account the synergistic effect observed in methyl ketone mixtures (*14*), the methyl ketones probably make some contribution to the flavor of Swiss cheese.

The alcohols identified have no direct influence on the flavor of the cheese because of their high thresholds, but they may be an index to the fermentation processes in each cheese. The alcohols also may contribute indirectly to flavor by formation of esters with fatty acids. Ethyl butanoate and methyl hexanoate, if above threshold concentration in cheese, may play a significant role in its flavor. Bills (*6*) found that the addition of 5 p.p.m. 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 flavor, but the two imported Emmenthal cheeses had an outstanding flavor. In general, the pattern for volatiles obtained by GLC for these cheeses (E and F, Table III) was much more intense than those obtained with the domestic cheeses. The higher carbonyl concentration, mentioned previously, might also be the result of the curing process which is different from that used with domestic cheese.

The quantitative data obtained for free fatty acids (*15*) and selected volatiles along with data published for free amino acids (*9*) were utilized to compound a synthetic flavor mixture. The fat from a typically flavored cheese was obtained by high speed centrifugation of the cheese (*15*). A mixture was prepared using this fat. Another sample was prepared using this fat and amino acids. Hence, standards with and without amino acids were used in comparison to synthetically compounded mixtures. The initial synthetic mixture contained the quantities of free fatty acids found in Swiss cheese (*11*) from acetic to octanoic. The higher acids were omitted from the mixture to avoid the soapy taste which Anderson (*3*) found in compounded Blue cheese flavor. Amino acids (*9*) and the average concentration of volatiles (Table III) also were added. On organoleptic evaluation, the mixture had a strong vinegar or acidic flavor but was somewhat reminiscent of Swiss cheese.

A second mixture was prepared in which the fatty acid concentrations were reduced to one half the value found in cheese. This reduced the harshness of the acid, but the sample still lacked a desirable Swiss flavor. The pH of this sample was 4.5. This value is considerably lower

Table III. Concentration (P.P.M.) of Selected Compounds in Swiss Cheese Volatiles

	Cheese Sample							
	Domestic				Imported			
Compound	A	B	C	D	E	F	Av. Concn.[a]	Av. % Dev.[b]
Dimethyl sulfide[c]	0.106	0.056	0.123	0.183	0.079	0.094	0.11	2.2
Diacetyl[c]	0.8	0.2	0.6	0.4	1.4	1.4	0.8	3.8
Acetaldehyde	2.0	0.6	1.8	1.8	1.1	1.0	1.4	2.4
Acetone	0.6	0.6	2.1	0.7	1.6	3.9	1.6	4.4
Butanone	0.7	0.1	0.1	0.1	0.2	0.6	0.3	4.5
2-Methylbutyraldehyde[c]	0.06	0.06	0.09	0.1	0.3	0.2	0.42	3.9
2-Pentanone[c]	0.06	0.09	0.06	0.09	1.36	4.24	0.98	1.7
2-Heptanone[c]	0.06	0.15	0.12	0.12	0.73	1.49	0.45	2.0
Ethanol	9.6	3.5	35.3	24.9	16.7	8.0	16.3	4.9
2-Butanol	0.5	0.1	0.1	0.1	0.7	1.0	0.3	3.1
1-Propanol	2.1	0.8	4.1	3.5	2.7	1.1	2.9	1.9
1-Butanol	0.8	1.1	1.1	0.6	0.4	0.2	0.7	4.5
Methyl hexanoate	4.1	0.3	1.8	0.8	1.7	0.1	1.5	3.5
Ethyl butanoate	0.1	0.1	1.0	1.0	0.2	0.2	0 6	1.2

[a] Calculated from average values determined for each cheese.
[b] Average per cent deviation of duplicates from their mean.
[c] Calculated from cheese fat.

than that found in natural cheese. Therefore, the pH of the second sample was adjusted with 2*N* alkali to 5.6 and the flavor was re-evaluated. This sample was judged to have a typical Swiss-like character and resembled the flavor of the natural cheese. The amounts of compounds used in this mixture are shown in Table IV.

A series of synthetic mixtures was prepared to evaluate the effects of free fatty acids, amino acids, and selected volatiles on flavor. The results of the evaluation are summarized in Table V and several general conclusions may be drawn from these evaluations. Fatty acids 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, free fatty acids, and selected volatiles were present and the pH adjusted to that 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. 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, which has been found to contain certain keto acids (*13*), may play a significant role in flavor. In the natural cheeses, peptides, which were absent from the synthetic mixture, would also be suspected to contribute to flavor.

Table IV. Compounds Used in Synthetic Swiss Cheese Flavor (P.P.M.)

Compound	Added to Mixture	Found in Cheese[a]
Fatty acids		
Acetic	1862	3724
Propionic	2960	5919
Butyric	165	329
2-Methylbutyric	50	100
3-Methylbutyric	6	13
Caproic	58	115
Caprylic	47	94
Amino acids		
Proline	6000	...
Glycine	1600	...
Serine	1950	...
Threonine	1950	...
Aspartic	2500	...
Glutamic	3000	...
Cysteic	760	...
Tryptophan	2200	...
Histidine	3700	...
Lysine	2200	...
Selected volatiles		
Dimethyl sulfide	0.11	0.11
Diacetyl	0.8	0.8
Acetaldehyde	1.4	1.4
Acetone	1.6	1.6
Butanone	0.3	0.3
2-Methylbutyraldehyde	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, Langler and Day (*15*) (sample D); amino acids Hintz *et al.* (*9*); selected volatiles, average concentrations from Table III of this study.

Table V. Organoleptic Evaluation of Synthetic Swiss Cheese Flavors

Synthetic Mixture	pH	Judges' Comment
Standard fat	[a]	Bland, lacked sweet taste, lacked full flavor
Standard fat and amino acids	[a]	Typical, full and sweet flavor
Free fatty acids[b] and volatiles	5.6	No Swiss character, acid flavor
Free fatty acids[b], volatiles,[b] and amino acids	5.6	Typical, full and sweet flavor

[a] Not determined.
[b] Quantities used shown in Table IV.

Literature Cited

(1) Am. Petroleum Inst., Chemical Thermodynamics Properties Center, Agricultural and Mechanical College of Texas, College Station, Tex., "Catalog of Mass Spectral Data," 1948 to date.
(2) Am. Soc. Testing Materials, Subcommittee IX, Committee E-14, "Uncertified Mass Spectra."
(3) Anderson, D. F., Day, E. A., J. Agr. Food Chem. **14,** 241 (1966).
(4) Bassett, E. W., Harper, W. J., *J. Dairy Sci.* **41,** 1206 (1958).
(5) Bennett, G., Liska, B. J., Hempenius, W. L., *J. Food Sci.* **30,** 35 (1965).
(6) Bills, D. D., Ph.D. thesis, Oregon State University, Corvallis, Ore., 1966.
(7) Friedel, R. A., Schultz, J. L., Sharkey, A. G., Jr., *Anal. Chem.* **28,** 926 (1956).
(8) Harvey, R. J., *J. Dairy Res.* **27,** 41 (1960).
(9) Hintz, P. C., Slatter, W. L., Harper, W. J., *J. Dairy Sci.* **39,** 235 (1956).
(10) Honkanen, E., Moisio, T., *Acta Chem. Scand.* **17,** 2051 (1963).
(11) Krett, D. J., LeBoyer, Daume, H. E., Stine, J. B., *J. Dairy Sci.* **35,** 480 (1952).
(12) Krett, D. J., Stine, J. B., *Ibid.*, **34,** 476 (1951).
(13) Kreula, M., Virtanen, A. J., *Acta Chem. Scand.* **11,** 1431 (1951).
(14) Langler, J. E., Day, E. A., *J. Dairy Sci.* **47,** 1291 (1964).
(15) *Ibid.*, **49,** 91 (1966).
(16) Libbey, L. M., Bills, D. D., Day, E. A., *J. Food Sci.* **28,** 329 (1963).
(17) Lindsay, R. C., Ph.D. thesis, Oregon State University, Corvallis, Ore., 1965.
(18) McFadden, W. H., Day, E. A., Diamond, W. J., *Anal. Chem.* **37,** 89 (1965).
(19) Morgan, M. E., Day, E. A., *J. Dairy Sci.* **48,** 1382 (1965).
(20) Patton, S., *Ibid.*, **47,** 817 (1964).
(21) Ryhage, R., von Sydow, E., *Acta Chem. Scand.* **17,** 2025 (1963).
(22) Sharkey, A. G., Jr., Schultz, J. L., Friedel, R. A., *Anal. Chem.* **28,** 934 (1956).

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