1977

A study of the chemical and flavor profiles of Swiss cheese

Stanley Leo Biede
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A study of the chemical and flavor profiles of Swiss cheese

by

Stanley Leo Biede

A Dissertation Submitted to the

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Signature was redacted for privacy.

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INTRODUCTION

Swiss cheese is described as having a unique nutty and sweet flavor. With Swiss being the third most popular cheese in terms of consumption in the United States, it is surprising that high quality flavor cannot be found in most commercial cheese produced in the United States.

Until recently very little work has been done to correlate the flavor of Swiss cheese with its chemical makeup. The advent of gas chromatography has allowed investigators to gain some insight into the chemical makeup of the flavor; however, due to the complexity of the flavor, attempts at reproduction have been unsuccessful.

Purposes were, first, to determine the chemical compounds which are important in Swiss cheese flavor. Secondly, to try to reproduce as much of the flavor as possible with synthetic mixtures, and finally to develop a simple method or methods which can be used to screen Swiss cheese microorganisms for the production of flavor compounds.

LITERATURE REVIEW

The sweet-nutty flavor of Swiss cheese has long intrigued investigators interested in the flavor of Swiss cheese. Langler et al. (36) recently attempted to characterize the flavor using gas chromatography and mass spectrometry. They were able to identify 54 compounds, but were unable to attribute a given flavor characteristic to any particular compound. They suggested the flavor was caused by interaction among the compounds present.

Early studies on Swiss cheese flavor indicated that the flavor was caused by a very few compounds. Propionic acid was reported to be the most important component by Babel and Hammer (3) because its development paralleled that of the flavor. They further reported that the sweetness was due to either the presence of propionic acid or its calcium or sodium salts. Later, Virtanen and Kreula (60) correlated the sweetness with the proline content.

In 1958, Kosikowski and Mocquot (26) proposed the "component balance theory" of cheese flavor, which supposes that the balance between a relatively small number of compounds, rather than any single compound, is responsible for the flavor of a cheese variety. Major contributors to Swiss cheese flavor include: fatty acids (27), aldehydes (4), ketones (33), amines (64), amino acids (60), peptides (57) and several miscellaneous compounds (33). These compounds are not unique to Swiss cheese, but the relative proportions found in Swiss make its flavor unique, and a shift in the proportion of any one compound can lead to an off-flavor or defect (14).

Fatty acids

Swiss cheese of good quality has a large amount of acetic and propionic acid, a relatively small amount of longer chained fatty acids and very little or no butyric acid (27). In 1904, Orla-Jensen (46) showed that good flavored ripened Swiss had a molar ratio of 2.3:1 for propionic to acetic acid. Krett and Stine (27) showed that Swiss cheese containing "normal" amounts of acetic acid but little or no propionic or butyric had a flat flavor. They also observed that if the amount of butyric acid was high, the flavor became objectionable. Krett et al. (28) later studied the development of fatty acids in Swiss cheese and found that ripened Swiss contained the following amounts of fatty acids, in mg/g of cheese: acetic 2.9, propionic 6.3, butyric 0.38 and 2.9 mg of higher acids (calculated as caproic acid). Hintz et al. (19) and Kurtz et al. (30) also determined the fatty acid content in Swiss and found amounts similar to those reported by Krett et al. (28).

In studying aqueous solutions of both propionic acid and propionate, Kurtz et al. (30) found the solutions lacked the sweet and nutty flavor characteristics of Swiss cheese as had been reported by Babel and Hammer (3). They did indicate that propionic acid was probably important in the flavor complex, but other compounds were responsible for the nutty and sweet flavors.

Langler (33) and Langler and Day (35) extensively studied the amounts of volatile and long-chained fatty acids in Swiss cheese. They found that high quality Swiss contained 2-methylbutyric acid whereas lower quality contained little, if any, and suggested that this acid might be important in Swiss flavor. Langler (33) also found caproic, caprylic and capric acids present in Swiss cheese in amounts above the flavor-threshold values reported by Patton (47). A comparison of the proportions of the esterified fatty acids of milk fat with those of the long-chained free fatty acids of Swiss cheese suggested these acids might arise from the nonspecific hydrolysis of the milk fat.

Aldehydes

Of the aldehydes present in fermented dairy products, acetaldehyde is the most prominent. Bassett and Harper (5) were the first to establish its presence in Swiss cheese. Langler (33) assumed acetaldehyde was important in the flavor because the average concentration in Swiss cheese was 1.4 mg/kg which was well above the flavor threshold of 0.4 mg/kg.

Other aldehydes found in Swiss cheese have been considered of less importance as flavor contributors. Branched chained aldehydes may possibly be intermediates in the production of fatty acids through amino acids (39), while aldehydes such as benzaldehyde and phenylacetaldehyde may come from the feed since they have been found in the volatiles of grass and corn silage (38).

Ketones

Methyl ketones were found in Swiss cheese by Langler (33). Their presence has been attributed to the high cooking temperatures used during manufacture (10). Langler and Day (34) showed that a mixture of methyl ketones had a synergistic effect on flavor. Perceptible flavors were evident in mixtures in which each ketone was present at a concentration below its average flavor threshold. Because of this, it was suggested that methyl ketones might affect the flavors of Swiss cheese.

Amino Acids

Amino acids have long been studied as important flavor components of Swiss cheese. They have been shown to be flavor enhancers (40), background flavors (25,38,55) and precursors for other flavor compounds. Virtanen et al. (61) attributed the sweet flavor of Swiss to the levels of proline present. Hintz et al. (19) found that both proline and propionic acid are necessary for good flavored Swiss cheese. Proline and hydroxyproline were shown by Virtanen and Kreula (60) to make up a large proportion of the amino acids in the serum. They concluded that these two amino acids may cause the sweet flavor. Additions of glutamic acid, which is sour, to skimmilk in concentrations found in ripened cheese, produced the typical pleasant taste of Swiss cheese (56). As Swiss cheese ripens, the amounts of basic amino acids decrease (32). The basic amino acids have been suspected as the cause of bitter flavored Swiss cheese (43). Mulder (43) reported that the acidic amino acids were present in greater amounts in Swiss cheese than in any other hard rennet cheese and concluded that the proportion of acidic to basic amino acids was important in the flavor. Due to the high amount of arginine in casein, it was felt that this amino acid should be a factor in Swiss cheese flavor; however, the amount present in Swiss has been shown to be substantially lower than in milk (61). Hintz et al. (19) found that the amounts of amino acids varied with the sample of cheese and also the age of the sample. As Swiss cheese aged, the amounts of cysteic acid, taurine, proline, lysine and histidine increased, while the level of glycine remained static. Aspartic acid, threonine-serine, asparagine-glutamine, glutamic acid, tyrosine-phenylalanine and tryptophan showed no relationship between amounts and age. In studying the relationship between sweet amino acids (glycine, alanine, proline, serine and threonine) and bitter ones, Dȳlanyan et al. (12) found that proline was present in amounts much higher than any other sweet amino acid, and that poor quality Swiss cheese had a bitter to sweet ratio of 2.33, while the ratio of high quality cheese was between 1.93 and 2.05.

Miscellaneous Compounds

Several investigators have reported the presence of acetoin and diacetyl in Swiss cheese (9,59). Cisiszar et al. (9) reported an average of 2.6 mg of diacetyl/kg of Swiss cheese, but no relationship between the amount present and the flavor could be demonstrated. Langler (33) showed levels to be somewhat lower; however, they were still above threshold and, thus, were thought to be important in the flavor of Swiss cheese.

Langler (33) reported the presence of seven alcohols in Swiss cheese. Of those present ethanol and 1-propanol were present in the highest amounts; however, the concentrations were found to be too low to be able to influence the flavor. Langler did indicate that their ability to form esters with fatty acids might influence the flavor.

Of the sulfur compounds present in Swiss cheese, dimethyl sulfide is the most important (33). Its concentration varies from 0.05 to 0.183 mg/kg in Swiss cheese (33,36) which is much greater than its flavor threshold of 0.024 mg/kg of milk fat (11).

Recently, Sloot and Hofman (50) discovered the presence of six pyrazines in Swiss cheese. They reported that while the alkylpyrazines are not responsible for key flavors of Swiss cheese, they could be considered to be contributors to the natural cheese flavor.

MATERIALS AND METHODS

Cheese Samples

Seven Swiss cheeses including 6 domestic and 1 foreign cheese were used in this study. Cheeses were selected to give a wide range of flavors.

Water and Chemicals

Water was double distilled and stored in glass to assure it would be free of any flavors or odors.

All chemicals used for chemical analysis were reagent grade. Chemicals used in flavor evaluations were either obtained chemically pure or purified when necessary.

Moisture and Fat Determination

Moisture and fat determinations were carried out according to A.O.A.C. procedures (2).

Fractionation of Cheese

The cheeses were fractionated according to the scheme shown in Figure 1. Approximately 4 kg of cheese was finely ground and packed into 50-ml polyethylene centrifuge tubes and centrifuged for 1 h at 30,000 x G in a Sorvall superspeed RC2-B automatic refrigerated centrifuge (Ivan Sorvall Inc., Newton, Conn.). After centrifuging, the oil layer was removed from the tube, weighed and stored at -20C for further analysis.

The remaining curd pellets were placed in a Waring blender, covered with redistilled petroleum ether (40-60C) and blended for 1 min. The pet ether extraction was repeated until all the residual oil had been removed.

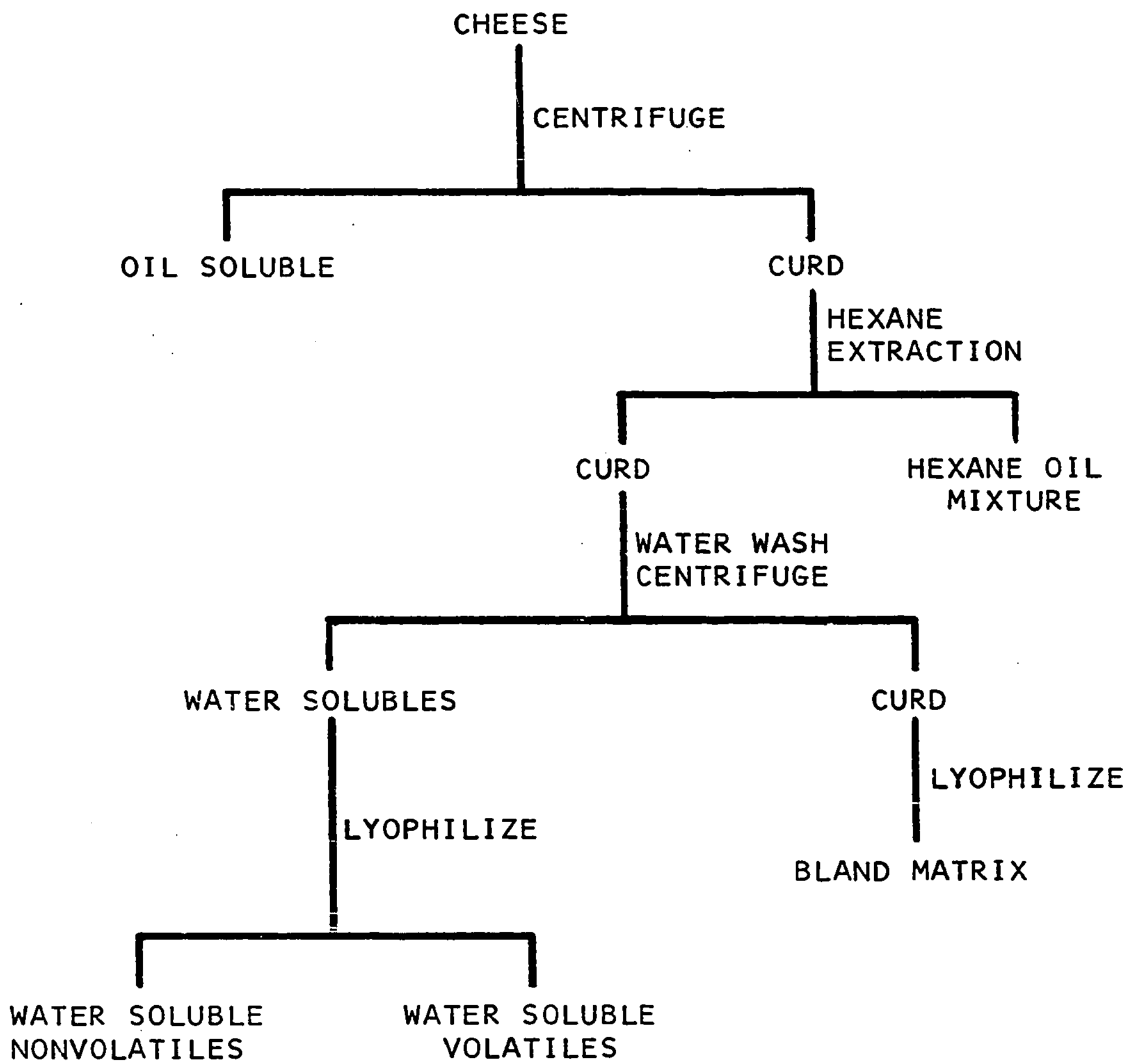


Figure 1. Scheme used for fractionation of Swiss cheese

This was determined by placing a drop of pet ether on a glass microscope slide, allowing the pet ether to evaporate and visually checking for an oil film. If no oil film was present, the samples were considered to be free of oil.

The oil-free curd was washed four times with a total amount of water equivalent to 800 ml of water/456 g of whole cheese. After each wash the slurry was centrifuged in a Westfalia LWA-205 laboratory separator (Centrico Inc., Englewood, N.J.) fitted with a chamber bowl. After the final wash and centrifugation, a portion of the washed curd was lyophilized using an all glass laboratory freeze drier fitted with dry ice-acetone collection traps. The lyophilized washed curd was stored at -20C for further analysis.

The water wash which was removed by centrifugation was lyophilized in the same manner as the washed curd. The resulting volatile distillates and residues were stored separately at -20C for further analysis.

Ammonia Determination

The concentration of ammonia in the water-soluble-volatile fraction was determined by the method of Folin and Bell (13).

Diacetyl Determination

One hundred milliliters of the water-soluble-volatile fraction was distilled and the first 10 ml of distillate were collected in an ice water bath. The concentration of diacetyl in the 10 ml of distillate was then determined using the method of Walsh and Cogan (62).

Free Fatty Acids

Isolation from whole cheese

One gram of cheese was acidified with 0.3 ml of concentrated H_2SO_4 and ground in a mortar and pestle with 5 g of silicic acid which had been dried for 72 h at 175C. This mixture was placed on the top of a elite column prepared according to the method of Wiseman and Irvin (65). The column was developed using 400 ml of 15% acetone in hexane which had been equilibrated as described by Wiseman and Irvin (65).

The eluate was extracted three times with 2% Na_2CO_3 using 1 ml for the first two extractions and 0.5 ml for the final extraction. For each extraction, the Na_2CO_3 and eluate were mixed for 30 min.

The Na_2CO_3 extracts were combined in a 35 x 12 mm screw capped vial, adjusted to pH 10 with 1 N NaOH, dried under nitrogen on a steam bath and then esterified.

Isolation from cheese fat

Approximately 0.5 g of fat was dissolved in 12 ml hexane in a 50-ml polyethylene centrifuge tube. The solution was extracted twice by mixing for 1 h with 1 ml of 2% Na_2CO_3. The Na_2CO_3 extracts were combined in a 35 x 12 mm screw capped vial, adjusted to pH 10 with 1 N NaOH, dried under nitrogen on a steam bath and esterified.

Isolation from the water-soluble-volatile fraction

One milliliter of the water-soluble-volatile fraction was placed in a 35 x 12 mm screw capped vial, adjusted to pH 10 with 1 N NaOH, dried under nitrogen on a steam bath and esterified.

Esterification and cleanup

The dried extracts were mixed with 200 µl of decanol and acidified with 35 µl of concentrated H_2SO_4. The vial was sealed with Teflon tape under the screw cap and heated for 2 h at 55C.

The reaction mixture was diluted with 500 µl of hexane and streaked on a 20 x 20 cm .75 mm silica gel G thin-layer plate. The vial was washed three times with 500 µl of hexane which was then streaked on the same silica gel plate.

The thin-layer plate was developed in 15% diethyl ether in hexane and sprayed with 2',4'-dichlorofluorescein. The ester band was scraped off the plate and put into a filtering funnel fitted with a fritted glass disc. The esters were eluted by washing the silica gel six times with 5 ml of diethyl ether. The ether was removed under nitrogen and the esters were resuspended in hexane.

Gas Chromatography of Esters

The esters from the water-soluble-volatile fraction were chromatographed on a F & M 810 gas chromatograph (F & M Scientific Corporation, Avondale, Pa.) equipped with a hydrogen flame ionization detector and on-column injection. The column was a 3.2 mm by 180 cm stainless steel tube filled with 15% Carbowax 20M on acid-washed Chromosorb W. Nitrogen was used as the carrier gas at 40 ml/min. The injector and column temperatures were 170C and that of the detector was 250C. Peaks were measured with a Disc integrator (Disc Instruments, Inc., Costa Mesa, Ca.).

The esters of the whole cheese and fat-soluble fraction were chromatographed on a Beckman GC5 gas chromatograph (Beckman Instruments, Fullerton, Ca.) equipped with a hydrogen flame detector and on-column injection. The column was a 3.2 mm by 130 cm stainless steel tube filled with 15% silicone GE SE-30 on acid-washed Chromosorb W. The carrier gas was nitrogen at 40 ml/min. The injector temperature was 170C and that of the detector, 250C. The oven temperature was held at 160C for 4 min and then the column was programmed to 250C in 8 min and held at 250C until the stearic acid peak had eluted. Peaks were measured using an Autolab 6300 Digital integrator (Autolab Division of Spectraphysics, Mountain Vein, Ca.).

Lactic Acid

Lactic acid was determined in 1 g of the water-soluble-nonvolatile fraction according to the procedure of Harper and Randolph (17).

Sugar Determinations

Glucose, galactose, and lactose were determined in 1 g of the water-soluble-nonvolatile fraction by the method of Hettinga et al. (18).

Salt Determination

The salt content of 3 g of the water-soluble-nonvolatile fraction was determined by the method described by A.O.A.C. (2).

Amino Acid Analysis

Twenty-five g of the water-soluble-nonvolatile fraction were resuspended in water and diluted to 250 ml. Enough 95% ethanol was added to the solution to make a 70% ethanol solution. The 70% ethanol solution was allowed to stand for 24 h at 4C and then centrifuged at 6900 x G for 15 min. The supernatant was decanted and the residue was resuspended in

100 ml of 70% ethanol and recentrifuged. The supernatant was decanted and the residue was lyophilized.

The supernatants were mixed and a 4-ml aliquot was placed in a 10-ml volumetric flask and dried under nitrogen. The dried material was resuspended in 10 ml of pH 2.2 citrate buffer, filtered through a Millipore filter and the amino acids were measured on a Beckman auto analyzer (Beckman Instruments, Fullerton, Ca.).

Organoleptic Analysis

A modification of the flavor profile method of Cairncross and Sjöström (8) was used for the sensory analysis of the whole cheese and each flavor fraction. An example of the rating form used is shown in Figure 2.

Four randomly numbered samples were presented to the panel at each session.

Calcium and Magnesium Determination

Approximately 1 g of the water-soluble-nonvolatile fraction was weighed into a porcelain crucible and ashed according to the procedure described by A.O.A.C. (2). The amounts of calcium and magnesium present in the ash were then determined by the method of Bird et al. (7).

Proteolysis Determination

The amount of proteolysis in the water-soluble-nonvolatile fraction was determined by a modification of the procedure of Kirimura et al. (23) according to the scheme shown in Figure 3. Approximately 5 g of the water-soluble-nonvolatile fraction was resuspended in 100 ml of water by blending for 2 min in a Waring blender. The solution was transferred to a graduated

SWISS CHEESE FLAVOR PROFILE

DATE ________________ PANEL MEMBER ____________

CHARACTER NOTE	SAMPLE NUMBER					
SWEET						
BURNED						
NUTTY						
BITTER						
LIPOLYZED						
ACID						
VOLATILE						
FERMENTED						
BUTTERY						

0 = NOT DETECTED

1 = VERY LOW

2 = LOW

3 = MEDIUM

4 = STRONG

Figure 2. Flavor profile rating form used for organoleptic analysis

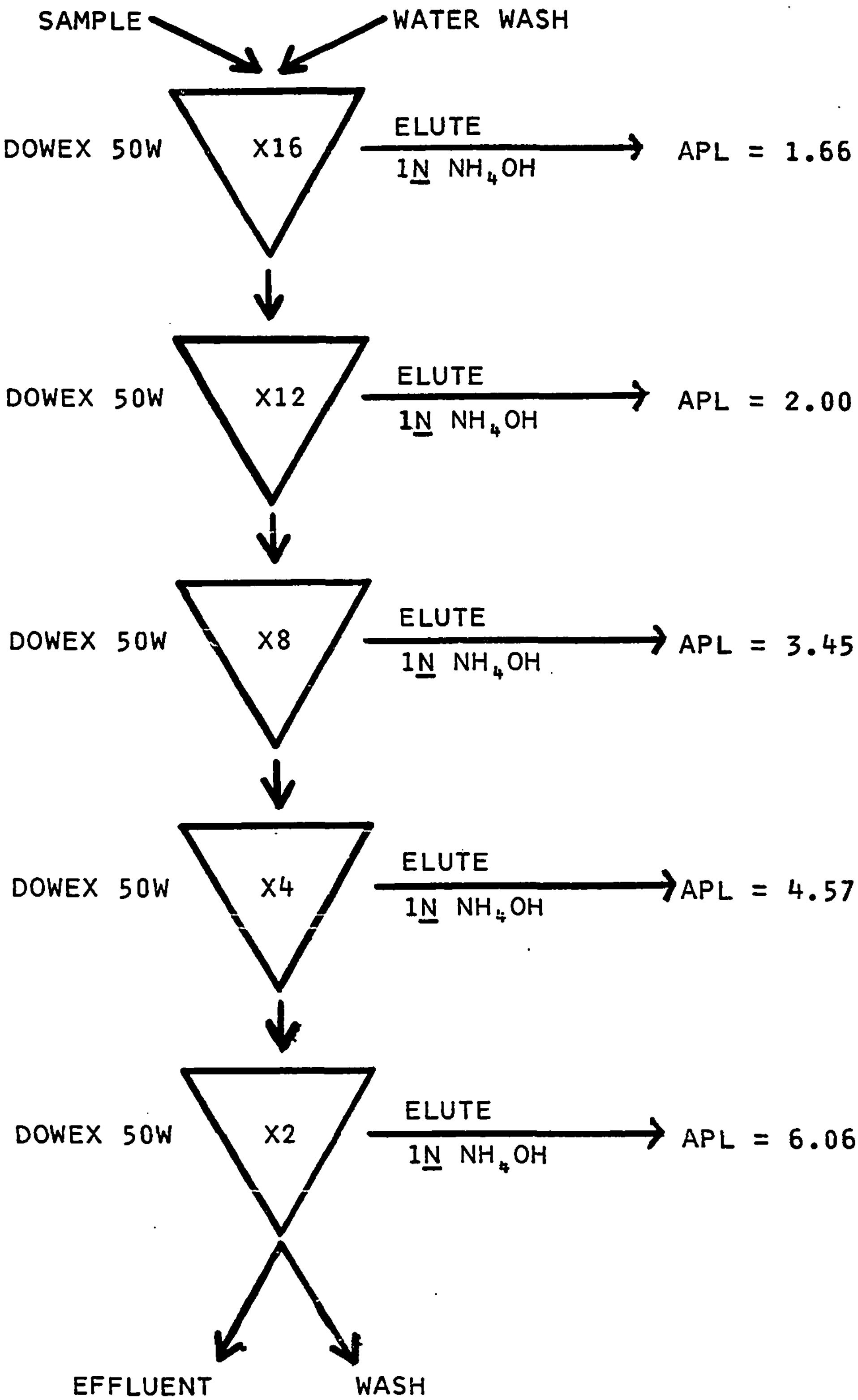


Figure 3. Scheme used to determine proteolysis in water-soluble-non-volatile fraction. [APL = average peptide length]

cylinder and made to a final volume of 150 ml. A 100-ml aliquot of the solution was passed through the Dowex 50W X 16 column. The effluent from the X16 column was passed through the Dowex 50W X 12 column and likewise with each succeeding column. When the effluent had passed through the Dowex 50W X 2 column it was collected and dried on a rotary evaporator. Each resin column was washed by passing 1 L of water successively through each column in the same order as the samples. After the wash had passed through the columns, it was collected and dried, and each column was eluted separately with 500 ml of 1 N NH_4OH. The eluates were collected and dried in the same manner as the effluent. The dried eluates, effluent and wash were weighed and the relative amount in each was used as an indication of proteolysis.

Distillation of Oil Fraction

Twenty-five grams of cheese oil were placed in the distillation apparatus shown in Figure 4. The pot was heated to 35C in a water bath and the vacuum held at 1-10 μ for 3 h. At the end of the 3 h period, the diffusion pump was cooled, the vacuum released and the condenser was transferred to a clean distillation pot in the apparatus shown in Figure 5.

The volatiles were transferred to trap 2 shown in Figure 5 under vacuum by removing the liquid nitrogen in the condenser and allowing it to warm to room temperature. After 2 h the vacuum was released and the contents of trap 2 were allowed to come to room temperature. Five milliliters of a 2% Na_2CO_3 solution were used to wash the inside of the trap and its fittings and to neutralize the contents of the trap.

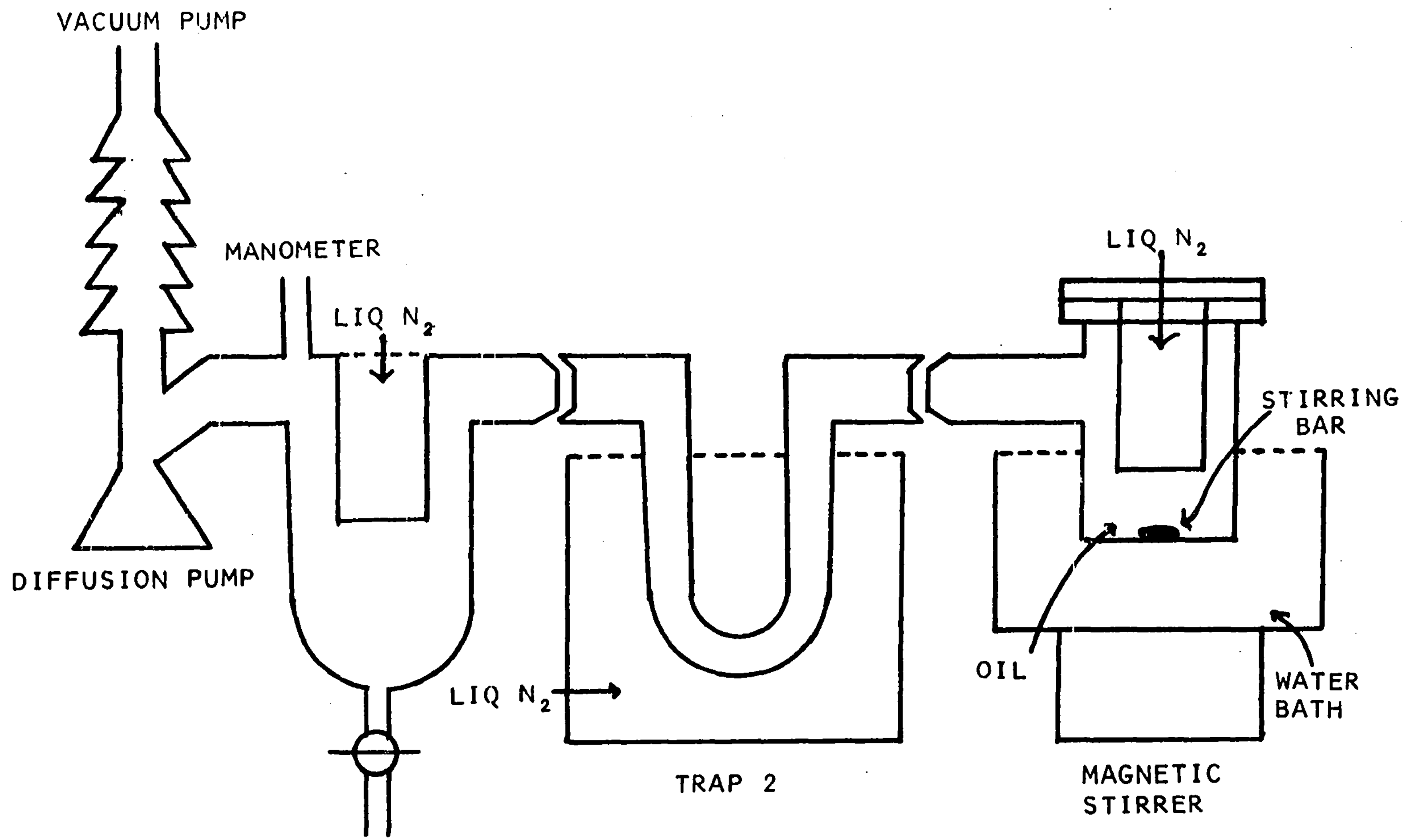


Figure 4. Molecular still used to distill cheese oil

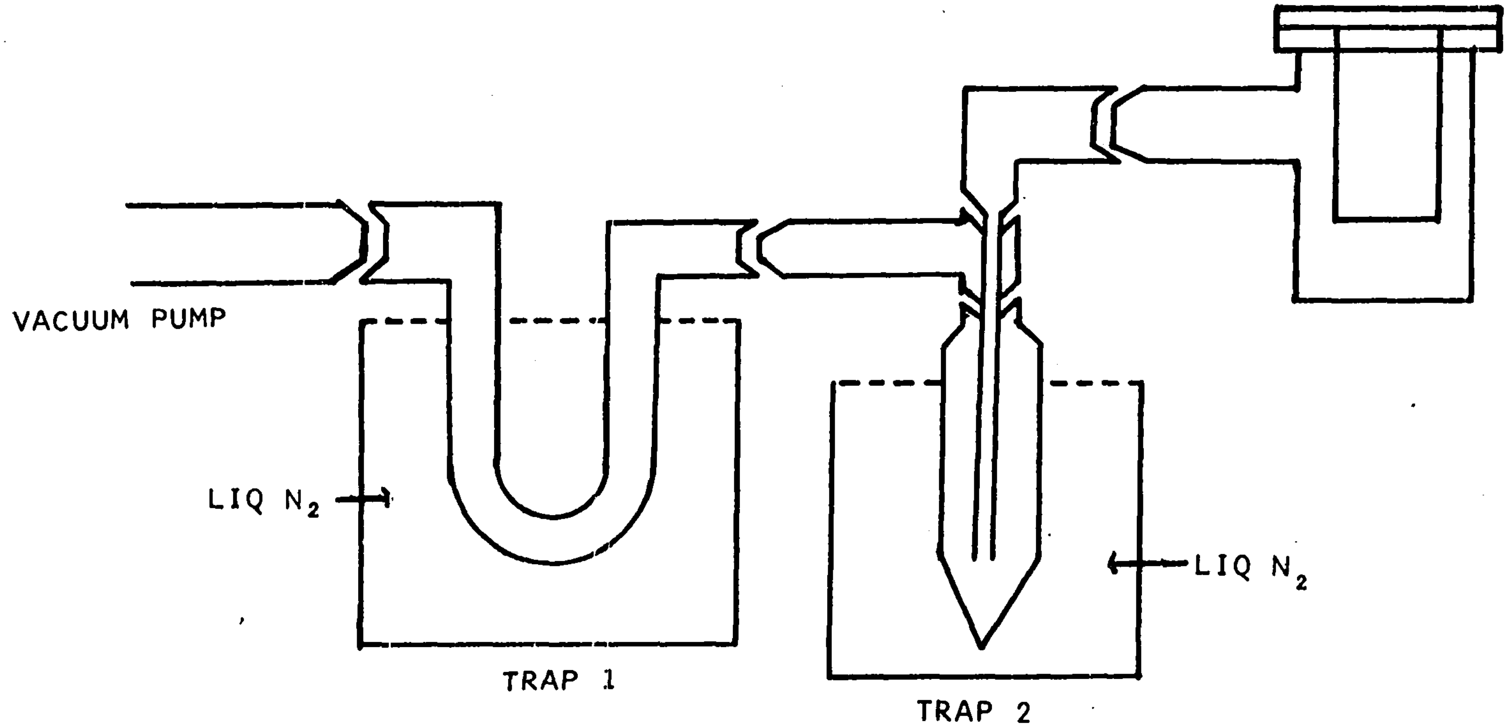


Figure 5. Apparatus used to transfer cheese oil distillate

The neutralized solution was passed through a 3.2 x 100 mm glass column filled with approximately 80 mg of Tenax-GC (Applied Sciences Laboratories, Inc., College Park, Pa.). Approximately 8 mg of the Tenax-GC was put into the first 10 mm of the 3.2 x 100 mm glass liner from the injection port of an Aerograph series 1500 gas chromatograph (Varian Instrument Division, Palo Alto, Ca.). The liner was placed in the injection port which was heated to 250C for 1 min and then removed from the injection port. The compounds eluted from the Tenax-GC were collected on a chromatography column consisting of a 3.2 mm x 180 cm glass column filled with 15% silicone GE SE-30 on Chromosorb W held at 55C. The column was fitted with a 25:1 effluent splitter which allowed one part to go to a hydrogen flame detector and 25 parts to pass out of the chromatograph for odor evaluation. The column temperature was held at 55C for 2 min after the Tenax-GC fitted liner was removed and then the programmer was turned on and the temperature allowed to rise at 8C/min to a limit of 200C. The carrier gas was nitrogen at 40 ml/min.

Peaks were tentatively identified by their odors and by comparison of retention times to those of pure compounds.

RESULTS AND DISCUSSION

Gross Composition of Whole Cheese

Moisture and fat determinations (Table 1) showed slight variations among cheeses in both the percent moisture and fat on the dry basis. These variations were not significant and the amounts present all lay within government standards (45).

The percentages of salt in samples D, E and F were significantly higher than the others. The amounts shown for all cheeses are higher than the supposed typical values shown in government standards (45), and without knowing the complete manufacturing history of the cheeses, it is impossible to explain the deviations from the standards.

The amounts of lactic acid shown in Table 1 are higher than those reported by Schormüller and Langner (49).

Flavor Analysis of Cheese and Fractions

Fractionation procedure

The fat-soluble fraction (FS) was obtained by centrifugation of the whole cheese. The amount of fat recovered ranged from 5 to 40% of the fat in the cheese. This is a lower recovery than those reported by McGugan and Howsam (42) using this technique, but the fat recovered was sufficient to allow organoleptic and chemical evaluation.

Fat remaining in the curd was removed by extraction with petroleum ether. This extract was not evaluated further, but it was supposed that the FS would have a similar composition and flavor.

Results reported later on the distribution of fatty acids between the fat and nonfat portions of cheese will show that the nonfat portion contained considerable amounts of fatty acids. Probably these fatty acids were removed from the nonfat portion of the cheese by the extensive washing with petroleum ether.

Table 1. Gross composition of cheeses used in this study

Cheese		Moisture	Fat on dry basis	Salt	Lactic acid	pH
A*[a]	Domestic	36.82[b]	44.98[b]	1.92[b]	7.44[c]	5.32
B*	Domestic	37.18	45.51	1.78	10.11	5.45
C*	Domestic	39.97	48.97	1.77	6.64	5.50
D**	Foreign	35.36	52.93	3.22	11.64	5.79
E***	Domestic	37.38	50.68	3.30	14.59	5.39
F****	Domestic	40.22	52.99	3.59	8.75	5.76
G****	Domestic	38.84	51.16	2.50	13.87	5.68

[a]Stars indicate cheeses obtained from the same manufacturer.

[b]Percent.

[c]mg/g cheese.

A water wash was used to separate the protein matrix from two additional flavor-bearing fractions. The water-soluble-nonvolatile fraction (WSNV) and water-soluble-volatile fraction (WSV) were obtained by lyophilizing the water wash. The WSNV was the residue left in the lyophilization flasks, and the WSV was the distillate collected in the cold traps.

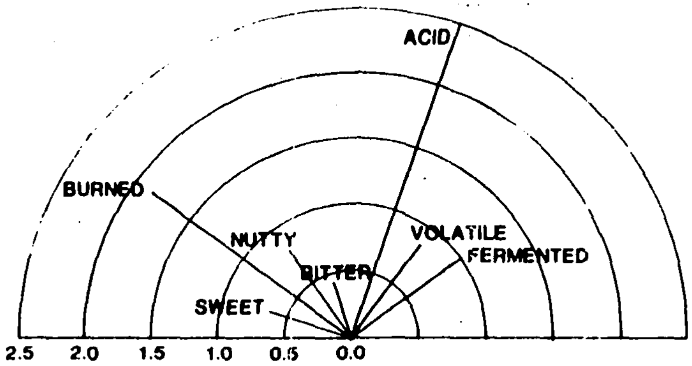
The water-insoluble protein matrix was dried and submitted to a taste panel for organoleptic analysis. The matrix was judged to be free of flavor.

Flavor profiles

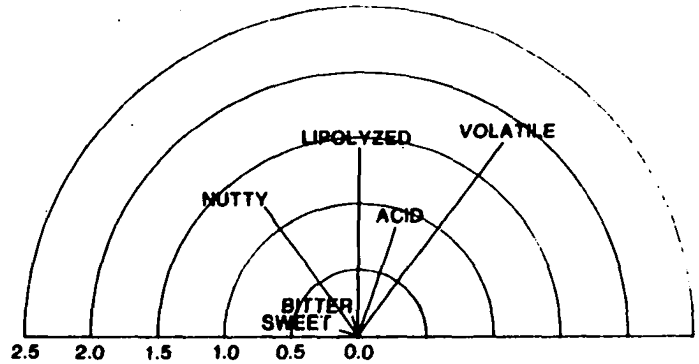
Graphic representation of the flavor profiles for the seven cheeses and fractions are shown in Figures 6-12. These profiles are the averages of three separate evaluations of each sample by seven judges trained in the use of the rating form shown in Figure 2. More detailed discussions of the character notes will follow; however, a brief description of the more unfamiliar terms may be helpful: Burned was a caramelized flavor or a flavor resembling that of lactic acid. Nutty was used to describe the overall "cheesiness" of the samples and resembled the flavor of mild Cheddar cheese. The volatile note usually was perceived just after the sample had been placed in the mouth and chewed only a few times. It was used to describe the extent to which the flavor seemed to quickly fill the mouth. Fermented was used by the flavor panel to describe any flavor defect or oddity not concerned with the lipolyzed or bitter notes. The buttery note was used to describe the flavor of diacetyl.

Analysis of variance (51) (Table 21 of the Appendix) showed that significant differences were observed among the samples for all flavors

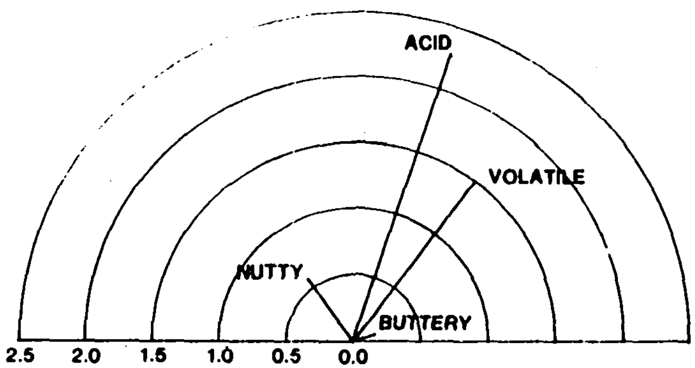
Figure 6. Flavor profiles of cheese A and its fractions



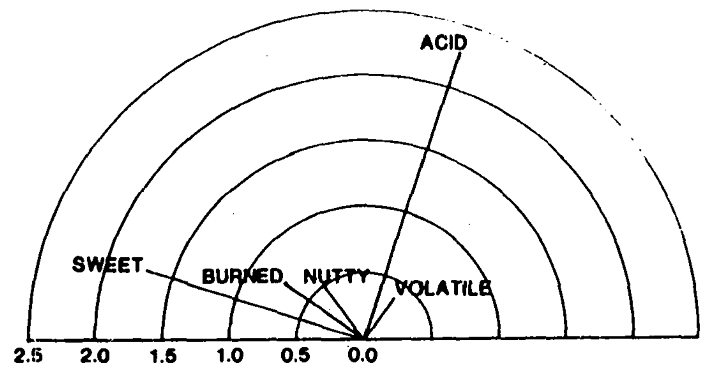
WHOLE CHEESE



FAT-SOLUBLE FRACTION

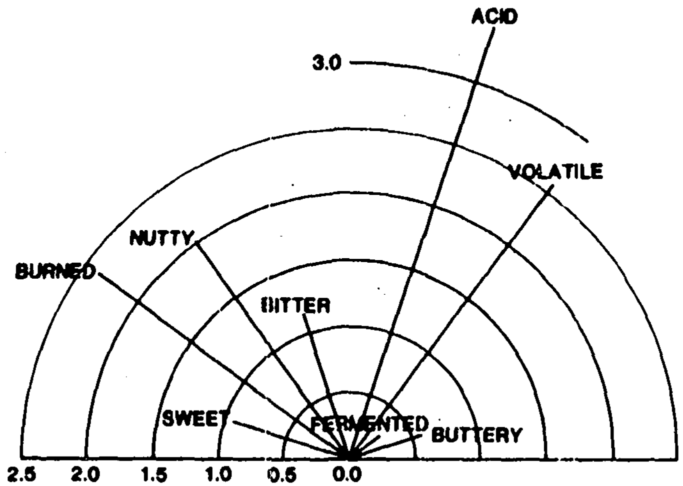


WATER-SOLUBLE-VOLATILE FRACTION

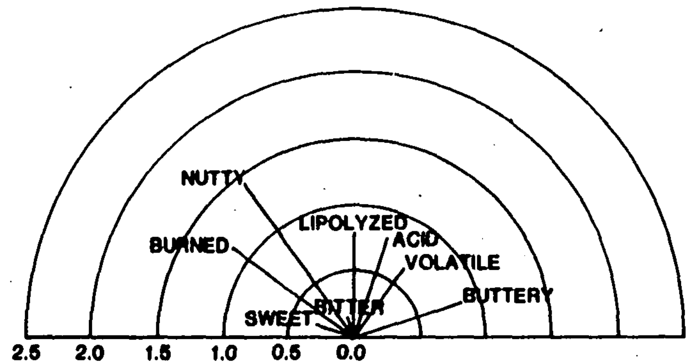


WATER-SOLUBLE-NON-VOLATILE FRACTION

Figure 7. Flavor profiles of cheese B and its fractions



WHOLE CHEESE



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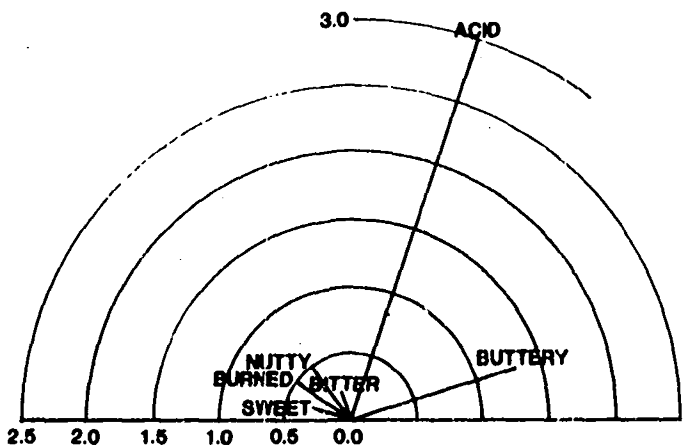
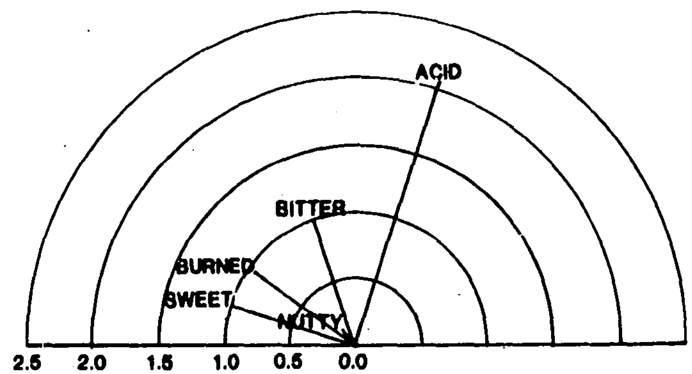
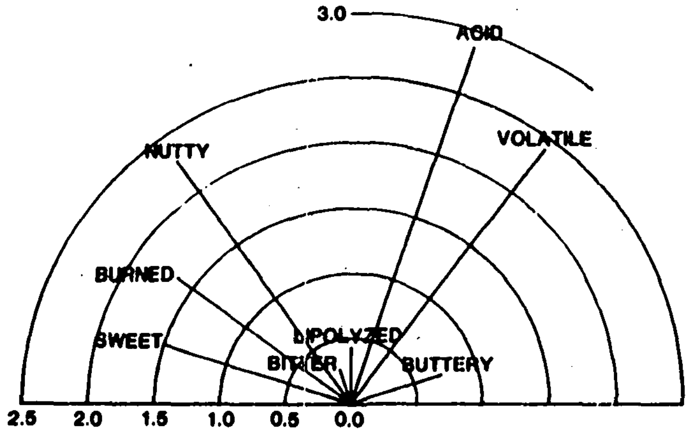


WATER-SOLUBLE-VOLATILE FRACTION

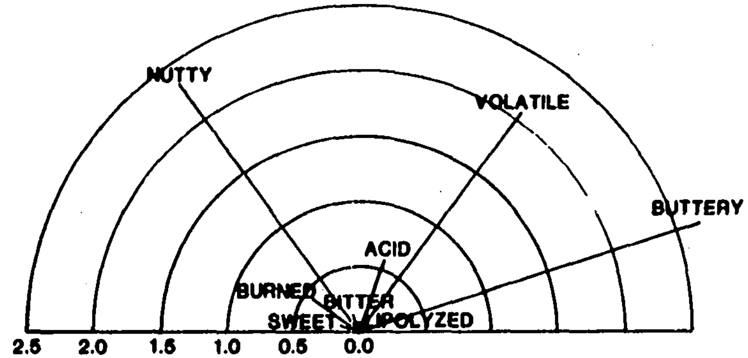


WATER-SOLUBLE-NON-VOLATILE FRACTION

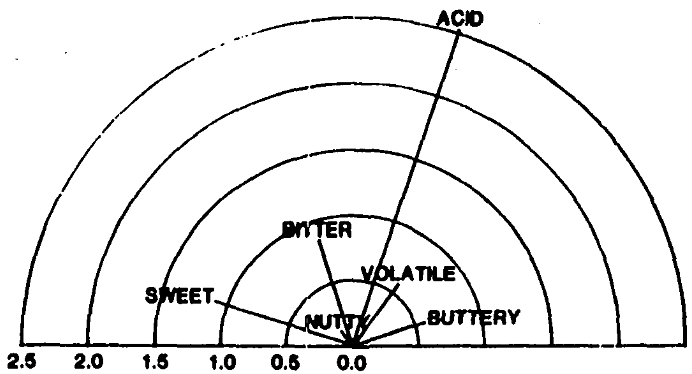
Figure 8. Flavor profiles of cheese C and its fractions



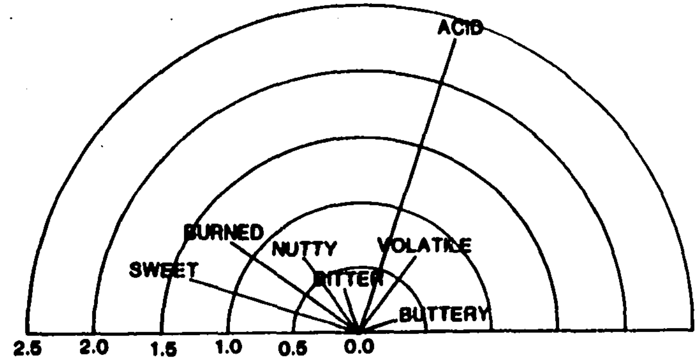
WHOLE CHEESE



FAT-SOLUBLE FRACTION

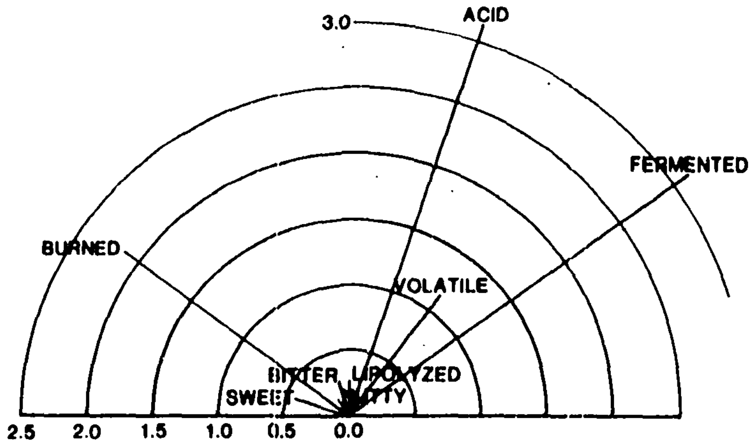


WATER-SOLUBLE-VOLATILE FRACTION

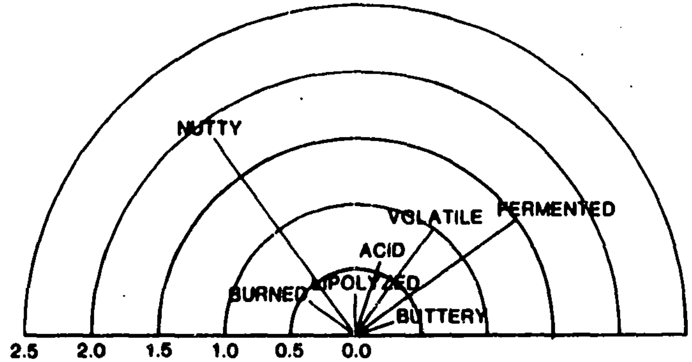


WATER-SOLUBLE-NON-VOLATILE FRACTION

Figure 9. Flavor profiles of cheese D and its fractions



WHOLE CHEESE



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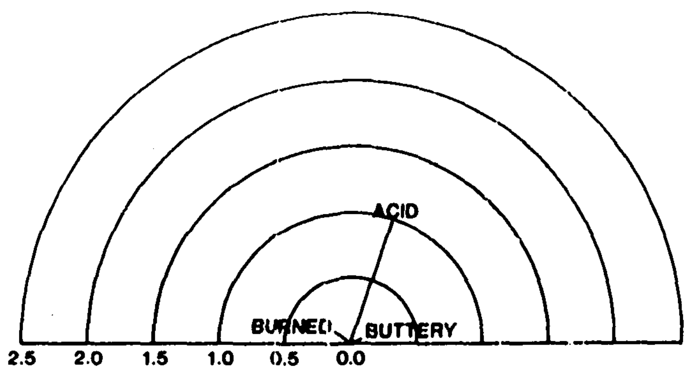
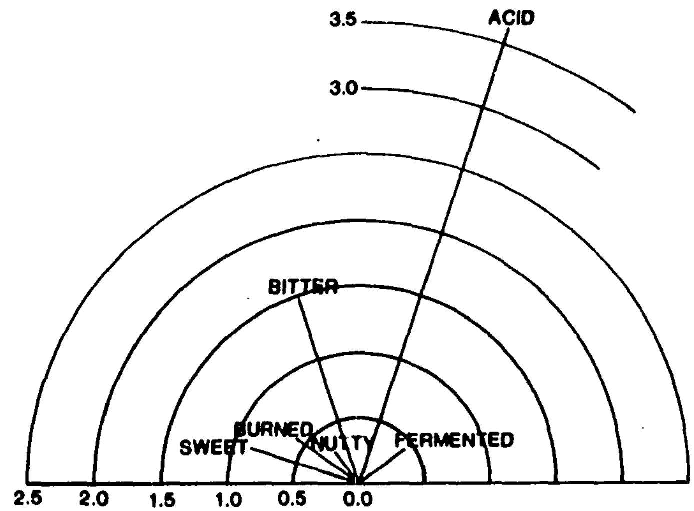
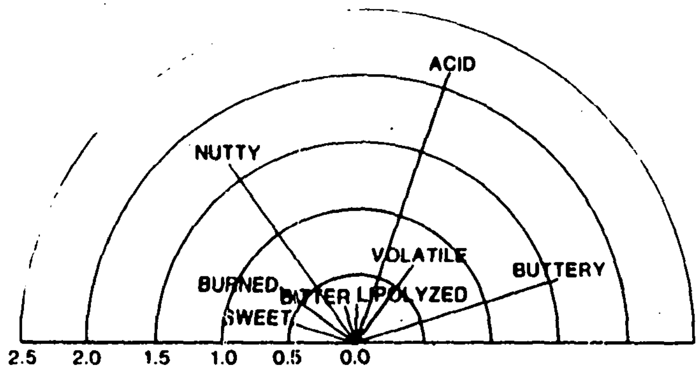


WATER-SOLUBLE-VOLATILE FRACTION

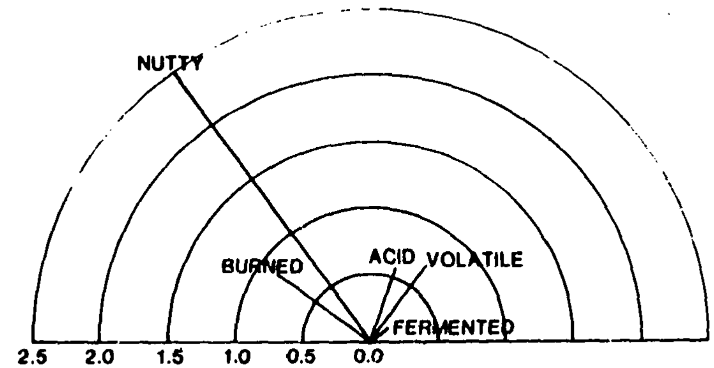


WATER-SOLUBLE-NON-VOLATILE FRACTION

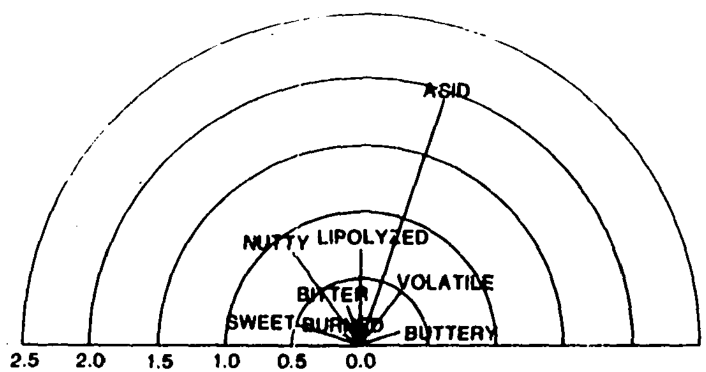
Figure 10. Flavor profiles of cheese E and its fractions



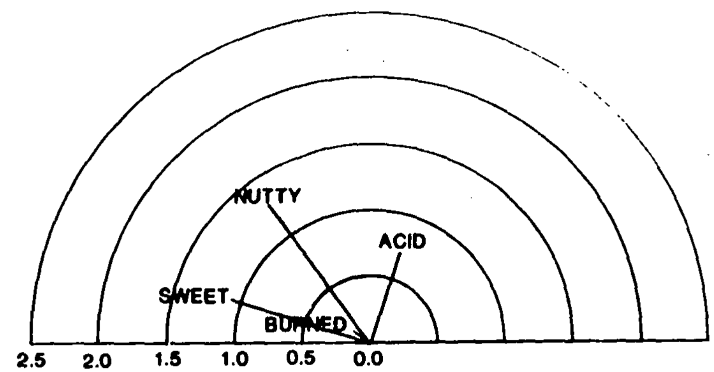
WHOLE CHEESE



FAT-SOLUBLE FRACTION

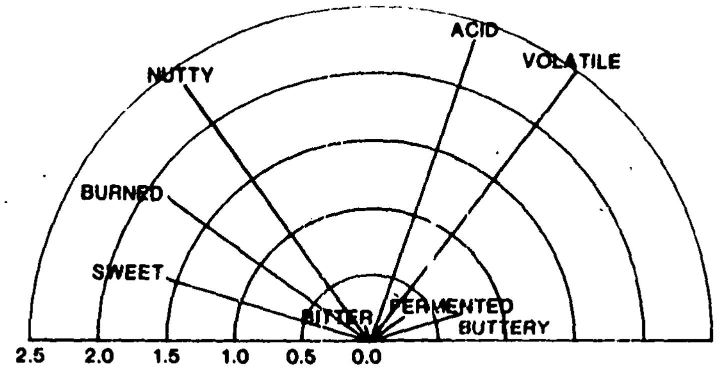


WATER-SOLUBLE-VOLATILE FRACTION

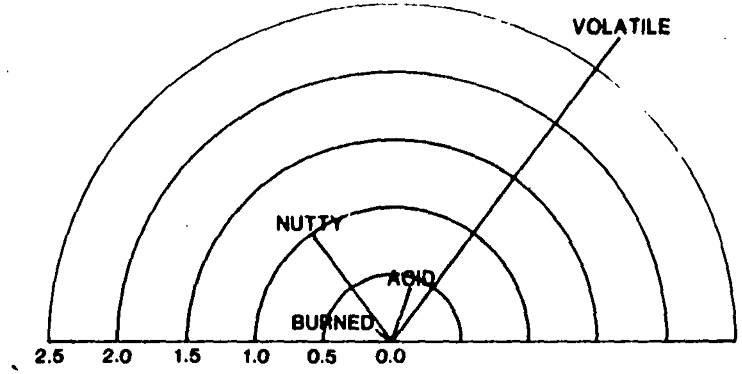


WATER-SOLUBLE-NON-VOLATILE FRACTION

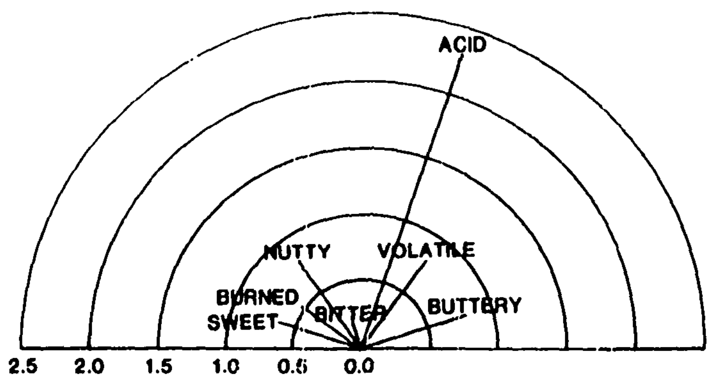
Figure 11. Flavor profiles of cheese F and its fractions



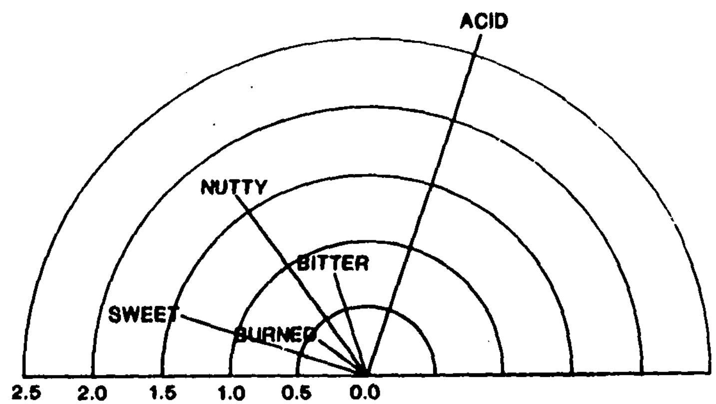
WHOLE CHEESE



FAT-SOLUBLE FRACTION

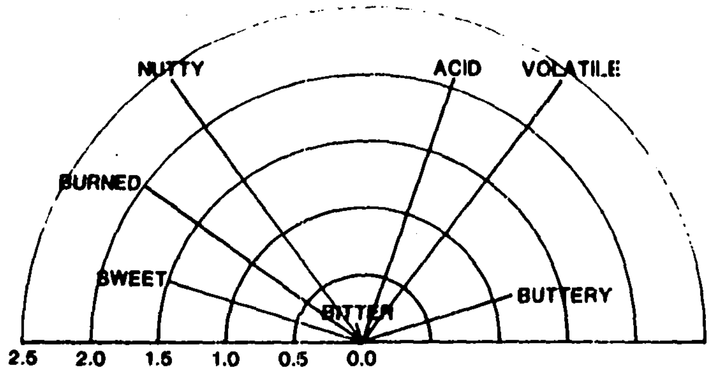


WATER-SOLUBLE-VOLATILE FRACTION

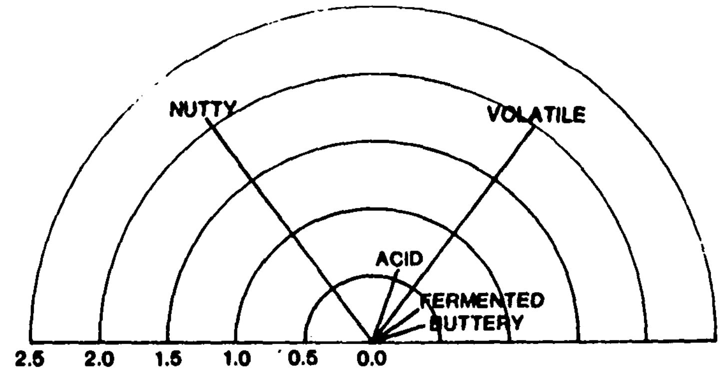


WATER-SOLUBLE-NON-VOLATILE FRACTION

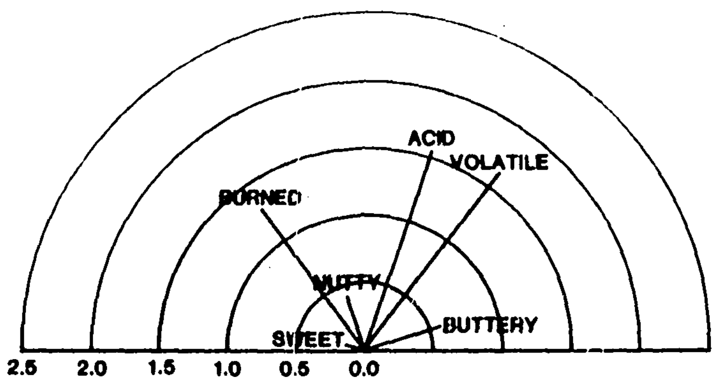
Figure 12. Flavor profiles of cheese G and its fractions



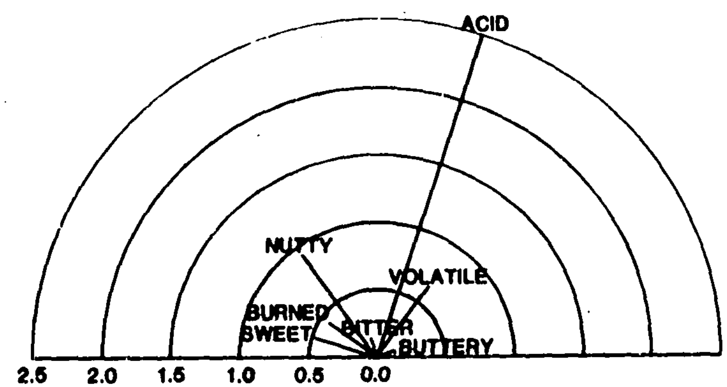
WHOLE CHEESE



FAT-SOLUBLE FRACTION



WATER-SOLUBLE-VOLATILE FRACTION



WATER-SOLUBLE-NON-VOLATILE FRACTION

except bitter. Significant judge X fraction interaction was found for the flavors sweet, nutty and bitter and a significant sample X judge interaction was found for sweet. These indicate that some judges may not have used these flavor descriptions consistently across fractions and samples. A more extensive analysis revealed that these inconsistencies were distributed among nearly all the judges, and it was not practical to eliminate any of the panel.

Results of a Duncan's multiple range test (54), on the means of the character notes for the whole cheeses and each of the fractions (Tables 2-9), showed great variation in the flavor panel's ability to distinguish different intensities in the flavor notes of samples. In most cases, the lack of significant differences among samples was due to the low intensities or absence of the character note. This was not true for the acid notes in the whole cheese (Tables 2 and 3) and FS (Tables 4 and 5) or the sweet note in the WSNV (Tables 8 and 9). In the case of the acid notes, lack of significant differences was probably due to the small range of variation in the samples. The lack of significant differences for the sweet note in the WSNV is probably due to the nature of the sweet flavor which was very subtle and easily masked by the other flavors. This perceptive difficulty was indicated by the high standard deviations for sweet in the WSNV.

The Duncan's test did show significant differences among the samples of whole cheese for burned, nutty, bitter, volatile, fermented and buttery. In the FS, burned, bitter, lipolyzed, volatile, fermented and buttery character notes had significant differences. In the WSV there were

Table 2. Means and standard deviations of preference and character notes for whole cheese

Cheese	Preference		Character note							
			Sweet		Burned		Nutty		Bitter	
	Mean	s.d.[a]	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.
A	1.69[b]	.60	.64	.63	1.86	1.07	.79	1.07	.43	.79
B	1.80	.77	.93	.73	2.54	.98	2.34	.93	1.14	1.46
C	2.79	.89	1.50	.87	1.64	1.03	2.30	.77	.29	.76
D	1.08	.28	.43	.79	2.14	1.46	.14	.38	.29	.76
E	2.14	1.03	.50	.50	.71	.76	1.64	1.18	.29	.76
F	3.31	.75	1.57	1.13	1.86	1.07	2.36	.48	.14	.38
G	3.00	.91	1.50	.96	2.00	1.29	2.43	.98	.14	.38

[a]Standard deviation.

[b]Intensity scale shown in Figure 2.

Table 3. Duncan's listings for means of preference and character notes of whole cheese

Preference	Character note			
	Sweet	Burned	Nutty	Bitter
F	F	B	G	B
G	G	D	F	A
C	C	G	B	C
E	B	F	C	D
B	A	A	E	E
A	E	C	A	F
D	D	E	D	G

Character note									
Lipolyzed		Acid		Volatile		Fermented		Buttery	
Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.
0.00	0.00	2.50	.96	.86	1.07	1.00	1.29	0.00	0.00
0.00	0.00	3.43	.45	2.57	.98	.29	.76	.57	1.13
.43	.79	2.89	.46	2.43	.79	0.00	0.00	.71	1.25
.29	.49	3.14	1.03	1.14	1.46	3.14	1.46	0.00	0.00
.29	.76	2.14	.69	.71	.95	0.00	0.00	1.89	1.32
0.00	0.00	2.36	.63	2.50	.76	.29	.76	.71	.76
0.00	0.00	2.07	1.24	2.43	1.40	0.00	0.00	1.14	1.07

Character note				
Lipolyzed	Acid	Volatile	Fermented	Buttery
C	B	B	D	E
D	D	F	A	G
E	C	C	F	C
B	A	G	B	F
A	F	D	E	B
F	E	A	C	D
G	G	E	G	A

Table 4. Means and standard deviations of character notes for fat-soluble fraction

Cheese	Character note							
	Sweet		Burned		Nutty		Bitter	
	Mean	s.d.[a]	Mean	s.d.	Mean	s.d.	Mean	s.d.
A	.14[b]	.38	0.00	0.00	1.21	1.29	.14	.38
B	.29	.76	1.19	1.43	1.43	1.27	.14	.38
C	.14	.38	.42	1.13	2.36	1.07	.14	.38
D	0.00	0.00	.42	.79	1.86	.38	0.00	0.00
E	0.00	0.00	.86	.90	2.50	1.32	0.00	0.00
F	0.00	0.00	.14	.38	1.50	1.19	0.00	0.00
G	0.00	0.00	0.00	0.00	2.07	1.43	0.00	0.00

[a]Standard deviation.

[b]Intensity scale shown in Figure 2.

Table 5. Duncan's listings for means of character notes of fat-soluble fraction

Character note			
Sweet	Burned	Nutty	Bitter
B	B	E	A
A	E	C	B
C	C	G	C
D	D	D	D
E	F	F	E
F	A	B	F
G	G	A	G

Character note									
Lipolyzed		Acid		Volatile		Fermented		Buttery	
Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.
1.43	1.51	.86	1.07	1.79	1.07	0.00	0.00	0.00	0.00
.79	.99	.78	.57	.64	1.18	0.00	0.00	.86	1.46
.07	.19	.57	.98	2.07	.61	0.00	0.00	2.69	1.60
.29	.49	.57	.98	1.00	1.00	1.50	1.19	.29	.76
0.00	0.00	.57	1.13	.71	1.25	.14	.38	0.00	0.00
0.00	0.00	.43	.53	2.79	.99	0.00	0.00	0.00	0.00
0.00	0.00	.57	.98	2.00	1.04	.43	1.13	.43	1.13

Character note				
Lipolyzed	Acid	Volatile	Fermented	Buttery
A	A	F	D	C
B	B	C	G	B
D	C	G	E	G
C	D	A	A	D
E	E	D	B	E
F	G	E	F	F
G	F	B	C	A

Table 6. Means and standard deviations of character notes for water-soluble-volatile fraction

Cheese	Character note							
	Sweet		Burned		Nutty		Bitter	
	Mean	s.d.[a]	Mean	s.d.	Mean	s.d.	Mean	s.d.
A	0.00[b]	0.00	0.00	0.00	.57	.53	0.00	0.00
B	.29	.49	.50	.87	.50	.65	.21	.39
C	1.09	1.08	0.00	0.00	.14	.38	.86	1.07
D	0.00	0.00	.14	.38	0.00	0.00	0.00	0.00
E	.50	.41	.14	.38	.86	.69	.29	.49
F	.64	.94	.57	.79	.79	.81	.21	.57
G	.14	.38	0.00	0.00	1.29	1.22	.43	.79

[a]Standard deviation.

[b]Intensity scale shown in Figure 2.

Table 7. Duncan's listings for means of character notes of water-soluble-volatile fraction

Character note			
Sweet	Burned	Nutty	Bitter
C	F	G	C
F	B	E	G
E	D	F	E
B	E	A	B
G	A	B	F
D	C	C	A
A	G	D	D

Character note									
Lipolyzed		Acid		Volatile		Fermented		Buttery	
Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.
0.00	0.00	2.29	.49	1.50	.87	0.00	0.00	.14	.38
0.00	0.00	3.00	.29	0.00	0.00	0.00	0.00	1.29	.76
0.00	0.00	2.53	.92	.57	.98	0.00	0.00	.57	.77
0.00	0.00	.96	.63	0.00	0.00	0.00	0.00	.07	.19
.71	1.25	1.93	.73	.50	.96	0.00	0.00	.29	.76
0.00	0.00	2.30	.40	.79	1.07	0.00	0.00	.79	.91
0.00	0.00	1.57	1.24	1.64	1.44	0.00	0.00	.57	1.13

Character note				
Lipolyzed	Acid	Volatile	Fermented	Buttery
E	B	G	A	B
A	C	A	B	F
B	F	F	C	G
C	A	C	D	C
D	E	E	E	E
E	G	B	F	A
F	D	D	G	D

Table 8. Means and standard deviations of character notes for water-soluble-nonvolatile fraction

Cheese	Character note							
	Sweet		Burned		Nutty		Bitter	
	Mean	s.d.[a]	Mean	s.d.	Mean	s.d.	Mean	s.d.
A	1.71	1.78	.71	1.11	.50	.65	0.00	0.00
B	1.00	1.53	.93	1.37	.14	.38	1.00	1.41
C	1.36	1.18	1.54	1.48	.71	.95	.36	.75
D	.86	1.07	.57	.79	.29	.76	1.14	1.68
E	1.07	.53	.14	.38	1.29	.91	0.00	0.00
F	1.43	1.27	.43	.79	1.64	1.38	.79	1.07
G	.50	.76	.43	1.13	.93	1.10	.14	.38

[a]Standard deviation.

[b]Intensity scale shown in Figure 2.

Table 9. Duncan's listings for means of character notes of water-soluble-nonvolatile fraction

Character note			
Sweet	Burned	Nutty	Bitter
A	C	F	D
F	B	E	B
C	A	G	F
E	D	G	C
B	F	A	G
D	G	D	A
G	E	B	E

Character note									
Lipolyzed		Acid		Volatile		Fermented		Buttery	
Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.
0.00	0.00	2.29	.81	.36	.94	0.00	0.00	0.00	0.00
0.00	0.00	2.07	1.27	0.00	0.00	0.00	0.00	0.00	0.00
0.00	0.00	2.36	.48	.71	1.25	0.00	0.00	.29	.76
0.00	0.00	3.64	.85	0.00	0.00	.43	1.13	0.00	0.00
0.00	0.00	.71	.39	0.00	0.00	0.00	0.00	0.00	0.00
0.00	0.00	2.64	1.03	0.00	0.00	0.00	0.00	0.00	0.00
0.00	0.00	2.50	.82	.64	1.11	0.00	0.00	.14	.38

Character note				
Lipolyzed	Acid	Volatile	Fermented	Buttery
A	D	C	D	C
B	F	G	A	G
C	G	A	B	A
D	C	B	C	B
E	A	D	E	D
F	B	E	F	E
G	E	F	G	F

significant differences in the sweet, nutty, lipolyzed, acid, volatile and buttery notes. And in the WSNV burned, nutty, bitter and acid had significant differences among samples. The importance of these differences will be discussed in more detail in later sections.

Cheese preferences

The whole cheeses were rated by the taste panel according to preference on a scale of 1 to 4 with 1 being least preferred. This rating showed that the most preferred cheeses were F and G and the least preferred was cheese D (Table 2). The whole cheese flavor profiles of F and G (Figures 11 and 12) showed them high and nearly equal in the intensity of positive character notes (sweet, burned, nutty, acid and volatile), while having little or no flavor defects present (bitter, lipolyzed and fermented). On the other hand, cheese D (Figure 9) was intensely fermented, and had very low intensities for the positive character notes other than acid and burned. The other cheeses (A, B, C and E) were less preferred than F and G, seemingly because of their uneven distribution of positive character notes and the occasional presence of off-flavors such as bitter, fermented and lipolyzed.

These results indicate that a balance between the positive character notes is necessary for the flavor of a good Swiss cheese and that a shift in the balance or the presence of a flavor defect can cause the cheese to be rated low.

<u>Prediction of whole cheese flavor from the fractions</u>

An attempt was made to predict the intensity of whole cheese flavors from the intensities observed in the fractions. To do this the following regression formula was developed:

$$Y_w = b_oX_o + b_sX_s + b_nX_n$$

where

Y_w = Intensity of character note in whole cheese.

X_o = Average intensity of character note in fat-soluble fraction.

X_s = Average intensity of character note in water-soluble-volatile fraction.

X_n = Average intensity of character note in water-soluble-nonvolatile fraction.

This formula was not successful in predicting the intensity of the character notes in the whole cheese. This is probably because the panel could not distinguish flavor differences among the samples for several important flavor notes (Tables 3-9). Also the character notes of the whole cheese correlate very poorly with those of the various fractions (Table 22 of the Appendix). Possibly the washing of the nonfat portion of the cheese with petroleum ether removed fatty acids bound to the protein and altered the flavors of the water-soluble fractions. It is also possible that the poor correlation is because the intensity of a given character note in the whole cheese depended upon interactions between several different character notes of each fraction as well as the interaction of the fractions themselves. This idea was not pursued further due to the complexity in determining the proper interactions.

Relation of Flavor to Composition

Water-soluble-volatile fraction

Flavor profiles The flavor profiles of the WSV (Figures 6-12) showed the only relatively intense flavor note present was acid, and results of the Duncan's test (Table 7) showed the flavor panel was able to distinguish differences among samples. The other character notes, with the exception of the fermented note, were present to varying extents. Duncan's tests also showed significant differences between samples in the intensities of the sweet, nutty, lipolyzed, volatile and buttery notes. The inability of the flavor panel to detect significant differences in the burned and bitter notes was due to the low amounts of these flavors present in this fraction. The only sample which had a lipolyzed flavor was sample E (Figure 10), and the intensity of the lipolyzed note in this sample was significantly higher than the other samples.

Chemical composition Chemical analysis of this fraction showed only five major components: diacetyl, ammonia, and acetic, propionic and butyric acids (Table 10). When this fraction was adjusted to pH 10, a faint odor resembling triethylamine was produced; however, no further analysis was made to confirm the presence of amine compounds.

Relationship between flavor profile and chemical composition Because the acid character note was dominant, we expected the acid content of the fraction to correlate with this character note, but no correlation between the amounts of acetic, propionic or butyric acid could be shown. Acidity was best explained by taking into account the total amount of free fatty acid and ammonia. By subtracting the molar amount of ammonia from

Table 10. Chemical makeup of the water-soluble-volatile fraction

Cheese	Diacetyl	NH_3	Acetic acid	Propionic acid	Butyric acid	Net[a] acid	pH
A	60[b]	110[c]	220[c]	670[c]	20[c]	6.44	4.50
B	100	110	410	840	30	12.05	4.75
C	560	100	400	680	30	10.32	4.61
D	100	70	210	350	100	5.36	4.92
E	580	50	110	70	170	2.22	4.32
F	300	60	170	230	30	2.75	4.55
G	100	70	200	350	10	4.05	4.42

[a] mM acids - mM ammonia.

[b] µg/1000 ml.

[c] mg/1000 ml.

the total molar amount of fatty acid, sample B, which has the most intense acid note, had the highest net amount of free acid. Samples D, E and G, which were weakest in the acid note, were among those having the least amount of free acid. This was not true for sample F, which was among the most acid in flavor and had one of the lowest amounts of free acid. These results, along with a high correlation between free acid and the acid note (r = .602), do support the idea that the acidity of the WSV fraction was caused by the amount of free acid present.

Of the other character notes present in the WSV, the sweet note was highly correlated (r = .791, Table 23 of the Appendix) with the amount of diacetyl present. There is considerable literature on the importance of diacetyl in Swiss cheese flavor (9,33,36), but no direct relationship between diacetyl and sweetness has been reported. Diacetyl has been reported to be important in the sweetness of apple cider (63) at very low concentrations. The amount of diacetyl present in the WSV is well above its threshold in a water solution (53), and it is reasonable that diacetyl would be correlated with sweetness.

The lipolyzed flavor of this fraction was highly correlated (r = .863) with the amount of butyric acid present. This relationship is to be expected, and it is supported by the work of Goudkov and Sharpe (15) who reported on the influence of butyric acid on lipolyzed flavor in Cheddar cheese.

Although the nutty, volatile and buttery character notes were found to be significantly different among samples and also relatively important in the flavor profiles of the WSV, the volatile note was the only flavor that could be correlated with the chemical composition. The negative correlation between the volatile note and butyric acid (r = -.629), while not significant, indicates that the presence of butyric acid repressed the perception of this flavor. This repression is probably due to a shift in the delicate balance of the volatile note by butyric acid.

Synthetic mixture A synthetic WSV fraction was made for each cheese using the components and amounts shown in Table 10 and adjusting the pH to that of the original WSV with .1 N NaOH. These synthetic mixtures were presented to the panel along with samples of the original WSV. The results of the flavor analysis is shown in Table 11. For the most part, the flavor panel was unable to determine significant differences between the profiles of the original and synthetic mixtures. Although the experiment was not tried, it is probable that some of the samples and synthetics were so similar that they could not have been distinguished in a triangle test. The character note which most often differed from the original was the acid note; however, the manner in which the acidity varied was not consistent among samples, and thus no explanation for the variation could be found.

Since the synthetic mixtures did not vary significantly from the original WSV, the inability to attribute the character notes of the WSV to the chemical makeup indicates that interactions of the chemical components, and not the components separately, are important for the flavors of this fraction.

Table 11. Comparisons of the original water-soluble-volatile fraction and the synthetic water-soluble-volatile fraction

Cheese	Type[a]	Character note								
		Sweet	Burned	Nutty	Bitter	Lipolyzed	Acid	Volatile	Fermented	Buttery
A	O	.31[b]	.50	.19	.23	0.00	2.06**	.33	0.00	0.00**
	A	.50	.42	.23	.43	0.00	1.08	.21	0.00	.34
B	O	.23	.56	.15	.08	0.00	1.50**	.21	0.00	.10
	A	.15	.46	.13	.28	0.00	1.98	.25	0.00	.04
C	O	.24	.28	0.00	.04	0.00	1.70	.06	0.00	.32
	A	.15	.24	.13	.20	0.00	1.67	.13	0.00	.36
D	O	.04	.08	0.00*	.65	0.00	.65**	.04	.42	.10
	A	.13	.06	.10	.27	0.00	1.02	.02	.04	.07
E	O	.17	.23	.36	.38	.08	1.58	0.00**	0.00	.43
	A	.42	.25	.38	.64	.21	1.58	.25	0.00	.33
F	O	.15	.33	.06**	.42	0.00	1.90	.23	0.00	.28
	A	.25	.25	.33	.36	0.00	1.65	.08	0.00	.25
G	O	.13	.40	.08	.21	0.00	1.75**	.02	0.00	.21
	A	.07	.21	0.00	.27	0.00	1.63	.04	0.00	.06

[a]O = original water-soluble-volatile fraction, A = synthetic water-soluble-volatile fraction.

[b]Intensity scale shown in Figure 2.

*Differences are significant at 10% level.

**Differences are significant at 5% level.

Water-soluble-nonvolatile fraction

Flavor profiles   As was the case with the WSV, the flavor profiles of the WSNV (Figures 6-12) showed the acid note to be the most dominant and intense flavor. Most of the sweetness of the whole cheese appeared in this fraction along with burned, bitter and nutty notes. This fraction was free of any lipolyzed character, and only one sample, sample D (Figure 9), had a fermented note. The Duncan's test (Table 9) showed the flavor panel was able to detect significant differences among samples in the burned, nutty, bitter and acid notes, but was unable to detect any differences in sweet, volatile, fermented or buttery. The lack of significant differences in the volatile, fermented and buttery notes was due mainly to their low intensities.

Chemical composition   Because of the importance which has been placed on the relationship of free amino acids and the flavor of Swiss cheese (1,19,37,57,60), free amino acids were determined in the WSNV (Table 12). This fraction is high in lysine, glutamic acid, proline, valine and leucine; and the relative amounts present are typical of those reported by Hintz et al. (19) and Antila and Antila (1) in whole Swiss cheese.

Table 13 shows the relative amounts of water-soluble proteins and peptides of various sizes. Proteolysis would be indicated both by an increase in the total water-soluble proteins and by increasing amounts of material that would penetrate the highly cross-linked resins. A proteolysis index (PI) was calculated by summing the amounts present in each fraction divided by its average peptide length as reported by Takeuchi and

Table 12. Free amino acid content of the water-soluble-nonvolatile fraction

	Cheese						
	A	B	C	D	E	F	G
Lysine	34.5[a]	34.5	24.6	54.9	24.3	52.2	43.8
Histidine	10.9	10.5	7.4	8.9	9.1	13.2	10.9
Arginine	15.2	ND[b]	ND	ND	ND	ND	ND
Aspartic acid	3.0	2.0	0.8	3.0	1.7	2.0	3.0
Threonine	9.7	6.5	5.5	11.8	4.6	9.5	12.8
Serine	18.6	14.7	13.0	20.5	16.7	15.1	18.4
Glutamic acid	50.9	52.6	40.1	76.1	34.5	62.5	70.9
Proline	34.5	33.8	28.6	55.3	23.7	38.7	49.2
Glycine	6.4	6.0	3.6	8.9	4.8	7.1	8.1
Alanine	11.5	11.7	6.9	11.9	7.1	11.7	13.4
Valine	24.7	22.2	17.4	32.9	15.4	26.6	29.3
Methionine	7.5	6.3	5.2	10.2	3.7	8.8	9.2
Isoleucine	15.7	12.0	10.8	12.3	10.0	21.1	22.4
Leucine	38.2	32.4	30.5	46.0	27.7	41.8	41.1
Tyrosine	10.2	7.0	8.1	2.7	6.6	9.7	7.9
Phenylalanine	19.4	18.8	15.1	23.9	13.2	22.9	22.9

[a]mg/g of the water-soluble-nonvolatile fraction.

[b]None detected.

Table 13. Quantities of peptides (mg/g) and proteolysis indices of the water-soluble-nonvolatile fraction

Cheese	Effluent	Wash	X16	X12	X8	X4	X2	PI[a]
A	1.73[b]	6.91	28.35	2.32	1.57	6.10	4.37	21.63
B	.92	5.67	13.67	3.97	1.38	9.88	.73	13.58
C	2.07	1.13	11.07	4.19	1.44	6.53	1.85	11.24
D	0.00	6.37	42.44	3.94	4.38	8.32	3.07	31.78
E	7.07	31.81	31.43	5.90	1.18	10.60	.44	28.60
F	2.54	20.94	30.78	6.67	2.27	11.74	3.81	28.14
G	3.03	15.50	41.19	10.59	2.27	4.53	3.41	34.22

[a]Proteolysis index = (effluent + wash)/9.77 + X16/1.66 + X12/2.00 + X8/3.45 + X4/4.57 + X2/6.06.

[b]mg/g cheese.

Yoshii (58). Using this criterion, samples D and G were relatively proteolyzed while samples A, B and C had little proteolysis.

Other selected components of the WSNV are shown in Table 14. The amounts of lactic acid and salt in this fraction represented approximately 95% of that found in the original cheese. Calcium and magnesium were measured because organoleptic analysis of this fraction implicated them in modification and production of several flavor notes. The amounts found in this fraction, when calculated on the whole cheese basis, are similar to those reported by Jacobs (21) for Swiss cheese.

Relationship between flavor profiles and chemical composition

The acid character note, which was the most prominent note in the WSNV, was most intense in cheese D (Figure 9), while cheese E (Figure 10) was the least intense. There are significant correlations between the acid flavor of this fraction and the amount of medium-sized (r = .821) and large peptides (r = -.860) (X8 and effluent proteolysis fractions, Table 13). This tends to indicate that the shorter peptides present in this fraction are mainly acidic in nature while the larger ones tend to be more basic; however, no proof for this was discovered. There is no correlation between either the pH or amount of lactic acid (Table 14) in this fraction, which would have been a more logical explanation for the acidity.

While there were no significant differences in sweetness among samples of this fraction, the sweet note was fairly intense. The most obvious explanation for the sweetness was the presence of residual sugars, either lactose, glucose or galactose. Measurement of these sugars by the method of Hettinga et al. (18) showed no detectable amounts. During

Table 14. Selected components of the water-soluble-nonvolatile fraction

Cheese	Lactic acid	Salt	Ca^{++}	Mg^{++}	pH
A	137[a]	3.54[b]	2.96[a]	0.39[a]	5.93
B	125	2.20	2.51	0.12	5.91
C	146	3.89	3.83	0.28	6.29
D	163	4.51	2.53	0.17	6.32
E	157	3.55	3.29	0.29	5.75
F	112	4.60	3.34	ND[c]	6.08
G	173	2.55	2.69	ND	6.06

[a]mg/g of the water-soluble-nonvolatile fraction.

[b]Percent.

[c]None detected.

determinations of the size distribution of peptides it was discovered that the sweetness of the whole fraction was destroyed by the fractionation. It seemed possible that this was caused by removal of Ca^{++} and Mg^{++} ions during the separation. Addition of the proper amounts of these ions to the fractions seemed to restore sweetness to the low molecular weight fractions (X16 and X12) but not others (Table 24 of the Appendix). Because the X16 fraction contains mainly amino acids and very small peptides, it was possible that sweet amino acids (proline, serine, threonine, alanine and glycine) were causing the sweetness. Mixtures of these sweet amino acids in the amounts shown in Table 12 were not sweet and additions of calcium and magnesium to the amino acid mixtures did not cause the sweetness to appear. This suggests that the interactions of calcium and magnesium with small peptides are responsible for the sweet flavor of the WSNV, and sweet amino acids, such as proline, are not responsible for the sweet flavor as has been claimed by several investigators (37,60,61).

Since the burned character note was used to describe a lactic acid flavor, it was felt that the amount of lactic acid present in the WSNV influenced the intensity of this note. But there was a negative correlation between the burned note and the amount of lactic acid ($r = -.747$) as well as all the other chemical analyses (Table 24 of the Appendix).

Biede (6) reported a relationship between the amount of proteolysis in Swiss cheese and the presence of a burned flavor which was described as brown sugar. Comparison of Tables 8 and 9 with 13 showed that samples which were the most intensely burned were also among the samples with the least amount of proteolysis. The ion exchange fractions which contained medium and higher molecular weight peptides (X8, X4, X2) were most intense in the burned note (Table 24 of the Appendix), but evidently in none of these fractions was the burned component pure enough to correlate with the flavor. This is in contrast to earlier work (6), which showed increases in proteolysis resulted in increased burned flavor in whole Swiss cheese. This relationship may only hold for whole cheese and not the WSNV.

The nutty flavor of the WSNV fraction was highly correlated with the amount of calcium present (r = .761) and also the amount of very large peptides present [effluent (r = .671) and wash (r = .780)]. This suggests that both large peptides and calcium were important in the nutty flavor. The nutty (cheesy) flavor of the WSNV fraction was probably a brothy flavor note that contributed to the nuttiness of whole cheese. It was different from the nutty flavor of the FS fraction. Table 24 of the Appendix indicates the brothy flavor was concentrated in the X12 and X16 ion exchange fractions, but the flavor does not correlate with the amounts of these fractions.

The bitter character note was more intense in the WSNV than in any other fraction. A bitter character note has long been associated with a flavor defect in cheese (44), especially in Cheddar. Mair-Waldburg and Sturm (41) found that bitter Swiss cheese had a very soft body, and attributed this defect to extreme proteolysis by Streptococcus faecalis var. liquefaciens. Since that time the presence of bitter flavor has been attributed more specifically to the production of bitter peptides (20,44) by either bacterial initiated proteolysis or the rennet used in manufacture. Because of this, the presence of a bitter note in the WSNV, which is high in amino acids and peptides, is expected. There was a slight but not significant correlation between the presence of medium-size peptides, as indicated by the X8 and X4 ion exchange fractions (r = .592 and .439, respectively), and the bitterness of the WSNV. These fractions by themselves were not considered bitter by the small panel that examined them (Table 24 of the Appendix). This may indicate that peptides alone are not responsible for the bitter flavor.

Cheese D (Figure 9) had the most intense bitter note in this fraction. It also had the highest content of lysine, valine, leucine and phenylalanine (Table 12), which are known to be bitter amino acids (23). This was thought to possibly be the cause for a definite astringent flavor in the X16 proteolysis fraction (Table 24 of the Appendix); however, a solution containing these amino acids in the amounts shown in Table 12 did not reproduce the astringent-bitter flavor. This indicates that small peptides are more important in producing bitter flavors and that their importance lies in the interaction with bitter amino acids.

Synthetic mixtures The nature of the WSNV is such that it was impossible to develop any type of synthetic mixture to compare to the original fraction. As has been discussed previously, solutions of free amino acids, lactic acid, calcium and magnesium did not reproduce the flavors present in this fraction, and the results have pointed to the importance of small peptides in the flavors of the WSNV.

Analysis to determine the actual peptides or proteins responsible for the flavors would be quite time-consuming and tedious. If one were to test the influence of each possible peptide of lengths reported to be contained in the various proteolysis fractions (58), the number of organoleptic tests would be very large. For example, combinations of the 16 amino acids shown in Table 12 would produce 240 different dipeptides, 4080 tripeptides and 65,530 tetrapeptides, with the number continuing to increase to 16^{10} for the number of decapeptides which could be present in the wash and effluent proteolysis fractions. Considering the results indicate that interactions of these peptides are important, the number of organoleptic analyses becomes phenominal.

Fat-soluble fraction

Flavor profiles Results of the flavor profiles of the FS (Figures 6-12) showed that the most prominent character notes of this fraction were nutty and volatile, and that all other character notes were present in varying degrees. The flavor panel, however, was unable to distinguish differences among samples in the intensities of the sweet, nutty, bitter or acid notes (Tables 4 and 5). These results also showed that cheese D (Figure 9) was significantly higher in the fermented note than any other cheese; likewise cheese C (Figure 8) was significantly higher in the buttery note and cheese A (Figure 6) was higher in the lipolyzed flavor. The fact that differences in the nutty note, which was a prominent flavor, could not be detected by the flavor panel is mainly caused by both the extreme intensities of the flavor and also in the variation of the panel members in using this term as is evidenced by the high standard deviations. The lack of significant differences in the other character notes can be best explained by their extremely low intensities.

Chemical composition Fatty acid analysis of free fatty acids of the FS is shown in Table 15. Very little acetic or propionic acid was found in this fraction as compared to the whole cheese (Table 16). Langler and Day (35) have reported the presence of C_{18} unsaturated fatty acids; however, the method used in this investigation did not resolve the unsaturated acids from the saturated ones, so the amounts reported in Table 15 include any unsaturated fatty acids that may be present.

The free fatty acids in the whole Swiss cheese are shown in Table 16. The amounts varied significantly among samples and are similar to those reported in the literature (19,22,33,35,49). These analyses showed small amounts of valeric and isovaleric acid to be present. Isovaleric has been reported to be present in Swiss cheese by Langler and Day (35), but there are no reports of valeric in Swiss cheese.

Distribution of fatty acids From the information obtained from fatty acid analysis of the whole cheese and FS, distribution coefficients between the fat and water phases in the whole cheese were calculated according to the following equations:

$$(A_f)F_f = A_o$$

where

A_f = mg fatty acid/g fat.

F_f = Fraction fat in whole cheese.

A_o = mg fatty acid/g cheese contributed by the fat.

Table 15. Fatty acid composition of the fat-soluble fraction

Acid	Cheese						
	A	B	C	D	E	F	G
Acetic	.099[a]	.091	.054	.069	.081	.083	.064
Propionic	.688	.329	.183	.224	.209	.251	.187
Butyric	.655	.461	.497	.394	.531	.526	.521
Caproic	.429	.305	.294	.229	.278	.271	.314
Caprylic	.349	.249	.198	.173	.179	.194	.190
Capric	1.054	.680	.508	.496	.438	.534	.448
Lauric[b]	1.463	.798	.572	.567	.503	.601	.510
Myristic[b]	3.801	2.176	1.746	1.563	1.508	1.651	1.538
Palmitic[b]	7.772	4.587	3.731	3.357	3.141	3.705	3.400
Stearic[b]	6.974	4.632	3.769	3.542	3.066	2.896	2.327

[a]mg/g oil.

[b]Includes unsaturated fatty acids of the same chain length.

Table 16. Fatty acid composition of whole cheese

Acid	Cheese						
	A	B	C	D	E	F	G
Acetic	2.29[a]	3.57	3.84	2.52	.79	3.05	4.37
Propionic	6.21	5.48	5.13	5.59	.59	3.12	6.13
Butyric	.94	.21	.69	.83	1.88	.83	.48
Iso-valeric	.07	.09	t[b]	.56	t	t	.10
Valeric	ND[c]	.01	.06	.06	.05	.01	.01
Caproic	.36	.51	.23	.27	.12	.22	.10
Caprylic	.22	.31	.15	.19	.11	.13	.11
Capric	.48	.70	.42	.46	.31	.28	.27
Lauric[d]	1.06	.66	.43	.48	.29	.27	.32
Myristic[d]	1.08	1.22	1.21	1.29	.71	.66	.69
Palmitic[d]	2.39	2.32	2.46	2.75	1.33	1.30	1.50
Stearic[d]	2.34	1.70	2.01	1.85	1.17	.95	.91

[a]mg/g cheese.

[b]Trace.

[c]Not detected.

[d]Includes unsaturated fatty acids of the same chain length.

$$A_c - A_o = A_{cf}$$

where

A_c = mg fatty acid/g cheese.

A_{cf} = mg fatty acid/g of oil-free curd.

$$A_{cf}/F_w = A_w$$

where

F_w = Fraction water in whole cheese

A_w = mg fatty acid/g cheese water.

A_o and A_w were converted to moles of fatty acid/L of oil and moles of fatty acid/L of water to give HA_o and C_w, respectively.

$$HA_w = \frac{C_w}{1 + \text{antilog}(pH - pK_a)}$$

where

HA_w = Moles of unionized free fatty acid/L of water.

pH = pH of whole cheese

pK_a = pK of fatty acid.

$$K = \frac{HA_w}{HA_o}$$

where

K = Distribution coefficient between water and oil phase.

The resulting distribution coefficients are shown in Table 17. If the distribution were determined by partition between oil and water, and pH, the coefficients in Table 17 should be constant for a given fatty acid.

Table 17. Distribution coefficients of fatty acids between the water and oil phases of Swiss cheese

Fatty acid	Cheese						
	A	B	C	D	E	F	G
Acetic	14.667[a]	15.500	30.625	9.182	5.000	8.846	19.900
Propionic	6.686	10.000	14.130	8.143	1.692	3.656	10.625
Butyric	.826	1.286	.509	.524	.732	.339	.073
Caproic	.382	.792	.208	.278	.046	.136	.002
Caprylic	.261	.563	.231	.273	.083	.008	.042
Capric	.123	.405	.259	.185	.083	.069	.042
Lauric	.309	.297	.222	.154	.044	.036	.044
Myristic	.003	.146	.146	.141	.032	.015	.016
Palmitic	.021	.120	.169	.139	.026	.007	.016
Stearic	.040	.040	.114	.052	-[b]	.001	-

[a]HA_w/HA_o.

[b]HA_w is negative.

Actually they varied over a wide range. Therefore, the ratio of A_W/A_O was calculated and is given in Table 18. A large ratio indicated that the fatty acid was bound in some manner in the water phase of the cheese (for the purpose of this discussion, the water phase is referring to the part of the whole cheese which is not lipid in nature).

The results in Table 18 show that the ratios for nearly all fatty acids $>C_4$ are high in cheeses B, C and D, low in cheeses E, F and G and moderate and varied for cheese A. This order of ratios is more or less the reverse of that for the proteolysis index (PI) (Table 13) and wash and effluent fractions. This seems to indicate a negative relation between the binding of fatty acids $>C_4$ in the water phase and the proteolysis of the cheese.

In general, as proteolysis increased, the binding of short-chained fatty acids (C_2 and C_3) in the water phase decreased, went through a minimum and then increased.

The interactions between fatty acids and protein in foods has been alluded to in a review article by Solms et al. (52). They indicated that the hydrocarbon chain of the fatty acid was the determining factor in how the protein interaction was accomplished and that interactions were greatest if the proteins were unfolded or their tertiary structures destroyed. They also indicated that the binding of long-chained fatty acids to proteins was mainly by hydrophobic bonding. This supports the results of this study in which long-chained fatty acids, which are hydrophobic, were bound less as proteolysis proceeded, a process which would diminish the hydrophobic areas of the protein.

Table 18. Ratios of fatty acids between water and oil phases of Swiss cheese

Acid	Cheese						
	A	B	C	D	E	F	G
Acetic	81.79[a]	137.31	240.00	109.57	30.38	112.96	218.50
Propionic	32.18	57.68	96.79	73.55	8.81	39.00	105.69
Butyric	5.19	9.03	4.79	6.19	11.06	4.94	2.98
Caproic	3.00	5.80	2.71	3.46	1.35	2.53	1.03
Caprylic	2.24	3.37	2.63	3.22	1.93	2.10	1.86
Capric	1.63	3.56	2.86	2.72	2.21	1.64	1.94
Lauric	2.59	2.86	2.59	2.49	1.80	1.41	2.03
Myristic	1.02	1.93	2.39	2.43	1.47	1.25	1.45
Palmitic	1.10	1.74	2.27	2.41	1.32	1.10	1.42
Stearic	1.20	1.27	1.84	1.54	.79	1.02	.75

[a] A_c/A_o.

<u>Miscellaneous volatiles</u>

A typical gas chromatogram of the neutral volatiles of the FS is shown in Figure 13. A comparison of the chromatograms for all seven cheeses showed twelve major odor-associated peaks in common. The samples varied in the area of the peaks and the intensity of the odors rather than the kinds of odors. Table 19 shows the relative areas of the odor-associated peaks.

The method of Hammond et al. (16) showed that the neglected bathroom and peanut odors were alkyl pyrazines, probably tri- and tetramethyl pyrazine. Reaction of the distillate with 2,4-dinitrophenylhydrazine (DNPH) removed the bread, aldehyde, ketone and soda cracker odors completely and reduced the intensity of the plantlike, tygon and sweet dusty odors. On this basis, these odors were tentatively classified as carbonyls. Preliminary investigations of swine house odors (E. G. Hammond, Dept. of Food Technology, I.S.U., unpublished results) showed that compounds which have retention times and odors similar to tygon and plantlike are long-chained unsaturated aldehydes (possibly 2,4-decadienal, 2-nonenal or 2,4-nonadienal). The method of Wong et al. (66), which is reported to separate lactones from other cheese constituents, showed that apricot and moldy orange may be lactones. This was also confirmed by the retention times of known lactones; however, more verification is needed.

The presence of lactones and carbonyls (4,33,36) as well as alkyl pyrazines (50) have been reported in Swiss cheese so odors in these groups were to be expected. The cresol odor and peak corresponded in retention time and temperature to a standard solution of p-cresol; however, there is no report in the literature of the presence of p-cresol in Swiss cheese.

Figure 13. Typical gas chromatogram of neutralized distillate from cheese oil. [Odors at the indicated letters were: A. bread; B. aldehyde; C. ketone; D, neglected bathroom; E. soda crackers - peanuts; F. plantlike; G. tygon; H. cresol; I. sweet dusty; J. apricot lactone; K. moldy orange; L. campfire - stale newspaper. Points at indicated numbers were: 1. insertion of Tenax tube; 2. removal of Tenax tube. Attenuation was 32×10^{-12}]

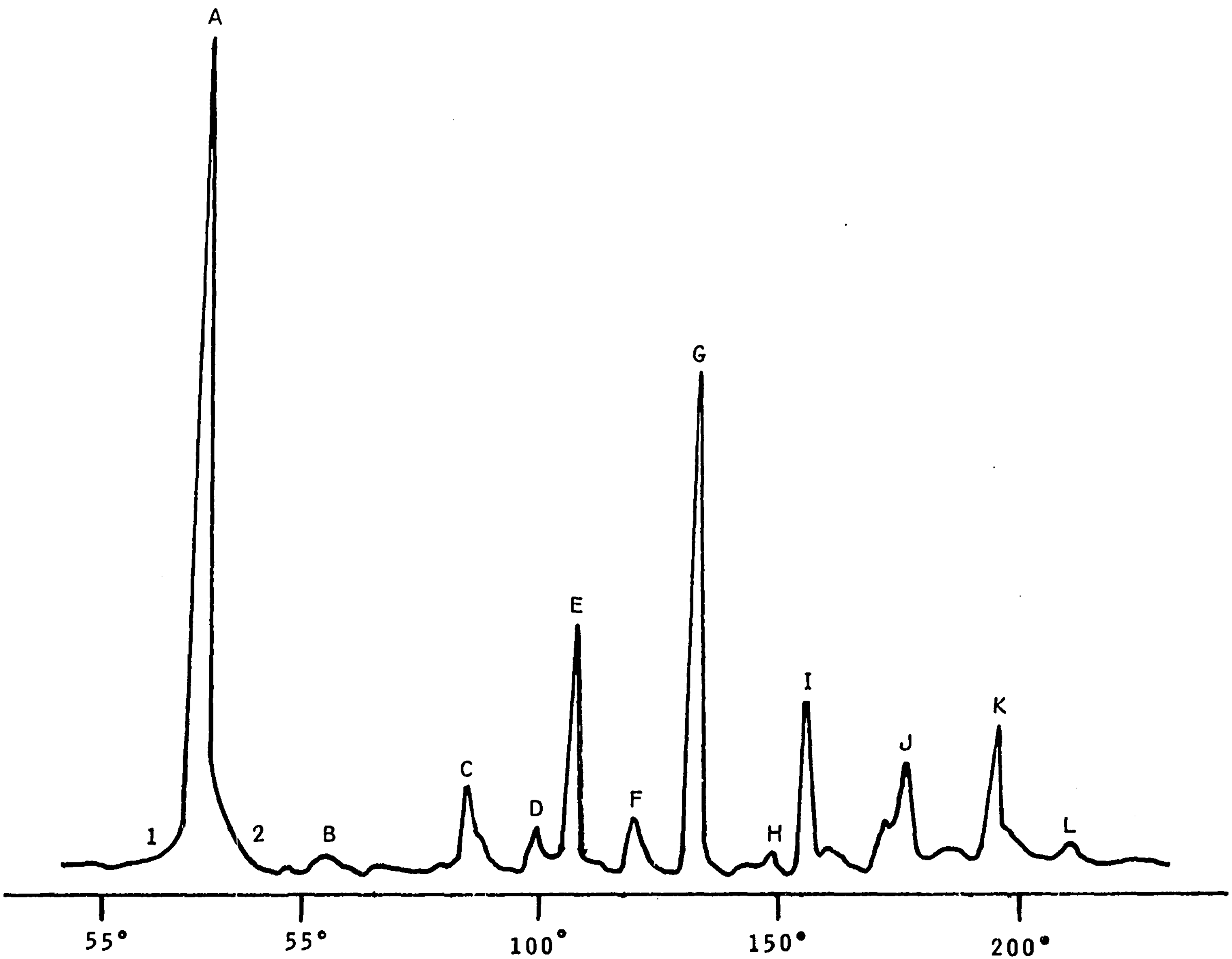

A
1
2
B
C
D
E
F
G
H
I
J
K
L
55°
55°
100°
150°
200°

Table 19. Relative peak areas[a] of distillate from fat-soluble fraction

Cheese	Odor				
	Bread	Aldehyde	Ketone	Neglected bathroom	Soda crackers-peanuts
A	4.38	NP[b]	.69	.41	1.07
B	.56	.24	2.56	.22	.64 1.28
C	9.78	.50	.50	NP	.55
D	212.29	.34	32.30	.53	27.26
E	555.68	1.23	14.33	4.79	28.49
F	57.6 79.36	1.84 1.84	16.18	1.31	9.02
G	85.81	10.03	8.18	.45	3.20

[a] cm^2/g oil.

[b] Odor present but no measurable peak.

Table 19. Continued

Odor						
Plantlike	Tygon	Cresol	Sweet dusty	Apricot lactone	Moldy orange	Campfire-stale newspaper
.62	2.77	NP	.95	.53	1.5	.32
NP	2.13	.11	.61	.30	1.46	.43
.18	NP	NP	.22	.12	NP	NP
12.29	63.92	2.20	13.81	1.37	15.16	1.01
10.79	26.16	NP	6.44	2.77	1.73	.76
9.24	22.65	NP	5.01	.96	12.27	NP
2.07	6.27	.43	1.76	.63	NP	NP

<u>Relationship between flavor profile and chemical composition</u> The most intense flavor notes of the FS were nutty and volatile. When the oil distillates were treated with sodium carbonate solution the nutty odor disappeared leaving only a much weaker aroma. This indicated the nutty aroma was caused by fatty acids, but the fatty acids were negatively correlated with the nutty note (Table 26 of the Appendix). Mineral oil samples containing C_2, C_3, C_4, C_6, C_8, C_{10} and C_{12} fatty acids in the amounts shown in Table 15 were presented to the flavor panel. These results (Table 20) showed the synthetic mixtures were described as nutty, but as a rule they were less nutty than the original and several of these comparisons differed enough to be significant. The nutty flavor appears to depend on an interaction among the fatty acids and other components of the oil-soluble fraction.

As with the nutty note, the volatile character note could not be correlated with the chemical composition of the FS (Table 25 of the Appendix). It had been hoped that because the volatile note was composed of many flavors, the constituents of the neutral distillate would correlate with this note; however, this was not the case. The synthetic FS mixture (Table 20) had an intense volatile note which, for most cheeses, was not significantly different from the original FS. This again points to the importance of the interactions of the free fatty acids in producing the volatile character note.

The lipolyzed flavor of the FS was found to correlate highly with the amount of all fatty acids present ($r > .73$ for all fatty acids) with the exception of butyric acid which was only moderately correlated ($r = .49$).

Table 20. Comparison of the original fat-soluble fraction and synthetic fat-soluble fraction

Cheese	Type[a]	Character note				
		Sweet	Nutty	Lipolyzed	Acid	Volatile
A	O	.14[b]	1.21	1.43	.86	1.79
	A	.44	.89	2.31	1.00	1.69
B	O	.14	1.68	.54**	.86	1.39
	A	.33	1.53	2.11	.58	1.08
C	O	.07	2.35*	.42**	.58	2.19
	A	.16	1.23	2.06	.25	1.75
D	O	.07	1.38	.15**	.62*	1.04
	A	.28	1.00	1.89	.25	1.14
E	O	.00	2.50**	.00**	.57	.71
	A	.44	.89	2.06	.64	1.36
F	O	.00	1.50	.00	.43	2.79*
	A	.19	1.36	1.88	.42	1.78
G	O	.00	2.07**	.00**	.57	2.00
	A	.28	.64	2.44	.69	1.75

[a]O = original oil-soluble fraction; A = synthetic oil-soluble fraction.

[b]Intensity scale shown in Figure 2.

*Differences significant at 10% level.

**Differences significant at 5% level.

This relationship is supported by the reports of Kuzdzal-Savoie (31), Kolar and Mickle (24) and Scanlan et al. (48) that fatty acids including long-chained fatty acids are important in producing a lipolyzed or rancid flavor in dairy products. The synthetic FS mixture was shown to be significantly higher in the lipolyzed note than the original FS. This indicates that the constituents of the neutral distillate are important in modifying the flavor and preventing the perception of the lipolyzed note.

Cheese D was the only whole cheese and FS to be intensely fermented (Figure 9). Gas chromatography of the neutral distillate (Table 20) showed cheese D had significantly larger peaks associated with the ketone, plantlike, tygon, cresol, sweet dusty, moldy orange and campfire - stale newspaper odors than the other cheeses, and the areas of these odor-associated peaks correlated with the fermented note (r = .84, .57, .87, .99, .86, .65 and .66, respectively). The absence of the fermented note in the synthetic FS mixture (Table 20) gives further support to the idea that the constituents of the neutral distillate are important in production of the fermented character note. These results also indicate that the fermented flavor is produced when a balance between the acid and neutral constituents of the cheese oil is disrupted.

Although the flavor panel was able to distinguish between differences in samples for the buttery note, no correlations between the constituents of the FS fraction and buttery could be shown.

Whole cheese

Flavor profiles The results of the flavor profile of the whole cheese (Figures 6-12) showed that the most prominent character note in all cheeses was acid. All other character notes were present in varying amounts. The Duncan's test (Table 3) showed the flavor panel was able to distinguish differences among cheese in the intensities of the burned, nutty, bitter, volatile, fermented and buttery notes. While the fermented note was determined to have significant differences among samples, cheese D (Figure 9) was the only cheese to have an intense fermented note. The inability to distinguish differences in the acid note was probably due to its high intensity in all cheeses. The lack of significant differences for the sweet flavor was due to the subtle nature of the sweet note, which was difficult for the panel to distinguish in the whole cheese. The absence of significant differences in the lipolyzed note was due to the very low intensities in the whole cheeses.

Relation between flavor profile and chemical makeup Swiss cheese has long been recognized as being a sweet cheese. As with the WSNV the sweetness in the whole cheese was thought to be due to the presence of residual sugars; however, none was found to be present. Babel and Hammer (3) suggested that the sweetness of Swiss was due to high volatile fatty acid content, namely acetic and propionic acids. There was a significant correlation (r = .796, Table 27 of the Appendix) between the sweetness in the whole cheese and the amount of acetic acid present, but no correlation could be shown with the amount of propionic acid.

The sweetness of whole cheese could not be correlated with proteolysis as the WSNV was. This is probably because the sweet flavor is subtle and easily altered by other flavors in the cheese. The sweetest cheeses tended to be those whose other flavors were intense.

The amount of lactic acid in the whole cheese was highly correlated (r = .720) with the burned flavor. Probably, like the WSNV, the whole cheese burned flavor depends on medium-sized peptides whose exact amounts cannot be measured.

There was a slight but not significant correlation (r = .546) between the amount of acetic acid in the whole cheese and the nutty character note, but no other fatty acid could be correlated. There was a relationship between the ratio of A_c/A_o for acetic and propionic acid and the nutty note. The cheeses which had the most intense nutty notes had high ratios. Likewise, the cheeses which were low in nutty notes had low A_c/A_o ratios. Kristoffersen and Gould (29) have reported on the importance of the ratios of fatty acids to each other in Cheddar cheese and similar comparisons were made on the whole cheese; however, no correlations were shown. These results, as do those with the FS fraction, indicate a relation between the short-chained fatty acids and the nutty flavor.

Of the whole cheeses, cheeses A and B (Figures 6 and 7) were significantly more bitter than the other cheeses. They were intermediate in proteolysis. There is a high but not significant negative correlation between the proteolysis index (PI) (Table 13) and the bitter flavor (r = -.62). This may indicate that the medium and longer peptides, which are somewhat bitter (Table 24 of the Appendix), are responsible for the bitter note in the Swiss cheese, and also supports the work of Mair-Waldburg and Sturm (41) on the relationship of proteolysis and bitter flavored Swiss cheese. Fatty acid analysis of the whole cheese (Table 16) showed a strong correlation between the amount of caproic, caprylic and capric acids (r =

.844, .914 and .902, respectively) and the bitter flavor. The reason behind this correlation is not clear since these acids are not known to be bitter.

The flavor profiles showed only three cheeses to be lipolyzed: cheeses C, D and E (Figures 8-10). The intensity in each cheese was weak and not significantly different from those cheeses which were not lipolyzed. No relationship between the lipolyzed note in the whole cheese and the fatty acid content could be found.

Although the acid note in the whole cheese was one of the more intense character notes, the panel could not distinguish significant differences in intensity and the flavor could not be correlated to either the chemical composition or the pH of the whole cheese (Table 1).

The whole cheeses were also quite intense in the volatile note, except for cheeses A, D and F (Figures 6, 9 and 10). It is proposed that the volatile note was low in cheese D because of its intense fermented note. There is a significant correlation ($r = .835$) between the intensity of the volatile note and the amount of acetic acid (Table 16). However, none of the other fatty acids could be correlated with this flavor note. The volatile note followed the same trend as nutty, in that those cheeses which were high in volatile notes also had high A_c/A_o for acetic and propionic acids (Table 18).

The fermented note in the whole cheese, like that in the FS fraction, seems to be caused by an imbalance between the fatty acids and other FS volatiles in cheese D. In none of the others was the fermented flavor significant.

The buttery note, like the acid note, could not be correlated to the chemical composition of the whole cheese. Cheeses C and E were rich in diacetyl and are among the most buttery, but cheese G which was also buttery was not particularly rich in diacetyl.

One must conclude that interactions play an important role in the whole cheese flavors.

SUMMARY AND CONCLUSIONS

The purpose of this investigation was to determine which compounds in Swiss cheese are important in its flavor and to see if the flavor of the cheese could be predicted from an analysis of the flavor compounds.

The results showed that the flavor of Swiss cheese depended upon a few classes of compounds: fatty acids, carbonyls, ions, lactic acid, lactones, pyrazines and peptides. The flavor of seven Swiss cheeses selected to cover as wide a flavor range as possible could be described adequately with a flavor profile of nine flavor notes. The flavor of the cheese could be divided conveniently into three fractions: an oil-soluble fraction, a water-soluble-volatile fraction and a water-soluble-nonvolatile fraction.

The nine flavor notes could sometimes be successfully correlated with the chemical analysis of some compounds or class of compounds, but in general it was not possible to predict the flavor of cheese from chemical analysis or from flavor observations on fractions. Seemingly this was because of the complex interactions that occurred among the flavor constituents. Predictions of the flavor from chemical analyses were limited also by inadequate techniques for analyzing the complex peptide fractions obtained from the cheese and incomplete identification of some oil-soluble flavors.

Relations between flavor notes and chemical analyses that were suggested were:

1. The sweetness of Swiss cheese which mostly went into the water-soluble-nonvolatile fraction was caused by an interaction of small peptides

with calcium and magnesium ions. Diacetyl was responsible for the sweetness found in the water-soluble-volatile fraction.

2. The burned flavor was found mostly in the water-soluble-nonvolatile fraction and was caused by short and medium length peptides.

3. The nutty flavor was composed of a fat-soluble and a water-soluble component. The fat-soluble component was caused primarily by fatty acids, but interaction with minor flavor constituents of the oil was also important. The water-soluble nutty was a brothy flavor note caused by small peptides that were recovered in the nonvolatile fraction.

4. A mixture of fatty acids was responsible for the lipolyzed flavor that was concentrated in the fat-soluble fraction. Butyric acid was the cause of lipolyzed flavor in the water-soluble-volatile fraction.

5. The acid flavor of the cheese was a complex interaction among many constituents, but the acid flavor of the water-soluble-volatile fraction was correlated with the amount of net free fatty acid after subtracting the ammonia present.

6. The volatile note was caused by interactions among fatty acids.

7. Unusually high levels of neutral volatiles in the fat-soluble fraction were responsible for the fermented note.

Because of the importance of fatty acids in the flavor, the distribution of fatty acids between the fat and nonfat phases of the cheese was studied. The occurrence of long-chain fatty acids in the nonfat phase could not be accounted for by the pH and suggested binding by the cheese protein. The extents of binding of fatty acids C_4 and greater decreased with proteolysis while those of acetic and propionic acids reached their minima in moderately proteolyzed cheese.

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APPENDIX

Table 21. Analysis of variance of flavor profiles

Source	d.f.	s.s.	m.s.	F	Probability of a larger F
			Sweet		
Sample	6	9.68	1.61	2.98	<.01
Fraction	3	37.18	12.39	22.94	<.01
Judge	6	11.67	1.96	3.61	<.01
Sample X judge	36	21.99	.61	1.13	<.05
Judge X fraction	18	25.84	1.44	2.67	<.01
Residual	126	67.83	.54		
			Burned		
Sample	6	11.13	1.85	2.37	<.05
Fraction	3	74.76	24.92	31.95	<.01
Judge	6	15.94	2.66	3.41	<.01
Sample X judge	36	27.85	.77	.99	>.05
Judge X fraction	18	17.79	.99	1.27	>.05
Residual	126	98.40	.78		
			Nutty		
Sample	6	31.35	5.22	6.00	<.01
Fraction	3	59.77	19.92	22.90	<.01
Judge	6	7.35	1.22	1.40	>.05
Sample X judge	36	29.79	.83	.95	>.05
Judge X fraction	18	34.81	1.93	2.22	<.01
Residual	126	109.15	.87		

Table 21. Continued

Source	d.f.	s.s.	m.s.	F	Probability of a larger F
			Bitter		
Sample	6	5.19	.86	2.15	>.05
Fraction	3	4.94	1.65	4.13	<.01
Judge	6	19.04	3.17	7.93	<.01
Sample X judge	36	13.78	.38	.95	>.05
Judge X fraction	18	16.15	.90	2.25	<.01
Residual	126	50.53	.40		
			Lipolyzed		
Sample	6	2.82	.47	1.57	<.05
Fraction	3	3.53	1.18	3.93	<.01
Judge	6	1.60	.27	.90	>.05
Sample X judge	36	9.61	.27	.90	>.05
Judge X fraction	18	4.49	.25	.83	>.05
Residual	126	37.86	.30		
			Acid		
Sample	6	17.17	2.86	3.29	<.01
Fraction	3	117.37	39.12	44.97	<.01
Judge	6	8.76	1.46	1.68	>.05
Sample X judge	36	21.66	.60	.69	>.05
Judge X fraction	18	22.81	1.27	1.46	>.05
Residual	126	109.70	.87		

Table 21. Continued

Source	d.f.	s.s.	m.s.	F	Probability of a larger F
			Volatile		
Sample	6	39.66	6.61	6.18	<.01
Fraction	3	78.39	26.13	24.42	<.01
Judge	6	3.41	.57	.53	>.05
Sample X judge	36	40.49	1.12	1.05	>.05
Judge X fraction	18	14.28	.79	.74	>.05
Residual	126	134.63	1.07		
			Fermented		
Sample	6	34.39	5.73	11.02	<.01
Fraction	3	13.69	4.56	8.77	<.01
Judge	6	2.96	.49	.94	>.05
Sample X judge	36	12.70	.35	.67	>.05
Judge X fraction	18	8.52	.47	.90	>.05
Residual	126	64.98	.52		
			Buttery		
Sample	6	21.12	3.52	4.51	<.01
Fraction	3	12.31	4.10	5.26	<.01
Judge	6	10.62	1.77	2.27	<.05
Sample X judge	36	22.80	.63	.81	>.05
Judge X fraction	18	18.38	1.02	1.31	>.05
Residual	126	98.57	.78		

Table 22. Whole cheese and fraction flavor profile correlations

	1[a,b]	2	3	4	5	6	7	8	9
1[a,b]	1.00								
2	.18	1.00							
3	.08	-.11	1.00						
4	-.01	-.20*	-.07	1.00					
5	.04	.05	-.22*	.14	1.00				
6	.07	.44**	-.32**	.03	.19*	1.00			
7	.37**	.14	.47**	-.06	-.04	.08	1.00		
8	-.21*	.33**	-.57**	-.08	.04	.28**	-.34**	1.00	
9	-.04	-.42**	.40**	.09	.04	-.38**	0.00	-.28	1.00
1[c]	-.01	-.01	-.14	-.01	-.09	.13	-.19*	-.01	-.07
2	-.15	.02	-.06	-.05	-.01	.02	.08	-.08	0.00
3	-.02	-.14	.16	.06	-.08	-.20	0.00	-.14	.15
4	-.02	-.24**	.04	.31**	-.09	-.13	.02	-.13	.21*
5	.01	.15	.01	-.03	-.03	.13	.11	-.11	-.17
6	.07	-.07	.11	.37**	-.17	-.18	.04	-.10	.21*
7	.34**	.06	.33**	-.09	.03	-.20*	.29**	-.18	.08
8	0.00	.22*	-.41**	-.20*	-.10	.11	-.22*	.45**	-.22*
9	.06	-.10	.20*	.01	.05	.24	.15	-.11	.09
1[d]	.11	-.12	.25**	-.15	.04	-.09	.14	-.30**	.01
2	-.20*	.06	.26**	-.18	-.08	-.15	.13	-.17	-.02
3	.08	-.02	.32**	0.00	-.15	-.17	.16	-.19	.05
4	.04	-.04	.20*	.07	.07	-.11	-.13	-.12	.29**
5	-.05	-.03	.01	-.08	.16	-.03	-.09	-.09	.37**
6	-.14	.11	.35**	0.00	-.02	.11	.22*	-.43**	.08
7	.09	-.21*	.20*	-.06	-.19	-.36**	.06	-.24**	.08
8	.26**	.24**	.05	-.06	-.05	-.01	.10	-.06	-.08
9	-.01	-.04	.18	.12	-.18	.11	.32**	-.16	0.00
1[e]	.17	.10	.04	-.20*	-.06	-.10	.14	-.04	-.17
2	.02	.04	.21*	.10	-.02	.14	.09	-.05	.10
3	-.01	-.31**	.12	-.04	-.02	-.27**	.05	-.24**	.29**
4	.13	-.04	-.15	.17	.06	-.03	-.02	.13	-.19
5	0.00	-.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6	-.05	.11	-.18	.01	.10	.12	.01	.33**	-.25**
7	.11	.07	.17	-.09	-.08	-.03	.04	-.15	-.11
8	-.14	.24**	-.17	-.06	-.05	.17	-.17	.21*	-.08
9	.11	-.27**	.23*	.17	-.06	-.07	.14	-.08	.30**

[a]1 = sweet; 2 = burned; 3 = nutty; 4 = bitter; 5 = lipolyzed; 6 = acid; 7 = volatile; 8 = fermented; 9 = buttery.

[b]Whole cheese.

[c]Fat-soluble fraction.

[d]Water-soluble-volatile fraction.

[e]Water-soluble-nonvolatile fraction.

*Significant at the 10% level.

**Significant at the 5% level.

1[c]	2	3	4	5	6	7	8	9
1.00								
-.11	1.00							
-.21	.06	1.00						
.14	-.04	-.04	1.00					
-.02	-.06	-.06	.04	1.00				
.04	0.00	-.15	.34**	.05	1.00			
-.28**	-.22*	.09	-.13	.07	-.04	1.00		
.06	-.81	-.16	-.10	-.13	-.14	-.22*	1.00	
.28**	-.04	-.01	.11	-.13	.08	.03	-.17	1.00
.08	.23*	.20*	-.09	-.11	.11	.14	-.24**	.41**
-.10	.33**	.26**	-.11	-.01	-.18	.12	-.15	-.09
-.13	-.21*	-.22*	-.04	.03	.03	.20*	-.17	-.19
-.10	.06	.31**	.20*	-.18	.04	.06	.09	.31**
-.04	.24**	.12	-.04	-.08	-.12	-.06	0.00	-.07
-.05	.18	-.01	.09	.11	-.19	.09	-.23*	.14
-.03	-.04	.20*	0.00	.05	.07	.24**	-.11	-.02
-.03	-.06	-.19*	-.03	-.06	.18	.08	.22*	-.05
.03	.32**	-.11	-.08	-.13	-.06	.02	-.02	.10
-.06	-.07	.06	.04	-.02	-.06	.31**	0.00	-.11
.06	0.00	-.10	.12	.03	.26**	.07	.01	.53**
.07	.03	.16	.20*	-.13	.07	.20*	-.29**	-.06
-.03	-.05	-.03	.05	-.09	0.00	-.09	.08	-.08
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
-.09	-.18	-.02	-.04	.11	-.05	.11	.44**	-.05
.08	.11	.14	-.07	.18	.11	.10	.07	.10
-.03	.23*	.02	-.03	-.06	-.09	-.16	.08	-.05
-.04	-.07	.12	.43**	-.08	.12	.11	-.07	.32**

Table 22. Continued

	1[d]	2	3	4	5	6	7	8	9
1[b]									
2									
3									
4									
5									
6									
7									
8									
9									
1[c]									
2									
3									
4									
5									
6									
7									
8									
9									
1[d]	1.00								
2	.32**	1.00							
3	-.19	-.18	1.00						
4	.08	-.02	-.21*	1.00					
5	-.10	-.07	0.00	.04	1.00				
6	.15	.26**	.01	.22	.06	1.00			
7	.17	.05	.06	.12	-.13	-.02	1.00		
8	-.07	-.05	.22*	-.05	-.02	-.04	-.09	1.00	
9	.19*	.19	.09	-.22*	-.11	.16	.05	-.08	1.00
1[e]	.14	.09	.01	-.10	0.00	.10	.17	.09	-.01
2	.24**	.10	-.05	.30**	-.12	.14	.16	.15	-.04
3	.24**	.20*	.16	-.02	.05	.02	.25**	-.10	.13
4	-.23*	-.07	-.07	-.03	-.09	-.13	-.14	.23*	0.00
5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6	-.17	-.01	-.15	.10	-.22*	-.10	-.06	.07	-.25**
7	.26**	.13	-.01	.29**	-.05	-.02	.37**	-.04	.10
8	-.07	.15	-.10	-.05	-.02	-.01	-.09	-.01	0.00
9	-.08	-.07	.05	.30**	-.03	.01	.04	-.02	.11

1[e]	2	3	4	5	6	7	8	9
1.00								
.05	1.00							
-.09	-.06	1.00						
-.01	-.13	-.24*	1.00					
0.00	0.00	0.00	0.00	1.00				
-.07	.16	-.10	.01	0.00	1.00			
-.03	.17	.08	-.15	0.00	.13	1.00		
-.13	.15	-.10	-.06	0.00	.18	-.04	1.00	
-.12	.09	.25**	-.08	0.00	.04	.12	-.02	1.00

Table 23. Correlations between chemical makeup and the flavor profile of the water-soluble-volatile fraction

Chemical component	Character note								
	Sweet	Burned	Nutty	Bitter	Lipolyzed	Acid	Volatile	Fermented	Buttery
Acetic acid	.336	.434	-.447	.236	-.522	.601	-.068	-.137	.454
Propionic acid	-.005	.158	-.436	-.101	-.610	.583	.160	-.167	.299
Butyric acid	.019	-.207	-.121	-.072	.863**	-.398	-.629	.335	-.441
Ammonia	-.073	.062	-.439	-.166	-.559	.584	.247	-.203	.174
Diacetyl	.791**	.422	-.025	.643	.626	.281	-.213	-.304	-.041

**Significant at the 5% level.

Table 24. Correlations between chemical makeup and the flavor profile of the water-soluble-nonvolatile fraction

Component	Character note								
	Sweet	Burned	Nutty	Bitter	Lipolyzed	Acid	Volatile	Fermented	Buttery
Lactic acid	-.783**	-.747*	.191	-.131	0.00	-.312	-.264	.173	-.296
Salt	-.022	-.680*	.608	.245	0.00	.023	-.667*	.382	-.513
Calcium	-.174	-.679*	.761**	-.179	0.00	-.638	-.468	-.288	-.323
Magnesium	-.364	-.054	-.178	-.426	0.00	-.573	-.180	.006	-.195
X16	-.456	-.771**	.235*	-.038	0.00	.310	-.176	.507	-.390
X12	-.675*	-.437	.536	-.216	0.00	-.070	.285	-.233	.211
X8	-.374	-.197	-.224	.592	0.00	.821**	-.228	.925**	-.202
X4	.216	-.328	.384	.439	0.00	-.266	.854**	.013	-.566
X2	.257	-.108	.118	-.063	0.00	.626	.252	.156	-.069
Effluent	-.048	-.508	.671*	-.675*	0.00	-.860**	-.048	-.048	-.027
Wash	-.147	-.828**	.780**	-.360	0.00	-.614	-.401	-.256	-.399

*Significant at 10% level.

**Significant at 5% level.

Table 25. Flavor description of proteolysis fractions by a panel of three judges

Cheese	Fraction	Description
A	X16	Slight sweetness, slight bitterness, strong brothy
	X12	Slight bitterness
	X8	Slight bitterness
	X4	Slight bitterness, slight saltiness
	X2	Slight saltiness
	Wash	Strong saltiness, strong astringency
	Effluent	Moderate saltiness, strong astringency
	Whole	Moderate sweetness, strong brothy
B	X16	Moderate sweetness, moderate to strong brothy, moderate astringency
	X12	Moderate burned, slight brothy
	X8	Slight burned
	X4	Strong burned
	X2	Slight burned
	Wash	Moderate burned, strong saltiness, moderate astringency
	Effluent	Slight saltiness, moderate burned
	Whole	Moderate sweetness, strong brothiness, slight burned
C	X16	Slight sweetness, slight astringency, strong brothy
	X12	Slight sweetness, moderate brothy
	X8	Slight brothy
	X4	Slight burned, slight brothy
	X2	Slight astringency

Table 25. Continued

Cheese	Fraction	Description
	Wash	Moderate astringency, strong saltiness
C	Effluent	Moderate astringency, slight saltiness
	Whole	Strong sweetness, moderate brothiness, slight acidity, moderate saltiness
	X16	Strong sweetness, moderate brothiness, slight burned, slight astringency
	X12	Slight sweetness, slight brothiness, slight burned
	X8	Slight astringency
	X4	Slight burned, slight astringency
D	X2	Slight burned
	Wash	Moderate burned, strong saltiness
	Effluent	Slight burned, slight astringency
	Whole	Moderate sweetness, moderate brothiness, slight astringency
	X16	Slight sweetness, strong brothiness, slight burned
	X12	Slight sweetness, slight burned, slight astringency
	X8	Slight burned
	X4	Slight burned, slight astringency
E	X2	Slight burned, slight astringency
	Wash	Slight brothiness, moderate astringency, strong saltiness
	Effluent	Moderate burned
	Original	Moderate sweetness, moderate nuttiness

Table 25. Continued

Cheese	Fraction	Description
F	X16	Moderate sweetness, strong brothiness, slight astringency
	X12	Slight sweetness, slight brothiness
	X8	Slight astringency, slight burned
	X4	Slight astringency, slight burned
	X2	Slight astringency
	Wash	Strong saltiness, moderate astringency, slight burned
	Effluent	Slight saltiness, slight astringency
	Original	Strong sweetness, moderate brothiness, slight saltiness, slight burned
G	X16	Slight sweetness, strong brothiness, moderate burned
	X12	Slight sweetness, slight brothiness, slight burned
	X8	Slight burned
	X4	Moderate burned
	X2	Weak burned
	Wash	Strong, salty, slight burned
	Effluent	Slight burned
	Original	Moderate sweetness, strong brothy, weak burned, slight astringency

Table 26. Correlations between the flavor profile of the fat-soluble fraction and the fatty acid makeup and distillate odor

	Character note								
	Sweet	Burned	Nutty	Bitter	Lipolyzed	Acid	Volatile	Fermented	Buttery
Fatty acids									
Acetic	.35	.16	-.74**	.24	.73*	.61	-.35	-.33	-.68*
Propionic	.40	-.25	-.73**	.54	.94*	.79**	-.08	-.27	-.39
Butyric	.06	-.47	-.30	.30	.49	.40	.05	-.65	-.68*
Caproic	.40	-.35	-.49	.60	.78**	.75**	.10	-.53	-.47
Caprylic	.57	-.18	-.71*	.70*	.95**	.86**	-.14	-.41	-.36
Capric	.52	-.20	-.76**	.66*	.96**	.83**	-.07	-.31	-.30
Lauric	.46	-.25	-.72*	.62	.95**	.82**	-.06	-.29	-.32
Myristic	.48	-.25	-.68*	.65	.95**	.83**	-.08	-.33	-.31
Palmitic	.47	-.28	-.71*	.64	.94**	.81**	-.04	-.33	-.32
Stearic	.60	-.04	-.64	.75**	.96**	.87**	-.20	-.24	-.11
Distillate odors									
Bread	-.55	.32	.62	-.60	-.42	-.32	-.42	.23	-.17
Aldehyde	-.42	-.44	.24	-.46	-.42	-.30	.21	.04	-.25
Ketone	-.66*	-.03	.14	-.77	-.40	-.53	.25	.84**	.72
Neglected bathroom	-.43	.33	.51	-.48	-.34	-.26	.45	-.11	-.44
Soda crackers, peanuts	-.61	.24	.43	-.67*	-.38	-.40	-.17	.61	.11

Plantlike	-.74**	.04	.26	-.81**	-.47	-.61	.15	.57	.02
Tygon	-.58	.03	.13	-.66*	-.29	-.41	.18	.87**	.35
Cresol	-.35	-.05	.03	-.41	-.11	-.17	.20	.99**	.60
Sweet dusty	-.60	.03	.16	-.67*	-.29	-.42	.15	.86**	.32
Apricot, lactone	-.64	.19	.48	-.68*	-.37	-.36	-.27	.25	-.27
Moldy orange	-.46	-.15	-.24	-.53	-.20	-.47	.51	.65	.25
Campfire, stale newspaper	-.13	.46	.11	-.25	-.12	.15	-.46	.66*	.23

*Significant at 10% level.

**Significant at 5% level.

Table 27. Correlations between fatty acids and lactic acid and the flavor profile of the whole cheese

Component	Character note								
	Sweet	Burned	Nutty	Bitter	Lipolyzed	Acid	Volatile	Fermented	Buttery
Acetic	.796**	.649	.546	.040	-.206	.016	.835**	-.221	-.214
Propionic	.302	.841**	-.160	.207	-.127	.406	.241	.321	-.722**
Butyric	-.667*	-.802**	-.260	-.400	-.006	-.399	-.720*	-.002	.491
Caproic	-.111	.385	-.268	.844**	.410	.767**	.113	.183	-.597
Caprylic	-.151	.393	-.318	.914**	.488	.782**	.075	.216	-.559
Capric	-.235	.307	-.287	.902**	.627	.779**	.072	.196	-.463
Lauric	-.442	.269	-.523	.469	-.056	.421	-.373	.268	-.674*
Myristic	-.283	.443	-.438	.469	.613	.810**	-.071	.521	-.605
Palmitic	-.279	.525	-.522	.347	.472	.733**	-.156	.612	-.702*
Stearic	-.533	.236	-.516	.298	.252	.551	-.355	.419	-.634
Lactic	.436	.720*	-.071	.000	.000	-.034	.098	.183	.227

*Significant at 10% level.

**Significant at 5% level.

Table 28. Percent amino acids in X16 fraction of water-soluble-nonvolatile fraction

Cheese	Percent total amino acids
A	31.13
B	22.49
C	22.28
D	39.76
E	21.21
F	35.48
G	37.29