AN ABSTRACT OF THE THESIS OF

ROY GARY ARNOLD (Name) for the DOCTOR OF PHILOSOPHY (Degree)

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Title: CHEMISTRY OF THE FLAVOR DETERIORATION OF STERILIZED CONCENTRATED MILK

Abstract approved: Dr. R. C. Lindsay

Deterioration of the flavor of sterilized concentrated milk (SCM) is recognized as the principal limiting factor to commercial acceptance of this product. Although a number of volatile compounds have been identified in SCM, quantitative information on these compounds is lacking. It is therefore difficult to ascertain the significance of these compounds. The purpose of this investigation was to determine the identity of additional flavor compounds of stored SCM and to determine the concentrations of the major flavor compounds.

Vacuum steam distillation was utilized to recover volatile flavor compounds from samples of SCM. The distillates were extracted with ethyl ether, and components of the ethereal flavor concentrates were separated by gas-liquid chromatography (GLC). Major components whose identity was unknown were collected from the GLC effluent, and were analyzed by capillary column GLC and mass spectrometry. A system for transferring trapped components

directly onto a capillary GLC column was developed.

2-Furfural, which had not previously been identified in SCM, and 2-furfurol, which had not been identified as a component of any stored milk product, were identified in stored SCM.

Commercial samples of SCM were placed in controlled storage, and subjected to flavor panel evaluation and a number of quantitative determinations at selected intervals of storage. The concentration of the odd-numbered n-methyl ketones, C_3-C_{11}, and of o-amino-acetophenone were determined by measuring the absorbance of their respective 2,4-dinitrophenylhydrazone derivatives. The concentration of 2-furfurol was determined by a gas entrainment, on-column trapping GLC technique. Acid degree values were obtained by titration of SCM milk fat. Hydroxymethylfurfural (HMF) values were determined by measuring the absorbance of the HMF thiobarbituric acid reaction product.

The methyl ketones and o-aminoacetophenone were found to exceed their flavor threshold concentrations after 13 weeks storage of SCM at 27° C. The concentration of 2-furfurol exceeded its threshold concentration after 26 weeks at 27° C, but not after 13 weeks. Acid degree values increased slowly, but did not reach significant levels through 26 weeks at 27° C. Hydroxymethylfurfural values increased slowly through the first 13 weeks at 27° C storage, followed by a marked increase during the second 13 weeks.

Heat degradation of thiamine was studied as a possible source of volatile flavor compounds. Heating of thiamine solutions in phosphate buffer at pH 6.7 resulted in the production of volatile components of potential flavor significance. The identity of these components was studied by gas entrainment, on-column trapping GLC, collection of components, and capillary column GLC in conjunction with mass spectrometry. The system developed for the transfer of trapped components directly onto the capillary GLC column was utilized.

Hydrogen sulfide, 2-methyl furan, 2-methyl thiophene and a compound which appeared to be a dihydro-2-methyl thiophene were identified as volatile heat degradation products of thiamine.

Chemistry of the Flavor Deterioration of Sterilized Concentrated Milk

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Assistant Professor of Food Science and Technology
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Head of Department of Food Science and Technology

Dean of Graduate School

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INTRODUCTION

Sterilized concentrated milk (SCM), a recently developed product, offers several advantages over other products designed for the preservation of milk. Among these advantages are non-refrigerated storage and transportation, removal of two-thirds of the bulk, ease of reconstitution, non-susceptibility to lipid autoxidation and absence of the intense caramel flavor associated with evaporated milk. SCM is sterilized by an ultra-high temperature, short-time procedure and then canned aseptically, whereas evaporated milk is sterilized in the can.

Commercial processes for the production of SCM have been developed, but acceptance of the product has been limited by flavor deterioration upon storage.

A number of flavor compounds have been identified in SCM. Quantitative information on these compounds is totally lacking, however, making it difficult to estimate the significance of these compounds. The objectives of this investigation were to determine the identity of additional flavor components which appeared to be of importance in the flavor of SCM, and to determine the concentrations of major flavor components of SCM.

During the course of the investigation, an interest in the heat degradation of thiamine (Vitamin B_1) as a possible source of volatile flavor compounds developed. An additional objective of the study was thus to determine the effects of heat on thiamine, and to identify any volatile flavor compounds which might be produced by thiamine degradation.

REVIEW OF LITERATURE

Flavor Deterioration of Sterilized Concentrated Milk

Deterioration of the flavor of sterilized concentrated milk (SCM) upon storage is recognized as the principal factor limiting successful commercial marketing of this product (Siebert et al., 1963; Bingham, 1964). Siebert et al. (1963) reported that samples of SCM were criticized as being stale after three months storage at 70° F. Graham (1965) noted that SCM retains its initial acceptable flavor for three to four weeks at 70-75° F, or for two to three months if the storage temperature is reduced to 40° F.

The seriousness of the problem becomes apparent when the logistics of national distribution of a canned product are considered. Graham (1965) pointed out that only 50% of the product can be expected to reach the ultimate consumer in three months, although a substantial part of this amount may not be consumed at that time. Seventy-five percent of the product might reach the consumer in six months, and 80 to 90% would be distributed by nine months. The remainder could be in distribution for nine months to two years. Implementation of a special distribution system for products of limited shelf life does not appear to be economically feasible.

The nature of the flavor which develops on storage of SCM is

difficult to describe. Stale is the term most commonly used, but there is little agreement as to the meaning of this term. Bassette (1958) expressed the opinion that the term, stale, indicates a degree of off flavor rather than a particular flavor sensation. Patel et al. (1962) referred to the flavor defect of stored SCM as an "old rubber flavor." Arnold (1966) found that experienced judges used the terms stale, scorched and caramelized most frequently in describing the flavor of stored SCM.

Flavor Compounds Identified in Stored Milk Products

A number of flavor compounds have been reported in various stored milk products. These compounds are listed in Table 1.

In addition to these compounds, certain workers have isolated flavor fractions which reportedly contained stale flavor components. In some of the earliest work on stale dry milk, Whitney and Tracy (1950) and Whitney, Paulson and Tracy (1950) reported that the stale components could be extracted with the fat from stale dried whole milk by organic solvents, and that these components could be recovered from the extracted fat by steam distillation. When added back to milk, the distillate imparted a stale flavor.

More recently, Mayer and Swanson (1966) reported the isolation of a fraction containing stale flavor components from SCM. These workers extracted freeze dried SCM with methanol, evaporated

Table 1. Compounds identified in various stored milk products

Compound	Milk Product in which Identified: Nonfat dry milk	Dry whole milk	Evaporated milk	SCM	Reference
n-aldehydes					
C_1, C_2, C_6-$C_8, C_{10}, C_{12}, C_{14}$	x				Bassette and Keeney (1960)
C_1-C_3, C_5-C_7, C_9, C_{10}, C_{12}		x			Parks and Patton (1961)
C_1-C_3, C_7		x			Nawar et al. (1963)
C_2			x	x	Patel et al. (1962; Dutra, Jennings and Tarassuk (1959)
C_2, C_3, C_5				x	Bingham (1964)
methyl propanal	x				Bassette and Keeney (1960)
3-methyl butanal	x				Bassette and Keeney (1960)
furfural	x	x			Bassette (1958); Parks and Patton (1961)
hydroxymethylfurfural			x		Potter and Patton (1956)
benzaldehyde		x		x	Parks and Patton (1961); Arnold, Libbey and Day (1966)
n-fatty acids					
$C_6, C_8, C_{10}, C_{12}, C_{14}, C_{16}$			x		Muck et al. (1963)
C_4, C_6, C_8, C_{10}				x	Arnold (1966)
diacetyl	x				Bassette and Keeney (1960)

Table 1. (continued)

Compound	Milk Product in which Identified: Nonfat dry milk	Dry whole milk	Evaporated milk	SCM	Reference
dimethyl sulfide				x	Patel et al. (1962); Bingham (1964)
δ-decalactone		x	x	x	Patton (1961); Keeney and Patton (1956); Muck et al. (1963); Arnold, Libbey and Day (1966)
δ-dodecalactone		x	x		Patton (1961); Muck et al. (1963)
δ-tetradecalactone			x		Muck et al. (1963)
γ-dodecalactone			x		Muck et al. (1963)
n-methyl ketones					
C_3-C_5	x				Bassette (1958)
$C_3, C_4, C_9, C_{11}, C_{13}$		x			Parks and Patton (1961)
C_3, C_5		x			Nawar et al. (1963)
C_3, C_5, C_7			x		Dutra, Jennings and Tarassuk (1959)
C_5, C_7			x		Wong, Patton and Forss (1958)
$C_5, C_7, C_9, C_{11}, C_{13}$			x		Muck et al. (1963)

Table 1. (continued)

Compound	Milk Product in which Identified: Nonfat dry milk	Dry whole milk	Evaporated milk	SCM	Reference
C_3, C_5				x	Patel et al. (1962)
C_3, C_6, C_7				x	Bingham (1964)
$C_3, C_5, C_7, C_9, C_{11}, C_{13}$				x	Arnold, Libbey and Day (1966)
acetophenone				x	Arnold, Libbey and Day (1966)
o-aminoacetophenone	x			x	Parks, Schwartz and Keeney (1964); Arnold, Libbey and Day (1966)
benzothiazole				x	Arnold, Libbey and Day (1966)
maltol			x		Potter and Patton (1956)
vanillin			x		Cobb et al. (1963)

the methanol, and extracted the residue with water. Organoleptic evaluation of the water extract indicated that the stale component was present. It was further reported that the fraction gave positive anthrone and ferricyanide reducing test and a negligible Kjeldahl nitrogen value. Paper chromatography indicated that the fraction was a complex mixture. The identity of the isolated components was not established.

Investigations to date have dealt primarily with the qualitative identification of compounds contributing to stale flavor. With the exception of the report of 3.2-4.0 ppb o-aminoacetophenone in three-year old stale nonfat dry milk (Parks, Schwartz and Keeney, 1964), quantitative data is not available on the compounds which have been identified. It is therefore difficult to assess the significance of these compounds in flavor deterioration of milk products.

The formation of lactones and methyl ketones has been suggested as the principal cause of stale flavor of SCM in a USDA report (USDA, 1964), but no supporting quantitative data were presented. Keeney (1965) has suggested that there is sufficient potential for lactone formation in milk to make the lactones significant in flavor defects, but that the methyl ketones probably do not contribute due to their high flavor thresholds.

Origin of Flavor Components

It is of interest to consider the origin of the flavor compounds identified in stored milk products. In so doing, it becomes apparent that a number of diverse chemical reactions take place during storage of a milk product.

The series of long chain n-aldehydes identified in dried milk products may be attributed to lipid autoxidation (Forss, Pont and Stark, 1955; Day and Lillard, 1960; Forss, 1967). It is significant that these aldehydes have not been found in SCM or evaporated milk. It is generally accepted that lipid autoxidation does not occur in sterile fluid-milk concentrates due to the reducing conditions established by the heat processing and the lack of oxygen in the can (Keeney and Patten, 1956; Keeney, 1965). Supporting evidence for this theory was reported by Sprecher, Strong and Swanson (1965). They found that the phospholipid fraction, which is generally very susceptible to oxidative attack, showed no change in a nine month old sample of SCM exhibiting an intense stale flavor.

The odd numbered n-methyl ketones are known to arise from hydrolysis and decarboxylation of β-keto esters normally present in milk fat (Ven, Begemann and Schogt, 1963). The mechanisms involved in the formation of 2-butanone and 2-hexanone have not been determined, but available evidence indicates that they arise in a

completely different fashion from the other methyl ketones (Parks, 1967).

Mattick, Patton and Keeney (1959) postulated that δ-decalactone originated from 5-hydroxy decanoic acid. Boldingh and Taylor (1962) subsequently presented evidence for the existence of 4- and 5-hydroxy acids in milk fat, and that these acids were the precursors of the γ- and δ-lactones. Wyatt (1966) found that the hydroxy acids were esterified to glycerol in milk fat, and that the lactone precursor existed as a hydroxy triglyceride. The necessity of heat and water for formation of lactones from their precursors was also demonstrated by Wyatt (1966).

Parks (1967) suggested that the presence of free fatty acids in stored fluid milk products may be accounted for by simple hydrolysis during storage. Tweig (1965) and Swanson (In Schultz, Day and Libbey, 1967, p. 310) maintain that there is definite evidence for the reactivation of lipase following high-temperature, short-time heat treatments such as those used in processing of SCM. Edmondson et al. (1966) found that lipase reactivation was negligible in stored samples of SCM, however.

Furfural, hydroxymethylfurfural, diacetyl, maltol, methyl propanal and 3-methylbutanal are the result of the heat treatment employed in producing these milk products (Parks, 1967). Methyl propanal and 3-methyl butanal can result from the Strecker

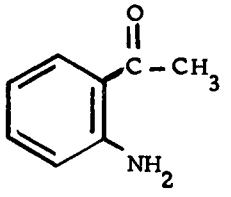
degradation of valine and leucine (Hodge, 1967). Hydroxymethyl-furfural, furfural, diacetyl and maltol are known products of sugar fragmentation and dehydration during the Maillard reaction (Hodge, 1953; Hodge, 1967).

Parks (1967) suggests that vanillin and benzaldehyde in milk products may be traced to the feed of the cow. Benzaldehyde has been observed in both corn and grass silage (Morgan and Pereira, 1962A and B). Richter (1952) reported that vanillin is a degradation product of lignin, suggesting that heat degradation of ligneous fragments from feed is a possible source of vanillin in milk.

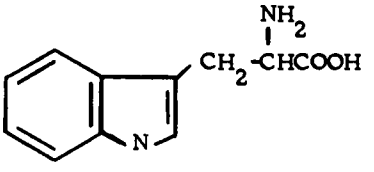
Methyl sulfide has been identified in fresh fluid milk (Patton, Forss and Day, 1956). Bingham (1964) found methyl sulfide in both fresh and stored SCM samples. Keenan and Lindsay (1967) have demonstrated the presence of an S-methyl methionine sulfonium salt, a precursor of methyl sulfide in milk. They suggest that the precursor enters into milk from feed.

The origin of o-aminoacetophenone has not been positively established, but it is believed to be derived from tryptophan, kynurenine or indican. Tabone, Magis and Troestler (1947) demonstrated the conversion of kynurenine to o-aminoacetophenone as under alkaline conditions. Tabone, Mamounas and Robert (1951) and Spacek (1954) subsequently found that alkaline degradation of tryptophan led to o-aminoacetophenone, with kynurenine appearing

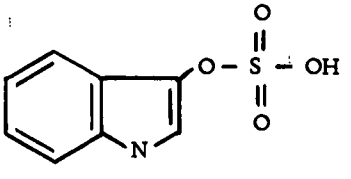
as an intermediate. Parks et al. (1967) recently reported evidence for the occurrence of kynurenine in fresh milk. Indican has also been reported in milk (Spinelli, 1946).



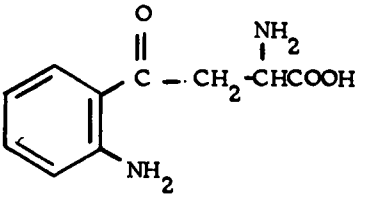
o-aminoacetophenone



tryptophan



indican (indoxyl sulfate)



Kynurenine

The origin of acetophenone has not been established. It has been suggested that this compound might arise by an alternate mechanism from the same precursors as those mentioned for o-aminoacetophenone (Arnold, 1966). Likewise, the origin of benzothiazole has not been established. Thiamine was suggested as a possible precursor of this compound (Arnold, 1966). This suggestion led to an interest in the heat degradation of thiamine, which is considered next.

Heat Degradation of Thiamine (Vitamin B_1)

Whole milk contains an average of 0.44 mg of thiamine per liter (Hartman and Dryden, 1965, p. 21). A certain percentage of this is lost during heat processing of milk. Hartman and Dryden (1965, p. 22) report the following losses of thiamine as a result of heat processing:

Pasteurization	
Holding method	5.5-25%
High-temperature, short-time	3-4 %
Sterilization	
In can	20-45%
Ultra-high temperature	4-5 %
Manufacture of evaporated milk	20-60%

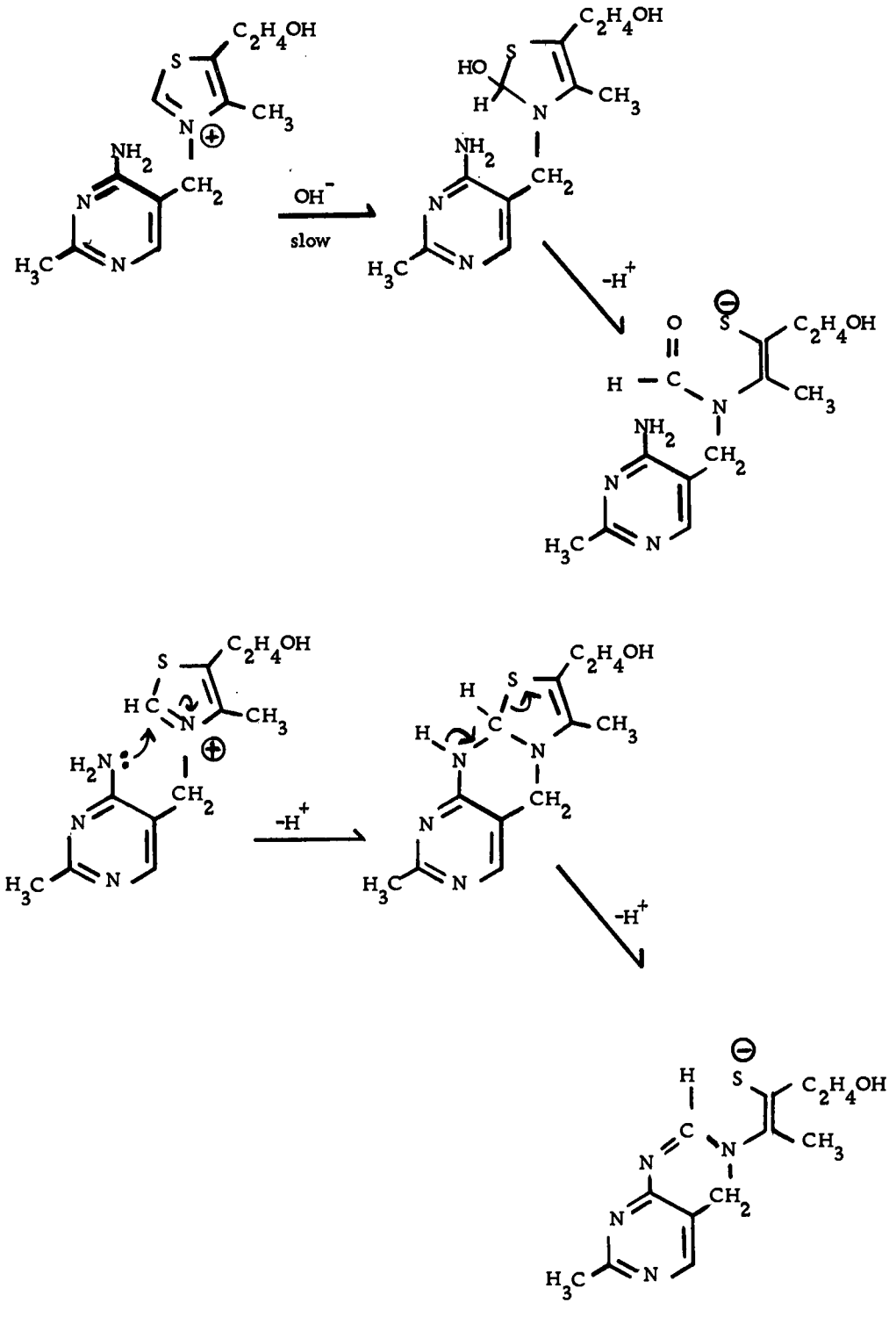
Thiamine is stable to heat under acid conditions, but becomes increasingly heat labile as the pH is increased. Booth (1943) observed the following percentage destruction of thiamine in boiling phosphate buffer:

pH	Loss of thiamine
3.6	15% in 3 hours
6.0	50% in 3 hours
6.8	90% in 3 hours
7.4	96% in 1 hours

Investigations related to the heat degradation of thiamine have been devoted principally to the loss of biological activity of the vitamin. Little has been reported concerning the heat degradation products. Obermeyer and Chen (1945) reported initial cleavage of thiamine to its pyrimidine and thiazole moieties under conditions encountered in bread baking. These workers reported that additional unknown degradation compounds were also formed, since only 30% of the pyrimidine portion and 67% of the thiazole portion released from thiamine were found as such in bread extracts.

Lholst, Busse and Baumann (1958) studied the products of thiamine resulting from the action of alkali. Using paper chromatographic methods, these workers identified the carbinol form of the vitamin, thiochrome, thiamine disulfide, two pyrimidine derivatives and two unknown products.

The following mechanisms, showing the effect of alkaline conditions (pH 9-10) on the structure of thiamine, have been proposed. The first scheme was described by Williams and Ruehle (1935) and Clark and Gurin (1935). The second was proposed by Maier and Metzler (1957). It is possible that these same structures may exist in smaller concentrations at lower pH values (pH 6.5-7.0), and that these structures might be early intermediates in heat degradation of thiamine.


C2H4OH
S
CH3
N
NH2
CH2
N
H3C
N
OH-
slow
HO
H
-H+
O
H
C
S
C2H4OH
CH3
H2N
-H+
-H+

EXPERIMENTAL PROCEDURE

Samples

Commercially processed samples of SCM were provided by A) Carnation Research Laboratories, VanNuys, California and B) Nodaway Valley Foods, Inc., Corning, Iowa. The latter product is currently being marketed under the trade name "Quilk." Both products were 3:1 concentrates. Processing parameters and composition data for the two products were as follows:

	A	B
Forewarming		
Temperature	*	265° F (129.5° C)
Time	*	15 sec
Sterilizing		
Temperature	273° F (134° C)	295° F (146° C)
Time	30.5 sec	3.5 sec
% fat	9.4%	9.8%
% total solids	34.0%**	34.3%**

*Information not provided

**Includes added Quadrafos, a polyphosphate, to inhibit gelation

Sample Storage Conditions

Samples of A and B processed in late 1966 were stored at 27° C for periods of 6, 13 and 26 weeks. Control samples were stored at 1° C. Fresh samples to serve as control samples for flavor panel evaluations were received bimonthly during the storage study period. In addition, a sample of A stored for 104 weeks (two years) at 1° C and a sample of B stored for 130 weeks (two and one-half years) at 27° C were available.

Flavor Panel Evaluations

Flavor scores on the fresh and stored SCM samples were obtained with a trained flavor panel consisting of 12 judges experienced in evaluating milk. Both the proposed American Dairy Science Association scorecard for concentrated and dried milks (shown below) and a nine point hedonic scale ballot (9 = like extremely; 1 = dislike extremely) were used. Flavor scores were averaged, and the most frequent criticisms on the ADSA scorecard were noted.

SCORE CARD FOR CONCENTRATED MILK

Name: _____ Date: _____

	Sample Number				
Astringent Sl. 9,8: def. 7,6: pron. 5,4					
Chalky Sl. 9,8: def. 7,6: pron. 5,4					
Cooked Sl. 9,8: def. 7,6: pron. 5,4					
Lacone Sl. 8,7: def. 6,5: pron. 4,3					
Scorched Sl. 6,5: def. 4,3: pron. 2,1					
Stale Sl. 6,5: def. 4,3: pron. 2,1					
Other: Name and intensity					
Flavor Score - may not be higher than lowest score from above. Score 10 for no criticism					

Identification of Flavor Components

Isolation of Components

In previous work on the identification of flavor components of SCM (Arnold, 1966), a rather complex isolation scheme was utilized. This scheme consisted of freeze drying the SCM samples, extraction of the fat and flavor components from the freeze dried product with an organic solvent, and recovery of the flavor compounds from the

extracted fat by vacuum steam distillation. Flavor compounds were then recovered from the distillate by ethyl chloride extraction.

In this work, an effort was made to simplify the isolation scheme by subjecting samples of SCM to vacuum steam distillation directly. Three liters of SCM were combined with two liters of boiled and cooled distilled water in a 12-liter distillation flask. The sample reached a maximum temperature of 33° C during distillation, and this temperature was maintained for two hours. Approximately two liters of distillate were collected in the first three cold traps, cooled by dry ice, dry ice-ethanol and liquid-nitrogen, respectively.

Following distillation, the cold traps were thawed and their contents combined. The distillate was saturated with sodium chloride and extracted with ethyl ether (reagent grade, treated for removal of peroxides by the method of Valseth (1953), and redistilled) in a liquid-liquid extractor, as described by Lindsay (1965, p. 70-71). The ethereal flavor concentrate solution was fractionally distilled to remove excess ethyl ether using a one x 60 cm fractionation column packed with glass helices and equipped with an electronically controlled fractional head condenser. The ether solution was concentrated to approximately 10 ml. Further concentration was accomplished by passing a stream of N_2 over the surface of the concentrate in a 20 ml test tube, and finally in a two ml tapered chromatographic tube. The final volume of sample for injection into a gas

chromatograph was less than 0.5 ml.

Gas Chromatographic Separation of Components

Components in the flavor concentrates obtained by this procedure were separated by temperature programmed gas-liquid chromatography (GLC). An Aerograph 204 equipped with a hydrogen flame detector and an effluent splitter was used for preliminary analysis of the flavor concentrates. One-eighth inch OD x 12 ft stainless steel columns packed with either 2 1/2% Apiezon L or 2 1/2% Carbowax 20M on 60-80 mesh acid and DMCS treated Chromosorb G were employed. The location of peaks corresponding to components previously identified in fresh and stale SCM was determined by comparing retention times with those of known compounds on the two columns, and by smelling the split effluent.

Trapping of Components

Unknown peaks in the chromatogram possessing odors which would appear to make them significant in milk off flavor were collected for mass spectral analysis. An Aerograph 90-P3 equipped with a thermal conductivity detector was used for collection of individual fractions. A 1/8-in OD x 4 ft stainless steel column packed with 2 1/2% Carbowax 20 M on 60-80 mesh acid and DMCS treated Chromosorb G was employed for separation of components. Manual

temperature programming was employed to enhance separation. Individual fractions of interest were trapped in 1.5 mm OD x 270 mm glass capillary tubes which were cooled with dry ice. Corresponding fractions were collected from three successive chromatograms of the flavor concentrates. The glass capillary tubes were sealed following collection. The trapped components were concentrated to one position in the sealed tube by cooling a localized area of the tube with dry ice while carefully warming the remainder of the tube with a small flame.

Mass Spectral Analysis

Capillary column GLC in conjunction with rapid scan mass spectrometry was used to identify components in the collected fractions. An F&M model 810 gas chromatograph equipped with a capillary injection splitter and a 300 ft x 0.01 ID capillary column coated with butanediol succinate (BDS) was employed for separation of the trapped components. The column was operated isothermally, the temperature selected depending upon the relative volatility of components in the particular fraction. Trapped fractions were taken directly from the sealed tube in a 10 μl syringe and injected directly into the injection splitter in those cases where sufficient volume of the fraction was available. When significant volume was not available, components were rinsed from the glass capillary tube with a minimum

volume of ether, and the resulting ether solutions were injected.

The total effluent from the capillary column was fed into the high vacuum ionization chamber of an Atlas CH-4 mass spectrometer (a nine-inch, 60 degree sector, single focusing instrument) equipped with a double ion source. The effluent from the capillary column was split in the ion source, with 50% going to the 20 eV source and the other 50% going to the 70 eV source. The 20 eV electron source, which operates at less than the ionization potential of the carrier gas (helium), was used as a GLC detector. Hence, total ionization resulting from the presence of organics in the carrier gas stream, was recorded. The 70 eV source produced fragmentation of the organics, and the fragmentation pattern resulting from a particular organic compound was recorded on a Honeywell 1508 Visicorder.

The following conditions were employed for the GLC-MS analysis of trapped components:

Column temperature	150° to 190° C
Column	BDS
Flow rate	one ml/min
Injector temperature	175° C
Range	10
Attenuation	x 10 initially
Filament current	20 eV source - 45 μA
	70 eV source - 12 μA

Electron voltage	20 eV and 70 eV
Accelerating voltage	3000 V
Analyzer pressure	1.5×10^{-6} Torr
Multiplier voltage	1.6 KV
Scanning speed	5 sec from m/e 25 to m/e 250

The collection procedure and subsequent analysis by combined GLC-mass spectrometry described above proved satisfactory for major components of the packed column chromatograms, but was unsatisfactory for minor components. This was primarily due to the fact that 99% of the collected sample was lost upon injection into the capillary injection splitter, which produces a 100:1 splitter effluent to column flow ratio. To overcome this sample loss, a capillary trapping, on-column injection system was developed in cooperation with Mr. R. A. Scanlan.

Capillary GLC On-column Injection System

Fractions from a packed column were collected in 0.76 mm ID x 15 cm stainless steel capillary tubing fitted with 1/16-inch Swagelok fittings at each end. The collection traps were sealed with 1/16-inch Swagelok caps after collection.

A schematic diagram of the on-column injection system is shown in Figure 1. The normal flow of carrier gas in the absence of a trap is through valve E. This is accomplished by opening valve E,

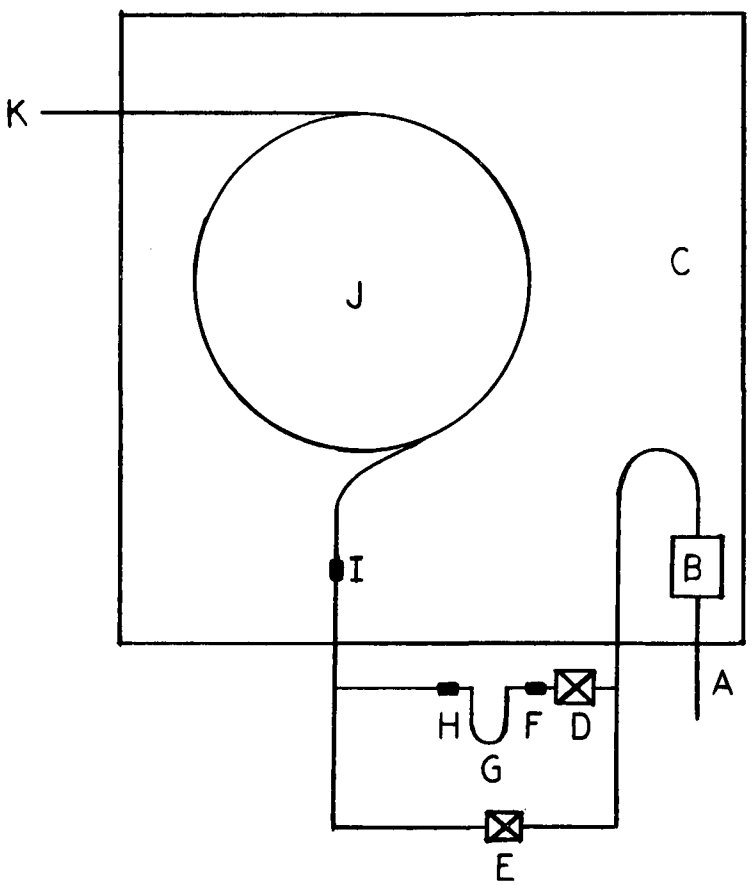


Figure 1. Schematic drawing of capillary trap on-column injection system. A. injection port; B. injection splitter; C. column oven; D. needle valve; e. toggle valve; F, H and I. 1/16-inch Swagelok union; G. capillary trap; J. capillary GLC column; K. to mass spectrometer.

closing valve D, and installing a Swagelok cap at union H.

The sequence of events involved in chromatographing trapped components was as follows. The capillary trap was attached at union F. The cap at union H was removed, permitting the outward flow of carrier gas and precluding the entrance of air into the inection system. Valve D was opened momentarily to flush air from the trap, which was still being cooled with dry ice. The free end of the trap was then attached to union H. The trap was heated with a heat gun, valve D was opened and valve E was closed. This directed carrier gas through the trap, resulting in transfer of trapped components directly onto the capillary GLC column.

A major precaution in using the technique was to avoid excessively large samples. Work with knowns indicated that approximately 10^{-6} grams was an ideal sample size. This amount could be approximated by comparing peak sizes of components trapped from packed GLC columns with peak sizes for known amounts of known compounds. The technique proved to be effective in obtaining sufficient quantities of minor components for mass spectral analysis. As will be shown later in the thesis, the technique was of value in obtaining mass spectra of components recovered by the gas-entrainment, on-column trapping procedure of Morgan and Day (1965).

Quantitative Studies

As indicated later in the thesis, the chromatograms of flavor concentrates from fresh and stored SCM were very similar. Few new peaks appeared in the stored SCM samples, and the ratio of component concentrations showed little change. It was observed, however, that in every case more flavor concentrate was obtained from the stored samples than from the fresh samples. Sufficient flavor concentrate was obtained from stored SCM samples for eight to ten GLC injections, but the fresh SCM yielded enough flavor concentrate for only one or two injections. This observation suggested that the differences in the volatile flavor components of fresh and stored SCM were more quantitative than qualitative. Hence, it was decided that quantitative data on some of the major flavor components should be obtained.

2-Furfurol

The chromatograms of the flavor extracts revealed that 2-furfurol was the major component in stored samples of SCM, and one of the major components of fresh SCM. It thus appeared desirable to collect quantitative data on this compound. Since useful color derivatives for 2-furfurol are not readily available, GLC was the method of choice. Stevens (1967) reported good success in

quantitative determination of the amounts of oct-1-en-3-ol and linalool, two relatively high boiling components, in snap bean liquor by the gas entrainment, on-column trapping technique of Morgan and Day (1965). These two compounds have boiling points of approximately 170 and 198-200° C, respectively. The boiling point of 2-furfurol is 170° C.

In searching for optimum conditions for quantitative measurement of 2-furfurol, it was determined that a 1/8-in OD x 10 ft column packed with 2 1/2% Carbowax 20 M on 60-80 mesh acid and DMCS treated Chromosorb G, operated isothermally at 120° C, achieved separation of 2-furfurol from other components of the samples. Under these conditions, 2-furfurol produced a sharp peak, allowing accurate measurement of peak height.

The following conditions were used for the gas entrainment, on-column trapping GLC technique employed for the quantitative determination of 2-furfurol:

Sample size	10 ml (whole milk concentration)
Purge time and rate	10 min at 10 ml/min
Water bath temperature	90° C
Instrument	Aerograph 1200, H_2 flame detector
Column	Carbowax 20 M
Column temperature	120° C

Flow rate	25 ml/min of N_2
Detector temp	200° C
Range	1
Attenuation	x 8 initially

A standard curve of 2-furfurol concentration plotted against recorder response (peak height) was constructed by adding known amounts of 2-furfurol to fresh whole milk and analyzing them by the gas entrainment, on-column trapping GLC technique. An internal standard was added in all samples to correct for variations in instrument sensitivity. 1-octanol was selected as the internal standard because it had a high boiling point (194° C), had sufficient water solubility, and was separated from 2-furfurol and other components by the Carbowax column. One ml of a 55 ppm solution of 1-octanol was added to the 10 ml sample in the purge bottle, resulting in a concentration of five ppm of 1-octanol in the 11 ml of sample. Recorder response for 2-furfurol could thus be corrected for variations in sensitivity, as reflected by variations in recorder response for 1-octanol.

The concentration of 2-furfurol in samples of SCM was determined by comparing corrected recorder response for the 2-furfurol peak with the standard curve.

Data regarding the flavor threshold of 2-furfurol in milk was not available. The average flavor threshold was thus determined by

presenting a series of coded samples containing varying concentrations of re-distilled 2-furfurol to a flavor panel of 10 judges, and asking the judges to indicate if they could detect the compound. A positive reference and a control sample were also provided. The number of positive responses was tabulated and plotted linearly against concentration. The average flavor threshold was considered to be the concentration at which the plot crossed the 50% positive response line, as suggested by Patton and Josephson (1957).

Methyl Ketones

Quantitative information on the series of odd-numbered n-methyl ketones, which have been identified in SCM as well as other stored milk products, was desired to determine if these compounds were present in sufficient concentration to significantly contribute to the flavor of SCM. In addition, knowledge of the concentration of the methyl ketones would be helpful in estimating the effects of storage on other flavor compounds. If the concentrations of the methyl ketones were known, comparison of GLC peak sizes of various components relative to the peak sizes of the methyl ketones would indicate whether or not these components were increasing on storage.

Solvent Purification. Solvents used in the quantitation of the methyl ketones were purified as follows.

Hexane and benzene: High purity hexane and reagent grade benzene were treated for removal of carbonyls by the method of Hornstein and Crowe (1962), and then redistilled.

Ethylene chloride: Ethylene chloride was distilled and stored over anhydrous potassium carbonate.

Chloroform: Chloroform was treated for removal of carbonyls by the method of Schwartz and Parks (1961), and redistilled. The redistilled chloroform was stabilized by the addition of 0.75% ethanol.

Nitromethane: Nitromethane was redistilled over boric acid.

Isolation of Methyl Ketones. The overall quantitation procedure used was similar to that described for fats and oils (Schwartz, Haller and Keeney, 1963). Milk fat was recovered from SCM samples by an adaptation of the procedure described by Harper, Schwartz and El-Hagarawy (1956). A 50 g sample of SCM was ground with 50 g of dry silicic acid in a mortar and pestle. The resulting powder was blended with 150 ml of hexane in a Waring Blendor for 10 minutes, and then packed in a 2.2 cm ID x 50 cm chromatography column. Hexane was then passed through the column. A total of 500 ml of hexane was used for each sample.

Formation of the 2,4-Dinitrophenylhydrazones (DNPH). A reaction column consisting of 0.5 g 2,4-dinitrophenylhydrazine, six ml of 85% phosphoric acid, four ml of distilled water and 10 g of

analytical grade Celite was prepared according to the procedure of Schwartz and Parks (1961). The hexane-fat solution was passed through this reaction column to convert the methyl ketones to their 2,4-dinitrophenylhydrazone derivatives.

Removal of Fat from DNPH's. The fat was removed from the hydrazone derivatives using a modified procedure of Schwartz, Haller and Keeney (1963). Seasorb 43 (magnesium oxide, unheated) and Celite 545 were used in a 1:2 ratio rather than a 1:1 ratio. Ten g of Seasorb 43 and 20 g Celite 545 were blended in hexane and poured into a 2.2 cm ID chromatographic column plugged at the bottom with glass wool. The column was packed with air pressure.

The fat-hydrazone mixture was dissolved in five ml of hexane and applied to the column. The column was subsequently washed with 150 ml of hexane, 100 ml of a 1:1 hexane-benzene mixture, and 150 ml of benzene. The hydrazones were eluted from the column with 150 ml of a 3:1 chloroform-nitromethane mixture. The chloroform-nitromethane solvent was removed from the eluent with a rotary evaporator.

Separation of DNPH's into Classes. The methyl ketone derivatives were separated from other hydrazones by the method of Schwartz, Parks and Keeney (1960). Fifteen g of Seasorb 43 (heated to 400° C/48 hr) and 30 g of Celite 545 (heated to 150°/24 hr) were blended with 150 ml of ethylene chloride. The resulting blend

was placed in a 2.5 cm ID x 60 cm chromatographic column, and the column was packed with air pressure. The hydrazone mixture from the previous step was taken up in four ml of ethylene chloride and applied to the column. The column was developed with ethylene chloride. The hydrazones of the methyl ketones (initial greenish-gray band) were collected. Excellent separation of the ketone derivatives from other hydrazone derivatives was achieved.

Separation of Ketone DNPH's into Individual Chain Lengths. A liquid-liquid partition column, as described by Day, Bassette and Keeney (1960), was prepared by blending 25 g of analytical Celite (heated to 160° C/24 hrs) with 250 ml of hexane equilibrated with nitromethane for 10 min, slowly adding 18.75 ml of nitromethane to the blendor, and packing the blend in a 2.0 cm ID chromatography column with air pressure. The methyl ketone derivatives were taken up in five ml of hexane equilibrated with nitromethane, and applied to the column. The column was developed with hexane equilibrated with nitromethane. The column eluate was monitored for absorbance at 345 millimicrons with a Vanguard Automatic Ultaviolet Analyzer used in conjunction with an automatic fraction collector. Absorbance and fraction number were recorded concurrently on a strip chart recorder. By matching tube numbers and absorption peaks, the individual members of the class were pooled and collected.

Determination of the Concentration of Individual Methyl Ketones. The concentrations of C_3, C_4, C_5, C_7, C_9 and C_{11} methyl ketone derivatives were determined by measuring their absorbance in chloroform at 363 millimicrons. The concentration of the C_{13} methyl ketone derivative was not determined, as it was not separated from an unidentified fore-peak on the partition column. The chain length of each fraction was tentatively assigned by its retention volume on the partition column, and was confirmed by the thin layer chromatographic technique of Libbey and Day (1964). Each sample of SCM was analyzed in duplicate.

Work by Langler (1963) and Anderson (1965) showed that recoveries from the columns used in this type of analysis were not as high as reported in the literature (Schwartz, Haller and Kenney, 1963). The percentage recovery of the individual ketones was determined by adding a standard mixture of C_3, C_5, C_7, C_9 and C_{11} methyl ketones to a steam stripped sample of fresh SCM. The resulting sample was analyzed as described above. Average percentage recovery of each ketone, as determined by duplicate analysis, was used in the calculation of the ketone concentrations in the SCM samples.

o-Aminoacetophenone

The determination of the concentration of o-aminoacetophenone,

which has been implicated as an important flavor compound in stale nonfat dry milk, was accomplished by a procedure similar to the isolation procedure of Parks, Schwartz and Keeney (1964).

Solvent Purification. Hexane and chloroform were purified as described for methyl ketone determinations.

Methanol, cyclohexane, cyclohexene, ethyl acetate and acetonitrile: Reagent grade lots of each of these solvents were redistilled prior to use.

N,N-dimethylformamide: Reagent grade N,N-dimethylformamide was used as such in the thin layer chromatography procedure.

Isolation of o-Aminoacetophenone. Milk fat was recovered by a procedure similar to that described for the methyl ketone procedure, but on a much larger scale. One thousand g samples of SCM, 1000 g of silicic acid and 4750 ml of hexane were used.

The hexane extract was washed three times with 200 ml of distilled water in a six l separatory funnel, and was then extracted with 200 ml of 10% HCl. The aqueous layer was collected, made alkaline with solid potassium carbonate, and back extracted with 500 ml of hexane.

Formation of the 2,4-Dinitrophenylhydrazone Derivative of o-Aminoacetophenone. A modified 2,4-dinitrophenylhydrazone reaction column was prepared using 200 mg of 2,4-DNPH reagent, 1.5 ml of 85% phosphoric acid, 0.5 ml of water and 2.0 g of analytical

Celite. The hexane solution from the previous step was passed through the reaction column, followed by 50 ml of hexane containing two ml of 2-nonanone. The column was washed with 100 ml of hexane. The o-aminoacetophenone derivative, possessing a basic amino group, remained on the acidic reaction column. The 2-nonanone reacted with excess reagent on the column, and the resulting derivative was eluted from the column with hexane.

The 2,4-DNPH derivative of o-aminoacetophenone was eluted from the column by stripping the column packing with 30 ml of methanol. Sixty ml of distilled water was added to the methanol, and the solution was made alkaline with solid potassium carbonate. The o-aminoacetophenone derivative was recovered by extraction of the methanol-water solution with 50 ml of chloroform. The chloroform fraction was dried for six hours with sodium sulfate, and the chloroform was subsequently evaporated on a rotary evaporator.

Removal of Residual Reagent from the Derivative. Attempts to remove residual reagent by the procedure described by Parks, Schwartz and Keeney (1964) proved unsuccessful in that the derivative moved with the reagent on the Celite-magnesia column. Substitution of benzene for chloroform as the eluting solvent for the reagent, followed by chloroform to elute the o-aminoacetophenone derivative improved the separation but a clean separation of derivative from reagent still was not achieved. This separation was

accomplished, however, by using a reaction column prepared without the 2,4-DNPH reagent. The column consisted of 2 g of analytical celite, 1.5 ml of 85% phosphoric acid and 0.5 ml of distilled water, and was prepared by grinding in a mortar and pestle.

The derivative from the preceding step was taken up in 5 ml of chloroform and placed on the phosphoric acid impregnated column. This was followed by 25 ml of chloroform to remove non-polar derivatives, 50 ml of hexane containing 2 ml of 2-nonanone to react with and remove residual reagent, and 100 ml of hexane to wash any 2-nonanone derivative from the column. The column was then unpacked with air pressure. The top one cm of column packing was removed and placed in 15 ml of methanol. The methanol solution was filtered, and the residue was washed with 15 ml of methanol. Sixty ml of distilled water was added to the filtrate. The solution was made alkaline with solid potassium carbonate and extracted with 50 ml of chloroform. The chloroform extract was dried with sodium sulfate for six hours, and the chloroform was subsequently evaporated. The resulting residue was free of unreacted reagent.

Partition Column Chromatography of the Derivative: The derivative from the preceding step was subjected to column partition chromatography on a 10 g Celite column using two percent ethyl acetate in methyl cyclohexane as the mobile phase and acetonitrile as the immobile phase, as described by Corbin (1962).

Determination of the Concentration of o-aminoacetophenone.

The concentration of o-aminoacetophenone was determined by measuring the absorbance of the 2,4-dinitrophenylhydrazone derivative in chloroform at 398 mμ. An average recovery value was determined by addition of a known concentration of o-aminoacetophenone to two samples of fresh SCM, and averaging the percentage recoveries at the completion of analysis. Single samples of each of the SCM samples were employed. Duplicate samples were not used because of the large quantities of solvent required and the time required for analysis. The values determined must therefore be considered as quantitative estimates.

The identity of the derivative was confirmed by comparison of the behavior of the derivative in the isolation scheme with known o-aminoacetophenone derivative, determination of the ultraviolet absorption maximum, and comparison with known derivative by thin layer chromatography. The thin layer solvent system was similar to that used in the paper chromatographic procedure of Sundt and Winter (1958). A seven cm x 19.5 cm silica gel Chromogram sheet (Eastman Kodak) was immersed in a 50:50 v/v acetone-N,N-dimethylformamide solution, and subsequently dried. Unknown fractions and known o-aminoacetophenone derivative were spotted two cm from one end. The Chromogram sheet was then suspended in a sealed chamber containing approximately a one cm layer of 5:3

cyclohexane-cyclohexene equilibrated with N,N-dimethylformamide. The Chromogram sheet was saturated with developing solvent by suspending it above the solvent layer for 18 hrs. Development required approximately six hours after the sheet was lowered into the solvent layer.

Authentic o-aminoacetophenone derivative was prepared by dissolving one g of 2,4-dinitrophenylhydrazine in 25 ml of 30% sulfuric acid, adding six ml of 30% sulfuric acid containing 0.7 g of o-aminoacetophenone, and allowing the mixture to stand overnight. Ten ml of water was then added, and the precipitate was recovered by filtration. The precipitate was washed with an alkaline potassium carbonate solution and warm ethanol, and was then recrystallized from chloroform.

Acid Degree Value

Reports in the literature concerning the possible reactivation of lipase and consequential development of rancidity in UHT sterilized products suggested that quantitative information on free fatty acid levels in stored SCM would be valuable. As a preliminary step to quantitative determination of individual free fatty acids, acid degree values were determined on fresh and stored samples of SCM to ascertain the effect of storage on total free fatty acid levels.

The method of Thomas, Nielsen and Olson (1955) was used.

Milk fat was recovered from reconstituted SCM samples by employing BDI reagent (30 g of Triton-X-100, 70 g of sodium phosphate, made to one l with water), heat and centrifugation. One ml of tempered fat was mixed with five ml of 4:1 hexane-n-propanol, and titrated with standardized 0.02 N potassium hydroxide to the phenolphthalein end point. Results were expressed as acid degree value, which is the number of ml of 1 N base required to titrate 100 g of fat.

Hydroxymethylfurfural (HMF) Value

Varying degrees of browning were noted in samples of SCM stored for long periods of time at 27° C The procedure of Keeney and Bassette (1959) for detecting early stages of browning in milk products was employed to ascertain the extent of browning in SCM.

Ten ml samples of reconstituted SCM and five ml of 0.3 N oxalic acid were mixed, heated in a boiling water bath for one hour and cooled. Five ml of 40% trichloroacetic acid (TCA) were added and the resulting solutions were mixed and filtered. One ml of 0.05 M thiobarbituric acid (TBA) was added to four ml of the filtrate, and the mixture was placed in a 40° C water bath for 40 min. The absorbance of the solution was measured at 443 mμ against a blank prepared in an identical fashion, substituting water for milk.

The principle of the test is that oxalic acid and heat convert

early intermediates of the non-enzymatic browning reaction to HMF, which is subsequently quantitated by measuring the absorbance of its TBA reaction product. HMF value is calculated from absorbance:

$$\mu \text{ moles HMF/l} = (\text{Absorbance} - 0.055)\ 87.5$$

Heat Degradation of Thiamine (Vitamin B_1)

Preliminary studies on the effect of heat on aqueous solutions of thiamine buffered to pH 6.7 with phosphate buffer indicated that compounds of flavor significance were formed. The odors resulting from these heat treatment were evaluated by a number of individuals. It was generally agreed that the odor resembled that of heated or boiled milk initially, but as heating of the thiamine solutions proceeded, an odor resembling that of stewing chicken was noted. These observations prompted an interest in the identification of compounds produced by heat degradation of thiamine.

Purification of Thiamine

U.S.P. grade thiamine hydrochloride was recrystallized from methanol. The crystals were collected on Whatman No. 2 filter paper, and were washed with ethanol and ethyl ether. Volatile contaminants were removed from aqueous 0.05 M solutions of thiamine by adjusting the pH of the solutions to 3.5, and purging with nitrogen for one hour in a boiling water bath. Thiamine, being heat stable at

pH 3.5, was not affected by this treatment.

Isolation of Volatile Components

The rapid detection of the odors resulting from heating of thiamine solutions at pH 6.7 suggested that the compounds responsible for the odors were reasonably volatile. Hence, the gas entrainment, on-column trapping technique of Morgan and Day (1965) was employed.

Ten ml quantities of 0.05 M thiamine buffered to pH 6.7 with phosphate buffer were placed in screw cap test tubes sealed with teflon caps and heated in a boiling water bath for 15 minutes. Previous work by Bills and Keenan (1967) had shown that volatile components were retained in these tubes when heated. The tubes were subsequently cooled, and the contents were transferred to a 20 ml screw cap vial containing 3.6 g of sodium sulfate, as described by Morgan and Day (1965)

The conditions employed for the gas entrainment, on-column trapping technique were as follows:

Sample size	10 ml
Purge time and rate	10 min at 10 ml/min
Water bath temperature	60° C
Instrument	Aerograph 1200, H_2 flame detector

Column	1/8-in OD x 10 ft, packed with 20% 1,2,3-tris(2-cyanoethoxy) propane on 80-100 mesh Celite
Column temperature	70° C
Column flow rate	25 ml/min N_2
Detector temperature	175° C
Range	0.1
Attenuation	x 8 initially

Control samples consisted of unheated 10 ml quantities of 0.05 M thiamine at pH 6.7. A second control consisted of 10 ml quantities of 0.05 M thiamine at pH 3.5, heated in sealed tubes in a boiling water bath for 15 minutes. Since thiamine is stable to heat at pH 3.5, peaks appearing in heated, pH 6.7 samples but not in heated, pH 3.5 samples are attributable to heat degradation products of thiamine.

Identification of Volatile Components

Components appearing in the gas entrainment, on-column trapping chromatograms of heated pH 6.7 samples, but not in the control samples, were trapped in 0.76 mm ID x 15 cm stainless steel traps. Trapped components were subsequently analyzed by capillary column GLC and mass spectrometry, using the on-column injection system previously described.

RESULTS AND DISCUSSION

Flavor Panel Evaluations

Flavor panel scores for the SCM samples are shown in Tables 2 and 3. Table 2 indicates the effect of storage at 27° C on average flavor scores. For both brands of SCM, the flavor scores decrease slightly after six weeks storage at this temperature, and show a marked decline after 13 weeks storage. Stale replaces cooked as the most frequently used criticism on the ADSA scorecard after 13 weeks at 27° C.

Table 2. Average flavor scores and principal criticism of samples of SCM stored at 27° C.

Commercial Sample	Weeks Storage 27° C	ADSA Ballot		Hedonic Scale Ballot
		Overall Score	Principal Criticism	
A-1	0	6.2	Cooked (60%)	4.9
	6	5.9	Cooked (60%)	4.0
	13	3.7	Stale (80%)	2.0
	26	3.0	Stale (90%)	1.9
B-1	0	7.1	Cooked (90%)	5.3
	6	5.7	Cooked (80%)	4.3
	13	3.6	Stale (80%)	1.9
	26	4.0	Stale (60%)	1.7
B-3	130	---	not submitted to panel; browning and gelation made sample unpalatable.	

Table 3. Average flavor scores of samples of SCM stored at 1° C.

Commercial Sample	Weeks Storage 1° C	Overall Flavor Score	
		ADSA Scorecard	Hedonic Scale Ballot
A-1	0	6.0	4.7
	16	6.2	4.9
	26	5.9	4.0
	36	5.9	5.0
A-2	0	5.5	4.6
	4	5.7	4.9
	13	6.8	5.2
	26	6.0	5.6
A-3	104	5.7	4.3
B-1	0	7.1	5.3
	21	7.1	5.2
	30	6.5	4.4
	40	7.1	6.2
B-2	0	6.0	4.7
	8	6.6	4.6
	18	6.7	5.2

Table 3 shows the effect of reducing the storage temperature to 1° C on flavor stability of SCM. Average flavor scores of samples stored at this temperature show no decrease through 21 weeks storage, and frequently show a slight increase. Very little decline in average flavor score is exhibited in samples stored as long as

104 weeks (2 years).

Identification of Components

Chromatograms of flavor concentrates obtained by steam distillation of fresh and stored (18 months at 27° C) SCM are shown in Figure 2. Peaks whose identity had previously been established (Arnold, Libbey and Day, 1966) are labeled in this figure. Two components which were trapped by the glass capillary method are indicated in the chromatogram of the stored SCM. These components were collected because of the obvious increase in peak size, and because of the odors emitting from the effluent splitter in these regions of the chromatogram.

The BDS capillary column chromatograms of the components collected in Traps 1 and 2 are shown in Figures 3 and 4. The mass spectrum of peak A, Trap 1, indicated that this peak was ethyl ether, which had been used to rinse the Trap 1 components from the glass capillary prior to injection. The mass spectra of peak B, Trap 1, and peak D, Trap 2, exhibited m/e 207 and 133 fragments, suggesting a silicone contaminant (Bieman, 1962, p. 172).

The mass spectrum of peak C, Trap 1, is shown in Figure 5. This spectrum compares favorably with the following published spectrum of 2-furfural (ASTM, n.d.): m/e 96 (molecular ion, 100%), 95 (92%), 39 (89%), 29 (31%), 38 (28%), 37 (19%), 40 (9%), 97 (6%),

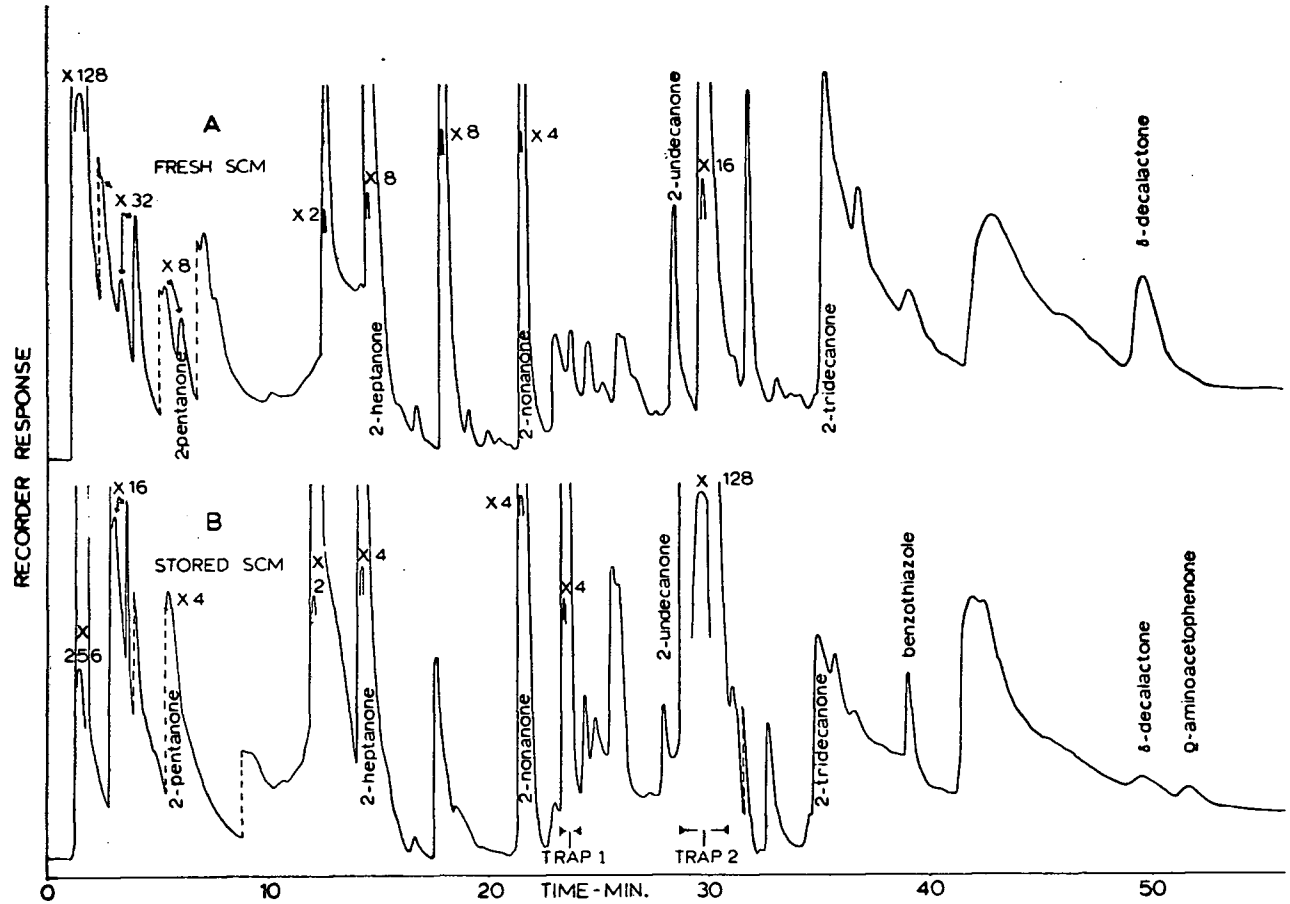


Figure 2. Chromatogram of flavor concentrate from A) fresh and B) stored samples of SCM; 2 1/2% Carbowax 20 M column, 70° C/4 min, 4° C/min to 200° C: trapped components designated

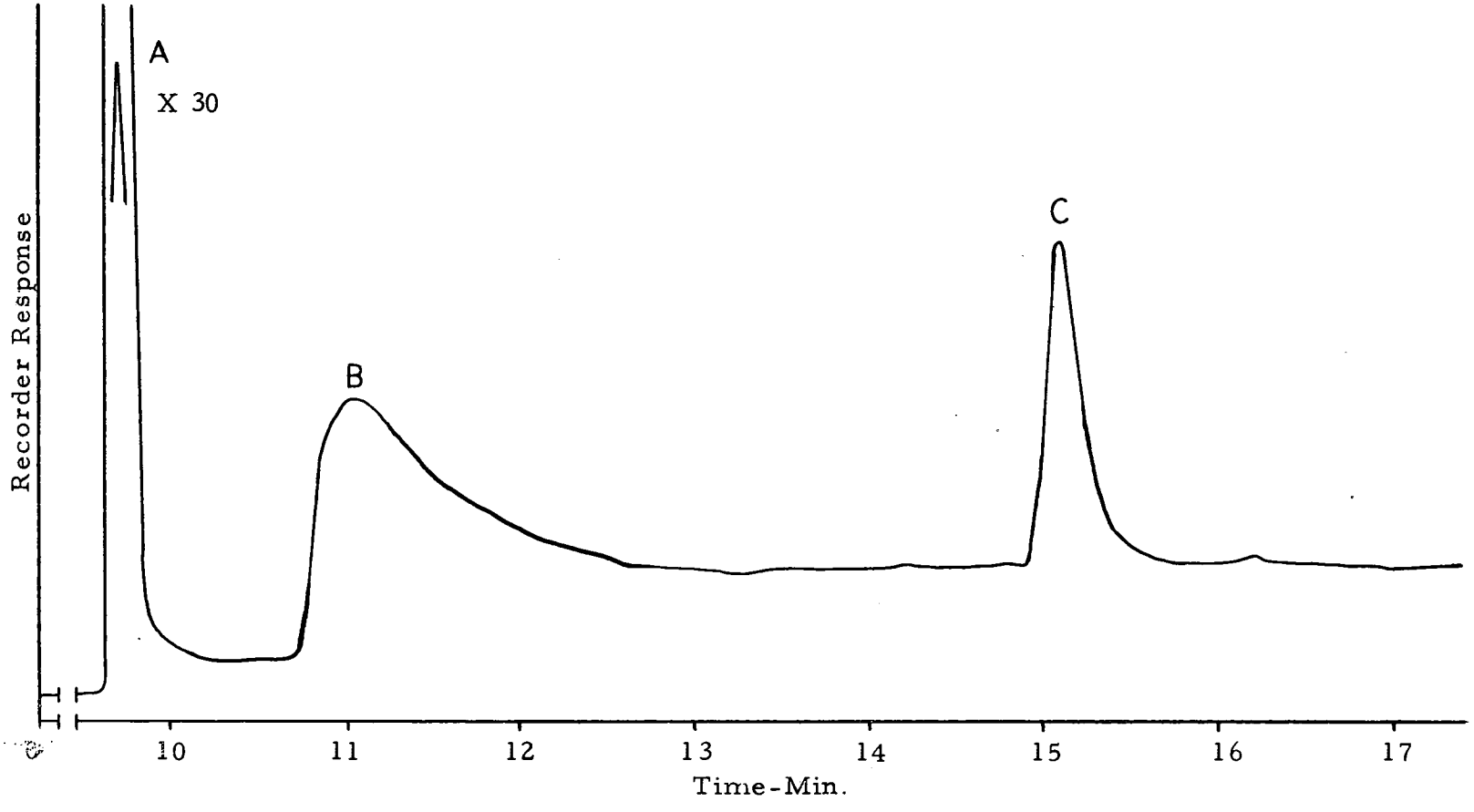


Figure 3. Chromatogram of the components of Trap 1 (from Figure 2); BDS capillary column, 150° C

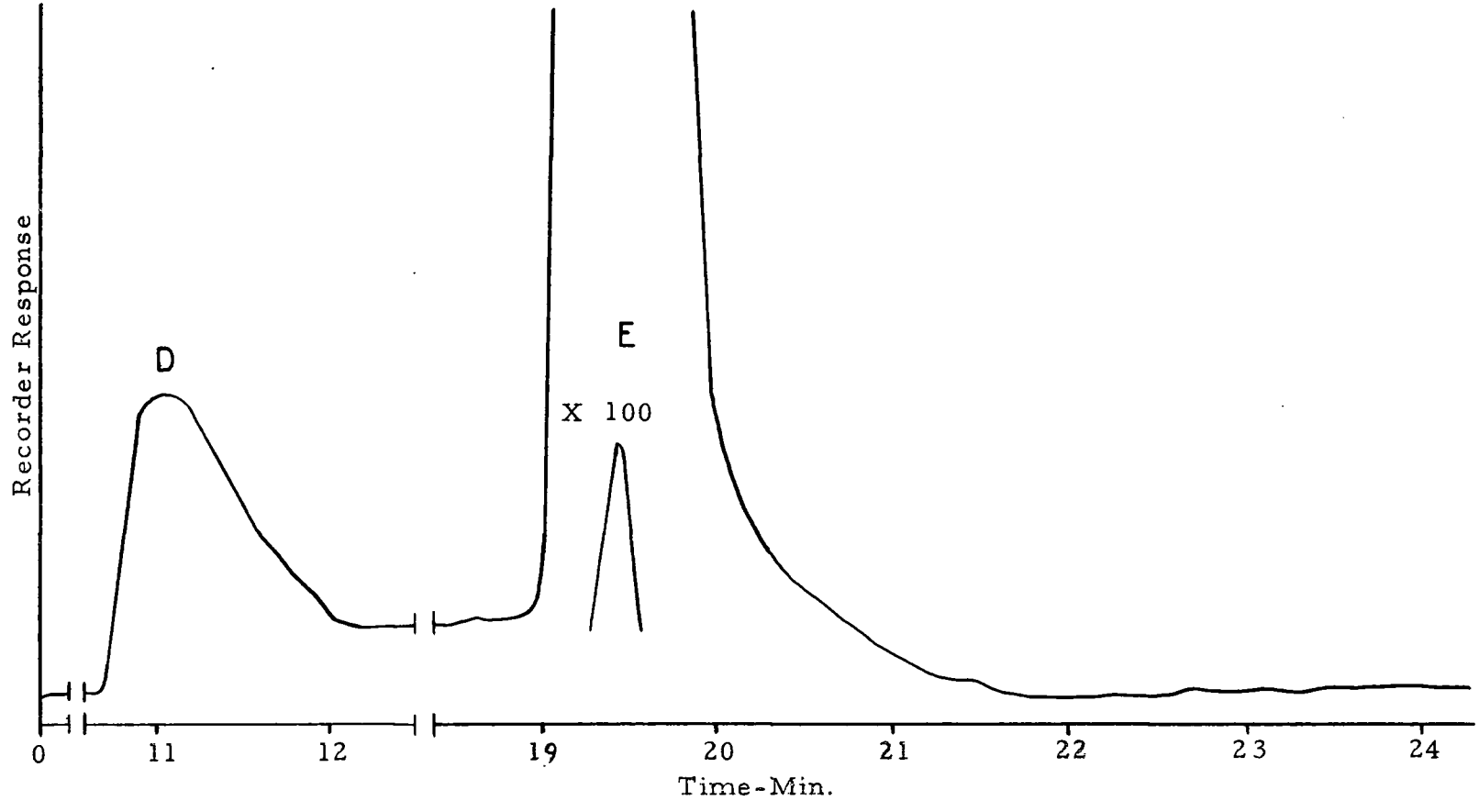


Figure 4. Chromatogram of the components of Trap 2 (from Figure 2); BDS capillary column, 150° C

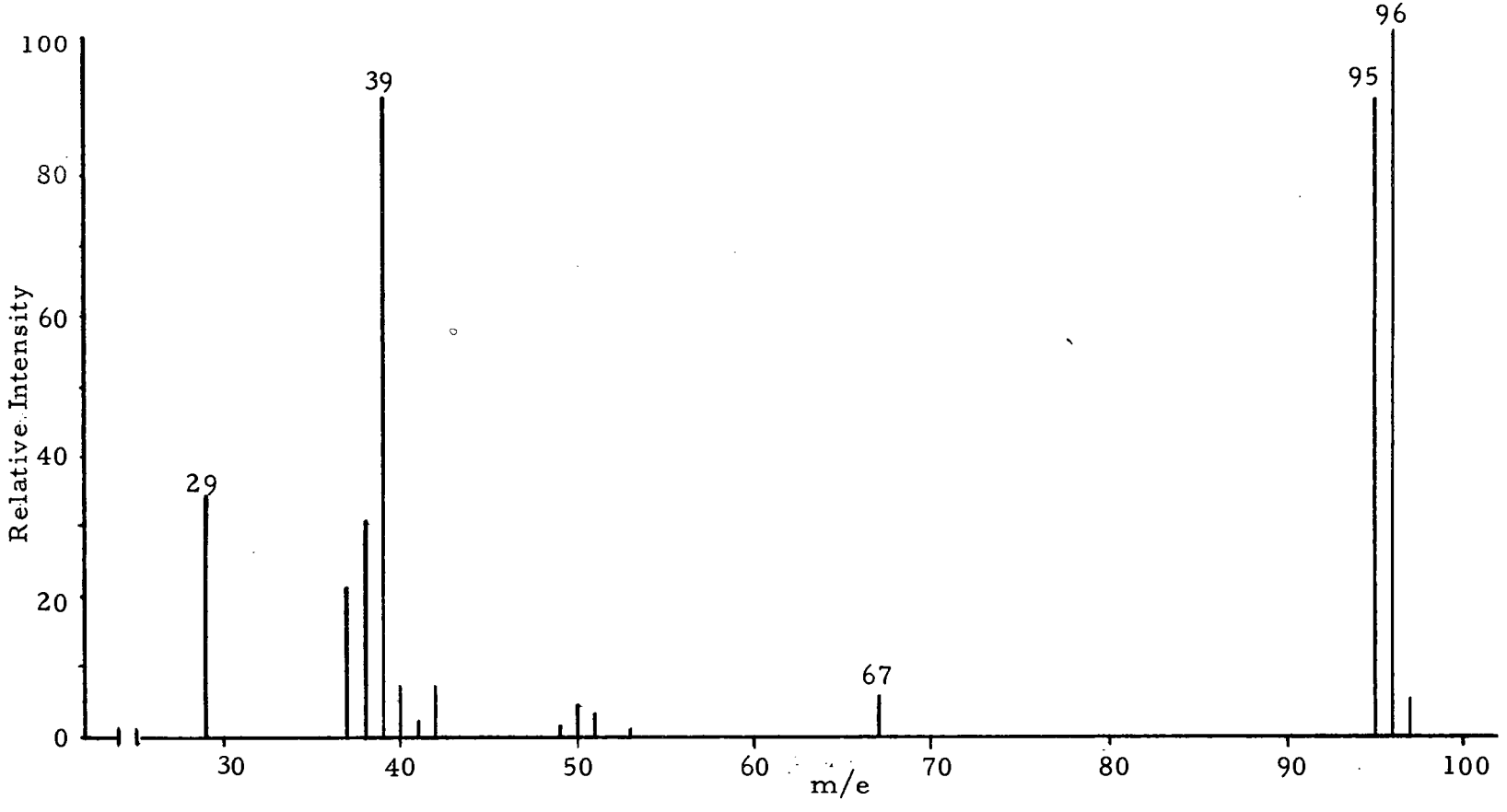


Figure 5. Mass spectrum of peak C, Trap 1

42 (7%), 67 (5%).

The mass spectrum of peak E, Trap 2, as shown in Figure 6, shows good agreement with the published spectrum of 2-furfurol (API, 1948 et seq.) : m/e 98 (molecular ion, 100%), 39 (97%), 41 (97%), 42 (77%), 53 (66%), 81 (66%), 29 (59%), 97 (55%), 27 (48%), 70 (34%), 69 (34%), 31 (29%), 51 (23%), 43 (21%), 52 (18%), 40 (16%), 44 (12%), 99 (6%).

The retention times of known samples of 2-furfural and 2-furfurol were determined on the packed Carbowax 20 M and the BDS capillary columns. The ratios of the retention time of the known compound to that of the corresponding unknown peak were as follows:

	Carbowax 20 M	BDS
2-furfural	1.00	1.03
2-furfurol	0.99	1.04

The two trapped peaks were therefore concluded to be 2-furfural and 2-furfurol, respectively.

Quantitative Studies

2-Furfurol

The GLC response curve for varying concentrations of 2-furfurol is shown in Figure 7. This curve shows a linear detector response over the range of concentrations studied. The fact that the

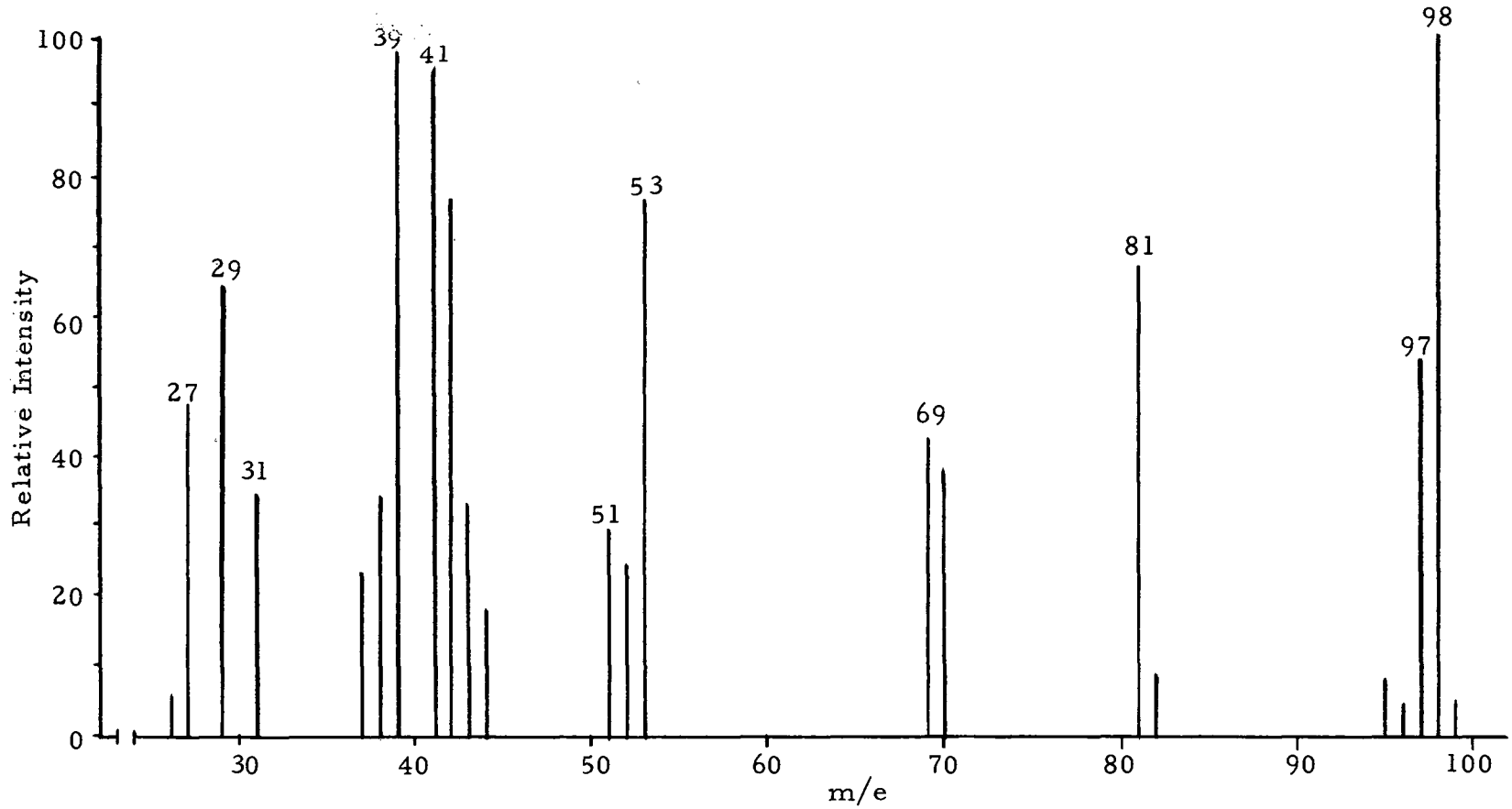


Figure 6. Mass spectrum of peak E, Trap 2

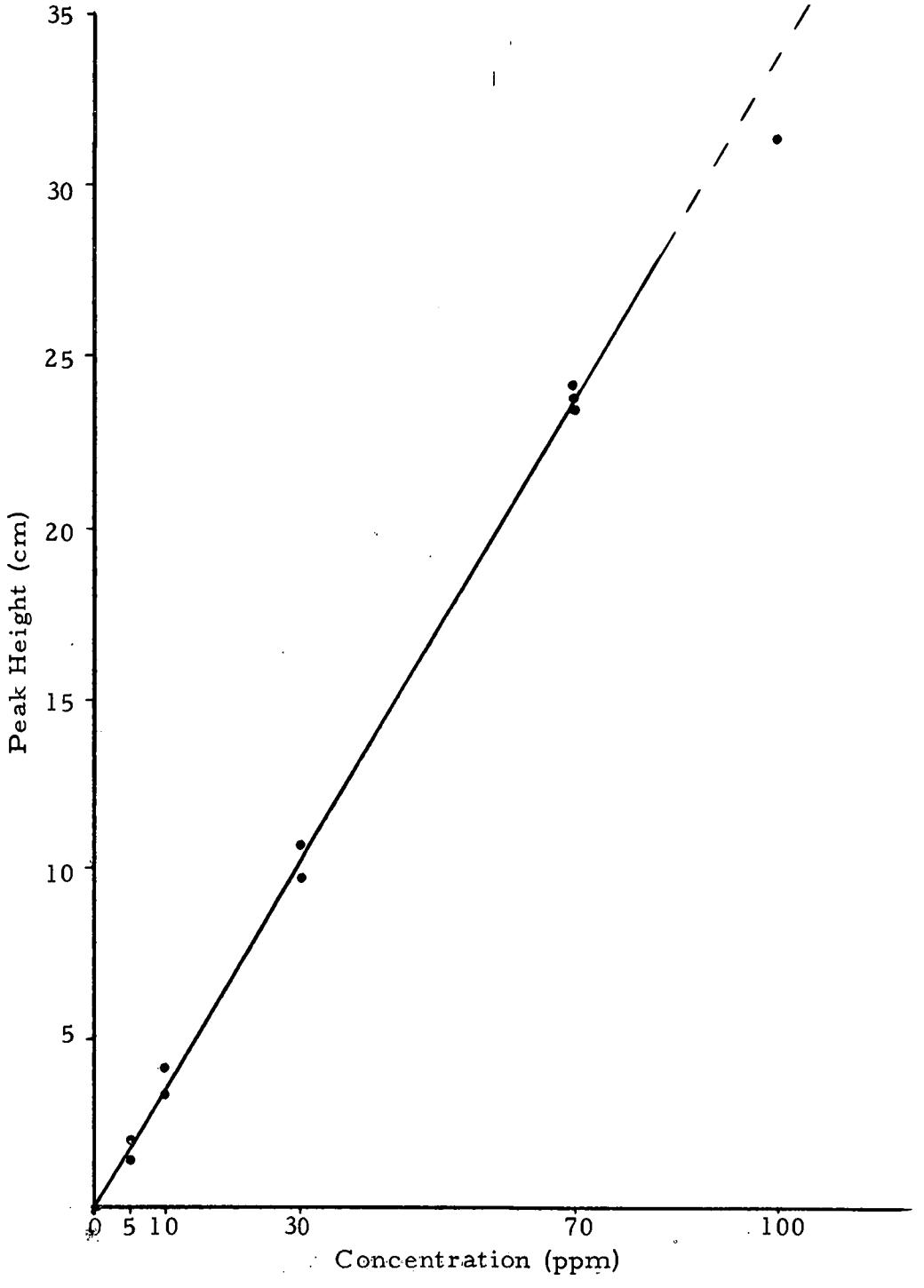


Figure 7. Recorder response with various concentrations of known 2-furfurol

two or more points determined for each concentration of 2-furfurol are in close proximity demonstrates the reproducibility of the technique.

The average concentration of 2-furfurol in samples of SCM, determined by the gas entrainment, on-column trapping GLC technique, are shown in Table 4. The concentration of this component increases upon storage at 27° C, showing a considerable increase after 26 weeks at this temperature.

Table 4. Average concentration of 2-furfurol in stored SCM, expressed as ppm on whole milk basis.

Commercial Sample	Weeks Storage at 27° C	ppm 2-furfurol
A-1	0	4.7
	13	7 0
	26	25. 3
B-1	0	3.5
	13	4 8
	26	15. 3
B-3	130	57.0

The significance of these levels of 2-furfurol is best appreciated by comparing them with the average flavor threshold (AFT) data. The AFT plot for 2-furfurol is shown in Figure 8. The AFT of 2-furfurol in milk was determined to be 10-12.5 ppm. It must then be concluded

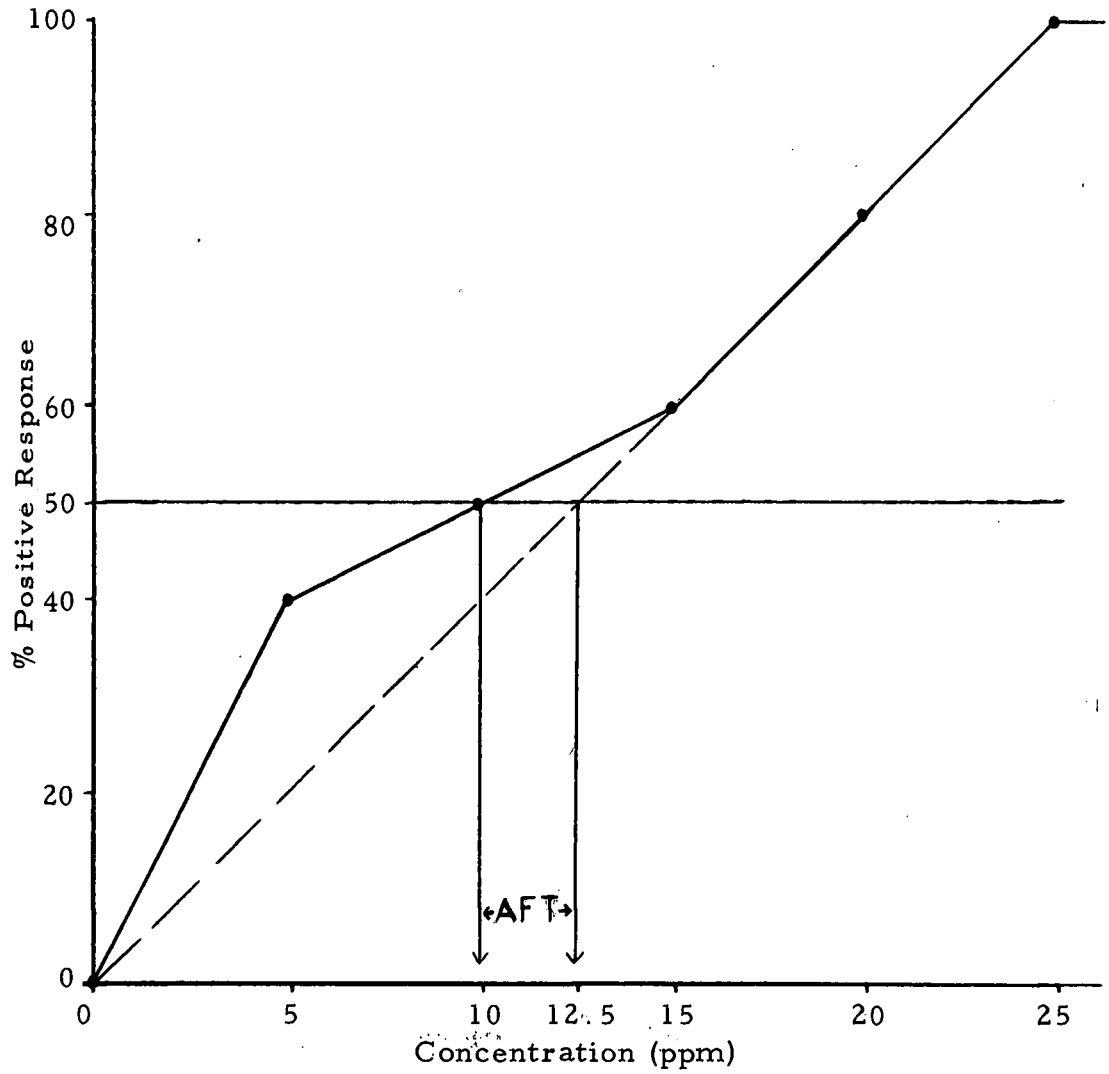


Figure 8. Flavor threshold curve for 2-furfurol in milk

that 2-furfurol contributes to the flavor of SCM after 26 weeks storage at 27° C. Considering the possibility of additive interaction between 2-furfurol and other compounds, levels less than 10-12.5 ppm might also be of significance. If this were the case, the levels of 2-furfurol found in samples stored for 13 weeks at 27° C, or even those found in fresh SCM might be of significance. The data suggests that this compound becomes increasingly important to the flavor of SCM as the product is stored.

2-Furfurol is a known product of non-enzymatic browning (Hodge, 1967, p. 472). The fact that 3.5 and 4.7 ppm 2-furfurol were found in the two fresh products suggests that some browning occurs during heat processing (forewarming, concentration and sterilization) of SCM. The increasing levels of 2-furfurol upon storage at 27° C are a reflection of the extent of non-enzymatic browning in the stored products. Further evidence for non-enzymatic browning in stored SCM is presented in the section on hydroxymethylfurfural (HMF) values.

Considering the relatively high levels of 2-furfurol found in this study, it is interesting to note that it is not among the list of compounds previously identified in stored milk products, as reviewed in Table 1. When the techniques used by the various workers are taken into consideration, the reason becomes apparent. The majority of these workers were looking specifically for carbonyl compounds

using carbonyl derivative reagents, or were using headspace GLC techniques. Both of these techniques would preclude the isolation of 2-furfurol. Two exceptions were the investigations of Muck et al. (1963) and Arnold, Libbey and Day (1966), who used solvent partitioning and solvent extraction techniques, respectively. In both cases, hydrocarbon solvents were used. It appears that 2-furfurol, being completely miscible with water, was not partitioned into the extraction solvent.

n-Methyl Ketones

The average recoveries of the individual methyl ketones at the completion of analysis were as follows:

n-methyl ketone:	C_3	C_5	C_7	C_9	C_{11}
Average % recovery:	31.6	43.0	45.7	46.1	45.7

These values were used in the calculation of the ketone concentrations in the milk samples. These recoveries are lower than those reported by Anderson (1966), but are similar to those of Langler (1963). It is possible that these recovery values might be higher than those achieved in actual analysis of SCM samples, since methyl ketones added to the product might not be associated or bound in the same manner as those occurring naturally in the product. This would result in low calculated values.

Results of the quantitative analysis of the C_3, C_4, C_5, C_7, C_9

and C_{11} n-methyl ketones in SCM are shown in Table 5. The data are given as average ppm of the respective ketone on a whole milk basis, as determined by duplicate analyses. The average percent deviation of the duplicates from their mean was as follows:

n-methyl ketone:	C_3	C_4	C_5	C_7	C_9	C_{11}
Average % deviation:	9.8	4.7	5.3	6.7	10.2	10.1

Table 5. Average concentrations of n-methyl ketones in stored samples of SCM, expressed as ppm on whole milk basis.

Commercial Sample	Weeks Storage at 27° C	Average ppm of n-methyl ketones					
		C_3	C_4	C_5	C_7	C_9	C_{11}
A-1	0	3.8	0.17	0.16	0.07	0.05	0.11
	6	4.1	----	0.27	0.24	0.10	0.24
	13	3.2	0.16	0.40	0.53	0.31	0.28
	26	3.5	0.19	0.51	0.59	0.37	0.38
A-3*	104	2.5	0.19	0.34	0.23	0.16	0.20
B-1	0	2.5	0.15	0.17	0.04	0.03	0.08
	6	2.2	----	0.20	0.15	0.12	0.14
	13	4.1	0.17	0.38	0.43	0.22	0.24
	26	2.4	0.16	0.45	0.57	0.47	0.34
B-3	130	3.5	0.13	0.27	0.52	0.22	0.23

*Stored at 1° C

The average flavor threshold of the individual methyl ketones, the average flavor threshold of the methyl ketone mixture (considering

additive interaction), and maximum attainable concentrations of n-methyl ketones in 4% milk, as reported by Langler and Day (1964), are presented in Table 6.

Table 6. Average flavor thresholds and maximum obtainable concentration of n-methyl ketones in 4% milk, as reported by Langler and Day (1964).

n-methyl ketone	AFT	Concentration at the AFT of the mixture	Concentration obtainable in 4% milk
C_4	79.5	0.13	0.52
C_5	8.4	0.20	0.80
C_7	0.7	0.38	1.52
C_9	3.5	0.18	0.72
C_{11}	15.5	0.20	0.80
C_{13}	18.4	0.46	1.84
Total	126.0	1.55	6.20

The concentrations of the n-methyl ketones found in stored SCM samples are well below their individual flavor thresholds, but are above the concentrations at the AFT of the methyl ketone mixture reported by Langler and Day (1964). The data thus indicates that the concentrations of the methyl ketones increase on non-refrigerated storage, reaching significant concentrations after 13 weeks storage at 27° C. Although the concentration of the C_{13} methyl ketone was not determined in this study, the fact that the other ketones are present in ratios similar to those reported by Langler and Day (1964)

suggests that the C_{13} methyl ketone would also exceed the concentration at the AFT of the mixture.

Another interesting observation is that the concentrations of methyl ketones does not increase to the maximum theoretical concentrations, even after 130 weeks storage at 27° C. The rate of increase in methyl ketone concentration appears to taper off between 13 and 26 weeks at 27° C.

o-Aminoacetophenone

The average recovery of o-aminoacetophenone at the completion of analysis was determined to be 39%. This value was used in calculating the concentration of o-aminoacetophenone in stored SCM samples. These concentrations are shown in Table 7.

Table 7. Concentration of o-aminoacetophenone in stored SCM, expressed as ppb on whole milk basis.

Commercial Sample	Weeks Storage	ppb o-aminoacetophenone
A-1	0	Not detected
	13	1.2
	26	2.4
B-1	0	Not detected
	13	1.5
	26	2.7

Comparing the values in Table 7 with the threshold for o-aminoacetophenone of 0.5 ppb (Parks, 1967) suggests that this compound reaches significant concentrations after 13 weeks storage at 27° C, and is of definite significance after 26 weeks at 27° C.

Acid Degree Values

Average acid degree values (ADV) for the SCM samples are shown in Table 8. These values show a gradual increase on storage at 27° C. The suggested interpretation of ADV is as follows (MIF, 1959, p. 324):

	ADV
Normal fresh milk	0.2 - 0.5
Experienced judges detect rancidity	1.3 - 1.6
Customer complaints expected	> 2.0

ADV determined for fresh raw and pasteurized milk, also presented in Table 8, are somewhat greater than the suggested values. Experience with the test in the OSU Dairy Products Laboratory has indicated that test results are commonly somewhat higher than the suggested values.

On the basis of the ADV results, it was concluded that free fatty acid levels do not increase to significant levels in SCM after as long as 26 weeks at 27° C. The steady gradual increase in ADV

suggests simple hydrolysis, as proposed by Parks (1967), rather than reactivation of the lipase enzyme, as suggested by some other workers.

Table 8. Average acid degree values of stored SCM.

Commercial Sample	Weeks Storage at 27° C	Acid Degree Value
A-1	0	0.71
	6	0.72
	13	0.81
	26	0.98
B-1	0	0.93
	6	1.06
	13	1.18
	26	1.33
Fresh raw milk		0.58
Fresh pasteurized milk		0.74

Hydroxymethylfurfural (HMF) Values

Average HMF values for the SCM samples are reported in Table 9. HMF values increase slowly through 13 weeks storate at 27° C, and show a dramatic increase after 26 weeks storage. This suggests the slow continuation of the heat initiated non-enzymatic browning reaction during the first 13 weeks, followed by a more rapid rate of reaction during the second 13 weeks. This roughly

corresponds to visual observation of the stored products, and also agrees with the quantitative results reported for 2-furfurol.

Table 9. Hydroxymethylfurfural (HMF) values of stored SCM, expressed as micromoles/liter on whole milk basis.

Commercial Sample	Weeks Storage at 27° C	Micromoles HMF Per Liter
A-1	0	0.96
	6	3.54
	13	4.02
	26	28.0
B-1	0	0.65
	6	1.70
	13	2.53
	26	17.10
B-3	130	29.15

Keeney and Bassette (1959) reported that HMF values ranging from 3.9 to 28.3 for nonfat dry milks, and values of 13.3 and 35.8 for evaporated milk and evaporated skim milk, respectively. They found that HMF values greater than 5.0 corresponded with cereal-like flavor defects in nonfat dry milk. Although comparison of HMF values between nonfat dry milk and evaporated milk is difficult, it appears that non-enzymatic browning has progressed to such an extent after 26 weeks storage of SCM at 27° C as to have a significant effect on flavor. The effect of non-enzymatic browning after 13 weeks

at 27° C is questionable.

Summary of Quantitative Studies

In summary, this investigation has shown that n-methyl ketones and o-aminoacetophenone reach significant levels in SCM after 13 weeks at 27° C, and that 2-furfurol exceeds its threshold concentration after 26 weeks at 27° C. Free fatty acids do not reach significant levels during this storage period.

Possessing this information, the question becomes one of steps which can be taken to prevent flavor deterioration of SCM. It is obvious that during the storage of SCM, a number of diverse chemical reactions, such as methyl ketone formation, lactone formation, non-enzymatic browning, etc., are taking place. The possibility of inhibiting these reactions might thus be a first suggestion. The formation of ketones and lactones can be prevented by removal of their precursors. This can be accomplished by separation of the fat from the product and steam stripping the fat. At best, this is a costly operation.

Possible inhibitors of non-enzymatic browning are discussed by Reynolds (1965, p. 245-262). Among these inhibitors are sulfites and bisulfites. These inhibitors have been studied in dried fruits and vegetables. Information concerning their effects on milk is not available. Laws regarding the addition of additives to milk would,

at present, prevent the use of any such inhibitor in SCM. The most readily available procedure for inhibiting browning of SCM is refrigeration. This conflicts with one of the economic advantages of this product, however.

A second possibility for overcoming the flavor deterioration of SCM, as has been suggested by Graham (1965), concerns the use of additives to improve, camouflage or mask flavors. Although this would require modification of laws relating to the use of additives in milk, it has distinct possibilities. It might be necessary to accept those changes which do occur in stored SCM and to seek suitable additives or flavorings which will minimize the flavor deteriorative effects of these changes. To date, no work has been reported along this line.

Heat Degradation of Thiamine

Hydrogen Sulfide Production

The distinctive odor of hydrogen sulfide was noted when tubes containing 0.05 M solutions of thiamine at pH 6.7 were opened following heating. A piece of filter paper dipped in a solution of lead acetate turned black when held over one of these opened tubes. No hydrogen sulfide odor or lead acetate reaction was detected with unheated solutions, or with heated 0.05 M thiamine solutions at pH

3.5

Further evidence for the production of hydrogen sulfide by heat degradation of thiamine was obtained during the gas entrainment, on-column trapping technique. The odor of hydrogen sulfide was detected during the purging step when the effluent end of the GLC column was disconnected from the hydrogen flame detector, indicating that hydrogen sulfide was being purged from the sample but was not being stopped in the cold trap section of the column. When the column effluent was bubbled through a solution of 5% zinc acetate and 1.5% sodium acetate, a white precipitate was formed and the odor of hydrogen sulfide disappeared. It was therefore concluded that hydrogen sulfide was one of the heat degradation products of thiamine.

Identification of Volatile Components

Chromatograms of heated and unheated 0.05 M thiamine solutions, pH 6.7, obtained by the gas entrainment, on-column trapping GLC technique, are shown in Figure 9. New peaks or peaks sizably increased as a result of heating are labeled 1-5 in the chromatogram of heated thiamine. Components trapped for mass spectral analysis are also indicated on this chromatogram.

The Carbowax 20 M capillary column chromatogram of the components of Trap I is shown in Figure 10. The mass spectrum of peak A, Trap I, exhibited a predominant m/e 207, with smaller m/e

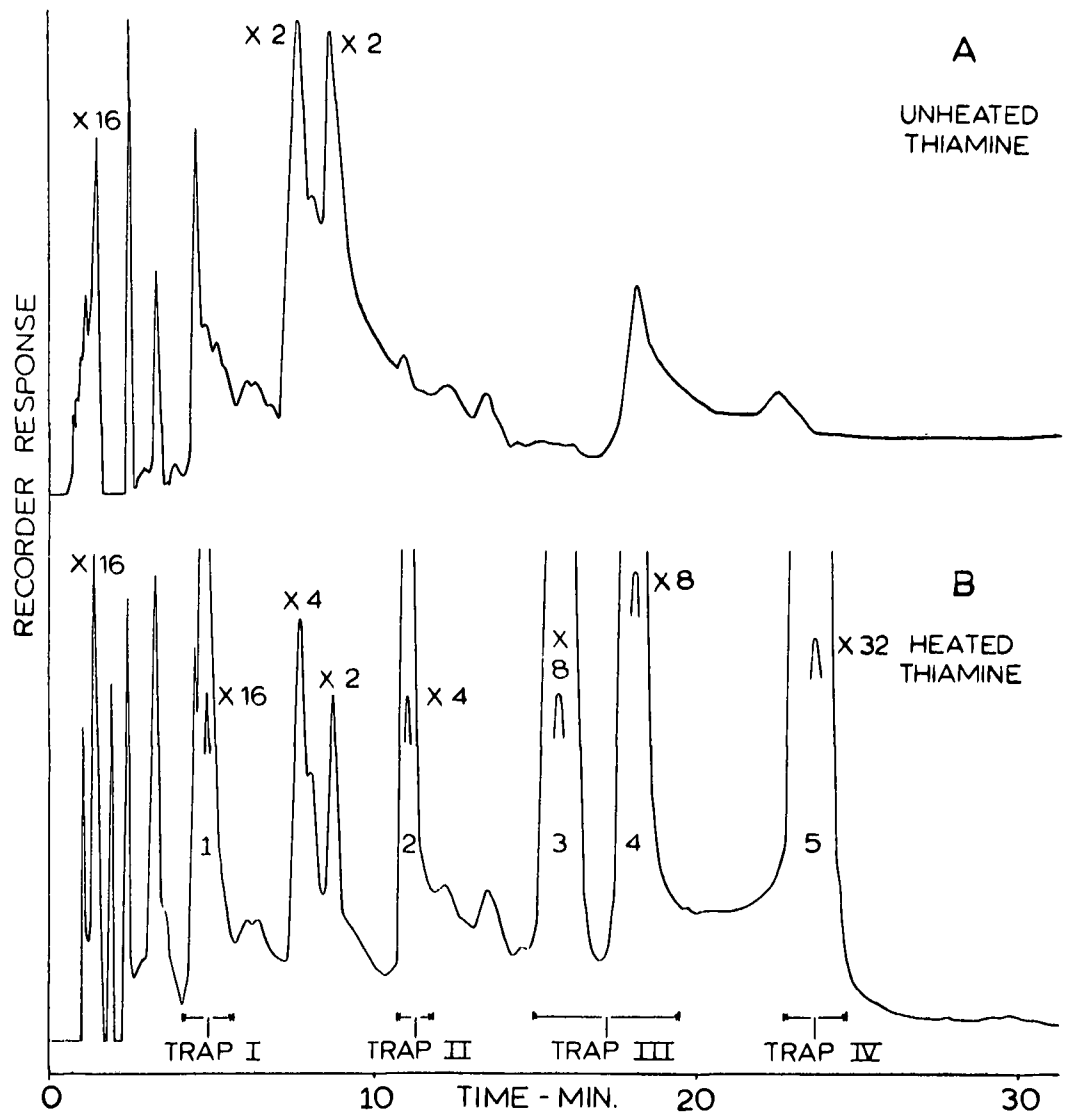


Figure 9. Gas entrainment, on-column trapping chromatograms of A) unheated and B) heated (15 min, boiling water bath) 0.05 M thiamine solutions, pH 6.7; 1,2,3-tris(2-cyanoethoxy) propane column, 70° C

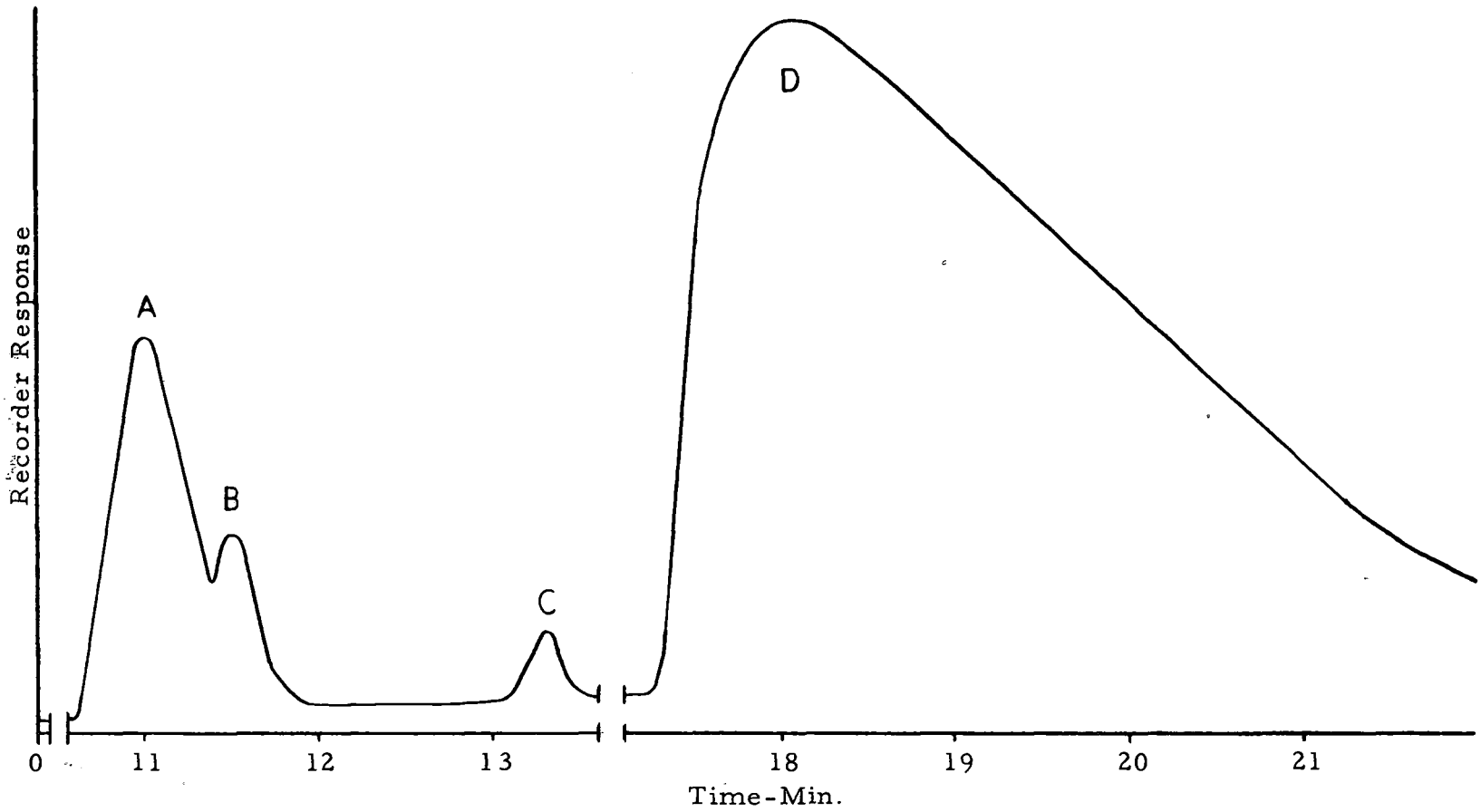


Figure 10. Chromatogram of the components of Trap I (from Figure 9); Carbowax 20 M capillary column, 80° C

133, 208 and 209. This suggests the commonly encountered silicone contaminant (Biemann, 1962, p. 172). This contaminant occurs frequently with the trapping and on-column injection technique. Peak C, with m/e 267, is another silicone contaminant (Biemann, 1962, p. 172). Peak D is due to water. The mass spectrum of peak B, Trap I, as shown in Figure 11, is in agreement with the published spectrum of 2-methyl furan (API, 1948 et. seq.) : m/e 82 (molecular ion, 100%), 53 (76%), 81 (62%), 39 (49%), 27 (38%), 50 (19%), 51 (18%), 29 (16%), 28 (14%), 26 (14%), 38 (11%), 52 (10%), 37 (9%), 83 (5.6%). The retention time of 2-methylfuran was determined on the 1,2,3-tris-(2-cyanoethoxy) propane and Carbowax 20 M columns. Relative retention times, known : unknown, were 1.00 on the Tris column and 1.01 on the Carbowax column. The identity of the unknown component was thus concluded to be 2-methylfuran.

The component in the Trap II region of the packed column chromatogram (Figure 9) could not be recovered in sufficient quantity by the trapping technique to obtain a mass spectrum.

The capillary column chromatogram of the components of Trap III is shown in Figure 12. The mass spectra of peaks E and F of this chromatogram indicated that they were due to ethyl ether and the silicone contaminant, respectively. Ethyl ether was also thought to be a contaminant, as this solvent was used to clean syringes in the same room in which the components were trapped for mass

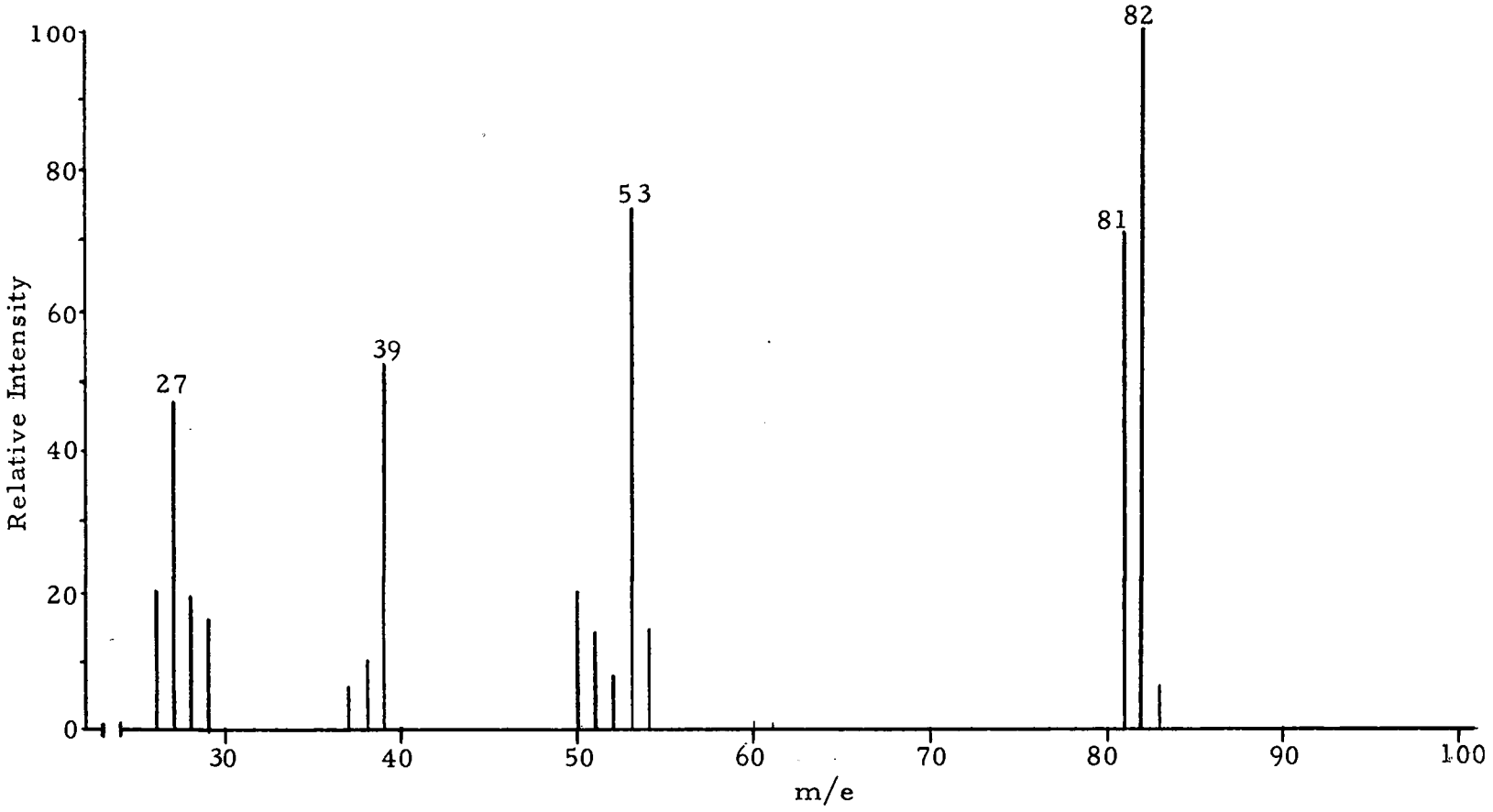


Figure 11. Mass spectrum of peak B, Trap I

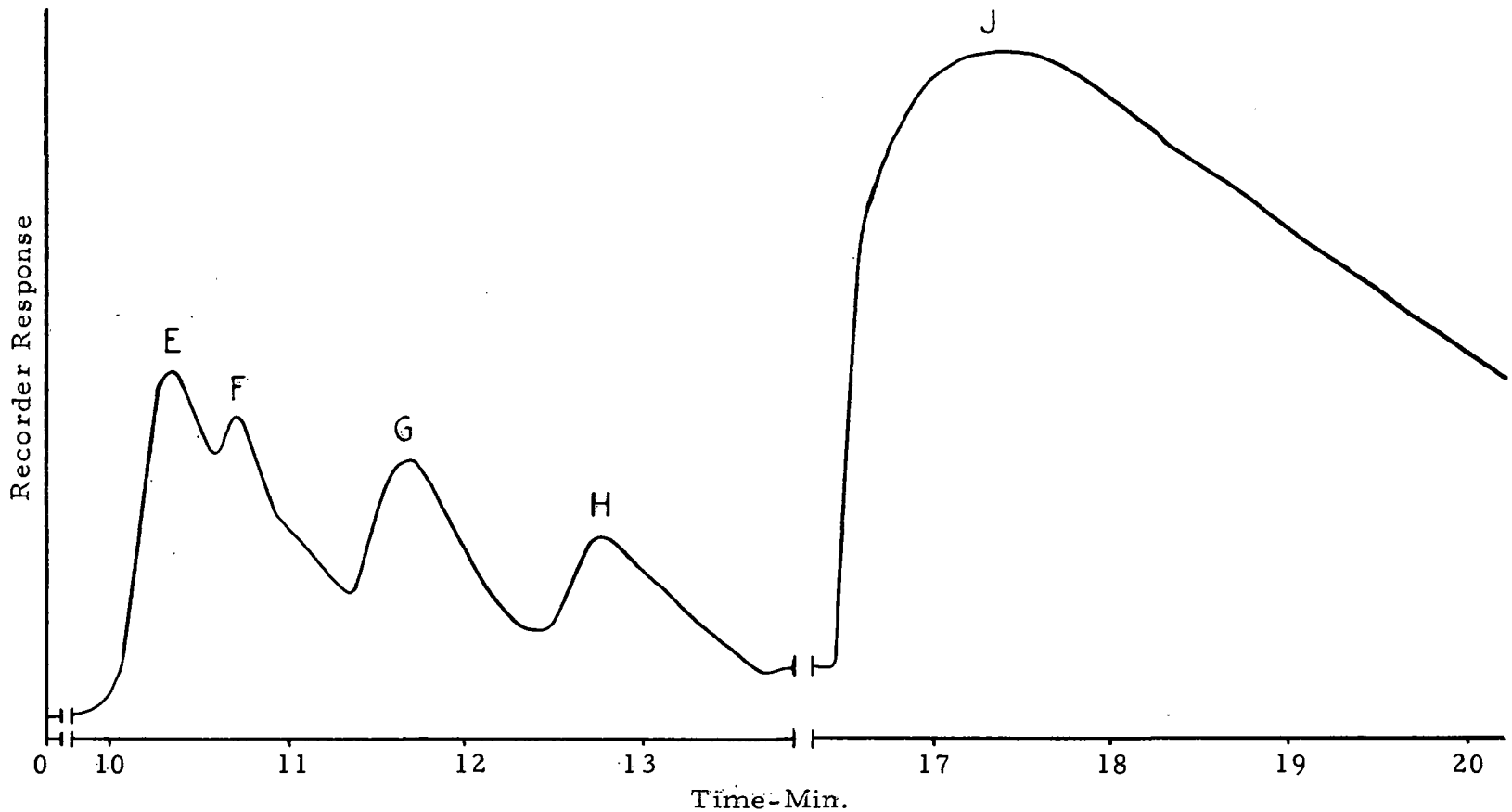


Figure 12. Chromatogram of the components of Trap III (from Figure 9); Carbowax 20 M capillary column, 80° C

spectral analysis. Peak J of Trap III is again due to water.

The mass spectrum of peak G, Trap III, is shown in Figure 13. This spectrum could not be interpreted as being due to any one compound. There appears to be a compound with a molecular ion of m/e 86. The spectra shows considerable hydrocarbon character, but the fragmentation pattern does not fit any of the isomeric C_6 hydrocarbons (mw 86). Retention times for C_6 hydrocarbons likewise showed no agreement. The retention time of 2-pentanone (mw 86) was within 7% of that of the unknown (peak 3, Figure 9) on the Tris column, suggesting that a C_5 carbonyl compound might be contributing to the spectrum. It was concluded that this spectrum is most likely due to two or more unresolved components whose identity cannot be established by the mass spectral data.

Peak H, Trap III, exhibited, in addition to m/e 267 from the silicone contaminant, the mass spectrum shown in Figure 14. This spectrum is in good agreement with the published spectrum of 2- or 3-methyl thiophene (API, 1948 et. seq; Budzikiewicz, Djerassi and Williams, 1964, p. 232). The spectrum of known 2-methyl thiophene is as follows: m/e 97 (100%), 98 (molecular ion, 55%), 45 (21%), 39 (13%), 53 (8.5%), 99 (7.4%), 27 (7%), 58 (6%), 69 (5%), 71 (4%), 100 (2.4%). The spectrum of 3-methyl thiophene is very similar, making it difficult to differentiate between the two isomers by mass spectrometry. Budzikiewicz, Djerassi and Williams (1964, p. 232)

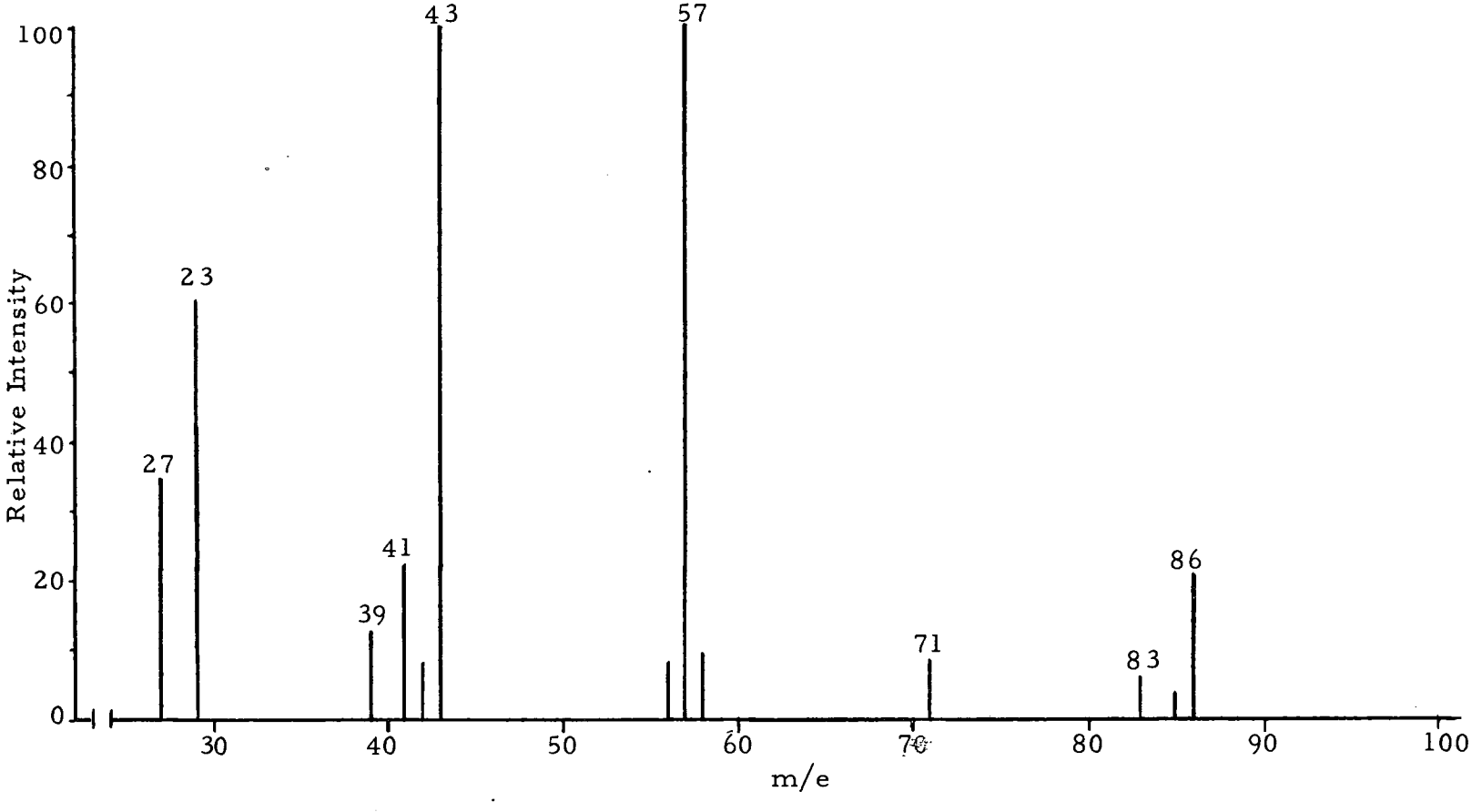


Figure 13. Mass spectrum of peak G, Trap III

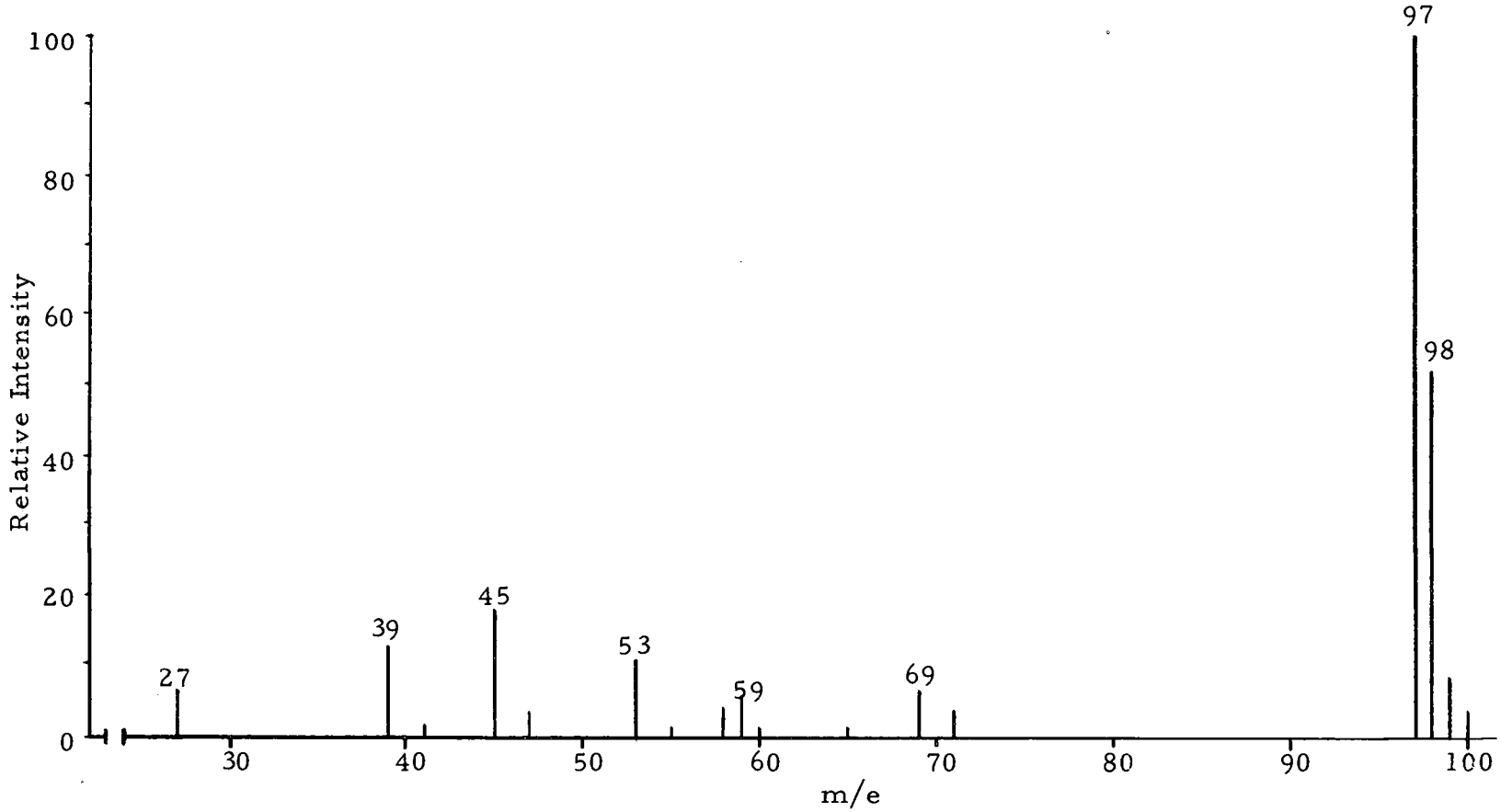


Figure 14. Mass spectrum of peak H, Trap III

point out that when the 2-position is occupied by a methyl group, the m/e 59 peak is 5-15% of the base peak (m/e 97). When the 2- and 5-positions are unoccupied, or are occupied by groups other than methyl, m/e 59 is only 1-5% of the base peak. In the spectrum of the unknown component, m/e 59 is 6% of the base peak, suggesting that the component is most likely 2-methyl thiophene. Further evidence for the identity of the unknowns was obtained by comparing the retention time of the unknown with that of 2- and 3-methyl thiophene. The ratio of retention times, known/unknown, were as follows:

	Tris column*	Carbowax column
2-methylthiophene	0.997	1.01
3-methylthiophene	1.30	1.17

*Unknown peak 4, Figure 9.

On the basis of this data, the identity of the unknown was concluded to be 2-methylthiophene.

The capillary column chromatogram of the components of Trap IV is shown in Figure 15. The mass spectra of peaks K and L showed m/e 207 and 267, respectively, indicating that they were due to the two silicone contaminants. Peak N was due to water.

Peak M of Trap IV was the largest peak encountered in the trapping of components for mass spectral analysis. The mass spectrum of this component is shown in Figure 16. No published

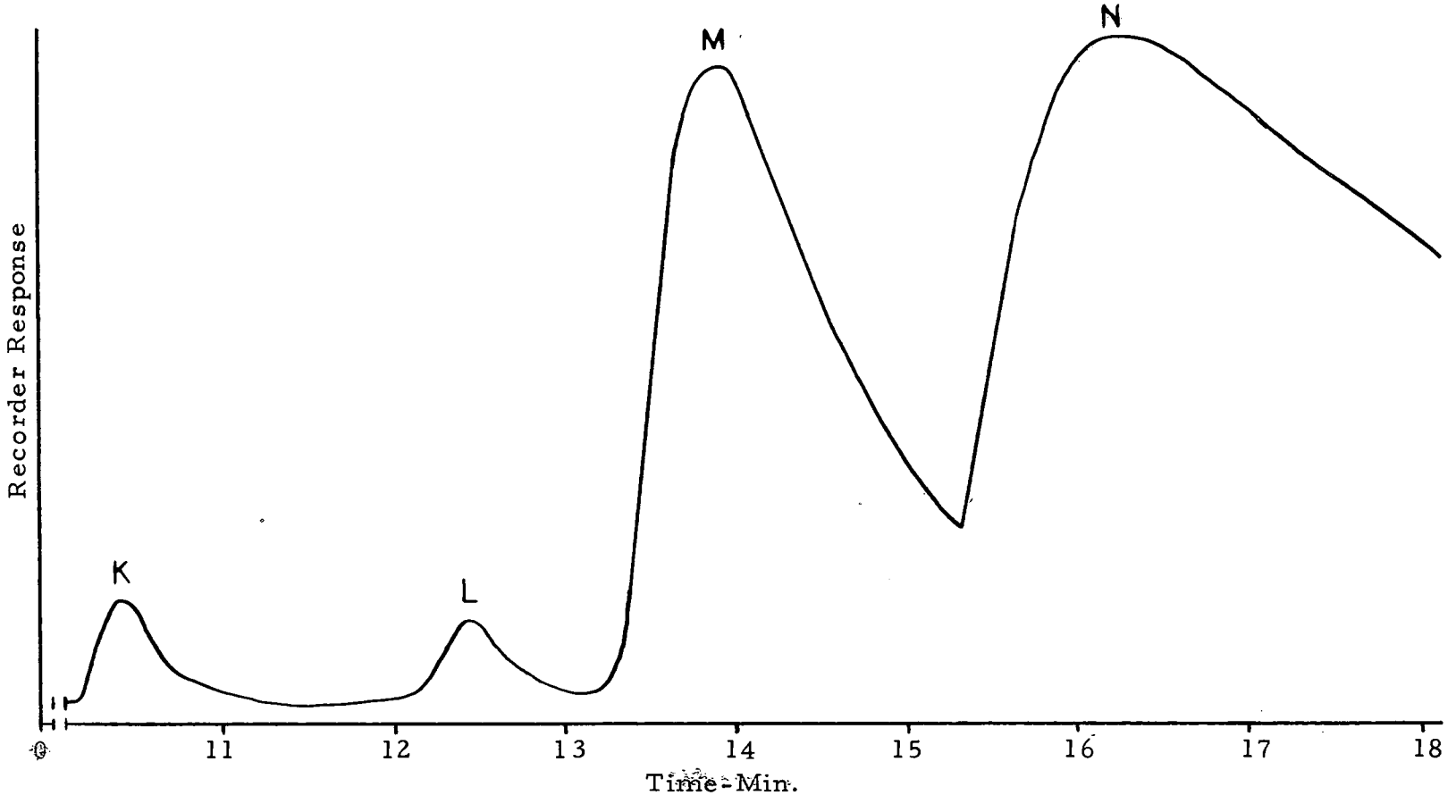


Figure 15. Chromatogram of the components of Trap IV (from Figure 9); Carbowax20 M capillary column, 80° C

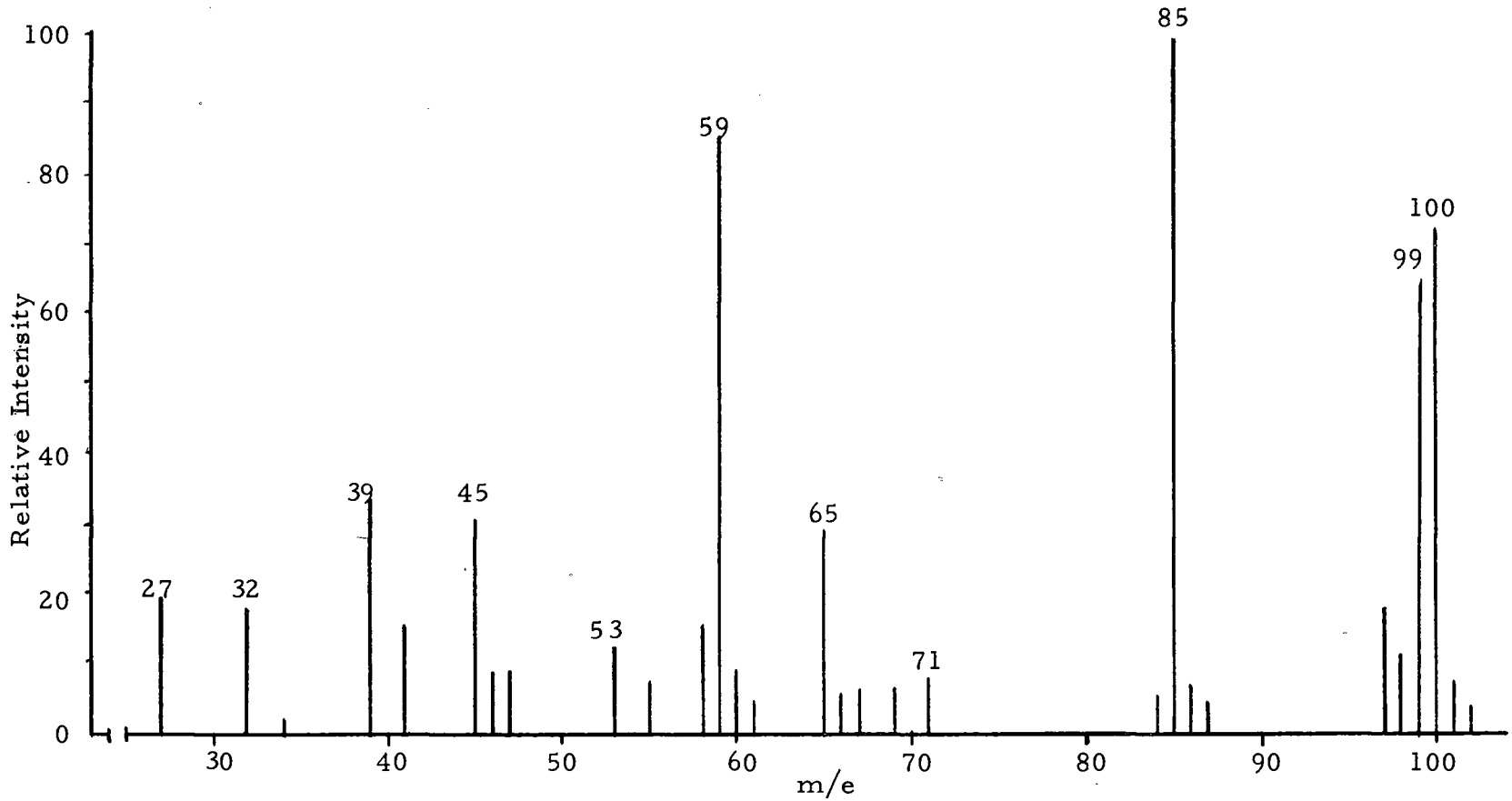
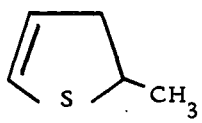
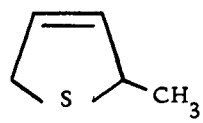


Figure 16. Mass spectrum of peak M, Trap IV

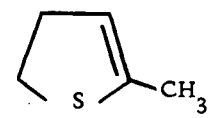
mass spectrum similar to that of the unknown could be located. A dihydro-methyl thiophene structure appeared to plausibly fit the data. Possible isomers of dihydro-2-methyl thiophene are shown below.



2,3-dihydro-
2-methyl-
thiophene



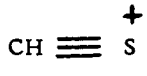
2,5-dihydro-
2-methyl-
thiophene



4,5-dihydro-
2-methyl-
thiophene

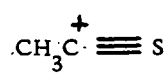
Similar isomers could exist for dihydro-3-methyl thiophene. The possible identification of the unknown as one of these structures was postulated after comparison of the mass spectrum with that of the 2-methyl thiophene. This spectrum shows a large p-1, which is characteristic of substituted furans and thiophenes in general. The molecular ion is two mass units higher than that of 2-methyl thiophene, suggesting the possibility of a similar compound with one less double bond. Other fragments prominent in the spectrum of the unknown, namely m/e 39, 45, 58 and 59 are frequently encountered in substituted thiophene type molecules, and are attributed to the following rearrangement ions (Budzikiewicz, Djerassi and Williams, 1964):

m/e 39



m/e 45

m/e 58



m/e 59

The large m/e 85 (the base peak of the spectrum) suggests the ready loss of a methyl group from the molecular ion. The spectrum of methyl tetrahydrofuran (API, 1948 et. seq.) shows p-15 as the base peak. Since the mass spectral behavior of furan and thiophene compounds is very similar (Budzikiewicz, Djerassi and Williams, 1964) the prominent p-15 fragment of the unknown would, by analogy, be consistent with the proposed partially reduced thiophene structure.

None of the isomeric dihydro-2- or 3-methyl thiophenes were available from chemical suppliers for comparison of retention times with that of the unknown. Birch and McAllan (1951) showed that reduction of 2- and 3-methyl thiophenes with sodium in liquid ammonia-methanol produced a mixture of unreduced methyl thiophene, 2,5- and 4,5-dihydromethylthiophenes and pentane thiols. The unreduced methyl thiophene and dihydromethylthiophenes could be recovered in the neutral fraction. This reaction was carried out with the 2- and 3-methyl thiophenes to determine if any of the reaction products had retention times similar to that of the unknown. The reaction was carried out as described below.

Five g of 2- or 3-methyl thiophene and 21 ml of absolute methanol were placed in a 100 ml three-neck flask equipped with a sealed stirrer and a dry ice cooled reflux condensor. The contents of the flask were cooled to -40° C with dry ice, and 20 ml of liquid ammonia was blown into the cooled, stirred solution. Two and one-half g of finely divided sodium metal was added during eight hours while the reaction temperature was maintained between -35 and -45°C. The reaction mixture was subsequently allowed to warm to room temperature overnight to evaporate ammonia. Thirty-five ml of ice water was added during vigorous stirring, while the temperature was maintained below 20° C. The neutral reaction products were recovered by extraction of the aqueous solution with two 25 ml quantities of isopentane. The isopentane was fractionally distilled using a Snyder floating ball type distillation column.

Gas-liquid chromatography of the isopentane extracts using a 1, 2, 3-tris-(2-cyanoethoxy) propane column revealed that there were three major components in the neutral fraction. One of these components was unreduced methyl thiophene, and the other two were presumed to be the two isomeric dihydromethyl thiophenes, as reported by Birch and McAllan (1951). One of the isomeric dihydro-2-methyl thiophenes had a retention time identical to that of the unknown component (peak 5, Figure 9) on the Tris column, while the second isomer had a slightly greater retention time. The retention times of the dihydro-3-methyl thiophenes were much greater than that of the unknown component.

The two dihydro-2-methyl-thiophene isomers were collected and mass spectra were obtained, as follows:

First isomer: m/e 85 (100%), 59 (82%), 100 (molecular ion, 77%), 99 (67%), 39 (41%), 45 (38%), 65 (28%), 58 (23%), 41 (21%), 53 (18%), 27 (18%), 101 (10%), 97 (10%), 71 (10%), 55 (7%), 98 (5%), 102 (2.5%).

Second isomer: m/e 85 (100%), 100 (molecular ion, 40%), 45 (34%), 39 (34%), 59 (27%), 65 (15%), 41 (15%), 27 (15%), 99 (11%), 97 (8%), 67 (8%), 58 (8%), 53 (8%), 98 (4%), 71 (4%), 101 (2%), 102 (1%).

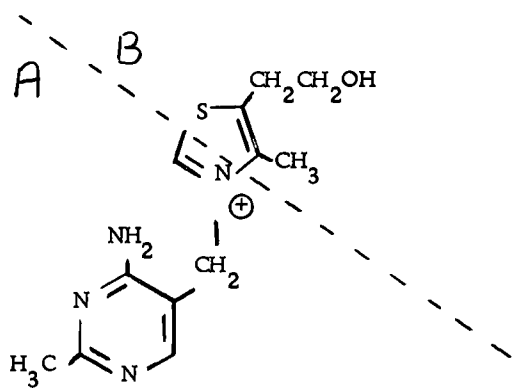
The mass spectrum of the first isomer is in excellent agreement with that of the unknown compound (Figure 16). On the basis of this information and the agreement of retention times, the unknown was concluded to be a dihydro-2-methyl thiophene.

The position of the double bond is difficult to establish from the mass spectral data. The m/e 85 fragment, resulting from the loss of the methyl group, is the base peak of both isomers. The fact that the first isomer does not lose methyl as readily as the second isomer suggests that the first isomer is the reaction product with the double bond in the 2, 3-position (4, 5-dihydro-2-methyl thiophene). Birch and McAllan (1951) reported that the boiling point of the 4, 5-dihydro isomer of 3-methyl thiophene is 8° C less than that of the 2, 5-dihydro isomer. Assuming that the isomers of dihydro-2-methyl thiophene show a similar pattern, the 4, 5-dihydro-2-methyl thiophene would be expected to have the lower boiling point and would therefore be the first isomer to elute from the GLC column. On the basis of this

reasoning, the unknown was tentatively identified as 4, 5-dihydro-2-methyl thiophene.

Mechanism of Thiamine Degradation

The mechanisms involved in the heat degradation of thiamine present an interesting problem for the theoretical organic chemist. It is apparent that the heat degradation of thiamine has a drastic effect on the thiazole portion of the molecule. The volatile products identified can be explained as thermal rearrangement and elimination



products of the "B" portion of thiamine, as pictured. Whether the thiazole ring opens first, followed by cleavage of the C_5H_8OS fragment, or whether the thiazole moiety is cleaved from the pyrimidine moiety and then undergoes further degradation is now known.

Flavor Significance

The flavor significance of the volatile components arising from heat degradation of thiamine is of interest. Hydrogen sulfide is

considered an important flavor compound in a number of foods. It has been implicated as a cause of the cooked flavor of heated milk.

Hodge (1967, p. 972) describes the odor of 2-methyl furan as "ethereal." This compound has been identified in coffee volatiles by a number of workers (Gianturco, 1967, p. 442). Merritt et al. (1963) suggested that 2-methyl furan is among the more significant compounds contributing to the characteristic coffee aroma.

The odor of 2-methyl thiophene is best described as "heated onion" or "sulfury." The dihydro-2-methyl thiophene possesses a similar, but much sharper, odor. The odor characteristics of these two compounds suggest that they could be important contributors to certain food flavors.

In summary, it would appear that the volatile compounds produced by heat degradation of thiamine may contribute to food flavors. These compounds may be of considerable importance in the flavors imparted during cooking of foods high in thiamine.

SUMMARY AND CONCLUSION

This investigation was concerned with the definition of the chemical changes occurring during storage of SCM leading to flavor deterioration of the product.

Flavor compounds of stored SCM were isolated by vacuum steam distillation, followed by ether extraction of the distillates. Volatile flavor compounds in the ethereal concentrates were separated by gas-liquid chromatography (GLC). Important components were collected from the GLC effluent, and were subsequently analyzed by capillary column GLC in conjunction with mass spectrometry. A technique for transferring trapped components directly onto a capillary GLC column was developed.

Commercial samples of SCM were obtained and stored under controlled conditions. Flavor panel evaluations and quantitative determinations of methyl ketones, o-aminoacetophenone, 2-furfurol, acid degree value and hydroxymethylfurfural values were carried out at storage intervals.

Concentrations of odd-numbered n-methyl ketones, C_3-C_{11}, and of o-aminoacetophenone were determined by measuring the absorbance of their respective 2,4-dinitrophenylhydrazone derivatives. The concentration of 2-furfurol was determined by the gas entrainment, on-column trapping GLC technique. Acid degree values

were determined by titration of SCM milk fat. Hydroxymethylfurfural values were determined by measuring the absorbance of the thiobarbituric acid reaction product.

The effect of heat on thiamine solutions at pH 6.7 was studied. Volatile compounds produced were isolated and separated by the gas entrainment, on-column trapping GLC technique. These components were trapped and analyzed by capillary column GLC in conjunction with mass spectrometry, using the system developed for transferring trapped components directly onto the capillary column.

The findings of this investigation were as follows:

1. 2-Furfural and 2-furfurol were identified as flavor components of stored SCM. These compounds had not previously been identified in SCM, and the latter product had not been identified in any stored milk product.

2. The concentration of 2-furfurol was found to increase upon storage at 27° C, and exceeded its threshold concentration after 26 weeks at this temperature but not after 13 weeks.

3. The concentrations of the odd-numbered n-methyl ketones, C_3-C_{11}, also increased upon storage, reaching levels in excess of their combined average flavor threshold (considering additive interaction) after 13 weeks at 27° C.

4. The concentration of o-aminoacetophenone reached levels in excess of its reported threshold concentration after 13 weeks of

storage at 27° C.

5. Acid degree values showed a steady gradual increase through 26 weeks of storage at 27° C, suggesting simple hydrolysis rather than lipase reactivation as the origin of free fatty acids in stored SCM. On the basis of the acid degree values, free fatty acid levels do not appear to have reached significant levels after 26 weeks at 27° C.

6. Hydroxymethylfurfural values increased slowly during the first 13 weeks of storage at 27° C, and then increased markedly during the second 13 weeks. This suggests slow non-enzymatic browning during early storage of the product, followed by a more rapid rate of browning.

7. The production of volatile compounds of potential flavor significance upon heating of thiamine solutions at pH 6.7 was demonstrated.

8. Hydrogen sulfide, 2-methyl furan, 2-methyl thiophene and a compound that appeared to be a dihydro-2-methyl thiophene were identified as volatile heat degradation products of thiamine.

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