GAS CHROMATOGRAPHIC-MASS SPECTROMETRIC ANALYSIS OF VOLATILES IN FLAVORS AND BIOLOGICAL FLUIDS

A Dissertation

Presented to

the Faculty of the Department of Chemistry

College of Arts and Sciences

University of Houston

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

---

by

David R. Douglas

November 1971

TO MY FATHER AND MOTHER

ACKNOWLEDGEMENT

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ABSTRACT

The volatile flavor components of two types of Cheddar cheese and a Cheddar cheese powder have been analyzed by gas-liquid chromatography and mass spectrometry. Techniques applied to prepare samples for analysis were the following: centrifugation of cheese with subsequent direct injection of the oil obtained, low temperature, vacuum distillations of cheese oil and whole cheese, and extraction of the oil with methanol. The compounds identified include ketones, aldehydes, alcohols, acids, esters, lactones, terpenes, alkanes, alkenes, alkylbenzenes, and some chlorinated compounds.

A technique is described in which the oil obtained by centrifugation of cheese at room temperature is injected directly into the gas chromatograph in order to compare the volatile flavor components of several types of cheese. Chromatograms are shown for Roquefort, Blue, Romano, Limburger, and Cheddar cheeses. The method requires only 1 to 3 g of cheese and gives semiquantitative data. Roquefort cheese has the highest concentration of flavor compounds with ca. 110 ppm for 2-heptanone, whereas the most concentrated volatile components in Cheddar cheese do not exceed concentrations of 10 ppm.

The volatile components of roast beef and roast beef drippings have been analyzed by gas-liquid chromatography (GLC) and mass spectrometry (MS). Samples were prepared by low temperature, vacuum distillation of meat and drippings followed by extraction of the distillates with diethyl ether. Major volatile components are alkanals, alk-2-enals and alka-2,4-dienals.

In addition to these compounds the meat samples show high concentrations of 3-hydroxy-2-butanone and γ-butyrolactone. Further classes of constituents are 2-n-alkylfurans, 2-alkanones, 3-alkanones, 2,3-alkadiones, pyrazines, primary and secondary alcohols, acids, γ- and δ-lactones, alkanes, aromatic compounds, sulfur compounds and acetylpyrrole. By comparing the lean meat portion and the fat portion of roast beef it was found that the fat is the prime site of the aldehydes.

Finally, volatile urinary components from human urine were analyzed using ether-continuous liquid-liquid extractions and gas-liquid chromatography plus gas chromatography and mass spectrometry. Chromatograms from different subjects showed remarkable similarities. Pathological states could possibly be reflected by changes in these chromatographic patterns.

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INTRODUCTION

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INTRODUCTION

The analysis of many naturally occurring materials divides itself into several areas. The material may be analyzed to determine which elements are naturally present, to determine contaminants or to determine the relative composition of the various components. Often the analysis of trace materials is important to elucidate the important compounds for flavor or odor. In trace analysis, minor components of a substance may be present in parts per million or less. They can be contaminants such as mercury in foods or lead in the atmosphere, or naturally occurring compounds which are responsible for the smell or taste of foods.

Trace analysis involves separation of detection of the trace compounds in the presence of the major components of the material. In the analysis (odors and flavors) of food the food itself consists of many complicated, high molecular weight chemicals, and the volatile compounds responsible for flavor are present in quite small amounts. As Hornstein and Teranishi report:

> Determining what volatile compounds are largely responsible for the flavor of a food is the approach many flavor chemists take to try to define flavor. Using instrumental techniques, flavor chemists have detected and identified trace amounts of volatile constituents of foods at an ever increasing rate.
>
> These amounts can be extremely minute. Our sense of smell is so sensitive, that we can detect skatol (3-methylindole) at a 3×10^{-11}% weight-to-weight concentration in air. Recently it was found, however, that skatol purified by zone refining is odorless. The ability of submicrogram amounts of volatiles to profoundly affect odor and thus flavor is apparent. Nevertheless, the increasing sensitivity of chemical instrumentation during the past decade has put the chemist and his nose in the same ball park.[1]

Therefore, volatile compounds in food may be present in extremely small amounts and still exert a considerable influence on the odor and flavor of the food.

To reproduce the natural flavors of foods it is necessary to determine the type of volatile compounds and their relative amounts. The magnitude of the problems involved in the determination of these trace components is illustrated in the following statement by Hornstein and Teranishi:

> The low thresholds for odoriferous compounds and the low concentrations of these compounds in food products make chemical analyses for flavor components difficult-isolation, separation, detection, and identification present formidable problems. Thousands of kilograms of a food product must be processed for milligram yields of strong-smelling oils. This isolation must be accomplished without destroying the compounds responsible for the characteristic aromas and without introducing interfering compounds from solvents, containers, tubing, lubricants, and the like and without introducing off-aromas from heat damage, air oxidation, and light-catalyzed decompositions.
>
> Using gas chromatographs with very low limits of detection submicrogram quantities can be separated and detected. Thus, a flavor chemist must have, or have access to, equipment to handle a tremendous range of quantities. He must face chemical engineering problems of isolating minute quantities from tons of material and also face ultramicrochemical analytical problems of separating and identifying odoriferous constituents of complex mixtures available only in small quantities.
>
> The recent activity and progress in flavor chemistry are partly due to the great advances in microanalytical techniques. Indeed, these advances make flavor investigations easier and faster, and in some cases, possible.[1]

In the present study, the first problem investigated was an analysis of the flavor of Cheddar cheese. In the course of experimentation several other types of cheese were analyzed for comparison to the Cheddar cheese.

A similar study was undertaken of the flavor of lean roast beef and fat drippings of roast beef.

Finally, an investigation of body fluids was made, with special emphasis on human urine from a variety of subjects including normal males and females using the analytical methods developed in the study of food flavors. These methods included extraction and vacuum distillation for separation of the volatile compounds. The volatile concentrate mixtures were then analyzed using gas chromatography and a combination of gas chromatography-mass spectrometry. Open tubular columns were used in the analysis due to their greater ability to separate these complex mixtures. In addition, open tubular (capillary) columns are compatable with the small size of the samples. Thus Cheddar cheese and body fluids lend themselves well to the analytical method outlined above.

CHAPTER II

THE VOLATILE FLAVOR COMPONENTS OF CHEDDAR CHEESE

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INTRODUCTION

Investigations of the volatile flavor components of several varieties of cheese[2] gave rise to a study of the volatiles in Cheddar cheese. Cheddar cheese was selected because it is one of the most popular cheeses and, in spite of previous studies,[3-16] many of its volatile constituents remain unidentified. Recent reviews are given by Forss and Patton,[17] Day,[18] and Forss.[19] Apparently attempts to duplicate the Cheddar cheese odor with compounds previously found were not successful. These failures may be due to incomplete analytical data.

In this investigation gas chromatographic separation with high resolution open tubular columns was combined with mass spectrometric identification. Two varieties of Cheddar cheese aged for 3 months and 24 months, respectively, and a powder made from a 24 month old Cheddar cheese were analyzed.

EXPERIMENTAL

Direct Chromatographic Analysis of Cheese Oil

Oil was obtained by centrifugation of cheese and chromatographed directly as described by Liebich et al.[2] A number of compounds were tentatively identified from their retention data.

Preparation of Cheese Flavor Concentrates by Combined Centrifugation, Distillation and Extraction

Flavor concentrates were prepared in a similar manner to that used by McGugan and Howsam,[8] Libbey et al.[10] and O'Keefe et al.,[16] with several variations. The cheese was grated and packed into 250 ml glass centrifuge tubes which were capped and allowed to stand in a water bath at 40°C for 10 min. This procedure was found to have no detectable effect on either the relative or absolute concentration of the volatiles. However, it greatly enhanced the yield of oil upon centrifugation because this temperature is slightly above the melting point of the cheese fat and allowed much lower speeds and shorter times for centrifugation to be used. The samples were centrifuged in an International Model K centrifuge for 30 min at 2000 rpm. The oil was then decanted into a 1000 ml flask. Usually, 1-2 Kg of cheese were required to produce 300 g of oil, which ultimately yielded 15-20 µl of the concentrated volatiles.

The decanted oil was vacuum distilled in an apparatus similar to that previously described.[8,10] Three traps were used with liquid nitrogen as the coolant. The first trap collected most of the high boiling components and the water, while the second trap collected the more volatile portion. A third trap was used to prevent contamination of the sample with pump oil vapors. A three-way vacuum stopcock was inserted between the vacuum pump and the third trap to break the vacuum. In this way possible atmospheric contaminants entering the system while the vacuum was released were also collected in the third trap. The vacuum (20 microns) was produced by either Welch Duo-Seal Model 1402 or 1406H vacuum pumps. Dow-Corning High

Vacuum stopcock grease was used to lubricate the ground glass joints of the assembly. The distillations gave optimum yields when run for 6-8 hrs.

At the end of the distillation period, the vacuum was broken, the Dewars of liquid nitrogen were removed from the traps, and 10-20 ml of diethyl ether were added to the first two traps to dissolve the flavor volatiles and prevent their evaporation. The ether used was anhydrous Baker Analyzed Reagent which had been previously tested chromatographically for purity. The distillate and ether from both traps were combined, the ethereal porition was decanted, and the aqueous distillate was extracted with 2 more portions of 10-20 ml of ether. The ether portions were then combined and concentrated by evaporation on a 40°C water bath. Blank distillations, extractions, and concentrations were made to insure that no components in the flavor mixture were due to other sources in the procedures employed.

Preparation of Cheese Flavor Concentrates by Combined Distillation and Extraction

Whole cheese was grated and subjected to the above distillation and extraction procedure, as was the spray-dried cheese powder. While the powder produced good results (15 µl of volatile concentrate from 500 g of cheese powder) the distillation of the whole cheese was not very favorable. Due to the relatively large amount of water in cheese, more ether was required for extraction of the distillate of the whole cheese than was required for the distillate of the oil, thereby increasing the possibility

for contamination of the sample and resulting in lower yields of the concentrate.

Extraction of Cheese Oil With Methanol

One hundred grams of cheese oil were extracted for 20 hrs with 80 ml of redistilled Baker Analyzed Reagent methanol in a continuous liquid-liquid extractor. The yellow extract containing some nonvolatile material was cooled to 0°C. This resulted in the formation of a white precipitate. The liquid was decanted and concentrated on a 60°C water bath to 20 ml. The extract was then cooled again, decanted from another porition of precipitate, and further concentrated until a residue of 200 µl remained. The white precipitate was discarded as it exhibited only a weak odor, and gas chromatographic tests revealed no significant amount of volatile components.

Gas Chromatographic Analysis

A Perkin-Elmer Model 900 gas chromatograph was used in this investigation. It was operated with prepurified nitrogen as the carrier gas, at an inlet pressure of 15 psi for 0.02 in., i.d., columns and 10 psi for 0.03 in., i.d., columns. The dimensions of the open tubular columns used and the temperature conditions were the following: 600 ft x 0.02 in., i.d., stainless steel coated with Dowfax 9N15, programmed from room temperature to 150°C at 2°C/min; 500 ft x 0.02 in., i.d., stainless steel coated with Emulphor-O, programmed from 70°C to 210°C at 2°/min; and 500 ft x 0.03 in., i.d., stainless steel coated with silicone oil SF 96, programmed from 60°C to 200°C at 2.5°/min.

Mass Spectrometric Analysis

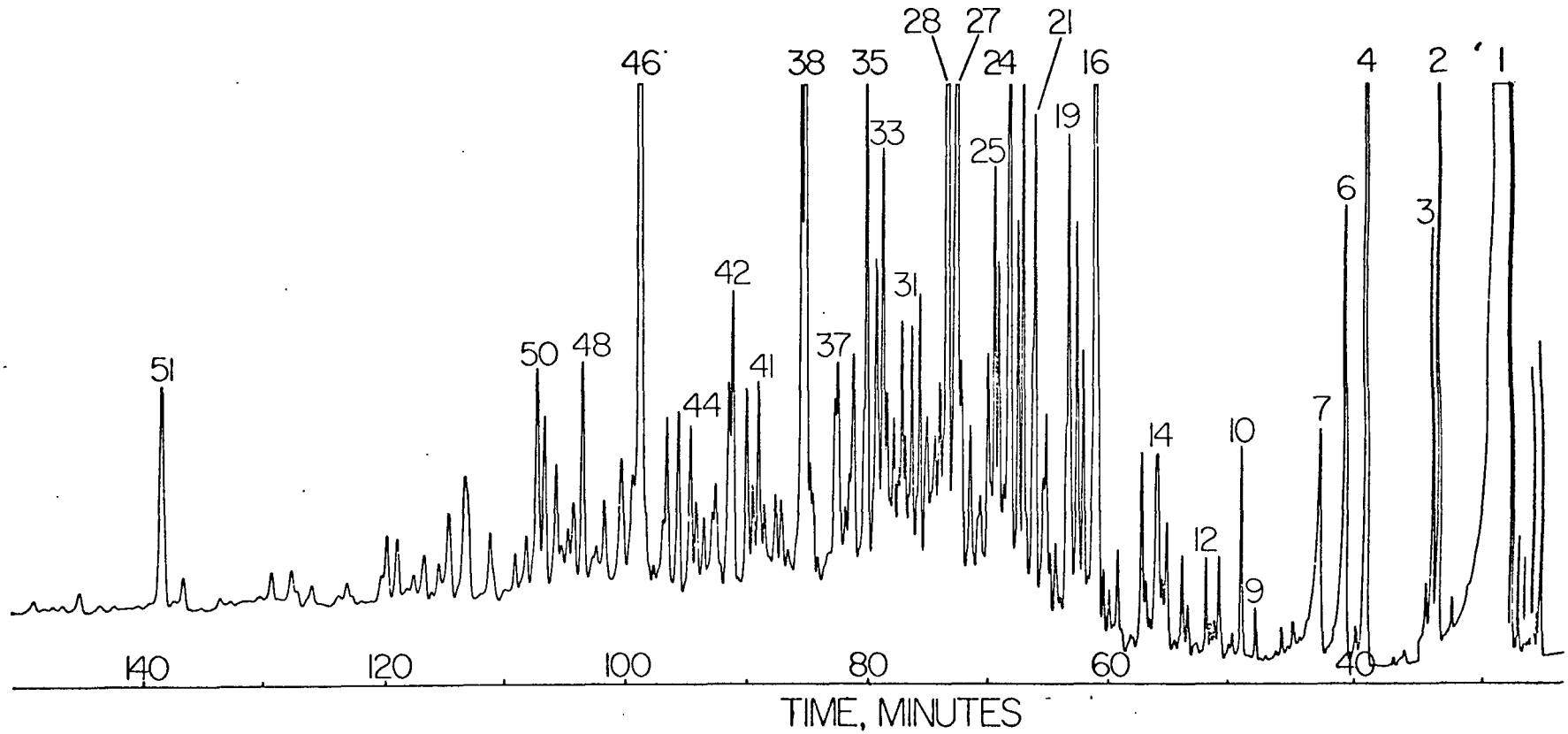
The flavor concentrates and the methanol extract were analyzed on an LKB 9000 gas chromatograph-mass spectrometer at 70 eV ionization energy, with an ion source temperature of 250°C and a separator temperature of 240°C. The columns used were the above Dowfax 9N15 and silicone oil SF 96 columns.

RESULTS AND DISCUSSION

Except for some tentative evaluations of constituents in cheese oil by injecting the oil directly into the gas chromatograph and by relying on the retention times, identifications were made with the flavor concentrates and the methanol extract.

Figures 1-3 show chromatograms of concentrates from two kinds of Cheddar cheese and a Cheddar cheese powder. The chromatograms of these three samples are quite comparable with chromatograms obtained by direct injection of cheese oil from the same cheeses.[2] However, due to the concentration effect they appear to be more complex because, in addition to the major components, many other constituents were detected. The concentrations of most of these minor components were sufficiently high to give good mass spectra. Dowfax 9N15 was chosen as the stationary phase for separation of the concentrates because preliminary studies had shown that this material gave the best separations, and that free fatty acids which otherwise would have obscured part of the chromatogram were not eluted from the column during the time of analysis. When the same column was used in the mass spectrometer, the free acids were not com-

Figure 1. Chromatogram of sample B, concentrated prepared by combined centrifugation, distillation and extraction from 3 month old Cheddar cheese.
Conditions: 600 ft x 0.02 in., i.d., Dowfax 9N15, temperature programmed from room temperature to 150°C at 2°C/min: inlet pressure 15 psi, attentuation 16, sample size 1.5 µl.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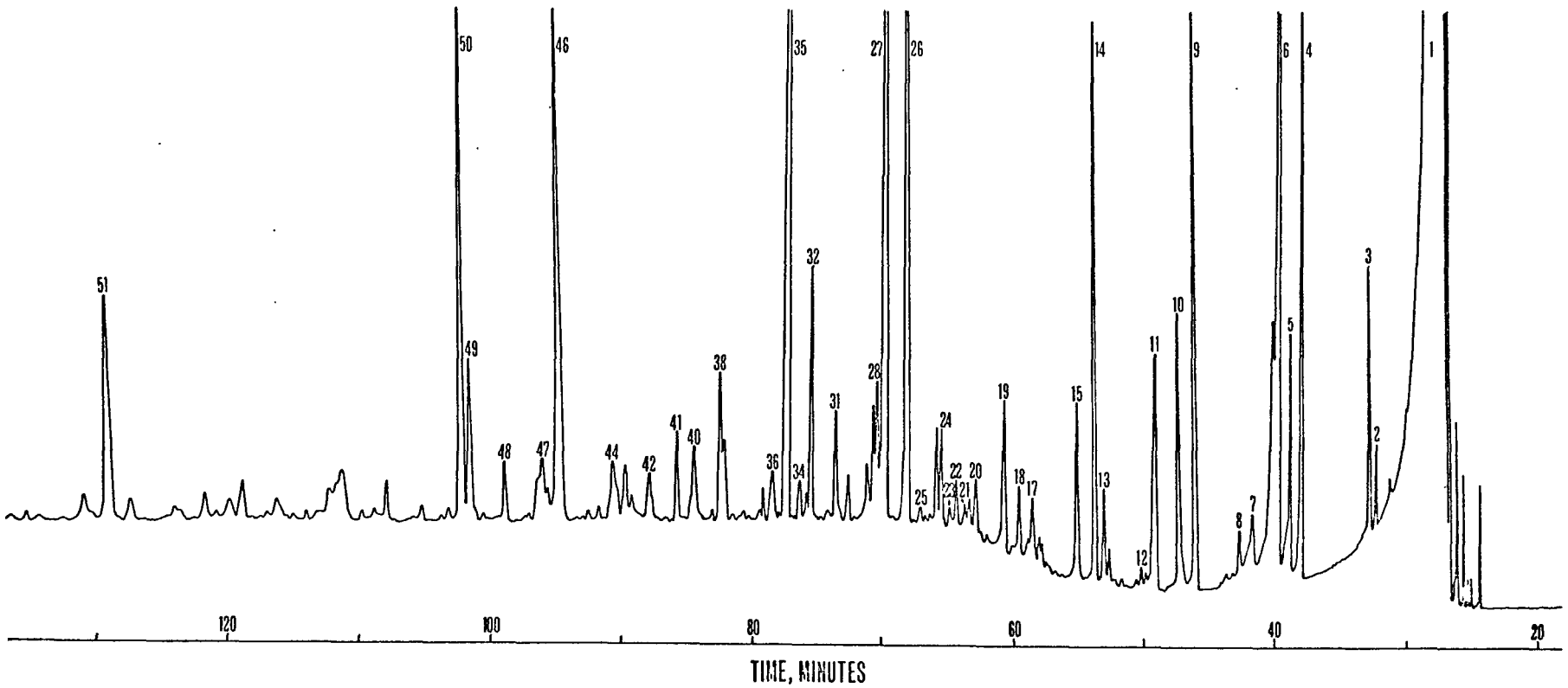
TIME, MINUTES

Figure 2. Chromatogram of sample C, concentrate prepared by combined centrifugation, distillation and extraction from 24 month old Cheddar cheese.

Conditions: 600 ft x 0.02 in., i.d., Dowfax 9N15, temperature programmed from room temperature to 150°C at 2°C/min: inlet pressure 15 psi, attentuation 16, sample size 1.5 µl.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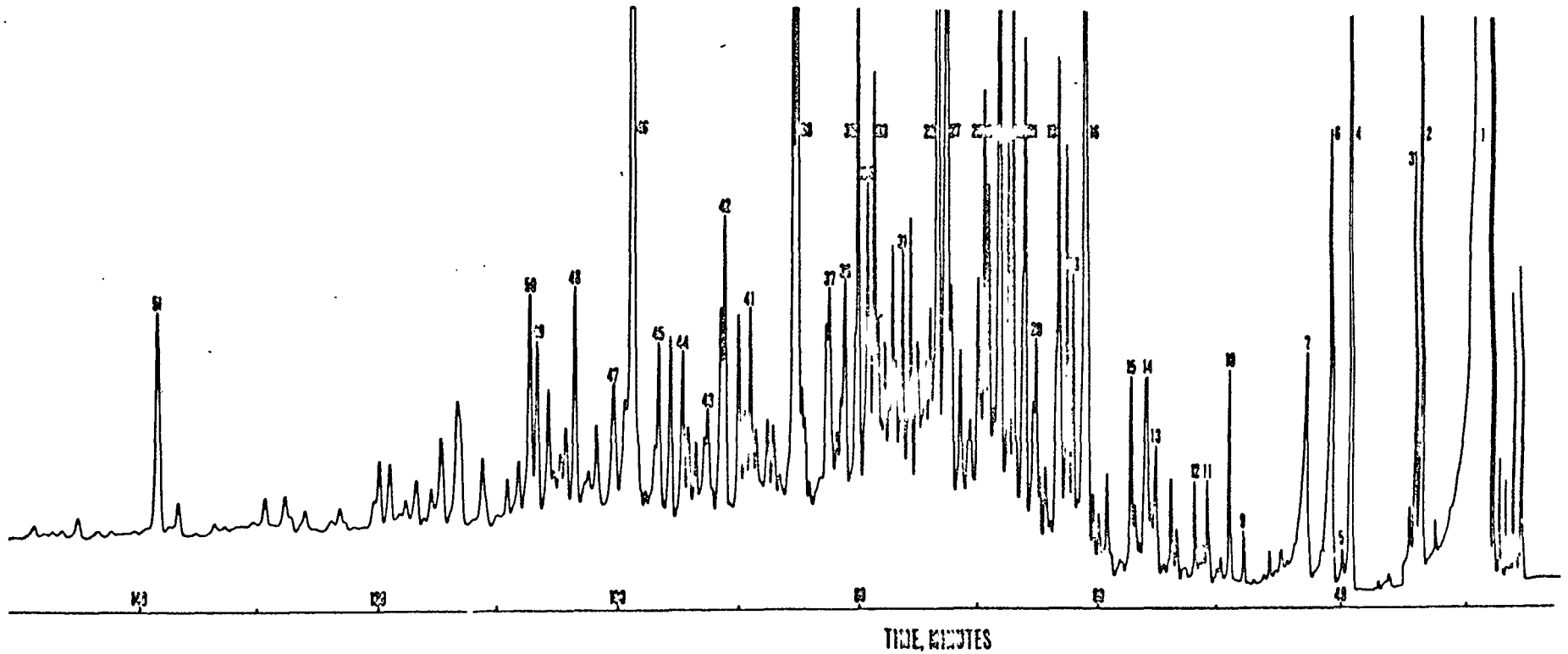
TIME, MINUTES

Figure 3. Chromatogram of sample P, concentrate prepared by combined distillation and extraction from 24 month old Cheddar cheese powder.

Conditions: 600 ft x 0.02 in., i.d., Dowfax 9N15, temperature programmed from room temperature to 150°C at 2°C/min: inlet pressure 15 psi, attentuation 16, sample size 1.5 µl.


TIME, MINUTES

pletely retained on the column due to the low pressure at the column outlet. However, the first of two large acid peaks was eluted later than 2-undecanone (peak 51) and did not interfere with the significant part of the chromatogram.

Each of the three samples shown in Figures 1-3 contain more than 150 compounds. The cheese powder sample exhibited the greatest complexity. All the compounds identified in the two fresh Cheddar cheeses and the cheddar cheese powder are listed in Table I. They include ketones, adehydes, alcohols, esters, alkanes, alkenes, aromatic hydrocarbons, terpenes, and some chlorinated compounds. Major components in the fresh cheeses are ethanol, 2-pentanone, ethyl butyrate, 2-heptanone, ethyl hexanoate, 2-nonanone and ethyl octanoate. Several significant differences can be observed between the two cheeses of different age. For example, peak 19 corresponding to n-butanol and 2-pentanol is one of the largest peaks in sample B and only a minor one in sample C. The concentration of aromatic hydrocarbons such as benzene, toluene, 1,4-dimethylbenzene, 1,3-dimethylbenzene, and n-propylbenzene is higher in sample B.

Spray-drying of cheese drastically changes the composition of the volatiles (Figure 3). It greatly increases the concentration of alkanes and alkenes. Most of these aliphatic hydrocarbons and many of the alkylbenzenes were not found in the fresh cheeses. In Table I several of the hydrocarbons are listed with their molecular formulas. However, no attempt was made to elucidate their complete structures. The concentrations of some esters such as ethyl butyrate, ethyl hexanoate and ethyl octanoate are reduced significantly.

TABLE I. Volatile Constituents of Cheddar Cheese and Cheddar Cheese Powder

Peak No.	Compound	Samples in which compound was identified	Identification
1	diethyl ether (solvent)	---	---
	acetaldehyde	A	R (tent.)
	ethyl mercaptan	A	R (tent.)
	propionaldehyde	A	R (tent.)
3	acetone	A,B,C,P	M,R
	isobutyraldehyde	A	R (tent.)
4	ethyl acetate	B	M,R
4	n-octane	B	M,R
5	2-butanone	B,C,P	M,R
6	isopropyl acetate	B	M,R
6	ethanol	B,C,P	M,R
	1-octene	B,C,P	M,R
7	2-methylbutanal	B,P	M,R
7	3-methylbutanal	B,P	M,R
8	benzene	B,C	M,R
	cis-1,2-dichloroethylene	B	M,R
9	2-pentanone	B,C,P	M,R
	methyl butyrate	B,C	M,R
10	n-nonane	B,P	M,R
10	chloroform	B	M,R

TABLE I. Continued

Peak No.	Compound	Samples in which compound was identified	Identification
20	camphene	B	M,R
	$C_{11}H_{22}$	P	M
21	ethylbenzene	B,P	M,R
22	$C_{11}H_{22}$	P	M
22	$C_{11}H_{24}$	P	M
23	1,4-dimethylbenzene	B,C,P	M,R
24	1,3-dimethylbenzene	B,P	M,R
	$C_{11}H_{22}$	P	M
	$C_{11}H_{24}$	P	M
	$C_{11}H_{22}$	P	M
25	β-pinene	B,C,P	M,R
	$C_{10}H_{20}$	P	M
26	5-methyl-2-hexanone	B,P	M (tent.)
27	1,2-dimethylbenzene	B,C,P	M,R
27	2-heptanone	B,C,P	M,R
28	chlorobenzene	B	M,R
	$C_{11}H_{22}$	P	M
28	n-undecane	B,C,P	M,R
28	methyl hexanoate	B,C	M,R
	$C_{11}H_{22}$	P	M
30	$C_{11}H_{22}$	B,P	M

TABLE I. Continued

Peak No.	Compound	Samples in which compound was identified	Identification
	$C_{12}H_{26}$	P	M
31	1-undecene	B,C,P	M
	$C_{12}H_{26}$	P	M
	n-propylbenzene	B	M
32	methylethylbenzene	B,C,P	M
34	limonene	B,C,P	M,R
35	trimethylbenzene	B,P	M
35	ethyl hexanoate	B,C,P	M,R
36	methylethylbenzene	B,C,P	M
	$C_{12}H_{24}$	P	M
	$C_{12}H_{26}$	P	M
	$C_{11}H_{22}$	P	M
	2-heptanol	B	M (tent.)
	2-methyl-1-pentanol	B	M (tent.)
37	trimethylbenzene	B,P	M
	2-octanone	P	M
	$C_{11}H_{20}$	P	M
38	n-dodecane	P	M
	diethylbenzene	P	M
	C_4-benzene	P	M
41	1-dodecene	P	M

TABLE I. Continued

Peak No.	Compound	Samples in which compound was identified	Identification
	C_4-benzene	P	M
42	$C_{12}H_{22}$	P	M
43	dimethylethylbenzene	P	M
	C_4-benzene	P	M
44	C_4-benzene	P	M
	dimethylethylbenzene	P	M
45	dimethylethylbenzene	P	M
46	2-nonanone	B,C,P	M,R
46	n-tridecane	P	M
	C_5-benzene	P	M
	C_5-benzene	P	M
	benzaldehyde	B,P	M
	C_5-benzene	P	M
50	ethyl-octanoate	B,C,P	M,R
	n-tetradecane	P	M
51	2-pentadecane	P	M
	n-pentadecane	P	M
	naphthalene	P	M
	ethyl decanoate	P	M,R
	$C_{15}H_{24}$	P	M
	n-hexadecane	P	M

TABLE I. Continued

Peak No.	Compound	Samples in which compound was identified	Identi-fication
	methylnaphthalene	P	M
	2-tridecanone	P,D	M,R
	2-pentadecanone	D	M,R
	δ-decalactone	D	M
	δ-dodecalactone	D	M
	δ-tetradecalactone	D	M
	δ-hexadecalactone	D	M
	butyric acid	B,P	M
	hexanoic acid	B,P	M
	octanoic acid	B,P	M
	decanoic acid	D	M,R
	dodecanoic acid	D	M,R
	tetradecanoic acid	D	M,R
	hexadecanoic acid	D	M,R
	methyl octanoate*	D	M
	methyl decanoate*	D	M
	methyl dodecanoate*	D	M
	methyl tetradecanoate*	D	M
	methyl pentadecanoate*	D	M
	methyl hexadecanoate*	D	M

TABLE I. Continued

Peak No.	Compound	Samples in which compound was identified	Identification
	methyl oleate*	D	M
	methyl linolate*	D	M
	methyl stearate*	D	M

A: oil of 3 month old Cheddar cheese, direct injection.

B,C: flavor concentrates from 3 month and 24 month old Cheddar cheese respectively, prepared by combined centrifugation, distillation and extraction.

D: methanol extract of 3 month old Cheddar cheese.

P: flavor concentrate from Cheddar cheese powder, obtained by combined distillation and extraction.

M: identified by mass spectrometry.

R: identified from retention data.

*: These methyl esters were found in the methanol extract. They may be artifacts formed by esterification of the free fatty acids with the methanol used.

Components not volatile enough to be distilled were identified from a methanol extract of the oil of 3 month old cheese. Ketones, lactones, methyl esters and acids found in this extract are also included in Table I. Free fatty acids up to C_{16} were eluted from the column (SF 96) in the mass spectrometer.

Attempts to simulate cheese flavor mixtures suggested that free fatty acids are the basis for any cheese flavor, which is in agreement with previously reported results.[9,17] The characteristic aroma of a certain type of cheese appears to be due to the proportions of the free fatty acids and, in particular, to the qualitative and quantitative composition of the mixture of the other volatile components. The relative concentrations of methyl ketones in Cheddar cheese are high, yet not as predominant as in Roquefort cheese. Several ethyl esters in samples B and C and n-butanol in sample B are of the same high concentration. Comparatively high amounts of the terpenes α-pinene, β-pinene, camphene, and limonene, not previously reported in Cheddar cheese were found in sample B. Langler et al.[20] identified α-pinene in Swiss cheese. It is surprising to find the large variety of alkenes, olefins and aromatic hydrocarbons especially in the cheese powder in which they appear to be responsible for an off-flavor. Some of the aromatic hydrocarbons have been previously reported in Cheddar cheese.[12,14] A number of alkanes, olefins and alkylbenzenes have been identified in Swiss cheese.[20] In addition, alkanes and alkenes are commonly found in irradiated foods.

CHAPTER III

COMPARATIVE CHARACTERIZATION OF DIFFERENT TYPES OF CHEESE BY GAS-LIQUID CHROMATOGRAPHY

CHAPTER III

COMPARATIVE CHARACTERIZATION OF DIFFERENT TYPES OF CHEESE BY GAS-LIQUID CHROMATOGRAPHY

INTRODUCTION

Various techniques have been applied to prepare samples of cheese volatiles for analysis. Direct injections of headspace vapor[21,22] were modified to incorporate concentration techniques utilizing a recycling, pumping system,[22] or a method which flushes the sample with nitrogen.[23] As an alternative to collecting the headspace vapor in dry ice/acetone cooled traps, a direct on-column trapping technique has been applied.[24,25] Frequently low temperature, vacuum distillations have been used. Cheese or cheese slurries[23,26-32] or cheese oil obtained by centrifugation of cheese[23,33-39] were distilled at about room temperature and a vacuum between 10^{-5} mm and 10 mm Hg. The distillate, usually collected on a cold-finger or in a series of 2 or 3 traps, was either directly chromatographed or extracted with a solvent to isolate the flavor components from the aqueous solution. A variation of this method was the injection of headspace vapor of the distillate. In some cases steam distillations were employed when carbonyl compounds were to be derivatized.[30,40,41] Although most solvents extract fat from the cheese, some investigators have used extraction procedures. Mixtures of cheese and Celite 545 were extracted with acetonitrile[42] or n-hexane.[24,43] Whereas the acetonitrile extract containing no significant amount of fat was injected directly into the gas chromatograph, the n-hexane extract was passed over a 2,4-dinitrophenyl-

hydrazine reaction column to isolate the carbonyl compounds from the fat. Methanol as a solvent for cheese extractions[23] necessitated a second extraction to remove the flavor compounds from the aqueous methanol solution. The solvent used was isopentane.

In this report a direct injection of cheese oil is described. This technique offers the following advantages over methods previously employed for cheese volatiles analysis:

a) It requires not more than 1 to 3 g of cheese.

b) The time necessary for preparation of a sample does not exceed 15 minutes.

c) It provides at least semiquantitative informations about the volatiles in any type of cheese.

d) Less chance exists for contamination or for any physical or chemical change in the flavor mixture.

EXPERIMENTAL

Some of the cheeses used in our laboratory were purchased locally, others were donated by Westreco, Inc., Marysville, Ohio. The following types were analyzed:

Imported Roquefort cheese (aged over 60 days)
Blue cheese (cured over 90 days)
Swiss cheese
Romano cheese
Limburger cheese
Several Cheddar cheeses
A spray-dried powder prepared from a two year old Cheddar cheese.

Sample Preparation

Depending on the fat content of the cheese, 1-3 g of cheese were cut into small pieces and packed into capped centrifuge tubes (5 ml tubes with Teflon-lined screw caps). The centrifugation was usually done in a Sargent Model S-15699 centrifuge for 10-15 minutes at room temperature and at ca. 4000 rpm. By heating the cheese sample to 60°C the centrifugations time could be reduced to 5 minutes or less. To obtain oil from cheese powder, approximately nine parts powder were mixed with one part water and the mixture was centrifuged.

Gas Chromatographic Analysis

To avoid loss of volatiles the cheese oil was injected immediately after centrifugation. The injection system was a Perkin-Elmer Model 900 injector without a splitter, which was designed for packed columns but was adapted for use with open tubular columns for this work. It contained glass liners 144 mm in length and 1.5 mm, i.d. The initial injector temperature was 190°C. After 10 minutes the injector heating was turned off. This period of time was sufficient to allow the volatile compounds to evaporate from the non-volatile oil, but not long enough to allow appreciable amounts of oil to enter the gas chromatograph column. After completion of each analysis the glass liner was replaced by a clean one. Used glass liners were cleaned easily with diethyl ether. The open tubular columns used for this investigation were the following: 500 ft x 0.02 in., i.d., stainless steel coated with Dowfax 9N15; 600 ft x 0.02 in., i.d., stainless steel coated with Dowfax 9N15; and 500 ft x 0.02 in., i.d.,

stainless steel coated with Emulphor-O. The columns were reversed by interchanging the column outlet with the inlet and conditioned overnight at a maximum temperature of 150°C to remove possible traces of oil from the front end of the column. The analyses were made on a Perkin-Elmer Model 900 gas chromatograph equipped with 2 flame ionization detectors. The carrier gas was prepurified nitrogen. The inlet pressure was 15 psi.

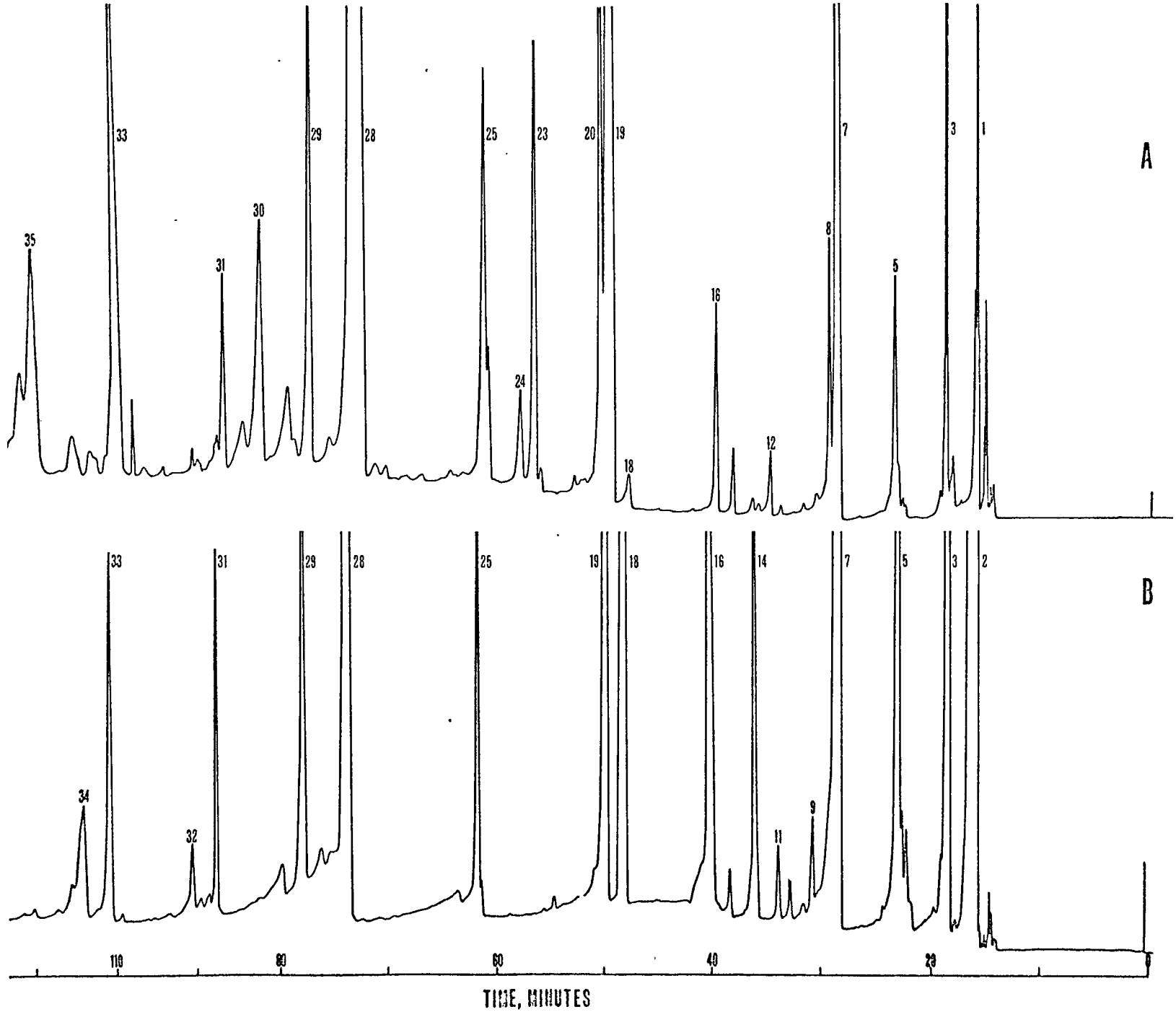
RESULTS AND DISCUSSION

Cheese oil has an odor typical of the whole cheese. The direct injection of oil represents a simple and rapid method of comparing the volatile flavor components of different types of cheese. Since no enrichment procedures such as distillation or extraction are applied, contamination is more easily prevented. The values for the relative and absolute concentrations taken from the chromatograms should be near the actual concentrations of the volatiles in cheese oil. Figures 4-6 show that each kind of cheese can be characterized by its specific chromatogram. Free fatty acids were not eluted from the columns during analysis time.

The volatile fractions of the various cheeses differ significantly with respect to qualitative and quantitative composition. No attempt was made to identify the volatile compounds in cheese oil. However, relying on mass spectrometric identifications made in several Cheddar cheese oil distillates, on retention data, and on previously reported results,[34,44] several peaks in the chromatograms can be considered at least as tentatively identified. The same number in different chromato-

Figure 4. Chromatograms of (A) Roquefort cheese, attenuation 16; (B) Blue cheese, attenuation 4 for 41 minutes, then attenuation 16.

Conditions: 500 ft x 0.02 in., i.d., Dowfax 9N15, temperature programmed from room temperature to 125°C at 2°C/min: inlet pressure 15 psi, sample size 10 µl of cheese oil.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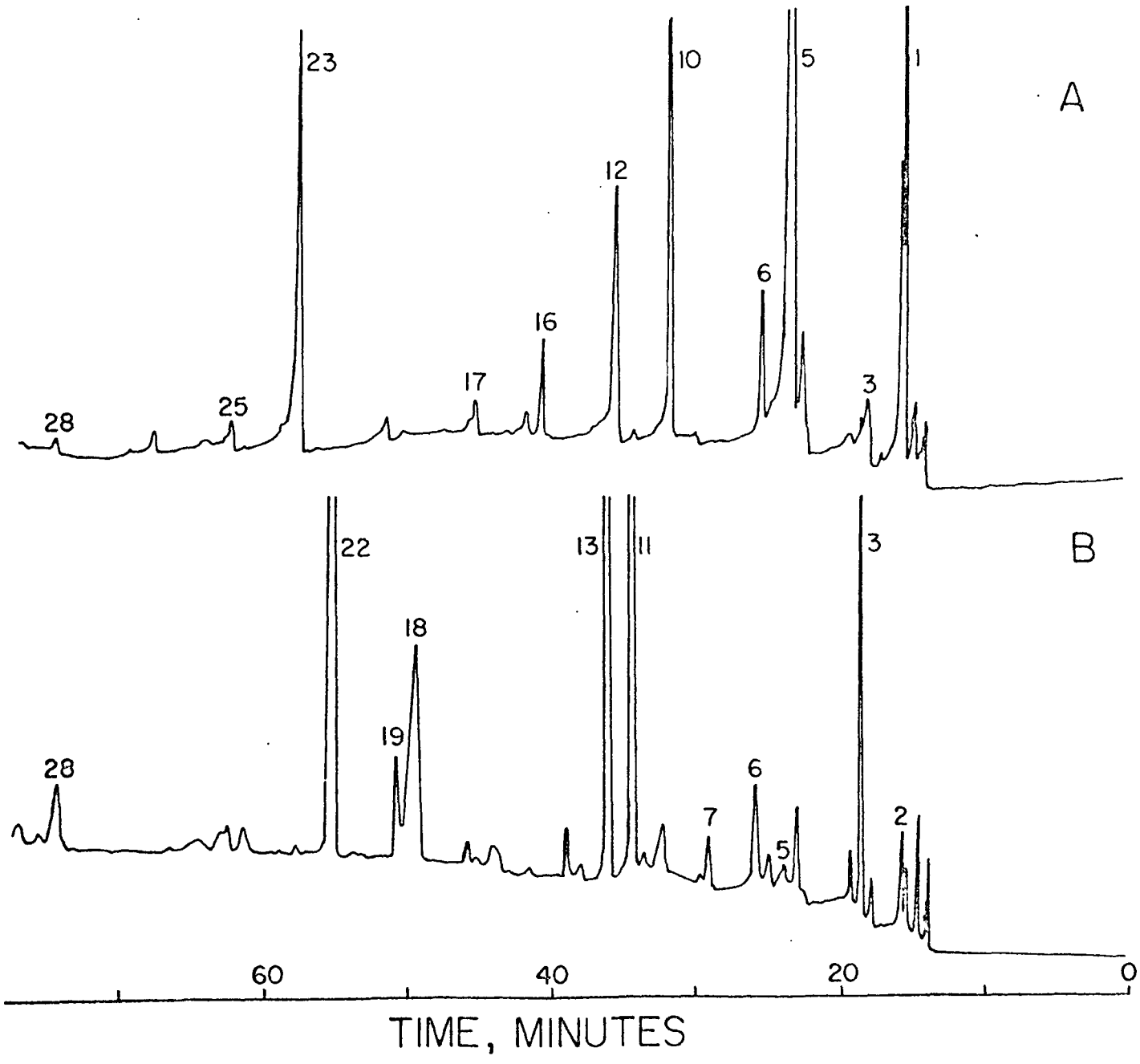
TIME, MINUTES

Figure 5. Chromatograms of (A) Romano cheese, attenuation 4; (B) Limburger cheese, attenuation 4.
Conditions: 500 ft x 0.02 in., i.d., Dowfax 9N15, temperature programmed from room temperature to 125°C at 2°C/min: inlet pressure 15 psi, sample size 10 µl of cheese oil.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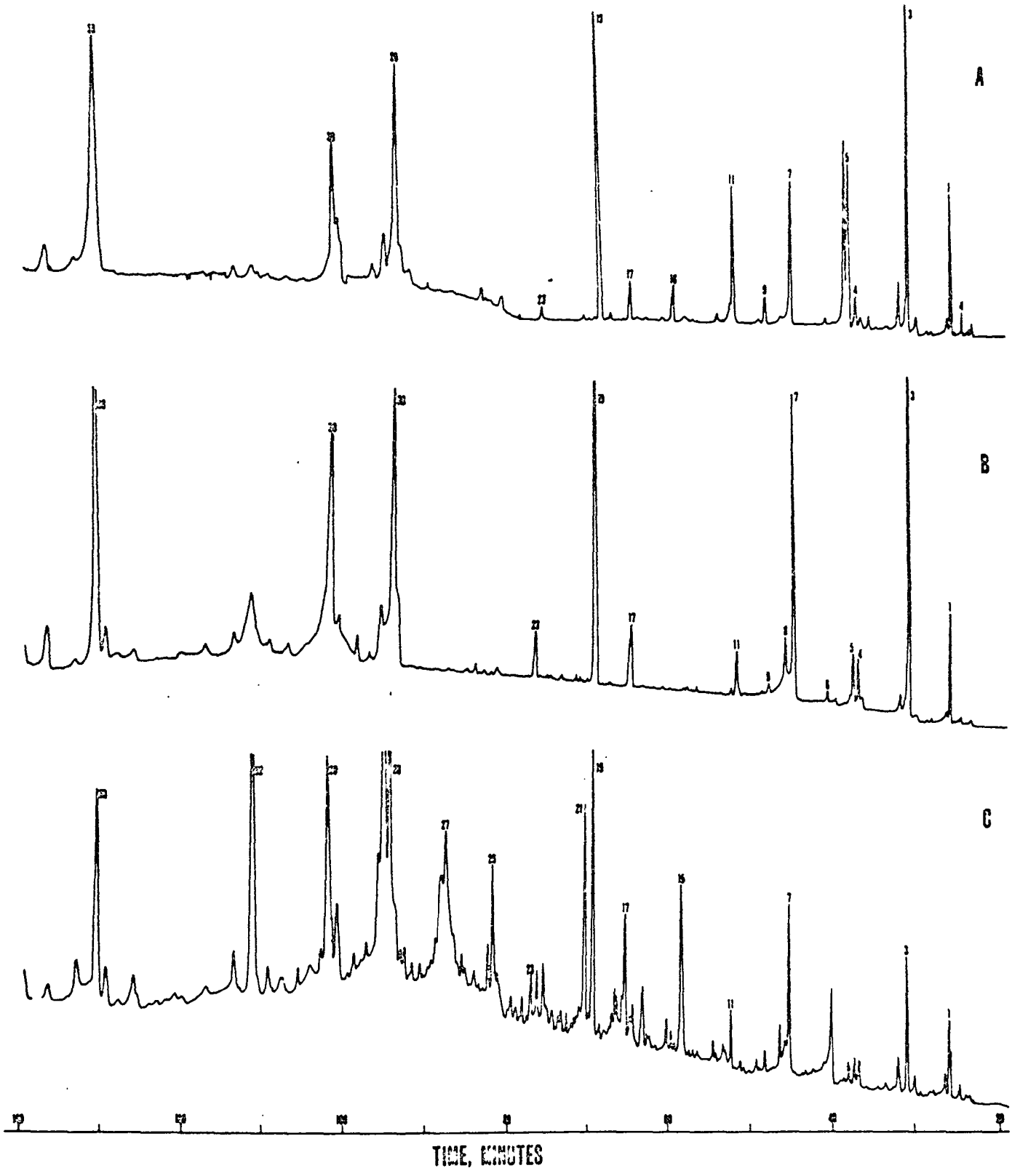
TIME, MINUTES

Figure 6. Chromatograms of (A) Cheddar cheese aged over 3 months; (B) Cheddar cheese aged 24 months; (C) Cheddar cheese powder from a 24 month old cheese. Attentuation 4 in all three chromatograms.

Conditions: 600 ft x 0.02 in., i.d., Dowfax 9N15, temperature programmed from room temperature to 150°C at 2°/min: inlet pressure 15 psi, sample size 10 µl of cheese oil.


A
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TIME, MINUTES

grams indicates the same compound. Cheese oils with the highest concentration of volatiles are Roquefort oil and Blue cheese oil. The concentration of 2-heptanone (peak 19) in Roquefort cheese oil as determined from standard injections and peak areas is ca. 110 ppm which is quite comparable with values reported by Schwartz et al.[43] Romano cheese and Cheddar cheese exhibit approximately 15-20 times lower concentrations of the volatiles than Roquefort. The largest peaks in the Cheddar cheese chromatograms correspond to concentrations of only ca. 5-10 ppm.

Roquefort cheese and Blue cheese are expected to have similar flavors, and this is reflected in their similar chromatograms. The largest peaks in both chromatograms are 3, 7, 19, 28, 33 corresponding to C_3, C_5, C_7, C_9 and C_{11} methyl ketones. Several more peaks are present in both types of cheese. There are, however, differences in other compounds. Roquefort shows relatively high concentrations of methyl butanoate, methyl hexanoate, ethyl butanoate, ethyl hexanoate, and ethyl octanoate (peaks 8, 20, 12, 23, and 30, respectively) which are hardly recognizable in the Blue cheese studied. On the other hand, Blue cheese contains large concentrations of compounds 2, 14, and 18. The chromatograms of Limburger and Romano are completely different from the Roquefort and Blue cheese chromatograms. It is surprising that Romano cheese does not appear to contain significant amounts of the otherwise common methyl ketones.

Direct cheese oil injection is also a very convenient method for controlling flavor changes occurring in connection with aging and processing. Figure 6 reveals several differences in the concentrations of

volatile components in Cheddar cheeses aged for over 3 months and 24 months, respectively. Further it shows the chromatogram of a Cheddar cheese powder, which was made by spray-drying 24 month old cheese. This powder contains numerous compounds not present at all in the cheese from which the powder was made or at least in much smaller concentrations.

The technique described in this work should be applicable to many other types of foods or other substances which contain either oil or fat which can be isolated by centrifugation.

CHAPTER IV

VOLATILE COMPONENTS IN ROAST BEEF

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VOLATILE COMPONENTS IN ROAST BEEF

INTRODUCTION

Many investigations have been devoted to the identification of volatile constituents of meat from several different species of animals. Studies of components in beef, pork, lamb and chicken have been reviewed by Hornstein and Crowe (1964),[45] Hornstein (1967),[46] Pippen (1967),[47] and Solms (1968).[48] The volatiles of boiled beef were investigated by Kramlich and Pearson (1960),[49] Yueh and Strong (1960),[50] Hornstein et al. (1960),[51] Bender and Ballance (1961),[52] Sanderson et al. (1966),[53] and Chang et al. (1968).[54] Tonsbeek et al. (1968)[55] and Copier et al. (1970)[56] identified compounds in beef broth.

This study was made to determine the volatile components of roast beef and roast beef drippings. Several concentrates were analyzed by GLC on open tubular columns and identified by mass spectrometry. It was also intended to compare the constituents in the lean meat portion and the fat portion of roast beef.

EXPERIMENTAL

The roast beef was prepared by Westreco, Inc., Marysville, Ohio. A rib roast weighing approximately 3 Kg was cooked without the addition of any condiment for 44 minutes per Kg at a temperature of 163°C. After cooking, the roast was cooled to room temperature, sliced, packed in aluminum foil, wrapped in a plastic bag, frozen in a dry ice chest and

sent to Houston by air. The drippings of the rib roast were collected, frozen and sent to Houston in the same parcel. Roast beef and drippings were kept in a freezer for 1-4 days before processing.

Preparation of concentrates of roast beef volatiles

Concentrates of volatile constituents were prepared by combined distillation and extraction. In a representative sample preparation, 1000 g of roast beef were cut into small pieces and distilled for 20 hours at a vacuum of 2 microns. The 3 liter round bottom flask containing the meat pieces was heated in a water bath at 55°C. The distillation apparatus used here has been described by Liebich et al. (1970).[57] Apiezon M grease was used to lubricate the ground glass joints of the assembly. After the 20 hour distillation period, the first trap had collected most of the aqueous sample, while the second trap contained no more than 2 ml. After removing the Dewars of liquid nitrogen and disconnecting the assembly, 30 ml of redistilled, anhydrous Baker Analyzed diethyl ether were added to the traps. Subsequently the traps were closed with ground glass stoppers and allowed to warm up. The combined contents of both traps were extracted for 24 hours with 80 ml of the above described diethyl ether in a continuous liquid-liquid extractor. By distillation of the ether extract at atmospheric pressure over a Vigreux column most of the solvent was removed and a sample of 1 ml was obtained. A further concentration of the extract was accomplished by leaving the sample vial uncapped at room temperature until approximately 35 µl remained. Blank distillations, extractions and concentrations were made to insure

that no components in the concentrate originated from contaminations. Samples from 500 g of the lean meat portion and 180 g of the fat portion of roast beef were prepared according to the same method.

Preparation of a concentrate of volatiles of roast beef drippings

The drippings from 6 pounds of roast beef amounted to approximately 120 g and contained about 70% fat. The entire amount of drippings was distilled under the same conditions as described for the meat and yielded 23 ml of aqueous distillate. After extraction with 60 ml of diethyl ether and concentration of the extract, 50 µl of a concentrated sample were obtained.

Gas chromatographic and mass spectrometric analysis

The GLC analyses were performed on a Perkin-Elmer Model 900 gas chromatograph. It was operated with prepurified nitrogen as the carrier gas at an inlet pressure of 14 psi. All samples were separated on a 500 ft x 0.02 in., i.d., stainless steel column coated with Dowfax 9N15 (Column A). The column was programmed from room temperature to 150°C at 2°C/min. Roast beef drippings were analyzed on an LKB 9000 gas chromatograph-mass spectrometer at 70 eV ionization energy, with an ion source temperature of 270°C and a separator temperature of 230°C. The GLC column used with this instrument was a 700 ft x 0.03 in., i.d., stainless steel column coated with Dowfax 9N15 (Column B).

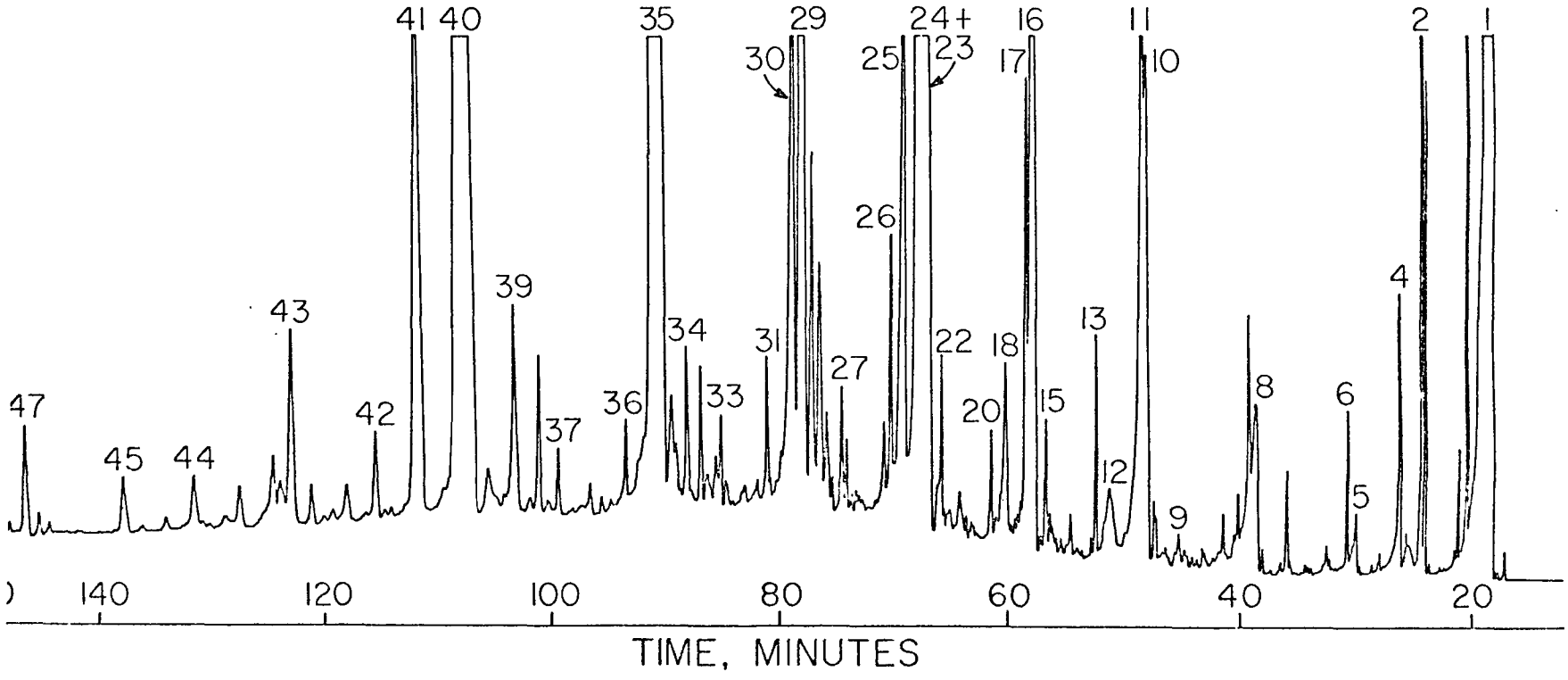
RESULTS AND DISCUSSION

The concentrated samples exhibited pleasant meaty odors with a somewhat fatty nuance for roast beef concentrates and slightly burned for the drippings. The odors of the drippings samples were considerably stronger than the meat odors and were distinctive of drippings.

Figures 7 and 8 show chromatograms of concentrates from drippings and roast beef, respectively. Odor differences are reflected in differences in the chromatograms. The concentrations of n-hexanal, 3-hydroxy-2-butanone and γ-butylrolactone (peaks 8, 12, 28) are much higher in meat than in drippings, while the drippings chromatogram shows higher concentrations for n-heptanal, n-octanal, 1-heptanol, 2-nonenal, 1-octanol, 2-decanal, 2-undecenal and 2,4-decadienal (peaks 10, 16, 25, 29, 30, 35, 40 and 41, respectively).

Many of the volatile constituents in roast beef and drippings could be identified by mass spectrometry. The chromatograms obtained with the 0.03 in. column on the LKB 9000 gas chromatograph-mass spectrometer were very similar to the chromatograms obtained with the 0.02 in. column on the Perkin-Elmer instrument. Checks on the identity of the compounds indicated by the spectra were made by comparing the gas chromatographic retention time of the component with that of the authentic compound, by using the reference spectrum from the literature, and in some cases by measuring the spectrum of the authentic compound. When standard compounds were not available for comparison, identifications were assigned if reference literature spectra were available and if the mass spectral fragmentation pattern and the retention time suggested a compound homologous to other components identified in roast beef or drippings.

Figure 7. Gas Chromatogram of a Flavor Concentrate Obtained from Roast Beef Drippings
Attenuation 256, sample size 0.3 µl.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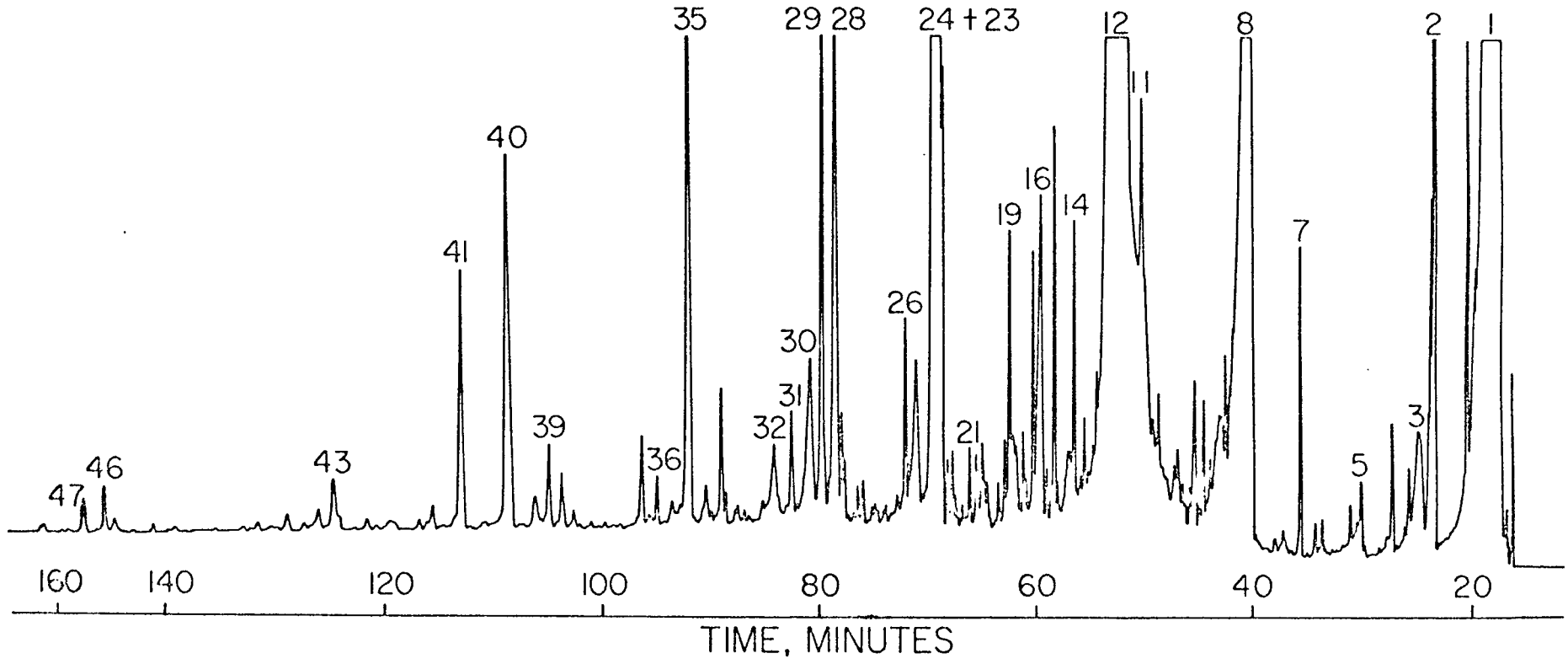
TIME, MINUTES

Figure 8. Gas Chromatogram of a Flavor Concentrate Obtained from Roast Beef
Attenuation 256, sample size 1.8 μl.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TIME, MINUTES

All the constituents identified in roast beef and drippings are listed in Table II. Peak numbers refer to numbers in the figures. Identified compounds which are not labeled appear in the chromatograms between two labeled peaks in the order given in the table. No numbers were assigned to these constituents because the experimental conditions for the GLC-MS analyses were different from the conditions for GLC-analyses resulting in slight changes in peak sizes and retention times. The chromatograms shown in the figures were recorded on the regular chromatograph. The main classes of compounds identified are alkanals, alk-2-enals, alka-2,4-dienals, 2-n-alkylfurans, ketones, 2,3-alkadiones, primary and secondary alcohols, acids, γ- and δ-lactones, alkanes, aromatic compounds, dimethyl disulfide, dimethyl sulfone and acetylpyrrole. Several of the compounds reported in this investigation have been found in other types of meat (Hornstein, 1967, Pippen, 1967; Solm, 1968; Nonaka et al., 1967;[58] Lillard and Ayres, 1969;[59] Hobson-Frohock, 1970[60]). Some low-boiling constituents not found in the concentrates from roast beef and drippings have been identified in boiled beef (Kramlich and Pearson, 1960;[49] Yueh and Strong, 1960;[50] Hornstein et al., 1960;[51] Bender and Ballance, 1961;[52] Sanderson et al., 1966[53]).

The carbonyl compounds seem to be the basis for the roast beef and especially the drippings volatiles. According to Hornstein and Crowe (1960,[61] 1963[62]) many of the aldehydes are formed by heating the fat portion of meat in air. This is in accordance with results obtained by comparing the volatiles of the lean meat portion with the fat portion of the same piece of roast beef. Unlike the lean meat, the fat shows

TABLE II. Volatile Constituents of Roast Beef and Roast Beef Drippings

Peak No.	Compound	Sample
1	Diethyl ether (solvent)	M,D
2	Ethyl acetate (solvent impurity)	M,D
3	Ethanol (solvent impurity)	M,D
4	2-Methylbutanal	M,D
4	3-Methylbutanal	M,D
	2,3-Butanedione	M
5	n-Pentanal	M,D
	n-Nonane	M,D
	n-Propanol	D
	2,3-Pentanedione	M
7	Toluene	M,D
	Dimethyl disulfide	M
8	n-Hexanal	M,D
	n-Decane	M,D
	n-Butanol	M,D
	1,4-Dimethylbenzene	M,D
	1,2-Dimethylbenzene	M
9	3-Heptanone	D
	n-Undecane	M,D
	2-Heptanone	M,D
10	n-Heptanal	M,D

TABLE II. Continued

Peak No.	Compound	Sample
	2-Hexenal	D
12	3-Hydroxy-2-butanone	M,D
12	1-Pentanol	M,D
13	2-n-Pentylfuran	M,D
	Trimethylbenzene	M
	Methylethylbenzene	M
14	3-Octanone	M,D
	Trimethylbenzene	M,D
	4-Heptanol (tent.)	D
	3-Heptanol	D
15	2-Octanone	M,D
	n-Dodecane	M,D
16	n-Octanal	M,D
17	2-Heptenal	M,D
	2,3-Octanedione	M
	Diethylbenzene	M
18	1-Hexanol	M,D
	Acetic acid	D
19	C_3-benzene	M
20	2-n-Hexylfuran	D
	n-Butylbenzene	M
	C_4-benzene	M

TABLE II. Continued

Peak No.	Compound	Sample
	Dimethylpyrazine (tent.)	D
	4-Octanol (tent.)	D
	3-Octanol	D
	2-n-Butoxyethanol	M
22	2-Nonanone	M,D
	n-Tridecane	M,D
23	n-Nonanal	M,D
24	2-Octenal	M,D
25	1-Heptanol	M,D
	Benzaldehyde	M,D
26	2-n-Heptylfuran	M,D
	C_4-benzene	M
	2,4-Octadienal (tent.)	M,D
	2,3-Butanediol	M,D
	2-Decanone	M,D
	n-Tetradecane	M,D
	n-Decanal	M,D
28	γ-Butyrolactone	M,D
29	2-Nonenal	M,D
30	1-Octanol	M,D
31	2-n-Octylfuran	D
	Butyric acid	D

TABLE II. Continued

Peak No.	Compound	Sample
	6(?)-Methyltetradecane	M
	Phenylacetaldehyde	D
32	2-Octen-1-ol	M
	n-Pentadecane	M,D
34	2-Undecanone	M,D
	γ-Hexalactone	D
	n-Undecanal	M,D
35	2-Decenal	M,D
36	2,4-Nonadienal	D
	δ-Valerolactone (tent.)	D
	γ-Heptalactone	D
	Dimethyl sulfone	M,D
	n-Hexadecane	M,D
39	2,4-Decadienal	M,D
	n-Dodecanal	M,D
40	2-Undecenal	M,D
41	2,4-Decadienal	M,D
	δ-Heptalactone	D
	n-Heptadecane	M,D
	Heptadecene	D
	Acetylpyrrole	D
	γ-Octalactone	D

TABLE II. Continued

Peak No.	Compound	Sample
43	2-Tridecanone	M,D
	n-Tridecanal	D
44	2-Dodecenal	D
	δ-Octalactone	D
45	2,4-Undecadienal	D
	n-Octadecane	D
47	γ-Nonalactone	M,D
	2-Tridecenal	D
	2,4-Dodecadienal	D
	δ-Nonalactone	D
	γ-Decalactone	D
	2-Pentadecanone	D

M: Compound was found in roast beef meat.

D: Compound was found in roast beef drippings.

high concentrations of several saturated and unsaturated aldehydes. The relatively low concentrations of n-heptanal and n-octanal in the meat fat as opposed to their higher concentrations in drippings are remarkable.

Several of the aldehydes found in roast beef, especially one or two isomers of 2,4-decadienal, have been identified in many other foods such as soybeans (Wilkens et al., 1970;[63] Wilkens et al., 1970[64]), potatoes (Buttery et al., 1970[65]), potato chips (Mookherjee, 1965[66]), carrots (Buttery et al., 1968[67]) and tea (Bondarovich et al., 1967[68]). It may be of interest to note that some volatile constituents in boiled beef were also determined. The compounds identified are listed in Table III. No attempt was made to prepare a synthetic mixture of the identified components in order to test its odor, since some of the required reference compounds were not available. Because of the complexity of the mixture, further investigations may be necessary to elucidate the complete nature of the flavor of roast beef.

TABLE III. Volatile Constituents of Boiled Beef

Dimethyl sulfone	Benzoic acid
Dimethyl disulfide	Benzyl alcohol
γ-Butyrolactone	Trimethylpyrazine
Methylbutyrolactone	Dimethylethylpyrazine
Phenol	Vinylguaiacol
Acetylpyrrole	Methylcinnamaldehyde
Dimethylpyrazine	Tetradecanal
Benzothiazole	Pentadecanal
Benzaldehyde	Heptadecanal

CHAPTER V

VOLATILE METABOLIC PROFILES IN HUMAN URINE

CHAPTER V

VOLATILE METABOLIC PROFILES IN HUMAN URINE

INTRODUCTION

Gas-liquid chromatography (GLC) has been successfully used to resolve many natural, multicomponent mixtures such as petroleum, essential oils, and flavors of foods, alcoholic beverages and fruits.[69-72] These mixtures are usually of high complexity and are frequently found to contain several hundred constituents. Biological fluids such as blood and urine also appear to exhibit a large number of components. These have been primarily identified as proteins, carbohydrates, steroids, amino acids, lipids, phenolic acids, purines, pyrimidines, etc., i.e., relatively nonvolatile constituents.[73-74] Little attention, however, has been paid to the more volatial chemicals in samples of biological origin.

In this investigation the volatile constituents in a number of urine samples were studied with respect to their variations in different individuals, their dependence on diet, and their identities. An extraction-distillation technique was employed to prepare concentrates of volatile, urinary components which gave reproducible results. The concentrates were analyzed by capillary column GLC and some compounds were identified by mass spectrometry (MS). These studies of urines of healthy persons were undertaken to explore the possibility of detecting certain pathological states by observing differences in the profile of the low-boiling compounds. Conclusive information concerning changes in pathological urines requires that the GLC patterns of normal urines be free from significant variance.

EXPERIMENTAL

Urine samples were collected from 6 male and 3 female subjects. Concentrates of volatile components were prepared in the following manner: 450 ml. of a 24-hour urine sample were neutralized with 8 g of sodium bicarbonate. The sample was then extracted for 24 hours with 80 ml of redistilled, anhydrous Baker Analyzed diethyl ether in a continuous liquid-liquid extractor. To remove the nonvolatile portion, the extract was vacuum distilled at room temperature. Apiezon M grease was used to lubricate the ground glass joints of the apparatus.[70] The ether and volatile components were collected in two traps cooled with liquid nitrogen. A vacuum of 15-20 mm Hg was applied until the distillation of the ether was completed. The rest of the sample was distilled for 3 hours at a vacuum of 2 microns. The solutions of urinary constituents collected in the two traps were combined. By slow and careful distillation at atmospheric pressure over a Vigreux column most of the solvent was removed, and a sample of 1 ml was obtained which was pipetted into a 2 ml sample vial uncapped at room temperature until approximately 15 µl remained. The concentrate was stored in a freezer until it was gas chromatographed. A blank for the procedure was run by substituting distilled water for urine in the sample preparation. The results showed that except for ethyl acetate and ethanol, no components in the concentrates were due to contamination. Ethyl acetate and ethanol are impurities in diethyl ether.

The GLC analyses were performed on a Perkin-Elmer Model 900 gas chromatograph. Prepurified nitrogen was used as the carrier gas at an inlet pressure of 14 psi. All samples were introduced into the chroma-

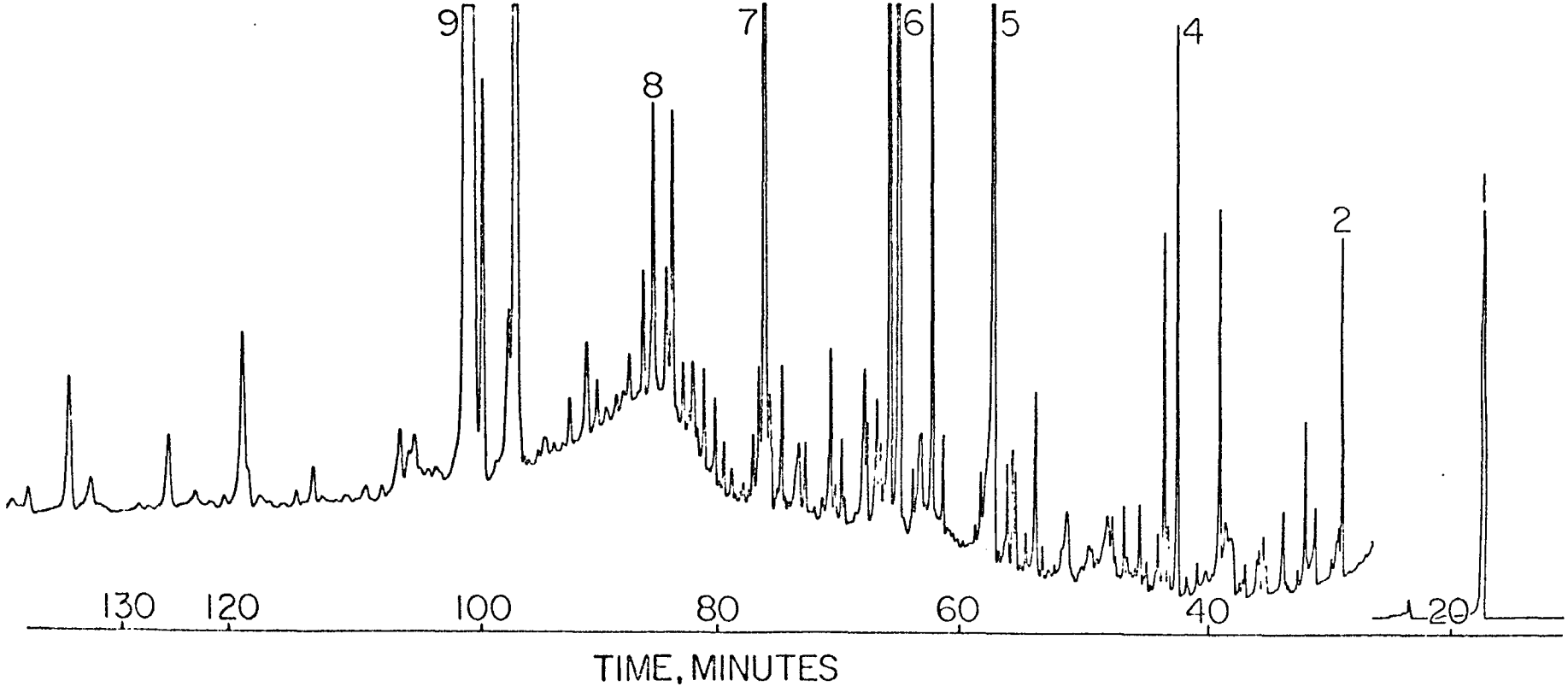
tograph by a direct injection procedure using a glass lined insert and separated on a 500 ft x 0.02 in., i.d., stainless steel column coated with Dowfax 9N15. The column was programmed from ambient temperature to 150°C at 2°C/min. Concentrates of urinary volatiles from male and female subjects were analyzed on an LKB 9000 gas chromatograph-mass spectrometer at 70 eV ionization energy, with an ion source temperature of 250°C and a separator temperature of 210°C. The GLC column used with this instrument was 700 ft of 0.03 in., i.d., stainless steel coated with Dowfax 9N15.

RESULTS AND DISCUSSION

Twenty urine samples from 9 different adults were analyzed. The experimental procedure described above was chosen because of its simplicity and reproducibility. Some alternative methods were examined which included high vacuum distillation of the whole urine. These required considerably more liquid nitrogen and longer distillation times. A procedure not incorporating a neutralization of the acids resulted in some cases in a broad acetic acid peak obscuring part of the chromatogram. It was observed, however, that all procedures gave comparable data. By dividing a urine sample into aliquot portions and processing them separately it was shown that the procedure employed gave reproducible results. Figures 9-11 depict that early portions of the chromatograms of samples from 3 subjects. A considerable number of components present in lower concentrations were eluted later.

Two urines of healthy individuals were analyzed by gas chromatography-mass spectrometry. The forty constituents which were identified are listed

Figure 9. Chromatogram of a concentrate of volatile urinary components from a sample of a male individual. Conditions: 500 ft x 0.02 in., i.d., Dowfax 9N15, programmed from room temperature to 150°C at 2°C/min., injector temperature 170°C, inlet pressure 14 psi N_2, sample size 2.5 µl.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TIME, MINUTES

Figure 10. Volatile urinary components in a sample from a female individual.

Conditions: 500 ft x 0.02 in., i.d., Dowfax 9N15, programmed from room temperature to 150°C at 2°C/min., injector temperature 170°C, inlet pressure 14 psi N_2, sample size 2.5 µl.

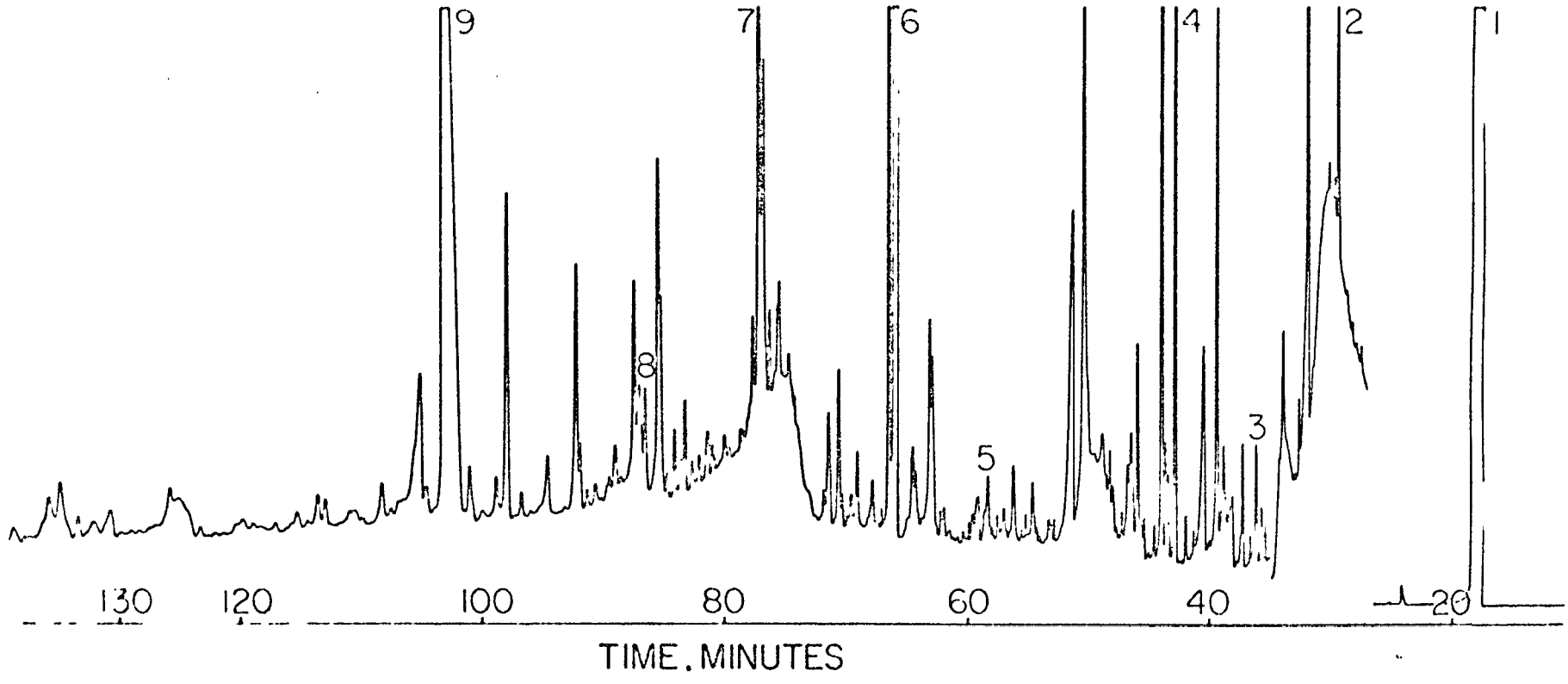
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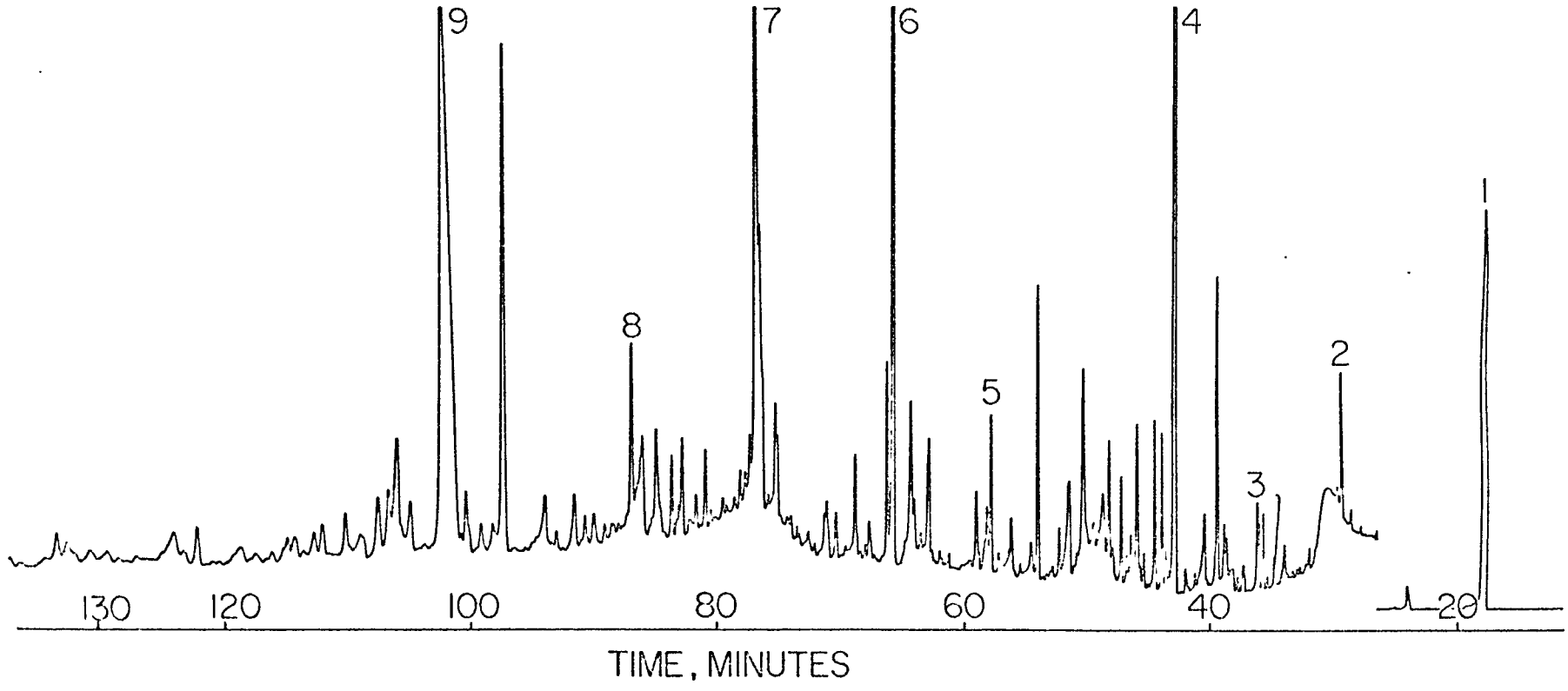
Figure 11. Volatile urinary components in a sample from another male individual.

Conditions: 500 ft x 0.02 in., i.d., Dowfax 9N15, programmed from room temperature to 150°C at 2°C/min., injector temperature 170°C, inlet pressure 14 psi N_2, sample size 2.5 µl.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TIME, MINUTES

in Table IV. The most characteristic components are diemthyl sulfone, pyrrole, 4-heptanone, allyl isothiocyanate, several alkyl furanes, ketones and lactones. An interesting compound with a molecular weight of 128 was tentatively identified as 4-methyl-5-hydroxyhexanoic acid lactone. Mass spectral data of this compound are as follows (relative intensities in parentheses): MW 128; 39 (21), 41 (48), 42 (55), 43 (60), 55 (24), 56 (100), 57 (22), 67 (3), 69 (7), 70 (9), 84 (27), 85 (6), 99 (2), 113 (2), 128 (2.5). The chromatograms show, especially when they are recorded at higher sensitivity, about three hundred peaks. Further studies should lead to additional identifications.

The chromatograms of the randomly selected twenty urine samples were quite revealing. It was suspected that dietary variations might cause drastic changes in the composition of the volatile fraction in normal urines. This would make it difficult for samples of pathological cases to be recognized. However, the chromatograms of all the samples studied show great similarities. The same result was obtained from the mass spectral data on the two samples which were analyzed. Concentrations of volatiles range from approximately 10 ng to 10 μg per 24 hr urine. Some of the components such as dimethyl sulfone, 4-heptanone and 2-pentanone invariably occur in high concentrations, while others such as pyrrole and allyl isothiocyanate are subject to more pronounced changes in concentration. In some cases differences up to an order of magnitude are observed. While some of these changes originate partly from variations in the diet, others may be characteristic of the individual. Urine samples from the same subject were collected in intervals of several days,

TABLE IV. Volatile Constituents in Human Urine

Peak No.	Compound	Sample
1	Diethyl ether (solvent)	
	3-Methyl-2-butanone	A,B
	2,3(?)-Dimethylfuran	A,B
	2,4-Dimethylfuran	B
2	2-Pentanone	A,B
	2-Methyl-3-pentanone	A
	3-Methyl-2-pentanone	A
	4-Methyl-2-pentanone	B
	1-Propanol	A,B
	2-Methyl-5-ethylfuran (tent.)	A,B
	Dimethyl disulfide	A,B
	3-Hexanone	A,B
3	2,3,5-Trimethylfuran	A,B
	2-Hexanone	B
	2-Methyl-1-propanol	A
	5-Methyl-3-hexanone (tent.)	A,B
	3-Penten-2-one	A,B
	4-Methylpent-3-en-2-one (tent.)	A

TABLE IV. Continued

Peak No.	Compound	Sample
4	4-Heptanone	A,B
	Cyclopentanone	A,B
	2-Methyltetrahydrofuran-3-one (tent.)	B
	3-Heptanone	A
	2-Heptanone	A,B
	3-Methylcyclopentanone (tent.)	A,B
	Limonene	B
	2-n-Pentylfuran	A,B
	4-Ethoxy-2-pentanone	A
	Cyclohexanone	A,B
	3-Octanone	B
5	Allyl isothiocyanate	A,B
	2-Octanone	B
	Acetic acid	A
6	Pyrrole	A,B
	Benzaldehyde	B
	2,3-Butanediol	A
7	γ-Valerolactone	A
	α-Terpineol	B
8	γ-Hexalactone	A
	Carvone	A
	δ-Hexalactone	A

TABLE IV. Continued

Peak No.	Compound	Sample
9	Dimethyl sulfone	A,B
	4-Methyl-5-hydroxyhexanoic acid lactone (tent.)	A
	p-Cresol	B

A: Concentrate of volatile, urinary compounds from a male subject.

B: Concentration of volatile, urinary compounds from a female subject.

days, two weeks, and three months during which time the diet must have changed. In spite of this, the chromatograms showed remarkable similarities. Indeed, the quantitative evaluation of a number of peaks in the urine chromatogram may lead to some form of "fingerprint." Pathological states could possibly be reflected in the profiles of the volatile urine compounds when they give rise to significant changes in the pattern of these compounds.

CHAPTER VI

CONCLUSION

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A group of closely-related methods have been devised which lead to the separation and identification of complex, volatile mixtures found in trace quantities in naturally occurring substances. The methods employ vacuum distillation, centrifugation, extraction (usually with diethyl ether), concentration, and gas chromatography, taking advantage of the efficiency and high separation power of open tubular columns to analyze the multi-component concentrates obtained. Combined gas chromatographic-mass spectrometric analyses were made to identify the compounds found. By using these procedures, it should be possible to analyze any substance which contains trace, volatile organic compounds contained in an aqueous or non-aqueous, liquid or solid matrix.

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