COMPARATIVE FLAVOR ANALYSIS OF THE BASIC VOLATILES OF ROASTED PEANUTS

By

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OF ROASTED PEANUTS

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TABLE OF CONTENTS

Chapter Page

I. INTRODUCTION. 1

II. HISTORICAL REVIEW 4

Volatile Compounds from Roasted Peanuts. 4
Pyrazine Compounds from Other Roasted Foods. 7
Quantitative Analysis of Volatile Compounds. 7
Some Peanut Quality Problems 9

III. EXPERIMENTAL METHOD AND MATERIAL. 11

Apparatus and Reagents 11

Vacuum Degassing System 11
Roasting Apparatus and Oil Removal. 14
pH Meter and Rotary Evaporator. 16
Gas Liquid Chromatograph. 16
Combination of Gas Liquid Chromatography-Mass Spectrometry 16
Reagents. 17

Peanut Samples 17

From Gold-Kist Co., Anadarko, Oklahoma. 17
From Department of Agronomy, Oklahoma State University. 17
From Department of Agricultural Engineering, Oklahoma State University 19

Procedure. 19

Roasting Peanuts and Pressing of Oil. 19
Collecting of Volatiles from the Oil. 23
Separation of Volatiles into Basic and Neutral-Acidic Fractions. 25
Concentration of the Basic Fraction 25
Quantitative Analysis by Gas Liquid Chromatography. 26
Identification of Compounds by Gas Liquid Chromotograph-Mass Spectrometer 26
Calculations Associated with Data 27

IV. RESULTS AND DISCUSSION 29

Chapter	Page

Design and Testing the Vacuum Degassing System Used for Collection of Volatiles. 29
Gas Liquid Chromatographic Analysis of Volatiles. . . 31
Evaluation of Analytical Procedure. 32
Peanut Flavor Analysis as Effected By Fertilization . 34

GLC Analysis 34
Identification of Unknown B. 40

Peanut Flavor Effected By Different Time of Planting. 40
Peanut Flavor Effected By Gas Treatment 45

GLC Analysis 49
Identification of the Unknowns 49

Absolute Concentrations of the Basic Volatiles of Roasted Peanuts 68
Three Minor Peaks on Chromatograms. 71

V. SUMMARY. 73

SELECTED BIBLIOGRAPHY 75

LIST OF TABLES

Table Page

I. Summary of Pyrazines Identified by Johnson in Basic Fraction of Roasted Peanut Volatiles. 5

II. Summary of Peanut Samples Related to the Experiment of Boron Deficiency in Fertilizer 18

III. Summary of Peanut Samples Planted and Harvested on Different Dates 20

IV. Summary of Peanut Samples Treated with Gaseous Atmospheres 21

V. Compounds in the Basic Fraction of Roasted Peanuts Identified by GLC 35

VI. Relative Concentration of Basic Fraction Constituents from Starr and Argentine Varieties of Spanish Peanuts . 36

VII. Relative Concentration of Basic Fraction Constituents from Peanuts Treated with Different Fertilizers 43

VIII. Relative Concentration of Basic Fraction Constituents from Dixie Peanuts Planted on Different Dates 46

IX. Relative Concentration of Basic Fraction Constituents from Spanhoma Peanuts Planted and Harvested on Different Dates 47

X. The Average Values from Table VIII and Table IX 48

XI. Relative Concentration of Basic Fraction Constituents from Peanut Sample Treated with Different Gaseous Atmospheres 56

XII. Estimated Concentration of 2,5- and 2,6-Dimethylpyrazines in the Roasted Peanut Samples 70

LIST OF FIGURES

Figure		Page
1.	Gas Liquid Chromatogram of the Basic Fraction of Roasted Peanuts Analyzed by Johnson (21).	6
2.	Photograph of the Vacuum Degassing System	12
3.	Schematic Drawing of the Vacuum System.	13
4.	Photograph of the Peanut Roasting Apparatus	15
5.	Flow Diagram of Experimental Procedure Used in the Analysis of Peanut Volatiles.	22
6.	Calculation of the Chromatographic Peak Area by Triangulation	27
7.	Calibration of McLeod Gauge with Thermocouple Gauge	30
8.	GLC Detector Response for Selected Pyrazine Standards . . .	33
9.	A Typical Gas Liquid Chromatogram of the Basic Fraction of Roasted Argentine Peanuts	37
10.	A Typical Gas Liquid Chromatogram of the Basic Fraction of Roasted Starr Peanuts	38
11.	Photograph of Peanut Samples Treated with Different Fertilizers	39
12.	Gas Liquid Chromatogram of Basic Fraction of Roasted Peanuts Fertilized with N, P, K..	41
13.	Gas Liquid Chromatogram of Basic Fraction of Roasted Peanuts Fertilized with N, P, K, B.	42
14.	Mass Spectra of Unknown B and 1, 2-Dichloroethane	44
15.	Photograph of Peanut Samples Treated with Different Gaseous Atmospheres	50
16.	Photograph of the Peanut Oil Collected from Samples Treated with Different Gaseous Atmospheres.	51
17.	Gas Liquid Chromatogram of Basic Fraction of Roasted	

Figure | Page

Peanuts Treated with Nitrogen. 52

18. Gas Liquid Chromatogram of Basic Fraction of Roasted Peanuts Treated with Carbon Dioxide. 53

19. Gas Liquid Chromatogram of Basic Fraction of Roasted Peanuts Treated with Dry Air 54

20. Gas Liquid Chromatogram of Basic Fraction of Roasted Peanuts Treated with Sulfur Dioxide (5%) and Nitrogen (95%). 55

21. Alternating Voltage Accelerator Tracing of Unknown 1S. . . . 58

22. Alternating Voltage Accelerator Tracing of Unknown 3N
(A) By m/e 78 and m/e 80. 59
(B) By m/e 79 and m/e 80. 60

23. Mass Spectrum of Unknown 1S. 61

24. Gas Liquid Chromatogram of a Standard Mixture of Pyrazine and Pyradine 62

25. Mass Spectra of Pyridine and Unknown 3S. 63

26. Mass Spectra of Unknowns 3S' and 2N. 64

27. Mass Spectra of Unknowns 4S and 3N 66

28. Mass Spectrum of Pyrazine. 67

29. Mass Spectra of Unknowns 5S and 1N 69

30. Gas Liquid Chromatogram of the Three Minor Peaks in Roasted Starr Peanuts. 72

CHAPTER I

INTRODUCTION

The flavor of food was appreciated for a long time before any research could be done to explain it on a scientific basis. Flavor is a complex sensation involving taste and odor, and is the result of interaction between chemical stimuli in the food and the sensory apparatus (1). On October 2, 1969, the Society of Flavor Chemists defined flavor as the sensation caused by any substance, natural or synthetic, taken in the mouth, which stimulates the senses of taste and smell (2).

The peanut is one of the finest and most valuable foods; it provides an abundant source of protein, vitamins and minerals at a modest cost. Heat processing or roasting, greatly improves the flavor and the texture of peanuts, and such products retain the nutritional value (3).

The pleasant flavor of the roasted peanut is enjoyed by many people. The roasted peanuts and roasted peanut products have a distinctive odor which may be attributed in part to certain pyrazine compounds (4, 5).

To gain a complete understanding of a food flavor, a three-pronged investigation is necessary:

(a) To isolate the volatile components from foods.

(b) To separate and to identify the volatile components.

(c) To determine and to quantitatively measure either absolutely

or relatively the component(s) which is (are) responsible for the flavor in the food.

The poor stability and the minute quantities of the volatiles complicate flavor analysis, especially for quantitative determination, so sensitive instruments such as the mass spectrometer, and the gas liquid chromatograph are essential to deal with the flavor research.

In a wide range of foodstuffs, differences in the composition of volatiles were reported among botanical varieties (8, 9, 10), geographical origins (11, 12) and food-handling conditions (13, 14). However, the differences were suggested mostly in the relative concentration of volatiles rather than in the presence or absence of certain component(s) (15, 10).

The purpose of flavor research is to improve the flavor in foods, to control the quality of foods and to formulate flavor imitations.

Roasted peanut flavor has been studied at Oklahoma State University since 1961. Several studies were successfully done on flavor precusors (16, 17, 18), the volatile components (4, 5, 19, 20, 21) of roasted peanut flavor, and the formation of alkylpyrazines in roasted peanuts (17, 22, 23). Most of these were qualitative studies.

There are three predominant types of peanuts -- Spanish, Runner and Virginia (48). Each type of peanut includes several varieties. All of the samples examined in the study were of the Spanish type.

The quality of flavor has well been recognized to be related to maturity, storage conditions and may also be related to fertilization, irrigation and location. However, no quantitative studies were available. The major objective of this research was to conduct quantitative

flavor analysis on peanut samples from a variety of treatments and contribute to a continuation of research on peanut flavor in this laboratory. It focused on the comparative examination of the volatile constituents in the basic fraction (pyrazines) of roasted peanuts representing different varieties, different fertilization levels, different storage conditions under different gaseous atmospheres and different time of planting and harvesting.

This study was mostly devoted to the relatively quantitative analysis of different samples by gas liquid chromatography. During the study some unknown compounds were found in certain samples, so an effort to identify these compounds by the combination mass spectrometer-gas liquid chromatograph was made.

CHAPTER II

HISTORICAL REVIEW

Volatile Compounds from Roasted Peanuts

Pickett and Holley (24) initiated research on roasted peanut flavor in 1952. Hoffpauir (25) published a short review a year later. No other report appeared until Mason (16) and Mason *et al* (19) isolated and identified some of the volatiles from roasted peanuts; the major components were 2,5-dimethylpyrazine and benzaldehyde.

Newell (17) and Newell *et al* (19) indicated that the precursors of the pyrazines found in roasted peanut flavor were amino acids and reducing sugars. Koehler (22) and Koehler *et al* (23) confirmed this finding and the latter authors elucidated the mechanism of their formation using radioisotopically labeled precursors.

The major advance in identification of roasted peanut volatiles was done by Johnson (4, 21) and Johnson *et al* (5, 20). Five pyrazines and several aldehydes, all of low molecular weight, were identified by these authors (4, 5, 20). Johnson (21) employed the combination mass spectrometer-gas chromatograph, ultraviolet spectroscopy, infrared spectroscopy and nuclear magnetic resonance spectrometry and identified the other components in roasted peanut aroma. He listed a total of 26 and 24 compounds identified in the basic fraction and the neutral fraction respectively. A summary and a chromatogram of the compounds identified by Johnson (21) in the basic fraction were shown in Table I and Figure 1 respectively.

TABLE I

SUMMARY OF PYRAZINES IDENTIFIED BY JOHNSON (21) IN BASIC FRACTION OF ROASTED PEANUT VOLATILES

Compound	MW	Component	Analyzed by: MS[1]	AVS-MS[2]	IR[3]	NMR[4]	GC-RT[5]	UV	Identification[6] P or T
2-Methyl-[a]	94	A	X			X	X		P
2,5-Dimethyl-[a]	108	B				X	X		P
2,6-Dimethyl-	108	C	X				X		P
2-Ethyl-	108	D	X	X			X		P
2,3-Dimethyl-	108	E	X				X		P
2-Ethyl-6-methyl-	122	F	X(HR)		X		X		P
2-Ethyl-5-methyl-	122	G	X		X		X		P
2-Ethyl-3-methyl-	122	H	X				X		T
Trimethyl-[a]	122	I	X			X	X		P
2,5-Dimethyl-3-ethyl-	136	J	X		X		X		P
2,3-Dimethyl-5-ethyl- 2,6-Dimethyl-3-ethyl-	136	K	X		X		X		T
2-Methyl-6-propyl-	136	L	X						T
2,6-Diethyl-3-methyl- 2,3-Diethyl-5-methyl-	150		X				X		T
2-Ethyl-3,5,6-Trimethyl-	150		X				X		T
Methyl-2,3-cyclopentane-	134	S	X(HR)						T
2-Isopropenyl-	120	T	X					X	T
Methyl isopropenyl-	134	W	X(HR)					X	T

1 - mass spectra (HR = High resolution); 2 - accelerating voltage switching mass spectra; 3 - infrared; 4 - nuclear magnetic resonance; 5 - gas chromatographic retention time; 6 - P = positive, T = tenative; a - previously identified in roasted peanut volatiles.

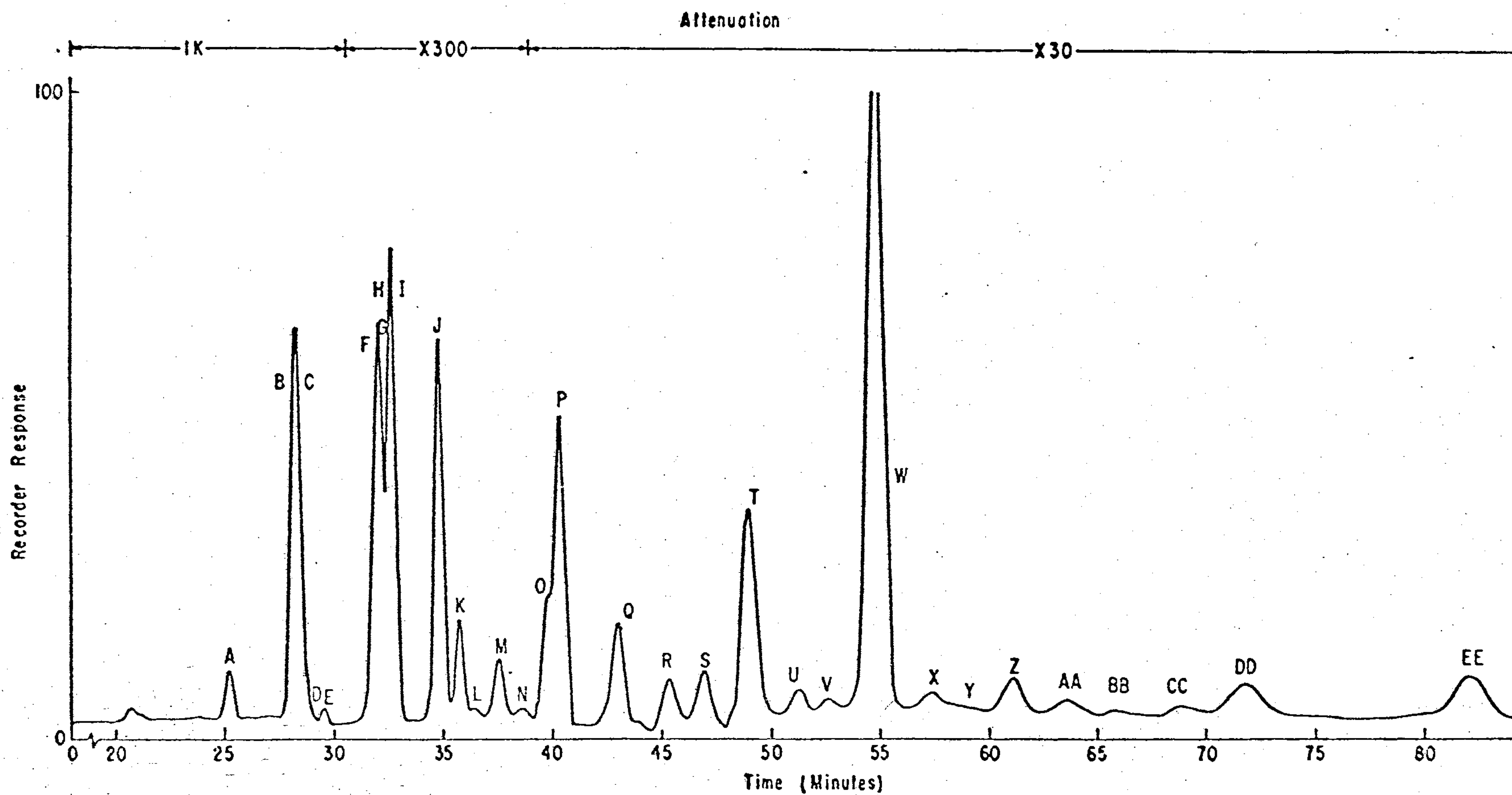


Figure 1. Gas Liquid Chromatogram of the Basic Fraction of Roasted Peanuts Analyzed by Johnson (21). For Identification of the Compounds see Tables I and V.

Pyrazine Compounds from Other Roasted Foods

Pyrazine compounds have been found not only in roasted peanuts but also in other roasted foods. Reichstein and Standinger (26), Stoll et al (27) Goldman et al (28) and Bondarovich et al (6) collectively identified 22 pyrazine compounds from coffee, and the last group of authors reported complete infrared and mass spectrometer data for these compounds.

Deck and Chang (29) identified 2,5-dimethylpyrazine as an "earthy, raw potato" flavor and estimated the concentration of this compound at about 10 p.p.m. in oil from potato chips.

Marion et al (30) Rizzi(31), van der Wal et al (32) and van Praag et al (7) collectively identified 11 alkylpyrazines in the basic fraction of cocoa.

Recently Wang and co-workers (33) identified 5 alkylpyrazines as the major basic volatile components from roasted barley.

Johnson (4) and Mason et al (5) and later Bondarovich et al (6) and van Praag et al suggested that the pyrazine compounds contribute the nut-like odor and play an important role in the flavor of a variety of roasted foods.

Quantitative Analysis of Volatile Components

Reports devoted exclusively to the quantitative analysis of the volatile components of roasted peanuts are very limited. An early study by Pickett and Holley (24) indicated that carbon dioxide and water vapor occurred in relatively high percentages in the roasted peanut volatiles. Young and Holley (34) compared the differences in volatiles resulting from storage and roasting of peanut varieties.

They concluded that the yield of peanut volatiles from roasting increased when nuts were stored after shelling, and they also pointed out that the effect was dependent on variety.

Koehler (22), the first author reporting absolute quantitative analysis of peanut flavor, determined chromatographically that 2-methylpyrazine was present in roasted peanuts at a concentration of 6 mg/kg.

Cobb (36) utilized the isotopic dilution method to measure the volatile quantity (benzaldehyde) in roasted peanuts. The roasted peanuts were slurried with water, then benzaldehyde-^{14}C was added to the slurry. After reduced-pressure distillation, 2,4-dinitro-phenyl-hydrazine was used to generate the corresponding 2,4-dinitrophenylhydrazones in the distillate. Separation of the labeled hydrazones from other material presented was achieved by thin layer chromatography, and the recovered material was analyzed by ultraviolet spectroscopy. The initial benzaldehyde was calculated from the formula proposed by Day et al (45).

Published papers dealing with the quantitative analysis of the volatiles of other foods are more frequent than those on roasted peanuts. Recently several groups of workers determined the percent of several volatiles in various foods. Wilson (37) quantitatively analyzed the volatiles from celery essential oil, Buttery et al (38) from carrots, Smith et al (11) from spearmint oil, and Stinson et al (39) from cherry essence. Brodnitz and Pollock (40) used the same method to determine if the composition of an onion oil varied from standard oil. Nelson et al (13) analyzed the tomato volatiles by a known standard (isobutyl acetate) which was added to the samples prior to extraction; the quantities corresponded to the standard were obtained. Most

of these quantitative studies took advantage of the technique of gas liquid chromatography.

Recently Biggers _et al_ (41) presented a computational method to rank the blends of coffee using gas chromatography and computer analysis. Two extreme varieties of coffee, the best one and the worst one, were chromatographically investigated, then two sets of chromatographic profiles and the ratio of selected peak heights were computerized to constitute a pattern. A comparison of the profiles, peak intensities and peak ratios between the pattern and the unknown sample was produced by the computer and used for predicting the quality of the sample.

Some Peanut Quality Problems

Harris (46, 47) found that growth and development of peanuts were influenced by boron, one of the minor elements. Recently Hallock (49) reported that deficiency of boron made peanut quality inferior and caused damage in the seed, namely, hollow heart or concealed damage.

Manbeck _et al_ (50) investigated the storage of peanuts. The research was initiated because peanut growers, lacking facilities to cure a large quantity, often delayed drying their freshly dug peanuts. Consequently, they had to send these peanuts to some place to be dried and stored. This group of workers intended to find out a way of storing peanuts for a short duration under varying atmosphere treatments, while keeping the peanuts from losing quality.

Woodroof (3) pointed out, that harvesting too early would result in a high proportion of immature nuts of low quality, and harvesting too late would lead to loss of peanuts from sprouting due to overmaturity. An investigation was initiated by the Department of Agronomy at Okla-

homa State University to determine if the interval between planting and harvesting influenced the peanut quality.

CHAPTER III

EXPERIMENTAL METHOD AND MATERIAL

A. APPARATUS AND REAGENTS

Vacuum Degassing System

The vacuum degassing system similar to the one used by Merritt (42), Mason (19) and Johnson (21) was modified and employed throughout this study. The system was composed of vacuum pumps, glass tower, cold traps, and pressure gauge as shown in Figures 2 and 3. Both a mechanical vacuum pump (Welch Dual Stage) and an oil diffusion pump were utilized to produce high vacuum. The former was rated at 0.1 micron and 140 liters per minute; the latter was three-staged and air-cooled.

The glass tower assembly was 147 cm high and was used for the oil degassing. A 500 ml reservoir was designed at the top of the glass tower to introduce the oil into the foaming chamber. The side arm tubing of the foaming chamber circulated the pressure above and below the oil level when foaming took place. From the foaming chamber the oil was conducted to the glass column. The flow rate of the oil was controlled by a teflon stopcock.

The glass column was constructed in a series of expansion bellows which the oil passed over. The expansion bellows provided the maximum surface area and made the oil degassing more efficient. The oil was collected in a 100 ml round bottom flask situated at the bottom of the

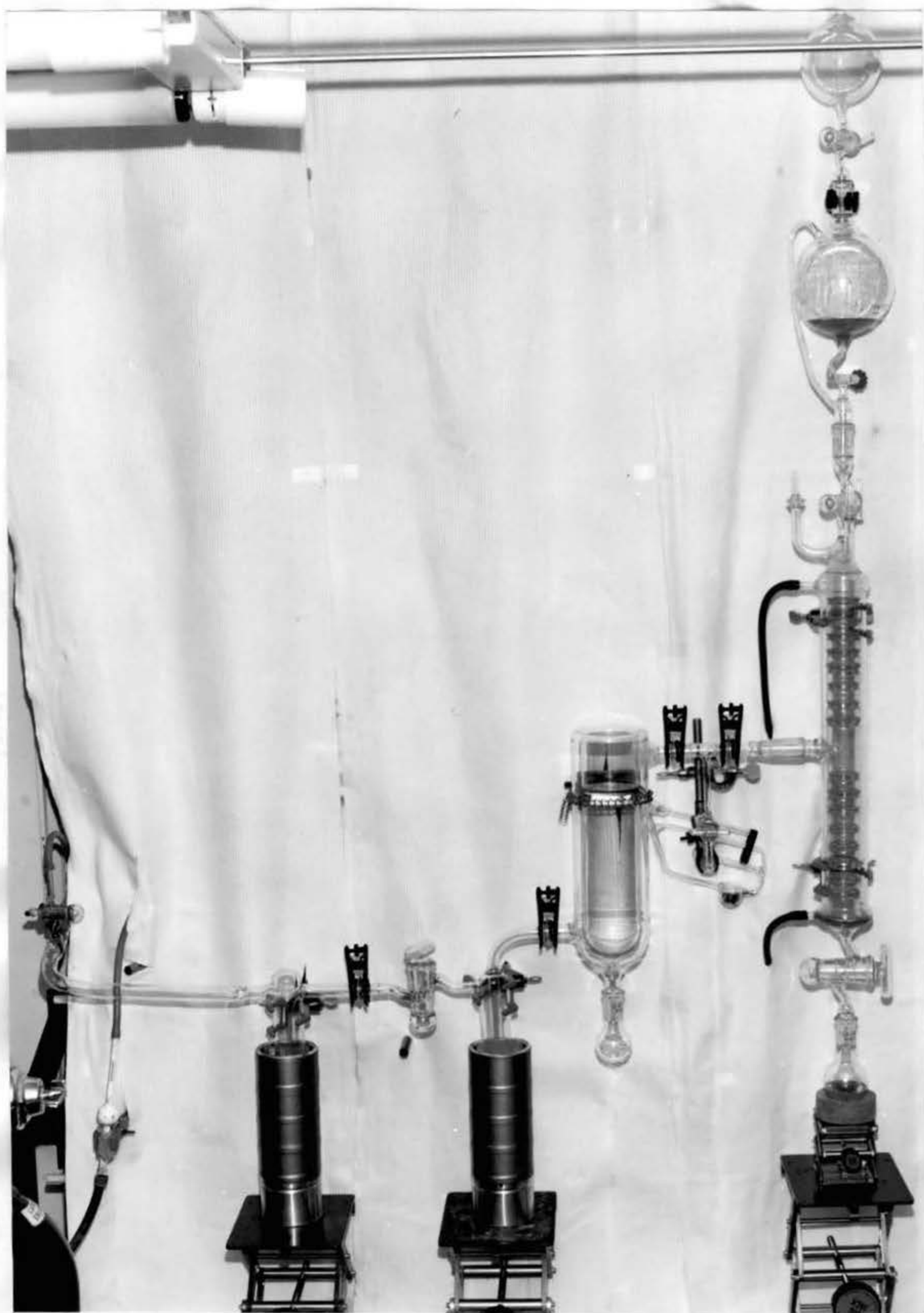


Figure 2. Photograph of the Vacuum Degassing System

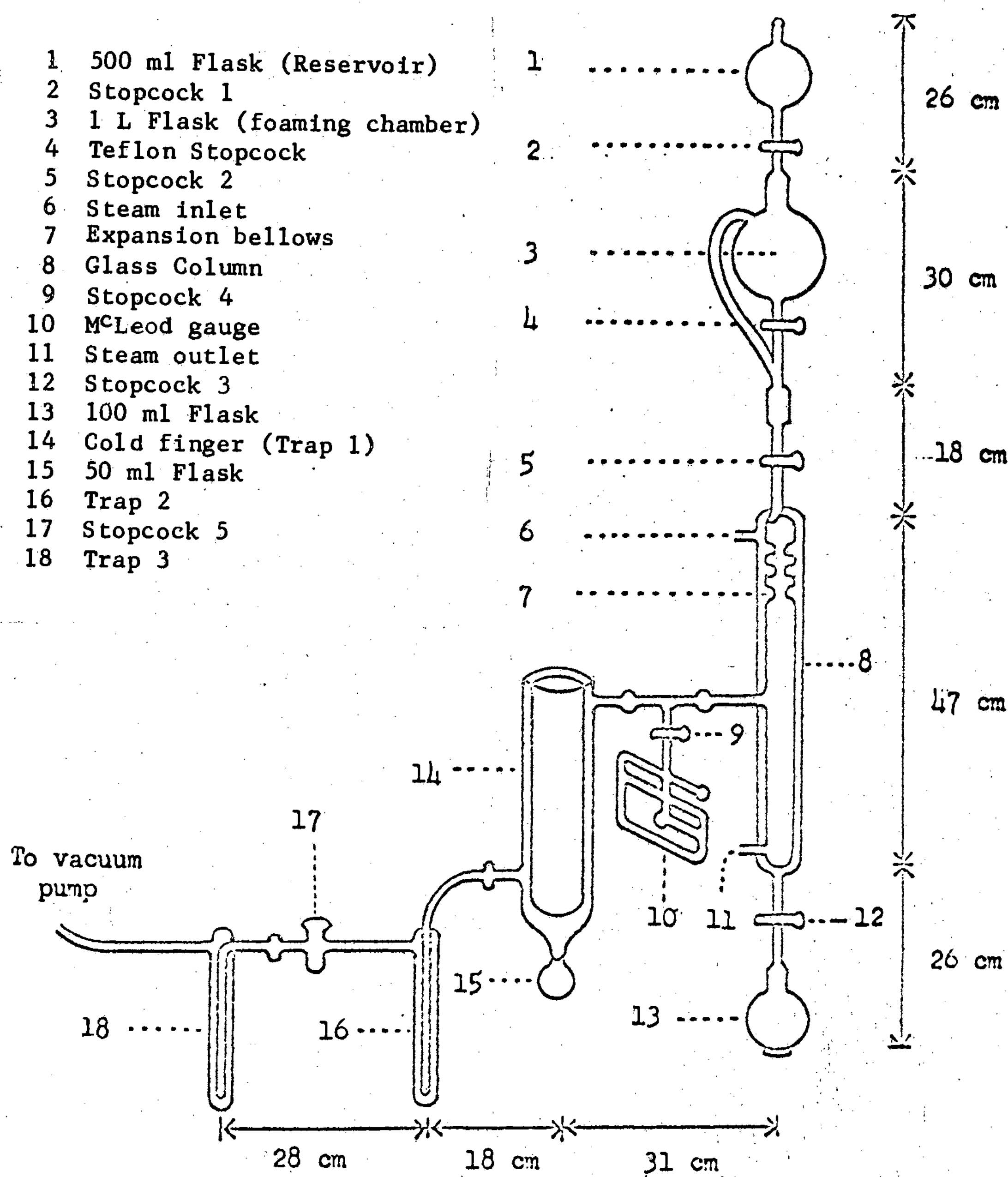


Figure 3. Schematic Drawing of the Vacuum Degassing System

glass tower. The glass column was jacketed with steam of about 90° C for accelerating the degassing effect.

A large cold finger (trap 1) and two cold traps (traps 2 and 3) were included between the glass column and the diffusion pump. Dewar flasks of liquid nitrogen were placed around the two cold traps, and the cold finger was filled about two-thirds full with liquid nitrogen. Most of the volatiles removed as condensate were collected by the 50 ml flask attached to the cold finger; the rest was collected by the trap 2. The trap 3 was used for preventing the diffusion oil from backing through the evacuated system and contaminating the volatiles.

A McLeod mercury gauge of the tilting type was set up perpendicularly between the cold finger and the glass column. Stopcock 4 was used to isolate the mercury in the gauge for keeping mercury from distilling out. Nitrogen gas in a cylinder was used to balance the pressure of the system with the atmospheric pressure before the collected condensate was removed from the system. Viton O-rings, size 28/15, were inserted on the ball joints of the system to improve the vacuum.

Roasting Apparatus and Oil Removal

A General Electric Deluxe rotisserie oven equipped with a cylindrical wire basket as shown in Figure 4 was used to roast the peanuts. The rotisserie oven generated 450° F maximum temperature and rotated 6 cycles per minute. The wire basket was 6 inches in diameter, by 13 inches long and could handle up to 1,500 gm of peanuts. A Gray Co. Universal electric timer was connected with the rotisserie oven. A high intensity lamp was placed outside the window of the oven for checking the color of roasting peanuts.

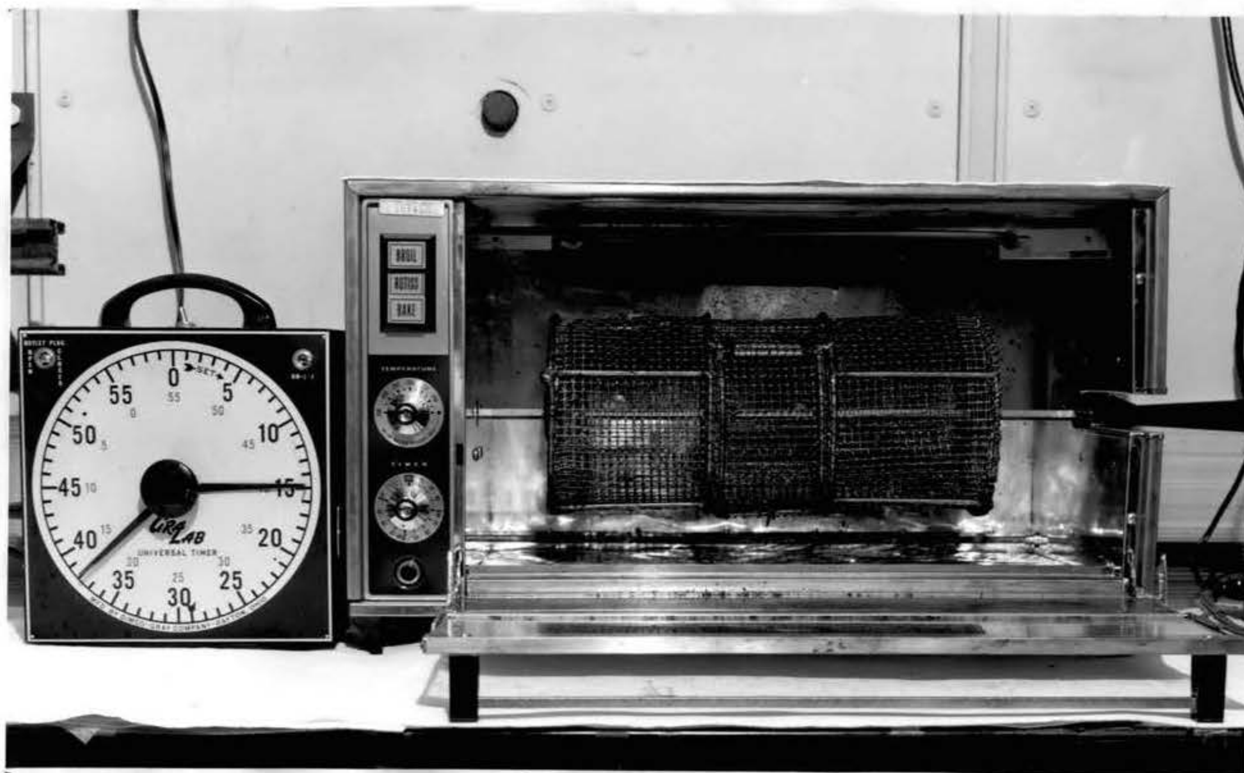


Figure 4. Photograph of the Peanut Roasting Apparatus

The oil of roasted peanuts was removed by a Carver laboratory press. The plate of the press was fitted with a filter cloth, 12 inches long and 12 inches wide, purchased from Arthur H. Thomas Co.

pH Meter and Rotary Evaporator

A Sargent Model DR single glass electrode pH meter was used to adjust the pH values of the collected condensate. The basic fraction was concentrated using a Buchler Instruments rotary evaporator. A stoppered mercury manometer was connected with the rotary evaporator for measuring the vapor pressure in the evaporator.

Gas Liquid Chromatography (GLC)

All the gas liquid chromatographic analyses were accomplished with a modified Barber Colman Model 5,000 gas liquid chromatograph (43) equipped with a hydrogen flame-ionization detector. Hydrogen was produced by a Milton Roy Model E-150 hydrogen generator. Helium was used as the carrier gas.

Combination of Gas Liquid Chromatography-Mass Spectrometry (GC-MS)

Mass spectral analyses were performed with a prototype of the LKB 9000 combination gas liquid chromatograph-mass spectrometer as described by Waller (43). It was constructed in 1966 in the laboratories of Dr. Ranger Ryhage at the Karolinska Institutet in Stockholm, Sweden. The spectral data, measured manually, were computerized with the IBM 350/50 computer and plotted with a Cal Comp Model 565 Plotter (44).

Reagents

Methylene Chloride, Spectranlyzed, (Redistilled) Fisher Scientific Co. Fair Lawn, Jersey.

Gas Chrom Q (100/120 mesh), Carbowax 20 M. Applied Science Laboratory, Inc., P. P. Box 140, State College, Pa.

Pyrazine and 2,6-Dimethyl-pyrazine, Aldrich Chemical Company, Milwaukee, Wisconsin.

Apiezon N Grease was supplied by Apiezon Products Limited, 8, York Road, London.

Pyridine, Fisher Scientific Co., Fair Lawn, Jersey.

B. PEANUT SAMPLES

Raw shelled peanuts were obtained from the following sources:

(i) Gold-Kist Peanut Co., Anadarko, Oklahoma

Sample 1, Argentine variety, was purchased from Gold-Kist Peanut Co. in 1969.

(ii) Department of Agronomy, Oklahoma State University

A total of twelve samples were donated by Department of Agronomy.

Sample 2, Starr variety, was used to compare with sample 1 in volatiles.

Two samples were used to relate boron deficiency to peanut flavor. Sample 3 was fertilized with three elements: N, P, and K in the ratio of 20:80:80 while sample 4 with four elements: N, P, K and B in the ratio of 20:80:40:40. Sample 3 had a high degree of non-uniformity in seed size, and it contained more immature kernels than Sample 4 (Table II).

Samples 5, 6 and 7 were planted and harvested on different dates

TABLE II

SUMMARY OF PEANUT SAMPLES RELATED TO THE EXPERIMENT OF BORON DEFICIENCY IN FERTILIZER*

Sample Description \ Sample No.	3	4
Elements in Fertilizer	N,P,K	N,P,K,B
Ratio of Elements	20:80:80	20:80:40:40
Immature Kernels	More	Less
Seed Size	High degree of non-unifority	Medium degree of non-uniformity

* Both samples were of Starr variety planted on 7/6/69 and harvested on 10/1/69 at Fort Cobb, Oklahoma.

and were used to investigate the effect of these variables on the volatile components (Table III).

Two groups of samples were concerned with the comparison of different periods of growth. For one group (samples 8, 9 and 10) the interval between planting and harvest was 120 days while for the other one (samples 11, 12 and 13) this interval was 160 days (Table III). The quantity of peanuts obtained for Samples 3 to 13 ranged from 400 to 800 gm.

(iii) Department of Agricultural Engineering, Oklahoma State University

Five samples (samples 14 to 18) were obtained from an experiment concerned with the effect of gas treatments on *Aspergillus flavus* spores. These samples were examined to determine the effect of gas treatments on volatiles. Samples 14, 15, 16 and 17 were innoculated with *A. flavus* spores and then exposed to a different gaseous atmosphere: nitrogen, carbon dioxide, dry air, or a combination of sulfur dioxide (5%) and nitrogen (95%) in containers at $35^\circ \pm 2^\circ$ F for fourteen days in storage. The last sample was an untreated control which was stored in atmospheric air at ambient temperature plus ten degrees for the same length of time (Table IV). All the five samples obtained ranged from 500 to 800 gm.

C. PROCEDURE

A flow diagram of the analysis procedure was shown in Figure 5.

Roasting Peanuts and Pressing of Oil

All peanut samples were stored at -12° C until roasted. One and a half hours in advance of roasting the peanut sample was placed in

TABLE III

SUMMARY OF PEANUT SAMPLES PLANTED AND HARVESTED ON DIFFERENT DATES

Sample No.	Variety	Dates Planted to Harvested	No. of Days	Location
5	Dixie	5/23/69 -- 9/20/69	120	Stratford, OKLA.
6	Dixie	5/29/69 -- 9/26/69	120	Stratford, OKLA.
7	Dixie	6/10/69 -- 10/8/69	120	Stratford, OKLA.
8	Spanhoma	5/22/69 -- 9/19/69	120	Fort Cobb, OKLA.
9	Spanhoma	5/29/69 -- 9/26/69	120	Fort Cobb, OKLA.
10	Spanhoma	6/10/69 -- 10/8/69	120	Fort Cobb, OKLA.
11	Spanhoma	5/22/69 -- 10/29/69	160	Fort Cobb, OKLA.
12	Spanhoma	5/29/69 -- 11/5/69	160	Fort Cobb, OKLA.
13	Spanhoma	6/10/69 -- 11/17/69	160	Fort Cobb, OKLA.

TABLE IV

SUMMARY OF PEANUT SAMPLES TREATED WITH GASEOUS ATMOSPHERES[a]

Sample No.	Code[b]	Temperature Level (C^o)	Gaseous Atmosphere	Odor	Color
14	CYN	35 ± 2	N_2	Normal	Slightly Darker
15	CYC	35 ± 2	CO_2	Normal	Normal
16	CYA	35 ± 2	Dry Air	Normal	Normal
17	CYS	35 ± 2	SO_2, 5% & N_2, 95%	Undesirably Sour	Very Light
18	Control	Ambient+10	Atmosphere	Normal	Normal

a - All samples were cured partially to about 20% moisture in field.

b - Codes used were as follows:
C - Cold temperature 35 ± 2° C.
Y - Some field curing (about 20% in moisture).
N - Nitrogen (100%)
C - Carbon dioxide (100%)
A - Dry Air.
S - Combination of Sulfur dioxide 5% and Nitrogen 95%.

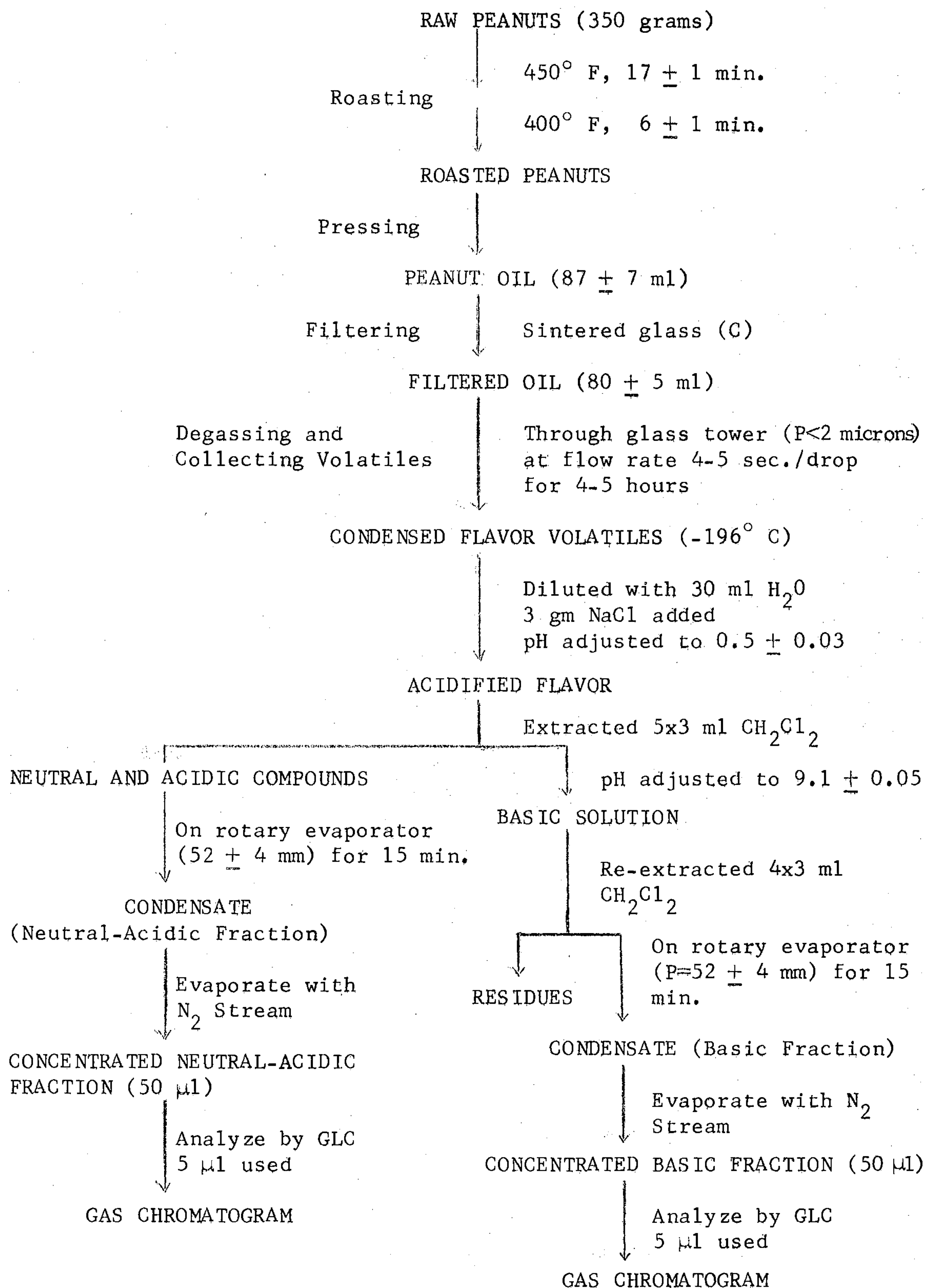


Figure 5. Flow Diagram of Experimental Procedure Used in the Analysis of Peanut Volatiles

ambient temperature (about 25° C). An average batch of 350 gm. was processed. The rotisserie oven was set at 450° F and warmed up for 30 minutes; then the whole peanuts without shells were placed in the wire basket and placed in the oven. A medium roast was desired and this point was judged initially by the color and the smell of the roasting peanuts. It required 16 to 18 minutes at 450° F to reach the medium roast stage, then an additional 5 to 8 minutes was required to complete the roasting procedure. This latter step varied according to the sample type. The roasted peanuts were immediately folded in aluminum foil to retard cooling and were carried to the pressing room. The press plate holder was lined with a piece of filter cloth and then filled with peanuts. The oil was pressed at 10,000 psi and collected with a 100 ml graduated cylinder. About 80 ml to 94 ml of oil was collected.

Collecting of Volatiles from the Oil

The pressed oil was filtered through a coarse Buchner funnel with a water aspirator to remove the small bits of testa which interfered with the passage of the oil through the stopcocks. The filtered oil was remeasured in another 100 ml graduated cylinder, and it was usually found that about 8 ml to 10 ml of oil was lost in the process of filtering. The volatiles were collected from the filtered oil by the vacuum degassing system.

Before the oil was transfered into the system, three preliminary steps were done: (a) the system was evacuated until a vacuum of lower than 2 microns of mercury was reached, (b) the glass column was heated completely with circulating steam and hot water, and (c) liquid nitro-

gen was added first to trap 3, then to trap 2, finally to trap 1, thus eliminating contamination by the diffusion oil. The peanut oil was slowly introduced into the foaming chamber from the reservoir by the glass stopcock 1. While the oil foamed for about ten minutes, some volatiles were quickly removed. The oil was then led to the heated glass column and the flow rate adjusted to 4 to 5 seconds per drop. The oil was degassed and the volatiles were removed by condensation on the upper area of the cold finger as the oil dropped along the expansion bellows into the collection flask. Four and one half hours were required for the oil to move entirely into the flask. It was found that one pass of oil through the glass tower at such a flow rate was sufficient to considerably deodorize the oil.

To remove the volatiles from the system the following procedure was used:

(a) Isolate the system by turning off stopcocks 2, 3 and 5.

(b) Turn off both pumps.

(c) Move the dewar of liquid nitrogen from trap 2 to the 50 ml flask under the cold finger.

(d) Warm up trap 2 and let the trace volatiles migrate to the cold finger and the 50 ml flask.

(e) Replace the liquid nitrogen in the cold finger with hot water in order to transfer the volatiles to the 50 ml flask.

(f) Heat the neck of the 50 ml flask with a hair drier until the flask could be rotated around the joint.

(g) Balance the system pressure with nitrogen (stopcock 5).

(h) Remove the 50 ml flask from the system, stopper, then store at -12° C.

When the volatiles of one sample were collected, the degassing system was taken apart. The grease on the glassware was wiped out as much as possible. Then the glassware was rinsed with the Skelly Solvent B, and put in a high-temperature oven at 1.100° F overnight to volatilize the oil residues.

Separation of Volatiles into Basic and Neutral-Acidic Fractions

The separation method was adapted from van Praag *et al* (7) and Johnson (21). The collected volatiles in the 50 ml flask were diluted to 30 ml with distilled water, transfered to a small beaker, and 3 mg of NaCl was added to the solution. When the NaCl was completely dissolved, the pH was adjusted to 0.5 $\pm$ 0.03 with concentrated hydrochloric acid. The acidified fraction was extracted 5 times with 3 ml of redistilled methylene chloride, separation of the methylene chloride layer (neutral-acidic fraction) from the aqueous fraction was obtained by the separating funnel and the neutral-acidic fraction was kept for further study. The pH of the aqueous fraction was readjusted to 9.1 $\pm$ 0.05 and the solution was reextracted 4 times with 3 ml of redistilled methylene chloride to produce the basic fraction. The basic fraction was transfered to a 50 ml round-bottomed flask, stoppered and stored at 2° C.

Concentration of the Basic Fraction

By means of a rotary evaporator, the basic fraction in the flask was concentrated at room temperature for 15 minutes. The pressure in the evaporator was regulated with a stoppered mercury manometer, which was connected to the evaporator. The pressure was kept at 52 $\pm$ 4 mm of

mercury during the experiment. Then the basic volatile condensate was transferred to a small vial and carefully evaporated by a stream of nitrogen to 50 μl. This small vial, 1.5 inches long, was prepared by sealing off the smaller end of a Pasteur pipette over a laboratory burner. The vial was calibrated at the 50 μl mark with distilled water. Only oven-dry vials were used.

Quantitative Analysis by Gas Liquid Chromatography

Analytical column similar to the one Johnson (21) used was prepared and employed. The column, 21 feet long and ¼ inch in diameter, was packed with 5% (W/W) Carbowax 20 M on base washed gas Chrom Q (35, 21). A sample (5 μl) of the concentrated basic fraction was removed from the vial and analyzed on the packed column in the gas liquid chromatograph under the following conditions: oven temperature programmed from 70° C to 170° C at 3° C/min; injection port temperature, 180° C; the detector temperature 275° C; helium flow of 40 ml/min.; compressed air pressure 20 psi; and hydrogen pressure 16 psi.

Identification of Compounds by Gas Liquid Chromatograph-Mass Spectrometer

The gas liquid chromatograph-mass spectrometer was used to identify some of the unknown compounds which were found on gas liquid chromatographic analysis of some of the peanut samples. All the spectral data were obtained under the following conditons: ionizing voltage - 20 electron volts, accelerating voltage - 3.5 KV, trap current 40 to 60 μ amps, electron multiplier voltage - 1.7 to 2.1 KV, source temperature - 310° C, separator temperature - 220° C and the scan speed for mass 0-200 - 3 to 5 seconds.

The alternating voltage acceleration (AVA) mass spectrometric method as proposed by Sweeley et al (51), was used to investigate partially resolved compounds. Two values of m/e were chosen and continuously recorded. The selection of these two values was based on the fact that the AVA technique could separate two ions only within 10% of the mass range.

Calculations Associated with Data

The chromatographic peak indicated the relative concentration of a compound or a mixture of compounds in the sample. The triangle method was used to estimate the peak area by drawing a straight line through the inflection point of each side of the curve as shown in Figure 6A. The area of the triangle was calculated by the formula:

$$\text{Area of peak} = \tfrac{1}{2}\ \text{Height} \times \text{Base}$$

For the overlapping peaks, each peak area was calculated by doubling each right triangle (Figure 6B).

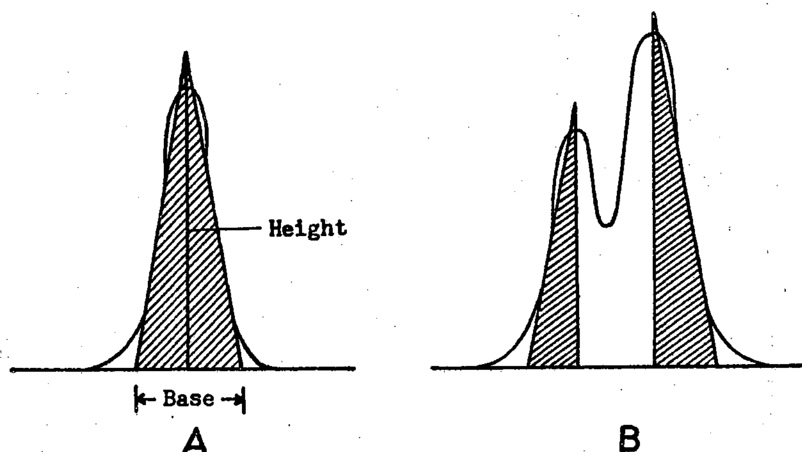


Figure 6. Calculation of the Chromatographic Peak area by Triangulation. A. Resolved Peak B. Over-lapping Peaks.

The calculation of the relative concentration for each peak was (i) to assign the highest peak, peak 2, as the base (100%) (ii) to divide each peak area by peak 2 area. When the concentration was less than 1% it was termed trace.

The peak standard deviation (S.D.) was estimated by the formula:

$$S.D. = \sqrt{\frac{(X-\bar{X})^2}{N-1}} \qquad (52)$$

where $\bar{X}$ is the arithmetic average of the individual observations X's from a certain peak, and N, the number of observations.

CHAPTER IV

RESULTS AND DISCUSSION

A. DESIGN AND TESTING THE VACUUM DEGASSING SYSTEM USED FOR COLLECTION OF VOLATILES

The vacuum degassing system used by earlier workers (42, 19, 21) was modified so that a more efficient flow over the heated bellows could be obtained, and so that the vacuum could be improved. Such improvements were necessary before routine flavor analysis could be made, since the earlier procedure was too time-consuming and inefficient. The modifications were:

(a) A teflon stopcock was added between the foaming chamber and the heated bellows. The glass stopcock used earlier was satisfactory for qualitative flavor analysis, but for the quantitative analysis the teflon stopcock permitted more accurate control of the oil flow rate into the glass bellows tower. A reproducible oil flow rate was found to be one of the important factors in obtaining reproducible results on GLC.

(b) A tilting type McLeod gauge was set up on the line between glass bellows tower and cold finger (trap 1) for measuring the pressure in the system. The gauge was calibrated with a Vactroionic Model DG-250 thermo-couple gauge, and a linear relation was obtained between these two gauges (Figure 7). The McLeod gauge could be isolated from the system by glass stopcock number 4. When a pressure of 2 microns

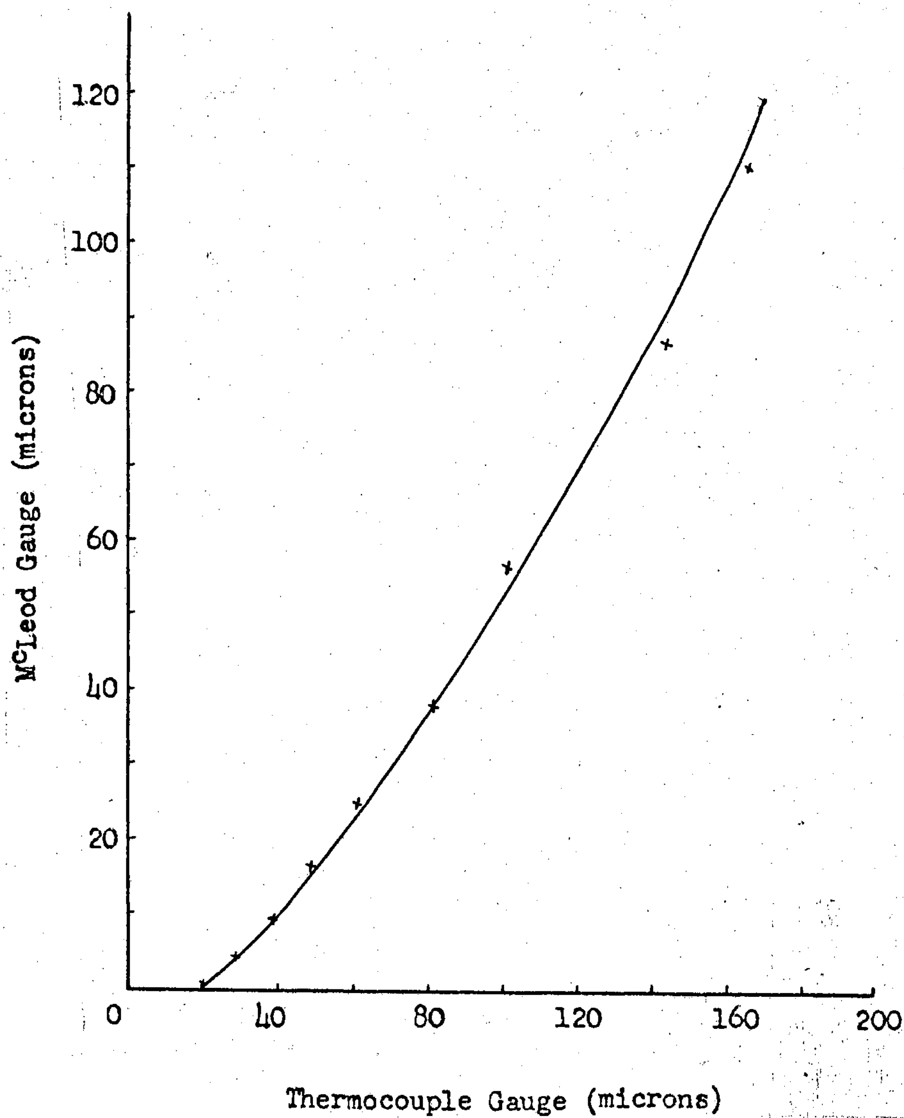


Figure 7. Calibration of McLeod Gauge with Thermocouple Gauge

was reached, the gauge was closed from the rest of the system; otherwise the collected volatiles were contaminated with mercury.

(c) All the joints were fitted with Viton O-rings to improve the seal.

(d) The capacities of the two flasks used for collecting the oil and the volatiles were reduced to 100 ml and 50 ml respectively for the convenience in rapid evacuation.

The vacuum degassing technique played a critical role in the whole analytical procedure. It was found that the vacuum pressure should be lower than 2 microns of mercury through the experiment. If leaks occurred in the system during degassing, the amount of volatile compounds collected varied. The oil flow rate also influenced the amount of volatiles collected. If it was too fast, small amounts of volatiles were collected and usually this was not enough for GLC analysis. Under this condition the yield of volatiles was not reproducible. Hence it appeared that the less volatile compounds were not being removed in the degassing process. The objective was to produce nearly odorless peanut oil after one pass through the glass bellows. A rate of 4-5 seconds per drop was found adequate to produce odorless peanut oil from the samples tested.

B. GAS LIQUID CHROMATOGRAPHIC ANALYSIS OF VOLATILES

After collection of the volatiles, it was found that the pH adjustment and the multi-extraction procedures needed to be done in a very consistent manner so that reproducible analytical data could be obtained. One of the greatest difficulties encountered in obtaining reproducible results was in the concentration technique used.

An effort was made to concentrate the basic fraction with a water bath at 45° C, but at least two disadvantages were revealed from this technique: (i) a loss of volatile compounds occurred and (ii) the process was time-consuming, requiring about two hours. Experiments using the rotary evaporator under controlled vacuum for partial concentration and a nitrogen stream for the final concentration step provided the best results with respect to obtaining reproducible quantitative analysis of the volatiles.

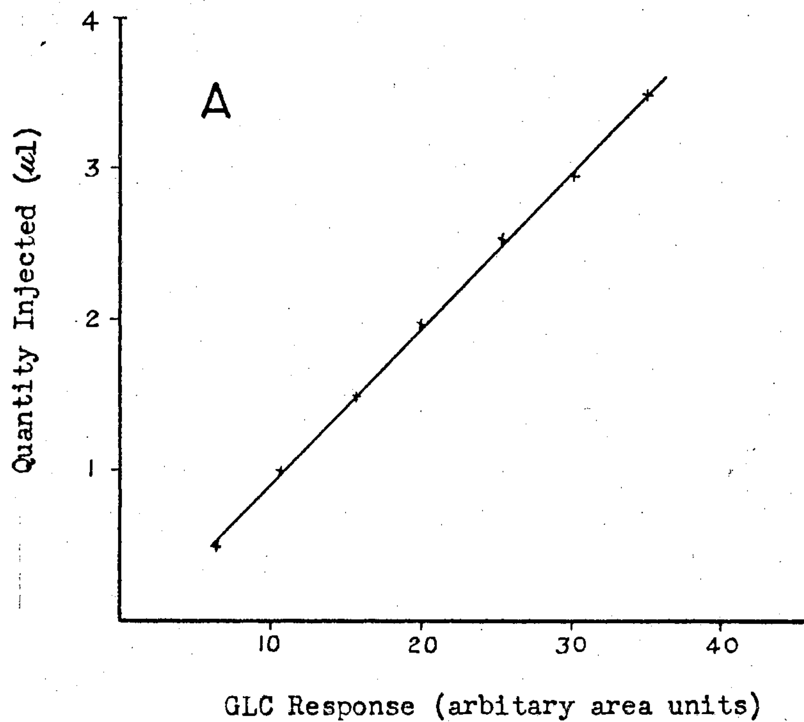
Originally, a smaller sample batch was expected to be used for the GLC analysis, because the sample available in the study was limited in amount. It was found that the GLC could not provide the significant chromatograms of volatiles unless more than 350 gm of peanuts in a batch was used. Hence, this amount of peanuts was chosen for oil GLC analyses.

An initial attempt was made to use the integrator for evaluating chromatograms, but it was found the integrator did not record proportionally when the base line moved up owing to the programming temperature. Consequently, the recorder pen was chosen to measure the peak area. Linear relation resulting between standards (pyrazine and 2,6-dimethylpyrazine) and the GLC detector response is shown in Figure 8.

The reproducibility of retention time was obtained by GLC of the standards, pyrazine and 2,6-dimethylpyrazine, as well as from samples. Standard pyrazine exhibited a reproducible retention time at 19.1 minutes and standard 2,6-dimethylpyrazine 24.2 minutes.

C. EVALUATION OF ANALYTICAL PROCEDURE

Argentine and Starr varieties were used as standards to test the procedure proposed and to provide an acceptable flavor analysis model.



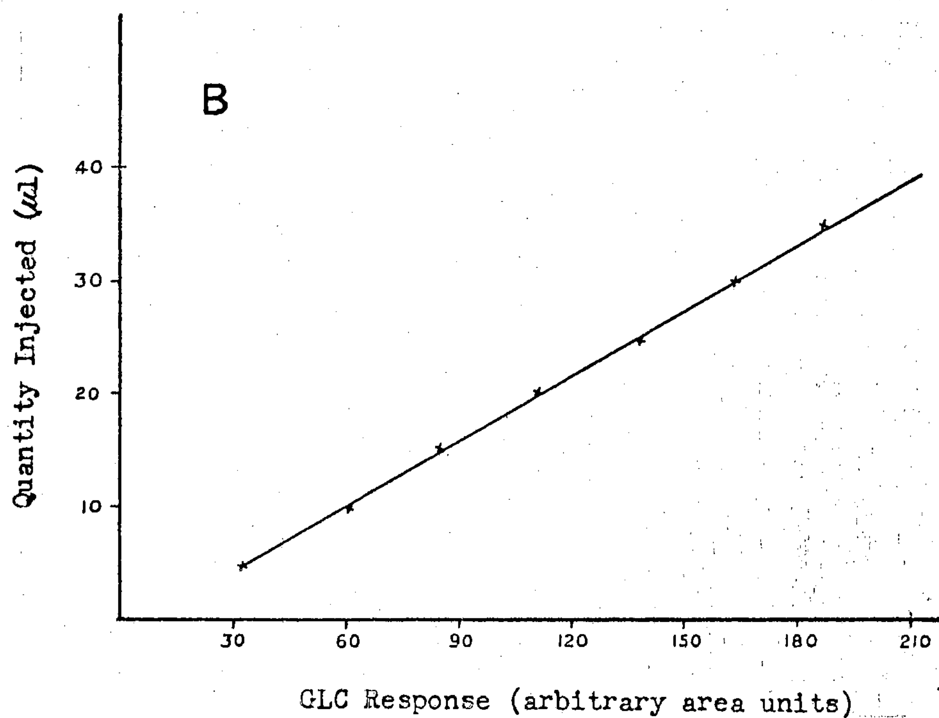


Figure 8. GLC Detector Response for Selected Pyrazine Standards. A. Pyrazine. B. 2,6-Dimethylpyrazine.

Under the final conditions selected, sample 1 (Argentine) and sample 2 (Starr) were analyzed several times. The results were shown in Tables V (retention time) and VI (relative concentration). The typical chromatograms of Argentine and Starr were shown in Figures 9 and 10 respectively.

It was found that all 16 peaks appeared in both varieties, but the quantities of the same peak varied. Slight differences were observed mainly in peaks 5, 6, 14 and 16. It is apparent that the difference between Argentine and Starr is quantitative rather than qualitative.

The calculated peak value by the triangulation method was about 4% less than the observed peak value (48). Obviously, the higher the retention time goes, the broader the peak appears, and the less accurate is the peak value obtained.

The area calculation for the overlapping peaks, such as peaks 4 and 5, was not so accurate as for the resolved peaks because it was hard to estimate precisely the triangle under the curve. As might be expected, peaks 4 and 5 had greater standard deviations than other peaks (Table VI).

D. PEANUT FLAVOR ANALYSIS AS EFFECTED BY FERTILIZATION

GLC Analysis

Flavor volatiles of samples 3 and 4 fertilized with N, P, K and N, P, K, B, respectively, were shown in Figure 11. When these two samples were brought into this laboratory, both samples had normal color and odor; but sample 3 had more immature kernels and some of the hearts were decolored. Hollow hearts were not found in sample 3. Probably, the damage caused by deficiency of boron was not serious.

TABLE V

COMPOUNDS* IN THE BASIC FRACTION OF ROASTED PEANUTS, IDENTIFIED BY GLC

Peak No.	Johnson Label(21)	Compound	Retention Time (Min.)
1	A	2-Methylpyrazine	21.5
2	B,C	2,5-Dimethylpyrazine, 2,6-dimethylpyrazine	24.2
3	D,E	2-Ethylpyrazine, 2,3-dimethylpyrazine	25.2
4	F,G	2-Ethyl-6-methylpyrazine, 2-ethyl-5-methylpyrazine	27.3
5	H,I	2-Ethyl-3-methylpyrazine, trimethylpyrazine	27.8
6	J	2,5-Dimethyl-3-ethylpyrazine	29.7
7	K	2,3-Dimethyl-5-ethylpyrazine, 2,6-dimethyl-3-ethylpyrazine	30.5
8	M	2,6-Diethyl-3-methylpyrazine, or 2,3-diethyl-3-methylpyrazine	32.0
9	N	2-Ethyl-3,5,6-trimethylpyrazine	32.8
10	P	$C_8H_{10}N_2$	34.2
11			36.2
12			38.2
13	S	Methyl-2,3-cyclopentanopyrazine	39.5
14	T	2-Isopropenylpyrazine	41.2
15			43.1
16	W	Methylisopropenlpyrazine	47.0

* Identified by Johnson (21) under similar conditions.

TABLE VI

RELATIVE CONCENTRATION OF BASIC FRACTION CONSTITUENTS FROM STARR AND ARGENTINE VARIETIES OF SPANISH PEANUTS

Starr (Sample 2)					Argentine Sample (1)			
Experiment				Peak No.		Experiment		
1	2	3	AVE ± S.D[c]		AVE ± S.D	1	2	3
30.0	28.5	30.3	29.6 ± 0.9	1	28.8 ± 0.8	29.0	29.5	28.0
100.0	100.0	100.0	100.0	2	100.0	100.0	100.0	100.0
7.2	7.0	7.4	7.2 ± 0.2	3	7.5 ± 0.3	7.3	7.8	7.3
23.7	26.3	25.7	25.2 ± 1.4	4	26.0 ± 1.4	27.3	25.5	25.1
29.0	30.3	31.4	30.2 ± 1.2	5	35.6 ± 0.8	34.7	36.3	35.8
14.8	15.5	15.5	15.2 ± 0.5	6	23.2 ± 1.1	23.3	22.1	24.2
5.4	5.3	5.3	5.3 ± 0.1	7	6.6 ± 0.4	6.1	6.9	6.8
2.9	2.8	2.4	2.7 ± 0.3	8	3.2 ± 0.5	3.6	2.7	3.7
1.1	Tr[c]	1.9	1.3 ± 0.5	9	1.3 ± 0.3	1.1	1.4	1.6
2.3	2.5	1.9	2.2 ± 0.3	10	1.7 ± 0.4	2.1	1.4	1.7
1.0	1.5	1.2	1.2 ± 0.3	11	Tr	Tr	Tr	Tr
Tr[b]	Tr	Tr	Tr	12	Tr	Tr	Tr	Tr
Tr	Tr	Tr	Tr	13	Tr	Tr	Tr	Tr
3.7	4.1	4.3	4.0 ± 0.3	14	5.0 ± 0.1	4.9	5.1	5.0
Tr	Tr	Tr	Tr	15	Tr	Tr	Tr	Tr
3.4	3.6	3.9	3.6 ± 0.3	16	5.7 ± 0.4	5.9	5.2	6.0

a - Peak 2 was used as the base (100%)
b - Tr = less than 1%
c - S.D = standard deviation

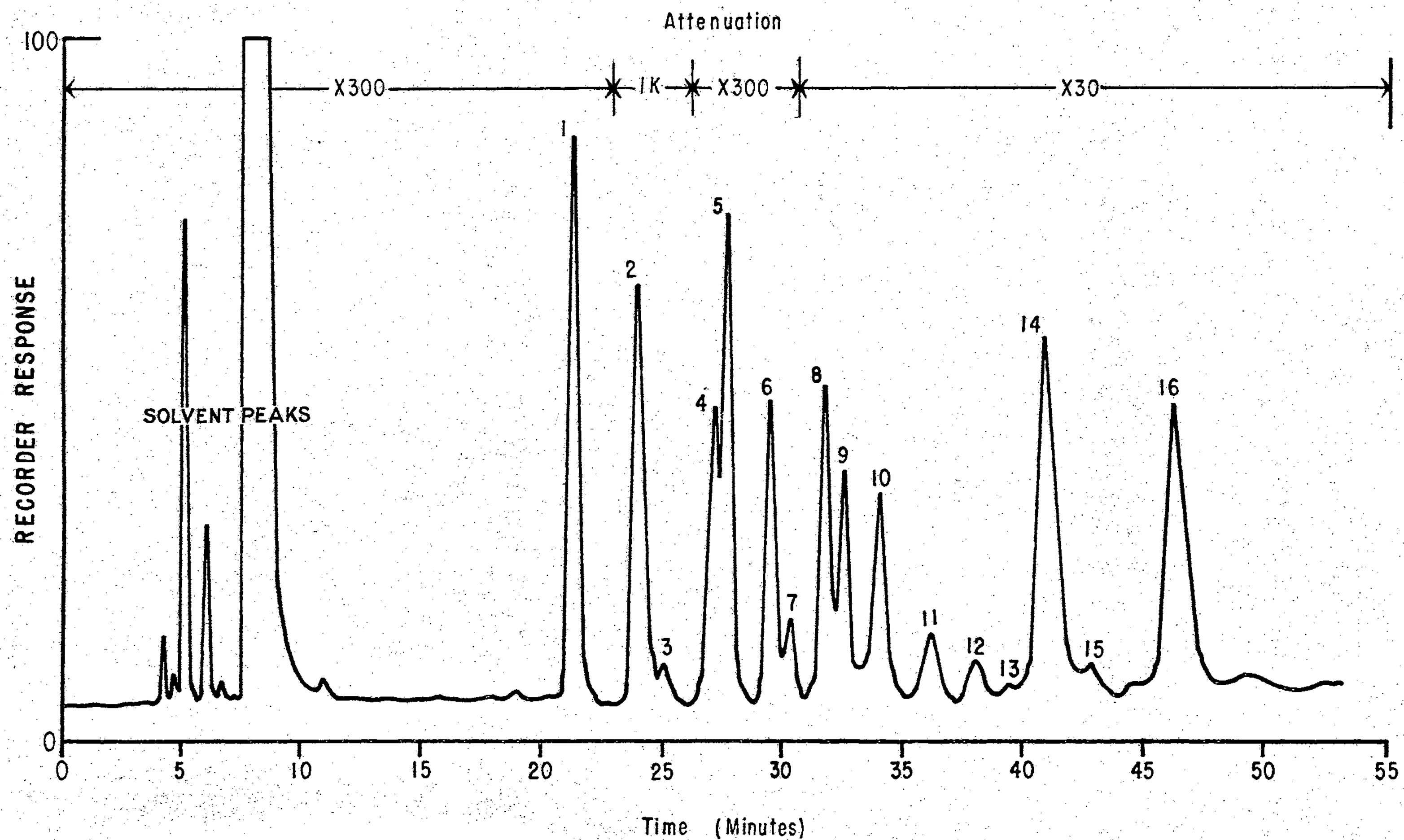


Figure 9. A Typical Gas Liquid Chromatogram of the Basic Fraction of Roasted Argentine Peanuts (For matching peak numbers with compounds see Table V).

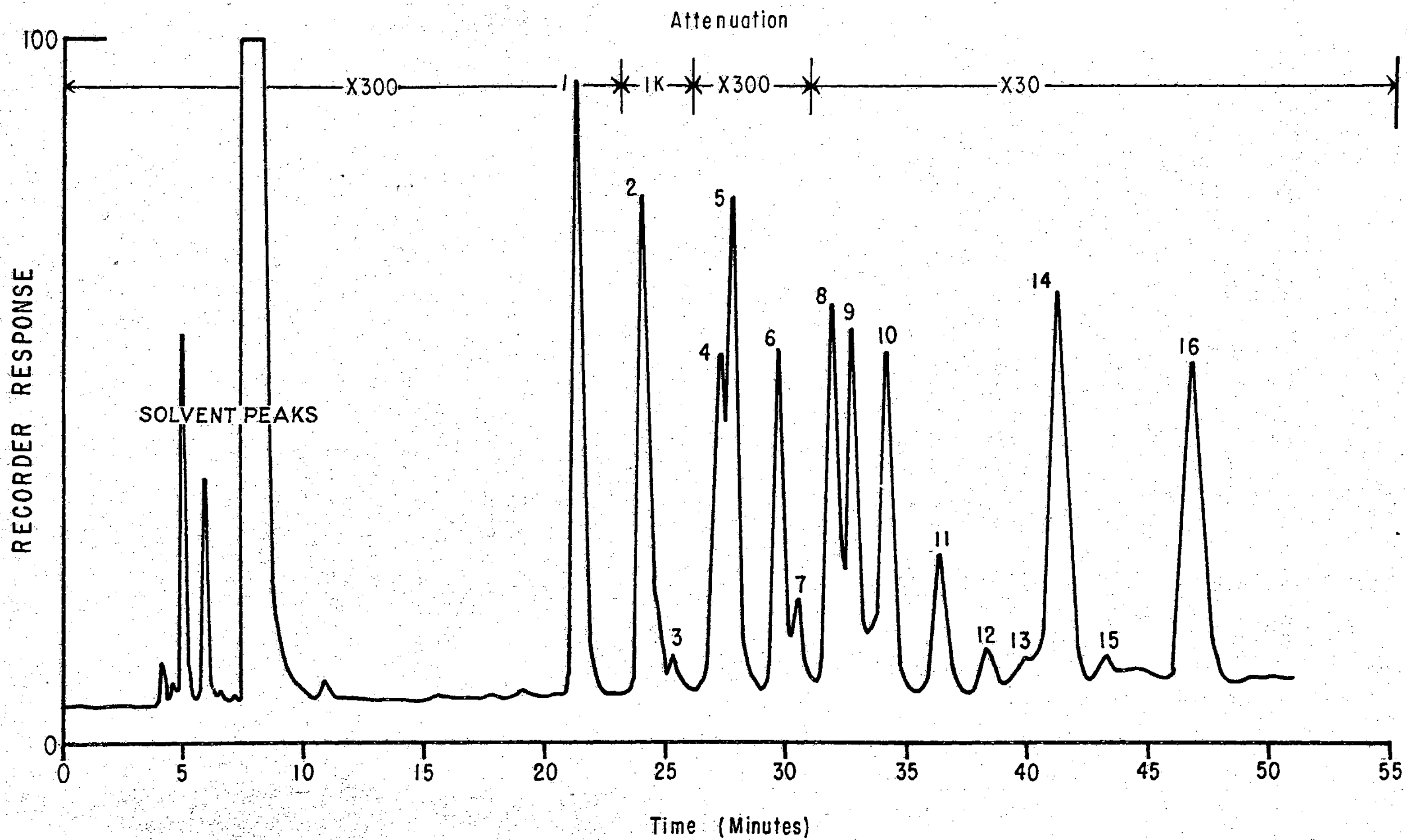


Figure 10. A typical Gas Liquid Chromatogram of the Basic Fraction of Roasted Starr Peanuts (For matching peak numbers with compounds see Table V).

Figure 11. Photograph of Peanut Samples Treated with Different Fertilizers. (Sample details, see Table II).

Figures 12 and 13 are the chromatograms obtained from samples 3 and 4 respectively under the conditions stated previously. The qualitative difference between them was that sample 4 had an additional peak (unknown B) at the retention time of 12.7 minutes. The relative concentrations for each components are shown in Table VII. It is obvious that peaks 11, 16, 6 and 7 are greater in sample 3 than in sample 4, the first one of the four peaks by about three-fold and the second one about 50%. Unknown B at a relative concentration of 12.5% to peak 2 is a new compound found only in the peanuts treated with boron in the fertilizer. The relative concentration of peak 1 is lower in sample 3 than in sample 4.

Identification of Unknown B

The mass spectrum of the unknown B is similar to that of 1,2-dichloro-ethane (Figure 14). The ten most abundant peaks in both spectra are m/e 62, 49, 27, 64, 26, 63, 98, 51, 61, and 100. Comparison of these ten most abundant peaks with those reported (54) strongly indicates that unknown B is 1,2-dichloroethane.

In the mass spectrum of the unknown B, molecular ions of m/e 98, 100 and 102 which express M, M + 2 and M + 4 are in the ratio of 54.6:38.9:0.65. This ratio is close to the one 56.8:37.1:0.61 reported by Beynon (53) for $C_2H_4Cl_2$.

E. PEANUT FLAVOR EFFECTED BY DIFFERENT TIME OF PLANTING

Samples 5, 6 and 7 of Dixie variety planted on different dates and harvested after 120 days (Table III) exhibited no differences in seed appearance among them. The patterns of the chromatograms of the volatiles for these three samples was very similar to those of sample 1 or

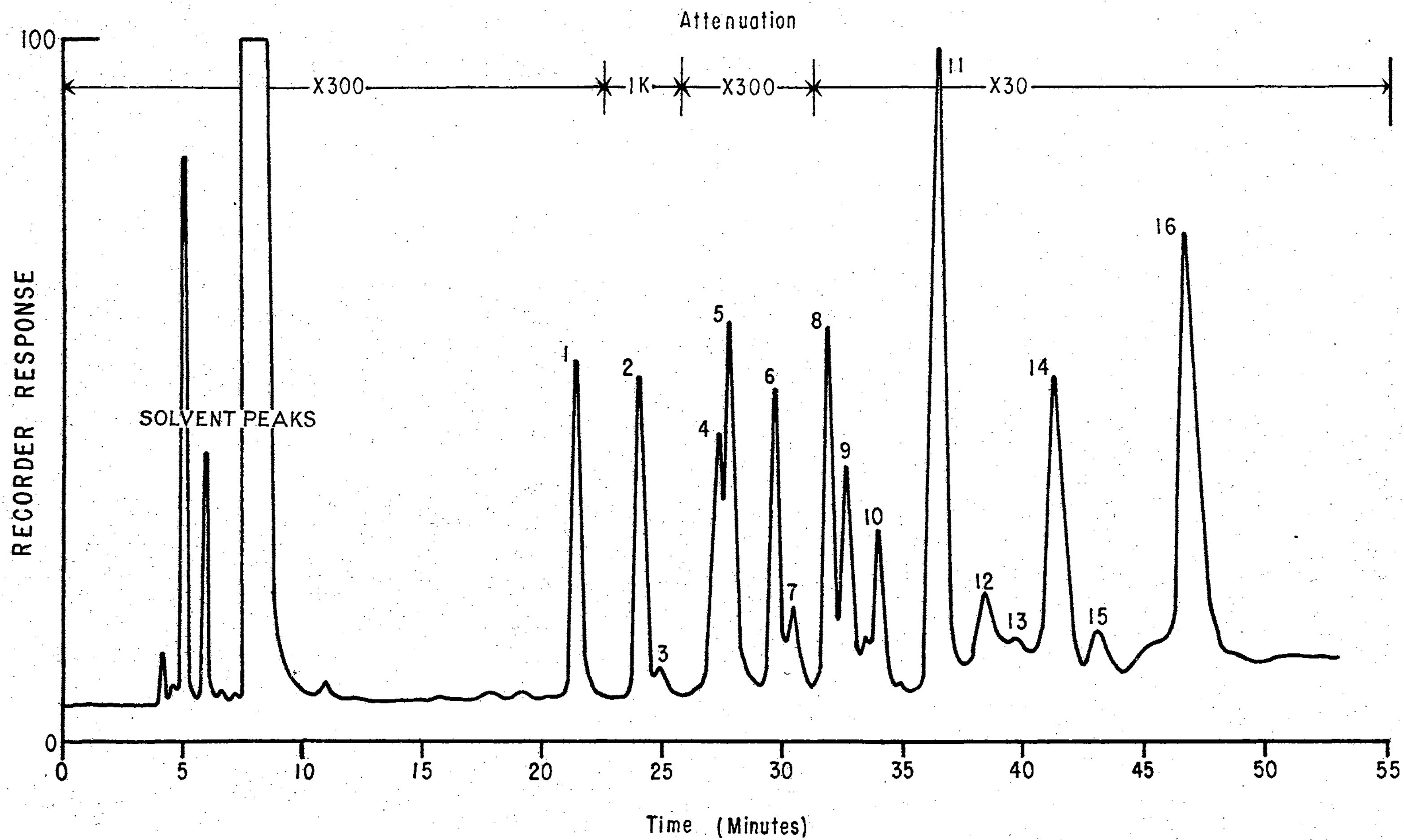


Figure 12. Gas Liquid Chromatogram of Basic Fraction of Roasted Peanuts Fertilized with N, P, K. (For matching peak numbers with compounds see Table V).

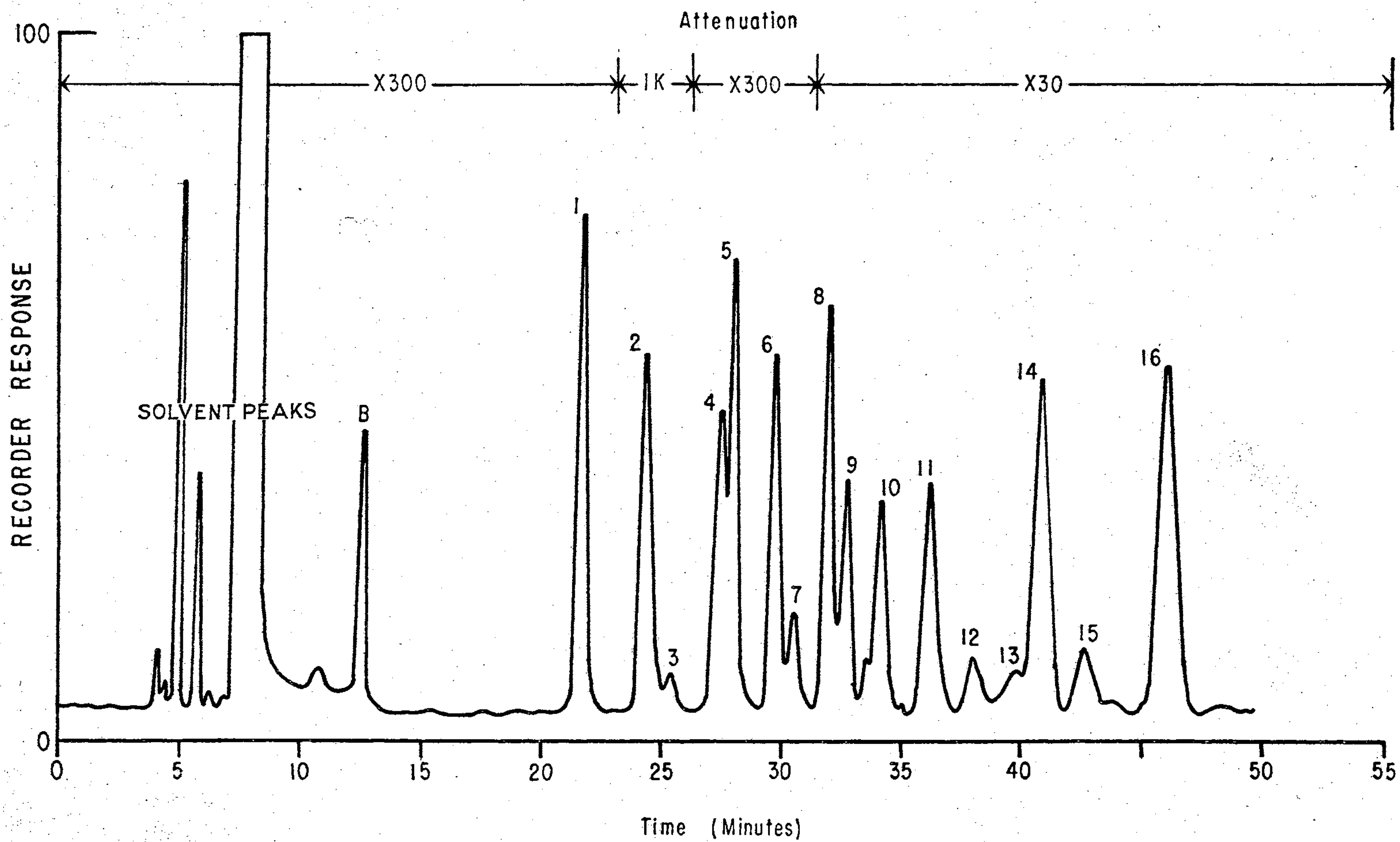


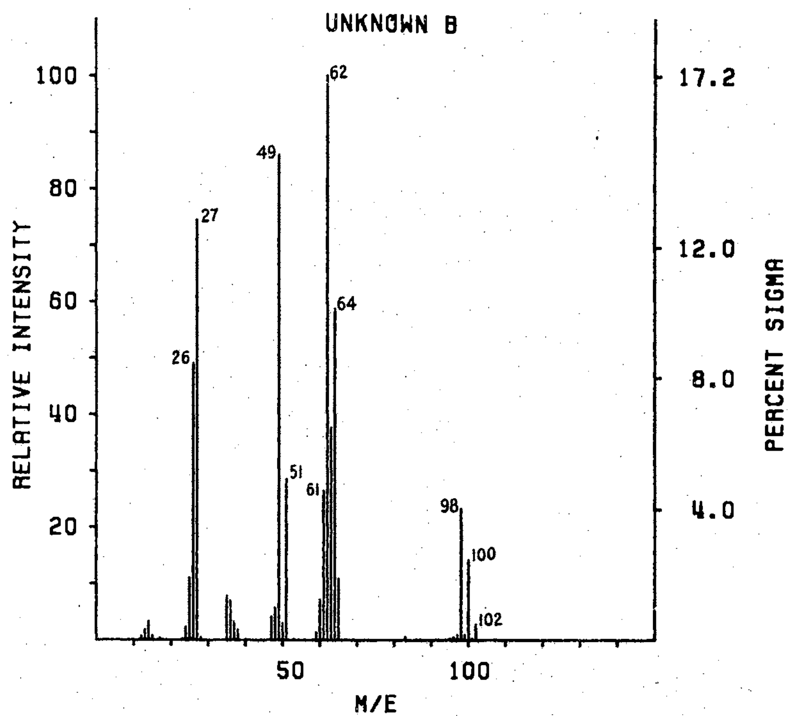
Figure 13. Gas Liquid Chromatogram of Basic Fraction of Roasted Peanuts Fertilized with N, P, K, B. (For matching peak numbers with compounds see Table V).

TABLE VII

RELATIVE CONCENTRATION OF BASIC FRACTION CONSTITUENTS FROM PEANUTS TREATED WITH DIFFERENT FERTILIZERS

Sample 3[a]	Peak No.	Sample 4[b]
30.8	1	34.1
100.0	2[c]	100.0
7.6	3	8.9
30.8	4	30.5
37.2	5	37.1
26.6	6	23.7
9.1	7	6.2
4.3	8	2.8
1.9	9	1.7
1.4	10	1.8
7.7	11	2.3
1.1	12	Tr[d]
Tr	13	Tr
4.9	14	4.1
Tr	15	Tr
7.5	16	5.2
	Unknown B[e]	12.5

a - Sample 3 was treated with N,P,K; see Table II.
b - Sample 4 was treated with N,P,K,B; see Table II.
c - Peak 2 was used as the base (100%).
d - Tr = less than 1%.
e - Retention time of the unknown was 12.7 minutes.



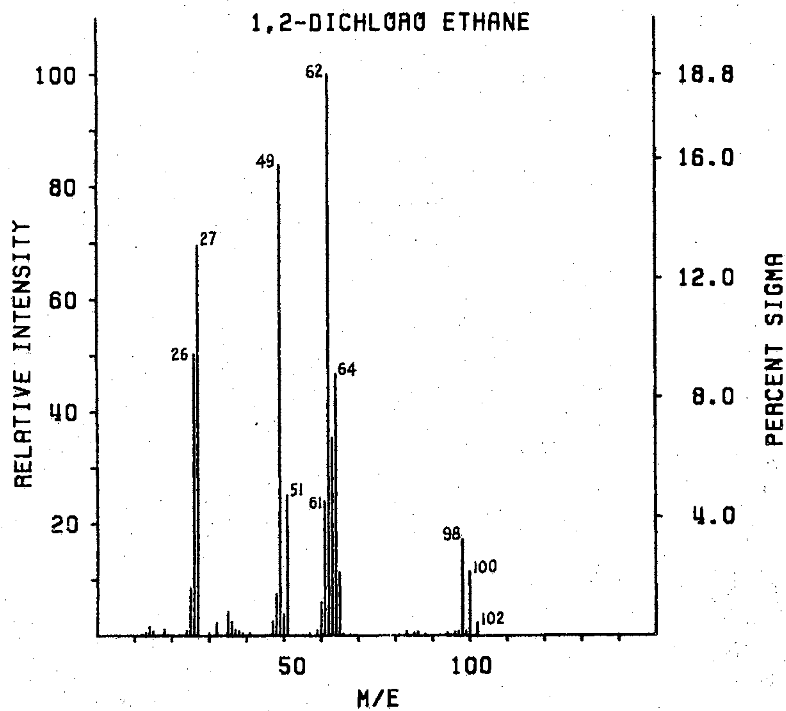


Figure 14. Mass Spectra of Unknown B and 1,2-Dichloro-ethane.

2. The relative concentrations of these samples are tabulated in Table VIII. It is apparent that the relative concentrations of each peak from the three samples are very similar. In view of these data, it may be considered that samples 5,6 and 7 have identical qualitative and quantitative composition of the volatile flavor components. The average concentrations for each peak of the three samples are shown in Table X.

In the six Spanhoma samples, although samples 8, 9 and 10 were harvested for 120 days after planting and samples 11, 12 and 13, 160 days after planting (Table IV), the seed appearance of all six samples was identical. The relative concentrations of the volatile components these samples are shown in Table IX, and the average value of each three samples in Table X.

It was found that these two varieties, Dixie and Spanhoma, looked alike in appearance, color and odor, but the seed size of Spanhoma was relatively larger than that of Dixie.

From Tables VIII, IX and X three conclusions may be drawn:

(i) The volatile variation among the three samples in each group was not great.

(ii) Comparison of "Spanhoma-120 days" with "Dixie-120 days" indicated that a quantitative difference in some volatile components existed from variety to variety.

(iii) Comparison of "Spanhoma-120 days" with "Spanhoma-160 days" indicated that the effect of the period of growth on volatile composition was not marked for most of the peaks. Only peak 5 revealed an appreciable difference between these two groups of samples.

F. PEANUT FLAVOR EFFECTED BY GAS TREATMENT

TABLE VIII

RELATIVE CONCENTRATION OF BASIC FRACTION CONSTITUENTS FROM DIXIE PEANUTS PLANTED ON DIFFERENT DATES[a]

Peak Number	Sample Number		
	5	6	7
1	27.4	27.7	24.1
2[b]	100.0	100.0	100.0
3	8.0	8.0	7.5
4	27.8	29.2	27.0
5	34.0	34.3	33.8
6	22.2	24.3	25.0
7	6.4	7.1	7.0
8	2.9	3.0	3.3
9	3.2	3.2	3.8
10	3.2	2.8	3.0
11	1.1	1.5	1.3
12	Tr[c]	Tr	Tr
13	Tr	Tr	TR
14	4.9	5.5	4.5
15	Tr	Tr	Tr
16	5.4	7.3	6.2

a - Dates planted and harvested were shown in Table III.
b - Peak 2 was used as the base (100%).
c - Tr = less than 1%.

TABLE IX

RELATIVE CONCENTRATION OF BASIC FRACTION CONSTITUENTS FROM SPANHOMA PEANUTS PLANTED AND HARVESTED ON DIFFERENT DATES

SAMPLES NO. (120 DAYS)				SAMPLES NO. (160 DAYS)		
8	9	10	Peak No.	11	12	13
25.8	27.2	27.2	1	24.9	26.0	24.9
100.0	100.0	100.0	2[b]	100.0	100.0	100.0
7.0	7.5	7.3	3	7.1	8.3	7.4
26.9	28.0	25.3	4	22.6	23.2	22.1
36.0	37.4	37.0	5	29.5	30.6	28.4
22.1	20.8	19.4	6	21.9	22.1	20.8
5.5	5.6	4.9	7	5.7	6.3	5.2
2.8	2.2	2.7	8	2.2	2.3	2.5
2.5	1.7	2.6	9	2.1	2.1	2.1
2.2	2.3	2.6	10	2.8	2.4	2.3
1.1	1.0	1.2	11	1.0	1.1	1.3
Tr[c]	Tr	Tr	12	Tr	Tr	Tr
Tr	Tr	Tr	13	Tr	Tr	Tr
3.9	4.2	4.0	14	3.5	2.9	3.1
Tr	Tr	Tr	15	Tr	Tr	Tr
5.1	4.5	4.3	16	4.9	4.3	4.5

a - Dates planted and harvested were shown in Table III.

b - Peak 2 was used as the base (100%).

c - Tr = less than 1%.

TABLE X

THE AVERAGE VALUES FROM TABLE VIII AND TABLE IX

Sample Variety And No. of Days[a]	Spanhoma 120 Days	Spanhoma 160 Days	Dixie 120 Days
Average Value From Sample No.	8, 9, 10	11, 12, 13	5, 6, 7
Peak Number	Ave. ± S.D.	Ave. ± S.D.	Ave. ± S.D.
1	26.7 ± 0.8	25.3 ± 0.6	26.4 ± 2.0
2	100.0	100.0	100.0
3	7.3 ± 0.3	7.6 ± 0.6	7.8 ± 0.2
4	26.7 ± 1.4	22.6 ± 0.6	28.0 ± 1.1
5	36.8 ± 0.7	29.5 ± 1.1	34.1 ± 0.3
6	20.8 ± 1.4	21.6 ± 0.7	23.8 ± 1.5
7	5.5 ± 0.4	5.7 ± 0.6	6.8 ± 0.4
8	2.6 ± 0.3	2.3 ± 0.2	3.1 ± 0.2
9	2.3 ± 0.5	2.1 ± 0.0	3.4 ± 0.3
10	2.4 ± 0.2	2.1 ± 0.3	3.0 ± 0.2
11	1.1 ± 0.1	1.1 ± 0.2	1.3 ± 0.2
12	Tr	Tr	Tr
13	Tr	Tr	Tr
14	4.0 ± 0.2	3.2 ± 0.3	5.0 ± 0.5
15	Tr	Tr	Tr
16	4.6 ± 0.4	4.6 ± 0.3	6.3 ± 1.0

a - No. of days between planting and harvesting

GLC Analysis

Samples 14, 15, 16, 17 and 18 stored in N_2, CO_2, dry air, SO_2 plus N_2 and conventional air respectively were described in Table IV. Sample 17 was very different from the other four samples in seed characters. It smelled sour and had a very light colored skin (Figure 15). While this sample was roasting, the sour odor was very strong. The oil collected still smelled sour and was dark brown in color (Figure 16). The volatiles collected contained some small black particles.

The chromatograms of these five samples were shown in Figures 17 to 20, and the tabular results in Table XI. (The chromatogram of sample 18, a control, is similar to the one of sample 1 or 2).

The only qualitative variation found occurred in the first 20 minutes of analysis. Three unknowns were observed in sample 14, and five unknowns in sample 17.

Generally speaking, the relative concentration varied from sample to sample, but the one of sample 17 was markedly different from the others. Peaks 1, 7, 11 and 14 were obviously higher and peaks 4, 5, 6, 16 lower than those in control.

On account of the fact that sample 17 revealed a sour odor and a dark brown color when roasting, and exhibited an unusual chromatographic pattern, peanuts stored in such a combination of SO_2 and N_2 probably provided one or more off-flavor characteristics. Peanuts stored in CO_2, N_2 and dry air showed no off-flavor.

Identification of the Unknowns

There were 3 unknowns (LN, 2N and 3N) presented in sample 14 (Figure 17), and five unknowns (1S to 5S) in sample 17 (Figure 20). Most

Figure 15. Photograph of Peanut Samples Treated with Different Gaseous Atmospheres. (Sample details, see Table IV).

Figure 16. Photograph of the Peanut Oil Collected from Samples Treated with Different Gaseous Atmospheres. (Sample details, see Table IV).

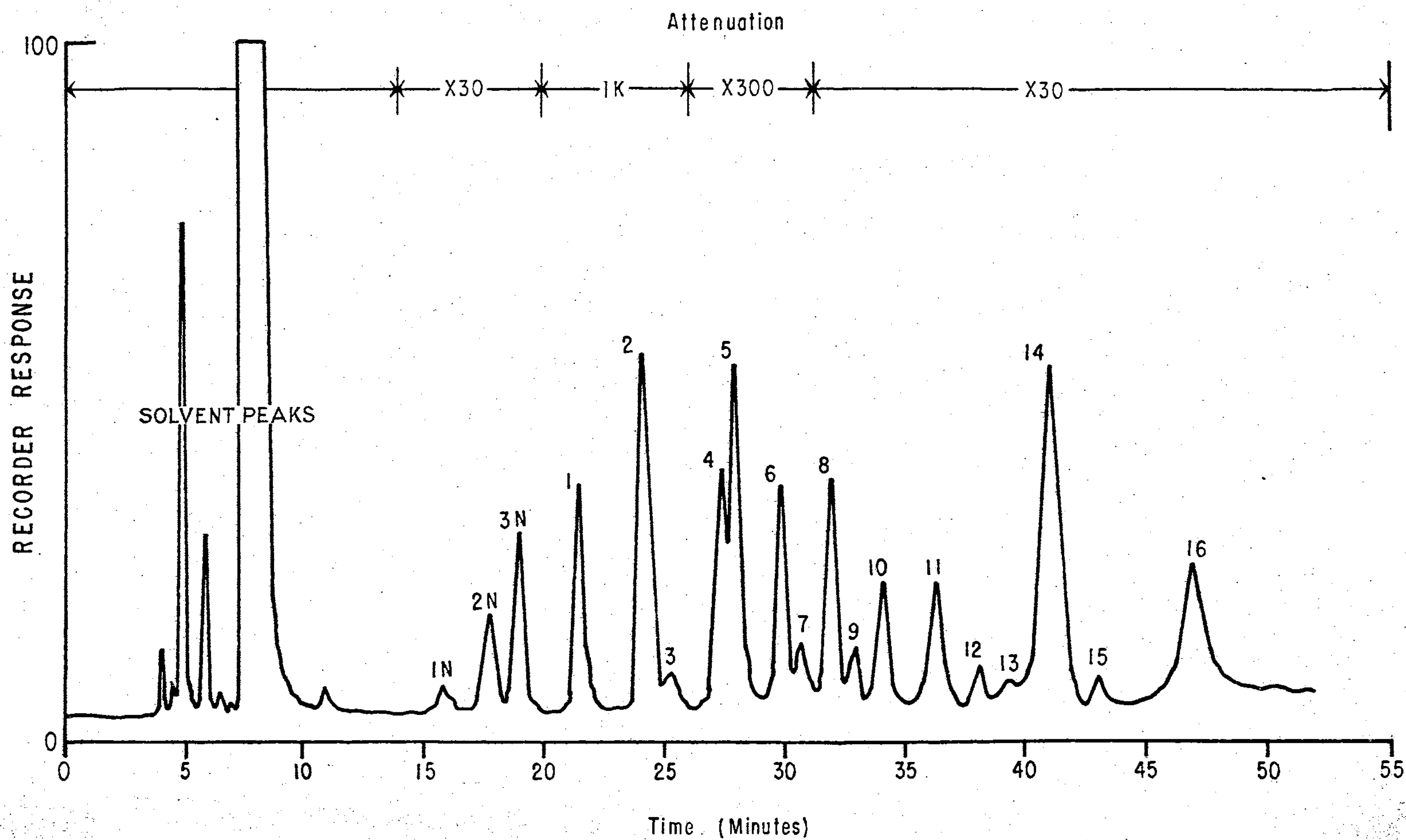


Figure 17. Gas Liquid Chromatogram of Basic Fraction of Roasted Peanuts Treated with Nitrogen. (For matching peak numbers with compounds see Table V).

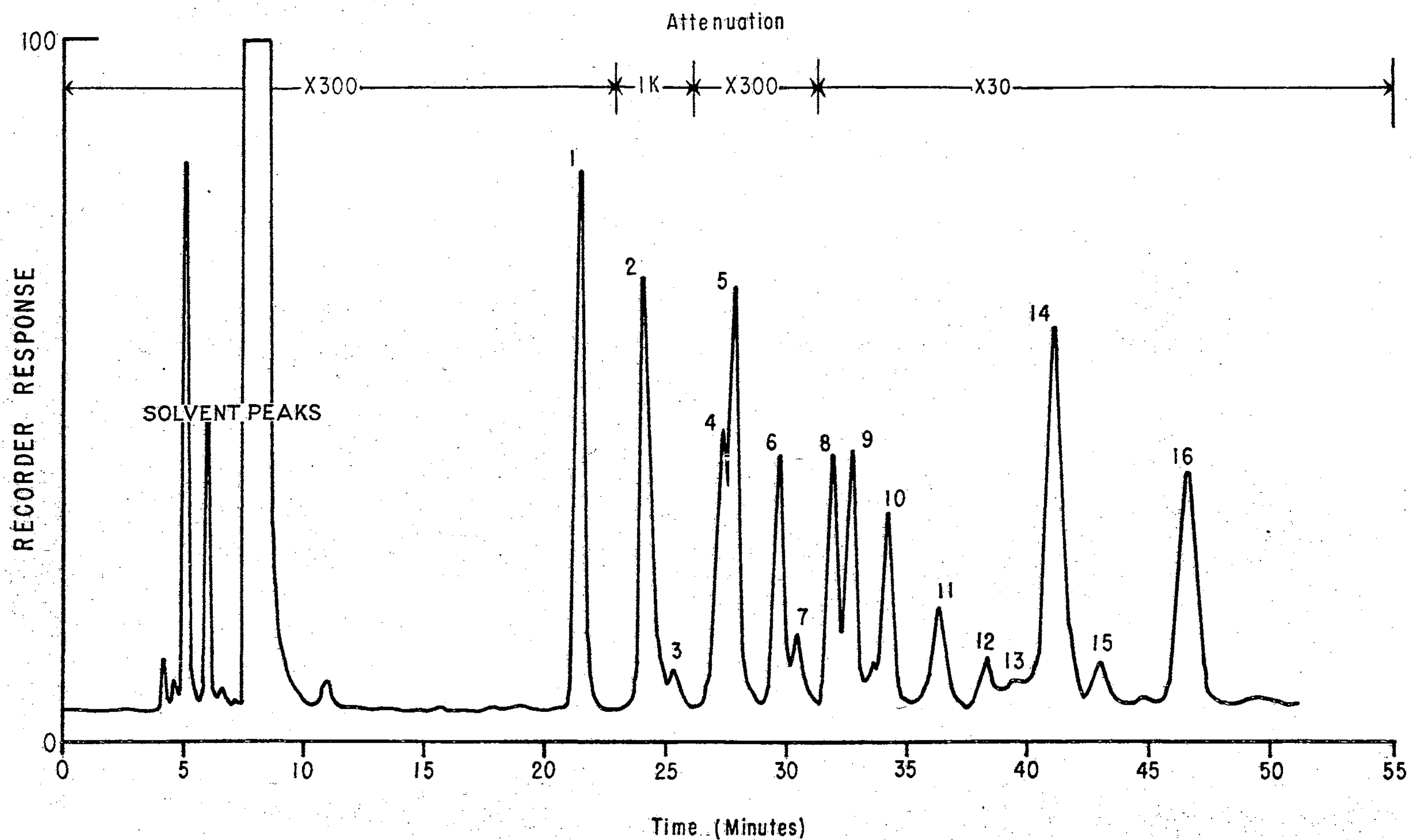


Figure 18. Gas Liquid Chromatogram of Basic Fraction of Roasted Peanuts Treated with Carbon Dioxide. (For matching peak numbers with compounds see Table V).

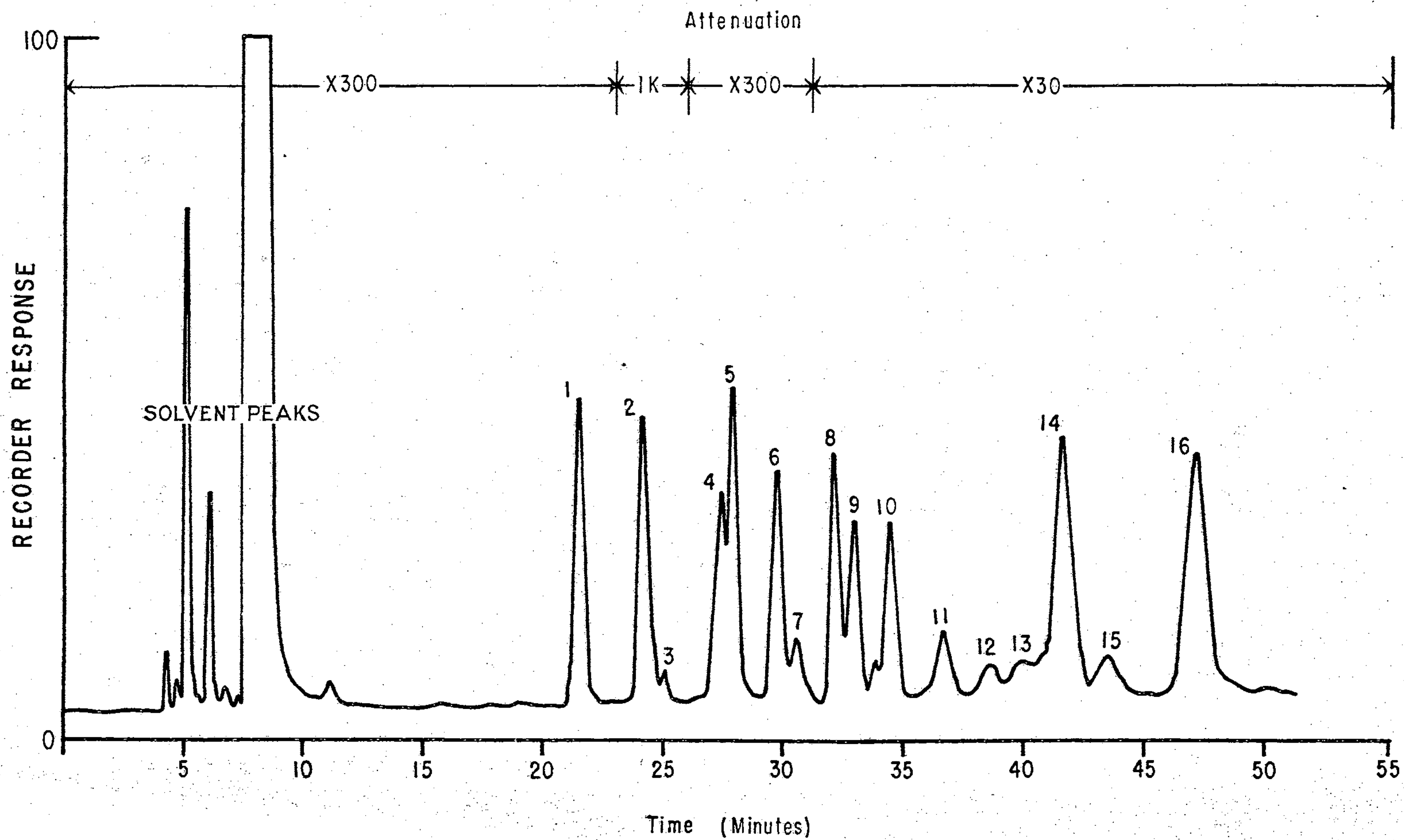


Figure 19. Gas Liquid Chromatogram of Basic Fraction of Roasted Peanuts Treated with Dry Air. (For matching peak numbers with compounds see Table V).

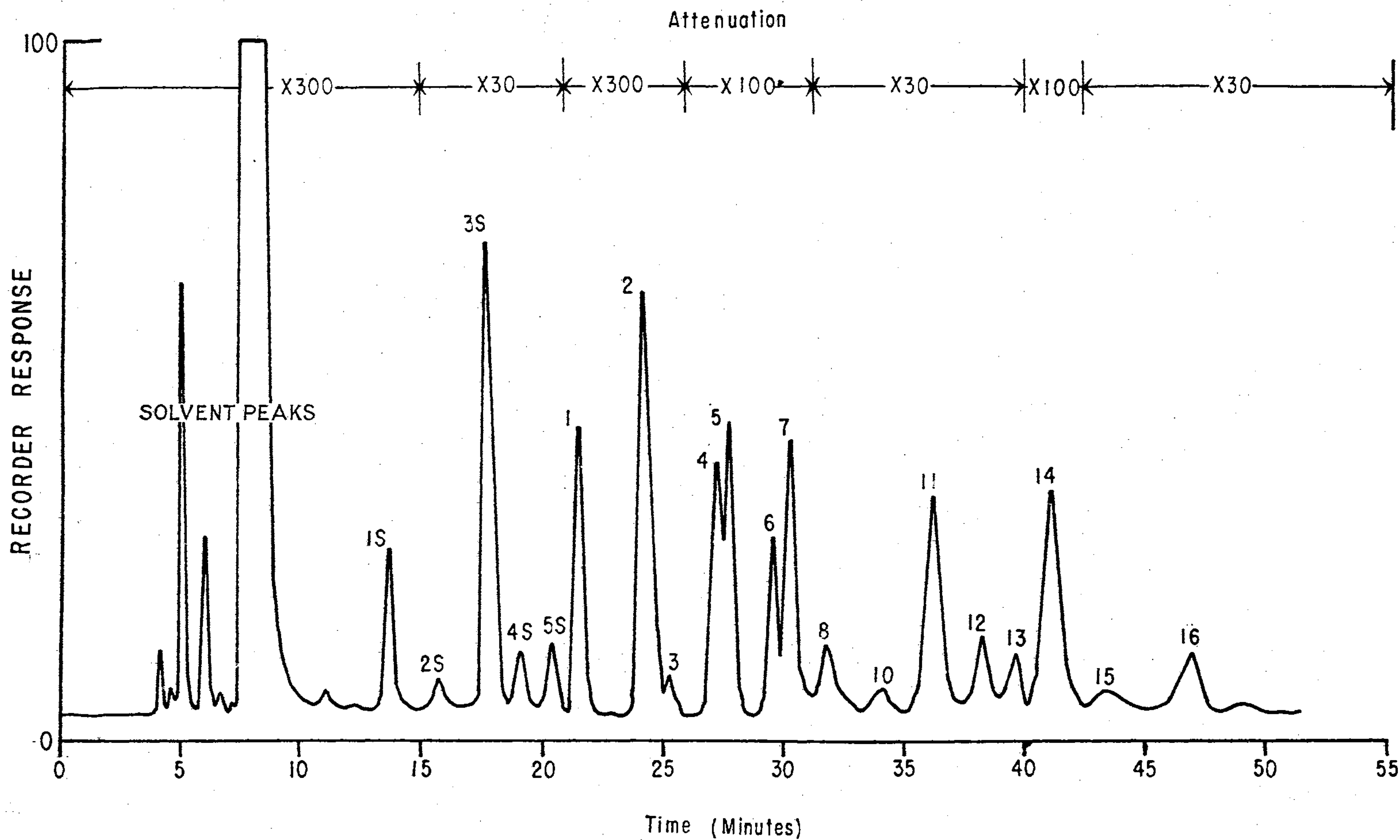


Figure 20. Gas Liquid Chromatogram of Basic Fraction of Roasted Peanuts Treated with Combination of Sulfur Dioxide (5%) and Nitrogen (95%). (For matching peak numbers with compounds see Table V).

TABLE XI

RELATIVE CONCENTRATION OF BASIC FRACTION CONSTITUENTS FROM PEANUT SAMPLES TREATED WITH DIFFERENT GASEOUS ATMOSPHERES

Peak Number	Sample Number[a]				
	14	15	16	17	18
1	54.5	29.1	32.9	44.0	32.9
2[b]	100.0	100.0	100.0	100.0	100.0
3	8.8	8.5	6.7	7.3	7.9
4	26.6	23.5	26.7	20.7	24.6
5	31.8	29.3	32.6	17.6	32.1
6	15.8	13.6	21.0	8.7	17.9
7	5.0	4.9	6.2	16.9	6.0
8	2.0	1.6	2.1	1.4	2.0
9	Tr[c]	1.8	1.9		1.6
10	1.2	1.3	1.9	Tr	2.2
11	1.2	Tr	Tr	6.0	1.8
12	Tr	Tr	Tr	1.9	Tr
13	Tr	Tr	Tr	1.8	Tr
14	4.9	4.0	4.4	23.5	3.9
15	Tr	Tr	Tr	Tr	Tr
16	2.2	2.9	5.0	2.2	4.0

Unknowns	Retention Time (min.)	14	15	16	17	18
1N	(15.8)	Tr				
2N	(17.8)	Tr				
3N	(19.1)	1.4				
1S	(13.7)				22.1	
2S	(15.8)				Tr	
3S	(17.8)				8.3	
4S	(19.1)				Tr	
5S	(20.5)				Tr	

a - Sample 14 was treated with Nitrogen
Sample 15 was treated with Carbon Dioxide
Sample 16 was treated with dry air
Sample 17 was treated with Nitrogen and Sulfur Dioxide
Sample 18 was treated with atmosphere air
For more details see Table IV

b - Peak 2 was used as base (100%)

c - Tr = less than 1%

of these peaks on the chromatograms are minor ones except 1S and 3S. The quantity of material in 2S was too low for a good mass spectrum to be obtained.

The alternating voltage accelerating (AVA) technique was used to determine if there was a mixture of compounds for each of the unknowns. The unknowns 1S, 2N and 3N were detected by this technique. The characteristic of a mixture shows the different retention time between these two values chosen, such as 1S in Figure 21, and 3N in Figure 22.

The mass spectrum of 1S was shown in Figure 23.

By examining the gas liquid chromatographic retention time on Figures 17 and 20, it was found that unknown 1N was similar to 2S (15.6 minutes), 2N to 3S (17.8 minutes) and 3N to 4S (19.1 minutes). Comparison of the retention time of these unknowns with that of standards indicated that pyridine was present in 2N and/or 3S, and pyrazine in 3N and/or 4S (Figure 24).

The mass spectrum of 3S (Figure 25) obtained on the top of the peak is identical to that of standard pyridine. The molecular ion 79 produced an abundant peak at m/e 52 by losing m/e 27 which was HCN.

The mass spectrum of 2N (Figure 26) did not show the presence of pyridine; instead, the molecular ion m/e 111 was found. The peak m/e 96 was associated with the loss of methyl and peak m/e 79 with the loss of CH_3OH. A partial fragmentation of unknown 2N was proposed as shown on the following page.

Other prominent peaks such as m/e 42, 43, 55 and 70 are not explainable unless much rearrangement is used. It would seem more desirable to obtain high resolution mass spectral data so that the

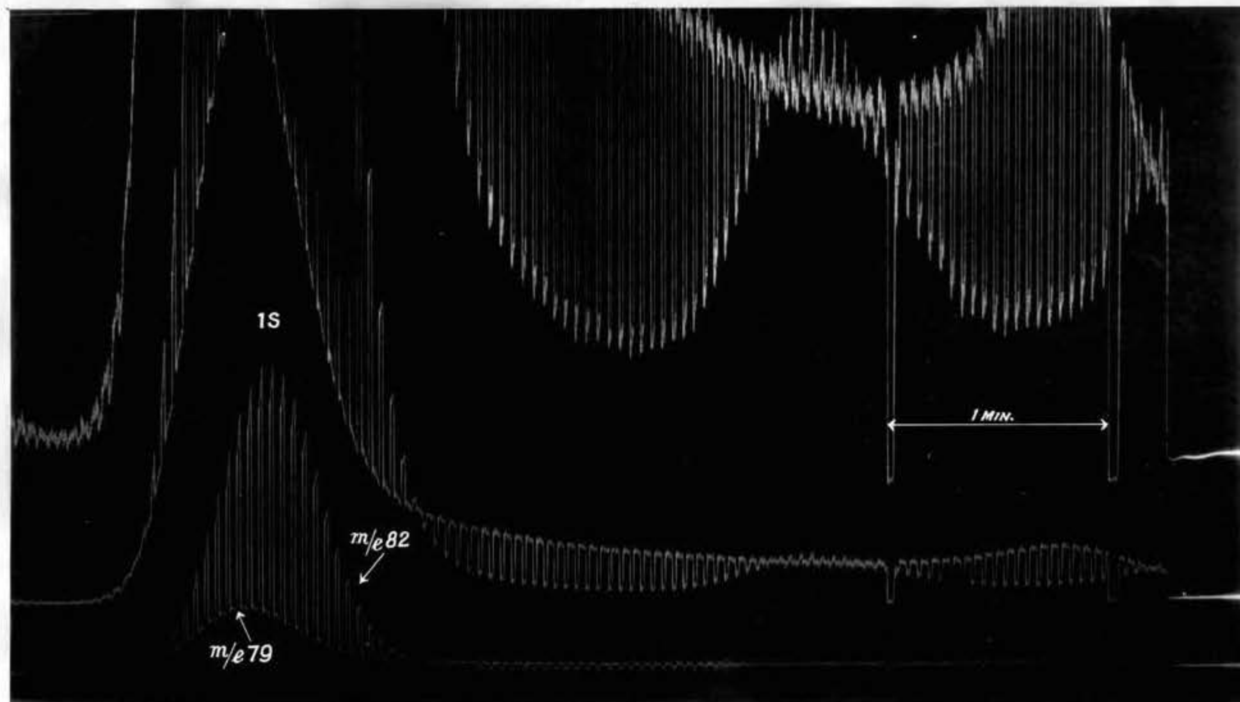


Figure 21. Alternating Voltage Accelerator Tracing of Unknown 1S.

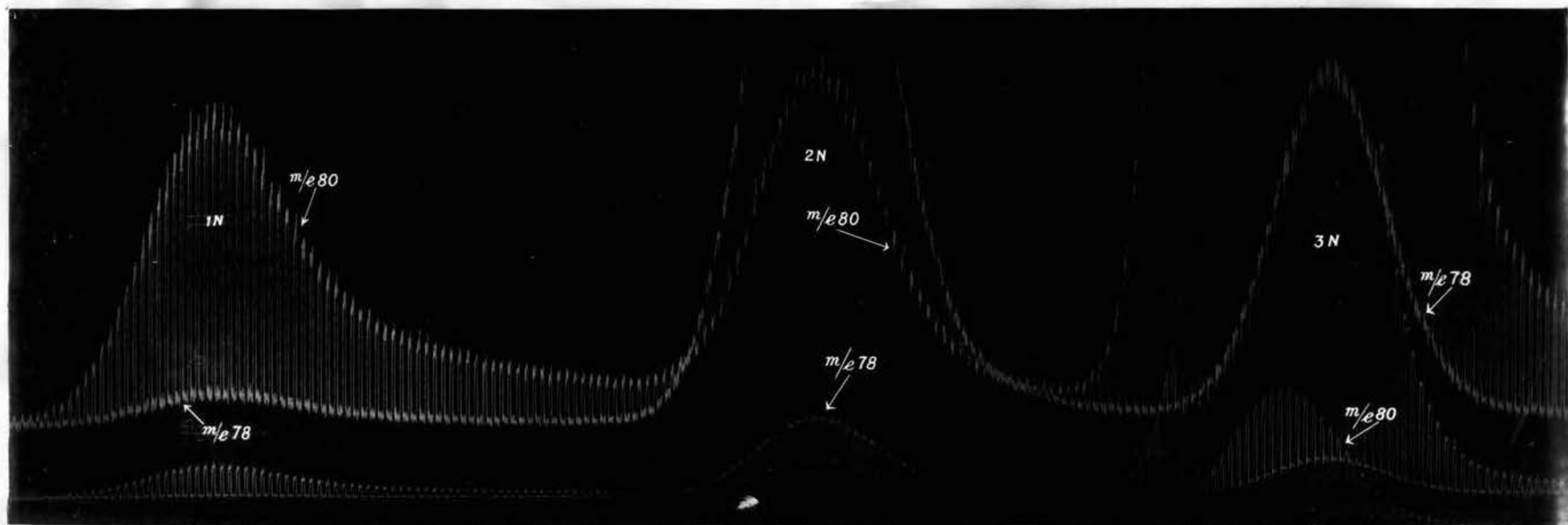


Figure 22. Alternating Voltage Accelerator Tracing of Unknown 3N. (A) By m/e 78 and m/e 80.

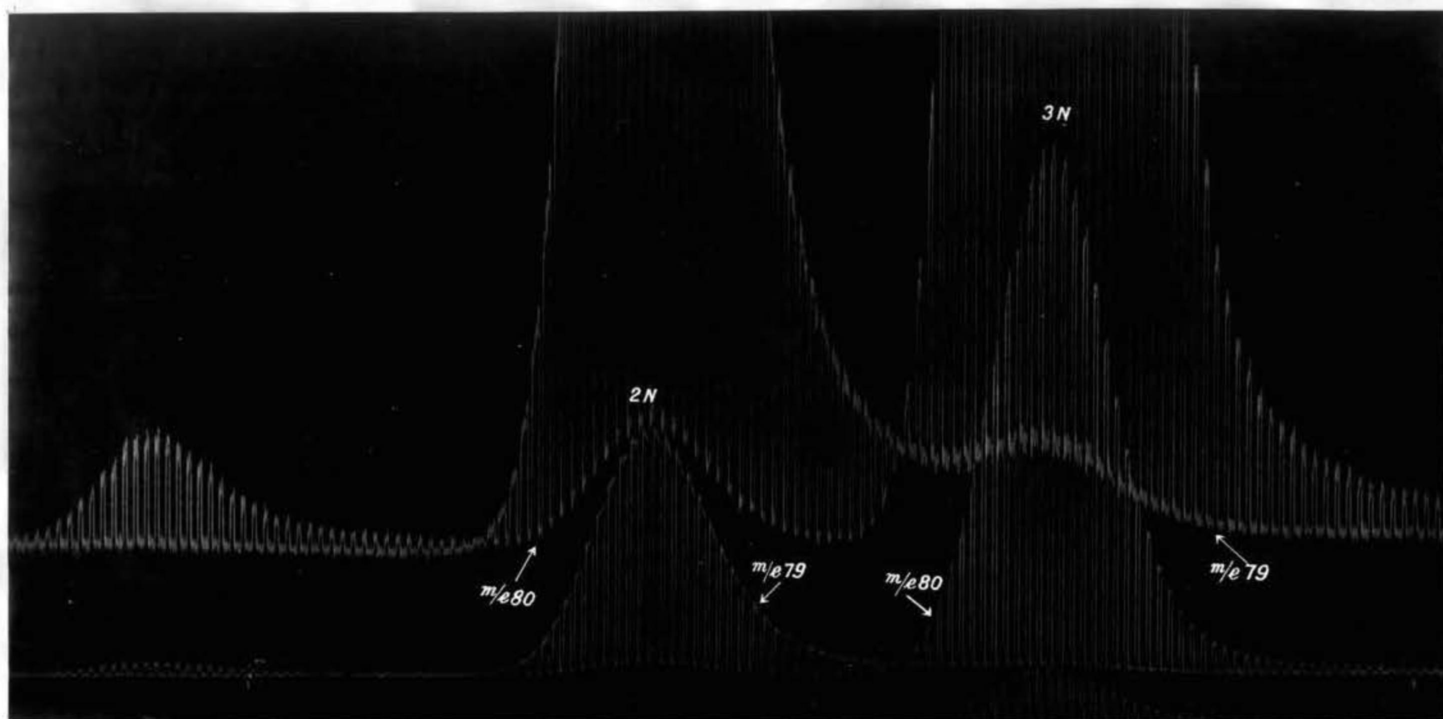


Figure 22. Alternating Voltage Accelerator Tracing of Unknown 3N. (B) By m/e 79 and m/e 80.

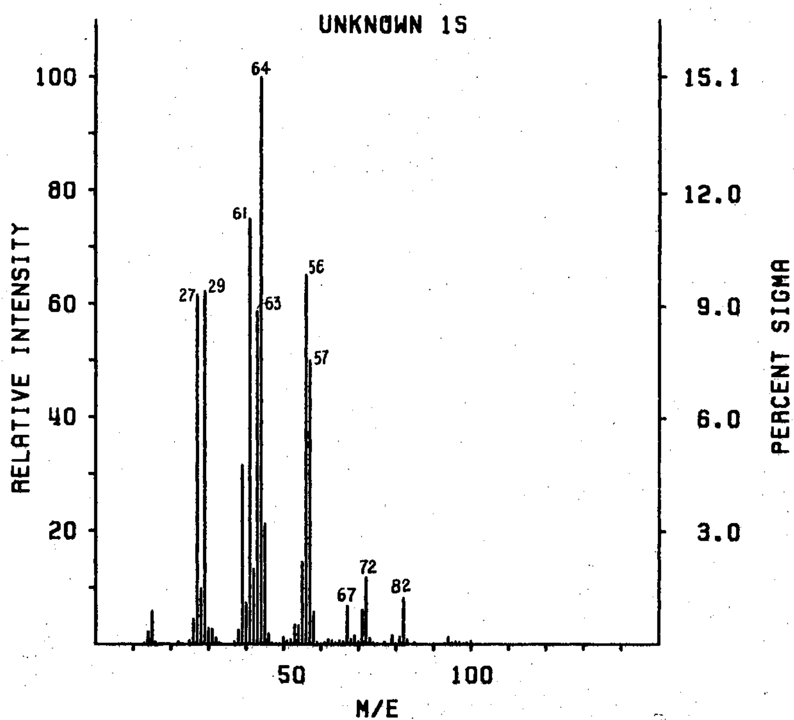


Figure 23. Mass Spectrum of Unknown 1S.

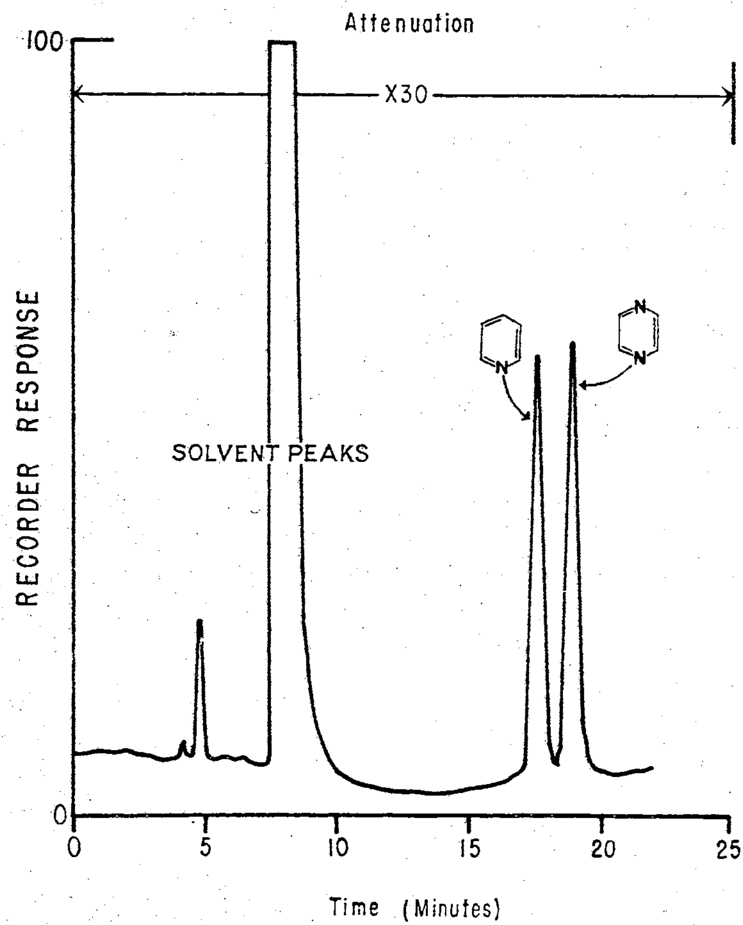
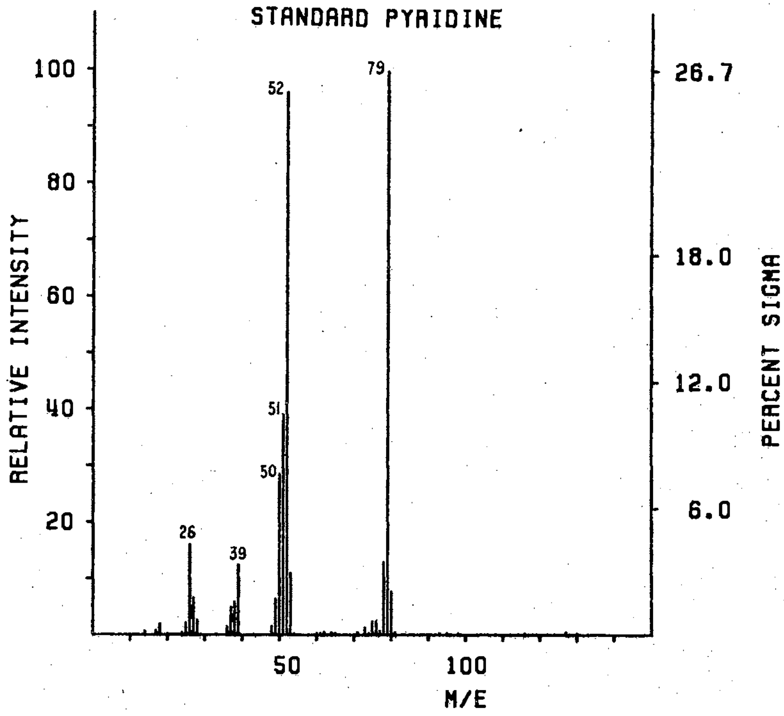


Figure 24. Gas Liquid Chromatogram of a Standard Mixture of Pyrazine and Pyridine.



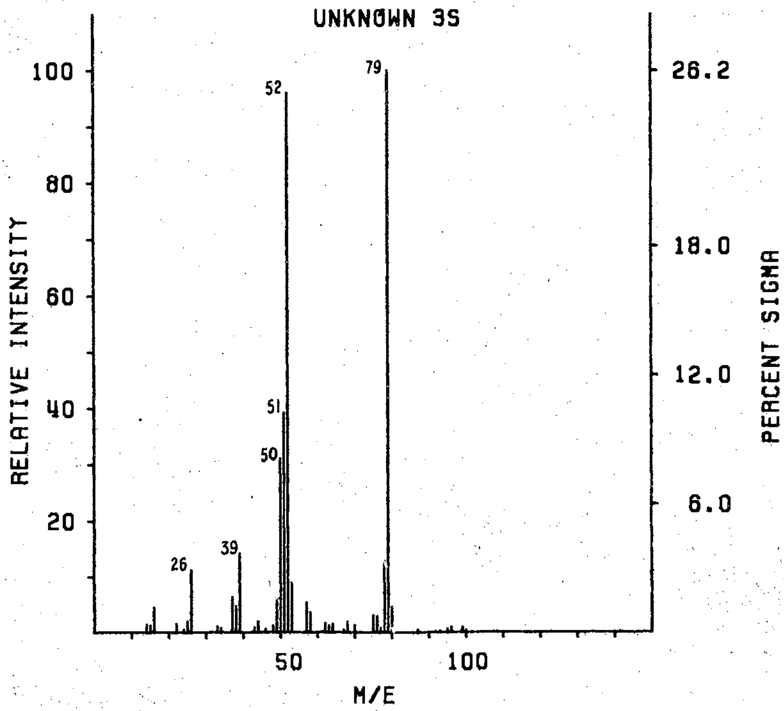
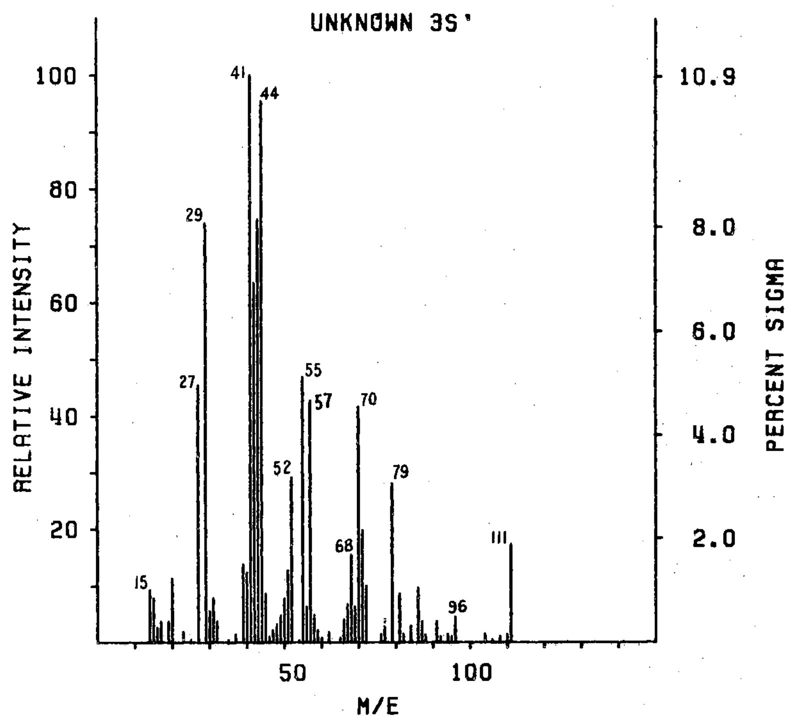


Figure 25. Mass Spectra of Pyridine and Unknown 3S.



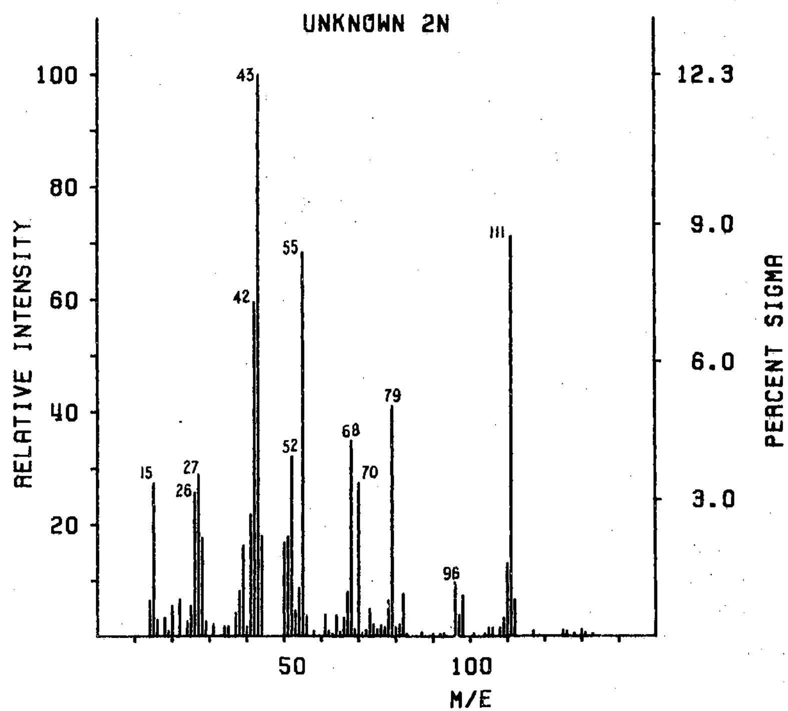
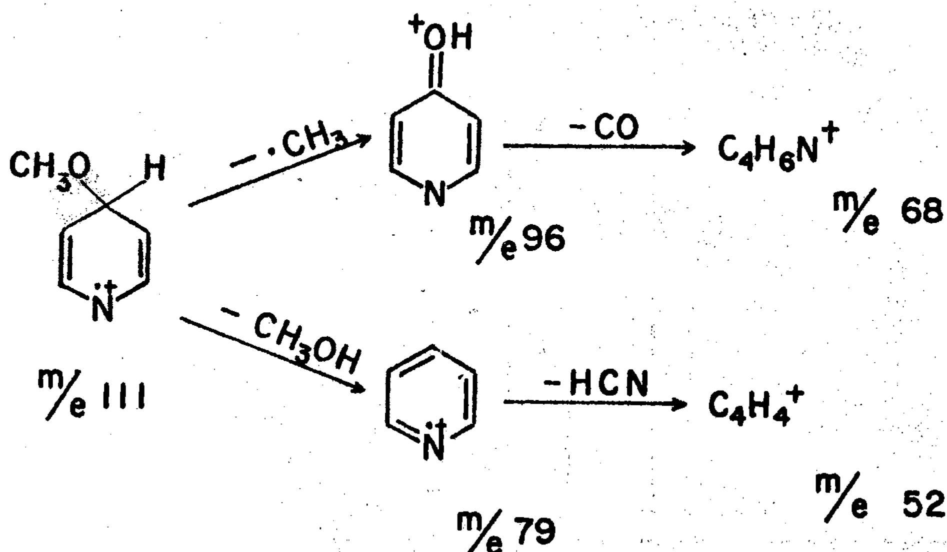


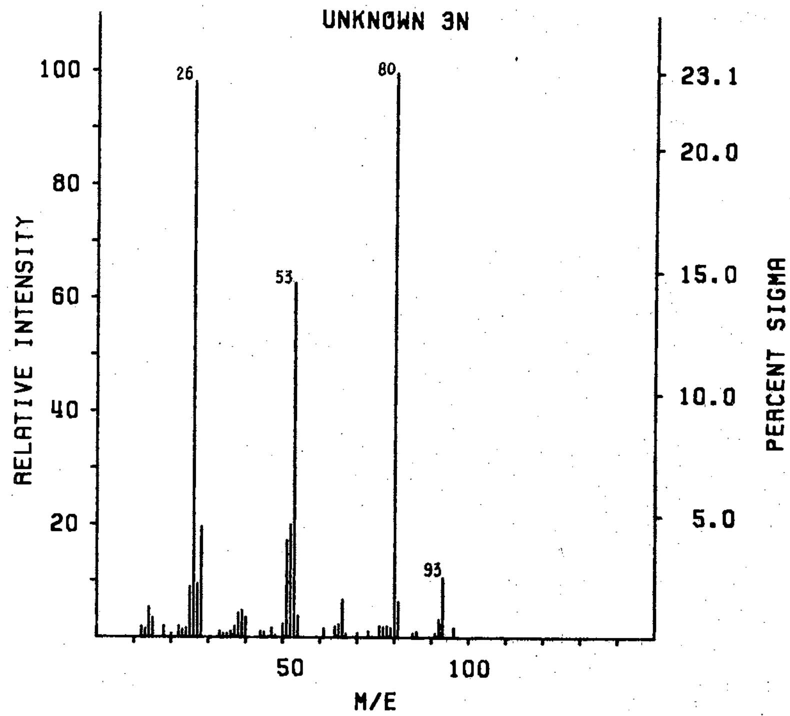
Figure 26. Mass Spectra of Unknowns 3S' and 2N.

elemental composition of the ion would be known and could seem an aid in spectra interpretation.



The mass spectrum of 3S' (Figure 26) was obtained from the rear shoulder of the peak 3S, the molecular peak appeared at m/e 111, which was the same as that of 2N.

The mass spectrum of 3N is similar to that of 4S (Figure 27) in some part. Both molecular ions showed at peak m/e 93, and three of the most abundant peaks at m/e 80, 53, and 26 were shown on both spectra. Comparison of these two spectra with that of standard pyrazine (Figure 28), showed that the character of pyrazine was expressed on these three peaks. According to the retention time, the three of the most abundant peaks (m/e 80, 53 and 26) and the evidence revealed by AVA technique, it could be assumed that pyrazine was one of the mixed compounds in the unknowns 3N and 4S, probably, the other compound of the



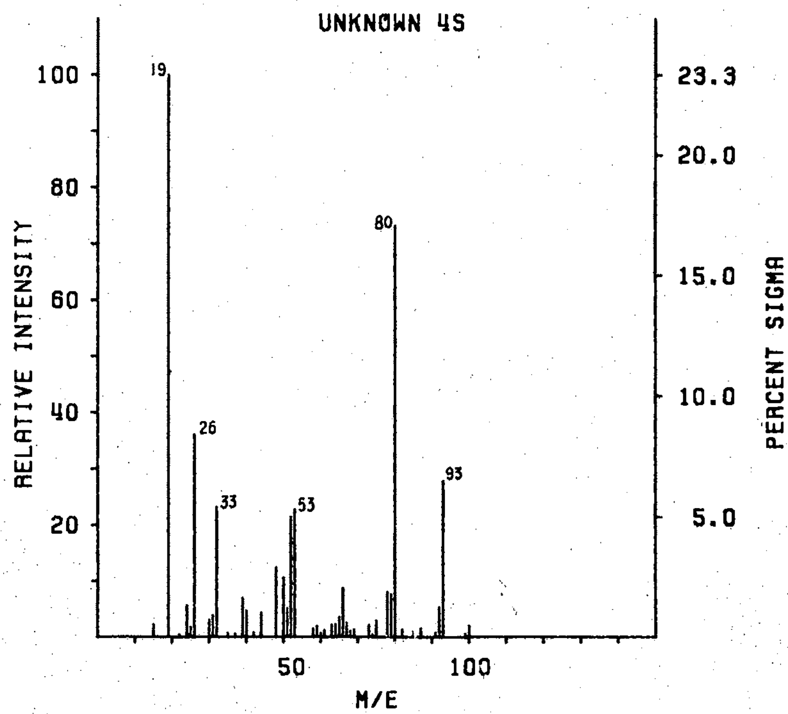


Figure 27. Mass Spectra of Unknowns 4S and 3N.

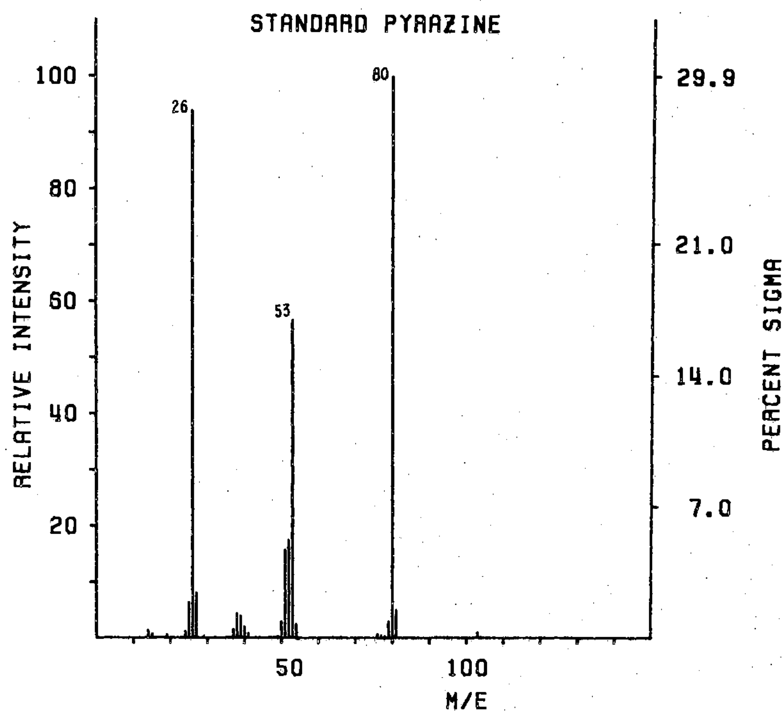


Figure 28. Mass Spectrum of Pyrazine.

mixture was in a minor amount with the molecular ion of m/e 93.

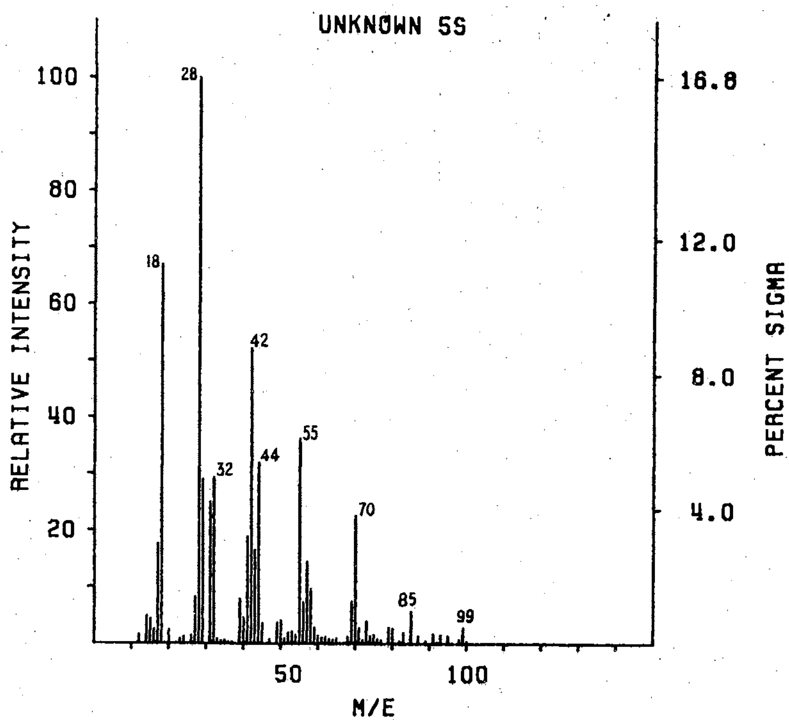
The mass spectra of 1N and 5S were shown in Figure 29.

G. ABSOLUTE CONCENTRATIONS OF THE BASIC VOLATILE CONSTITUENTS OF ROASTED PEANUTS

Peak 2 was used not only to calculate the relative concentrations of the volatiles as shown in Tables VI, VII, VIII, IX and XI, but also to estimate the absolute concentrations of the volatiles of roasted peanuts. Peak 2 was an unresolved peak composed of 2,5- and 2,6-dimethylpyrazines; so, either one of the 2 standard compounds could be used for the estimation of the absolute quantity of either or both of these compounds. In this study, standard 2,6-dimethylpyrazine was selected as a reference to measure the linear relation between the peak area and the amount injected (Figure 8.B). Data for all samples are shown in Table XII. Using peak 2 as a base, the absolute concentration of any other peak shown on the chromatograms can be obtained, however, the molar response of the detector varies with each compound, consequently such data would not be highly accurate.

By this procedure, dimethylpyrazine was estimated to be present at a concentration of 1.1 mg/kg in roasted Argentine or Starr peanuts. Most samples had 0.9 $\pm$ 0.1 mg/kg except sample 17, which had been treated with SO_2 plus N_2. The latter sample had only 40% of the standard and a definite off-flavor was also observed. The expected results would be higher than those reported above.

In Table XII, dimethylpyrazine absolute concentrations expressed in mg/kg were calculated from the concentrated volatiles in the small vial and represented only a fraction of the total volatiles in roasted



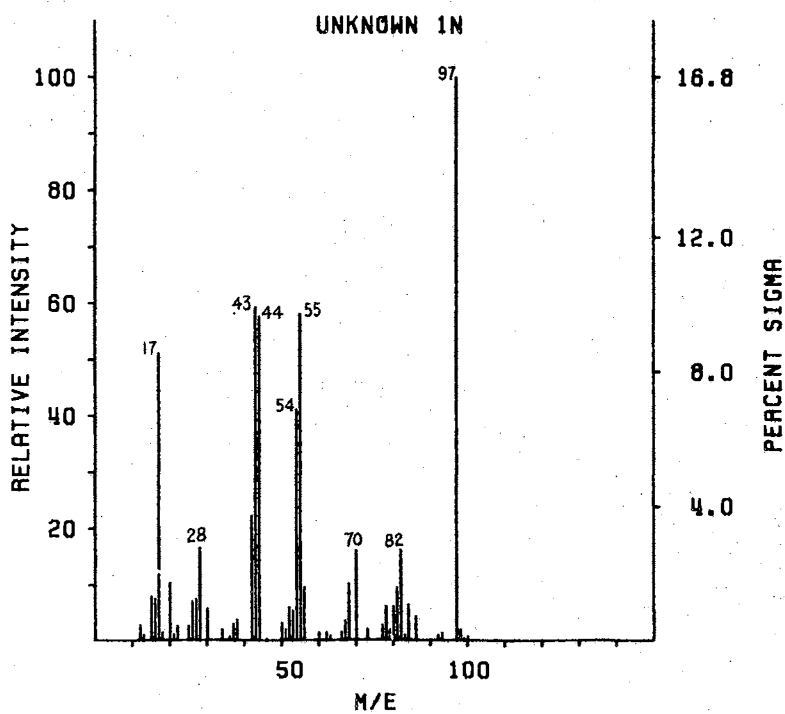


Figure 29. Mass Spectra of Unknowns 5S and 1N.

TABLE XII

ESTIMATED CONCENTRATION OF 2,5- and 2,6-DIMETHYLPYRAZINES IN THE ROASTED PEANUT SAMPLES[a]

Sample No.	Arbitrary area units	μg/5μl[b]	μg/50μl[c]/350gm	mg/kg.
1	191[d]	35	350	1.1
2	202[d]	37	370	1.1
3	140	25	250	.8
4	168	31	310	.9
5	160	29	290	.9
6	188	34	340	1.0
7	160	29	290	.9
8	158	29	290	.9
9	162	30	300	.9
10	181	33	330	1.0
11	158	29	290	.9
12	152	28	280	.9
13	163	30	300	.9
14	169	31	310	.9
15	202	37	370	1.1
16	134	25	250	.8
17	71	13	130	.4
18	167	31	310	.9

a - sample details, see Tables II, III and IV and Chapter III.
b - amount injected on column.
c - initial volume used in GLC analysis.
d - Average of three runs.

peanuts because the experimental procedure resulted in some volatiles being lost in the following ways:

(i) When pressing, some oil still remained in the pressed peanuts and some was lost in the filter cloth and on the press plate holder.

(ii) During filtering, about one tenth of the filtered oil was lost.

(iii) When concentrating on the rotary evaporator and by nitrogen some volatiles were lost.

If the problems cited above can be eliminated or if the amount of loss can be determined, then the calculated concentrations should be more accurate than those in Table XII.

H. THREE MINOR PEAKS ON CHROMATOGRAMS

When all samples were completed, all the chromatograms suggested the traces of three minor peaks at the retention time of 15.6, 17.8 and 19.1 minutes. The evidence of the existence of these three peaks (peaks a, b and c) was achieved by attenuation of 30 (Figure 30). Then an attempt was made to review some previous findings: (i) three pairs of unknowns (1N and 2S, 2N and 3S' and 3N and 4S) occurred also at the correspondent retention time, (ii) both 3N and 4S contained pyrazine compound, (iii) the molecular ion of 2N appeared to be the same as that of 3S'. Consequently, it may be proposed that in addition to the 16 main peaks (peaks 1 to 16) on the chromatograms, the three minor peaks are also the peanut volatile constituents and they are related with the three pairs of unknowns. It may be concluded that small amounts of pyridine and pyrazine are always present in roasted peanut flavor.

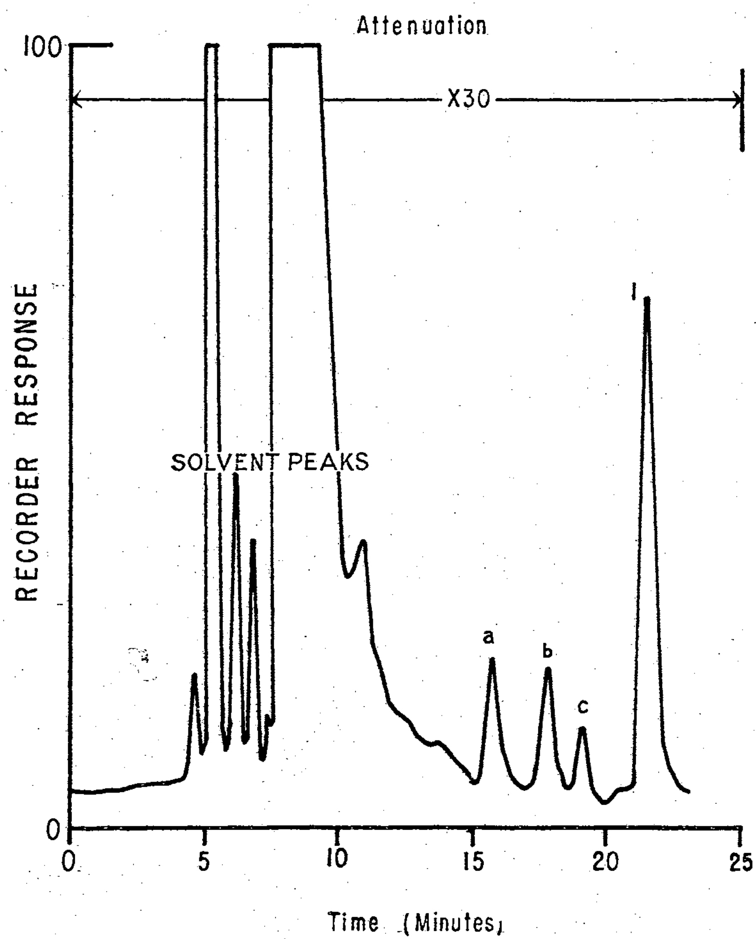


Figure 30. Gas Liquid Chromatogram of the Three Minor Peaks in Roasted Starr Peanuts.

CHAPTER V

SUMMARY

The purpose of the study was to examine comparatively the volatile constituents in the basic fraction of roasted peanuts from various samples. Most of the study was concerned with the relative quantitative analysis of these samples and the identification of some of the unknowns found in certain samples.

The vacuum degassing system was modified and tested for its ability to remove sufficiently the flavor volatiles from the roasted peanut oil. The selected procedure was capable of producing highly reproducible results.

The peanut varieties examined appeared to differ mostly in the relative concentrations of volatiles rather than in the presence or absence of certain component(s).

The peanuts fertilized with an additional element, boron, produced an unknown component which was identified as 1,2-dichloroethane by the combination gas liquid chromatography and mass spectrometry. The relative concentrations of peak 11, 6 and 7 on the chromatogram in this sample were found less than those in the sample fertilized without boron.

Peanuts stored in combination of SO_2 and N_2 were different from peanuts stored in N_2, CO_2 or dry air in relative volatile concentrations and seed appearance.

Three unknowns (1N, 2N and 3N) were shown in the sample treated with N_2, and five unknowns (1S to 5S) in the sample treated with SO_2 plus N_2. By means of alternating voltage accelerating technique, 1S and 3S were confirmed to be two mixtures. 3S was positively shown to be pyridine, while 4S and 3N were similar in part of their mass spectra which indicated that pyrazine existed in each peak. The mass spectrum on the shoulder of 3S showed the molecular ion at m/e 111, which was the same as 2N. A partial fragmentation pattern of 2N was postulated.

No significant differences in the relative volatile concentrations were found from peanuts harvested 120 days after planting at different dates in growing season. A slight effect of the length of time of growth to the relative volatile concentration was detected.

The absolute concentrations of 2,5- and 2,6-dimethylpyrazines were calculated from standard 2,6-dimethylpyrazine and this concentration was estimated to be 1.1 mg/kg in roasted Argentine or Starr peanuts, and 0.4 mg/kg in the sample treated by SO_2 plus N_2, which showed positively an off-flavor.

All the chromatograms indicated that in addition to the 16 main peaks, the three minor peaks in the first 20 minutes of analysis were also the peanut volatile constituents. Although more than 3 peaks were detected, it may be concluded that small amounts of pyradine and pyrazine are always present in roasted peanut flavor.

SELECTED BIBLIOGRAPHY

(1) Amoore, J. E., in The Chemistry and Physiology of Flavor, Schultz, Day and Libbey, ed. AVI Publishing Co. Westport, Conn. (1967).

(2) Anonymous, Food Technology, 23, 1360 (1969).

(3) Woodroof, J. G., Peanuts: Production, Processing, Products AVI Publishing Co., Westport, Conn. (1966).

(4) Johnson, B., "Isolation and Identification of Some Volatile Constituents of Roasted Peanuts," M.S. Thesis, Oklahoma State University, 1966.

(5) Mason, M. E., Johnson, B., Hamming, M. C., J. Agr. Food Chem., 14, 454 (1966).

(6) Bondarovich, H. A., Friedel, P., Krampl, V., Renner, J. A., Shephard, F. W., and Gianturco, M. A., J. Agr. Food Chem., 15, 1093 (1967).

(7) Van Praag, M. Stein, H. S. and Tibbett, M. S., J. Agr. Food Chem., 16, 1005 (1968).

(8) Wolford, R. W., Attaway, J. A., Alberding, G. E., and Atkins, C. D., J. Food Sci., 28, 320 (1965).

(9) Flath, R. A., Forrey, R. R., and Teranishi, R., J. Food Sci., 34, 328 (1969).

(10) Swain, A. P., Peterson, R. F., and Stedman, R. L., J. Sci. Food Agr., 17, 349 (1966).

(11) Smith, D. M., Skakum, W., and Levi, L., J. Agr. Food Chem., 11, 268 (1963).

(12) Self, R., in "Recent Advances in Food Science," Vol. 3, Leith, J. M., and Rhodes, D. N., eds., Butterworths, London, 1963. p. 170.

(13) Nelson, P. E., and Hoff, J. E., J. Food Sci., 34, 53 (1969).

(14) Stevens, K. L., Flath, R. A., Lee, A., Stern, D. J., J. Agr. Food Chem., 17, 1102 (1969).

(15) Flath, R. A., Black, D. R., Forrey, R. R., McDonald, G. M., Mon, T. R., and Teranishi, R., J. Chrom. Sci., 7, 508 (1969).

(16) Mason, M. E., and Waller, G. R., J. Agr. Food Chem., 12, 274 (1964).

(17) Newell, J. A., "Precursors of Typical and Atypical Roasted Peanut Flavor," Ph.D. dissertation, Oklahoma State University, 1967.

(18) Newell, J. A., Mason, M. E., and Matlock, R. S., J. Agr. Food Chem., 15, 767 (1967).

(19) Mason, M. E., "Procedures in Studying and Factors Influencing the Quality and Flavor of Roasted Peanuts," Ph.D. dissertation, Oklahoma State University, 1963.

(20) Mason, M. E., Johnson, B., and Hamming, M. C., J. Agr. Food Chem. 15, 66 (1967).

(21) Johnson B. "Chemical Characterization of Roasted Peanut Aroma" Ph.D. dissertation, Oklahoma State University, 1970.

(22) Koehler, P. E., "Formation of Alkylpyrazine Compounds and their Role in the Flavor of Roasted Food," Ph.D. dissertation, Oklahoma State University, 1969.

(23) Koehler, P. E., Mason, M. E., and Newell, J. A., J. Agr. Food Chem. 17, 393 (1969).

(24) Pickett, T. A., and Holly, K. T., Peanut Roasting Studies, Georgia Expt. Sta. Tech. Bull. No. 1 (1952).

(25) Hoffpauir, C. L., J. Agr. Food Chem., 1, 668 (1953).

(26) Reichstein, T. and Standinger, H., "The Structure and Components of Food," Vol. IV, John Wiley and Sons Inc., N. Y., 1939, p. 152.

(27) Stoll, M., Winter, M., Gautschi, F., Flament, I., Willhalm, B., Helv. Chim. Acta, 50, 628 (1967).

(28) Goldman, I. M., Seibl, J., Flament, I., Gauschi, F., Winter, M., Willhalm, B., Stoll, M., Helv. Chim Acta, 50, 694 (1967).

(29) Deck, R. E., and Chang, S. S., Chem. Ind., 1343 (1965).

(30) Marion, J. P., Muggler, C. F., Viani, R., Bricont, J., Reymond, D., and Egli, R. H., Helv. Chim. Acta, 50, 1509 (1967).

(31) Rizzi, G. P., J. Agr. Food Chem., 15, 549 (1967).

(32) Van der Wal, B., Sipma, G., Kettenes, D. K., Semper, A. Th. J., Rec. Trav. Chim., 87, 238 (1968).

(33) Wang, P. S., Kato, H., Fujimaki, M., Agr. Biol. Chem., 33, 1775 (1967).

(34) Young, C. T., and Holley, K. T., Comparison of Peanut Varieties in Storage and Roasting, Georgia Expt. Sta. Tech. Bull. N. S. 41 (1965).

(35) Auda, H., Juneja, H. R., Eisenbraun, E. J., Waller, G. R., Kays, W. R., and Appel, H. H., J. Am. Chem. Soc., 89, 2476 (1967).

(36) Cobb, W. Y., J. Food Sci., 34, 466 (1969).

(37) Wilson, C. W. III, J. Food Sci., 34, 535 (1969).

(38) Buttery, R. G., Seifert, R. M., Guadagni, D. G., Black, D. R., Ling, L. C., J. Agr. Food Chem., 16, 1009 (1968).

(39) Stinson, E. E., Dooley, C. J., Fillpic, V. T., Hills, C. H., J. Food Sci., 34, 246 (1969).

(40) Brodnitz, M. H., and Pollock, C. L., Food Technol, 24, 78 (1970).

(41) Biggers, R. E., Hilton, J. J., Gianturco, M. A., J. Chrom. Sci. 7, 453 (1969).

(42) Merritt, C. Jr., Bazinet, M. L., Sullivan, J. H., and Robertson, D. H., J. Agr. Food Chem., 11, 152 (1963).

(43) Waller, G. R., Proc. Okla. Acad. Sci. 47, 271 (1968).

(44) Li, H. Y. Walden, J., Etter, D. and Waller, G. R., Proc. Okla. Acad. Sci., 48, 250 (1969).

(45) Day, E. A., Basette, R., and Keeney, M., J. Dairy Sci., 43, 463 (1960).

(46) Harris, H. C., Peanut Farmer, July, 1965, p. 8.

(47) Harris, H. C., Fla. Agr. Expt. Sta. Bull. 494 (1952).

(48) James, A. T., and Wheatley, V. R., Biochem. J. 63, 269 (1956).

(49) Hallock, D. L., Peanut Farmer, July, 1966.

(50) Manbeck, H. B., Moseley, Y. C., Barnes, G. L., and Nelson, G. L., "Controlled Atmosphere for Short Duration Storage of Peanuts Before Drying," Annual Meeting of Am. Soc. Agr. Engin., June, 1969.

(51) Sweeley, C. C., Elliott, W. H., Fries, I., and Ryhage, R., Anal. Chem. 38, 1549 (1966).

(52) Snedecor, G. W., and Cochran, W. G., Statistical Methods, 6 ed., The Iowa State University Press, Ames, Iowa (1968).

(53) Beynon, J. H., Saunders, R. A., and Williams, A. E., The Mass Spectra of Organic Molecules, Elserver Publishing Company, New York, (1968).

(54) Cornu, A., and Massot, R., eds., Compilation of Mass Spectral Data, Heyden and Son Limited, Paris, France (1966).

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