OKLAHOMA STATE UNIVERSITY LIBRARY
OCT 12 1970

CHEMICAL CHARACTERIZATION OF

ROASTED PEANUT AROMA

Thesis Approved:

George R. Waller
Thesis Adviser

Roger E. Koeppe

E. J. Eisenbraun

George V. Odell

Glenn A. Todd

D. Durham
Dean of the Graduate College

762366

ACKNOWLEDGEMENTS

The author gratefully acknowledges the encouragement and counseling of his major adviser, Dr. George R. Waller, during the course of these studies and the preparation of this dissertation. Thanks are extended to Dr. G. V. Odell for his encouragement and interest and to Dr. E. J. Eisenbraun, Dr. G. W. Todd and Dr. R. E. Koeppe for their helpful suggestions.

The author thanks Dr. A. L. Burlingame, Dr. D. H. Smith, Mr. James Wilder, Mr. Fred Walls and Miss Martha Petrie for access to the high resolution mass spectrometer and willing assistance during its use.

The author wishes to thank Mr. Kieth Kinneberg for assistance and instructions in mass spectrometry.

A very special thanks goes to his wife, Charlotte, for her encouragement, understanding and assistance during the years of this study.

Finally, the author acknowledges the support of a National Defense Education Act Fellowship and the Biochemistry department for financial support. He is also indebted to the Department of Biochemistry for facilities and equipment furnished for these studies.

TABLE OF CONTENTS

Chapter		Page
I.	INTRODUCTION	1
II.	LITERATURE REVIEW	4
	Introduction	4
	Peanut Volatile Constituents	5
	Precursors of Peanut Aroma	8
	Simularities of Pyrolyzed Foods	9
	Isolation and Identification of Volatile Flavor Components	10
III.	MATERIALS AND METHODS	13
	Introduction	13
	Apparatus and Reagents	13
	Roasting Apparatus	14
	Oil Recovery	14
	Vacuum Degassing	14
	Reagents	15
	Procedures	15
	Preparation of Peanuts for Analysis	15
	Concentration of the Volatiles	16
	Fractionation by Ion Exchange Chromatography	17
	Fractionation by Extraction Techniques	17
	Results and Discussion	18
IV.	CHARACTERIZATION OF THE BASIC FRACTION OF ROASTED PEANUT AROMA	23
	Introduction	23
	Apparatus and Reagents	24
	Apparatus	24
	Reagents	25
	Procedures	26
	Separation of Basic Fraction	26
	Gas Chromatography-Mass Spectrometry	27

TABLE OF CONTENTS (Continued)

Chapter		Page
	Infrared and Ultraviolet Spectroscopy	28
	Results and Discussion	29
	Gas Liquid Chromatography	29
	Mass Spectrometry	30
	Low Resolution	30
	High Resolution Mass Spectra	31
	Accelerating Voltage Switching Mass Spectrometry	31
	Mass Spectral Data (Low Resolution and High Resolution	32
	Ultraviolet Spectroscopy and Gas Chromatographic Retention Time	37
	Infrared Spectroscopy	38
	Gas Chromatographic Retention Time Indices	39
V.	CHARACTERIZATION OF THE NEUTRAL FRACTION OF ROASTED PEANUT AROMA	65
	Introduction	65
	Apparatus and Reagents	65
	Apparatus	65
	Reagents	66
	Procedures	66
	Results and Discussion	67
	Results	67
	Mass Spectra (Low and High Resolution)	68
	Gas Chromatographic Retention Times and Order	74
	Discussion	75
VI.	SUMMARY	94
SELECTED BIBLIOGRAPHY		96

LIST OF TABLES

Table		Page
I.	Gas Chromatographic Retention Time and Ultraviolet Spectroscopic Data	42
II.	Infrared Data on Pyrazines Isolated from Basic Fraction of Roasted Peanut Aroma and Appropriate Standards	43
III.	Gas Chromatographic Relative Retention Time Indices, Calculated as I_E Values*, of Compounds Identified in Basic Fraction of Roasted Peanuts and Some Standards	44
IV.	Summary of Pyrazines Identified in Basic Fraction of Roasted Peanut Volatiles	45
V.	Summary of Compounds Identified in the Neutral Fraction and Methods used to Confirm their Identity	78
VI.	Gas Chromatographic Data Comparing Relative Retention Times and Order of Some Neutral Fraction Components and Standard Compounds	79

LIST OF FIGURES

Figure		Page
1.	Vacuum Degassing System	20
2.	Flow Diagram of Fractionation Procedure	21
3.	Flow Diagram of Fractionation Procedure	22
4.	Gas Liquid Chromatographic Analysis of the Basic Fraction	47
5.	Gas Liquid Chromatographic Analysis of the Basic Fraction	49
6.	Capillary Gas Liquid Chromatographic Analysis of the Basic Fraction	51
7.	Combination Gas Liquid Chromatographic-Mass Spectrometric Analysis of the Basic Fraction	53
8.	Combination Capillary Gas Liquid Chromatographic-High Resolution Mass Spectrometric Analysis of the Basic Fraction	55
9.	Accelerating Voltage Switching Mass Spectrum of Peaks B, C, D, and E	56
10.	Mass Spectra of Components C and D and Standards	57
11.	Mass Spectra of Components E and F and Standards	58
12.	Mass Spectra of Components G and H and Standards	59
13.	Mass Spectra of Components J and K and Standards	60
14.	Mass Spectra of Components L and M and Standards	61
15.	Mass Spectra of Components N, P and S and a Standard	62
16.	Mass Spectra of Components T, U, W and X	63
17.	Mass Spectrum of Component Z	64
18.	Combination Capillary Gas Liquid Chromatographic Mass Spectrometric Analysis of the Neutral Fraction	81

LIST OF FIGURES (Continued)

Figure		Page
19.	Mass Spectra of Scans H and 8 and Standards	82
20.	Mass Spectra of Scans 9 and 10 and Standards	83
21.	Mass Spectra of Scans 12 and 18 and Standards	84
22.	Mass Spectra of Scans 22 and 46 and Standards	85
23.	Mass Spectra of Scans 52 and 62 and Standards	86
24.	Mass Spectra of Scans 66 and 68 and Standards	87
25.	Mass Spectra of Scans 74 and 78 and Standards	88
26.	Mass Spectra of Scans 82B and 83 and Standards	89
27.	Mass Spectra of Scans 87 and 89 and Standards	90
28.	Mass Spectra of Scans 92 and 93 and Standards	91
29.	Mass Spectra of Scans 96 and 101 and Standards	92
30.	Mass Spectra of Scans 103 and 105 and Standards	93

CHAPTER 1

INTRODUCTION

Roasted peanuts (Arachis hypogaea) possess a unique and widely enjoyed flavor. Raw peanuts have a different and unique flavor (1,2). The process of roasting (pyrolysis) subjects the peanut to internal temperatures of 130°-150° C (3) during which the typical roasted peanut flavor is produced. A knowledge of the volatile components is deemed a necessary guide for ascertaining appropriate changes in preprocessing, processing, storage and marketing techniques to improve flavor and control quality, thereby ultimately to increase consumer acceptances. This is not only true for roasted peanuts and peanut products but of the other pyrolytically processed foods, such as cocoa and coffee, where duplication of a natural flavor or modification of the flavor of a natural food product is considered desirable.

The isolation and identification of volatile constituents of foods contributes to an understanding of flavor chemistry in the following ways:

(1) Accumulates basic knowledge concerning the kinds of compounds that are involved in food aroma.

(2) Aids in the identification of flavor precursors.

(3) Compliments the biologists investigation of olfaction and taste and the mechanism of action of the odor and taste

response.

(4) Aids in quality control of foods and food products.

(5) May lead to the discovery of compounds which may ultimately be combined for the creation of imitation flavors.

(6) Discovery of trace volatile constituents which may have very low thresholds or are present in subthreshold concentrations, but exhibit a synergistic effect thereby contributing to the odor of the food.

It was for these reasons that a study of the volatile components of roasted peanuts was initiated.

The objectives of the research project follow:

(1) To develop methods of isolation and fractionation of the volatile constituents (aroma) of roasted peanuts.

The low concentration and threshold value of volatile compounds associated with flavor make chemical analysis for flavor compounds difficult. Concentration and isolation of trace components become important in analysis and must be accomplished without destroying the aroma compounds and without introducing artifacts.

(2) To determine gas chromatographic parameters necessary for complete resolution of the complex mixture of aroma constituents into individual components.

To separate the complex aroma concentrate maximum resolution is needed. To simplify the separation, pre-fractionation techniques are often employed. The high resolution needed can be met using capillary gas chromatography, but the inherent low capacity can become a limiting factor in this analysis.

(3) To identify as many volatile components as possible by

using the combination gas chromatograph-mass spectrometer. Mass spectrometry provides the most information per quantity of compound, and is therefore the method of choice for identification of odoriferous compounds. The combination gas chromatograph-mass spectrometer is widely used in flavor analysis since it permits identification of many components in a complex mixture to be made. Both low and high resolution mass spectrometers are employed.

CHAPTER II

LITERATURE REVIEW

INTRODUCTION

The sensory receptors are the detectors which inform us of physical and chemical changes in our environment. According to Aristotle, man is said to possess 5 primary, or major senses: sight, hearing, touch, smell, and taste. Many animals cannot hear, a few have no visual perception, but all forms of animals react to chemical stimuli. Man has at least three different senses which respond to chemical stimuli: taste, smell and the so-called common chemical or pain sense (4). Lepkovsky (5) states that sensory stimuli: 1) make possible the recognition of food; 2) make it possible for the animal to choose its food in accordance with its need; 3) initiate appropriate responses in the viscera, making them ready for digestion of the meal; 4) are important in the cessation of eating since they promote satiety; and 5) make possible the pleasure that is anticipated from eating. As noted by Pfaffmann (6) the senses of taste and smell possess one unique property; they can and do instigate strong acceptance or rejection responses. Thus one becomes aware of the intimate association of the senses of taste and smell with man's eating habits and of the economic, nutritional and esthetic values of food flavor.

As Brozek (7) noted "Flavor is a complex sensation, with taste,

aroma, and feelings as three categories of components."

Moncrieff (8) states, the factor which has the greatest influence is odor or aroma. If the odor is lacking, then the food loses its flavor and becomes chiefly bitter, sweet, sour or saline. Texture and astringency are also involved depending on the food product. The word flavor has been reserved to mean the overall sensation resulting from the impact of the food on the chemical sense receptors in the nose and mouth, according to Self (9).

The apparent olfactory thresholds for the most powerful odors are about 10,000 times lower than the lowest taste thresholds (3), emphasizing the important role of aroma in flavor.

The characteristic differentiating flavors of foods may be attributed primarily to volatile constituents, since the contribution of taste represents only qualities of sweetness, sourness, saltiness, and bitterness (7).

This literature review will cover what is known about roasted peanut flavor, in terms of the volatile constituents, precursors of volatile constituents, mechanisms of production of volatiles, and analytical techniques used in the study of food flavors.

Peanut Volatile Constituents

Studies on raw peanut volatile constituents are very limited. Pattee *et al.* (1) isolated and identified eleven volatile components from high temperature cured off-flavor peanuts as a means of defining the curing systems which produce the off-flavor. He used gas chromatographic retention times and functional group analysis to identify formaldehyde, acetaldehyde, ethanol, acetone, isobutyraldehyde, ethyl acetate, butyraldehyde, isovaleraldehyde, 2-methylvaleraldehyde, methyl

butyl ketone, hexaldehyde and 2- or 3-methylbutanal and furfural, tentatively. Pattee et al. (2,10) and Singleton et al. (11) have identified some volatile components of normal raw peanuts and related the changes in the volatile profile with enzymatic activity during maturation and curing temperature. Five major volatile compounds were identified; acetaldehyde, methanol, pentane, ethanol, and hexanol. Volatile profiles produced by peanuts cured at 22°, 35°, 45°, and 50° C. were studied in relation to evaluations of flavor and aroma by a taste panel. Acetaldehyde, ethanol, and ethyl acetate were implicated as associated with flavor deterioration. Increases in these were detected by evaluations of aroma and flavor by the taste panel.

Studies on the volatile components of roasted peanuts are limited but are much more extensive than those on raw peanuts. The earliest published findings on roasted peanut aroma were in 1952 by Pickett and Holley (3) and a review in 1953 by Hoffpauir (12). The volatile components found included a large quantity of carbon dioxide, aldehydes, furfurals, ammonia, sulfur compounds, and diacetyl.

Mason et al. (13, 14) reported, a decade later (1963) renewed efforts to reveal the chemical identities of the components of roasted peanut flavor and its precursors. Benzaldehyde and 2,5-dimethylpyrazine were identified as major components of a steam distillate of roasted peanuts. Also tenatively identified were acetylene, hydrogen cyanide, propiolonitrile, tetrahydrofuran, methyl pyrrole, methane thiol, butanal, allyl ethyl ether, and 2-methylpropanal.

Young and Holley (15) recently compared differences in peanut varieties on storage and roasting, determining volatiles as total carbonyls, total dicarbonyls, hydrogen sulfide, CO_2, and mercaptans.

Their results showed that total volatiles from roasting tend to increase as quality declines, with the increase generally greatest in the total carbonyl fraction.

Mason et al. (16) utilized ultraviolet spectroscopy, nuclear magnetic resonance and mass spectrometry to identify volatile components from roasted peanuts separated by preparative gas chromatography. Identified were N-methyl pyrrole, 2-methylpyrazine, 2,5-dimethylpyrazine, trimethylpyrazine, a methylethylpyrazine and a dimethylethylpyrazine. The pyrazine compounds were reported to possess a "roasted aroma". They reported in a second paper (17) the identification of acetaldehyde, isobutyraldehyde, benzaldehyde, phenylacetaldehyde and tentatively 2- and 3-methylbutanals and 3-methyl-2-butanone by regeneration of the 2,4-dinitrophenylhydrazone derivatives onto a combination gas chromatograph mass spectrometer, by ultraviolet data and thin layer chromatography of the 2,4-dinitrophenylhydrazone derivatives. They also reported that the low molecular weight aldehydes contributed some of the harsh aroma normally noted from warm freshly roasted peanuts, while phenylacetaldehyde contributed to the sweet bouquet of typical roasted peanut aroma.

Brown et al. (18) used solvent extraction techniques to separate and isolate aroma and flavor constituents of roasted peanuts. They reported the identification of 12 acids (10 were fatty acids C_2-C_7), hexanal, 2,4-decadienal, β-sitosterol, 2-oxooctanal and a dihydroxynaphthaleneacetic acid. Their approach was to examine large quantities of peanuts, but the problem of extracting components not involved in the aroma of roasted peanuts occurs.

Aldehydes and ketones are common constituents of many food aromas.

They arise primarily by Strecker degradation (19) of amino acids and via lipid degradation (20). Pyrazines are much less common food aroma constituents, being found only in foods which are processed by pyrolysis.

Pyrazines have been reported first in coffee (21) and more recently in cocoa (22,23,24,25,26), roasted peanuts (16), potato chips (27), and many additional ones in coffee (28, 29, 30, 31, 32), Koehler (33) gives an excellent review of the occurence of alkyl pyrazines in food and their formation in model systems.

Precursors of Peanut Aroma

In 1952, Picket and Holley (3) were the first to report the changes in concentration of various compounds within the peanut during roasting. They had earlier noted that oil and starch remained unchanged, while total sugars decreased (34). These workers observed decreases in amino acids and carbohydrates during roasting and implied that these were flavor precursors. More recently (1964), Mason and Waller (13) reported work on the isolation and localization of roasted peanut flavor precursors. From roasting studies, a semi logarithmic plot of time versus 1/Temperature was found to be linear, showing that the formation of flavor components was solely a function of energy. This observation lead them to conclude that flavor formation resulted from intramolecular reactions in which the close proximity of another molecule was not necessary. Isolation of the precursor by extraction with 1M NaCl, centrifugation and gel filtration was followed by roasting of fractions after each step of the isolation. The 1M sodium chloride soluble portion of ground defatted peanuts was roasted and found to have typical roasted peanut aroma. The soluble portion, 45%

of the original dry weight of the fat-free peanuts, was separated by gel filtration on Sephadex G-25. Individual fractions were lyophilized and roasted. Peanut aroma was observed from fractions containing only molecules of low molecular weight. The other fractions, mostly protein, produced little or no aroma.

The same authors also reported the protein body-aleurone grain fraction as the specific location of flavor precursors and implicated the aleurone grains more strongly than the protein bodies.

Newell (35) reported results from this laboratory on precursors of typical and atypical roasted peanut flavor. He observed significant decreases in amino acids and carbohydrates during roasting. An equation was derived which related amino acid flavor precursor concentration to subjectively measured flavor of roasted peanuts. From calculations of this equation, aspartic acid, glutamic acid, glutamine, asparaging, histidine and phenylalanine were associated with the production of typical peanut flavor. Precursors of a typical flavor were found to be threonine, tyrosine, lysine and an unknown amino acid. Model systems, using the amino acids associated with typical aroma and carbohydrates, were found to yield most of the same volatile compounds known in roasted peanut aroma.

Simularities of Pyrolyzed Foods:

Hoffpauir (12) noted the similarity of the volatiles produced from roasting coffee and peanuts. In view of the similarities of the morphology (36), roasting processes and flavor precursors (37, 35), cocoa beans and peanuts, Newell (35) suggested that pyrazines would be found in the volatile components of roasted cocoa beans and be quantitatively important components. Almost simultaneously, Rizzi(23) and

later Marion et al. (24), Filiment et al. (25), van Praag et al. (22), and van der Wal et al. (26) collectively reported some 21 pyrazine compounds identified among the volatile components of cocoa beans. van Praag et al. (22), and Mason et al. (16), reported "nut-like" aroma from alkyl pyrazines.

Alkylpyrazines are common, quantitatively significant components of roasted coffee, cocoa and peanuts. Non-enzymatic browning reaction products resulting from roasting or pyrolyzing foods have been identified as major components of the aroma of these foods. One of the primary events occurring during roasting of peanuts or cocoa beans is the Millard reaction between amino acids and reducing carbohydrates (35). Hodge (38) divided the browning reaction (Millard) into three stages: 1) the initial colorless stage involving sugar-amine condensation and Amadorii rearrangement; 2) the intermediate stage characterized by dehydration reactions to give furfurals and/or reductones, production of sugar degradation products, and Strecker degradation (conversion of α-amino acids to aldehydes containing one less carbon atom and carbon dioxide); 3) compounds formed during the intermediate stage are thought to polymerize to unsaturated, colored, nitrogen containing polymers. The chief reactions were aldol condensations, aldehyde-amine polymerization and formation of heterocyclic nitrogen compounds such as pyrazines, pyrroles, pyridines, and imidazoles.

Mechanisms for the formation of pyrazines in model systems are postulated by Koehler (39) Newell (35) and van Praag (22).

Isolation and Identification of Volatile Flavor Components

Extensive research has been conducted in the past decade in the area of identification of food aroma constituents. The wealth of

papers in the area can be related to the use and availability of modern analytical instrumentation. From the advent of gas chromatography (40, 41) (1952) growth in the field of flavor chemistry has increased with the use of more refined gas chromatographic techniques, capillary (Golay) (42, 43) columns, mass spectrometry, combination gas chromatography-mass spectrometry (44, 45, 46), and capillary gas chromatography-high resolution mass spectrometry (47). Also quite important in this growth was improved infrared and nuclear magnetic resonance instruments and techniques.

A number of techniques are routinely employed to concentrate the aroma. The most commonly used methods involve variations of direct solvent extraction (18, 48, 49), steam distillation (50, 51), vacuum degassing (29), aroma stripping at both positive and reduced pressures (51, 52, 53), derivatization of components (52), the direct analysis of head-space gases (54, 55, 56), and many variations and combinations of the mentioned techniques.

Tang and Jennings (51) compared several methods of preparing apricot essence concentrates to permit the detection of any artifact production. They were unable to distinguish differences in the aromas of the different concentrates. However, the essence prepared by direct extraction of the fruit and molecular distillation was characterized by having the largest relative amount of higher boiling components. Vacuum steam distillations yielded essences with larger relative amounts of lower boiling components.

Probably no aroma concentration method is universally superior. The method of choice will vary with the particular problem. Gianturco (28) observed that steam distillation of roasted coffee was useful for

orientative studies, but not a suitable method of obtaining samples for identification work. He preferred to strip the volatile fraction at low temperature and pressure from the oil obtained by pressing the roasted beans. An extensive solvent extraction procedure for roasted peanut volatiles has been utilized by Brown et al. (18).

CHAPTER III

MATERIALS AND METHODS

Introduction

The concentration of most food aroma constituents is a few parts per million or less. This presents a definite problem for the flavor chemist. The aroma must be concentrated so that the instruments employed in identification are able to detect the components. The nose is somewhat more sensitive than the most sensitive instrument.

Two variations of the vacuum degassing technique were employed in concentrating the volatile components of roasted peanuts. The first was the vacuum degassing of a finely ground slurry of roasted peanuts in glycerol as defined by Mason et al. (16), except mineral oil was substituted for glycerol. This substitution was made when it was feared that the glycerol might react with components in the peanuts to produce volatile products. This procedure was time consuming and inefficient, therefore the degassing of homogenates was abandoned.

The second procedure involved vacuum degassing of peanut oil, pressed from whole roasted peanuts, over a steam heated manifold (Figure 1). This was a modification of the method used by Mason (14).

Fractionation of the collected condensed volatiles was by two methods; 1) ion exchange chromatography 2) pH adjustment and a solvent extraction similar to the procedure of van Praag (22).

Apparatus and Reagents

Roasting Apparatus

The roasting of the peanuts was performed in a General Electric rotisserie oven fitted with a cylindrical wire basket designed specifically for this purpose. The basket was constructed of wire mesh and was 6 inches in diameter and 13 inches long. Homogenation of the peanuts was accomplished in a one quart jar attachment of the Sorvall Omnimixer.

Oil Recovery

Whole roasted peanuts were pressed with a Carver laboratory press to obtain the oil.

Vacuum Degassing

The vacuum degassing system consisted of a Welch dual stage vacuum pump rated at 0.1 micron and 140 liters per minute, a three stage oil diffusion pump to obtain a high vacuum, two small cold traps and a large cold finger trap in series, and either a 12 liter flask or the glass column shown in Figure 1. A 1 liter reservoir was placed at the top allowing introduction of the oil into the next chamber, which served as a "foaming" chamber. In the "foaming" chamber there was equal pressure above and below the oil level, so that foaming takes place here, rather than when entering the heated molecular still, thus allowing the oil flow to be more easily regulated through the stopcock at the base of the "foaming" chamber.

The glass tower consists of a series of expansion bellows, which maximize degassing of the oil. Finally a 1 liter collection flask is at the bottom of the molecular still. Liquid nitrogen was used to cool

each of the three cold traps. The large cold finger, (Trap #1), was quite efficient but was backed up by the smaller middle trap, (Trap #2). The first trap, (Trap #3), prevented back diffusion of the diffusion pump oil.

The collected condensate was separated into two fractions by extraction of an aqueous solution at high and low pH's. The pH was adjusted using a Sargent Model DR single glass electrode pH meter.

Reagents

Paraffin oil. White, heavy (Saybolt viscosity 335/350) Fisher Scientific Co., Fair Lawn, New Jersey.

Methylene Chloride. Certified spectranalyzed. Fisher Scientific Co., Fair Lawn, New Jersey.

Ion Exchange resin. Amberlite CG 120 (strongly acidic). Mallinckrodt Chemical Works, St. Louis, Mo.

Sodium Chloride, sodium citrate, sodium hydroxide and all other chemicals were ACS Reagent Grade.

Procedures

Preparation of Peanuts for Analysis

Good flavored Spanish peanuts (air dried), were roasted, in 4 pound batches, in an oven as previously described. Approximately one hour was required to reach a golden brown (medium roast) stage. They were room temperature cooled, split in a laboratory splitter and the testa and germ removed. The cotyledons were homogenized with mineral oil as described by Mason *et al.* (16) and Johnson (54).

Whole roasted peanuts were pressed while still hot, at 10,000 psi in ½ pound batches to obtain the oil. Approximately 500 mls of oil were obtained from 3½ pounds of roasted peanuts.

Concentration of the Volatiles

The procedure for vacuum degassing of a roasted peanut-paraffin oil slurry was as described by Mason et al. (16), except paraffin oil was used instead of glycerol.

The volatiles were condensed from the pressed oil by passing it through the system, previously described (Figure 1). The system was evacuated and liquid nitrogen placed in all three traps. A fritted glass funnel was situated on top of the reservoir and filled with peanut oil. The stopcock was turned to allow the reservoir to be evacuated sufficiently to pull the oil through the filter, removing small bits of testa which interfered with oil passage through stopcocks. With all stopcocks opened to the vacuum, the reservoir stopcock was opened slowly to allow a slow steady flow into the foaming chamber. The oil foamed considerably as dissolved air and the more volatile components were rapidly removed. The flow of oil was discontinued after the foaming reservoir was about 3/4 filled. The oil was then metered through the foaming chamber stopcock onto the heated glass tower at approximately 1 drop per second. The oil was quickly degassed as it dropped from one expansion bellow to the next lower and finally into the collection reservoir. The large stopcock at the bottom was closed after all the oil passed through, the reservoir was removed and the oil was passed through a second time to approach complete removal of the aroma constituents. The volatiles were collected

by isolating the system from the pump at the large stopcock between the two small cold traps. The liquid nitrogen bath was removed from the center cold trap, and the condensate cryogenically pumped to the large cold finger. A liquid nitrogen bath was then placed on the small collection tube at the bottom of the large cold finger and the liquid nitrogen removed from the cold finger, which allowed the condensate to be transfered into the collection tube. The collection tubes were stoppered and stored at -12° C.

Fractionation by Ion Exchange Chromatography

The collected condensate from a single batch of roasted peanuts was extracted with 20 mls of methylene chloride (see flow diagram in Figure 2). The CH_2Cl_2 extract was then extracted 3 times with 25 mls of 1N HCl to remove basic and acid soluble components and to eliminate the traces of paraffin oil which were collected. The acid extract was then placed on an Amberlite CG-120 column (equilibrated with pH 2.2 1M citrate buffer) 1 inch in diameter packed to a height of 10 inches. The acid extract was followed with 1 liter of citrate buffer to wash through all the neutral and acidic components. The basic fraction was then stripped from the column with 300 ml of 2N NaOH solution. The basic column eluate was extracted 3 times with 25 mls of methylene chloride and stored at -12° C. This methylene chloride extract was reduced to about 1 ml volume on a rotatory evaporator, just prior to gas chromatographic analysis.

Fractionation by Extraction Techniques

A more simple and rapid fractionation technique was employed and

was similar to that described by van Praag et al (22), see figure (3) for flow diagram. The condensate was diluted to 100 ml with deionized water, 10 grams of NaCl added and the pH was adjusted to 0.5 by slowly adding concentrated HCl. The acidified solution was extracted 5 times with 5 ml volumes of methylene chloride, to obtain the neutral fraction. The pH was then readjusted to 8.5 and the solution was again extracted 4 times with 5 ml volumes of methylene chloride to obtain the basic fraction. Both fractions were stored at -4^{o} C in a glass stoppered 50 ml round-bottomed flask. Immediately prior to analysis the volume was reduced to 1 ml on a rotary evaporator whereby the temperature was kept near 0^{o} C by solvent evaporation.

Results and Discussion

Good quality Spanish seed peanuts were used in all experiments. The testa and germ were removed after roasting and before homogenation. In later experiments the oil was pressed from whole roasted peanuts while still hot. The oil possessed a typical roasted peanut aroma, and was nearly odorless after two passes through the degassing system

About 1 ml of condensate was collected from 500 ml of oil, compared to approximately 2 ml from homogenation of 2 lb. of peanuts, the difference being due to the lower water content of the oil compared to the homogenate. The condensate in both cases was mostly water.

Comparison of the two techniques of volatile condensation, showed the oil method more efficient. The pressed oil technique was favored because of the contaminating traces of mineral oil appeared in the condensate obtained from the peanut oil homogenate. Also the possi-

bility of continued roasting and browning and hence additional production of volatile compounds was greater in the homogenate, since sugars, amino acids, protein, etc. are still present. However the oil became darker after two passes through the heated glass tower. It was assumed that no significant reactions took place in the oil medium while passing through the 100° steam heated system, since it was well below the 130°-150° roasting temperature (Pickett and Holley 1952) and flavor precursors presumably were in the pressed peanut residue (35).

Comparison of fractionation by ion exchange chromatography or by extraction showed the latter to be much less time consuming and more reliable. Both methods introduce the possibility of additional reaction of volatile components and the production of flavor artifacts when they are exposed to the very low pH conditions during extraction. This point will be discussed further in chapter V.

Volume reduction of the neutral and basic fractions on the rotary evaporator necessarily resulted in some loss of the more volatile aroma components. The greatest loss was from the neutral fraction. Headspace analysis was required to obtain the more volatile components. This involves analyzing the vapors above a flavor condensate.

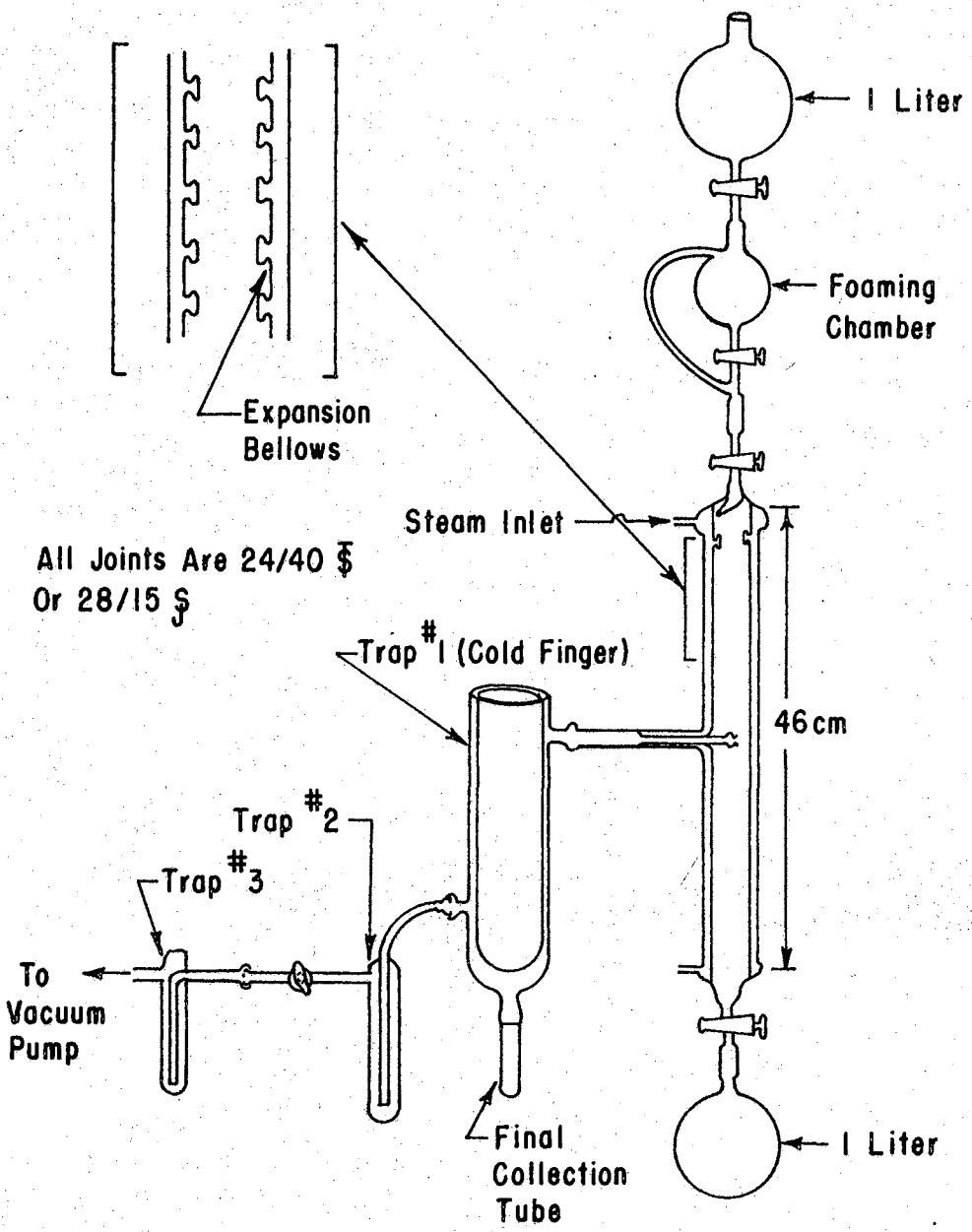


Figure 1. Vacuum Degassing System

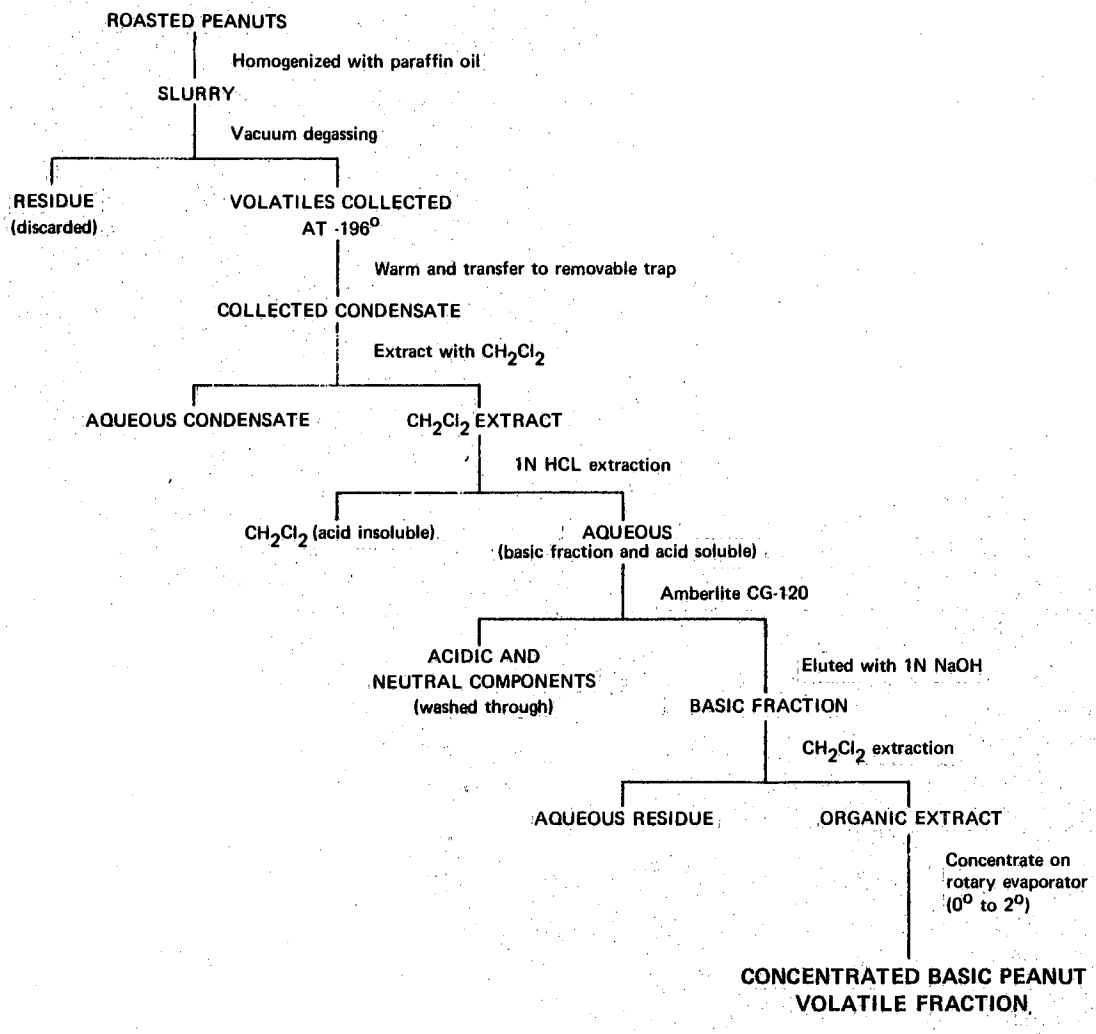


Figure 2. Flow Diagram of Fractionation Procedure

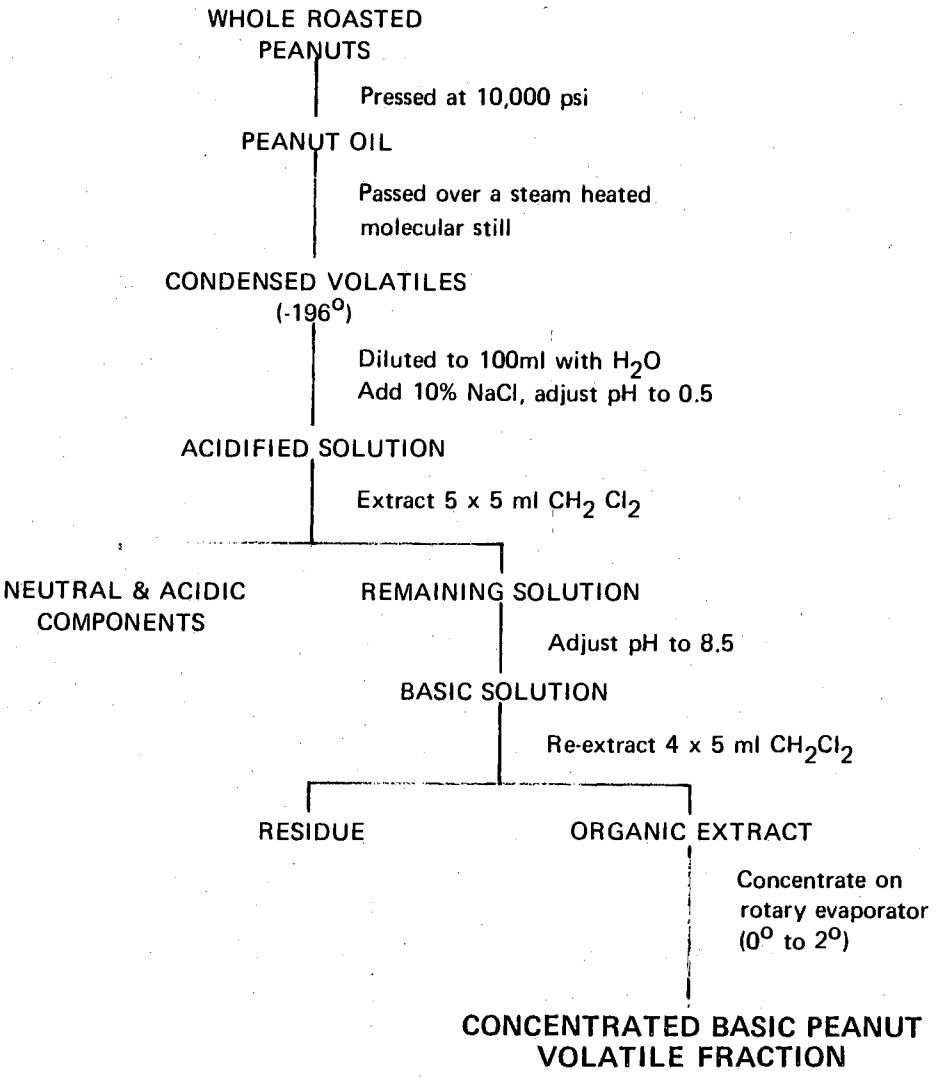


Figure 3. Flow Diagram of Fractionation Procedure

CHAPTER IV

CHARACTERIZATION OF THE BASIC FRACTION OF ROASTED PEANUT AROMA

Introduction

Alkylpyrazine compounds have been identified in aromas of roasted or pyrolyzed food products. Coffee, (28, 31, 32) cocoa (22, 23), potato chips (27) and peanuts (16), have been reported to contain alkylpyrazines. In addition two bacterial sources: a) a strain of Bacillus subtilus which produces tetramethylpyrazine, a compound considered to be responsible for the characteristic odor of Natto (57), which is a fermented soybean product consumed in some Asian countries, and b) a mutant of Corynobacterium glutamicum which accumulates high concentrations of tetramethylpyrazine in the medium (58) and an important component (3-isobutyl-2-methoxypyrazine) of the volatile oil of green bell peppers (59) are known to yield alkylpyrazines. The latter is the only known source of pyrazine production by a higher plant. Generally pyrazines are products of a nonenzymatic browning reaction (Millard) between amino acids and carbohydrates of pyrolytically processed foods (35, 38). A nut-like odor has been attributed to this class of compounds (16, 19).

The quantitatively major pyrazines of roasted peanuts have been reported from this laboratory; however, many other less abundant pyra-

zine compounds were indicated as constituents of roasted peanut aroma. These might contribute synergestically to the overall aroma, or they may have extremely low odor thresholds, as does 2-methoxy-3-isobutylpyrazine (2 parts per trillion), and are therefore potentially important in the overall flavor of roasted peanuts. Consequently the identification of the minor components of the basic fraction of roasted peanut aroma was undertaken.

Apparatus and Reagents

Apparatus

Gas chromatographic analyses were performed on a modified Barber-Colman Model 8000 gas chromatograph equipped with a hydrogen flame-ionization detector. Preparative gas chromatography was performed on a Perkin Elmer Model 801 gas chromatograph equipped with a dual hydrogen flame ionization detector and a 4:1 post-column stream splitter.

Low-resolution mass spectrometry was obtained on a prototype of the LKB 9000 combination gas chromatograph-mass spectrometer (GC-MS), which was constructed at the Karolinska Institutet, in Stokholm, Sweden in the laboratories of Dr. Ragnar Ryhage and was described by Waller (60). All spectra were obtained under the following conditions: ionizing voltage of 70 electron volts, 3.5 KV accelerating voltage, 40 μ amps trap current, 1.7 to 2.1 KV electron multiplier voltage, source temperature of 310° C, separator temperature of 220° C, and at scan speeds so that mass 0-200 was scanned in 3-5 seconds. The gas chromatographic tracing was a recording of the total ionization current obtained from the collector plate in the analyzer tube. Spectra were counted, measured and corrected manually. The data were intro-

duced into the IBM 360/50 computer used to drive a Cal Comp Model 565 Plotter which plotted the mass spectra, Li et al. (61).

High resolution mass spectrometric analyses were conducted at the Space Sciences Laboratories, University of California, Berkeley, California. Spectra were obtained from a modified Associated Electrical Industries, Ltd MS 902 high resolution mass spectrometer equipped with a direct on-line data acquisition system connected to a Sigma 7 computer and with a capillary gas-liquid chromatographic column elutions directly into the ion source.

Infrared spectra were obtained on a Perkin Elmer Model 427 recording spectrometer.

Ultraviolet spectra were obtained in a Bausch and Lomb Spectronic Model 505 recording spectrometer.

Reagents

Gas Chrom Q (100/120 mesh), Carbowax 20M, and OV-17. Applied Science Laboratories, Inc., P. O. Box 140, State College, Pa.

Carbowax 1540, Union Carbide Chemicals Co., New York 17, New York.

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

2,6-Dimethylpyrazine and 2,3-dimethylpyrazine were purchased from Aldrich Chemical Company, Milwaukee, Wisconsin.

2-Methylpyrazine and 2,5-dimethylpyrazine were obtained from Wyandotte Chemicals Corp., Wyandotte, Michigan. 2-Ethylpyrazine and 2-Ethyl-6-methylpyrazine were gifts of the research division of Wyandotte Chemicals.

2-Methyl-6-propylpyrazine, 2 ethyl-3,5-dimethylpyrazine and 2-ethyl-

3,6-dimethylpyrazine were gifts from Firminich and Cie, Geneva, Switzerland.

2,6-Diethyl-3-methylpyrazine and 2,3-diethyl-5-methylpyrazine were gifts from the Corporate Research Department of the Coca-Cola Company, Atlanta, Georgia.

Other pyrazine standards used were synthesized in this laboratory by Philip Koehler (33).

Procedures

Separation of Basic Fraction

The alkylpyrazines found in the aroma of roasted peanuts presented a difficult problem of resolution into individual components by gas chromatography. Initial attempts to separate the components (most of which were identified as alkylpyrazines) in the basic fraction were effected with a 15% (w/w) Carbowax 20M analytical column. Additional attempts utilized a 4% OV-17 analytical column with operational parameters as follows: injection port temperature-145°, oven temperature programmed from 75° to 170° at 3°/min, and a helium flow of 42 ml/min. Figure 4 is the chromatogram obtained from an aliquot of the basic fraction analyzed in this manner.

Continued efforts involved the use of a column packed with 5% (w/w) Carbowax 20M coated on base washed Gas Chrom Q. The Gas Chrom Q was washed 3 times with methanol saturated with potassium hydroxide and once with methanol as described by Auda *et al.* (62). Figure 5 is the chromatogram obtained under the following conditions: temperature programmed from 70° at 3°/min to 170°, injection port temperature 175°, and helium flow of 40 ml/min.

Bondarovich et al. (31) pointed out the difficulty of the separation of some of the pyrazines from their isomers, which was also encountered in the work reported in this thesis. Consequently a .02 in (i.d.) X 500 ft stainless steel capillary column coated with Carbowax 1540 and KOH (63) was employed. Figure 6 shows the chromatogram obtained by temperature programming from 65° to 165° at 2°/min.

Compounds for infrared and ultraviolet analysis were collected from a preparative column packed with base washed Gas Chrom Q coated with 15% Carbowax 20M. The following operational parameters were employed: temperature programmed from 70° to 170° at 4°/min, and a helium flow of 60 ml/min. Preparative gas chromatography was performed on a Perkin-Elmer Model 801 gas chromatograph equipped with a dual hydrogen flame ionization defector and a 4:1 post column stream splitter, allowing collection of the majority of the eluting components. The splitter exit was adapted with a female Luerlock fitting to which a 17 gauge x 7 inch Luerlock needle could be quickly attached. The needle was inserted inside a 6 inch long section of 3 mm o.d. glass tubing with one end sealed and both tube and needle were immersed in a liquid nitrogen bath.

Retention time data was obtained on the capillary column previously described. The column was programmed from 80° to 200° at 2 degrees per minute with a flow rate of 10 ml per minute.

Gas Chromatography-Mass Spectrometry

Low resolution mass spectral data was obtained on the combination gas chromatograph-mass spectrometer (GC-MS) previously described.

GC-MS analysis of the basic fraction is shown in Figure 7. A 15%

Carbowax 20M column was used under the following operational parameters: injection temperature 175°, column temperature programmed from 70° to 170° at 3°/min. and helium flow of 30 ml/min. The vertical slash marks along the tracing indicate the points at which a mass spectrum was taken.

A post column gas adder was required for GS-MS analysis using the capillary column. This was necessary because the jet separators of the LKB instrument have optimum efficiency at 25-30 ml/min flow rate of helium, thus helium gas was added to increase the flow up to optimum separator efficiency.

For high resolution analysis, the samples were separated on the previously mentioned 500 ft x 0.02 in (i.d.) capillary column with elution directly into the ion source of the MS 902. The adaption was made by using the oven of a Perkin-Elmer Model 270 combination GC-MS, passing the last six feet of the capillary column through a heated 6 foot x 0.25 in copper tubing leading from the oven to an on-off valve very near the ion source of the MS 902. The valve was connected by an all glass heated inlet directly into the ion source. The copper tubing, the valve box, and glass inlet were maintained at 200° or above to minimize cold spots. Flow rate through the column was between 1.5 and 2 ml/min and the column was temperature programmed from 70° to 170° at 2°/min. A recorder was attached to the total ion current output of the MS 902 to obtain a tracing of the components as they eluted from the column as shown in Figure 8.

A technique of Sweeley, et al. (64) for the determination of unresolved compounds in gas chromatographic effulents was utilized. This technique employs an accelerating voltage alternator such that a con-

tinous recording of two values of m/e, separated by not more than 10% of the mass range, can be obtained. This technique was used to indicate the presence of trace amounts of unresolved alkylpyrazines.

Infrared and Ultraviolet Spectroscopy

Components collected from the preparative gas chromatographic column in the needle-glass tube traps as described above were dissolved in 75 microliters (µl) of carbon disulfide. This was concentrated to about 1/3 with a stream of nitrogen at room temperature, and transferred with a 50 µl volume NaCl infrared liquid microcell. This was then analyzed in a Perkin-Elmer Model 427 infrared spectrometer. Ultraviolet spectra were obtained by washing the individually trapped components from the needle-glass tube traps with 1 ml of 95 percent ethanol into a 1 ml quartz cuvette.

Results and Discussion

Gas Liquid Chromatography

Considerable difficulty was encountered in resolving the isomers of the several alkylpyrazines using gas liquid chromatography. Carbowax 20M appeared the best but gave very high background at temperatures above 180° C, which complicated the mass spectra obtained from GC-MS analysis.

Figure 4 is the chromatogram obtained from an OV-17 liquid substrate column. The possibility of using this support was lost when the resolution was not obtained along with the desirable lower column bleed. Note the lack of the resolution of the 4 isomers of alkylpyrazines at molecular weight 108 (23-25 minutes) and the 4 isomers at 122

(28-30 minutes).

The use of lower percentage coating (5%) and base washing the solid support (Gas Chrom Q) yielded both lower column belled and improved resolution. Comparison of Figure 5 to Figure 4 shows the improved resolution.

The chromatogram in Figure 7 shows the excellent resolution obtained with a 500 ft x 0.02 in (i.d.) Carbowax 1540-KOH column. Especially note the resolution of peaks B, C, & D, as compared to the same peaks in Figure 5, similarly for peaks F, G, H, & I.

GC-MS analysis of an aliquot of the basic fraction of roasted peanut volatiles confirmed the presence of the previously reported pyrazines and revealed the presence of a number more. Figure 7 is the chromatogram obtained from a GC-MS analysis using the 15% (w/w) Carbowax 20M column previously described. Peaks A (see Figure 5), B and I were previously identified as 2-methyl-, 2,5-dimethyl-, and trimethylpyrazines, respectively. Also peaks F & G and J & K not previously resolved were reported as a methylethylpyrazine, probably 2-ethyl-5-methyl, and a dimethylethylpyrazine, respectively.

Figure 6 is the gas chromatographic tracing obtained from an aliquot of the basic fraction on a 500 ft x 0.02 in (i.d.) Carbowax 1540-KOH column. The letters above each peak correspond to those in figures 5 and 6. GC-MS analysis was also obtained using this capillary column for resolving the mixture.

Mass Spectrometry

Low Resolution All the mass spectra were obtained by GC-MS analysis. This procedure has the inherent problem of column bleed background

which were substracted as accurately as possible. The column bleed tends to increase as a component elutes from the gas chromatographic column especially when large peaks emerge. Also mass spectra obtained under rapid scanning conditions sometimes tend to be slightly weighted toward one end of the spectrum. This depends on the rate of increase or decrease of the component emerging from the chromatographic column during the scan interval. This was minimized by scanning at or near the apex of the peaks of the emerging components when possible. For the above reasons the mass spectral data obtained from GC-MS analysis does not always exactly match their corresponding standard spectra. However in most cases the differences are negligible.

High Resolution Mass Spectra Due to the experimental nature of the GC-HRMS analysis and the limited access to the instruments, only one attempt at analysis of the basic fraction was possible. As with most experiments, the first analysis is usually less than optimum. The GC-HRMS data suffers from two problems: 1) excess sample which over loaded both the GC capillary column and the MS 902 which resulted in failures in the computer storage and/or processing of the data. Thus the most abundant components did not have high resolution data available, 2) due to excess sample and quite probably a "cold spot" in the connection between capillary column and the MS 902, considerable trailing and bleeding of the more abundant components occurred. Fortunately these more abundant components which trailed out were known allowing these fragments to be effectively substracted out.

However, some useful and reliable data were obtained and are included with the low resolution spectra.

Accelerating Voltage Switching Mass Spectrometry

Before the use of the capillary column in the analysis of the basic fraction of roasted peanut volatiles, some quantitively minor pyrazine components were unresolved from the more abundant adjacent compounds. Hence, the technique of Sweeley (64) previously described was employed. Mass spectral and gas chromatographic retention time data indicated that 2,5-dimethyl-, 2,6-dimethyl, ethyl- and 2,3-dimethylpyrazine were present but the first three were unresolved. In order to prove the presence of ethylpyrazine, the technique of accelerating voltage switching mass spectrometry was utilized. The instrument was set to switch approximately 2 times per second between m/e 107 and 108 yielding a continuous recording of the relative intensities of the two fragments. Ions 107 and 108 were chosen because ethyl pyrazine is unique in that it has m/e 107 as the base peak or 100% peak with the 108 at 70%, compared to the other 3 isomers which have ions at m/e 107 at the 5% level and m/e 108 is the base peak for all three. Hence a significant increase of 107 relative to 108 would strongly indicate the presence of ethyl pyrazine. The unresolved mixture was collected by preparative gas chromatography and analyzed as described on the GC-MS instrument. Figure 9 is the oscilliscope recording of the described experiment. Observation of the tracing labeled X 1, shows that m/e 108 goes off scale and then returns back on scale and as it decreases an increase of 107 relative to 108 is observed in the region labeled X. Hence the presence of ethylpyrazine was strongly suggested.

Mass Spectral Data (Low Resolution and High Resolution)

Mass spectra of components C and D (refer to Figure 6 for component

designations) along with standard spectra of 2,6-dimethylpyrazine and ethylpyrazine are shown in Figure 10. Excellent agreement is obvious between component C and 2,6-dimethylpyrazine, however that component D appears to be a mixture is indicated by fragment ions at m/e 108, 42, 40 and 39 all of which are prominent in the preceeding and following components (2,6-dimethylpyrazine and 2,3-dimethylpyrazine, respectively). Figure 11 compares standard mass spectra of 2,3-dimethylpyrazine, and 2-ethyl-6-methylpyrazine to components E and F. Also high resolution mass spectra (HRMS) scan 30 (Figure 8) of the basic fraction (BF) is included with component F. The spectra of component E compares closely with 2,3-dimethylpyrazine and the spectra of component F has only negligible differences with 2-ethyl-6-methylpyrazine. HRMS scan 30 shows the composition of component F ($C_7H_{10}N_2$) to be consistent with that of 2-ethyl-6-methylpyrazine. Bondarovich *et al.* (31) have published the mass spectra of a number of alkyl substituted pyrazines. Reference to published data will be made for aid in interpretation of spectra in those cases where standard data are not available. From metastable calculations and HRMS scan 30 (BF), the m/e 121 ion yields 94 by a loss of C_2H_3, the ethyl substituted pyrazines formed by loss of the ethyl group, the ring carbon, adjacent nitrogen and a hydrogen ($C_3H_6N^+$).

Mass spectra of components G and H are shown together with those of standards 2-ethyl-5-methylpyrazine and 2-ethyl-3-methylpyrazine in Figure 12. The spectra of component G is essentially identical to that of 2-ethyl-5-methylpyrazine. Ions below m/e 37 may or may not be included in the spectra but are not of any great significance for this study. The spectra of component H is very similar to that of 2-ethyl-

3-methylpyrazine. However there appears to be some trimethylpyrazine mixture since these two components are not resolved. Evidence for trimethylpyrazine is the presence of slight excess m/e 122 and 81; nevertheless, m/e 56 (absent in trimethylpyrazine), 67 and 121, below 5% in trimethylpyrazine, all point strongly to the presence of 2-ethyl-3-methylpyrazine.

Figure 13 shows mass spectra of components J & K along with standard spectra of 2,5-dimethyl-3-ethylpyrazine, 2-ethyl-5,6-dimethylpyrazine and HRMS scan 41. The spectra of J is identical to that of 2,5-dimethyl-3-ethylpyrazine. The HRMS data confirms the expected elemental composition. As is common for aromatic compounds the molecular ion(M^+) minus one is very intense for alkylpyrazines with an ethyl or larger side chain, m/e 121 results from the loss of a methyl group(M-15), while m/e 108 is produced by the fragmentation 136 to 108 (m^* 85.8, 136-108; m^* = metastable ion) a loss of C_2H_4, the ethyl side chain. The spectra of K compares closely to the literature values of 2-ethyl-5,6-dimethylpyrazine, but comparison to the spectrum of 2-ethyl-3,6-dimethylpyrazine shows m/e 56 is 42%, while that of K is 17%, higher than the 8.5% literature value of 2-ethyl-5,6-dimethylpyrazine. Also ions at m/e 54, 53, 52, and 42 of K are intermediary between the two standard isomers. The evidence strongly favors a mixture of 2-ethyl-5,6-dimethylpyrazine and 2-ethyl-3,6-dimethylpyrazine as component K.

The mass spectra of compounds L and M are shown in Figure 14 along with standard mass spectra of 2-methyl-6-propyl pyrazine and 2,6-diethyl-3-methyl pyrazine. The spectrum of L is very similar to that of 2-methyl-6-propylpyrazine. Large M-15 and M-28 fragments

located at m/e 121 and 108 are characteristic cleavage products of a propyl substituted compound. The background is relatively high in this spectrum. However, it is quite evident that L is a methyl propyl substituted pyrazine isomer. Component M yielded a mass spectrum very close to that of 2,6-diethyl-3-methyl pyrazine. The relatively low M-15 and M-28 rules out the possibility of component M being a propyl substituted pyrazine and also the fragments are too low to be 2,3-diethyl-3-methylpyrazine. This leaves only one other possible isomer 2,5-diethyl-3-methylpyrazine which was unavailable as a reference, but would be expected to fragment like the 2,6-diethyl-3-methyl isomer. Therefore component M was assumed to be either 2,6- or 2,5-diethyl-3-methylpyrazine.

Figure 15 contains mass spectra of compounds N, P, and S together with HRMS scan 55 and 67 (BF). and the standard spectrum of 2-ethyl-3,5,6-trimethylpyrazine. The mass spectrum of compound N has many similarities to that of 2-ethyl-3,5,6-trimethylpyrazine. Particularly the high ions m/e 122, 67, 54, 53 and 39 which are not nearly as abundant in spectra of 2,6-diethyl-3-methylpyrazine and 2,3-diethyl-5-methylpyrazines. Fragments from molecular ion 154 also add to the complexity. High resolution mass spectra gives the molecular ion composition of $C_8H_{10}N_2$ for P, two hydrogens less than a corresponding saturated analog indicating either a double bond or ring side chain.

Using literature spectra as references for interpretation of component P, (31, 65) the high M-1 is indicative of one of two types of substituted pyrazines; 1) ethyl side chains or 2) alkyl substituted vinyl side chains. The presence of considerable m/e 54 (C_3H_4N) suggested this is analogous to the loss of C_3H_6N via the ion transition

121 to 56 for a ethyl substituted pyrazine, probably by the transition of 133 to 54 losing the vinyl side chain minus one hydrogen plus its ring carbon and adjacent nitrogen (C_3H_4N). Thus this compound appears to be consistent with a vinyl and ethyl disubstituted pyrazine. This spectrum also contains ions at m/e 164, 163 and 149 suggesting a five carbon saturated derivative of pyrazine at molecular weight 164.

Component S had a molecular weight of 134 and composition $C_8H_{10}N_2$ also indicating either unsaturation or a ring. The two apparent features of this spectra are the intense 119 ion (M-15) and the intensity of M-1 being less than the molecular ion. The first feature points to a very labile methyl group and the second is not consistent with standard spectra of vinyl substituted pyrazines, hence a cyclic side chain is postulated. The loss of 15 mass units (CH_3) must come from a labile methyl on the cyclic side chain since a methyl on the ring is rarely cleaved. A 119 to 92 ion transition represents a loss of 27 mass units (HCN) which is a characteristic fragmentation pattern for alkylpyrazines. Also a 92 to 78 transition would be feasible representing a loss of CH_2.

Mass spectra of components T, U, W, and X are shown in Figure 16. All four of these components exhibit characteristic fragmentation patterns of pyrazines but have two less hydrogens than the saturated derivatives. HRMS scan 76 and 85 show molecular formulas which confirm this supposition.

The mass spectrum of component T shows it to have a molecular weight of 120 and a large M-1 ion at 119. Additional information is necessary before a structure can be postulated.

Compound U exhibits a mass spectral fragmentation pattern similar

to component S, a M-1 relatively intense but less than the molecular ion and a 100% ion at M-15 (m/e 133); however, U is a next higher analog of S being 14 mass units heavier.

Component W appears to be a 1 carbon higher analog of T, with molecular weight of 134, and a similarily intense M-1 ion but lesser parent ion.

The mass spectra of component X shows a molecular ion of 148 with a M-1 base peak at 147.

Figure 17 is the mass spectrum of component Z and HRMS 91 (BF). The data show Z to have a molecular weight of 148 with a molecular composition of $C_9H_{12}N_2$. This spectrum is similar to those of T and W and its higher molecular weight (14 mass units) suggest an analog.

The lack of standard mass spectra of the unsaturated or cyclic derivatives of pyrazine permits only tentative structure postulations.

Ultraviolet Spectroscopy and Gas Chromatographic Retention Time.

Preparative gas chromatographic separation and collection of components T and W permitted UV analysis of these two components. Table 1 shows the results of this analysis along with UV and gas chromatographic data of a number of standard pyrazines.

Examination of the wave length maximum of the various pyrazines shows a bathochromic shift in wave length as the number of ring substituents increases, and is unaffected by the size of the substituent. However, if the substituents are unsaturated, thereby extending the conjugation, a large bathochromic shift is observed. Compare ethyl pyrazine, λ_{max} of 266 mμ, and vinylpyrazine, λ_{max} of 285 mμ, and also the second substituents bathochromic effect, 2-ethyl-6-methylpyrazine 275 mμ λ_{max} compared to 2-methyl-5-vinylpyrazine 290 mμ λ_{max}.

Literature values of gas chromatographic retention times along with retention indices from Table III are included in Table I. Retention times increase with increasing molecular weight except with unsaturated side chains. The increase polarity due to these substiuents results in much longer retention times, compare 25.5 minutes for vinylpyrazine and 28.1 minutes for 2-ethyl-6-methylpyrazine to 33.8 minutes for 2-methyl-5-vinylpyrazine.

Consequently, component T appears to be a mono-substituted pyrazine with a three carbon unsaturated side chain. Component W seems to be a next higher analog, a disubstituted pyrazine with a methyl and three carbon unsaturated side chain. Comparison of the mass spectra to the literature spectra of 2-(trans-1-propenyl) pyrazine and 2-methyl-6-(trans-1-propenyl) pyrazine shows sufficient variation that isopropenyl- and methyl isopropenyl pyrazine were postulated as the structures for T and W, respectively.

The shorter retention time of S relative to W of the same molecular weight suggested a cyclic side chain with an easily lost methyl group (base peak in mass spectra M-15). Hence S was postulated to be a methyl-2,3-cyclopentanepyrazine. Ultraviolet spectra for components P, U, S, X and Z were not obtained.

Infrared Spectroscopy

Infrared spectra was obtained on components F, G and J.

Table II shows the characteristic absorption bands of an unresolved mixture of G & G compared to standard data from 2-ethyl-5-methyl- and 2-ethyl-6-methylpyrazine. Bands observed in the mixture were unique to both of above mentioned standards. Also shown is the very closely matched spectra of component J and 2,5-dimethyl-3-ethyl-

pyrazine.

Gas Chromatographic Retention Time Indices

Comparison of retention time indices of standards with the components of the basic fraction of roasted peanut volatiles was used along with mass spectra to confirm their identities. I_E values are calculated by linear interpolation of the retention time of the unknown between retention times of a series of methyl esters of normal carboxylic acids used as internal standards. This was a modification of the procedure of van den Dool and Kratz (66) and was particularly useful under temperature programmed conditions. The I_E value of each internal standard is arbitrarily given the value of the carbon number of the acid of the ester. Thus methyl heptanoate and methyl octanoate had values of 7.00 and 8.00 and any component that comes between is reported as decimal fraction of the distance between the two. The I_E values of most of the components of the basic fraction of roasted peanut aroma along with appropriate standards are given in Table III. Excellent agreement between standards and the corresponding unknowns were obtained (ie. + or - 0.02).

The pyrazine compounds identified in the basic fraction of roasted peanut aroma are tabulated in Table IV along with the technique used to confirm their identity. Components A, B and I have previously been reported as constituents of roasted peanuts volatiles. Also reported were a methylethylpyrazine and a dimethylethylpyrazine.

Six alkyl substituted pyrazines not previously reported in roasted peanuts are now positively identified along with tenative identification of 9 more pyrazines.

Eight pyrazines with vinylic or cyclic side chains were detected

and are reported, structures for three were postulated (S, T & W). A total of 23 pyrazines are reported in the basic fraction of roasted peanut aroma.

The role of pyrazines in roasted peanut aroma has not been clearly defined. Nevertheless, their significance cannot be dismissed because of their abundance and individual and collective aromas. Alkyl substituted pyrazines have been reported as having a "nutty" (16), nutlike (22), and roasted odor (33).

This author describes the odor of the basic fraction as having a sweet "nutty" or "nut like" aroma, but not "pea-nutty" as such. It was observed that upon acidification of the total aroma condensate, the characteristic roasted peanut aroma was lost. Immediately upon increasing the pH above 7 the characteristic peanut aroma was restored.

Some sensory evaluation of pyrazines has been reported by Koehler in his Ph.D. dissertation (33). His work was directed towards obtaining a specific evaluation of the role of pyrazine compounds in roasted peanut flavor. Odor thresholds and quantitative data were obtained for the more abundant pyrazines reported in roasted peanuts. Odor thresholds ranged from 26.5 ppm for 2-methylpyrazine to 0.85 ppm for a methyl-ethylpyrazine, with 2,5-dimethylpyrazine, the major pyrazine in roasted peanuts, having a threshold value of 17 ppm.

Quantitative data obtained in this study showed 6 mg of 2-methylpyrazine and 11 mg collectively of the 4 molecular weight 108 isomers of pyrazine per kilogram of roasted peanuts.

These limited results indicate that most of the pyrazines are present at concentrations near or below the odor thresholds. Most likely synergistic effects occur. Synergistic effects between two or

more compounds in solution can reduce the threshold of detection of the mixture considerably below that of either component (4). The interaction of two non-reactive compounds to yield an aroma quite unlike either of the pure components is a possibility that cannot be overlooked. The complexity of assessing the significance of a compound or group of compounds in relation to the overall response must be considered.

Significant evidence for an important role of pyrazines in foods and food products is afforded by commercial interest in using pyrazines in synthetic flavors. A methyl pyrazine with either a $-SCH_3$ or $-OCH_3$ substituent has been patented as imparting a nut-like flavor to foodstuffs (67). Also, acetylpyrazine and methylacetylpyrazine have been patented (68) for use in imparting a popcorn flavor to foodstuffs. A large flavor and fragrance company has recently developed imitation peanut flavor (69).

In summary, the results of this work established that pyrazines are an important integral part of typical roasted peanut aroma.

TABLE I

GAS CHROMATOGRAPHIC RETENTION TIME AND ULTRAVIOLET SPECTROSCOPIC DATA

	MW	λ_{max}^{EtOH} (mμ)	$R_{T(Lit.)}^{a}$	I_{E}^{b}
Pyrazine	80	261	19.3	6.32
Methyl-	94	266	22.1	6.90
Ethyl-	108	265	25.5	7.51
2,5-Dimethyl-	108	270	24.9	7.42
2-Ethyl-6-Methyl	122	270	28.1	8.08
Trimethyl-	122	277	29.0	8.21
2,5-Dimethyl-3-Ethyl-	136	278	30.9	8.57
Tetramethyl-	136	278	----	8.88
Vinyl-	106	285	30.7	----
2-Methyl-5-vinyl-	120	290	33.8	----
S	134	---	----	10.30
T	120	282	----	10.54
W	134	287	----	11.21

a) Data from Bondarovich *et al.* (31) obtained on a 13 feet x 0.25 inch 20% Carbowax 20 M column.

b) Retention time Indices from Table III.

TABLE II

INFRARED DATA ON PYRAZINES ISOLATED FROM BASIC FRACTION

OF ROASTED PEANUT AROMA AND APPROPRIATE STANDARDS.

CHARACTERISTIC INFRARED BANDS (in wave numbers CM^{-1})				
Components F & G[1]	2-Ethyl-5-methyl-[2] Pyrazine	2-Ethyl-6-methyl-Pyrazine	Component J	2,5-Dimethyl-3-ethylpyrazine
1379	1380	1377	1315	1317
1355	1349		1270	1271
1310	1310		1249	1249
1271		1271	1170	1170
1251	1251		1047	1048
1230		1230	1005	1005
1163	1162	1164	957	957
1063	1063	1063	892	891
1036	1037		855	855
1021		1021		
973	973	973		
892	892			
873		873		

1) Spectra of isolates were ran in CS_2
2) Standards ran as liquid films

TABLE III

GAS CHROMATOGRAPHIC RELATIVE RETENTION TIME INDICES, CALCULATED AS I_E VALUES*, OF COMPOUNDS IDENTIFIED IN BASIC FRACTION OF ROASTED PEANUTS AND SOME STANDARDS

Standard	I_E Value	Unknown	I_E Value
2-Methylpyrazine	6.90	A	6.89
2,5-Dimethylpyrazine	7.42	B	7.42
2,6-Dimethylpyrazine	7.48	C	7.47
2-Ethylpyrazine	7.53	D	7.51
2,3-Dimethylpyrazine	7.65	E	7.63
2-Ethyl-6-methylpyrazine	8.04	F	8.04
2-Ethyl-5-methylpyrazine	8.09	G	8.08
2-Ethyl-3-methylpyrazine	8.20	H	8.21
Trimethylpyrazine	8.20	I	8.21
2,5-Dimethyl-3-ethyl-pyrazine	8.58	J	8.57
2,6-Dimethyl-3-ethyl-	8.76	K	8.76
2-Methyl-6-propyl-	-----	L	8.88
		M	8.9-9.00
2,6-Diethyl-3-methyl-	9.06		9.04
2,3-Diethyl-3-methyl-			9.15
2-Ethyl-3,5,6-trimethyl-	9.26	N	9.27
		O	9.33
		P	9.43
		S	10.30
		T	10.54
		W	11.21
		X	11.45
		Y	11.79

*Modified procedure of van den Dool and Kratz (66) see procedures for discussion.

TABLE IV

SUMMARY OF PYRAZINES IDENTIFIED 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	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(HR)		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 4. Gas Liquid Chromatographic Analysis of the Basic Fraction

Conditions were as follows:

Column - 24' x 1/4" coiled glass

Column Packing - 4% OV-17 on Gas Chrom Q

Column Temperature - 75° to 170° at 3°/min

Inlet Temperature - 145°

Carrier Gas - Helium

Detector - Flame Ionization

Flow Rate - 42 ml/min

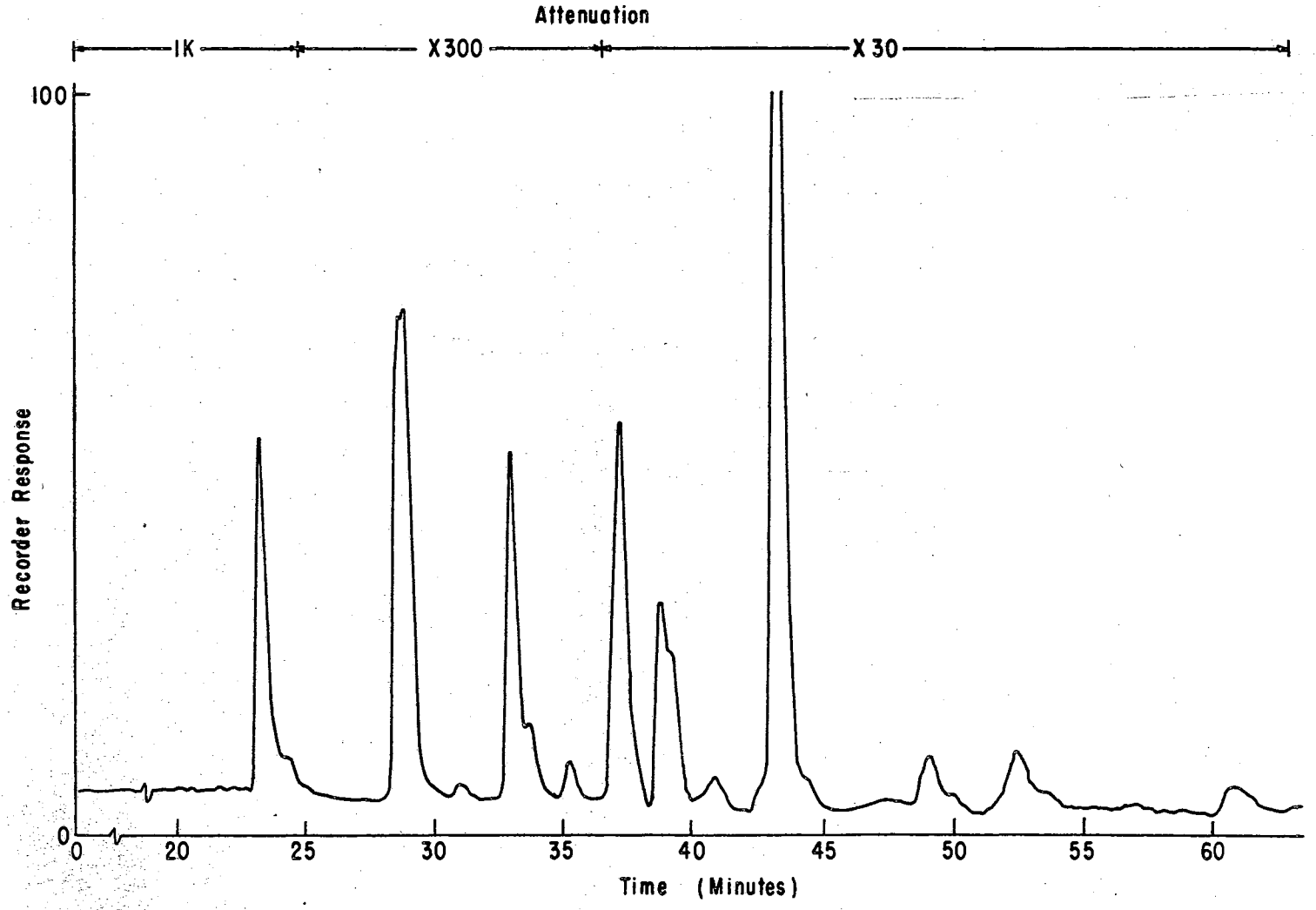

Attenuation
1K
X300
X 30
100
0
Recorder Response
0
20
25
30
35
40
45
50
55
60
Time (Minutes)

Figure 5. Gas Liquid Chromatographic Analysis of the Basic Fraction

Conditions were as follows:

Column - 21' x 1/4" coiled glass

Column Packing - 5% Carbowax 20M on base washed Gas Chrom Q

Column Temperature - 70° to 170° at 3°/min

Inlet Temperature - 175°

Carrier Gas - Helium

Flow Rate - 40 ml/min

Detector - Flame Ionization

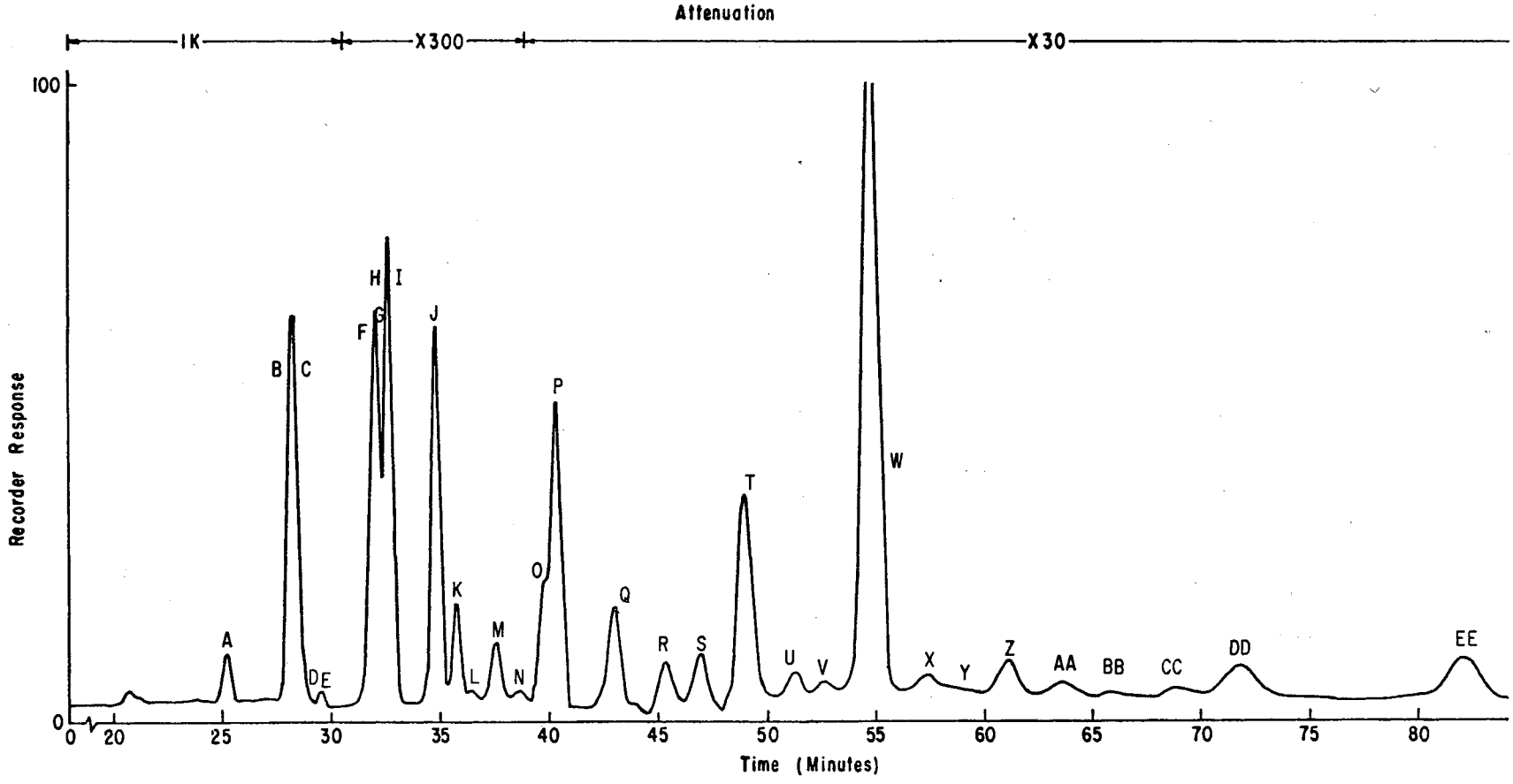

Attenuation
1K
X300
X30
100
0
Recorder Response
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P
Q
R
S
T
U
V
W
X
Y
Z
AA
BB
CC
DD
EE
0
20
25
30
35
40
45
50
55
60
65
70
75
80
Time (Minutes)

Figure 6. Capillary Gas Liquid Chromatographic Analysis of th

Basic Fraction

Conditions were as follows:

Column - 500' x 0.02" stainless steel

Column Packing - coated with Carbowax 1540 + KOH

Column Temperature - 65° to 165° at 2°/min

Inlet Temperature - 180°

Carrier Gas - Helium

Flow Rate - 2.8 ml/min

Detector - Flame ionization

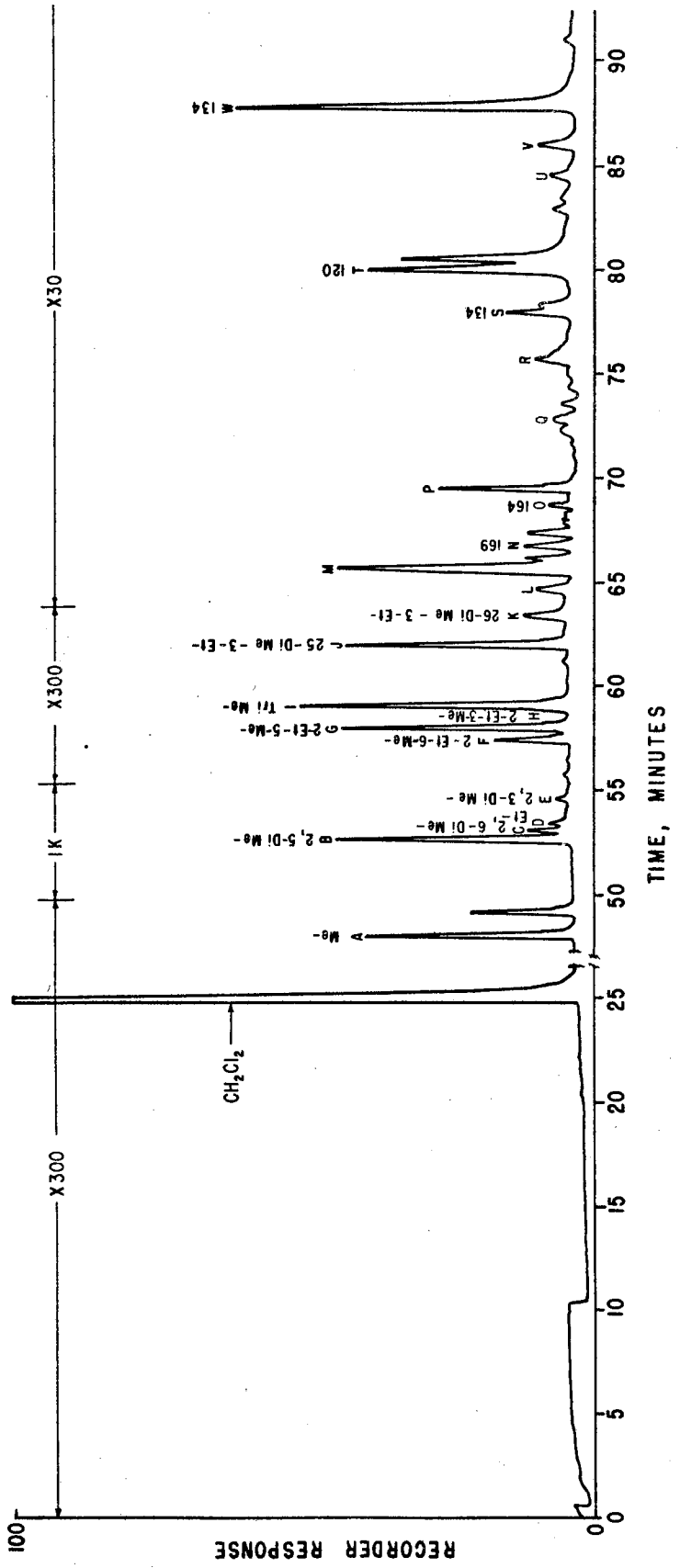

RECORDER RESPONSE
TIME, MINUTES
100
0
0
5
10
15
20
25
50
55
60
65
70
75
80
85
90
X300
1K
X300
X30
CH_2Cl_2
A Me-
B 2,5-Di Me-
C Et-, 2, 6-Di Me-
D
E 2,3-Di Me-
F 2-Et-6-Me-
G 2-Et-5-Me-
H 2-Et-3-Me-
I Tri Me-
J 25-Di Me -3-Et-
K 26-Di Me - 3-Et-
L
M
N 169
O 164
P
Q
R
S 134
T 120
U
V
W 134

Figure 7. Combination Gas Liquid Chromatographic-Mass Spectrometric Analysis of the Basic Fraction

Conditions were as follows:

Column - 17' x 1/4" coiled glass

Column Packing - 15% Carbowax 20M on base washed Firebrick

Column Temperature - 70° to 170° at 3°/min

Inlet Temperature - 175°

Carrier Gas - Helium

Flow Rate - 30 ml/min

Detector - Mass spectrometer

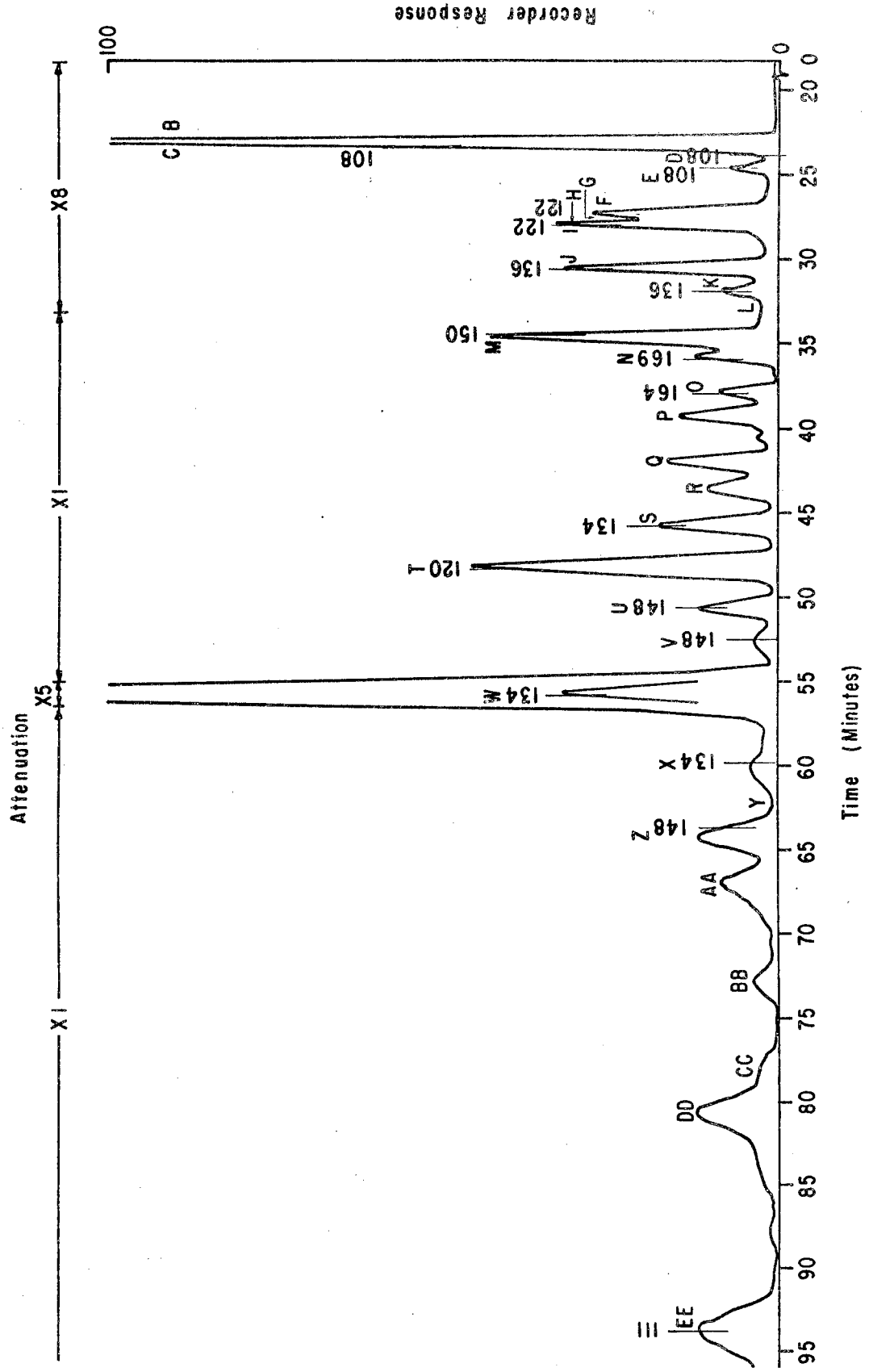

Recorder Response
100
0
Attenuation
X8
X1
X5
X1
Time (Minutes)
0 0
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
C
B
108
108
D
108
E
F
H
G
122
I
122
J
136
K
136
L
150
M
169
N
164
O
P
Q
R
134
S
120
T
148
U
148
V
134
W
134
X
Y
148
Z
AA
BB
CC
DD
EE
III

Figure 8. Combination Capillary Gas Liquid Chromatographic-Mas Spectrometric Analysis of the Basic Fraction

Conditions were as follows:

Column - 500' x 0.02" stainless steel

Column Packing - coated with Carbowax 1540 + KOH

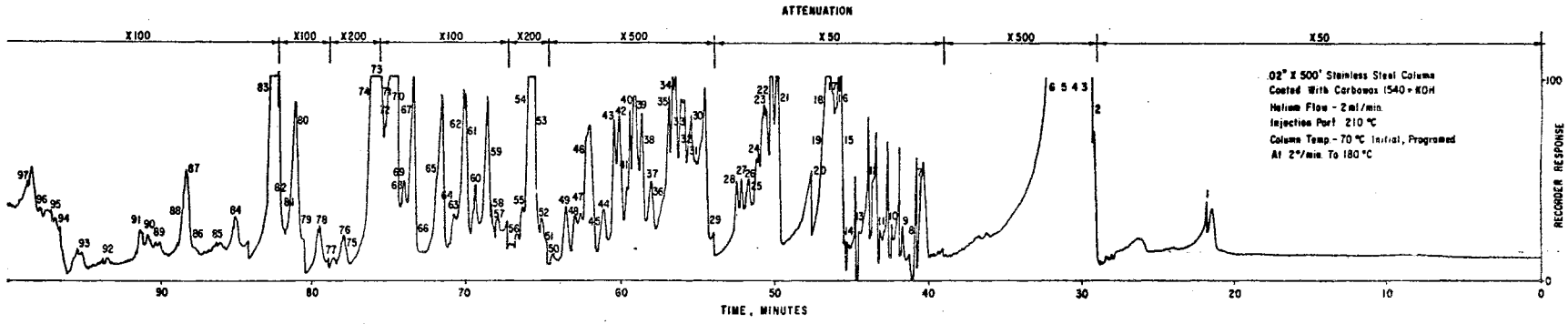
Column Temperature - 70° to 180° at 2°/min

Inlet Temperature - 210°

Carrier Gas - Helium

Flow Rate - 2 ml/min

Detector - High resolution mass spectrometer


ATTENUATION
X100
X100
X200
X100
X200
X500
X50
X500
X50
.02" X 500' Stainless Steel Column
Coated With Carbowax 1540 + KOH
Helium Flow - 2ml/min.
Injection Port 210 °C
Column Temp - 70 °C Initial, Programed
At 2°/min To 180 °C
RECORDER RESPONSE
100
0
90
80
70
60
50
40
30
20
10
0
TIME, MINUTES

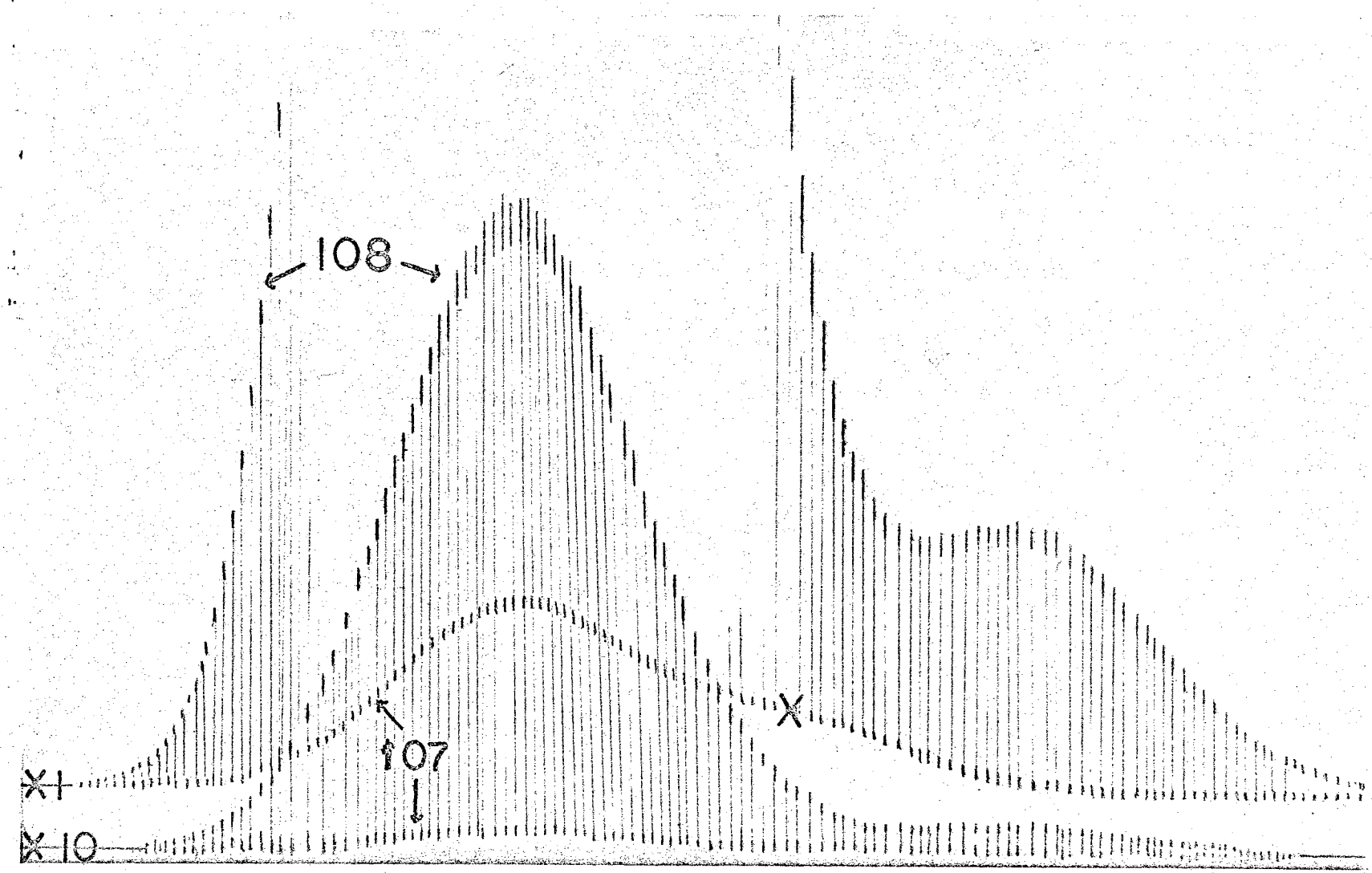


Figure 9. Accelerating Voltage Switching Mass Spectrum of Peaks B, C, D and E.

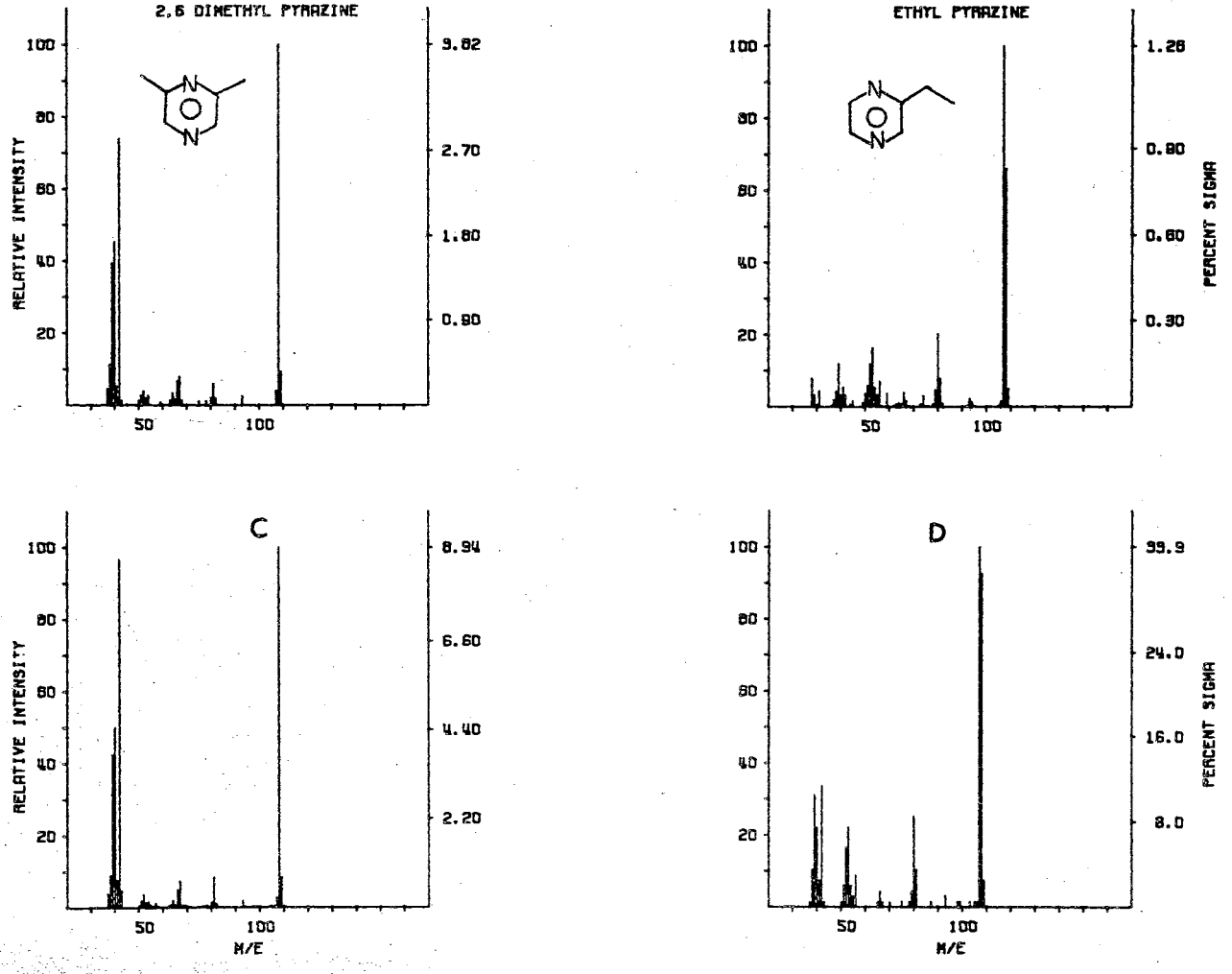


Figure 10. Mass Spectra of Components C and D and Standards (1175-9; 1175-10-11).

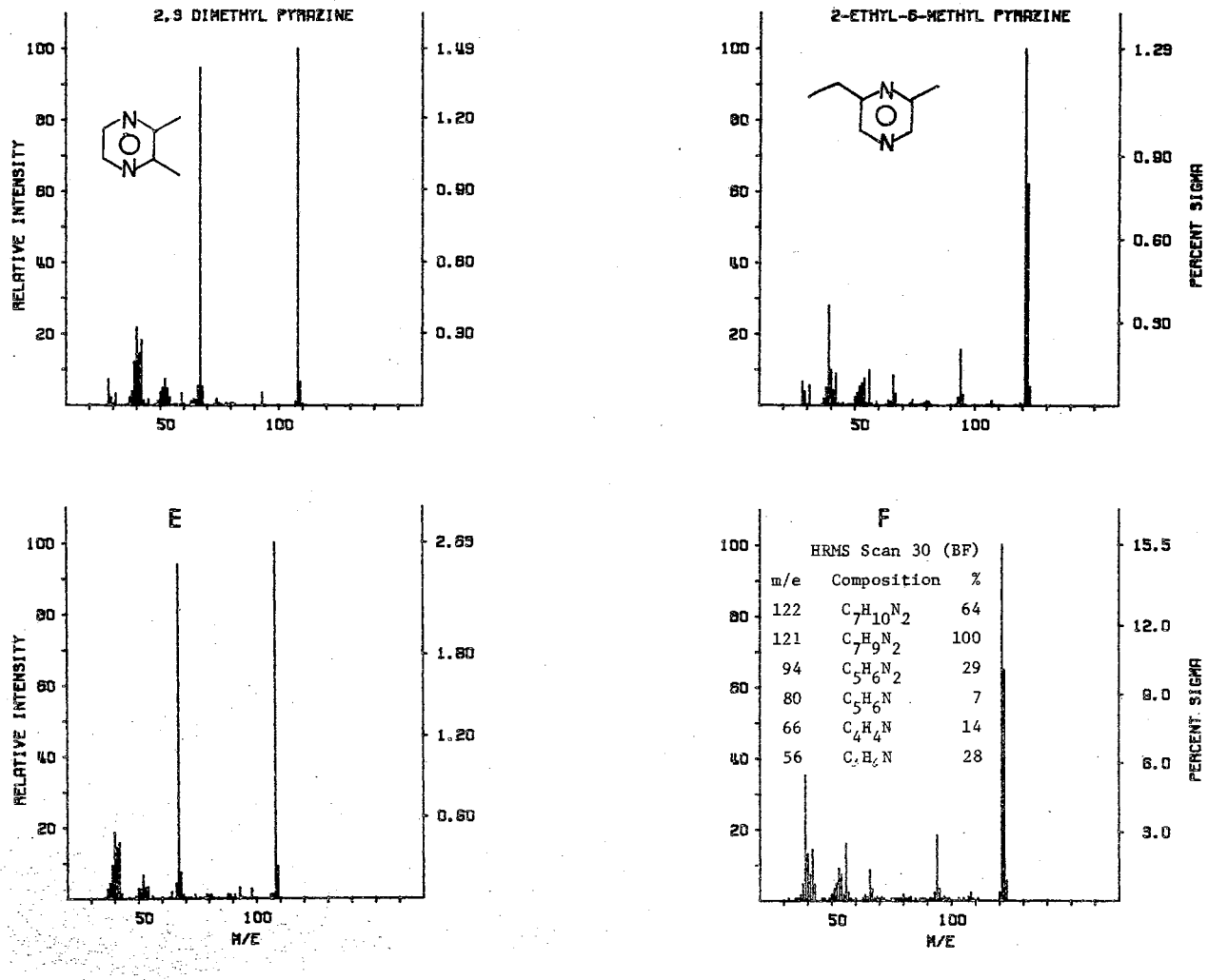


Figure 11. Mass Spectra of Components E and F and Standards (854-20-21; 1175-13).

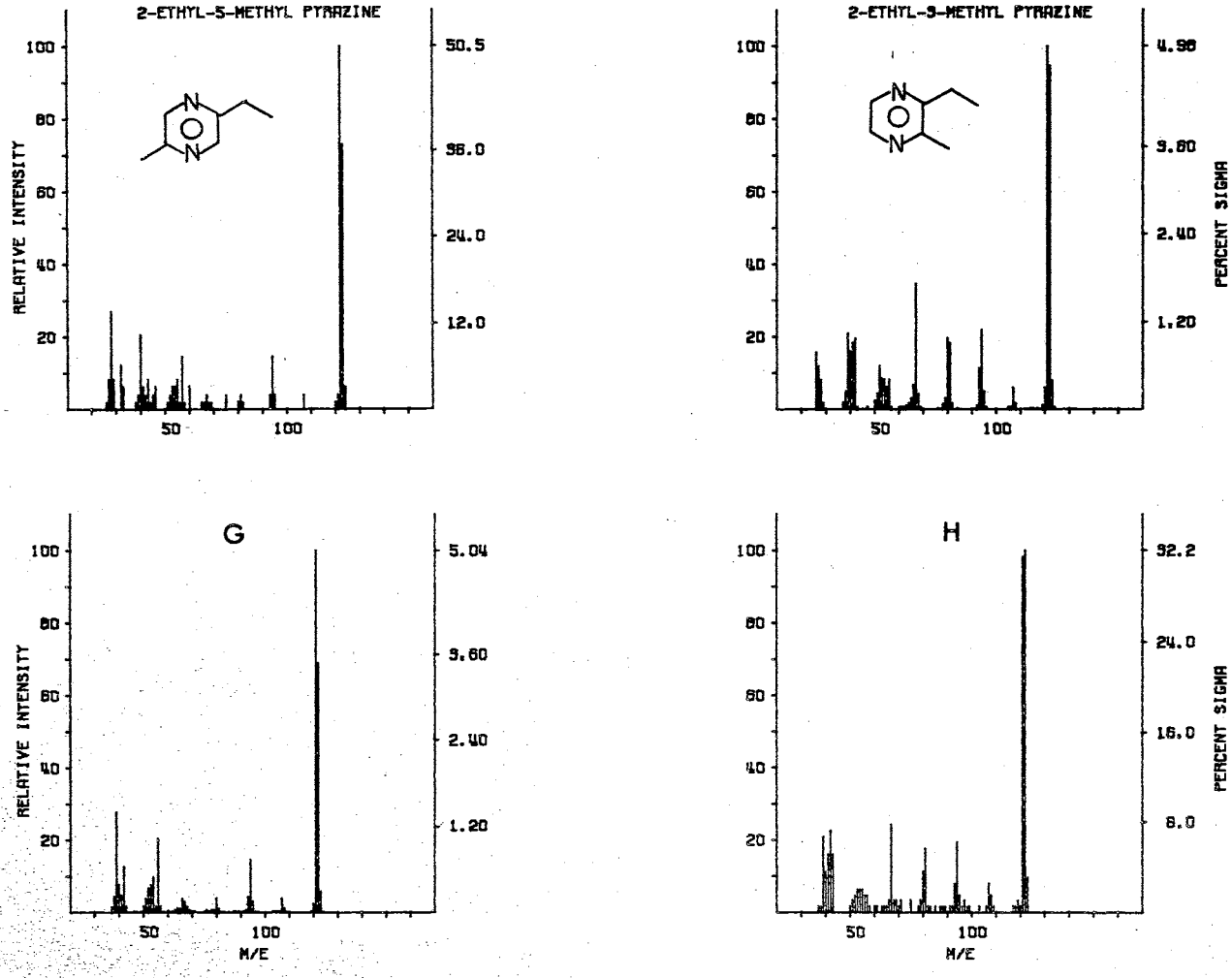
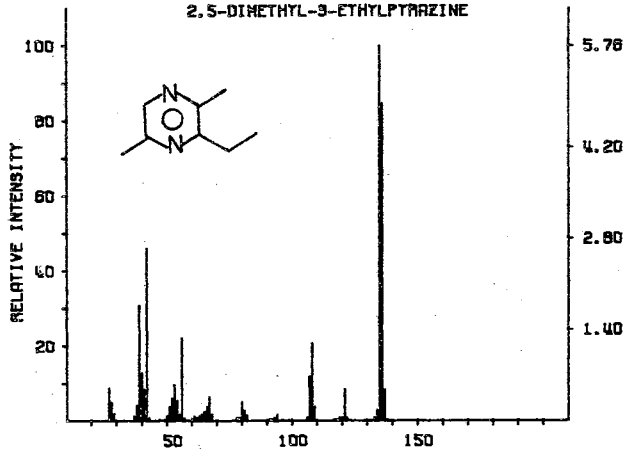
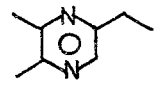


Figure 12. Mass Spectra of Components E and F and Standards (1175-14; 1175-15).

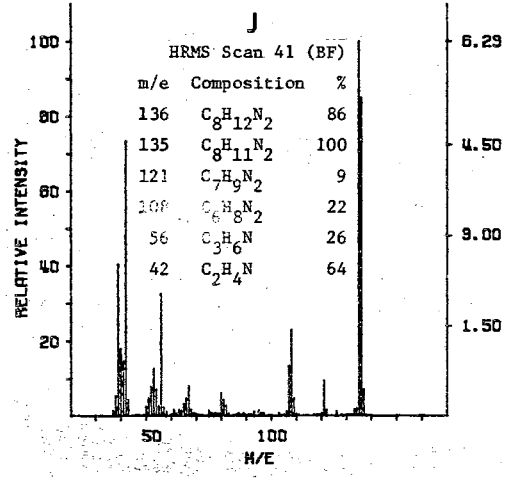


2-Ethyl-5,6-dimethylpyrazine



m/e	Intensity
136	80.3
135	100.0
108	19.3
80	7.3
56	8.5
54	33.8
53	21.1
52	13.6
42	40.8
39	31.0

Standard from van Praag et al. (22)



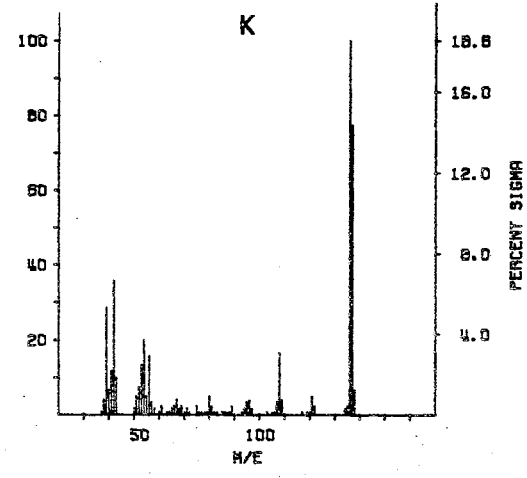


Figure 13. Mass Spectra of Components J and K and Standards (1175-18; 1175-19).

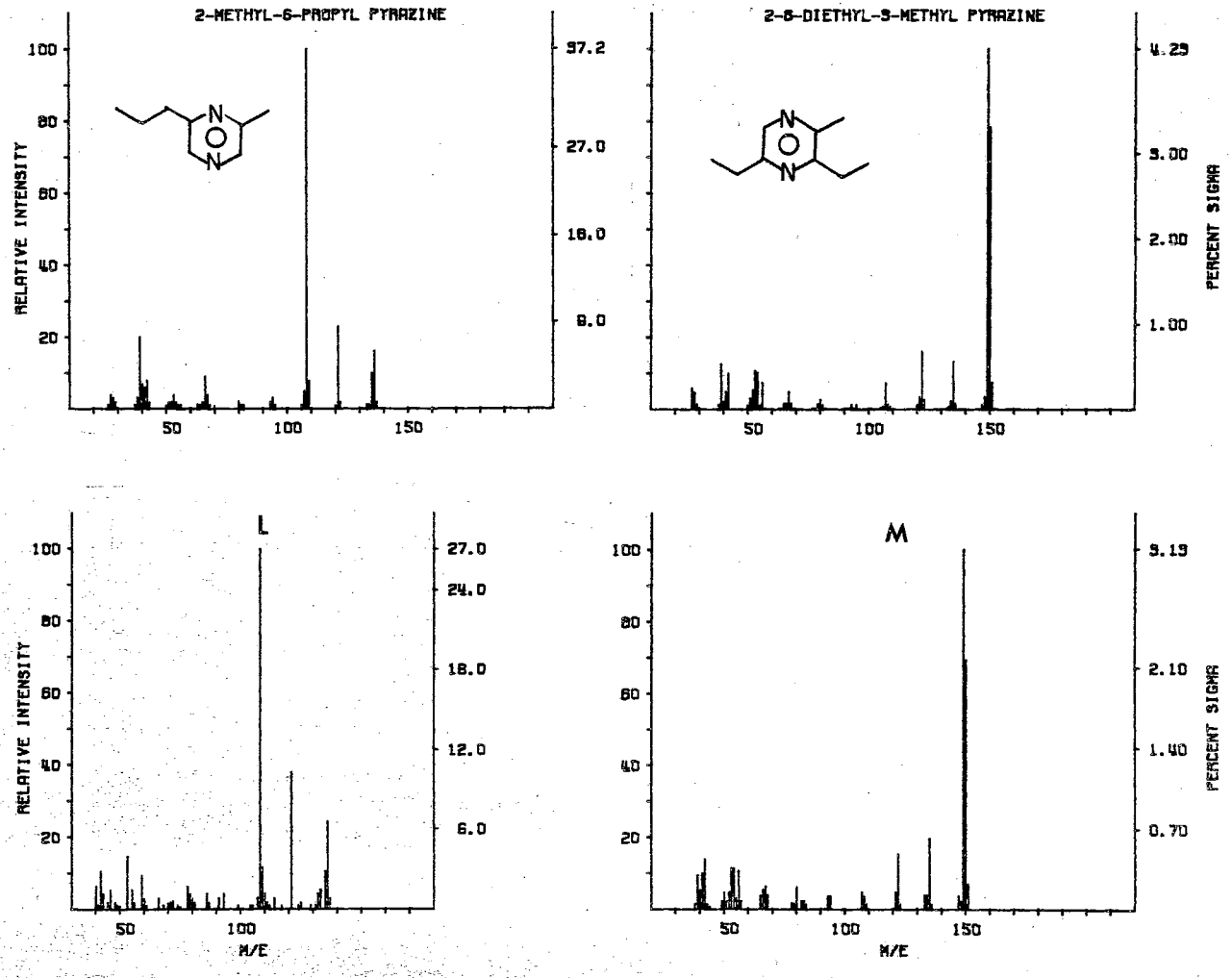
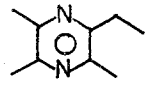


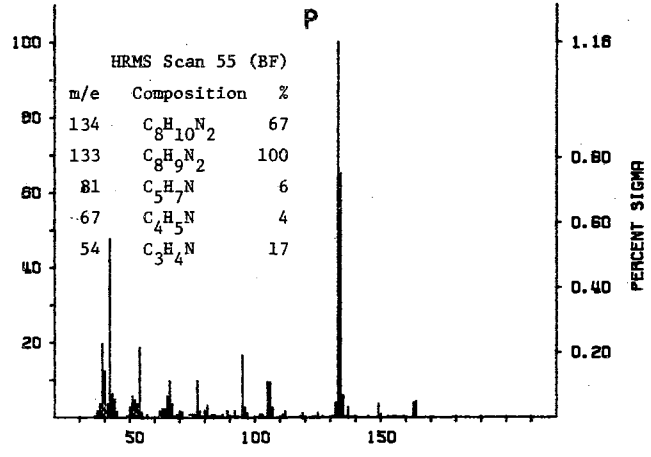
Figure 14. Mass Spectra of Components L and M and Standards (1219-11-17).

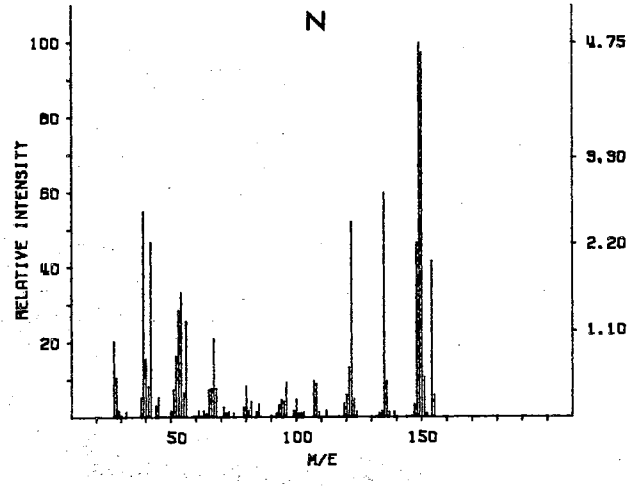
2-Ethyl-3,5,6-trimethylpyrazine



m/e	Intensity
150	94.9
149	100.0
122	20.5
67	19.6
56	17.8
54	28.0
53	38.3
42	32.2
41	17.7
39	26.6

Standard from van Praag et al. (22)





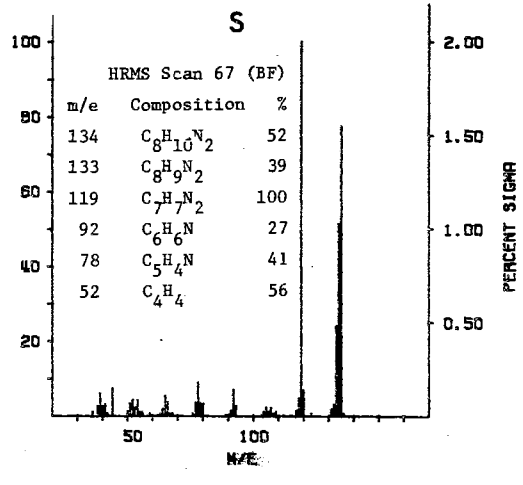


Figure 15. Mass Spectra of Components N, P and S and a Standard (1219-11).

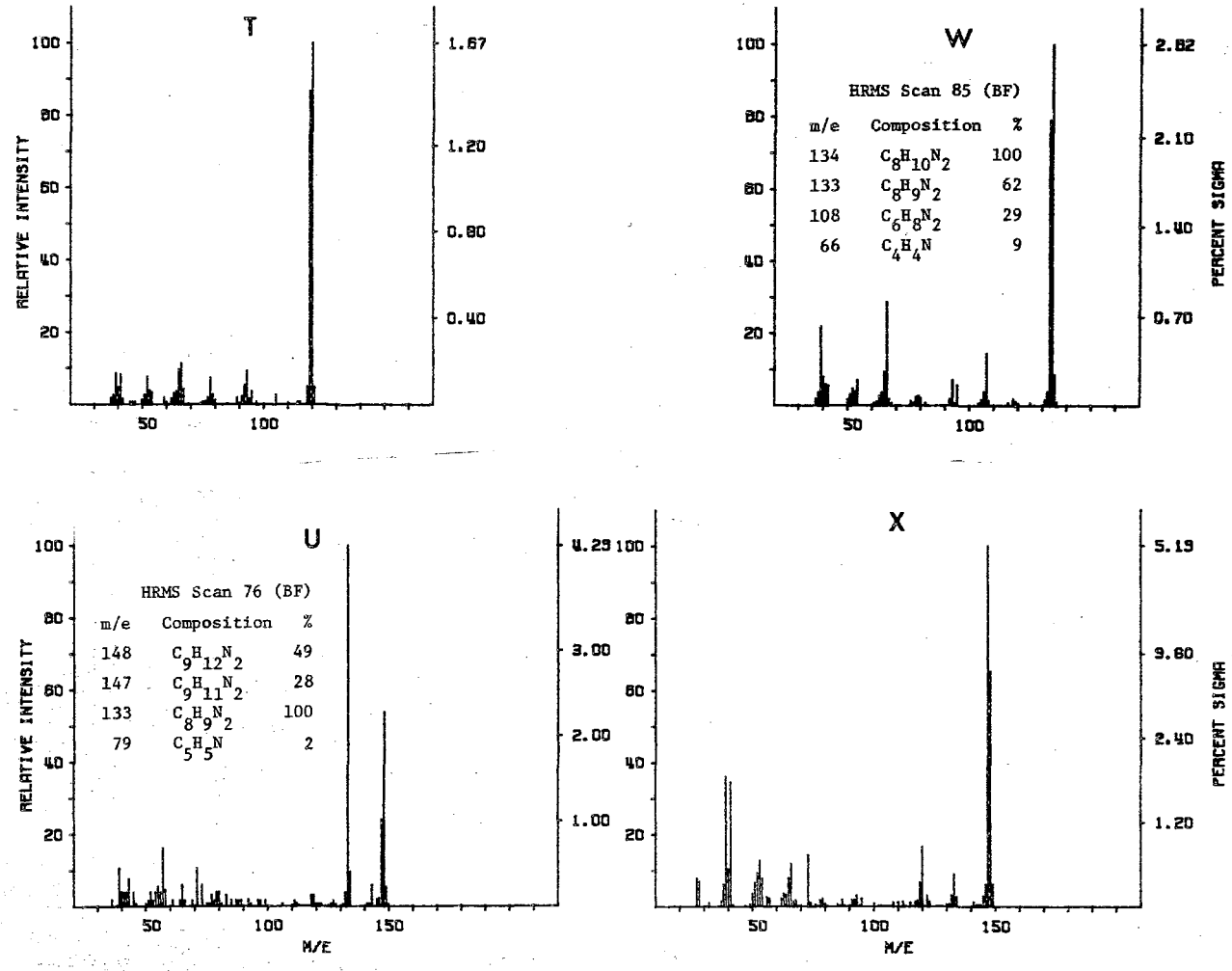


Figure 16. Mass Spectra of Components T, U, W and X (854-60-59;62-63;67-68;1329-33-34).

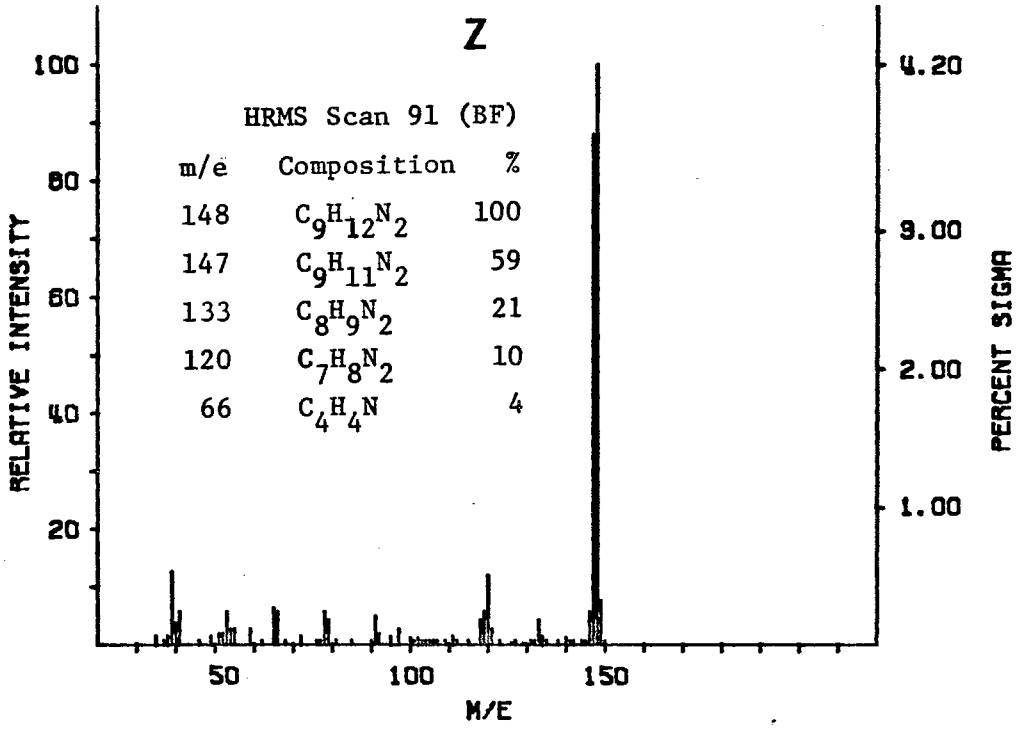


Figure 17. Mass Spectrum of Component Z (854-73-74).

CHAPTER V

CHARACTERIZATION OF THE NEUTRAL FRACTION OF ROASTED PEANUT AROMA

Introduction

The major aldehydes and ketones from roasted peanut aroma have been reported (17). Pickett and Holley (3) were the first to report that carbonyl compounds were constituents of roasted peanut aroma.

Although the quantitative major aldehydes and ketones have been reported and implicated as playing a very important contributory role to the overall flavor of roasted peanuts, many less abundant aldehydes and ketones are present and should not be overlooked as potential important contributors to roasted peanut aroma. It was the objective of the work presented in this chapter to identify as many components as possible, thus extending the chemical characterization of the neutral fraction of roasted peanut aroma.

Apparatus and Reagents

Apparatus

High and low resolution mass spectrometers employed in analysis of the neutral fraction are the same as described in chapter IV. Gas chromatography columns and instruments used are as previously described, with the analysis restricted to the Barber Colman Model 8000 gas chromatograph and the Carbowax 1540 capillary column.

Reagents

2-Amylfuran, 4-methyl-3-penten-2-one, N-methyl-2-pyrrolaldehyde, 5-methyl-2-pyrrolaldehyde, N-methyl-5-methyl-2-pyrrolaldehyde, N-(2-furfuryl) pyrrole, and 2-acetylthiophene, were a gift from the Corporate Research Department of the Coca-Cola Company Atlanta, Georgia.

5-methyl furfural, thiophene-2-carboxaldehyde, 2-acetylpyrrole, 2-phenyl-2-butenal, 4-methyl-2-phenyl-2-pentenal, 5-methyl-2-phenyl-2-hexenal and 2-methyltertrahydrofuran-3-one, were a gift from International Flavors and Fragrances, Union Beach, New Jersey.

Gas chromatographic supports and stationary phases used are described in Chapter IV.

Procedures

The dilute neutral fraction in methylene chloride was reduced in volume on an rotary evaporator near 0^{o} until the volume was about 1 ml. A portion of this was then placed in a tapered glass tube. This tube was prepared by pulling off the small end of a disposable Pasteur pippette over a laboratory burner, forming a pointed cavity inside. Approximately 2 inches of the tapered end was removed and used to contain about 0.5 ml of the concentrated neutral fraction. This was carefully evaporated to about 1-2 µl using a gentle stream of nitrogen and warming the tip occasionally with the fingers. Up to 0.5 µl could easily be removed from this container and analyzed on the capillary column. This concentration technique was found necessary to get the complex neutral fraction sufficiently concentrated to detect all of the constituents. Most of the more volatile components were lost

during this concentration procedure, most of which were known from previous identification.

Headspace analysis was performed to analyze the most volatile aroma components. This was accomplished by placing one half of the total aroma condensate (approx. 0.5 ml) from 4 pounds of roasted peanuts into a 8 mm (i.d.) x 15 cm tube sealed at one end and fitted with a gas tight septum at the other end. Approximately 25 mg of NaCl was added to the condensate in the tube to decrease the solubility of the organic components. A "headspace" analysis was performed by warming the tube with a heat gun to about 50° C, injecting with a gas syringe into the tube a quantity of helium gas equal to the amount to be removed, moving the plunger up and down 2-3 times to mix the gasses and with-drawing the gas sample (1-4 mls). This was immediately injected into a gas chromatographic column and analyzed in the normal manner.

Results and Discussion

Results

Figure 18 is a GC-MS total ion current tracing of 0.4 µl of highly concentrated neutral fraction of roasted peanut volatiles. The gas chromatography column employed was the Carbowax 1540 + KOH capillary column previously described. Each vertical line on the tracing is numbered consecutively and is the point at which a mass spectrum was taken.

High resolution mass spectra were obtained on a number of the components in the neutral fraction. The combination MS 902 capillary column previously described was employed. The percent intensities of ions reported with the HRMS data are not expected to fit closely those

of low resolution mass spectra for 3 reasons; 1) HRMS were obtained using a 12 second scan time (0-300 mass units) thus intensities could be greatly affected by rapid increase or decrease of column effluent 2) Intensities were rounded off to nearest whole numbers and 3) doublet information is not included since the HRMS were used primarily for comformation of existing low resolution mass spectra and not for fragmentation studies.

Mass Spectra (Low and High Resolution)

The spectra presented in this chapter are designated with a number which corresponds to a numbered vertical line on the total ion current tracing in Figure 18.

Standard spectra for toluene and 2-butanone together with spectrum H, mass spectrum #8 and HRMS scan 9 (NF) are shown in figure 19. The spectrum of H. obtained from a headspace analysis, compared to that of 2-butanone show the two to be quite similar. A significant M-15 (M- abbreviation for molecular ion) and M-43 is characteristic of methyl ketones representing losses of CH_3 and C_2H_3O, respectively. Spectrum 8 closely matches that of toluene, with the high resolution data confirming the correct compositions of the ions.

Figure 20 contains the mass spectra of methyl disulfide, hexanal, spectra 9 and 10 and HRMS scans 14 and 21. Spectrum 9 is nearly identical to that of methyl disulfide. The high M+2 ion (96) is strong indication of a sulfur compound and its intensity suggests the presence of 2 sulfur molecules. This is because of the natural abundance of S^{34} (4.2%). Also m/e 79, a large M-15 ion, has associated with it m/e 81, 2 mass units greater than 79, which also points to a sulfur containing compound. HRMS data confirms the composition as $C_2H_6S_2$ and reveals

the M-15 (79), M-30 (64), and M-47 (47), ions as resulting from losses of CH_3, CH_3 twice, and CH_3S respectively.

Spectrum 10 is nearly identical to the standard mass spectrum for n-hexanal. The base peak at m/e 44 is a result of β cleavage with transfer of a single hydrogen atom yielding $C_2H_4O^+$, which was confirmed by HRMS and also identifies m/e 100 as $C_6H_{12}O$. The spectrum is characteristic for a long straight chain aldehyde.

Figure 21 shows the mass spectra of 4-methyl-3-penten-2-one and 2-amylfuran together with spectra 12 and 18. Spectra 12 is identical to that of 4-methyl-3-penten-2-one. No HRMS data are available but the spectra can be recognized as that of a methyl ketone by the large M-15 at m/e 83 and M-43 at m/e 55 corresponding to loss of CH_3 and C_2H_3O respectively. Spectra 18 and 2-amylfuran appear to be related compounds. However on close examination it is found that 18 was from a very small peak hence the background becomes greater. The base peak of m/e 81 suggest that 18 is the spectrum of a substituted furan. Close comparison of the spectra reveal small M-15, M-29, M-43, and large M-57 ions at m/e values 123, 109, 95, and 81 respectively which would account for the expected cleavage of an amyl side chain. An ion is present at m/e 67 which is the mass of a furan ring less one hydrogen. A m/e 53 ion is characteristic of substituted furans formed by the loss of CO from m/e 81. In spite of the high background the spectrum 18 is considered to be 2-amylfuran; however, confirming evidence is needed.

Standard mass spectra of 2-methyl-tetrahydrofuran-3-one and 2-furfural are shown in Figure 22 along with spectra numbers 22 and 46 and HRMS scans 52 and 100. Spectrum 22 closely matches that of 2-

methyl-tetrahydrofuran-3-one. HRMS scan 52 which corresponds to spectrum 22 shows molecular composition of $C_5H_8O_2$, and a large M-28 peak at m/e 72 due to loss of CO. The base peak has a composition of C_2H_3O arising from the loss of m/e 44 (the methyl substituent, ring carbon 2 with its hydrogen atom and the ring oxygen which looses a hydrogen to give m/e 43, according to Viani et al (70). Spectrum 46 is very similar to the spectrum of 2-furfural, except a high m/e 43 is present which is most likely a background peak or error in substracting out the background. HRMS scan 100 confirms the molecular composition as $C_5H_4O_2$, the same as for 2-furfural. Also m/e 67 (M-17) results from a loss of HO, while m/e 43 and 39 have compositions of C_3H_7 and C_3H_3.

Standard mass spectra of methylfurylketone and 5-methylfurfural are shown in Figure 23 together with spectra numbers 52 and 62 from the neutral fraction. Spectrum 52 is quite similar to that of methylfuryl ketone. Both have the characteristic M-15 base peak at m/e 95 and M-43 peak at m/e 77 representing losses of CH_3 and C_2H_3O fragments, respectively. Ions at m/e 68 and 39 are characteristic of furan spectra (71). The parent ion at 110 is somewhat less intense than the standard spectrum, whereas the ions at m/e 39 and 43 are more intense. 5-Methylfurfural and spectrum 62 have very similar mass spectra. Both have the molecular ion as base peak with a large M-1 and a M-29 at m/e 81 due to loss of CHO. The large fragment ion at m/e 53 being characteristic of alkyl substituted furans.

Mass spectra of peaks 66 and 68 and standards N-ethylpyrrole-2-carboxaldehyde and N-methylpyrrole-2-carboxaldehyde are shown in Figure 24. The spectrum of peak 66 contains a mixture of compounds or high background peaks but predominantly a spectrum not unlike that of

the only available mass spectrum of N-ethylpyrrole-2-carboxaldehyde. The parent ion at 123 is the base peak for both spectra with the M-29 being the next most intense for each spectrum. The third most intense ion at m/e 39 agrees with both, followed by 108, 122, and 106 all having similar intensities. The large M-15 ion would be expected from the N-ethyl portion of the molecule and a 14% ion at m/e 80 would be expected from a N alkyl substituted pyrrole. Although the spectrum for 66 suggest a mixture of compounds and the standard information is minimal, there is very good evidence for 66 being N-ethylpyrrole-2-carboxaldehyde. The spectra of peak 68 compared very closely with the standard spectra of N-methylpyrrole-2-carboxaldehyde. The HRMS scan 130 shows the composition of the molecular ion to be C_6H_7NO. A M-29 ion at m/e 80 corresponds to loss of the aldehyde group, CHO.

Figure 25 includes standard spectra of furfuryl alcohol and thiophene-2-carboxaldehyde along with spectra of peak 74 and 78 and HRMS scan 135. Comparison of the spectra of furfuryl alcohol and peak 74 shows some distinct similarites. The intense peak at 39, 41, 42, 95 & 96 indicated some impurities, however looking at the HRMS scan 135 and spectra 74 there is no doubt of the presence of furfuryl alcohol. Intense ions at 98, 97, 81, 70, and 53 all agree with the standard spectrum. The relative intensities in the HR spectra are quite close to the standard except for 41 which could be due to background. The ion at 81 results from a loss of HO (M-17) and ion 70 is from a loss of CO (M-28). Spectrum 78 compares closely with the standard spectra of thiophene-2-carboxaldehyde except for a small amount of impurity due to a mixture. The parent ion is at 112 and a relatively high M + 2 is present in the spectrum indicating the presence of sulfur. A signifi-

cant ion at m/e 83 corresponds to a loss of CHO (M-29). Ions at 39, 50 and 58 also compare favorably.

In Figure 26 is shown mass spectra of peaks 82B, and 83 along with their corresponding HRMS scans 148 and 149. Also shown is standard mass spectra of napthalene and acetyl-2-thiophene. Spectrum 82B and HRMS 148 together show a compound whose molecular ion is at m/e 128 with a composition of $C_{10}H_8$. Ions at 102, 75, 64 and 51 also all compare favorably to the standard spectra of napthalene. Background is quite high in spectrum 82B as evidenced by intense ions below m/e 50. Spectra 83 and HRMS 149 taken together and compared with the standard spectra of acetyl-2-thiophene show this compound to be unmistakably acetyl-2-thiophene. The large M + 2 peak associated with both the parent ion (126) and M-15 (111) is a strong indication of a sulfur containing compound. The large M-15 ion at 111 is characteristic of a methyl ketone spectrum. The HR data gives the molecular compositon to be C_6H_6OS with a loss of CH_3 to give m/e 111. Also 83 is formed from a loss of C_2H_3O (M-43) which corresponds to a loss of the acetyl group, and is the characteristic ion of the thiophene ring minus one hydrogen.

Figure 27 compares spectra 87 and 89 to the standard mass spectra of N-(2-furfuryl) pyrrole and 3-phenylfuran. Spectrum 87 very closely matches that of N-(2-furfuryl) pyrrole. The large peak at 81 corresponds to cleavage between the two rings at the C-N bond leaving the positive charge with the methylene furan ring (M-66). Peaks at 39 and 53 are characteristic cleavage products of both furan and pyrrole rings (71). Spectrum 89 compares quite favorably with the literature spectrum for phenyl-3-furan. The large M-29 at m/e 115 is quite like-

ly due to a loss of CHO from the furan ring. Both spectra contain a peak at 57.5 due to a doubly charged ion at 115.

Standard mass spectra of 2-phenyl-2-butenal and 4-methyl-2-phenyl-2-pentenal together with spectra 92 and 93 are shown in Figure 28. Spectrum 92 is essentially identical to the standard spectra of 2-phenyl-2-butenal. The base peak at m/e 117 would be expected from a loss of CHO (M-29), and the large peak at m/e 91 is characteristic of alkyl phenyl compounds. Spectrum 93 shows distinct similarities to the standard spectrum of 4-methyl-2-phenyl-2-pentenal. Ions at 115, 117 and 146 are much more intense than in the standard, leading one to suspect a mixture with the close eluting peak 92, 2-phenyl-2-butenal, which has its three most intense ions at these three values. If this is the case, spectrum 93 is probably that of 4-methyl-2-phenyl-2-pentenal or a cis or trans isomer.

Figure 29 shows spectra 96 and 101 along with standards 2-acetylpyrrole and pyrrole-2-carboxaldehyde. The large M-15 ion at m/e 94 and large M-43 ion at 66 correspond to losses of CH_3 and C_2H_3O which are expected from the fragmentation of the acetyl group. Also the ion at m/e 66 is characteristic of a pyrrole ring minus one hydrogen. Comparison of spectrum 101 to the standard spectrum of pyrrole-2-carboxaldehyde leaves no doubt that 101 is pyrrole-2-carboxaldehyde. Both have the large M-1 and M-29 corresponding to losses of H and CHO. The ions at m/e 66 and 39 are characteristic of a pyrrole compound, resulting from a pyrrole ring minus one hydrogen and loss of HCN from 66 to yield 39 (C_3H_3).

The spectra of peaks 103 and 105 and standard spectra of 5-methyl-2-phenyl-2-hexenal and 5-methyl-2-pyrrolaldehyde are shown in Figure

30. Spectrum 103 is quite similar to the standard spectrum of 5-methyl-2-phenyl-2-hexenal. There is some background contamination in 103, however most of the more intense ions compare closely. The possibility of cis or trans isomers is suggested by another similar spectrum with the same parent ion which was also obtained but not included here. Spectra of 105 and 5-methyl-2-pyrrolaldehyde appear to be coincidental. The ion at m/e 80 corresponds to loss of CHO (M-29) while the ions at m/e 53 and 39 are characteristic of a pyrrole compound.

Gas Chromatographic Retention Times and Order

Gas chromatographic relative retention indices were obtained as described in Chapter IV on some of the components of the neutral fraction and available standards. Included in Table V are those components whose retention times agree with standard compounds. Under the heading GC-RT, an I_E indicates positive correlation of the I_E values of a specific standard and unknown compound. I_E values were not obtained on all of the components, thus order of retention and relative retention data from the literature was utilized in some cases as added proof of identity.

Table VI lists the compounds, whose I_E values were not obtained, along with retention time data of these compounds. These data are used to show approximate relative retention times and order of retention of isomers and homologs. The first column of data (R_T^a) shows retention times taken from the GC-MS analysis tracing shown in Figure 17. The second column (R_T^b) shows the retention times of some standard compounds on the previously described capillary column. The conditions employed were different from those used in Figure 17, hence the data is useful primarily as approximate relative retention times as well as

their order of separation. The third column (R_T^C) is taken from the literature and represents values obtained on a carbowax 20 M column.

Both furfural and 5-methylfurfural were identified and can be used as internal reference points. Methylfuryl ketone standard and the compound in spectrum 52 both have retention times a little less than half way between the values for furfural and 5-methylfurfural, a fact that provides additional evidence for spectrum 52 being methylfuryl ketone. Although N-ethylpyrrole-2-carboxaldehyde was not available the retention time for spectrum 66 fits the published standard values. Examination of the values in Table VI show the value for spectrum 66 and a literature value of N-ethylpyrrole-2-carboxaldehyde to be between 5-methylfurfural and furfuryl alcohol. The compound in spectrum 68 also falls in this range eluting closer to furfuryl alcohol than the compound in spectrum 66 and the value of N-methylpyrrole-2-carboxaldehyde also is in this range. These observations provide additional evidence that spectrum 66 is N-ethylpyrrole-2-carboxaldehyde and spectrum 68 is N-methylpyrrole-2-carboxaldehyde.

The retention time data reveals the decreased polarity of N-alkyl substituted pyrroles by their earlier elution time as compared with the unsubstituted nitrogen in the ring of pyrroles. Comparison of the retention times of the two isomers, N-methylpyrrole-2-carboxaldehyde and 5-methylpyrrole-2-carboxaldehyde, dramatically confirm these observations. Spectrum 105 appears to be 5-methylpyrrole-2-carboxaldehyde based on its very long retention time which compares favorably with standard values.

Discussion

Twenty four compounds are reported as constituents of the neutral

fraction of roasted peanut volatiles. Twenty have not been previously reported as constituents of roasted peanut aroma and the four previously reported were tentative identifications.

The major monocarboxyls of roasted peanut volatiles have been reported as acetaldehyde, a 2-methylpropanal, 2- and 3-methylbutanal, benzaldehyde and phenylacetaldehyde (17). It was suggested that these compounds could be formed by Strecker degradation of amino acids.

The following furans were identified: 2-amylfuran, 2-furfural, methylfuryl ketone, 5-methylfurfural, furfuryl alcohol, N-(2-furfuryl) pyrrole and phenyl-3-furan. Many of these are common constituents of food aromas. All listed except 2-amylfuran are reported as occuring in roasted coffee in a review paper by Soffelsma et al. (72). These types of components are formed by dehydration, rearrangement and degradation of sugar moieties during the browning process of the system.

The following 6 pyrroles were reported from this work: N-ethylpyrrole-2-carboxaldehyde, N-methylpyrrole-2-carboxaldehyde, N-(2-furfuryl)pyrrole, 2-acetylpyrrole, pyrrole-2-carboxaldehyde and 5-methylpyrrole-2-carboxaldehyde. All of these have been identified in roasted coffee (72). Both furans and pyrroles have been reported as volatile constituents of roasted cocoa beans (22). Pyrroles are known to be products of the Maillard reaction. Kato and Fujimaki (73) investigated the browning reaction between D-xylose and amino compounds. They found a number of N-substituted pyrrole-2-aldehydes in the browning mixture.

Three sulfur containing compounds were reported and include carbon disulfite, thiophene-2-carboxaldehyde and 2-acetylthiophene. All three are reported constituents of roasted coffee (72).

The identification of toluene and naphthalene appears to be an

unusual finding in food aromas and it is possible that they could be solvent impurities, however both of these hydrocarbons and many more are reported by Stoll et al. (32) as constituents of coffee aroma. 2-Methyltetrahydrofuran-3-one is also reported as a volatile component of coffee aroma (70).

Three phenyl-alkenals were found and are 2-phenyl-2-butenal, 4-methyl-2-phenyl2-2pentenal (tentative) and 5-methyl-2-phenyl-2-hexenal. These unusual compounds have been reported in the aroma of roasted cocoa beans by van Praag et al. (22). They reported that these were dehydrated aldol condensation products of phenylacetaldehyde and acetaldehyde, isobutyraldehyde and isovaleraldehyde. All four of these aldehydes are abundant in roasted peanut volatiles and are probably reactants which form the phenyl-alkenals. The author suggests the possibility of the phenyl-alkenals being artifacts since both the cocoa and peanut volatiles were fractionated by acidification of the total volatile condensate to below pH 1 with HCl. This acidic condition could catalyze condensation and/or dehydration reactions between aldehydes. That these phenyl-alkenals are not artifacts in cocoa aroma is supported by organoleptic evaluation of 5-methyl-2-phenyl-2-hexenal by van Praag et al. (22) as possessing a deep bitter persistant cocoa note. Two distinct compounds of molecular weight 188 were obtained, presenting the likelyhood of cis and trans isomers. van Praag et al. (22) made similar observations.

As might be expected, the neutral fraction possessed a coffee-like aroma when highly concentrated. The dilute neutral fraction has a very sweet roasted aroma and is of major significance in the overall flavor of roasted peanuts.

TABLE V

SUMMARY OF COMPOUNDS IDENTIFIED IN THE NEUTRAL FRACTION AND METHODS USED TO CONFIRM THEIR IDENTITY

Compound	Spectrum #	Confirmed By: MS	HRMS	GCRT	P or T
2-Butanone	H	X	X		T
Toluene	8	X	X		P
Methyl disulfide	9	X	X		P
n-Hexanal	10	X	X		P
4-Methyl-3-penten-2-one	12	X			T
2-Amylfuran	18	X			T
2- Methyl-tetrahydrofuran-3-one	22	X	X	I_E	P
2-Furfural	46	X	X	I_E	P
Methylfuryl ketone	52	X			T
5-Methylfurfural	62	X		I_E	P
N-ethylpyrrole-2-carboxaldehyde	66	X	X		T
N-methylpyrrole-2-carboxaldehyde	68	X			T
Furfuryl alcohol	74	X	X		P
Thiophene-2-carboxaldehyde	78	X		I_E	T
Naphthalene	82B	X	X	I_E	P
Acetyl-2-thiophene	83	X	X	I_E	P
N-(2-furfuryl)pyrrole	87	X		I_E	P
Phenyl-3-furan	89	X	X		P
2-Phenyl-2-butenal	92	X		I_E	P
4-Methyl-2-phenyl-2-pentenal	93	X			T
2-Acetylpyrrole	96	X		I_E	P
Pyrrole-2-carboxaldehyde	101	X		I_E	P
5-Methyl-2-phenyl-2-hexenal	103	X		I_E	P
5-Methyl-2-pyrrolaldehyde	105	X			T

TABLE VI

GAS CHROMATOGRAPHIC DATA COMPARING RELATIVE RETENTION TIMES AND ORDER OF SOME NEUTRAL FRACTION COMPONENTS AND STANDARD COMPOUNDS

Compound	R_t^a	R_t^b	R_t^c
Furfural	59	36.7	17
Methylfuryl ketone	63	39.7	19.5
5-Methylfurfural	69	44.1	23.5
N-ethylpyrrole-2-carboxaldehyde	73		26.6
N-methylpyrrole-2-carboxaldehyde	74.5	47.1	
Furfuryl alcohol	78	49.6	29.0
2-Acetylpyrrole	107	68.6	52.8
Pyrrole-2-carboxaldehyde	110	71.8	
5-Methylpyrrole-2-carboxaldehyde	117		60.0

a) values from Figure 17
b) 0.02 in x 500 ft Carbowax 1540+KOH column; flow - 19 ml/min; programmed at 3^{o}/min from 70 to 190^{o}
c) literature data from Tatum et al. (74)

Figure 18. Combination Capillary Gas Chromatographic-Mass Spec trometric Analysis of the Neutral Fraction

Conditions were as follows:

Column - 500' x 0.02" stainless steel

Column Packing - coated with Carbowax 1540 + KOH

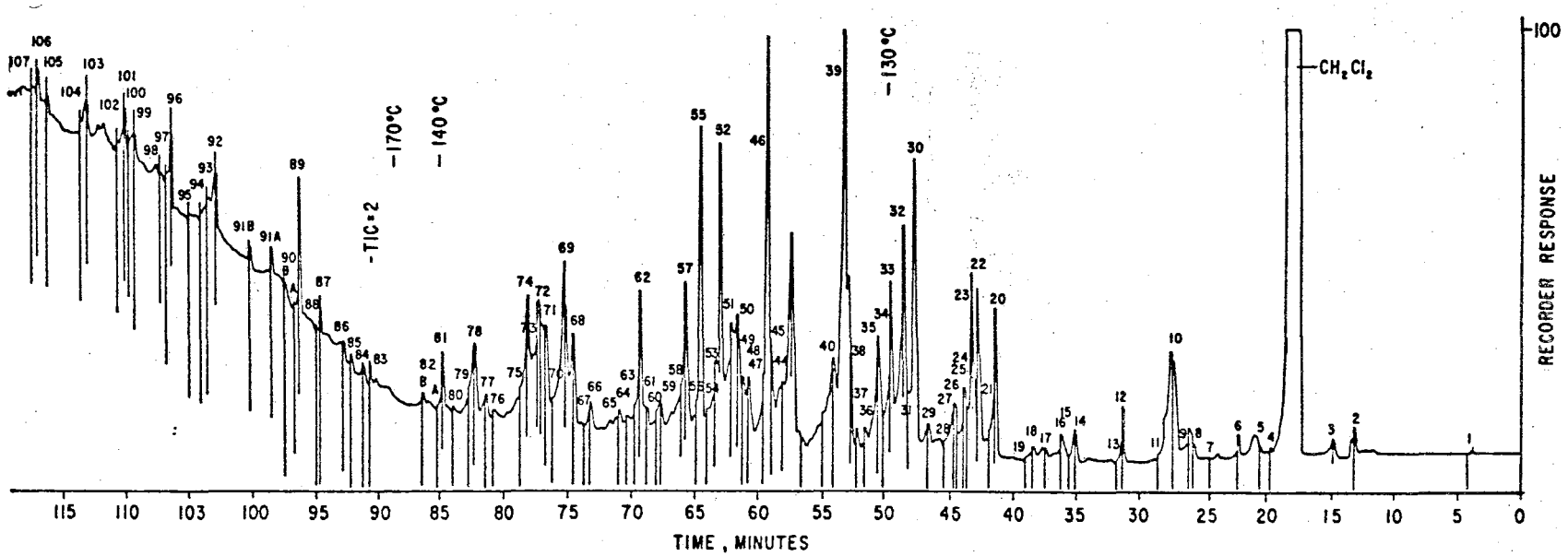
Column Temperature - 40° initially, manually increased at varic intervals as shown on the chromatogram

Inlet Temperature - 200°

Carrier Gas - Helium

Flow Rate - 3 ml/min with a post column gas adder at 20 ml/min

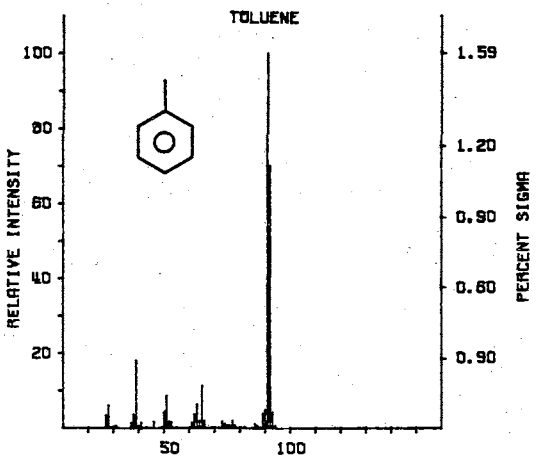
Detector - Mass spectrometer

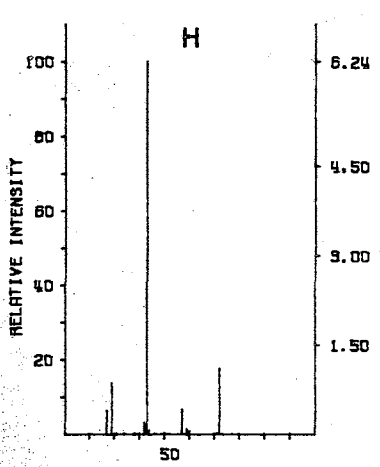

-TIC÷2
-170°C
-140°C
-130°C
CH2Cl2
100
RECORDER RESPONSE
TIME, MINUTES
115 110 103 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5 0

2-Butanone

m/e	Percent
72	16.1
57	6.3
44	2.4
43	100.0
42	5.1
29	23.6
27	15.3

ASTM Certified
Mass Spectra





HRMS Scan 9 (NF)

m/e	Composition	%
92	C_7H_8	73
91	C_7H_7	100
65	C_5H_5	11
51	C_4H_3	4
39	C_3H_3	7

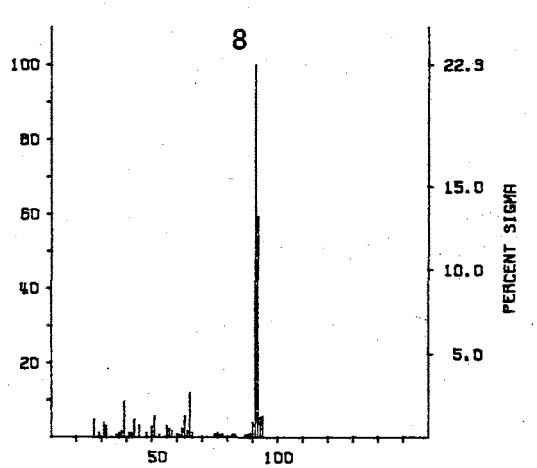


Figure 19. Mass Spectra of Scans H and 8 and Standards

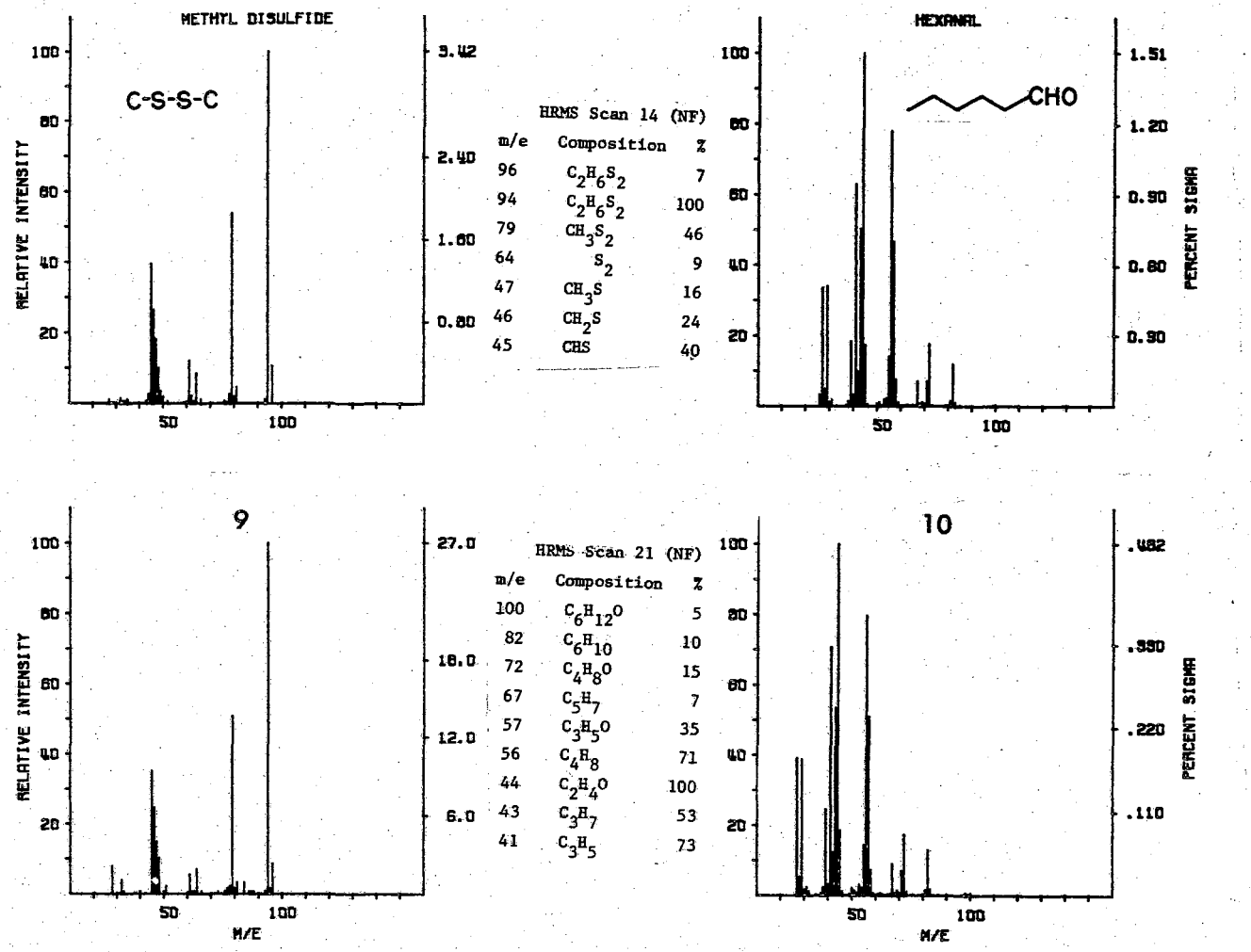


Figure 20. Mass Spectra of Scans 9 and 10 and Standards (1223-11; 1228-10-11).

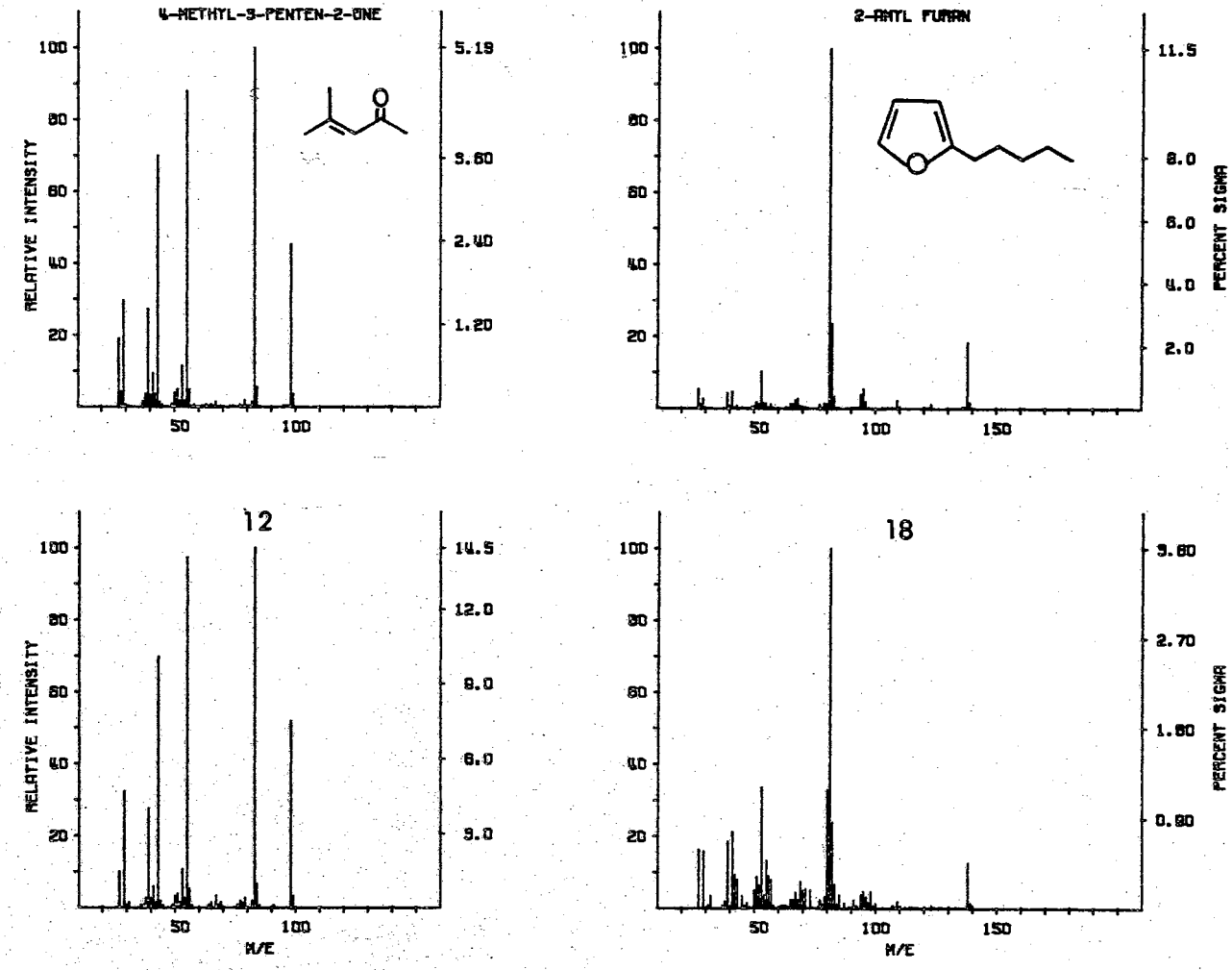


Figure 21. Mass Spectra of Scans 12 and 18 and Standards (1223-14-15; 1228-18-19).

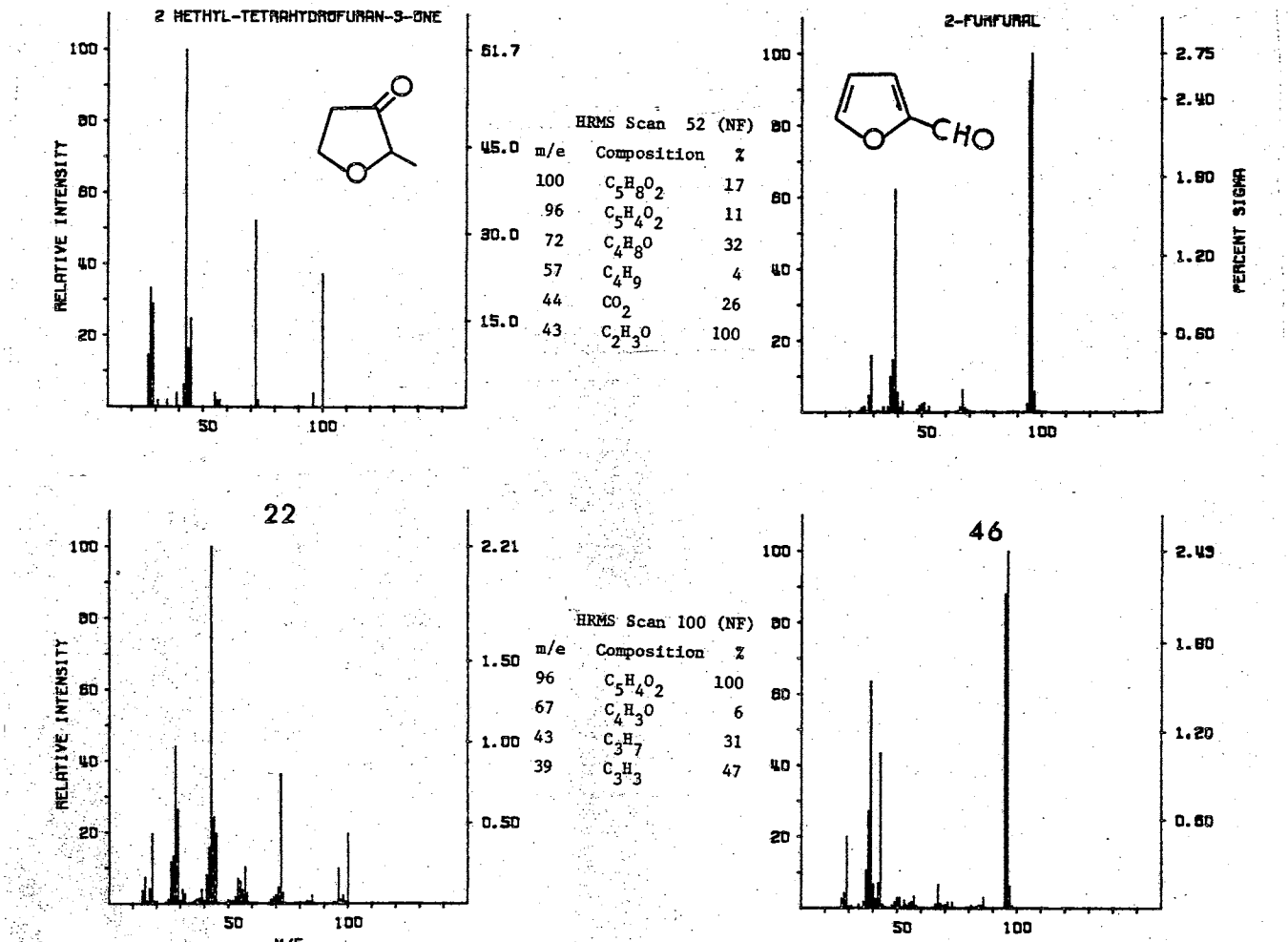


Figure 22. Mass Spectra of Scans 22 and 46 and Standards (1228-22; 46-47).

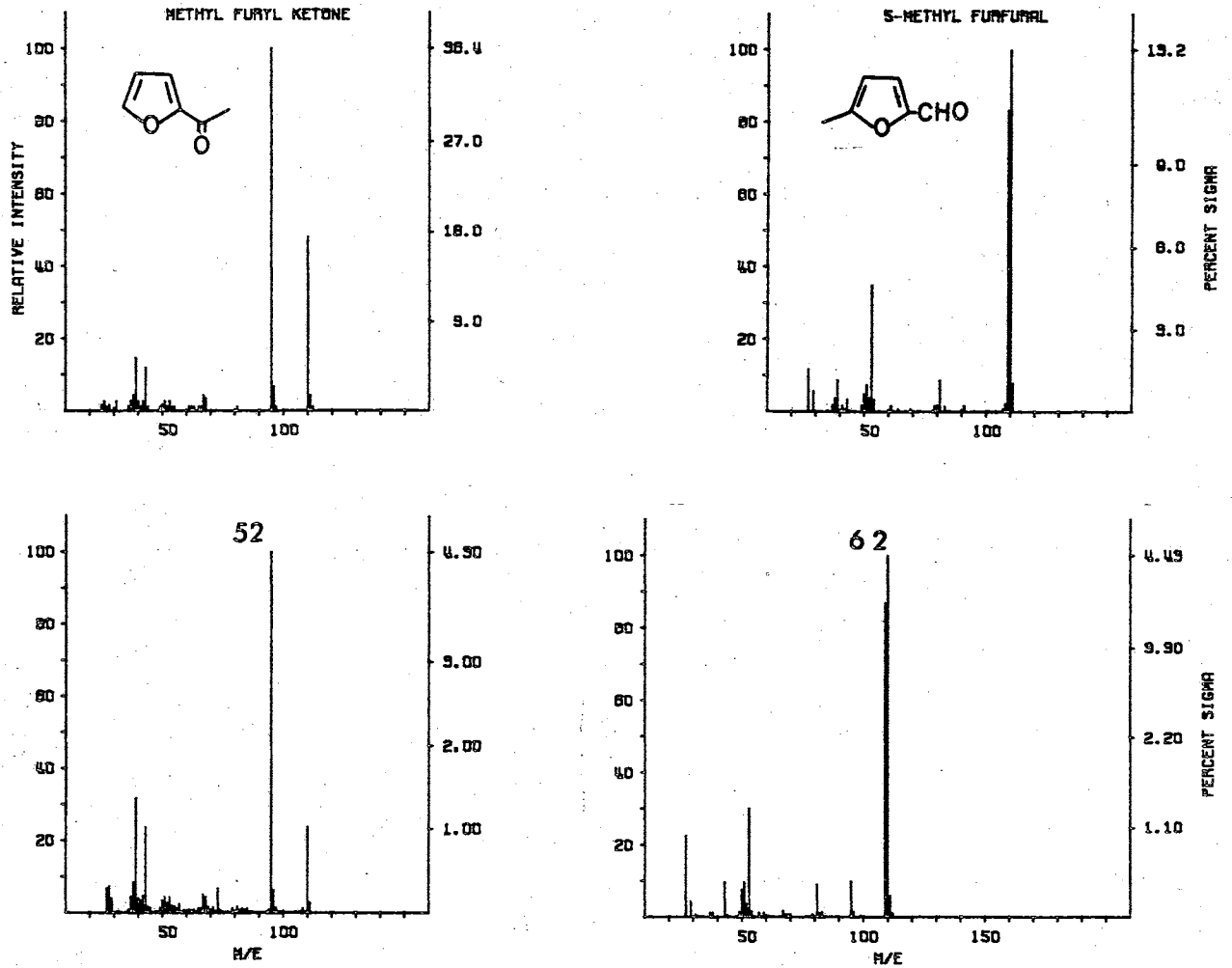


Figure 23. Mass Spectra of Scans 52 and 62 and Standards (1228-52-53; 62-68).

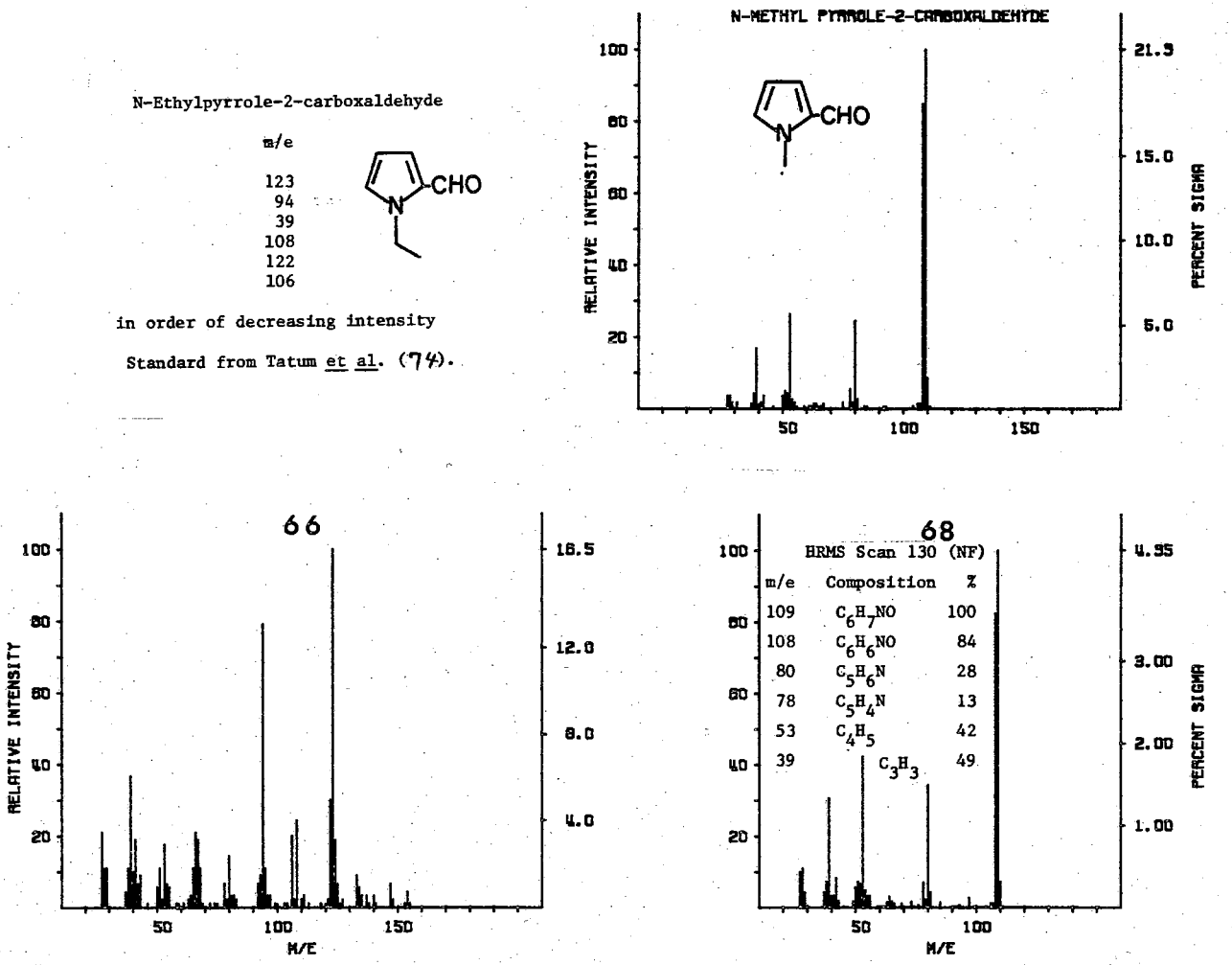


Figure 24. Mass Spectra of Scans 66 and 68 and Standards (1288-66-67; 68).

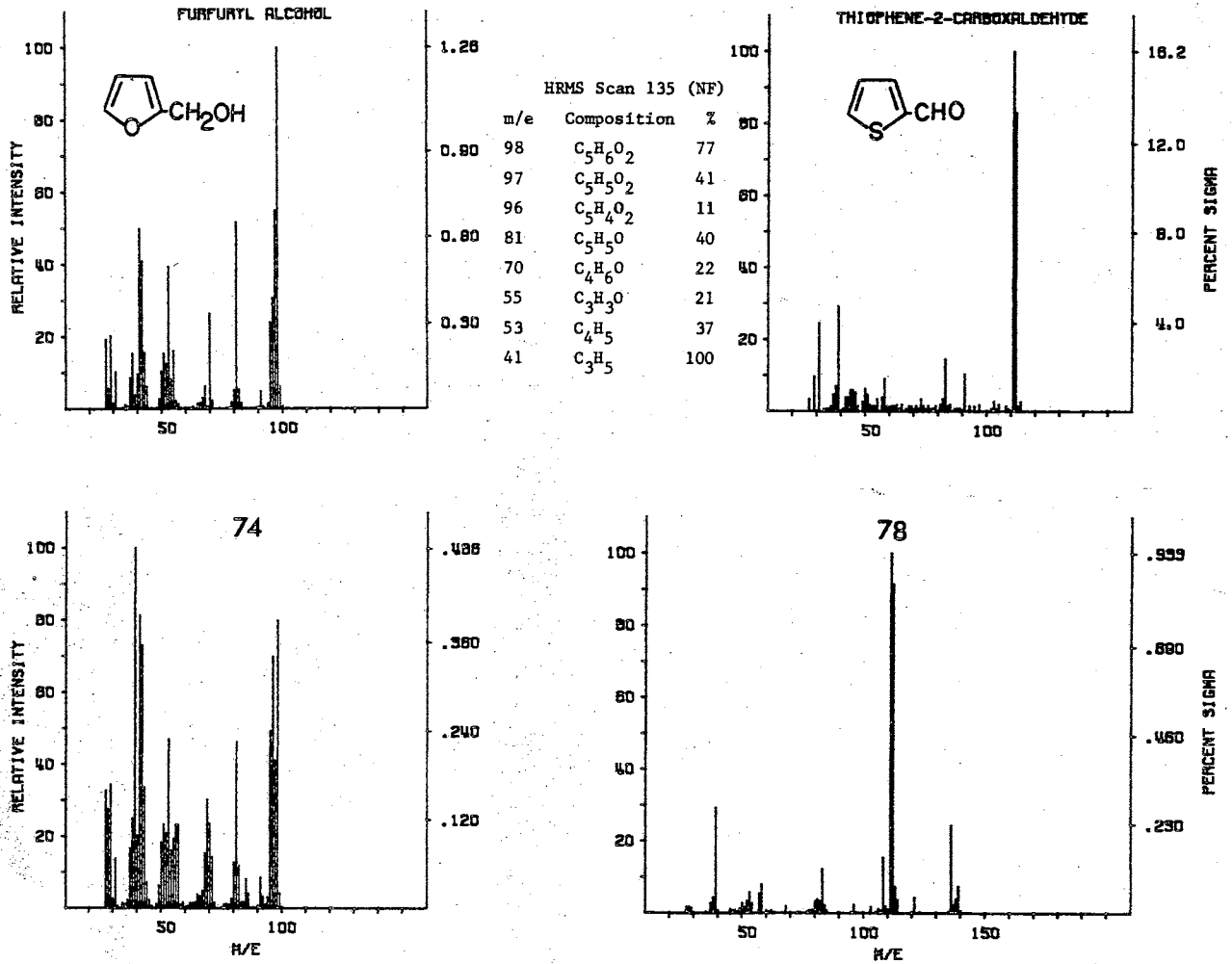


Figure 25. Mass Spectra of Scans 74 and 78 and Standards (1228-74-75; 1223-59-58).

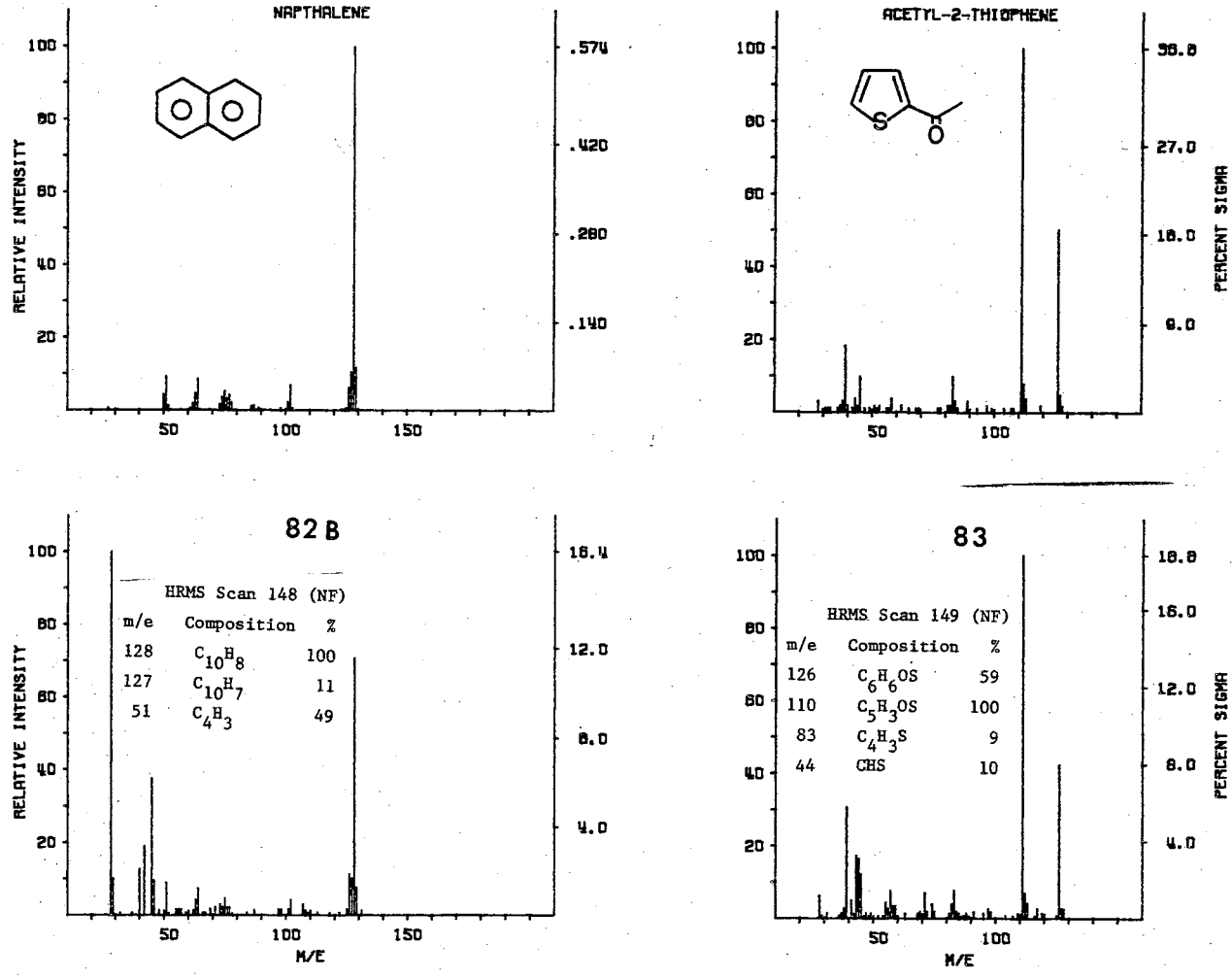
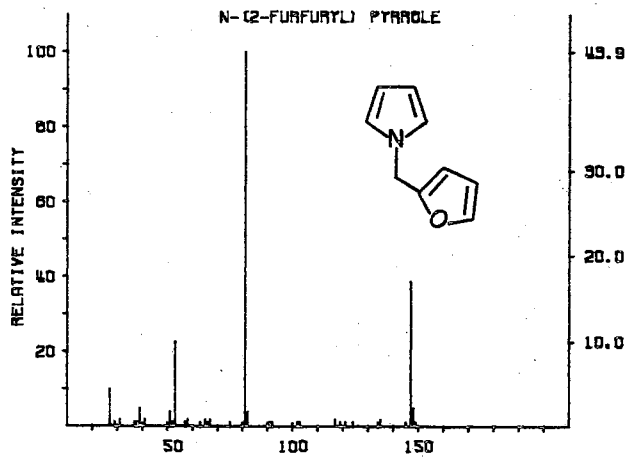


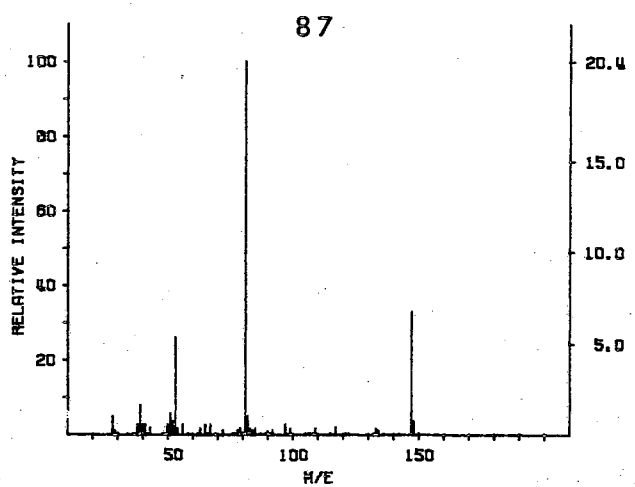
Figure 26. Mass Spectra of Scans 82B and 83 and Standards (1228-82B-82A; 83-82A).



Phenyl-3-furan

m/e	Intensity
144	100
115	79.0
89	8.5
72	4.9
63	8.8
57.5	1.8
51	3.5
39	5.3

Standard from Stoll et al. (32)



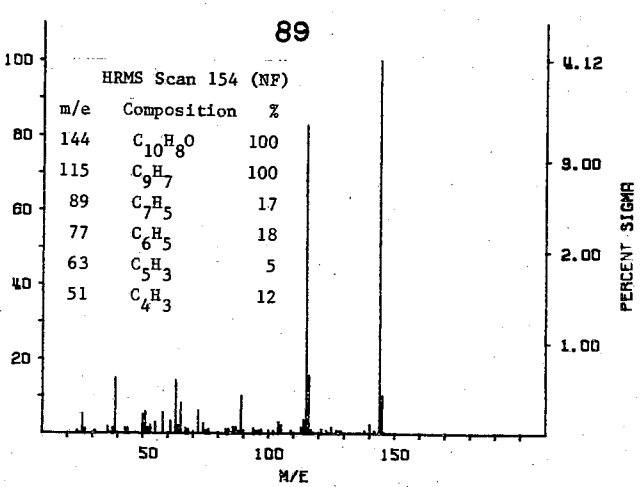


Figure 27. Mass Spectra of Scans 87 and 89 and Standards (1228-87-88; 1223-62).

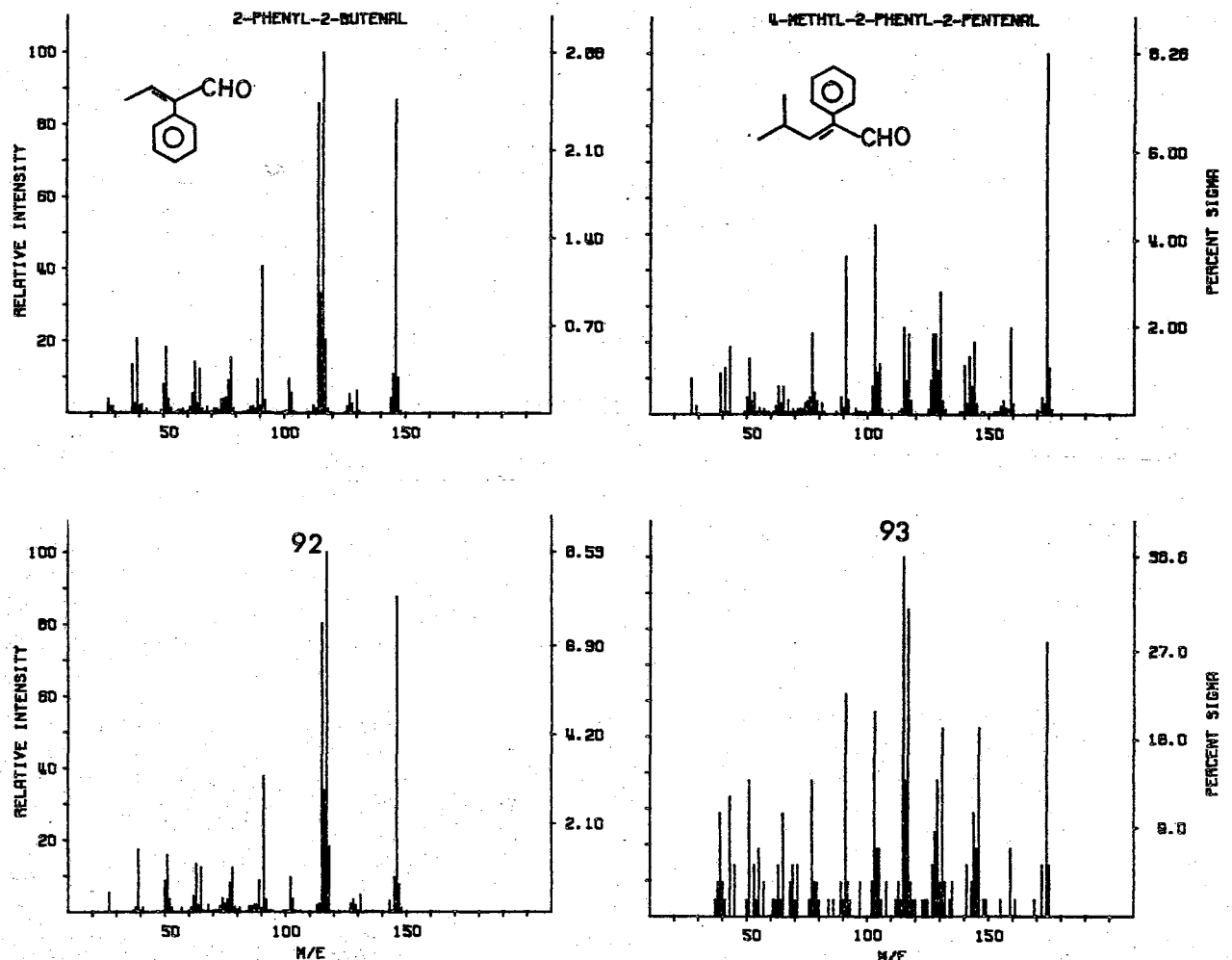


Figure 28. Mass Spectra of Scans 92 and 93 and Standards (1223-64-65; 1228-93-94).

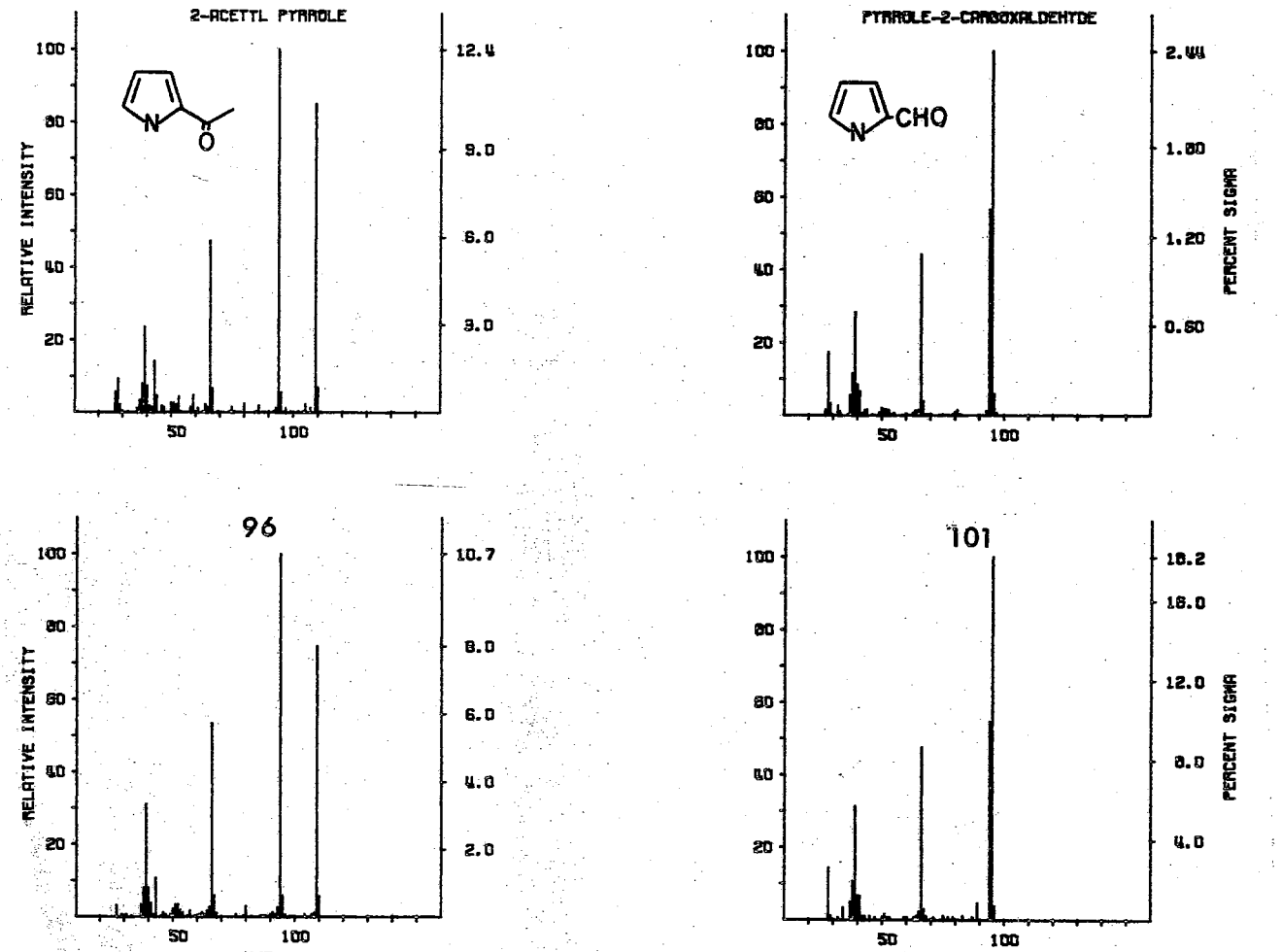


Figure 29. Mass Spectra of Scans 96 and 101 and Standards (1228-96-97; 1223-72).

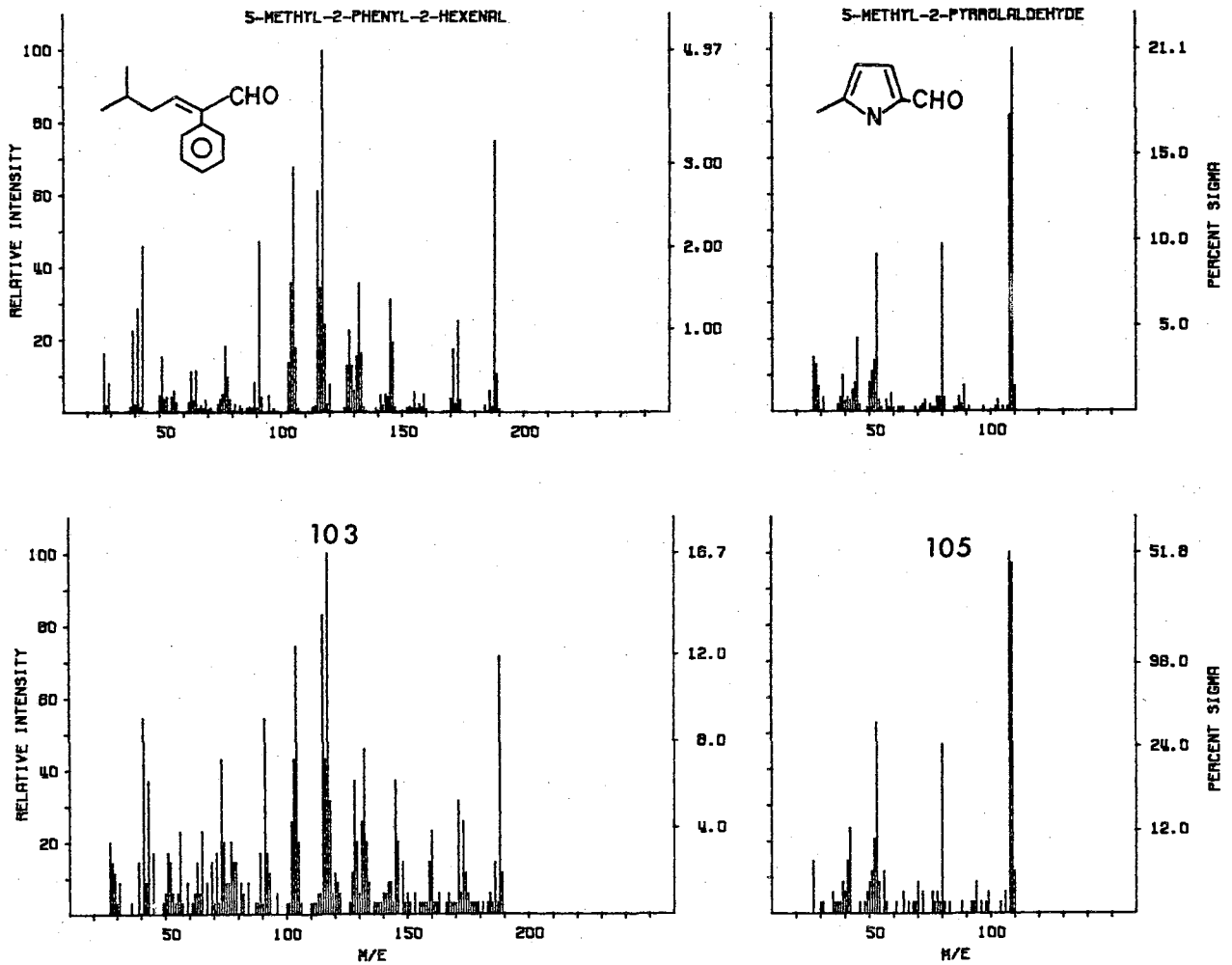


Figure 30. Mass Spectra of Scans 103 and 105 and Standards (1228-103; 105-107).

CHAPTER VI

SUMMARY

The proposed study was to isolate and identify volatile constituents of roasted peanuts. This involved a vacuum degassing for the isolation of the volatile compounds followed by a pH and solvent fractionation. Modern micro analytical instruments were used for identification. These included capillary gas chromatography, combination gas chromatography-mass spectrometry (GC-MS), infrared (IR) and ultra-violet spectroscopy (UV), and combination capillary gas chromatography-high resolution mass spectrometry.

The aroma components of roasted peanuts were concentrated and collected by vacuum degassing of the oil pressed from whole roasted peanuts. The oil was slowly introduced onto steam heated glass bellows where the aroma components were evolved more rapidly and swept through cold traps where they were condensed and later collected. The condensate was separated into basic and neutral fractions by solvent extraction at acidic and basic pHs.

Gas chromatographic conditions were developed which would resolve the complex mixtures sufficiently for analysis on GC-MS. Initial GC-MS analyses were performed utilizing Carbowax 20 M packed glass columns. Later a 500 ft x 0.02 in (i.d.) stainless steel capillary column was coated with Carbowax 1540 + KOH and used throughout the remainder of the study. Identifications were accomplished by relative

retention indices, GC-MS, capillary GC-MS, capillary GC-high resolution MS and UV and IR spectroscopy.

A total of 26 alkyl substituted pyrazines were found in the basic fraction of roasted peanut aroma. Eight of these contained cyclic or unsaturated alkyl substituents. The structure of three were postulated. The probable precursors of pyrazines and their significance in the overall flavor response was discussed.

Twenty four components of the neutral fraction of roasted peanut volatiles, not previously reported from roasted peanuts, were determined. The techniques used in their identification were similar to those used in identifying components in the basic fraction. Included in these compounds were 7 furans, 6 pyrroles, 3 sulfur compounds, an aldehyde, a ketone, 3 phenyl alkenals and miscellaneous compounds. Their significance and probable modes of formation are discussed.

SELECTED BIBLIOGRAPHY

1. Pattee, H. E., Beasley, E. O., and Singleton, J. A., J. Food Sci., 30, 388 (1965).

2. Pattee, H. E., Singleton, J. A., and Cobb, W. Y., J. Food Sci., In press (1969).

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

4. Amerine, M. A., Pangborn, R. M. and Roesser, E. B., Principles of Sensory Evaluation of Food, Academic Press, New York, 1965.

5. Lepkovsky, S., Food Technology, 17, 14 (1963).

6. Pfaffmann, C., Preface In Physiological and Behavioral Aspects of Taste, Kare, M. R., and Halpern, B. P., eds. University of Chicago Press, Chicago, Ill. (1961).

7. Brozek, J., Am. J. Clin. Nutrition, 5, 332-343 (1957).

8. Moncrieff, R. W., The Chemical Senses, 1st ed., John Wiley & Sons, Inc., New York (1946).

9. Self, R., in The Chemistry and Physiology of Flavor, Schultz, Day, and Libbey, eds. AVI Publishing Co., Westport, Conn. (1967).

10. Pattee, H. E., Singleton, J. A., Johns, E. B., & Mullin, B. C., Being submitted.

11. Singleton, J. A., Pattee, H. E., and Johns, E. B., Being submitted.

12. Hoffpauir, Carroll L., J. Agri. Food Chem., I, 668 (1953).

13. Mason, Michael E., and Waller, George R., J. Agri. Food Chem. 12, 274 (1964).

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

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

16. Mason, M. E., Johnson, Bobby and Hamming, Maynard, J. Agri. Food Chem. 14, 454 (1966).

17. Mason, M. E., Johnson, Bobby, and Hamming, M. G., J. Agri. Food Chem. 37, 760 (1967).

18. Brown, Bruce, A. Konigsbacher, Kurt, S., Ellison, Frank E., and Mann, Godfrey E., J. Food Sci. 33, 595 (1968).

19. Schonberg, A., and Moubasher, R., Chem. Rev. 50, 261 (1952).

20. Day, E. A., Food Tech., 19, 1585 (1965).

21. Reichstein. T. and Standinger, H., British patents 246454, and 260960.

22. van Praag, Michel, Stein, Herbert S., and Tibbetts, Merrick S., J. Agri. Food Chem. 16, 1005 (1968).

23. Rizzi, G. P., J. Agri. Food Chem. 15, 549 (1967).

24. Marion, J. P., Muggler-Chavan, F., Viani, R., Bricout, J., Reymond, D., Egli, R. H., Helv. Chim. Acta, 50, 1509 (1967).

25. Flament, I., Willhalm, B. Stoll, M., Helv. Chim. Acta, 50, 2233 (1967).

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

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

28. Gianturco, M. A., in Schultz, H. W., Day, E. A., and Libby, L. M., The Chemistry and Physiology of Flavors, AVI Publishing Co., Westport, Conn. (1967).

29. Merritt, Charles, Jr., Bazinet, M. L., Sullivan, J. H., and Robertson, D. H., J. Agri. Food Chem. 11, 152 (1963).

30. Reymond, D., Mueggler-Chavan, F., Viani, R., Vuataz, L., and Egli, R. H., J. of Gas Chrom. 4, 28 (1966).

31. Bondarovich., H. A., Friedel, P., Kranpl, V., Renner, J., Shephard, F. W., Gianturco, M. A., J. Agri. Food Chem. 15, 1093 (1967).

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

33. Koehler, Philip E., Formation of Alkylpyrazine Compounds and Their Role in the Flavor of Roasted Foods, (Ph.D. Dissertation, Oklahoma State University, 1969).

34. Pickett, T. A., Some Effects of Heat Treatment of Peanuts, Georgia Experiment Station Cir. 142, (1943).

35. Newell, Jon A., Precursors of Typical and Atypical Roasted Peanut Flavor, (Ph.D. Dissertation, Oklahoma State University, 1967).

36. Forsyth, W. G. C., Quesnel, V. C., in Advances in Enzymology, F. F. Nord, ed., Vol. XV. pp. 457, New York and London, Interscience Publishers ----- John Wiley and Sons (1963).

37. Pinto, A. and Chichester, C. O., J. Food Sci. 31, 726 (1966).

38. Hodge, J. E., J. Agri. Food Chem. 1, 928 (1953).

39. Koehler, P. E., Mason, M. E., and Newell, J. A., J. Agri. Food Chem. 17, 393-6 (1969).

40. James A. T., and Martin, A. J. P., Analyst, 77, 915 (1952).

41. James A. T., and Martin, A. J. P., Biochem. J. (London), 50, 679 (1952).

42. Golay, M. J. E., Gas Chromatography, V. J. Coates et al., eds. Academic Press, New York, 1958, p. 1.

43. Zlatz, A., and Kaufman, H. R., Nature 184, 2010 (1959).

44. Watson, J. Throck and Biemann, Klaus, Anal. Chem. 37, 845 (1965).

45. Ryhage, R., Anal. Chem. 36, 759 (1964).

46. McFadden, W. H., Teranishi, R., Black, D. R., Day, J. C., J. Food Sci. 28, 316 (1963).

47. Merritt, Jr., C., Bazinet, M. L., and Yeomans, W. G. J. Chromat. Sci. 7, 122 (1969).

48. Tonsbeck, Christian H. T., Plancken, Arie J., and Weerdhof, Teun V. D., J. Agri. Food Chem. 16, 1016 (1968).

49. Flath, R. A., Black, D. R., Guadagni, D. G., McFadden, W. H., and Schultz, T. H., J. Agri. Food Chem. 15, 29 (1967).

50. Buttery, R. G., Seifert, R. M., Guandagni, D. G., Black, D. R., and Ling, L. G., J. Agri. Food Chem. 16, 1009 (1968).

51. Tang, C. S. and Jennings, W. G., J. Agri. Food Chem. 15, 24 (1967).

52. Gasco, L., Barrera, R., and de la Cruz, F., J. of Chromatog. Sci. 7, 228 (1968).

53. Tang, C. S., and Jennings, W. G., J. Food Sci. 16, 252 (1968).

54. Johnson, Bobby R., Isolation and Identification of Some Volatile Constituents of Roasted Peanuts, (M. S. Thesis, Oklahoma State University, 1966).

55. MacKay, D. A. M., Lang, D. A., and Berdick, M., Anal. Chem. 33, 1369 (1961).

56. Jennings, Walter G., J. Food Sci. 30, 445 (1965).

57. Kosuge, T. and Kamiya, H., Nature 193, 776 (1962).

58. Demain, A. L., Jackson, M. and Trenner, N. R., J. of Bacteriology 94, 323-326 (1967).

59. Buttery, R. G., Seifert, R. M., Lundin, R. E., Guadagni, D. G., and Ling, L. C., Chem. and Ind. 490 (1969).

60. Waller, George R., Proc. Okla. Acad. Sci. 47, 271-292 (1968).

61. Li, H. Y., Walden, J., Etter, D., and Waller, G. R., Proc. Okla. Acad. Sci. 48, 250-257 (1969).

62. 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).

63. Cieplinski, E. W., Anal. Chem. 38, 928, (1966).

64. Sweeley, C. C., Elliott, Witt, Fries, I., and Ryhage, R., Anal. Chem. 38, 1549-1553 (1966).

65. Biemann, K., Mass Spectrometry: Organic Chemical Applications, McGraw-Hill, New York (1962).

66. van den Dool, H., and Kratz, P. D., J. Chromatog. 11, 463 (1963).

67. Japanese Patent No. 5189, 1967. (Food Techn. 21, 1118 (1967).

68. U. S. Patent No. 3,402,051, 1968.

69. Fact Finder & Action Guide section, Food Techn. 23, 478 (1969).

70. Viani, R., Muggler-Chavan, F., Reymond, D. and Egli, R. H., Helv. Chim. Acta 48, 114 (1965).

71. Beynon, J. H., Saunders, R. A. and Williams, A. E., The Mass Spectra of Organic Molecules, Elsevier Publishing Co. New York (1968).

72. Stoffelsma, J., Sipma, G., Kettenes,D. K., and Pypker, J., J. Agr. Food Chem. 16, 1000 (1968).

73. Kato, Hiromichi, and Fujimaki, Masao, J. Food Sci. 33, 445 (1968).

74. Tatum, J. H., Shaw, P. E., and Berry, R. E., J. Agr. Food Chem. 15, 773 (1967).

VITA

Bobby Ray Johnson

Candidate for the Degree of

Doctor of Philosophy

Thesis: CHEMICAL CHARACTERIZATION OF ROASTED PEANUT AROMA

Major Field: Biochemistry

Biographical:

Personal Data: Born near Oakwood, Oklahoma, October 30, 1941, the son of Mr. and Mrs. Albert Ray Johnson. Married Charlotte A. Fauchier of Oakwood, Oklahoma in Oakwood, Oklahoma, August 26, 1962.

Education: Attended the public schools of Oakwood, Oklahoma, and graduated from Oakwood High School; received the Bachelor of Science degree from Oklahoma State University in 1963 with a major in Agricultural Biochemistry; received the Master of Science degree in Biochemistry, in July 1966; completed requirements for Doctor of Philosophy degree in May, 1970.

Professional Experience: Graduate research assistant, Department of Biochemistry, Oklahoma State University, September 1963, to July, 1966; Chemistry Instructor at Oklahoma Christian College, school year 1966-67; Biochemistry Instructor at Oklahoma State University, academic year of 1967-68; NDEA fellow 1968-1969.

Professional and Honorary Societies: Phi Eta Sigma, Alpha Zeta, Phi Lambda Upsilon, Sigma Xi, American Chemical Society and Institute of Food Technology.