FACTORS RESPONSIBLE FOR FLAVOR AND OFF-FLAVOR DEVELOPMENT IN PECANS

By

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CHAPTER I

INTRODUCTION

The pecan, Carya illinoensis (Wang.) K. Koch, is a member of the family of Juglandaceae, genus, Carya. Other members of this family include the hickory and the walnut (1).

When botanists first began the study of trees in North America (about 1800), the pecan was discovered as a new species, found nowhere else in the world. It was native to the bottom lands of the Mississippi River, north to southern Illinois and west through Arkansas, Louisiana, and the eastern portions of Texas and Oklahoma (1). There is some evidence that the pecan tree may be the only native crop tree planted by the Indians before the white man came (2). In 1846, the pecan tree was first grafted by a Louisiana slave and it was from this date that intelligent interest began in pecan culture (3). The first successful propagation was performed by William Nelson in 1882 when he began the propagation of pecans in his nursery. Gradually, prime varieties were developed, propagated, and made available to the planters. Finally, by 1909, the pecan had risen to second place in values of American nut crops (1).

The uses of pecans in the food industry are innumerable. Not only is the pecan popular with the housewife (i.e. pecan pies, etc.), it is a large commercial commodity as well. Industries buy pecans, especially the smaller nuts, for use in cookies and other confections.

Nutritionally, pecans are an excellent source of Vitamin E as well

as several essential fatty acids. Pecans are an ideal fat source for persons with coronary difficulties due to the absence of saturated fatty acids with more than 18 carbon atoms.

Over the years, the pecan has become a major factor in the income of Oklahoma farmers. In total production, Oklahoma rose from 7th in the nation in 1966 to first in 1967 with a crop of 50 million pounds. The price per pound for Oklahoma pecans in 1967 was $0.31 setting the value of the crop in excess of 15 million dollars (4). Economically, this is about 30% of Oklahoma's horticulture output.

In consideration of the importance of this crop to the state, this study was undertaken to investigate flavor and rancidity in pecans. The objectives of this work were as follows: (1) to isolate and identify components related to flavor in pecans, (2) to study off-flavor development in pecans and factors in both the saponifiable and non-saponifiable lipid portions of the oil that may be responsible for increasing or decreasing the oxidative stability of the nuts, and (3) to investigate genetic and/or environmental effects on the chemical composition of the pecan oils.

CHAPTER II

LITERATURE REVIEW

Much of the research on pecans has been conducted by established classical methods before 1950. In recent years, Woodroof's research group has contributed greatly to the study of the pecan and factors associated with its keeping quality. Woodroof and Heaton (5) have published a study in which they extensively reviewed the literature regarding pecan development, varieties, shell development, storage, packaging, utilization and the chemical aspects of the nut.

Two excellent papers were published before 1950 dealing with the chemical changes occurring in the pecan during maturation. Hammar and Hunter (6) studied changes in nitrogen, oil and ash (and minerals including Ca, Mg, P, and K) during the filling period from August 25 to harvest (in Georgia). They concluded that the most critical period during the filling process was between August 25 and September 15, during which time, 85% of the dry weight of the nut was formed. Thor and Smith (7) had conducted a more thorough study of this process ten years earlier in 1935. They initiated sampling five weeks after full bloom (around the middle of May) and followed the nut development through maturity. Each week they determined dry matter, total nitrogen, ash, oil, free fatty acids, sugars and polysaccharides and presented the data in graphical form. They have shown that the pecan development is characterized by two growth periods. The first, from blossoming until late August, is

characterized by shuck and shell development. During the second period, mid-August through mid-September, kernel filling was of major importance.

Since all of the oil, protein, and polysaccharides are formed in a one month period, the precursors from which these constituents are formed must be rapidly translocated from other parts of the tree during this period. During the time of oil and protein synthesis, there is a considerable loss of sugar from the fruit. No free fatty acids are found during this period, so there is apparently no accumulation of these components before triglyceride formation (7).

Much research seeking to minimize oxidative deterioration of pecans during storage has been conducted. Medlock (8) observed that pecans could be stored several years at 0^{o} C. and low humidity. He also observed that when pecans were exposed to ammonia gas, a blackening of the skin ensued. Woodroof and Heaton (9) also noted this darkening effect of ammonia but found no detectable rancidity. Pecans act as "vapor scavengers" (10) and are best stored in places free from undesirable odors. Isbell (11) stored pecans for two years at 0^{o} C. and relatively high humidity and found no perceptible changes in flavor character. Woodroof reported that by heating pecans to just over 180^{o} C. for three minutes, oxidative enzymes could be denatured resulting in an improved nut; the only drawback was that a slight roasted flavor resulted (12). Warchaw and Whitehead (13) refined pecan oil and found it comparable to other high grade oils, such as olive oil. They found that no rancidity developed after storage at room temperature for twelve months. The oil could be used in such diversified products as salad dressings, cold creams, and even cooking fats. This oil had the following composition: oleate,

77.8%; linoleate, 15.8%; myristate, 0.04%; arachidate, 0.09%; palmitate, 3.2%; stearate, 1.8%; cholesterol, 0.28%; and lecithin, 0.5%.

Woodroof and Heaton (5) have investigated the relationships between fats and fatty acids and the characteristic flavor and off-flavor in pecans. They report that the oil content of pecan varieties varied from 55 to 75% of the nut's weight and was primarily dependent upon the degree of maturity when harvested. The demand was greatest for nuts with a high oil content, as these were usually the highest in flavor. They also observed a highly significant negative correlation between oil content and increasing unsaturation (as measured by the iodine value). Oleic acid in pecans varied from 46.1 to 83.4% while linoleic acid varied from 10.7 to 47.8%. In this article, Woodroof lists kernel yield, shell thickness, and stability of the unshelled nuts; halves per pound, oil, moisture, and stability of the meats; iodine and thiocyanogen values of the oils; and the oleic, linoleic, and saturated fatty acids of over fifty-three varieties of pecans. Differences in saturation of the oils are directly related to filling or maturity. In general, the best developed kernels had more oleic acid and less linoleic acid; therefore a poorly filled pecan would be an excellent source of linoleic acid.

CHAPTER III

NATURAL FLAVOR OF PECANS

Introduction

Pecans, unlike many other nuts, are not usually roasted in order to be flavorful; they have their own characteristic natural flavor. This flavor is a combination of five qualities: (1) sweetness, (2) lipid nature of the nut, (3) texture, (4) astringency and (5) a characteristic aroma. It is this characteristic aroma that will be investigated here.

Reagents and Apparatus

Reagents

Gas Chrom Q, Carbowax 20M, OV-17. Applied Science Laboratories, State College, Pa.

Methylene Chloride, Ethyl Acetate, Potassium Hydroxide, Potassium Carbonate, Certified. Fisher Scientific Co., Fair Lawn, N. J.

Diethyl Ether. J. T. Baker Chemical Co., Phillipsburg, N. J.

Molecular Seive 3A and 5A, Helium, Nitrogen, Compressed Air. Linde Division, Union Carbide Co., Houston, Texas.

Apiezons N and T. James G. Biddle and Co., Plymouth Meeting, Pa.

Apparatus

Gas chromatographic (GLC) analyses were performed on a modified -

Barber Coleman Model 5000 equipped with a hydrogen flame detector as described by Waller (14). The oven on this instrument is circular and the columns can interchangeably be used on the combination gas chromatograph--mass spectrometer (GC-MS). Hydrogen gas for the flame was provided electrolytically with an Elhygen, hydrogen generator.

The low resolution mass spectrometer used was a prototype of the LKB-9000 combination gas chromatograph-mass spectrometer, constructed at the Karolinska Institute, Stockholm, Sweden, in the laboratories of Dr. Ragnar Ryhage, as described by Waller (14). Spectra were obtained under the following conditions: ionizing voltage, 70 eV (11 eV was used for low voltage spectra); accelerating voltage, 3500 V; trap current, 60 or 20 μamps; electron multiplier voltage, 1700 V (also 1900 and 2100 V when greater sensitivity was required); source temperature, 310° C.; scan speed, 5; paper speed, 4 (i.e. net result is a scan from mass 0 to 200 in 3 to 5 seconds). The gas chromatographic tracing was a measure of the total ionization current obtained from the collector plate in the analyzer tube. Spectra were plotted by a Calcomp Model 565 plotter driven by an IBM 360/50 computer as described by Li, et al. (15).

Nuts were chopped with a mechanical nut chopper or ground in a hand meat grinder. Oil was pressed from the nuts with a Carver Laboratory Press.

Gas chromatographic columns were packed with the aid of a fluidized bed apparatus which rolls the packing in a stream of hot (70° C.) nitrogen gas to coat each particle evenly (16).

An Omnimixer was used to blend pecans with mineral oil and glycerol.

The vacuum system consisted of a Welch Dual Stage Vacuum Pump rated at 0.1 μ and 140 liters/min. and a three stage oil diffusion pump.

Procedures

High Vacuum Falling Film Flash Evaporation

The most common methods of removing volatile flavor components from foods are solvent extraction and high vacuum stripping of volatiles. Since the pecan has an oil base, the latter method is the most applicable. A typical falling film flash evaporator is pictured in Figure 1. The main components are: (1) a reservoir for containing the oil or slurry, (2) a long surface for degassing, (3) cold traps for collecting the volatiles, (4) collection tubes for concentrating the volatiles, and (5) a vacuum source. This particular apparatus had a pump for continually recirculating the mixture, although a pump was not required. The pump worked best in a position immediately below the flask. The major problem with this apparatus was the tendency to form air bubbles in the lines. The procedure for degassing a finely ground slurry of peanuts has been described by Mason, et al. (17) and earlier by Herz and Chang (18). The apparatus in Figure 2 is a simplified version of a falling film flash evaporator. The main advantage was the addition of a degassing chamber for removal of most of the air before reaching the condensers. This allowed a higher vacuum to be maintained.

Transferring Volatiles Directly to a GLC Column

A modification of Day's (19) procedure for the transfer of volatile components directly from a trap to a GLC column has been used. Figure 3 shows the system used in this research. The trap containing the volatiles was connected to the injection end of a column with a U-bend. The column was then evacuated, the liquid nitrogen moved from the trap

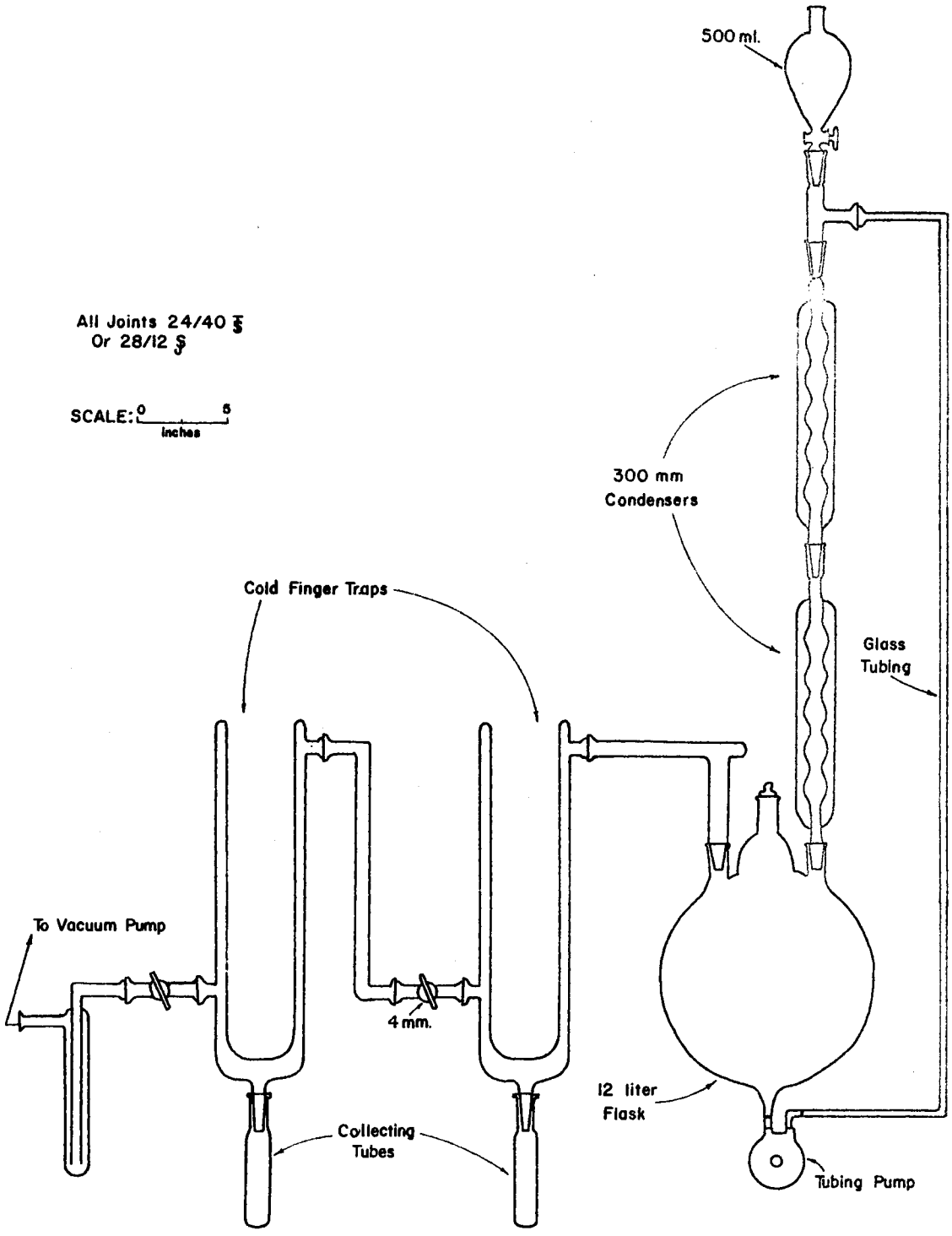


Figure 1. Falling Film Flash Evaporator With Recirculating Pump

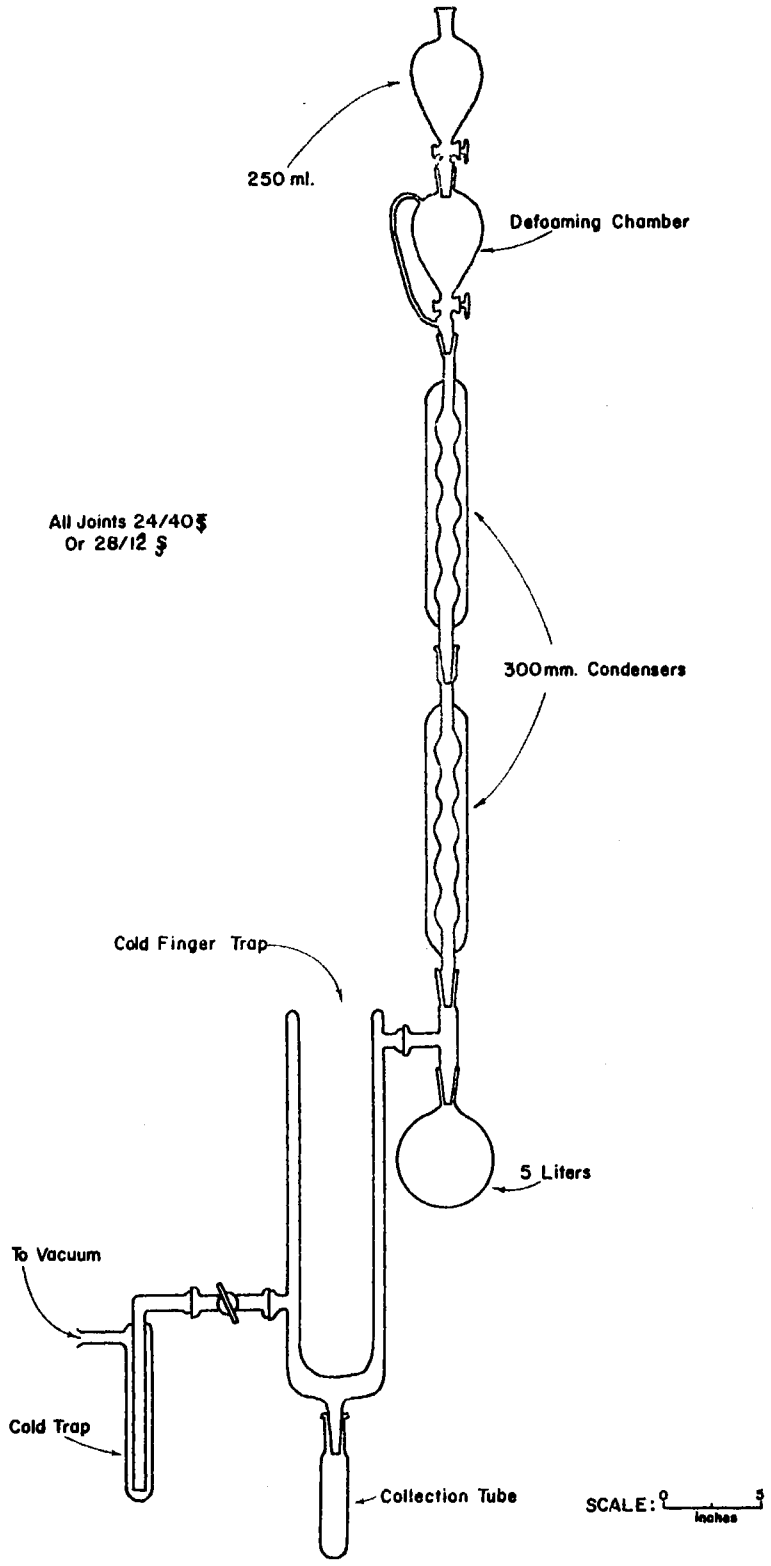


Figure 2. Falling Film Flash Evaporator

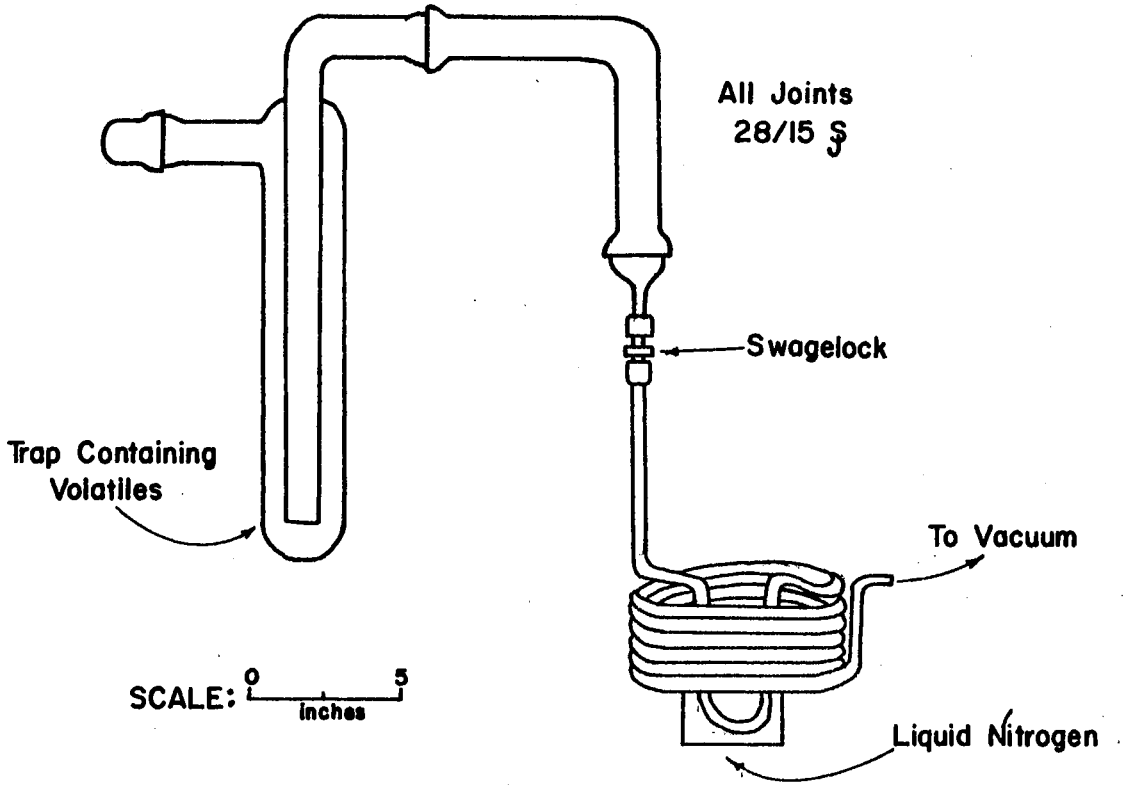


Figure 3. Transfer of Volatiles Directly From a Trap to a GLC Column

to the U-bend in the column and the trap warmed. The volatiles were then cryogenically pumped to the U-bend. Helium was bled in through the end of the column until the system was filled with helium. The column was then connected to a gas chromatograph, the liquid nitrogen on the "U" replaced with boiling water, and the chromatogram obtained. Figure 4 shows a U-bend that doubles as a trap for collecting volatiles. The stopcocks were manipulated so that once the volatiles were in the trap, they could be transferred to a column and GLC analysis accomplished as previously described.

Steam Distillation

The steam distillation apparatus used is shown in Figure 5. The basic components are: (1) a steam generator, (2) a flask for holding the pecans, (3) a condensor, (4) a collection flask, and (5) a series of traps for collecting the lower boiling components. A salt-ice bath was used in the first trap, dry ice and ethanol in the second, and liquid nitrogen in the third. These traps were employed mainly because there was a pleasant pecan-like odor emanating from the system. Distillation was continued until about one liter of water had been collected. No heat was used on the twelve liter flask, as earlier experiences showed a tendency towards roasting.

Sweeping Volatiles

The apparatus for sweeping volatiles from pecans is shown in Figure 6. Theoretically by grinding pecans to a fine state and filling the flask to maximum capacity, one should be able to sweep a considerable portion of the flavor components from the pecans. The aroma of the gas

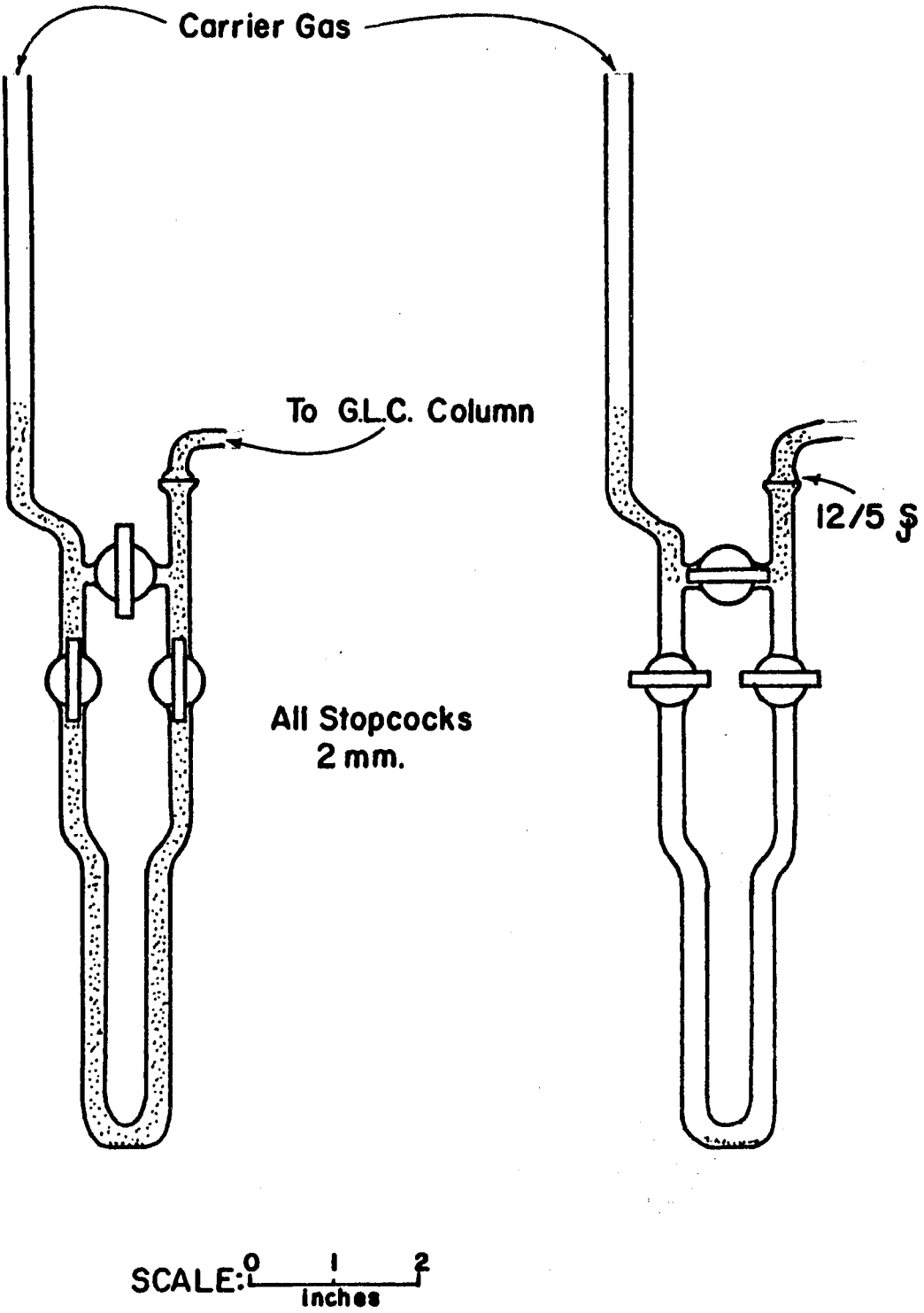


Figure 4. Combination Trap and GLC Flash Heater

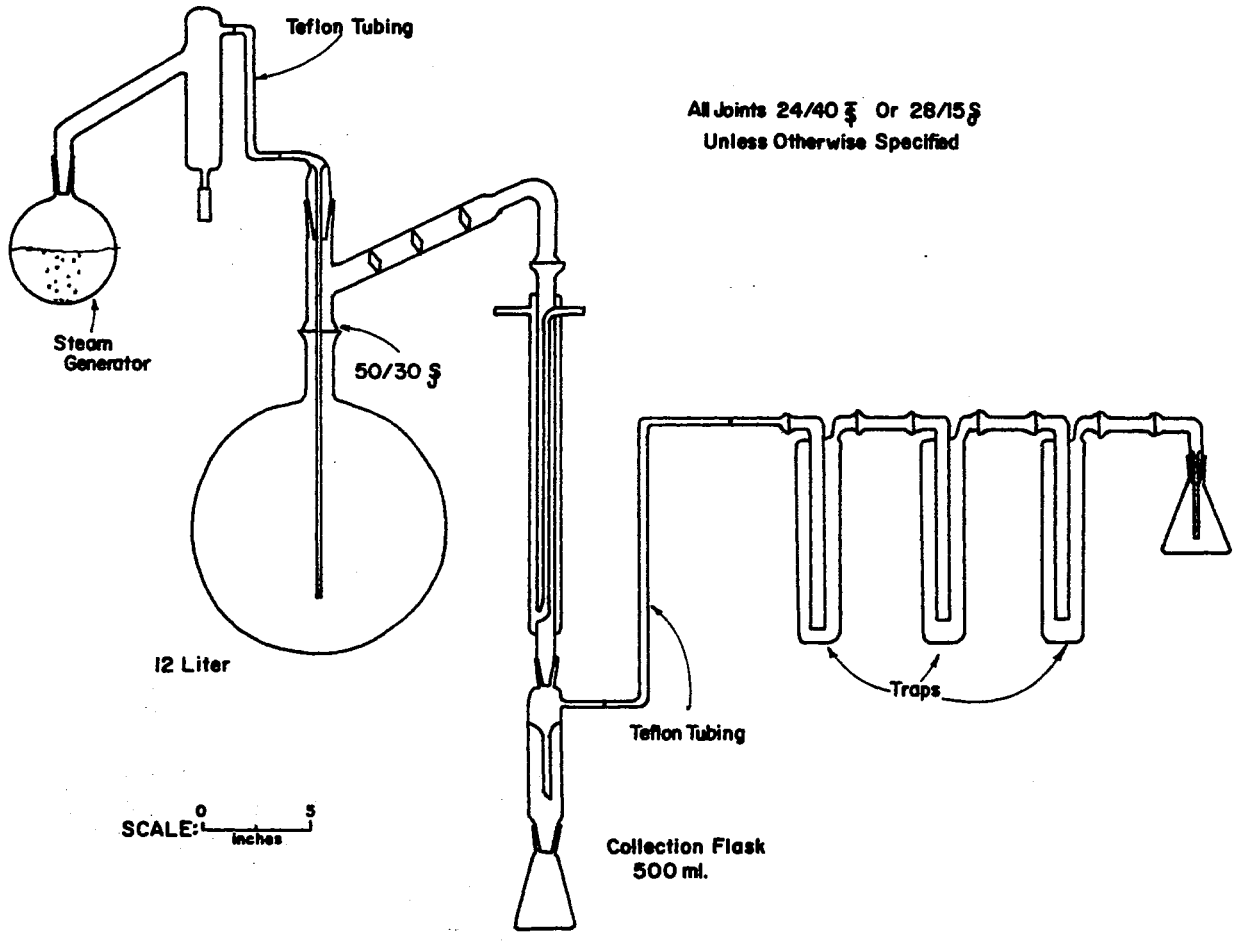


Figure 5. Steam Distillation Apparatus

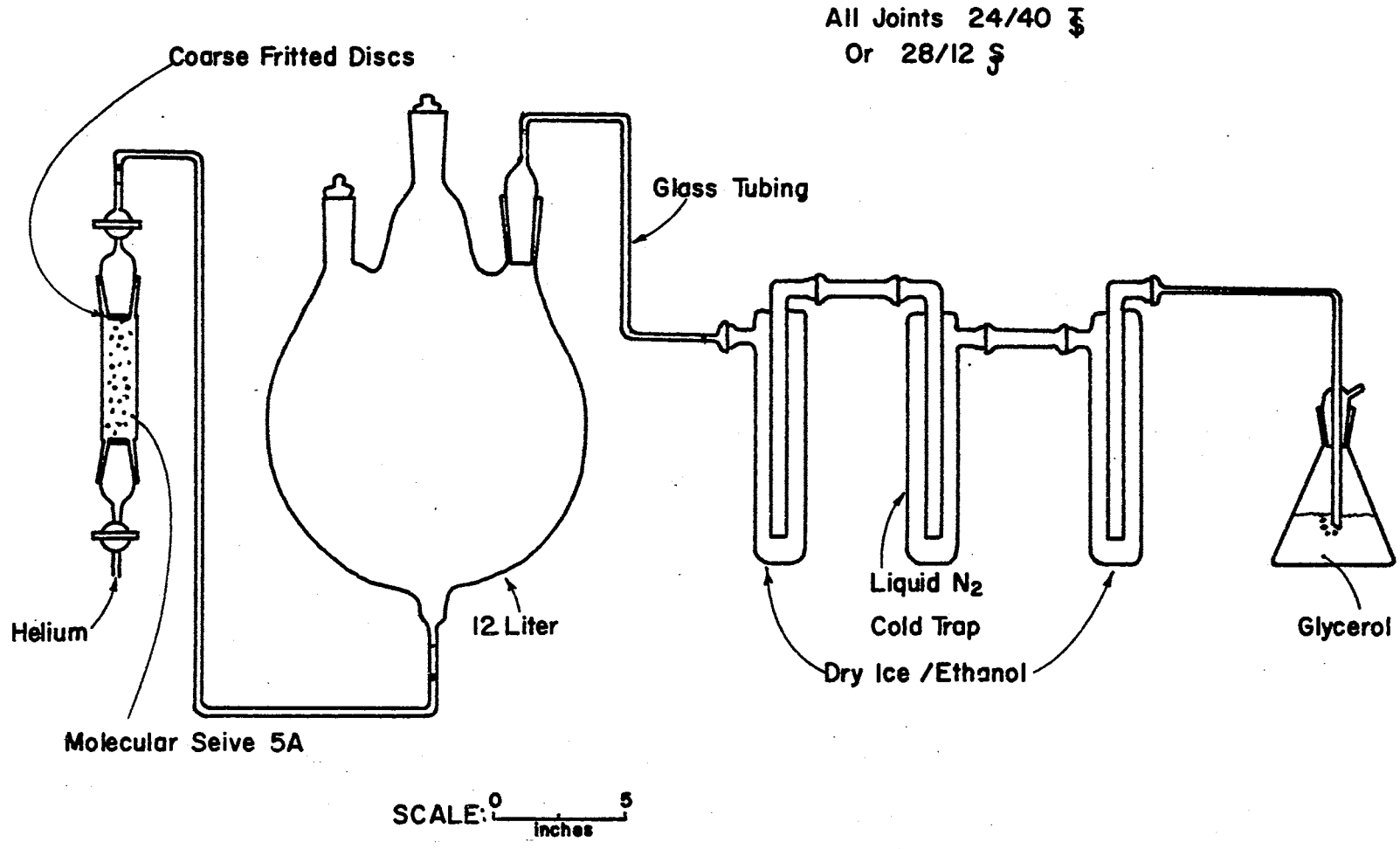


Figure 6. Apparatus for Trapping Volatiles Swept From Pecans

coming from the pecans indicated that this was feasible. Three traps were placed in line with a twelve liter flask; the first and third contained dry ice and ethanol, and the second contained liquid nitrogen. At the end of the train of traps, the gas was bubbled through glycerol to prevent back diffusion of vapors from the atmosphere. Nitrogen was initially used in this system, but since it liquified in the second trap, helium was used thereafter. The gas was initially passed over Molecular Sieve 5A to remove impurities. Rubber tubing was used at first but due to contamination by the tubing, the final apparatus was glass and teflon.

Pecan Aroma From Skin and Hull Wastes

Workers at the Georgia Experiment Station (20) have reported that high quality pecan aroma and flavor have been recovered from pecan skin and hull waste. In following their procedure, the skins and hull wastes (separately) were extracted with water and filtered; the aqueous extract was extracted with ethyl acetate (used by the Georgia workers), diethyl ether, and methylene chloride. These extracts were then concentrated and analyzed both organoleptically and by the combination GC-MS unit.

Vacuum Sublimation

The apparatus shown in Figure 7 was designed by this author and was one of the more successful methods for removing the flavor components. The main body consisted of a glass cylinder with a ridge at the bottom. Inserted in this cylinder was a smaller piece of glass tubing containing many small holes; this piece contained spacers and fitted over the ridge

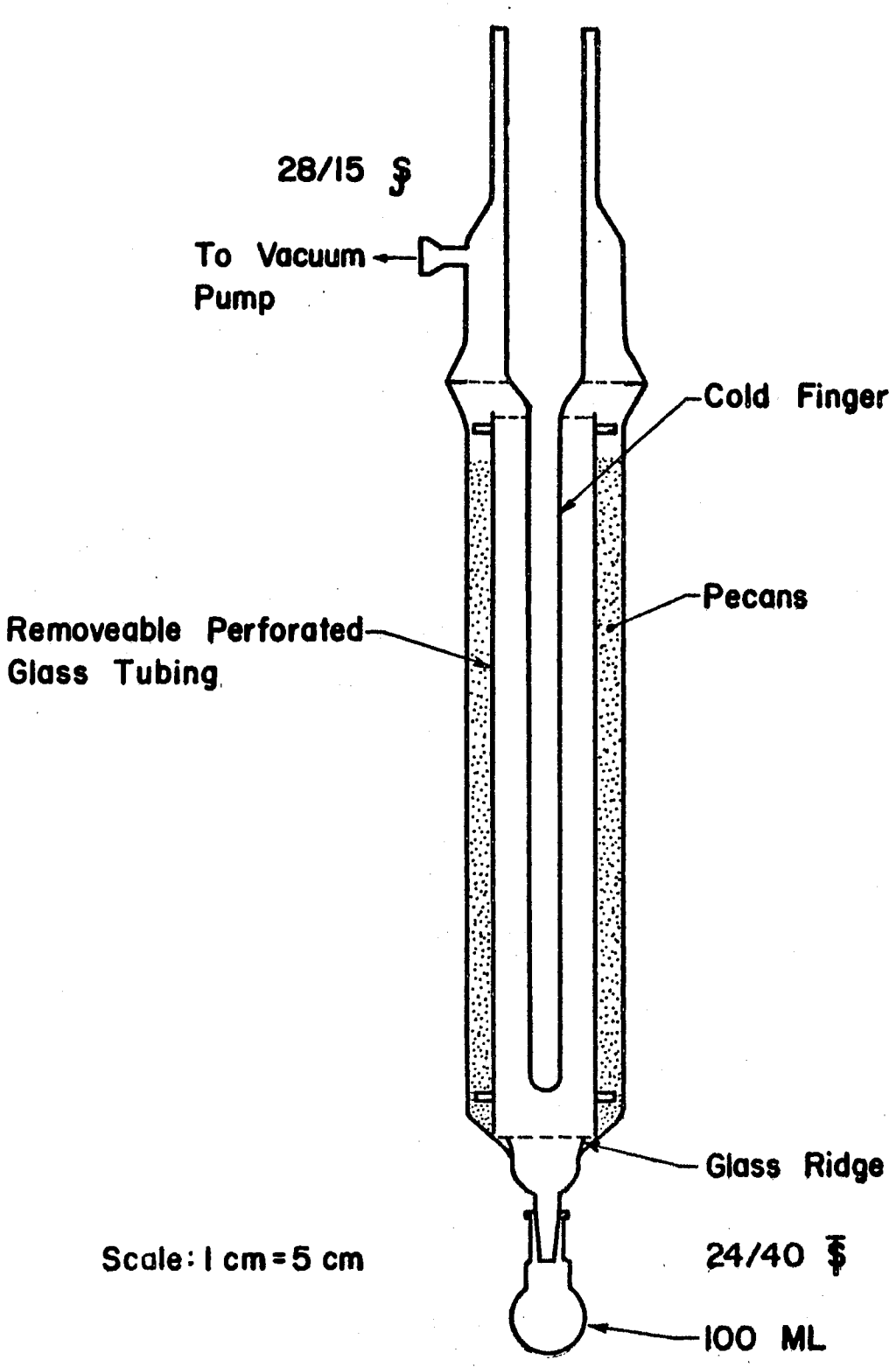


Figure 7. Vacuum Sublimation-Distillation Apparatus

at the bottom of the large cylinder. The air space between these two pieces was filled with ground pecans. The cold finger was then inserted into the inner chamber and filled with liquid nitrogen. A vacuum (35 μ) was applied to the system and the volatiles were distilled to the cold finger. After six hours, the vacuum source was removed and nitrogen gas bled into the system. The cold finger was then removed from the system, washed with methylene chloride, scraped, and further extracted. The condensate had a pleasant nutty aroma. This condensate was concentrated and subjected to combination GC-MS analysis.

Results and Discussion

Most of the early research was concerned with localization of the flavor components in the pecans. If the oil was extracted in a Soxhlet, the resulting meal was odorless and tasted like sweet flour. The oil had a greasy aroma but no typical pecan aroma. When pecans were blended in paraffin oil and centrifuged, the oil was odorless and the meal had a very strong pecan-like odor. Hence, in the high vacuum experiments, a ground pecan slurry was used.

High Vacuum Technique--Falling Film Flash Evaporator

Pecans were ground (in paraffin oil), and the slurry, which has a strong pecan aroma, was passed down the falling film flash evaporator. The volatiles collected initially smelled like pecans, but this aroma disappeared very rapidly due to either oxidation or volatilization of components, the net result being a beef broth aroma. It was hoped that on column trapping would eliminate these two problems. When the volatiles were collected, they were transferred, still in a vacuum, to the

U-bend in the column. Identification of the following twelve compounds was accomplished through combination GC-MS: pentane, diethyl ether, n-hexane, methylcyclopentane, benzene, methylcyclohexane, toluene, trichloroethylene, Freon 11, xylene, chloroform and carbon tetrachloride. Many of these may have come from the degassed paraffin oil or may be artifacts from the solvent. In any event, since there was not enough compound isolated by these techniques for identification, other techniques were selected.

Steam Distillation

In the experiment with steam distillation, the material from the dry ice-ethanol trap was transferred directly to a column. To minimize contamination, pecans that had not been stored in the vicinity of organic solvents were used, as oilseeds tend to pick up any aromas present. Upon combination GC-MS analysis of the trap, three compounds were isolated: dimethyl disulfide, methanol, and acetaldehyde, but whether they were truly part of the natural aroma or just heat-produced artifacts is still questionable.

Sweeping

Pecans were swept with helium for 24 hours. If the effluent coming from the flask of pecans was disconnected, a strong pecan-like aroma was observed. When the trap was reconnected, this aroma could not be detected coming from the other side of the trap. Hence the volatiles were being trapped. Subsequent GC-MS analysis showed that there was insufficient compound present for mass spectral analysis. A similar technique, head space analysis, gave no results.

Pecan Aroma From Skin and Hull Wastes

This was one of the more successful experiments in that the aroma of the extract had some pecan-like qualities. Hull wastes gave a caramel, brown sugar-like aroma, whereas the pecan skins gave a typically pecan aroma. The ethyl acetate extract was quite pecan-like, while the ether extract had an oxidized aroma, much like beef broth. This same aroma had been observed in the past. These fractions were then subjected to combination GC-MS analysis. The GLC tracing, column conditions and mass spectra are given in Figure 8. The vertical slash marks along the tracing indicate the points at which a mass spectrum was taken. The top chromatogram represents the ether extract, concentrated; the center chromatogram is the same as the top, further concentrated to remove more solvent; the bottom chromatogram represents the ethyl acetate concentrate. The reason for reporting both the first and second chromatograms is that air was mistakenly used to concentrate the sample. Instead of both major peaks increasing proportionally, the first peak decreased in intensity, while the second increased, indicating possibly an oxidation of one compound to the other. This view was supported by the oxidation problems encountered in the latter stages of the research. Component a had an M^+ 206, while component b had an M^+ 208, indicating possibly a hydroquinone to quinone oxidation. Component c had the same spectra as b, and component d had an M^+ 222. Component e was present in low concentration and had an M^+ 220 and a base peak at m/e 205. The spectrum of component e was similar to those of β-ionol and butylated hydroxyanisole. The author does not wish to speculate further on the structure, except to say that in regard to the base peaks, the 109 peak in component a could be from a substituted hydroquinone and the 108 peak in

Figure 8. Combination Gas Chromatographic - Mass Spectrometric Analysis of Flavor Components Obtained From Pecan Skins

Column conditions were as follows:

Column - 10' x $\frac{1}{4}$'' glass

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

Column Temperature - 160°C.

Injection Port Temperature - 200°C.

Separator Temperature - 210°C.

Carrier Gas - Helium

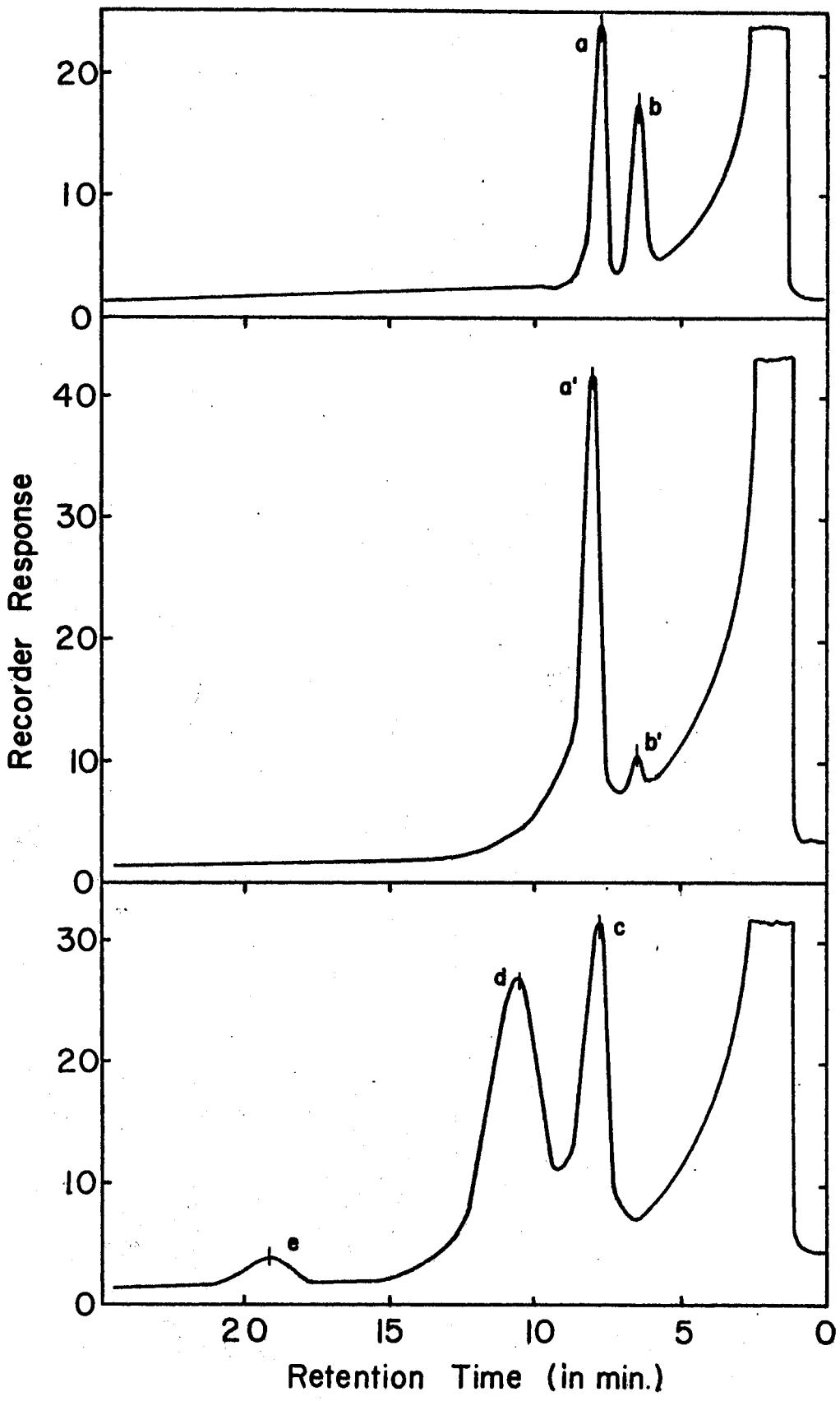
Flow Rate - 21 ml./min.

Top = Ether concentrate of aqueous pecan skin extract

Center = Top concentrated two-fold

Bottom = Ethyl acetate concentrate of aqueous pecan skin extract

Mass Spectrometer Log Number - 1736; Code 26 (i.e. author's personal file)


a
b
a'
b'
c
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e
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Retention Time (in min.)
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15
10
5
0

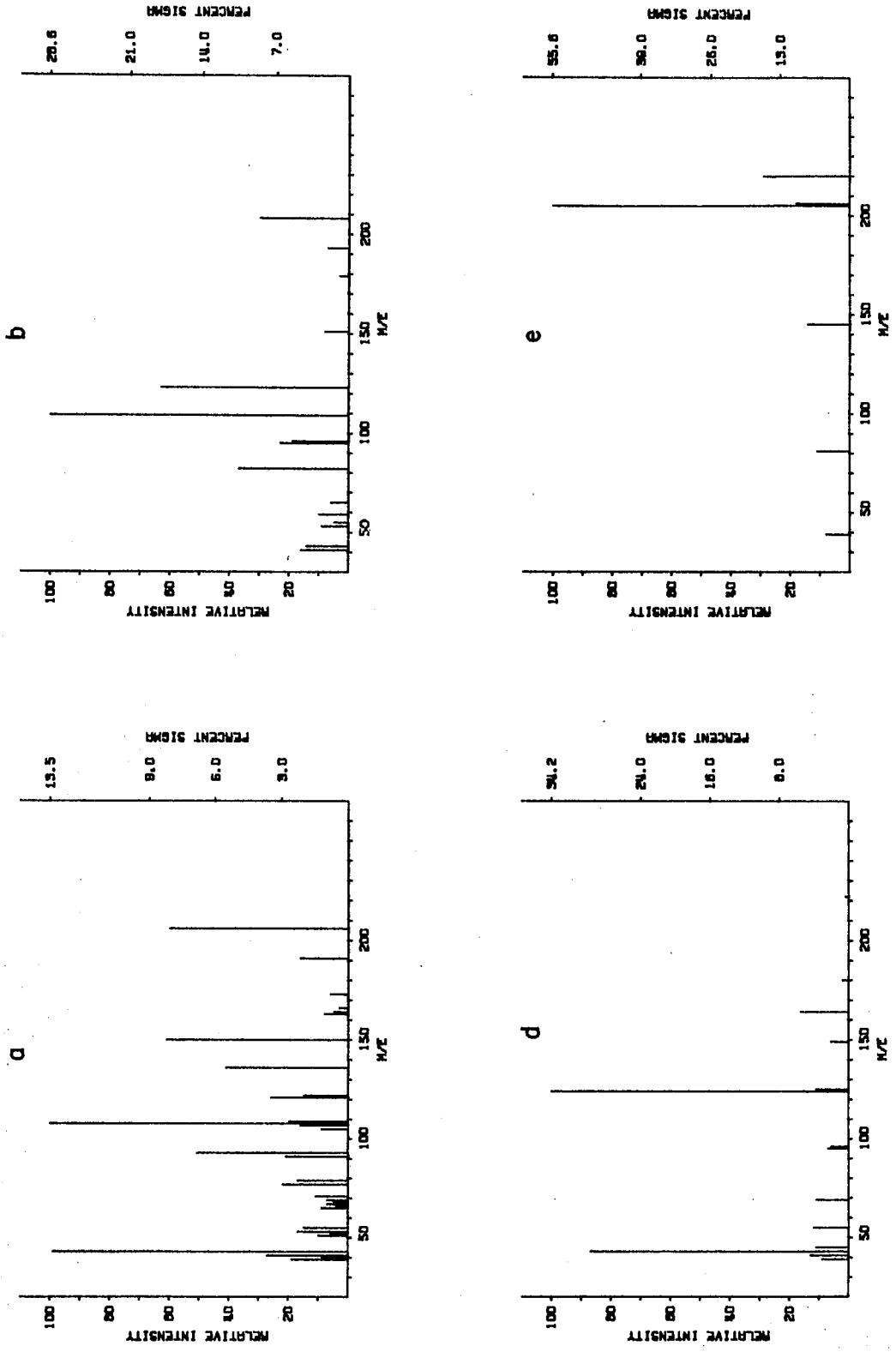


Figure 8 (Continued)

component b could be from a substituted quinone. If pyrogallol was added to the aqueous extract of the skins, the odor was enhanced, lending credibility to the argument.

When this experiment was repeated, sufficient material could not be obtained for mass spectral identification. This procedure was not pursued further because pecan skins were very difficult to obtain and the concentrate was greasy and tended to clog the syringes. The greater number of skins that were extracted, the oilier the concentrate.

Vacuum Sublimation

The first time vacuum sublimation was attempted, solid injection GLC (21) was used for analysis, as the components appeared relatively non-volatile, but no results were obtained. It was noted that a pleasant pecan-like aroma was attained with this procedure. This method was ignored for a while, since attention was being given the method using pecan skins. Several months later this technique was again tried, with the modification that a different column and method of injection were used.

The method of concentrating the extract may have been the reason for the results obtained. The methylene chloride was evaporated (100 ml initial volume), and the sample was concentrated to 5 to 10 μl (a factor of 10^4), of which 5 μl was injected. This was accomplished using a stream of nitrogen gas and taking the volume to about 1 to 2 ml. The material was then transferred to a modified Pasteur pipette (the end removed and sealed in a flame, forming a cone shaped well) which allowed the material to be concentrated into such a small volume. Some of the volatiles may have been lost during evaporation but due to the low boil-

Figure 9. Combination Gas Chromatographic - Mass Spectrometric Analysis of the Volatile Components From Raw Pecans

Column conditions were as follows:

Column - 20' x $\frac{1}{4}$'' glass

Column Packing - 20% Carbowax 20M on Gas Chrom Q

Column Temperature - Programmed, 65 - 158° C. @ 2°/min.

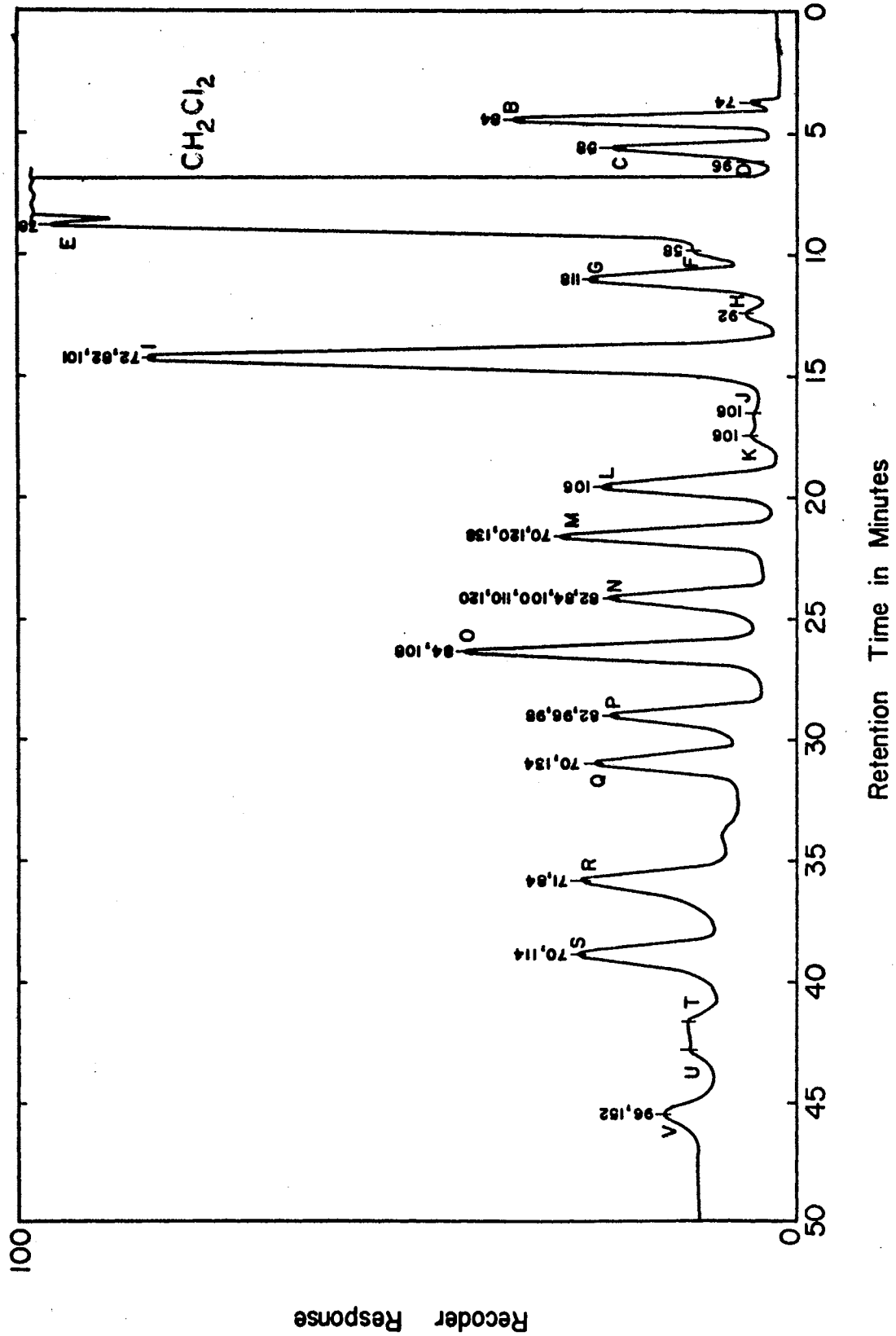
Injection Port Temperature - 180° C.

Separator Temperature - 200° C.

Carrier Gas - Helium

Flow Rate - 20 ml./min.

Mass Spectrometer Log Number - 1396; Code - 32


Recoder Response
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Retention Time in Minutes
CH_2Cl_2
74
B 84
C 58
D 96
E 76
F 58
G 118
H 92
I 72,82,101
J 106
K 106
L 106
M 70,120,138
N 82,84,100,110,120
O 84,108
P 82,96,98
Q 70,134
R 71,84
S 70,114
T
U
V 96,152

ing point of methylene chloride, the loss would have been minimal.

An OV-17 column was first used for the GLC analysis but poor peak resolution resulted. A carbowax column was used next and the results seemed satisfactory. The GLC tracing along with column conditions is shown in Figure 9. Mass spectra were obtained at 70 eV of each peak, but several had what appeared to be two M^+ ions. Consequently after obtaining the first chromatogram, the remaining 5 μl was injected and all spectra were taken at 11 eV. The slash mark at the top of the peak indicates the point at which a spectrum was taken. The numbers at the top of each peak indicate possible M^+ ions observed in the low voltage spectra. Unfortunately, many of these symmetrical peaks appeared multi-component; but it was still possible to speculate as to the identification of several compounds. The major component, peak I, appeared to contain primarily hexanal. Other prominent peaks at m/e 44, 57, 72 and 82 tend to support this conclusion. Peak P contains furfural (M^+ 96); this was apparent due to the two strong peaks of about equal intensity at m/e 95 and 96. Peak S showed an intense ion at m/e 99, which dwarfed most of the remaining peaks. A small peak at m/e 114 was noted and it was assumed to be the parent ion. No further identification was possible due to the poor separation. Capillary column GC-MS would be the best solution to this problem.

The mixture was then analyzed on a 500' x .02" capillary column which was prepared by Dr. Bob Johnson for use on roasted peanut volatiles. The GLC tracing (along with column conditions) and mass spectra shown in Figure 10 were obtained from this experiment. Mass spectral reference standards are given in Appendix D (22). The added resolution from this column tripled the number of compounds observed and made pos-

Figure 10. Combination Capillary Column Gas Chromatographic - Mass Spectrometric Analysis of the Volatile Components From Raw Pecans

Column - 500' x .02" stainless steel capillary

Column Packing - Carbowax 1540

Column Temperature - Programmed, 65-170° C. @ 1°/min.

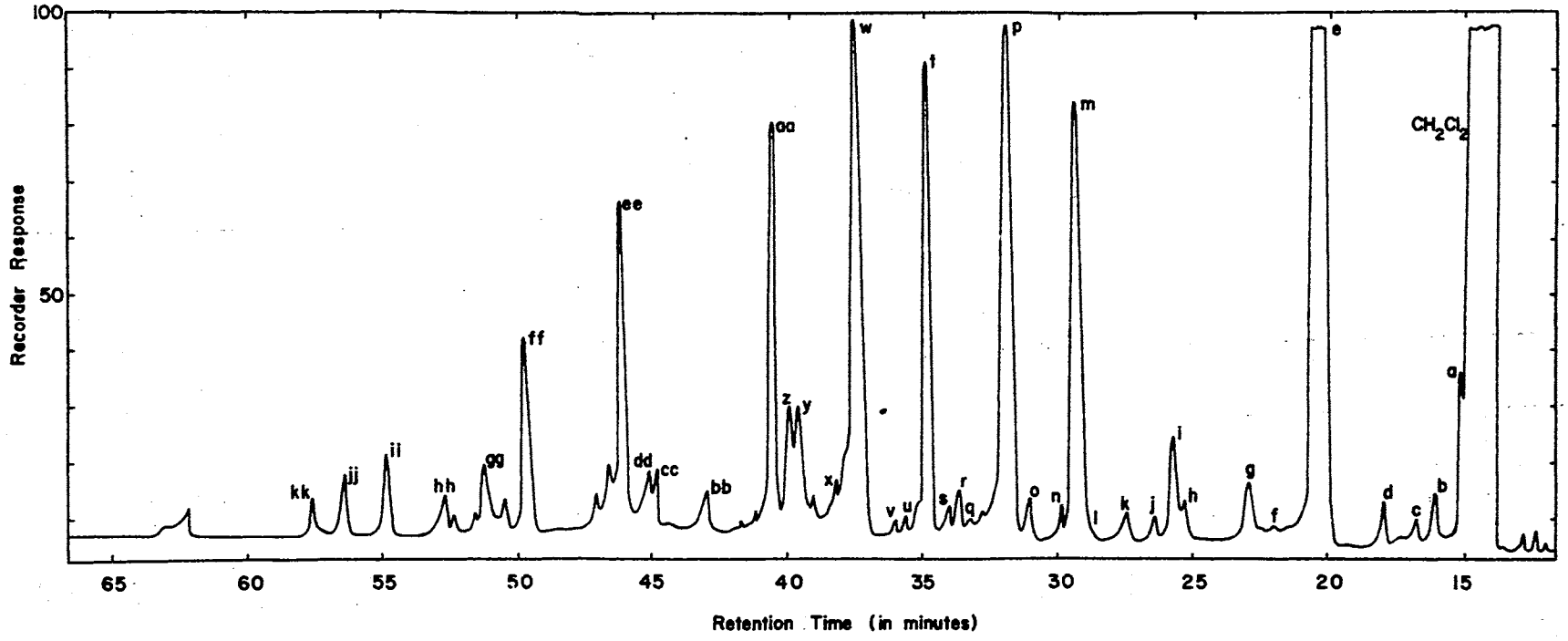
Injection Port Temperature - 190° C.

Separator Temperature - 200° C.

Carrier Gas - Helium

Flow Rate - 3.7 ml/min (calc.)

Mass Spectrometer Log Number - 1748; Code - 41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t
s
r
q
p
o
n
m
l
k
j
i
h
g
f
e
d
c
b
a
CH_2Cl_2
65
60
55
50
45
40
35
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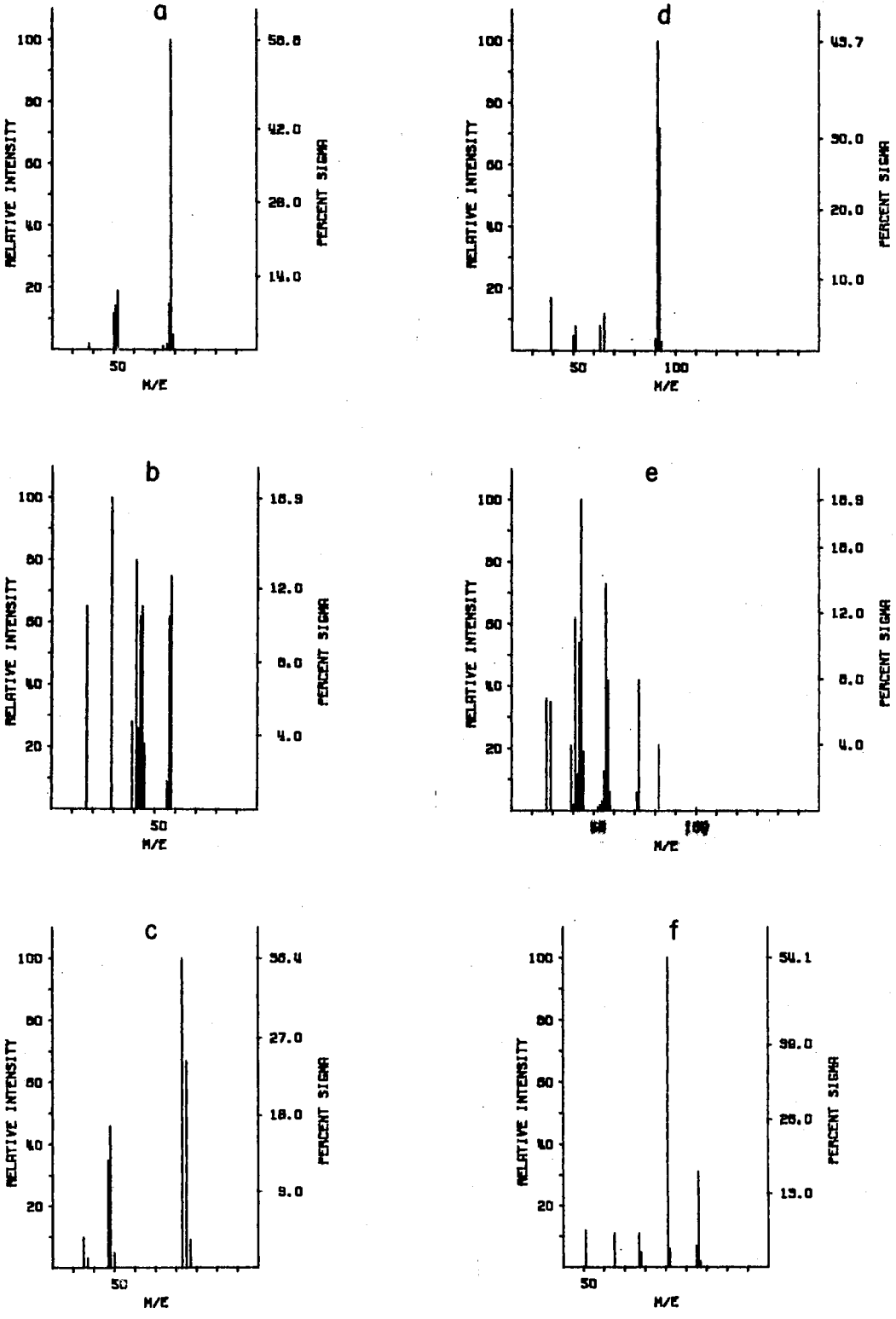
20
15
Retention Time (in minutes)



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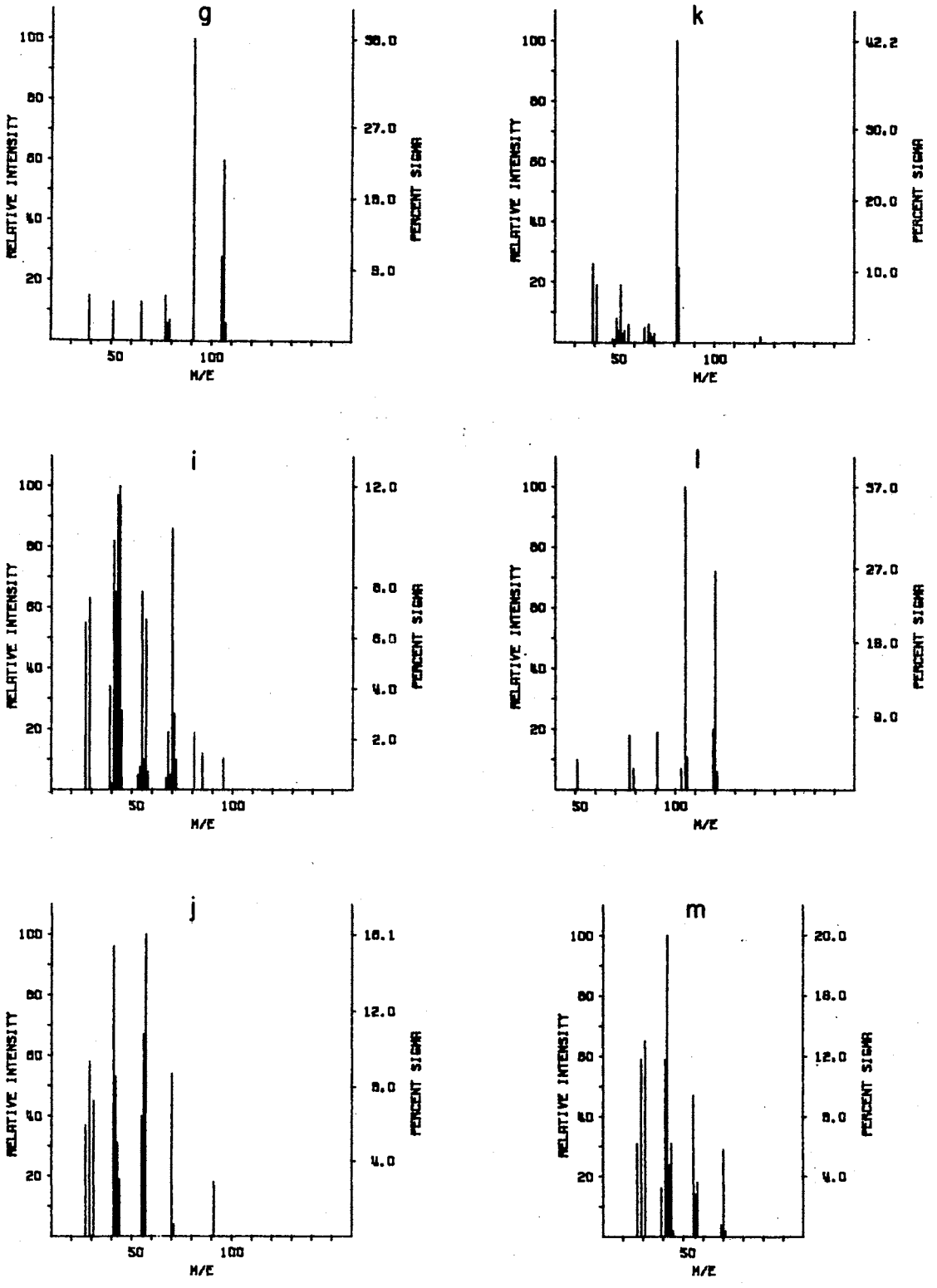
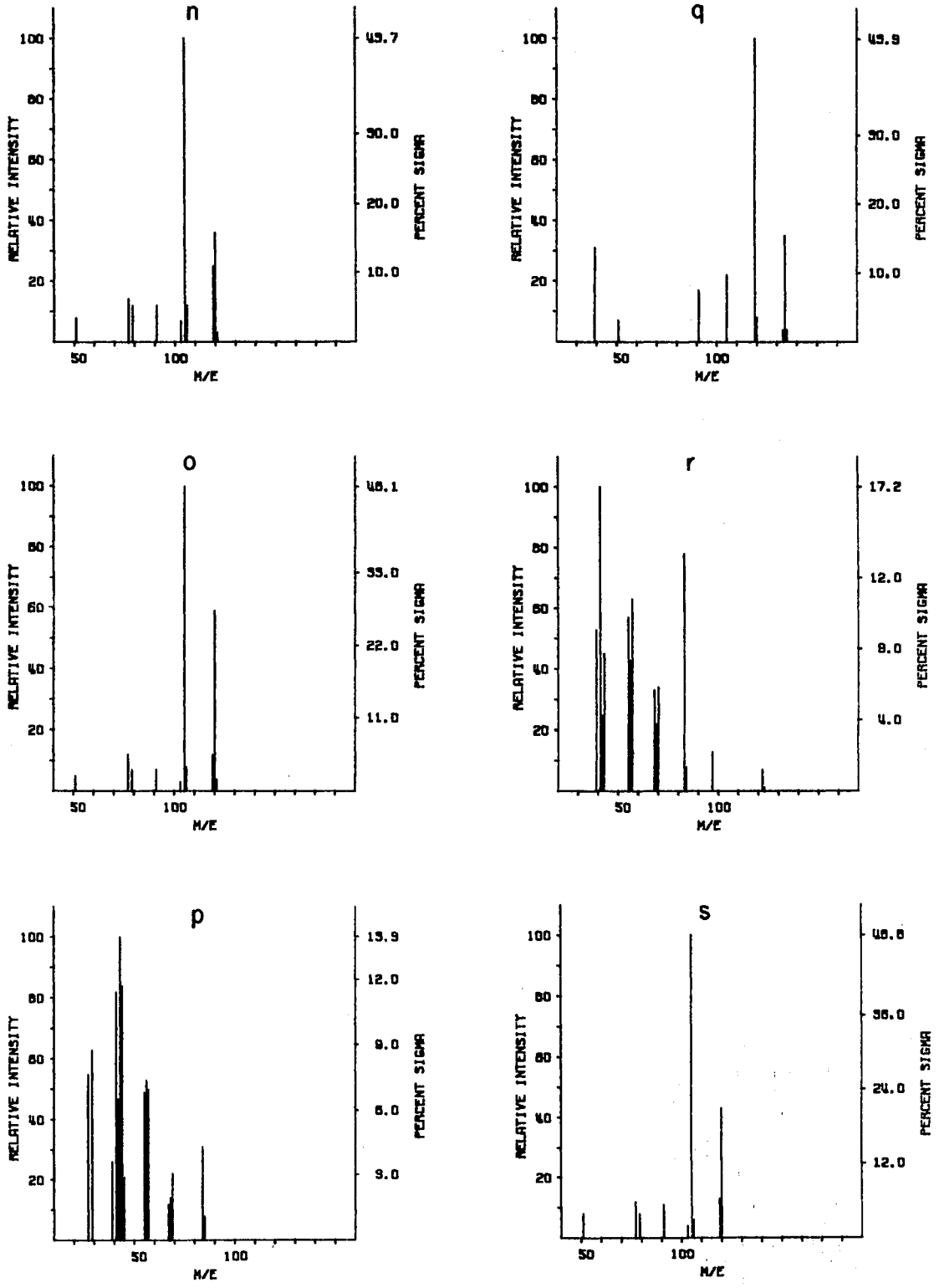


Figure 10 (Continued)



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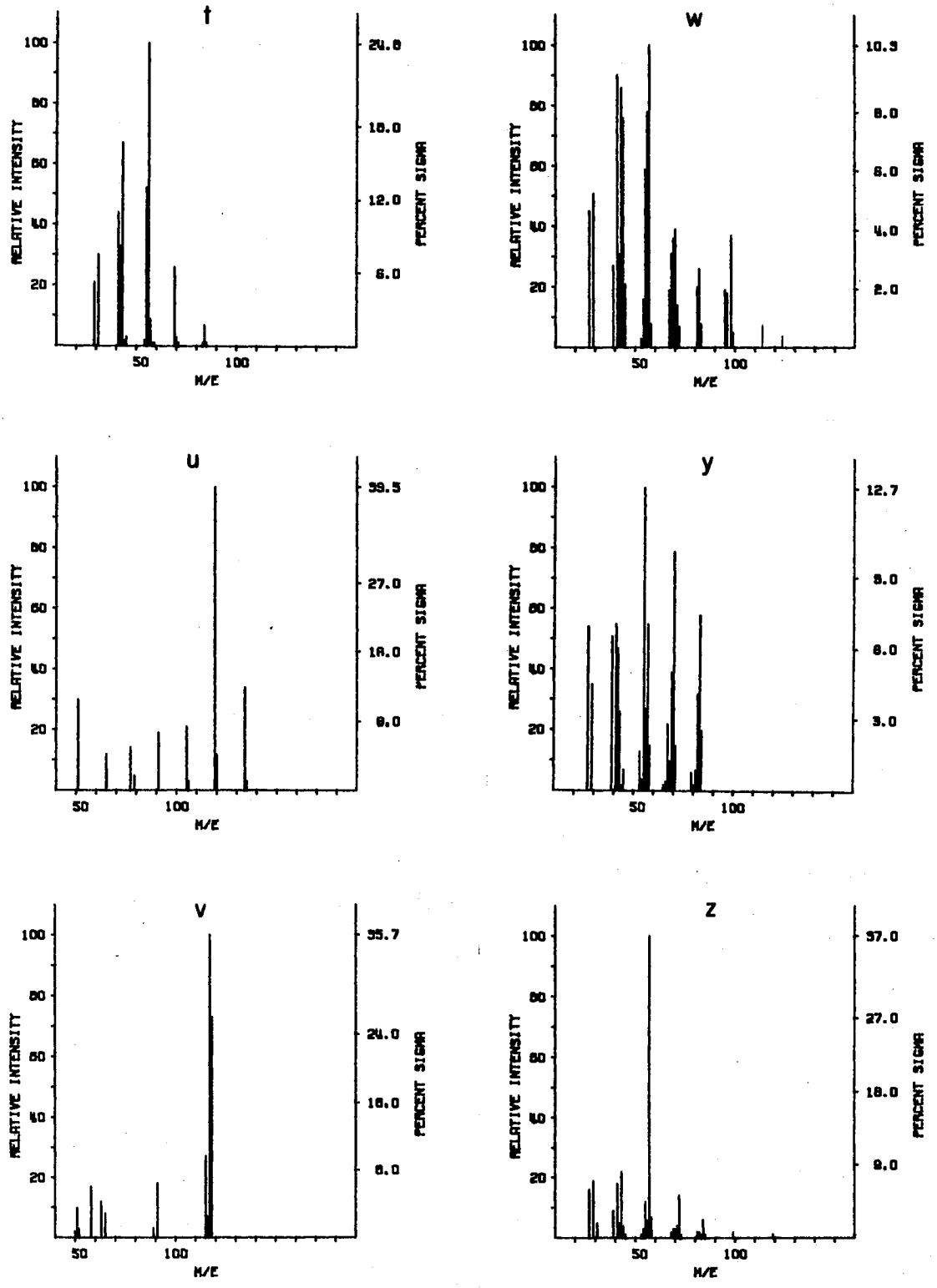


Figure 10 (Continued)

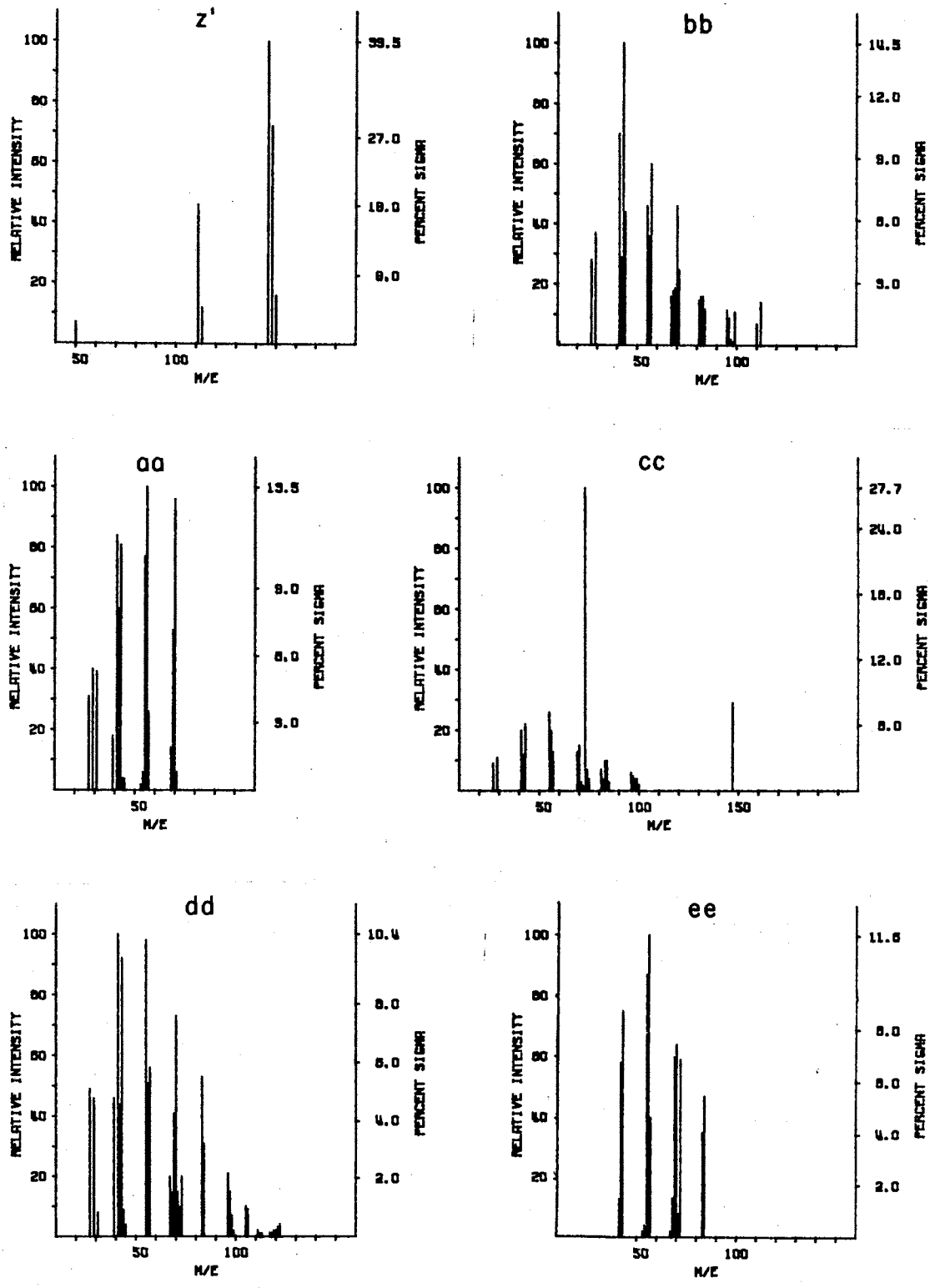


Figure 10 (Continued)

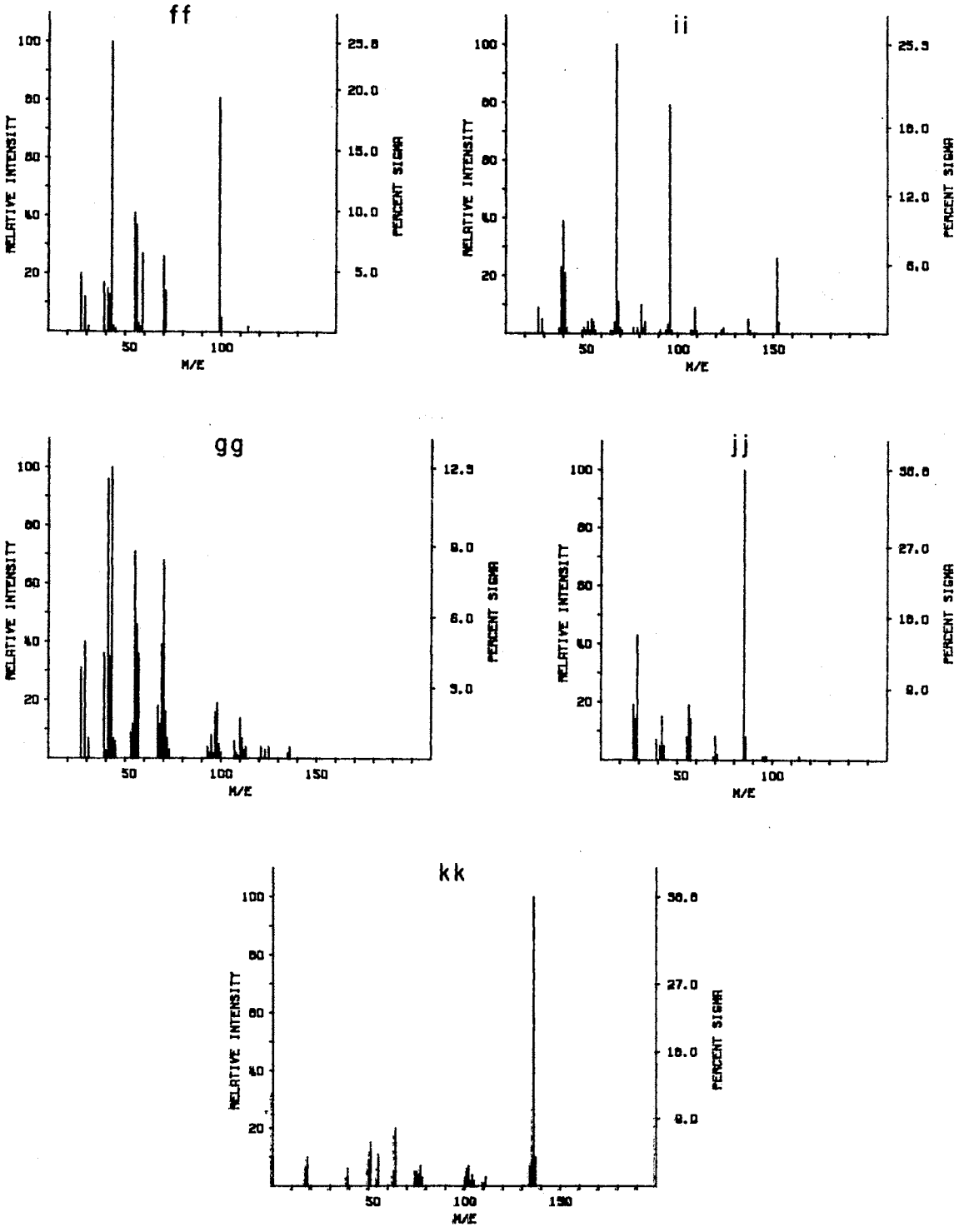
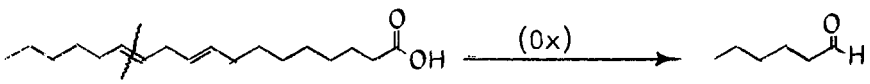


Figure 10 (Continued)

sible the identification of about two-thirds of the major components present. The identifications presented here are tentative.

Components a through d were probably solvent impurities. They were identified as follows: a, benzene; c, chloroform; and d, toluene. Component b was not identified.

Component e was identified as n-hexanal and represented the compound present in the highest concentration in the extract. This was first thought to be a mixture of several components due to the abundance of fragment peaks with even m/e values (i.e. 44, 56, 72, 82). Hexanal undergoes elimination of water to produce m/e 82 (M^+-18) and ethylene to produce m/e 72 (M^+-28) as well as β-cleavage to yield ions at m/e 44 and 56. Hexanal might be expected to be present, as it would be one product of linoleate oxidation as depicted below:



Linoleic Acid Hexanal

Components f and g were probably solvent impurities from the methylene chloride, since they were identified as ethylbenzene and xylene respectively. Component h was not identified due to interference of background peaks.

Component i did not have a distinguishable parent ion. The peak with the greatest m/e in this spectrum was at m/e 85. The spectrum most closely resembled heptanal, another product of fatty acid oxidation. The M^+ in heptanal would be at m/e 114; the peak at m/e 85 would represent the loss of -CHO from the parent ion. As in hexanal, the base peak was at m/e 44. The remainder of the spectrum closely resembles

that of n-heptanal.

Component j was a minor component and was not identified. A primary alcohol was indicated by the moderately intense peak at m/e 31. The molecular ion was not present and the general fragmentation pattern did not give a clue as to the structure.

Component k had an M^+ 138 and the spectrum was very similar to that of amylfuran as published by Johnson (23). Most furans arise from the pyrolysis of sugars, but this particular compound probably originated from the oxidation of linoleate, followed by cyclization. Component l apparently was another solvent impurity. It had an M^+ 120 and the spectrum most closely resembled that of 1,3,5-trimethylbenzene. Component m, another major component, had an intense peak (R.I. 69%) at m/e 31 indicating another primary alcohol. The spectrum most closely resembled that of 1-pentanol. No M^+ (m/e 88) was present, but there were peaks at m/e 70 (M^+-H_2O) and m/e 57 ($M^+=CH_2-OH^+$). The base peak was at m/e 42 (M^+-46) and could arise from the loss of water and ethylene with the subsequent formation of propene (m/e 42).

Component n had an M^+ 120. This component was tentatively identified as a methylethylbenzene. Component o had the same molecular weight as component n, and was assumed to be another solvent impurity. The spectrum most closely resembled 1,2,3-trimethylbenzene.

Component p was another aldehyde, n-octanal. In addition to the hydrocarbon fragments present in the spectrum (m/e 29, 43, and 57), typically diagnostic peaks were found at m/e 44 and 84 (M^+-44) both of which resulted from β-cleavage of the aldehyde with hydrogen migration to produce $CH_2=CH-O^+H$. Peaks representing loss of water (m/e 110) and loss of ethylene (m/e 100) were less than 1% R.I. which is characteris-

tic of alphatic aldehydes.

Peaks q, r, and s were additional minor components of this fraction. Component q was an alkyl benzene (M^+ 134). The spectrum which it most closely resembled was 1,4-dimethyl-2-ethylbenzene. Component s (M^+ 120) was another trimethylbenzene. Component r had an M^+ 122 and was not identified.

Component t was a major constituent of the concentrate. The moderately intense peak at m/e 31 suggested that it was a primary alcohol. As pentanol was eluted from the column at 29 minutes, the next alcohol expected would be n-hexanol. The spectrum agrees very well with that of n-hexanol. The base peak in n-hexanol, m/e 56, results from loss of water and ethylene yielding 1-butene. The molecular ion was not present but a peak at m/e 84 (M^+-18) was observed.

Components u and v appeared to be more solvent impurities. Component u had a spectrum which closely resembled 1,2-dimethyl-4-ethylbenzene. The most intense ions of component v were m/e 117, 118 and 115 suggesting the presence of dihydroindane.

Component w was a major component, n-nonanal. Diagnostic peaks present were, as in other aldehydes, a result of β-cleavage with hydrogen migration producing ions at m/e 44 and 98. The base peak is at m/e 57.

Components y and z were not identified although their spectra are included for reference. A minor component under the z peak (designated z') was identified, however. This minor component was an isomer of dichlorobenzene, another solvent impurity.

Components aa and bb appear not to be artifacts from the solvent. Component aa is an alcohol, n-heptanol. Component bb is n-decanal.

The spectra of these compare favorably with their respective standards. Components cc and dd were not identified. Component cc had a base peak at m/e 73. Component dd had a small peak at m/e 31 suggesting a primary alcohol.

Component ee was identified as n-octanol. There is a peak of medium intensity at m/e 31 and the rest of the spectrum along with the approximate elution time tended to confirm this identification. Components gg, hh and kk were not identified.

All of the products identified at this time could result from the oxidation of oleate and linoleate triglycerides. It is the opinion of this author that a large portion of typical pecan aroma is due to this slight oxidation. There is, however, a sweetish aroma that blends with this oxidized aroma to produce the typical pecan sensation. This portion of the aroma may be due to a combination of components ff, ii, and jj, none of which were identified.

The spectrum of component ff was characterized by two strong peaks at m/e 43 (100% R. I.) and 99 (81% R. I.). There was a very small peak at m/e 114 which may have been the molecular ion. The only compound that exhibits a significant peak at m/e 99 is 2,5-hexadione but the remainder of the spectrum did not agree with the spectrum of hexadione.

Component ii had an M^+ 152 and a base peak at m/e 68. This compound was not identified but had a spectrum similar to mono-oxygenated monoterpenoids (22)..

In component jj, over 40% of the total ionization was due to the base peak, m/e 85. This component had a molecular ion at m/e 114. The rest of the spectrum compares favorably with γ-caprolactone.

TABLE I

SUMMARY OF COMPOUNDS TENTATIVELY IDENTIFIED FROM RAW PECANS BY COMBINATION GAS CHROMATOGRAPHY-MASS SPECTROMETRY

Peak	M^+	Compound
e	100	Hexanal
i	114	Heptanal
k	138	Amylfuran
m	88	1-Pentanol
p	128	Octanal
t	102	1-Hexanol
w	142	Nonanal
aa	116	1-Heptanol
bb	156	Decanal
ee	130	1-Octanol
jj	114	γ-Caprolactone

Summary

The characteristic aroma of pecans was investigated. Five methods were applied to the isolation of the volatile organic compounds which comprised this fraction; the most successful technique was vacuum sublimation. Four low molecular weight alcohols, five low molecualr weight aldehydes, and a lactone were the major components of natural pecan aroma. These compounds could have resulted from the oxidation of unsaturated fatty acids. In addition the spectrum of a compound (ii) that may be significant in pecan aroma was obtained but the compound could not be identified.

CHAPTER IV

ROASTED FLAVOR

Introduction

For centuries man has known that roasting is necessary for the full development of the flavor of many foods. It is only in the last decade, however, that food researchers have shown that the flavor characteristics of many diverse foods are due to a relatively few chemical substances. Pyrazines and related compounds have been found to be ubiquitous in roasted flavors and represent perhaps the most significant discovery resulting from this research.

Mason (17) identified n-methylpyrrole, 2-methylpyrazine, 2,5-dimethylpyrazine, and several other alkylated pyrazines from roasted peanuts. Pyrazines have since been found in many other roasted foods, including cocoa (24, 25, 26, 27, 28), potato chips (29) and coffee (30, 31, 32, 33, 34) and may be responsible for the "taste" of such non-food commodities as cigarettes (35). Johnson (23) and Koehler (36) have written reviews on the occurrence of pyrazines in foods, mechanisms of their formation, and significance in flavor. Shu (37) has recently investigated the volatile components in peanuts subjected to different fertilization, curing and harvest dates.

Reagents and Apparatus

Reagents

The reagents used in this portion of the research are listed under reagents in Chapter III.

Apparatus

Pecans were roasted in a General Electric rotisserie oven fitted with a wire basket with a hinged door for adding and removing nuts. This basket was cylindrical in shape and made of wire mesh, 6 inches in diameter and 13 inches long.

Oil was pressed from the pecans using a Carver Laboratory Press. One liter of oil was used for vacuum degassing.

The vacuum source consisted of a Welch Dual Stage vacuum pump, coupled with a three-stage oil diffusion pump, as described in Chapter III. Ground glass joints were greased with Apiezon N (for normal temperatures) or Apiezon T (for high temperatures, e.g. 100°C. or more).

Mass spectra were plotted by a Calcomp Model 565 Plotter, California Computers, Inc., Anaheim, California, as described earlier.

Procedures

Roasting Pecans

Pecans were roasted at 400°F. until the centers were light brown (30 - 40 min, depending on the quantity of nuts used).

Volatile Collection Using High Vacuum Techniques

Pecans that were roasted in the rotisserie were pressed to remove

the oil. The aroma-rich oil was stripped of its volatile components with the apparatus shown in Figure 11. This apparatus was designed to place the oil that was being stripped of volatiles as close to the cold finger as possible. The oil was first placed in the upper reservoir, then slowly added to the lower reservoir, or the defoaming chamber. The oil remained in the defoaming chamber until bubbling ceased, at which time it was drained into the ridge at the top of the degassing apparatus. Ideally, the oil will fill this ridge and spill over, coating the entire outer wall, but this was not the case. Due to orientation of the apparatus and surface tensions, the oil ran down the inner side in three to six channels. The apparatus was wrapped with a heating tape and heated to 120°C. to assure efficient removal of volatiles. When the oil reached the bottom of the degassing apparatus, it was diverted to the one liter flask to the side. After the degassing operation was complete, the liquid nitrogen was removed from the cold finger and placed under the 100 ml round bottom flask. Warm water (about 40°C.) was added to the cold finger and volatiles were cryogenically pumped to the round bottom flask. Nitrogen gas was slowly bled into the system until atmospheric pressure was reached; the flask was removed and the contents extracted as described below.

Fractionation of Volatiles

The flow diagram depicted in Figure 12 was used to separate the volatiles into various components. First methylene chloride was added to the flask containing the volatiles. The contents were then transferred to a separatory funnel and acidified with an equal volume of 0.1 N HCl. The aqueous layer was extracted with three one-half volumes

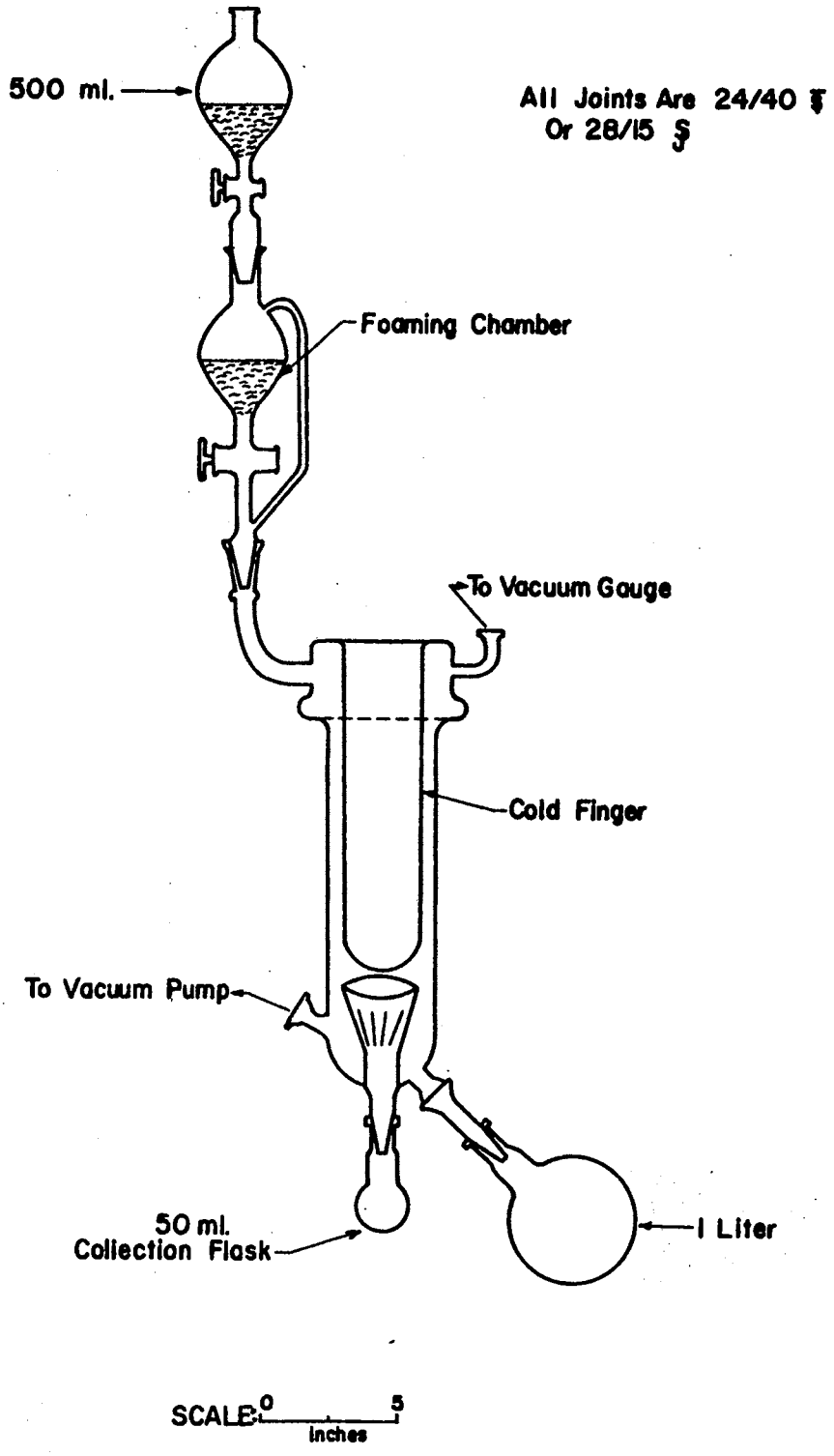


Figure 11. Apparatus for the Isolation of Flavor Components From Roasted Pecans

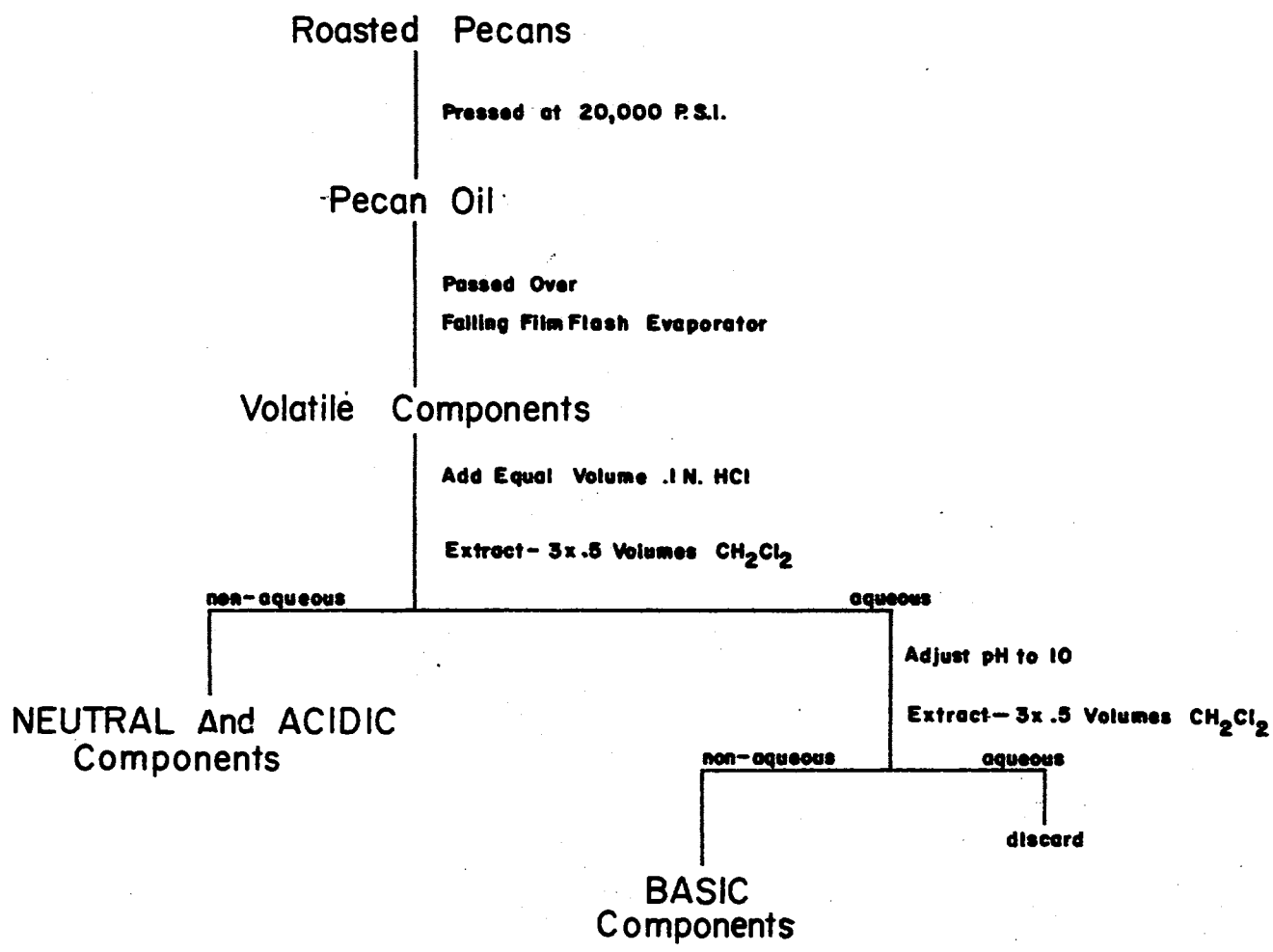


Figure 12. Flow Diagram of the Fractionation Process

of methylene chloride and the extracts were pooled to give the neutral components. The pH of the aqueous portion was increased to 10.0 and again this fraction was extracted with three one-half volumes of methylene chloride. These extracts were also pooled giving the basic fraction. This material was concentrated for GLC analysis by evaporating the solvent in a stream of nitrogen, then sealing off a disposable pipette and removing the last portion of solvent. This allowed concentration of 50 ml to less than 50 μl.

Roasting Under Nitrogen

It was necessary in one experiment to roast nuts under a nitrogen atmosphere. The apparatus shown in Figure 13 was designed for this purpose. It was simply a long glass tube about two inches in diameter wrapped with a heating element. Nitrogen gas was passed through the system during the roasting process which continued for one hour at 120°C.; then volatiles from the three traps were combined, dried with K_2CO_3 and analyzed by combination GC-MS. It might also be mentioned that the nuts were placed in the roaster overnight at 45°C., under a stream of nitrogen, to remove all oxygen prior to roasting.

Gas Chromatography--Mass Spectrometry

Gas chromatographic analyses were performed on a modified Barber Coleman 5000, equipped with a hydrogen flame detector. Column conditions used are given in Figure 14. The column was prepared according to the procedure of Cieplinski (39). Seven and one-half grams of Carbowax 20M were dissolved in 100 ml of methylene chloride; 3.5 g of NaOH were added to 100 ml methanol; 2 ml of the methanol/NaOH were added to the

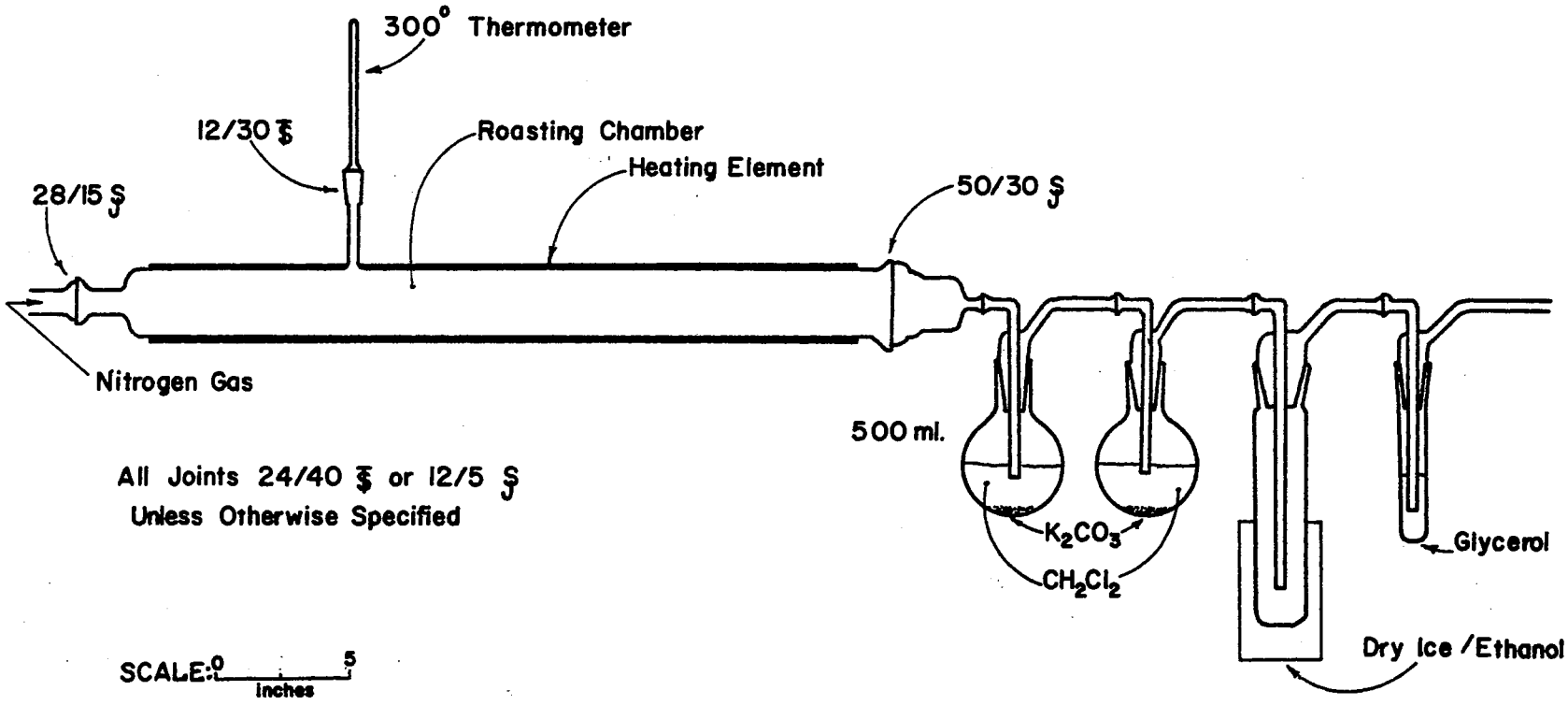


Figure 13. Apparatus for Roasting Under a Nitrogen Atmosphere

Carbowax solution; this final mixture was then used to coat the column. This solution made the liquid phase basic and reduced tailing of the pyrazines.

Low resolution mass spectrometry was performed on an LKB-9000 as described in Chapter III.

Results and Discussion

Analysis of the Basic Fraction

The GLC tracing of the basic fraction along with the mass spectra of the peaks are given in Figure 14. The mass spectra of several pyrazines are shown in Appendix C for comparative purposes. Due to column bleed, many of the less intense ions in the smaller peaks were not reported. Slash marks through the GLC peaks indicate points at which spectra were taken. Numbers at the top of the GLC peaks indicate possible molecular ions.

Component aa had an M^+ 69 indicating the presence of at least a single atom of nitrogen in the molecule. The base peak occurred at m/e 41. The remainder of the spectrum was fairly simple. The mass spectrum of pyrrole (standard), M^+ 67, indicated that component aa may be a dihydropyrrole.

Component bb had an M^+ 84, suggesting that it contained two or no nitrogens. A peak at m/e 43 (M^+ - 41) was present, which in pyrazines is a characteristic loss of acetonitrile; but without high resolution data, further identification was not possible. The base peak was at m/e 41, and as in piperazine, there was a strong peak at m/e 44. This compound might be a tetrahydropyrazine, but this is highly speculative.

In component cc, the base peak and M^+ was 79. Lack of fragmenta-

Figure 14. Combination Gas Chromatographic - Mass Spectrometric Analysis of the Components of the Basic Fraction of the Volatile Components From Roasted Pecans

Column conditions were as follows:

Column - 20' x $\frac{1}{4}$" glass

Column Packing - Carbowax 20M (5%) on Gas Chrom Q

Column Temperature - Programmed, 70-150° C. @ 3°/min.

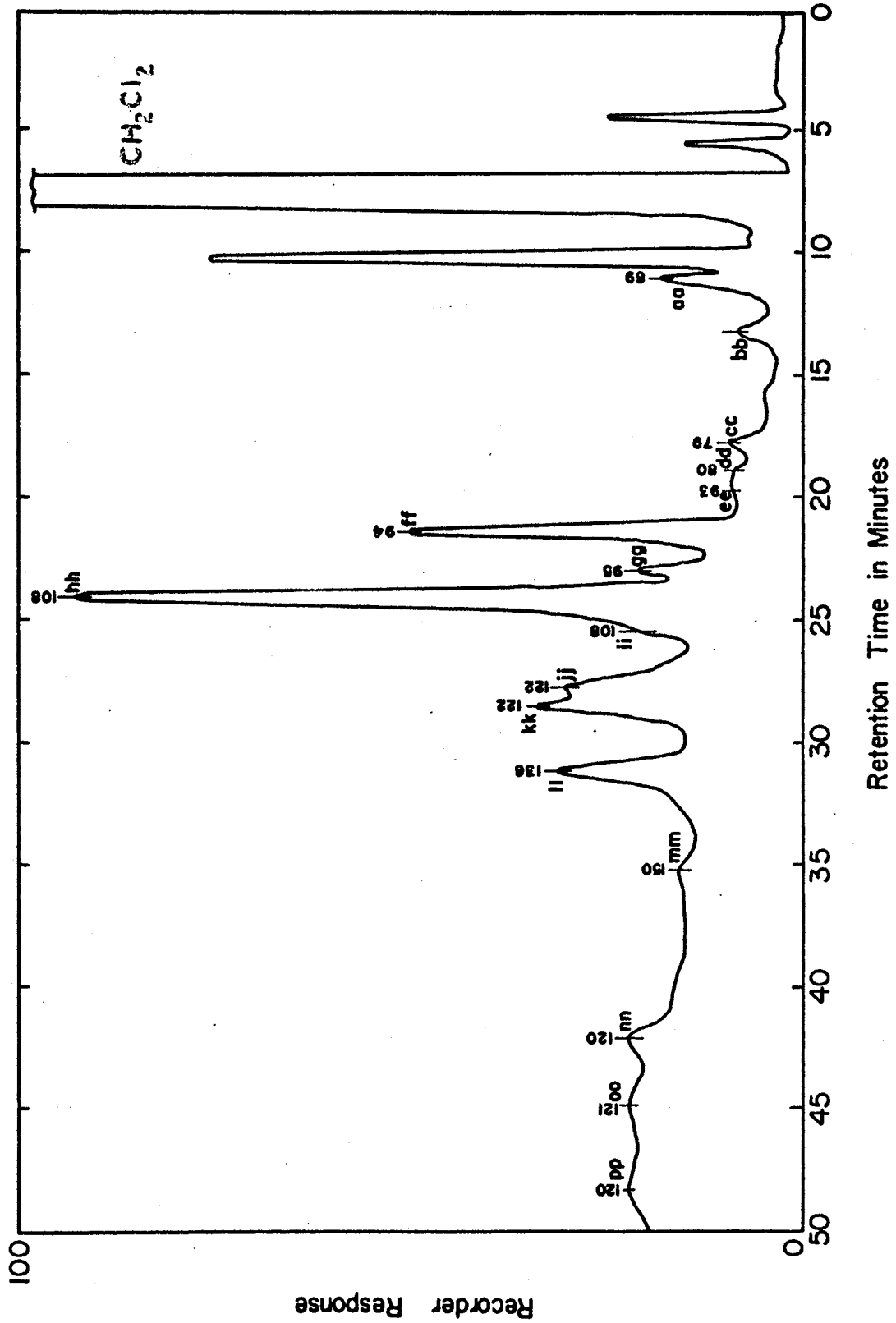
Injection Port Temperature - 190° C.

Separator Temperature - 210° C.

Carrier Gas - Helium

Flow Rate - 20 ml/min

Mass Spectrometer Log Number - 1397; Code - 33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jj
kk
ll
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pp
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93
94
95
108
108
122
122
136
150
120
121
120
0
5
10
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20
25
30
35
40
45
50
100
0
Recorder Response
Retention Time in Minutes

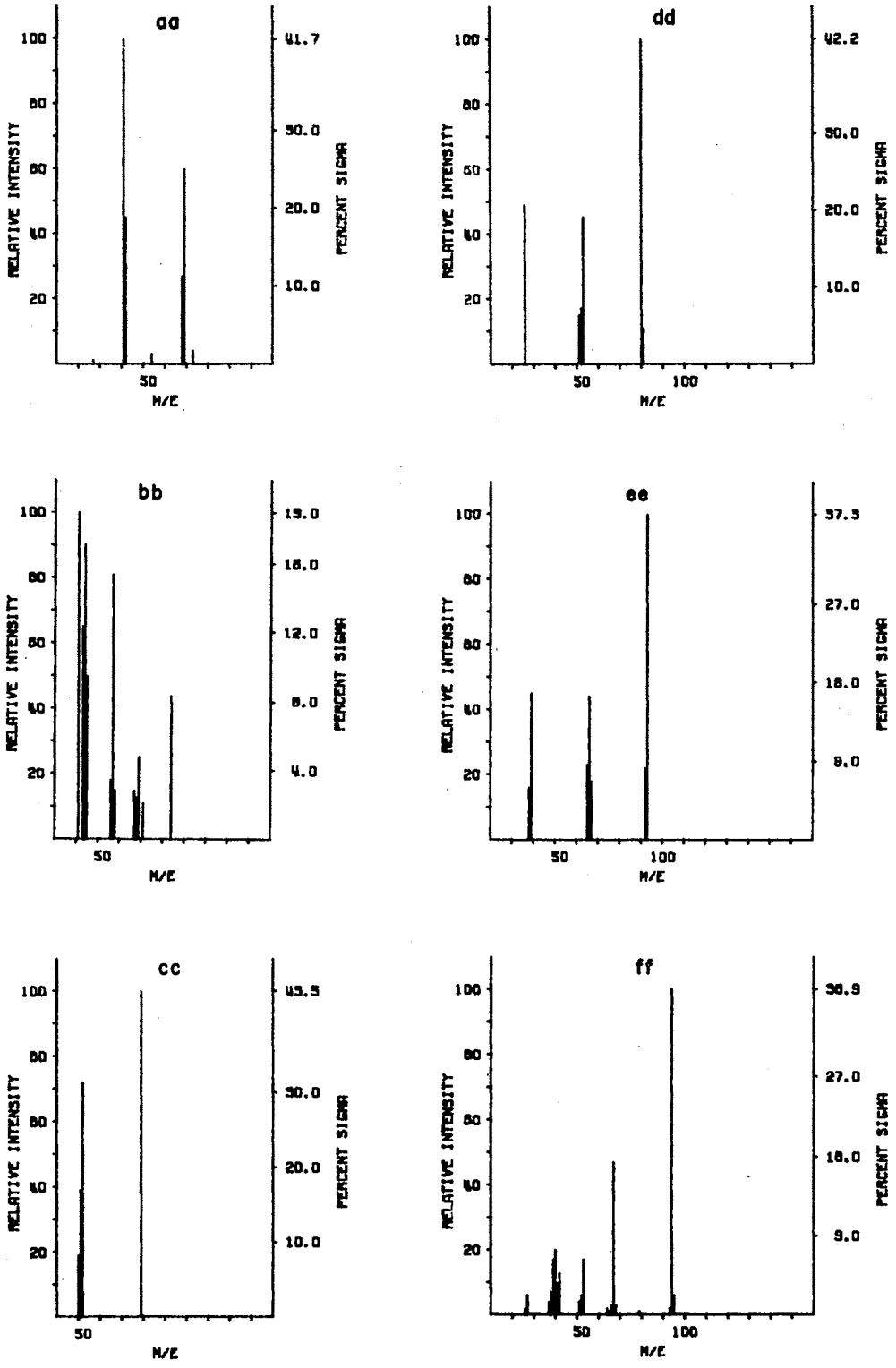


Figure 14 (Continued)

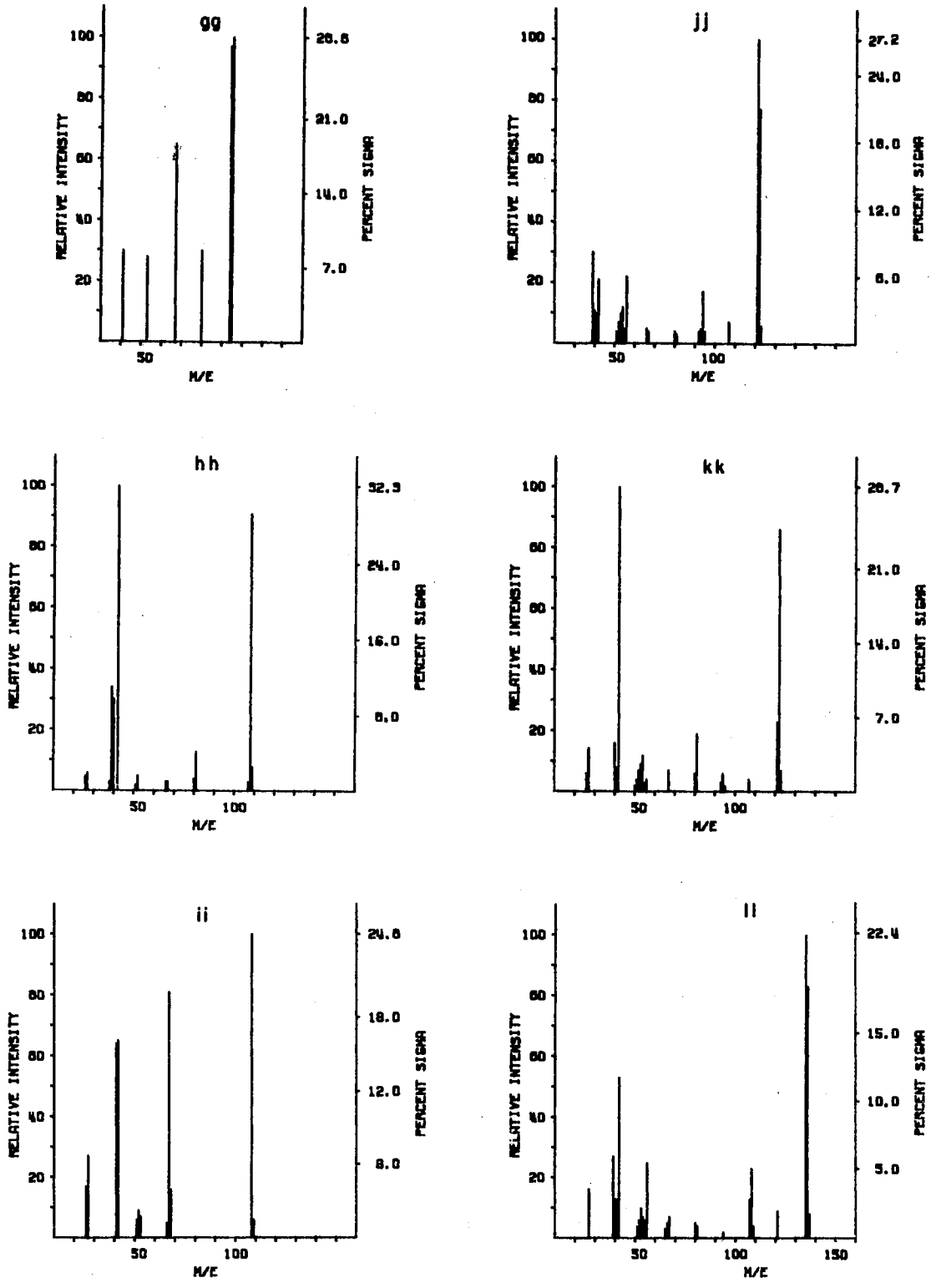


Figure 14 (Continued)

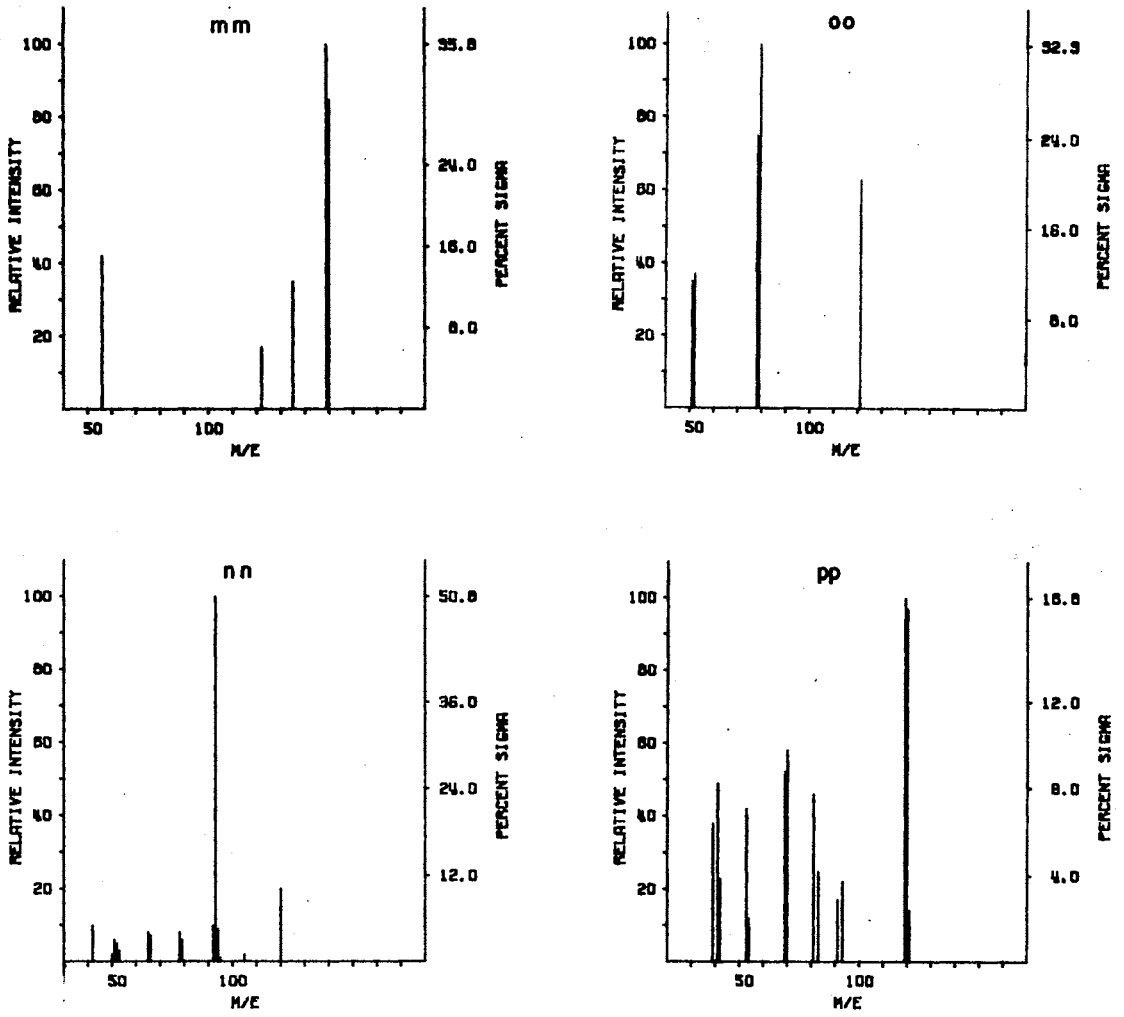


Figure 14 (Continued)

tion and the intensity of the molecular ion indicated the stability of an aromatic ring. The other intense peak present was at m/e 52 (M^+-27) which represented loss of HCN (39). The mass spectrum of pyridine compared very favorably with that of component cc.

Components dd and ee were poorly resolved on the gas chromatograph. However, a close examination of the two spectra reveals that the two components have very few common peaks. Component ee has an M^+ 93, 14 units higher than pyridine, suggesting a methylpyridine. The mass spectrum closely resembled that of 2-methylpyridine as well as the 3- and 4-substituted isomers. Component ee may be one or a combination of the three isomers. Component dd had an M^+ 80 with intense peaks at m/e 53 and 27. This component was pyrazine. The peak at m/e 53 represented loss of HCN from the pyrazine ring.

Component ff was the second most abundant component of this fraction. It had an M^+ and base peak 94. The second most intense peak arose from loss of HCN from the molecule. Component ff was methylpyrazine.

Component gg had a base peak and M^+ 95. The spectrum in general, was characteristic of a dimethylpyrrole, although the exact isomer was uncertain. Upon comparison with reference spectra (40) the 2,4-dimethyl isomer was the most likely. This isomer had a peak at m/e 41 (as did component gg) which was absent in the 2,5-isomer.

Peak hh, M^+ 108, contained a mixture of 2,5-dimethylpyrazine and 2,6-dimethylpyrazine. Both retention times and mass spectra were very similar, so it was difficult to tell which was present in the highest concentration. On the side of peak hh, a small shoulder existed which was denoted component ii. This component also had an M^+ 108; however in

component ii, there was a marked elevation of the peak at m/e 67. Upon comparison with the four pyrazine isomers of M^+ 108, 2,3-dimethylpyrazine was the only isomer that had a significant amount at m/e 67. Hence, it was concluded that a small amount of this pyrazine was present.

Peaks jj and kk were not totally resolved, but several spectra were taken of each peak in order to get a spectrum ofeach that was essentially void of the other component. These two components are isomeric with M^+ 122. Possibilities for these two components include: trimethyl-, 2-methyl-3-ethyl-, and 2-methyl-6-ethylpyrazines. The mass spectrum of the trimethylpyrazine differed noticeably from that of methylethylpyrazine; however, the spectra were not good enough for the resolution of the positional isomers. The mass spectrum of trimethylpyrazine was characterized by a large M^+ (also the base peak) whereas the base peak in the methylethyl isomers was 107. On this basis, component jj was tentatively identified as a methylethylpyrazine and component kk as trimethylpyrazine.

Component ll, M^+ 136, had intense m/e 135 and 136 peaks. This rules out the possibility of a propyl- or butylpyrazine as these longer side chains fragment to a larger extent reducing the intensity of the parent ion. Tetramethylpyrazine had essentially no m/e 135, whereas in component ll, m/e 135 was the base peak. The overall spectrum compared more favorably with 2,6-dimethyl-3-ethylpyrazine than with diethylpyrazine. Thus, component ll was a positional isomer of dimethylethylpyrazine.

Component mm had an M^+ 150 and was either 2,3,5-trimethyl-6-ethylpyrazine or a dimethylethylpyrazine. It was difficult to distinguish anything beyond the four or five most intense ions due to the size of

the peak.

Components nn and pp are also isomers, each with an M^+ 120. These compounds may be either methylvinylpyrazines or propenylpyrazines. Component nn had a very weak M^+ and the base peak is m/e 93. This suggested that the vinyl group was probably not in conjugation with the ring. This may indicate 2-propenylpyrazine. Component pp, on the other hand, had a base peak at m/e 119, indicating a conjugated double bond. The mass spectrum of this component was similar to that of the standard isopropenylpyrazine (23), although the lack of sufficient compound made this uncertain.

Component oo, M^+ 121, was not identified. The components of the basic fraction identifed are summarized in Table II.

Analysis of the Neutral Fraction

The GLC tracing of the neutral fraction is shown in Figure 15 along with column conditions and the mass spectra of the individual components in order of occurrence. Spectra are not listed if two or more intense apparent molecular ions are found under a single peak. The mass spectra of several reference standards are listed in Appendix D. The vertical slash mark through the top of the GLC peaks indicates the point at which a mass spectrum was taken. Components a, b and c were hexane, octane, and acetone and methylethyl ketone, the last two under peak c.

Component d was tentatively identified as either a hydrocarbon (i.e. a hexane) or 2-pentanone. Since hexane eluted before the solvent on this particular column, this component was probably 2-pentanone. The base peak at m/e 43 is loss of $CH_3\overset{+}{C}O$, and an M^+-15 at 71 is present. Component e was chloroform; the spectrum contained the typical C_{35}/C_{37}

TABLE II

SUMMARY OF COMPOUNDS IDENTIFIED IN THE BASIC FRACTION OF ROASTED PECANS

Peak	M^+	Compound
aa	69	Dihydropyrrole
bb	84	Not Identified
cc	81	Pyridine
dd	80	Pyrazine
ee	93	Methylpyridine
ff	94	Methylpyrazine
gg	95	Dimethylpyrrole
hh	108	2,5-(and 2,6-)Dimethylpyrazine
ii	108	2,3-Dimethylpyrazine
jj	122	Methylethylpyrazine
kk	122	Trimethylpyrazine
ll	136	Dimethylethylpyrazine
mm	150	2,3,5-Trimethyl-6-ethyl- or Methyldiethylpyrazine
nn	120	2-(2-Propenyl)pyrazine
oo	121	Not Identified
pp	122	2-iso-Propenylpyrazine

Note: All Identifications Are Tentative

Figure 15. Combination Gas Chromatographic-Mass Spectrometric Analysis of the Components of the Neutral Fraction of the Volatile Components From Roasted Pecans

Column conditions were as follows:

Column - 20' x $\frac{1}{4}$" glass

Column Packing - Carbowax 20M (5%) on Gas Chrom Q

Column Temperature - Programmed, 65-160°C. @ 2°C./min.

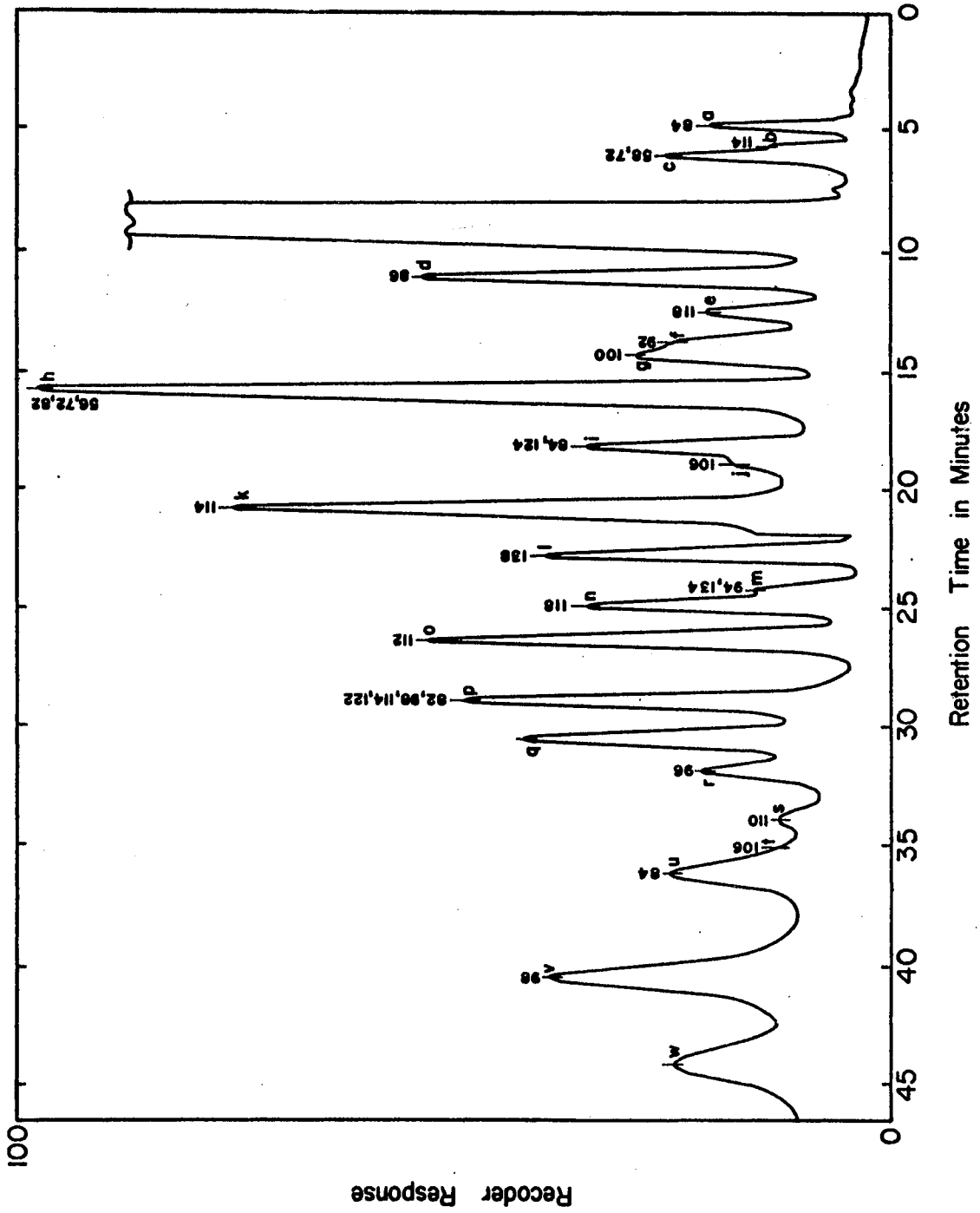
Injection Port Temperature - 185°C.

Separator Temperature - 210°C.

Carrier Gas - Helium

Flow Rate - 20 ml./min.

Mass Spectrometer Log Number - 1397; Code 33


Recoder Response
100
0
0
5
10
15
20
25
30
35
40
45
Retention Time in Minutes
a 84
b 114
c 56,72
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e 86
f 118
g 82
h 100
i 56,72,82
j 64,124
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s 110
t 106
u 84
v 98
w

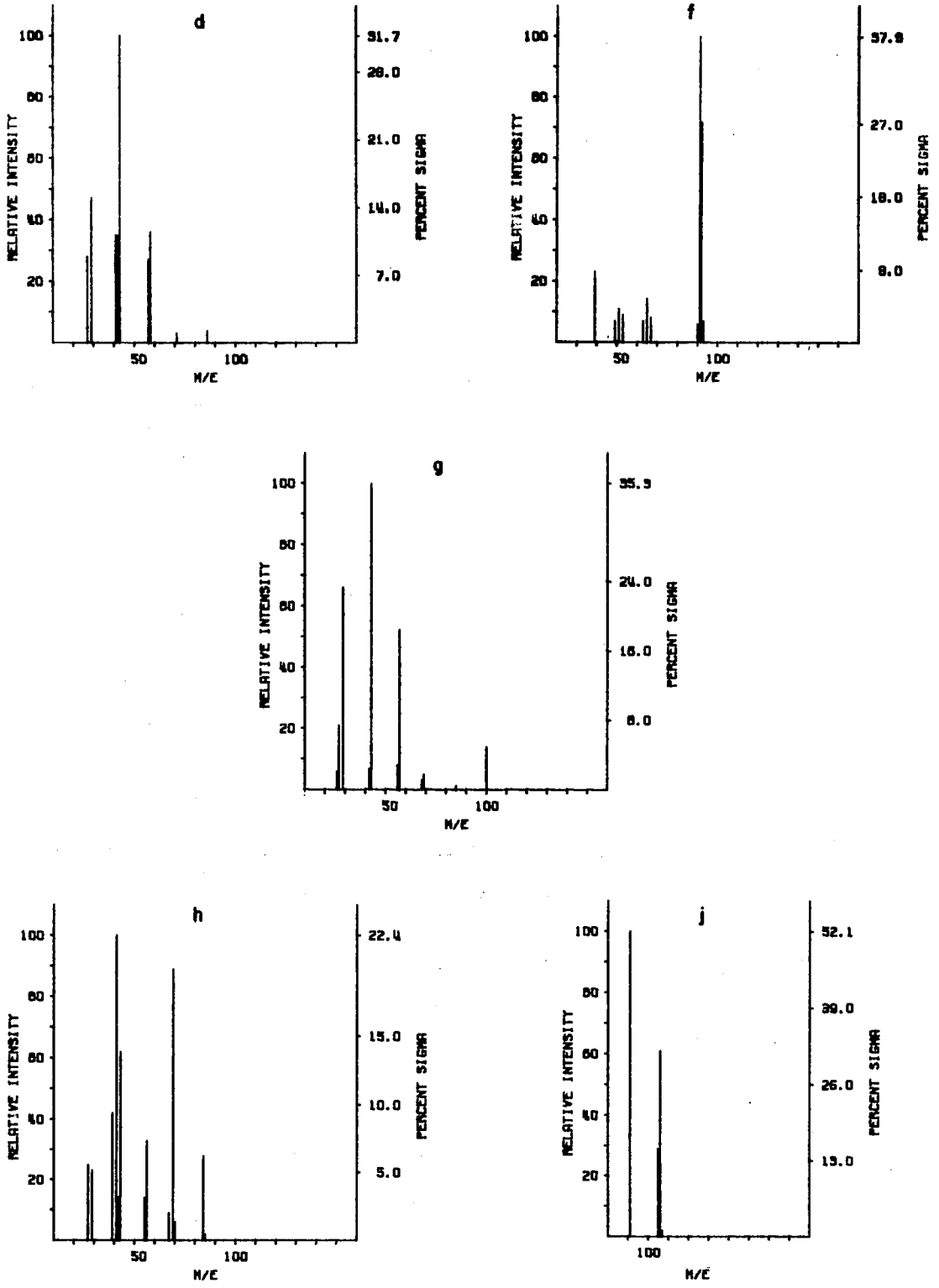


Figure 15 (Continued)

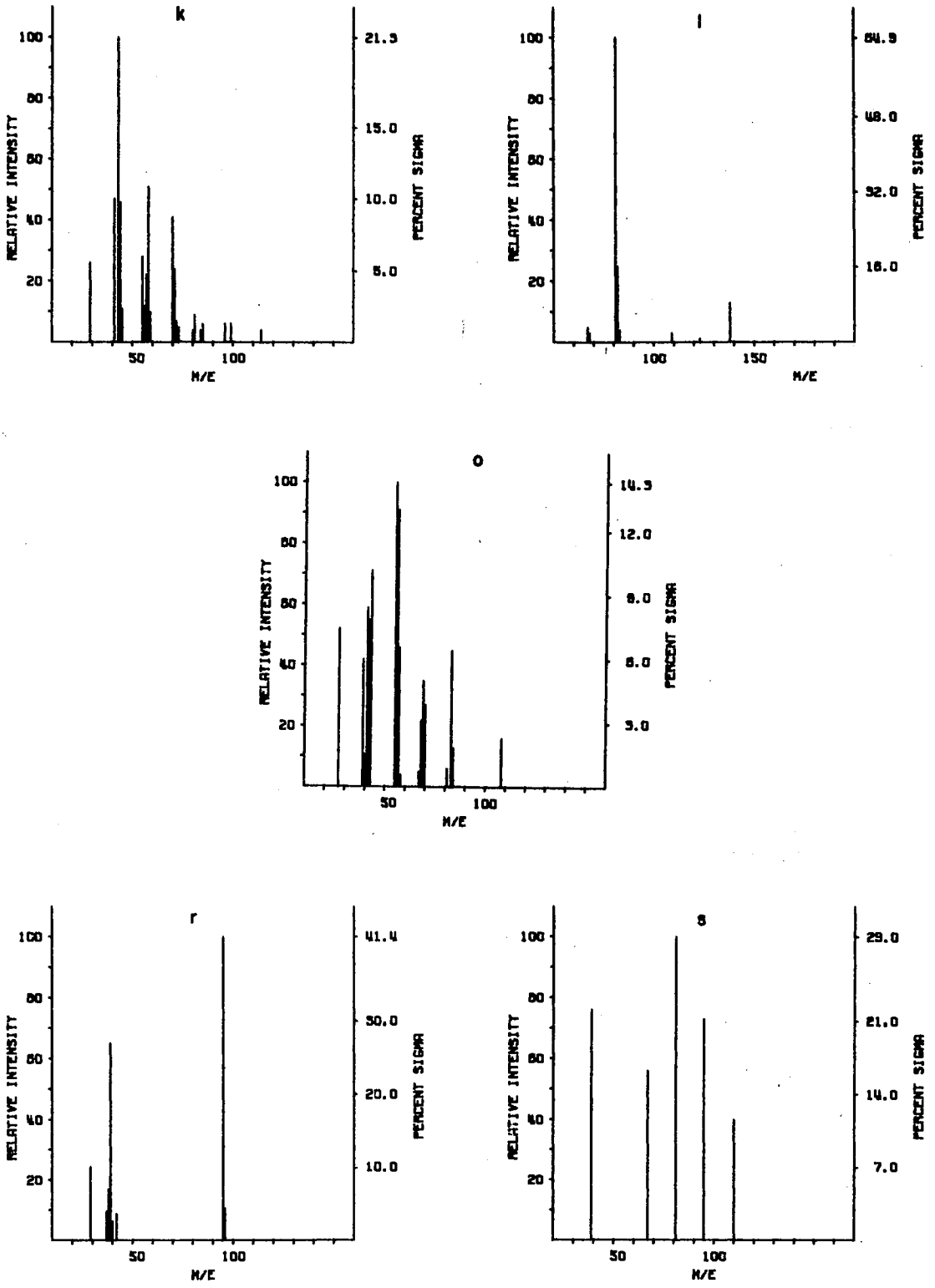


Figure 15 (Continued)

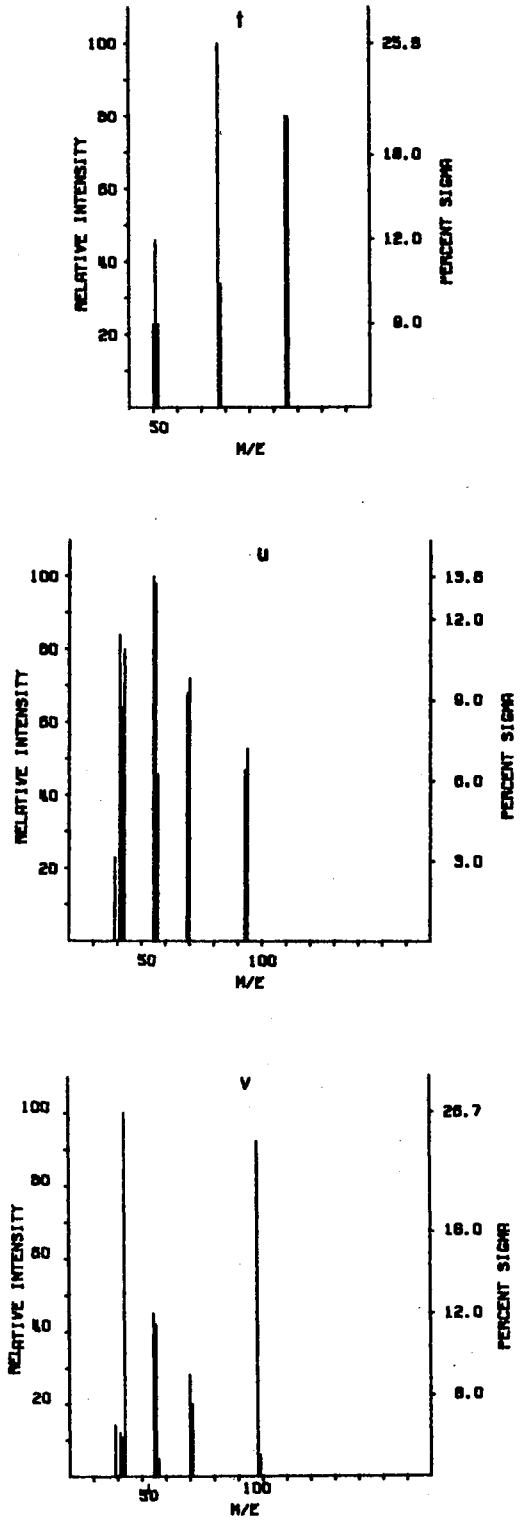


Figure 15 (Continued)

ratio indicative of halogenated hydrocarbons. Component f had an M^+ 92 and a base peak at m/e 91, characteristic of toluene. Component g was a methyl ketone with an M^+ 100; after examining the spectra of several isomers, the spectrum of 3-methyl-2-pentanone most agreed with component g. Components h and i appeared to have several components and nothing definitive could be said about their spectra. In component j, the tropylium ion was the base peak, with an M^+ 106 and a strong 105 peak. This indicated a xylene.

Component k was 2-heptanone. The two most intense peaks were m/e 43 and 58. 2-Heptanone was the only compound with an M^+ 114 that had an intense peak at m/e 58. The rest of the spectrumalso compared favorably with component k.

Component l had an M^+ 138 and a base peak at m/e 81. There was very little additional fragmentation indicating some aromaticity present in the structure. The peak at m/e 81 is characteristic of furans and results from the β-cleavage of an aliphatic side chain from the molecule. The portion cleaved is a C_4H_9 moiety making component l amylfuran. The spectra was very similar to an amylfuran standard published by Johnson (23). Components m through q were not identified.

Component r had three intense peaks at m/e 96, 95 and 39, with very little other fragmentation. This is characteristic of furfural. Component s was not identified. Component t was benzaldehyde. The base peak in the spectrum was m/e 77 or a benzene molecule less a hydrogen atom. The next most intense peaks were 106 and 105. Benzaldehyde has an M^+ 106 whereas m/e 105 results from the loss of the aldehydic hydrogen atom (39). Components u and v were not identified.

Further characterization of these fractions are being continued in

this laboratory.

Roasting Under Nitrogen in Search of Dihydropyrazines

This experiment was conducted to determine if dihydropyrazines are produced in the roasting process. Peanuts were used in this experiment due to their lower oil content. Koehler (36) has produced pyrazines by sugar and amino acid reaction, while Juneja (41) has synthesized pyrazines by condensing dicarbonyls with diamines to form dihydropyrazines which are then oxidized to pyrazines. These dihydropyrazines have a typical roasted aroma, some smelling like popcorn and others very nutty. Their existence, even transient, may be of great significance in roasted foods. Gianturco (42) stated that they would be difficult to chromatograph and that their existence is marginal.

Three of these dihydropyrazines were successfully chromatographed (GC) and the mass spectra are given in Figure 16 along with the respective pyrazines. In the 2,3-dimethyl isomers the lack of aromaticity is reflected in the decrease of the M^+ ions and increase of the fragmentation, all of which are two units higher in the 2,3-dimethyl dihydro form than in the respective pyrazine. This same trend is present in the other compounds.

If oxygen is necessary for the production of pyrazines (i.e. the final oxidation to pyrazines from an intermediate), then the exclusion of oxygen during roasting should show (1) the prevention of pyrazine production and (2) dihydropyrazine production. To test this hypothesis, the nuts were roasted in a nitrogen atmosphere. The apparatus shown in Figure 13 was used for the roasting. Nitrogen gas was constantly passed through the roaster. At first it was intended to use acid to trap the

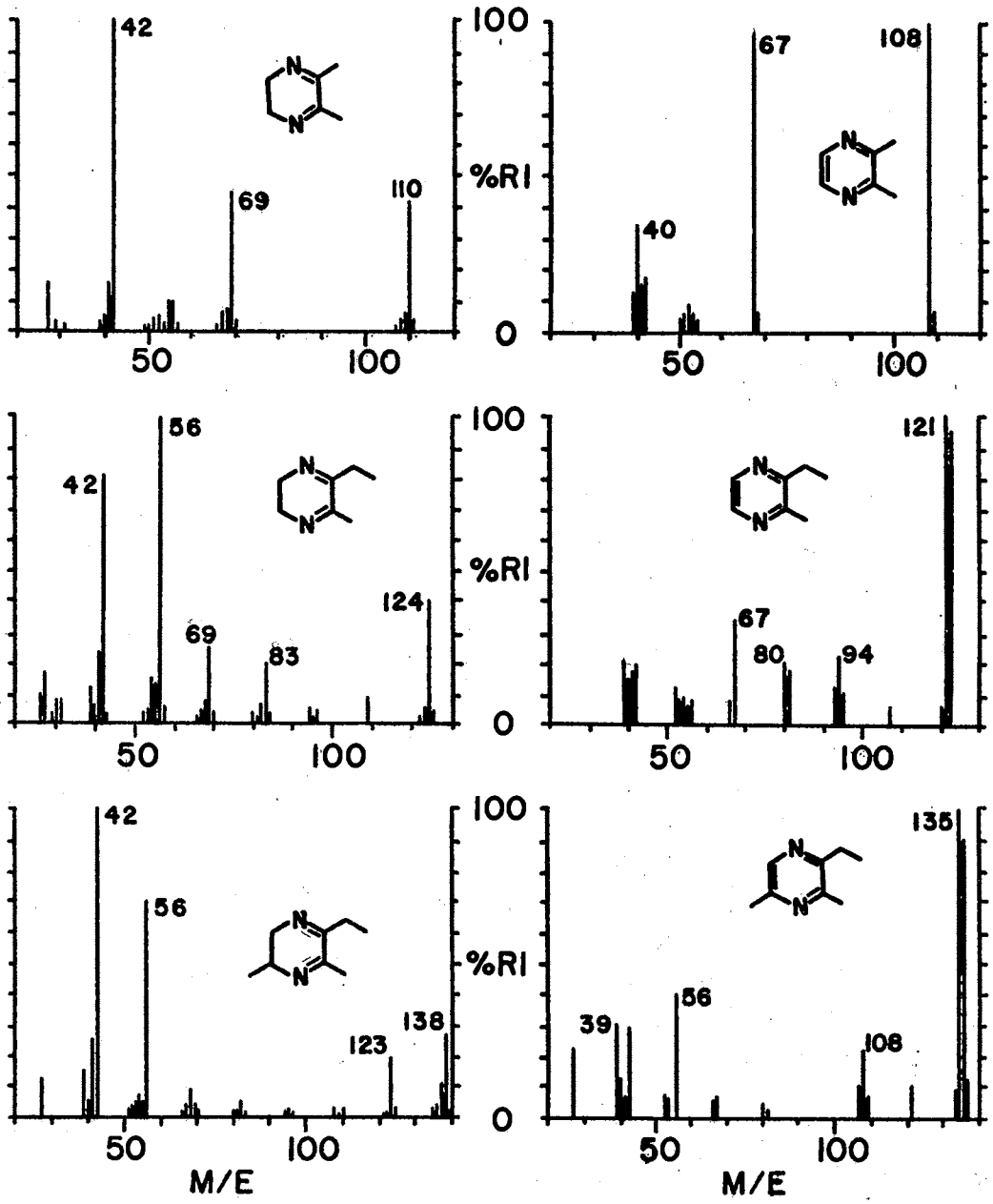
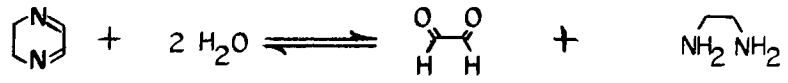


Figure 16. Mass Spectra of Three Dihydropyrazines and Each Corresponding Pyrazine. (MS Log Number 1505, Code 38)

basic components, but dihydropyrazines are unstable below pH 6, undergoing the following dissociation reaction:



Hence, the material in the trap was methylene chloride containing a gram of potassium carbonate to maintain a high pH. The GLC tracing along with column conditions and mass spectra are shown in Figure 17.

Tentative identification of the components present are given in Table III. Most of the components isolated were either oxygen (furans) or nitrogen (pyrazines, pyrroles) heterocycles. The pyrazines must be formed exclusively from amino acids and sugars with the fat acting as a solvent. While there are absolutely no aldehydes, ketones or other fat oxidation products formed, the formation of pyrazines and furans was still observed, the latter coming from pyrolysis of sugars. The formation of both the oxygen and nitrogen heterocycles present must therefore be a reaction not requiring molecular oxygen.

Upon a closer examination of the spectrum, component L, tentatively identified as a furan, could actually be a dihydropyrazine, probably the 2,5-dimethyl isomer, which is the most abundant of the three pyrazines. Neither this compound nor the spectrum are available for comparison.

An interesting aspect of this experiment was the organoleptic tests. When Dr. Clyde Young, an authority on the quality of roasted peanuts, was asked to taste the nuts that were roasted under nitrogen and nuts of the same variety roasted under normal conditions, he could tell no difference in the flavor. In light of this finding, one should speculate as to the importance to roasted flavor, if any, contributed by the

Figure 17. Combination Gas Chromatographic - Mass Spectrometric Analysis of the Volatile Components From Peanuts Roasted Under Nitrogen

Column conditions were as follows:

Column - 20' x $\frac{1}{4}$'' glass

Column Packing - 15% Carbowax 20M on Gas Chrom Q plus .07% KOH

Column Temperature - 135°C. for 35 min. then 165°C.

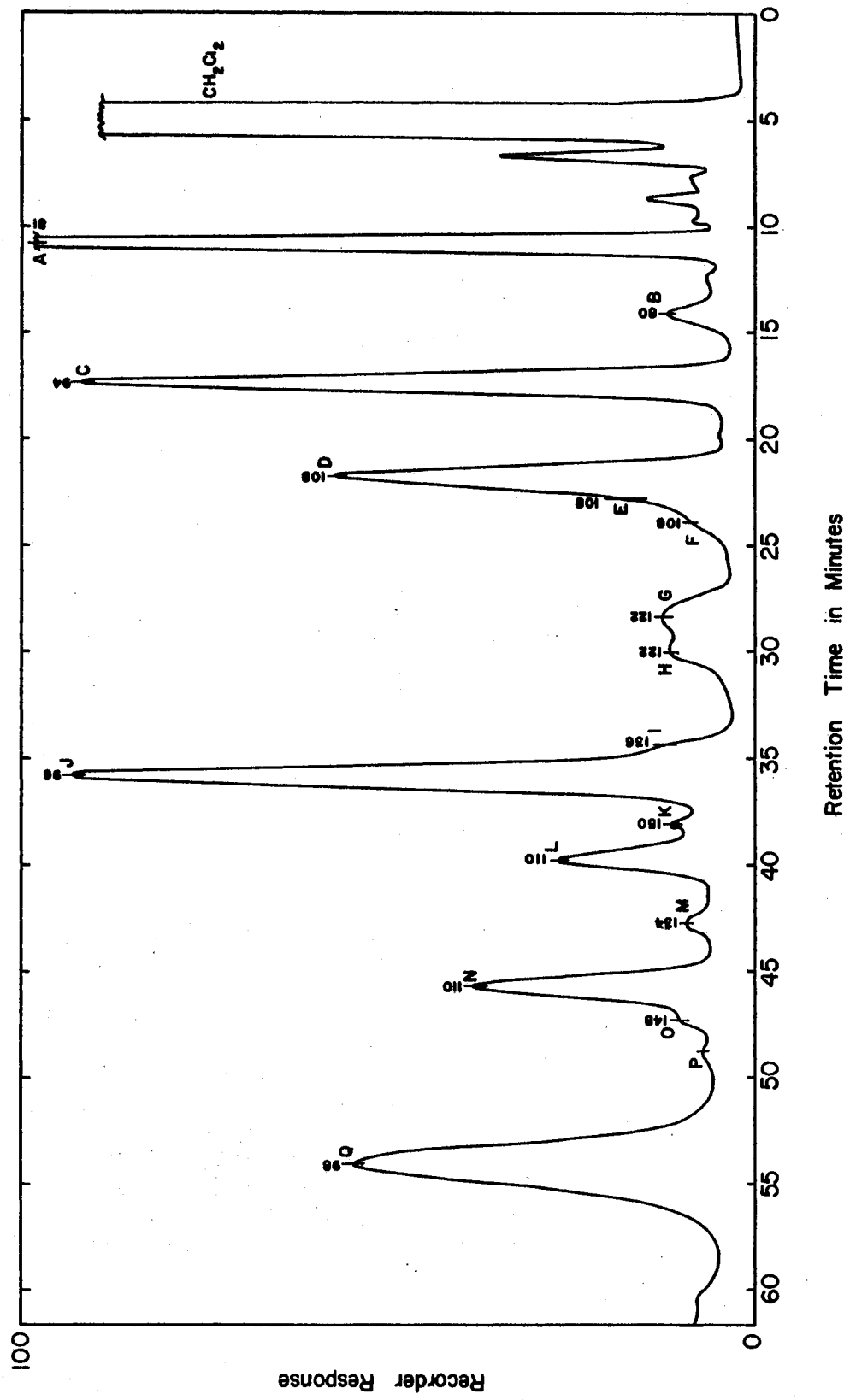
Injection Port Temperature - 195°C.

Separator Temperature - 210°C.

Carrier Gas - Helium

Flow Rate - 20 ml./min.

Mass Spectrometry Log Number - 1535; Code 40


Recorder Response
100
0
Retention Time in Minutes
0
5
10
15
20
25
30
35
40
45
50
55
60
CH_2Cl_2
A 18
B 60
C 84
D 108
E 108
F 108
G 122
H 122
I 136
J 96
K 150
L 110
M 134
N 110
O 148
P
Q 96

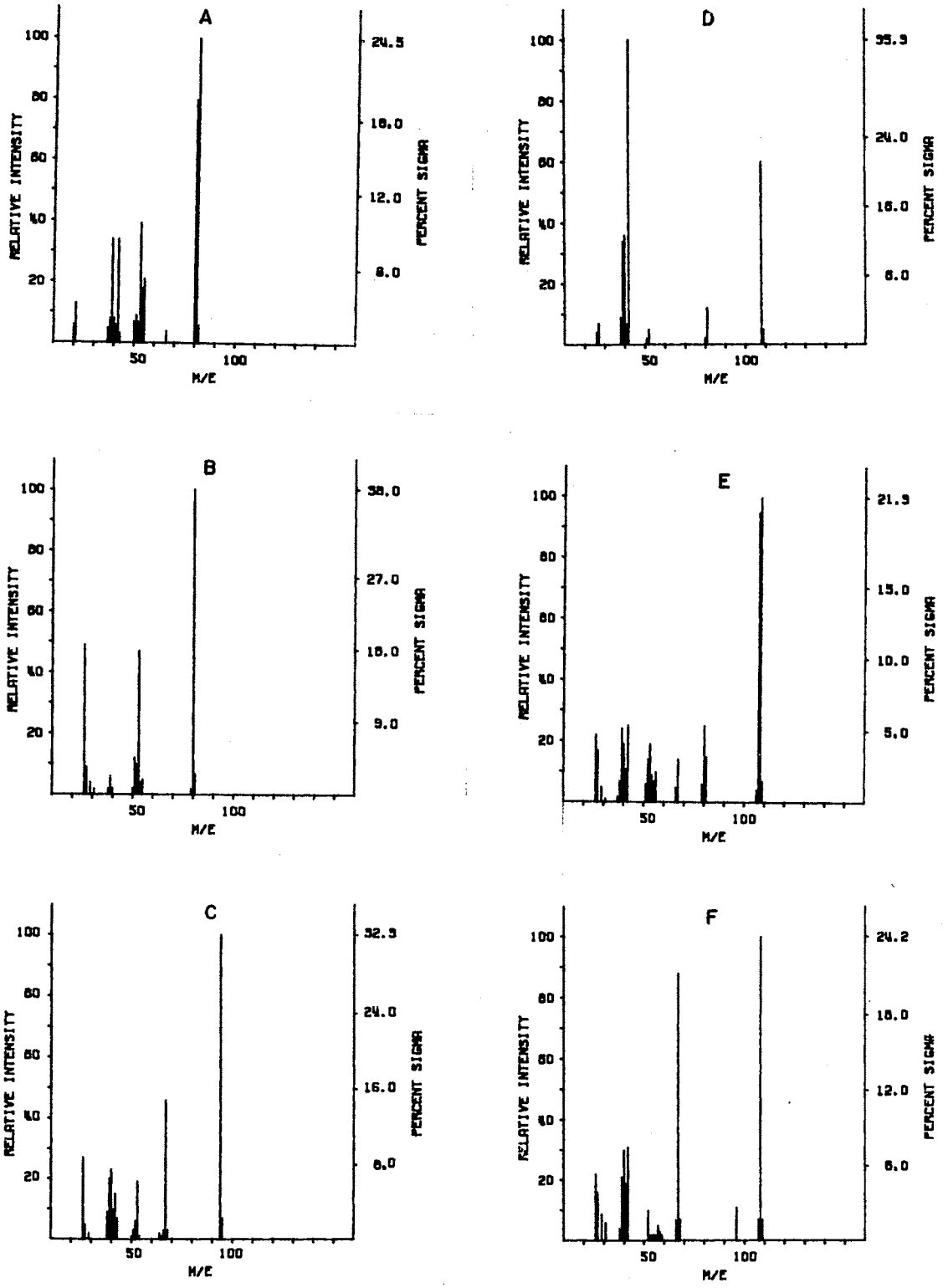


Figure 17 (Continued)

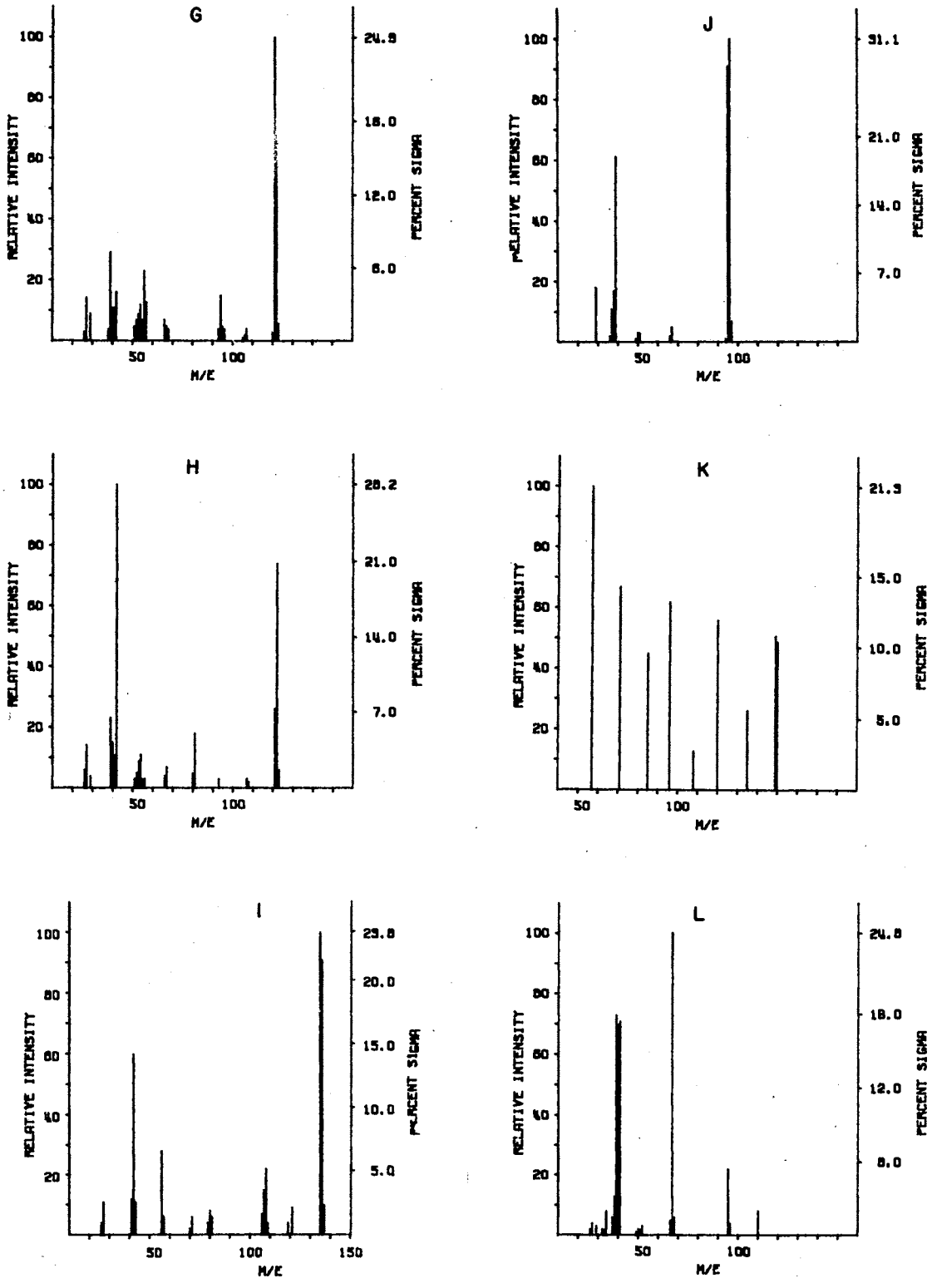
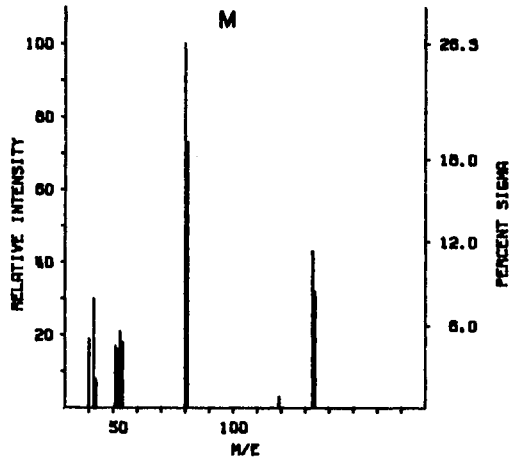
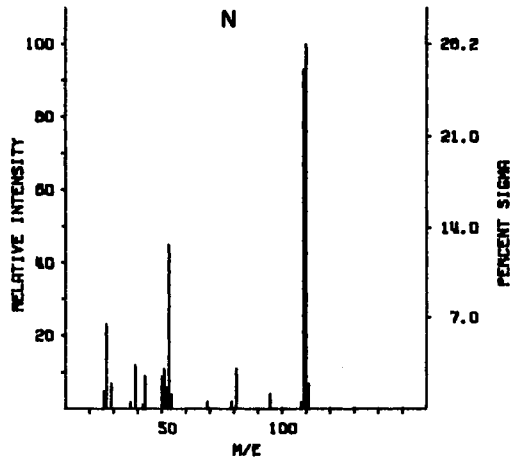


Figure 17 (Continued)





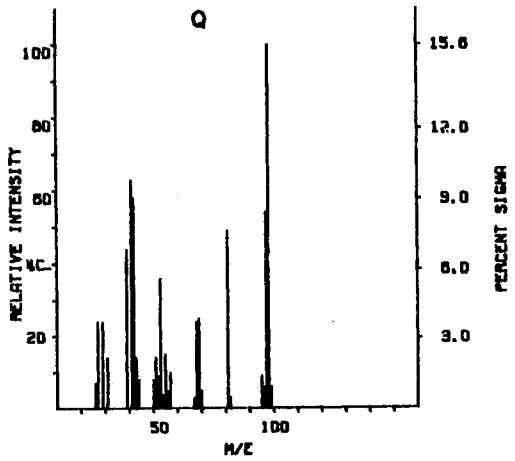


Figure 17 (Continued)

TABLE III

SUMMARY OF COMPOUNDS IDENTIFIED FROM PEANUTS ROASTED UNDER A NITROGEN ATMOSPHERE

Peak	M^+	Component
A	81	n-Methylpyrrole
B	80	Pyrazine
C	94	2-Methylpyrazine
D	108	2,5-(and 2,6-) Dimethylpyrazine
E	108	2-Ethylpyrazine
F	108	2,3-Dimethylpyrazine
G	122	Methylethylpyrazine
H	122	Trimethylpyrazine
I	136	Dimethylethylpyrazine
J	96	Furfural
K	150	Methyldiethylpyrazine
L	110	Not Identified
M	134	Dimethylvinylpyrazine
N	110	Methylfurfural
Q	98	Furfuryl alcohol

Note: All Identifications Are Tentative.

carbonyls and acids.

Summary

The volatile components responsible for roasted pecan aroma were partially characterized. Two pyridines, ten pyrazines and a pyrrole were tentatively identified in the basic fraction of the concentrate. A dihydropyrrole may be present but no spectra of a reference compound was available for comparison.

If molecular oxygen is required for pyrazine production then exclusion of oxygen during the roasting process might encourage dihydropyrazine formation if they are truly intermediates in pyrazine production. Volatiles isolated from peanuts roasted under a nitrogen atmosphere included ten pyrazines, a pyrrole, two furfurals, furfuryl alcohol and a compound as yet unidentified that may be 2,5-dimethyldihydropyrazine. No aldehydes, ketones or other fat oxidation products were present.

Subsequent comparison of these nuts with nuts roasted normally indicated that they were organoleptically indistinguishable. Hence the role of pyrazines and furans in peanut flavor is supported whereas the role of carbonyls is left in doubt.

CHAPTER V

OXIDATION STUDIES OF PECAN OILS

Introduction and Literature Review

In a 1955 survey, it was shown that fats accounted for 42% of man's caloric intake (43). Unlike proteins and sugars, however, fats are subject to auto-oxidation, producing by-products often harmful to the consumer. Thus, many investigators began studying (1) the methods by which fats oxidize, (2) ways of preventing this oxidation and (3) the effect of both oxidized fat and antioxidants on the growth of laboratory animals.

Although there has been much research on the lipid composition of the pecan (44, 5) very little has been reported on the oxidative stability of pecan oils. The relative stability of pecan kernels and unshelled nuts has been reported by Woodroof (5). Pyriadi has shown a relationship between tocopherol content and stability of pecan oils (45). Godkin showed that the development of rancidity in pecans could be retarded by coating the nuts with an antioxidant (e.g. tocopherol or ascorbic acid) (46).

The oxidation of pecan oils follows a generally accepted mechanism of auto-catalytic oxidation, shown in Figure 18 (47). The three basic steps in the process are the same as for any free radical mechanism: (1) initiation, (2) propagation, and (3) termination. The process is initiated by the formation of a free radical ($R^{\cdot}$) by either light, heat,

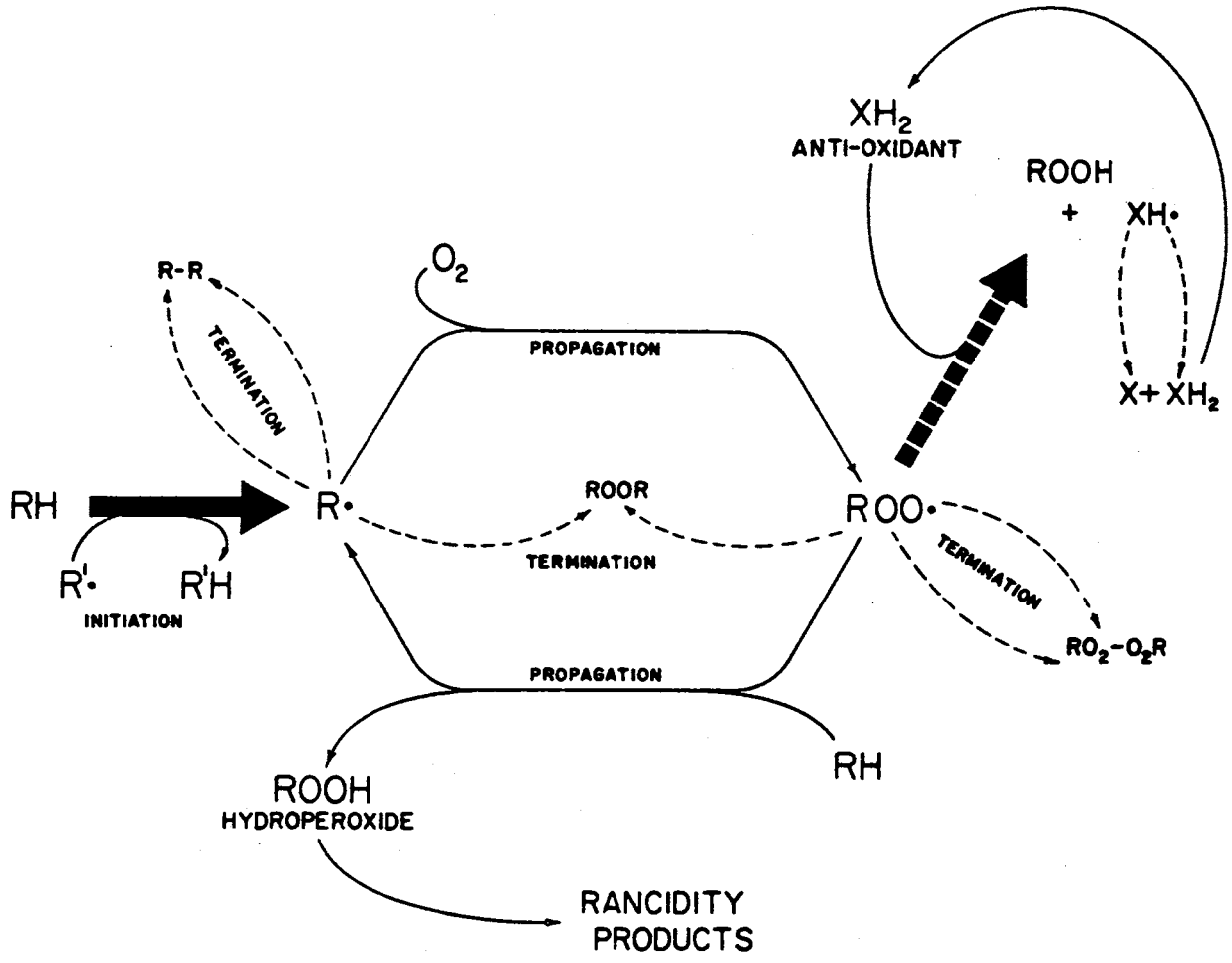


Figure 18. Graphical Scheme for Fatty Acid Oxidation

pro-oxidants, enzymes, or other biological catalysts. This radical then abstracts a hydrogen atom from an unsaturated fatty acid, giving a more stable allyl radical (R·), as shown below.

$$R = CH_3\text{-}(CH_2)_6\text{-}CH\text{-}CH{=}CH\text{-}(CH_2)_7\text{-}COOH$$

This radical (R·) propagates the reaction by reacting with oxygen to yield a peroxide radical (R-OO·). The peroxide radical completes the propagation step by abstracting another hydrogen atom from an unsaturated fatty acid yielding a hydroperoxide and another allyl radical. The cycle is repeated until a termination step is reached (dotted lines) in which radicals react forming a non-radical end product. The probability of termination by the mechanism shown is statistically unlikely, but it does present a possible mode of action for antioxidants.

Bollard and ten Have (48) found that an antioxidant acts as a radical trap by reacting with peroxide radicals, forming hydroperoxides, using the antioxidant as a hydrogen donor. An antioxidant may thus break the propagation cycle, preventing formation of hydroperoxides which might break down into alcohols, aldehydes, ketones, acids, and hydrocarbons, some of which are responsible for the "rancid" odor of fats and oils (49,50). In addition to hydroperoxide formation, linolenic acid radicals can form polymers which may be the precursors of reversion flavor in soybeans (51).

It has long been recognized that aldehydes, ketones, acids and hydrocarbons (C_4 - C_9) are largely responsible for the rancid aroma of fats and oils. Hvolby (52) has determined taste threshold values for aldehydes, ketones, and carboxylic acids using coconut oil as a dilution

solvent. The aldehydes had considerably lower threshold values than either ketones or carboxylic acids of the same chain length. Iwata, et al. (53) studied the nature and chemical composition of and evaluated organoleptically the rancid flavors of soybean, corn, and olive oils. Chromatographic analyses indicated that the volatile components were primarily from linoleate degradation; the characteristic (green, fishy) flavor of soybean oil was due to enhancement of the basic off-flavor with oxidation products of linolenate, whereas the "heavy" flavor of rancid olive oil was due to some oxidation products of oleate.

Pattee, et al., (54) have isolated twenty-one compounds from high temperature cured off-flavored raw peanuts. Eleven of these have been identified as follows: formaldehyde, acetaldehyde, ethanol, acetone, isobutyraldehyde, ethyl acetate, n-butanal, 3-methylbutanal, 2-methylpentanal, 2-hexanone, and n-hexanal. Swarthout (55) subjected rancid nut meat kernels to a high vacuum, then a water wash, followed by dehydration to remove products of rancidity. Moser, et al., (56) working with oils containing linolenate (crambe, mustard seed and rapeseed), have evidence that if linolenic acid is present in an edible oil, it is the primary precursor of typical off-flavor.

On the other hand, mild oxidation of certain oils may be necessary for the typical flavor of an oil. Pokorny and co-workers (57) have shown that the characteristic aroma of rapeseed oil produced by high temperatures is due to the oxidation of erucic, oleic, linoleic, and linolenic acids to carbonyls. McGlamery and Hood (58) have shown that pecans heated to an internal temperature of 176^{o} F. and cooled rapidly were less rancid than untreated controls, suggesting an involvement of oxidative enzymes in the rancidity development.

There are many factors present in nuts and oils that affect the development of off-flavors. Holman (59, 60) has isolated, purified, and crystallized a lipoxidase enzyme from soybeans. The enzyme is inhibited by α-naphthol and, ironically, by α-tocopherol. The substrate for the enzyme is linoleate. Other fatty acids act as competitive inhibitors in that they have an affinity for the active site, yet do not act as substrates. At low temperature and pH 9, the oxidation of linoleic acid by the enzyme yields totally conjugated hydroperoxides. Kwapiewski (61) has investigated the effect of sulfur-containing amino acids on the autoxidation of edible oils. Methionine, cystine, and cysteine all had slight antioxidant activities in rape, soybean, and olive oils. Nedoma (62) has reported the influence of natural colorants on the stability of purified oils. The highest antioxidative effect was achieved with gossypol (a toxic phenol) and some effect with β-carotene. A positive pro-oxidative effect was not shown with any coloring component. The pro-oxidative action of free fatty acids in the autoxidation of fats and oils was investigated by Popov (63). As little as 0.1% free fatty acid decreased the induction period of autoxidizing fat. The mechanism suggested for this phenomenon comprises formation of a complex between hydroperoxides and the fatty acid which results in acceleration of the reaction of the hydroperoxide yielding free radicals. Matsushita (64) investigated the antioxidant and pro-oxidant abilities of 111 biologically and physiologically active substances. Anti- and pro-oxidant activities were measured using linoleic acid as a substrate in borate buffer at pH 9. Aminopurine, resorcinol and rutin had the strongest activities. In a more applied study with peanuts, Cosler (65) found that a coating of acetylated monoglycerides prevents oil loss and mois-

ture gain.

There are many different methods available for determining rancidity in oils. The Kreis test was the first chemical test used in the determination of rancidity (66). This is a colorimetric test based on the reaction of aldehydes and ketones produced in the rancidity process with the phloroglucinol reagent. Lea (67) developed the peroxide method by which the peroxides oxidize iodide to free iodine which is measured titrimetrically. The standard A.O.C.S. method for analysis of oxidizing fats and oils is the active oxygen method (68) in which air is bubbled through an oil at 2.3 ml/sec with the oil at a temperature of 97.8° C. The oil is then checked for rancidity by one of the methods mentioned earlier. Foresti (69) studied rancidity development in oils spectrophotometrically. Rancidity in olive oils was found proportional to the absorbances at 225 mμ and 280 mμ . These absorbances are believed caused by the presence of saturated aldehydes and dienals as well as by α-, β -unsaturated aldehydes.

Sidwell (70) has investigated the use of 2-thiobarbituric acid as a reagent for measuring rancidity. The 2-thiobarbituric acid condenses with malonaldehyde (an oxidation product of linoleate) producing a pink color which is analyzed spectrophotometrically at 530 mμ . Recent work has shown that the TBA reagent gives orange colors with aldehydes and dienals (71). The absorption maximum of more than forty TBA-aldehyde complexes has been reported (72).

Not only is the TBA method a good method for measuring the aldehydes produced, but it also shows excellent correlation with rancidity development as measured organoleptically. Dugan (73) has shown good correlation between the TBA method and off-flavor in butterfat. Keans-

ton, et al., (74) compared the TBA test with the peroxide method, the Kreis test for aldehydes, and the degree of unsaturation. The TBA test paralleled the other methods for methyl linoleate and linolenate oxidation but was negative for methyl oleate. On the other hand, the TBA method was the most sensitive of the four methods, provided the limitations of the reaction were considered. Sedlacek (75) used the TBA method as a good indicator of edibility in walnuts while peroxide value determined storage ability. Although any of the aforementioned methods can follow the development of rancidity, none are foolproof. Pohle (76), while studying the relation of peroxide value and TBA value to the development of undesirable flavor characteristics, concluded that the flavor score cannot be estimated for any given fat from either the peroxide value or the TBA value. Either can be used to follow the development of off-flavors in a given product, but the relative level may vary from product to product.

A rapid method for the evaluation of rancidity has been reported by Arya et al. (77). In this method, the refractive index of the oil is used as a criterion for measuring rancidity. As the oil auto-oxidizes and peroxides are formed, the refractive index increases. Although the authors found that this method works for safflower oil, it was found to give erratic results with pecan oils and proved to be highly unsatisfactory.

Reagents and Apparatus

Reagents

Pecan oils, pressed from over 80 varieties of pecans, were obtained by Mr. Herman Hinrichs, O.S.U. Horticulture Department. Most of the

pecans were grown at the Oklahoma Agricultural Experiment Station farms across the state. Several varieties were obtained from the U.S. Pecan Field Station, Brownwood, Texas. Nuts from the 1967, 1968, and 1969 crops were used in the experiments.

Diethylene Glycol Succinate, Gas Chrom Q (100/120 mesh). Applied Science Laboratories, Inc., P. O. Box 440, State College, Pa.

Methyl Palmitate, Methyl Stearate, Methyl Oleate, Methyl Linoleate, and Methyl Linolenate. Analabs, Inc., 80 Republic Drive, North Haven, Connecticut.

Petri Dishes, internal diameter of 90 mm., height, 15 mm. Each dish was made of approximately the same gauge glass. Kimbell Products, Owens Illinois, Toledo, Ohio.

2-Thiobarbituric Acid (TBA). Eastman Organic Chemicals, Distillation Products Industries, Rochester, N. Y.

TBA Solution. Dissolve 0.67 gram of 2-Thiobarbituric Acid in distilled water with the aid of a steam cone, transfer to a 100 ml. volumetric flask, cool, and make to volume (70).

TBA Reagent. Mix equal portions of the TBA solution and glacial acetic acid. Make this fresh daily (70).

Benzene, thiophene free, and Glacial Acetic Acid, A.C.S. Certified Reagent Grade. Matheson Chemicals, Norwood, Ohio.

Benzene, Sodium dried. To 1 liter of reagent grade benzene, add about 50 grams of sodium metal, in small chunks.

2,2-Dimethoxypropane - Redistilled (76-79^{o} C.). Dow Chemical Co., Midland, Michigan.

Methanolic HCl. Methanol is dried for 1 hr. with molecular seive 3A. This dried methanol is filtered through sodium sulfate into a

bottle. Hydrogen Chloride gas, initially bubbled through concentrated sulfuric acid to remove all traces of water, is then bubbled into the methanol until the normality is 2.8 by titration.

Molecular Seive 3A. Linde Division, Union Carbide Co., Houston, Texas.

Norit A, Decolorizing Carbon. Pfanstiehl Laboratories, Inc., Waukeegah, Illinois.

Whatman No. 1 and No. 42. W. and R. Balston, Ltd., England.

Skelly, F. Skelly Oil Co., Kansas City, Mo.

β-Carotene, Crystalline. Sigma Chemical Co., St. Louis, Mo.

Hydrogen Chloride Gas. The Matheson Co., Inc., LaPorte, Texas.

Apparatus

Gas chromatographic analyses were performed on a Perkin Elmer 801 Gas Chromatograph, equipped with a hydrogen flame detector. Preparative gas chromatography was performed on a Varian-Aerograph Model 711 Gas Chromatograph with a hydrogen flame detector and a 49:1 stream splitter. Collection tubes were constructed by Wayne Adkins, Scientific Glass Blower, Oklahoma State University.

Low resolution mass spectral analyses on the fatty acid methyl esters were performed on the LKB-9000, previously described in Chapter III.

Optical density readings for the TBA tests were taken on a Beckman Model D.U. Spectrophotometer equipped with a Gilford power supply and photomultiplier and linear readout detector.

Oils were pressed from good quality Stuart pecans with a Carver Laboratory Press through a linen filter press using up to 20000 psi. Approximately 50% of the weight of the starting sample was obtained as

oil.

In the TBA analyses, solutions were mixed for 2 minutes with a Vortex Mixer, purchased from Scientific Industries, Inc., Queen Village, N. Y.

Procedures

Oxidation Studies

The active oxygen method (68), a relatively standard procedure for measuring oxidation of oils, was not used in our experiments; rather thirty grams of oil was added to uniform size petri dishes and placed in an oven. This method was much more convenient and not as time consuming. The temperature regulator was adjusted so the temperature in the oven was maintained at 66-68° C. Each day a one gram sample of the oil was removed from each petri dish (requiring the temperature to be lowered for about one hour out of 24) and subjected to the thiobarbituric acid (TBA) test. Most of the oils had a break point (i.e. time at which the oil suddenly becomes rancid) of twelve to eighteen days, so the volume of the oil usually decreased to about fifteen milliliters by the end of an experiment. The analysis was spectrophotometric and will be described later. A representative plot of data is shown in Figure 19. The last portion of the graph, in which the slope increases very rapidly, is extrapolated to the line connecting the initial points on the graph. At the point where these two lines intersect, the value of the X-axis is taken and denoted the "keeping time" of the oil.

Decolorized Pecan Oil

Five hundred grams of pecan oil was mixed with 500 ml of redis-

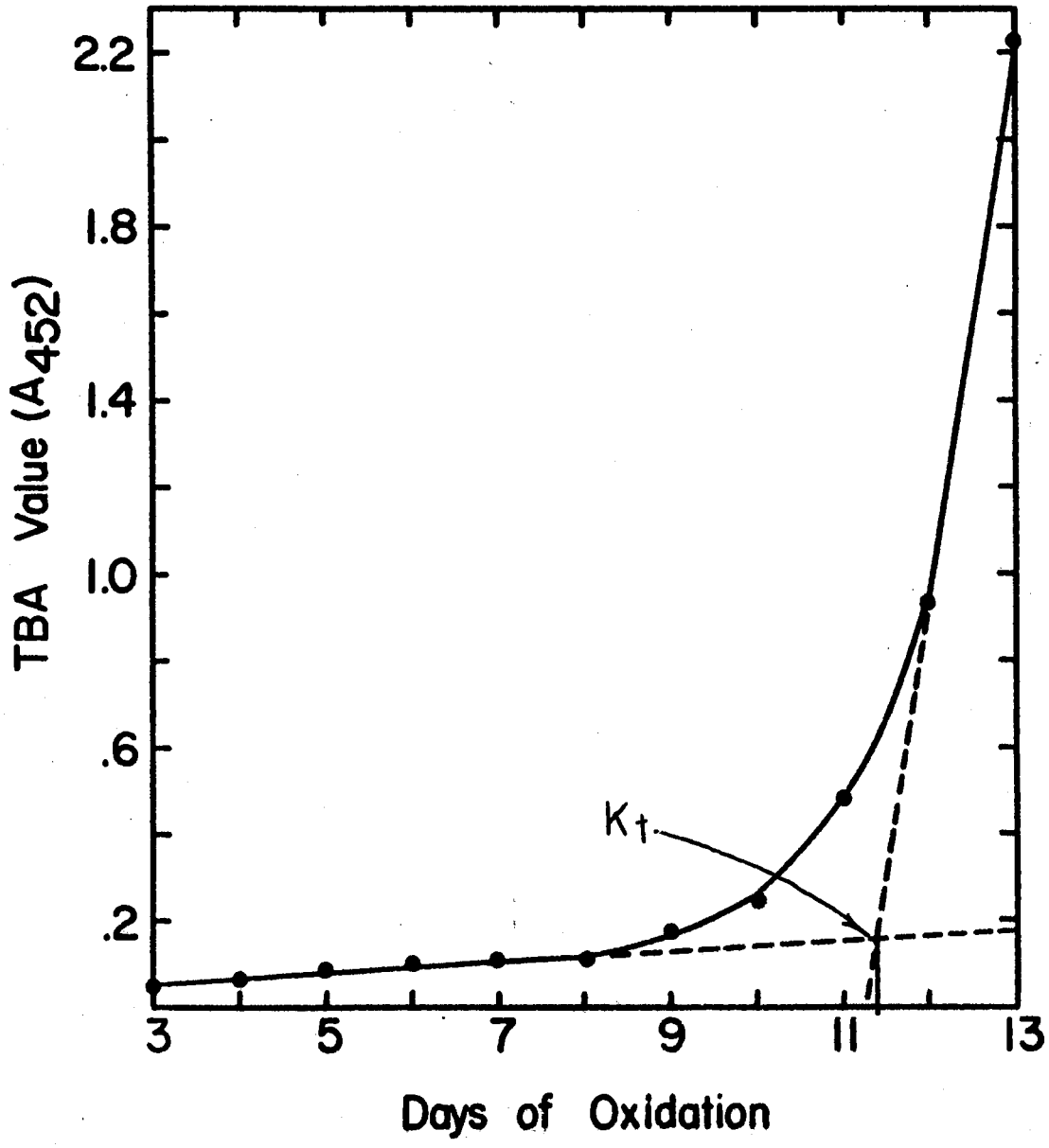


Figure 19. Sample Calculation of the Keeping Time (K_t) of a Pecan Oil.

tilled Skelly F. Twenty grams of Norit A Decolorizing Carbon was added to the mixture and was mixed for twelve hours using a magnetic stirrer and bar. After twelve hours of decolorizing, the crude mixture was filtered with Whatman no. 1 fluted filter paper. This removed more than 99% of the carbon. The material was then filtered through Whatman no. 42 filter paper. The pentane was removed under vacuum on a rotary evaporator at 40° C. The resulting colorless oil is referred to as the "stripped" oil throughout this chapter.

Thiobarbituric Acid Analyses

The method chosen for following the oxidation of the pecan oils was the thiobarbituric acid test as described by Sidwell, et al. (70). In this procedure, three grams of oil was dissolved in 10 ml of either benzene or carbon tetrachloride in a Maisel-Gierson tube, and 10 ml of TBA reagent was added. The mixture was then shaken for four minutes, transferred to a separatory funnel, and the bottom layer drawn off into a test tube. The test tube was immersed in a boiling water bath for thirty minutes, cooled, and the absorbance at 530 mμ was read against distilled water. This method was modified in several ways to decrease the time of the analyses. It was found that one gram of oil was satisfactory. It was necessary to make this modification because the oxidation experiments take 12-25 days which would require up to 80 gm of oil. The sample sizes of the pecans available necessitated the use of smaller samples. (Some pecan samples gave only about 40 gm of oil.) Secondly, the mixture to be shaken was added to a 30 ml capacity test tube and the material was mixed on a Vortex mixer for two minutes. This gave much more adequate mixing of the two phases; and mixing longer than two min-

utes did not increase the TBA values appreciably. Finally, the top layer (nonaqueous) was removed first with a pipette, then with a disposable Pasteur pipette. Thus, it was not necessary to transfer from test tube to separatory funnel several times, increasing both chances for error and amount of glassware to be cleaned. The TBA method was chosen over the peroxide value method as the latter required five grams of oil.

Fatty Acid Analyses

The fatty acid compositions of the individual pecan oils were determined according to the procedure of Mason and Waller (78), modified by Young and Waller (79). In this variation of the method, two drops of oil were dissolved in 4 ml of sodium dried benzene, to which 0.1 ml of 2,2-dimethoxypropane had been added. Then 0.5 ml of 10 ± 2% methanolic HCl was added, followed by mixing; the samples were allowed to stand overnight. The next morning, the samples were evaporated to remove the methanol and the HCl, and 2 ml of the sodium dried benzene was added to dilute the esters. One microliter of this mixture was then injected onto the gas chromatograph. A typical separation is shown in Figure 20. (The column conditions are given in the legend of this figure.) The vertical slash marks indicate a point at which a mass spectrum was taken. This method for fatty acid analyses was highly satisfactory for fatty acid composition of triglycerides, as the 2,2-dimethoxypropane drove the transesterification to completion. The only critical point in the method was dryness of the reagents. If the methanolic HCl became wet, phase separation would occur upon mixing with the other components. If this occurred, incomplete esterification would result with preferential esterification of the more highly unsaturated fatty acids. The

Figure 20. Gas Liquid Chromatographic Analysis of the Fatty Acids From Pecan Oils

Column conditions were as follows:

Column - 6' x $\frac{1}{4}$" glass

Column Packing - Diethylene Glycol Succinate (20%) on Gas Chrom Q

Column Temperature - 182°C. (160°C. with MS)

Injection Port Temperature - 240°C.

Detector Temperature - 130°C. (MS-Separators-210°C.)

Carrier Gas - Nitrogen (He with MS)

Flow Rate - 60 ml./min. (20 ml./min. with MS)

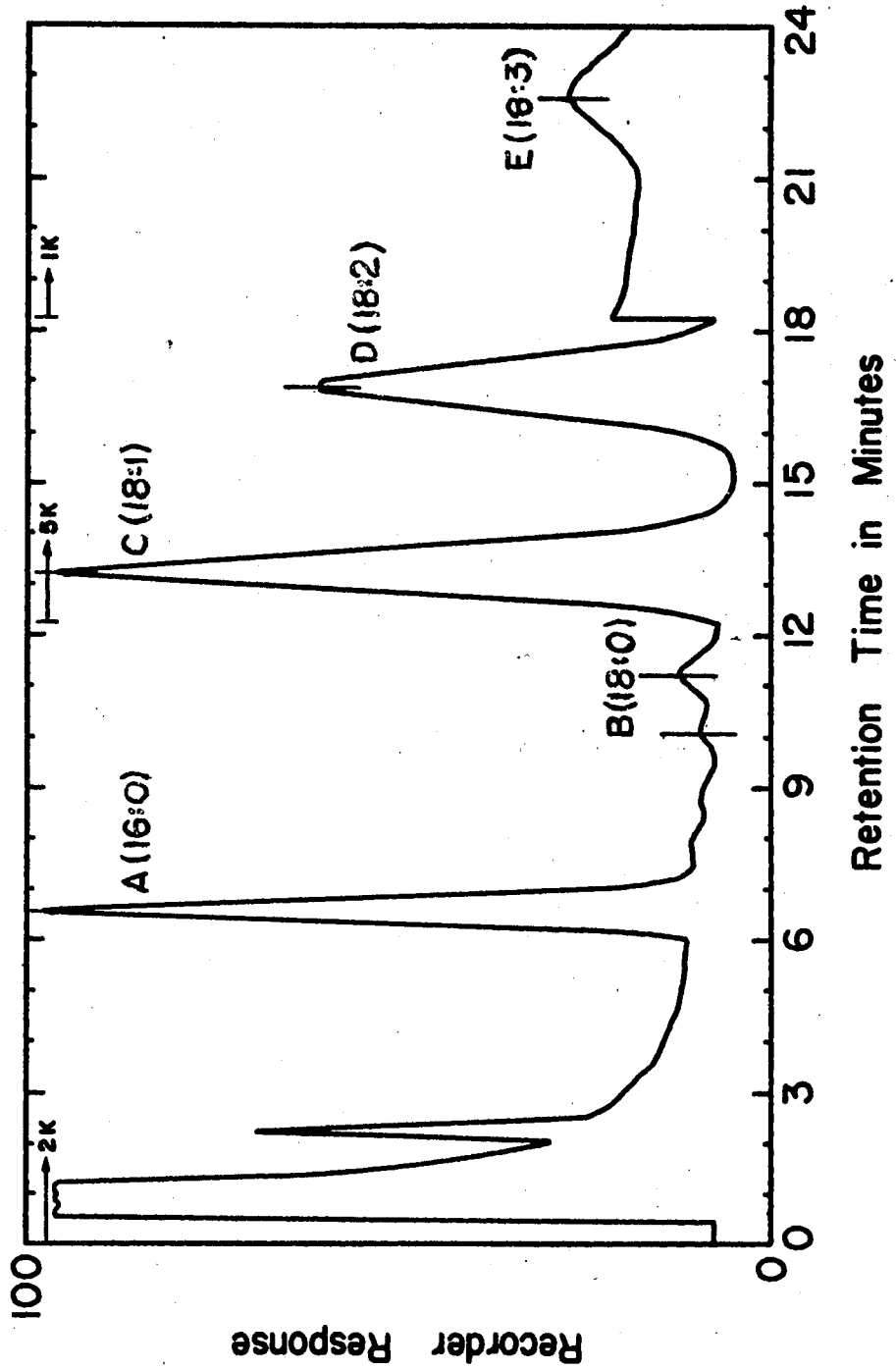
Detector - Hydrogen Flame

Hydrogen Pressure - 20 PSI

Air Pressure - 40 PSI

Instrument - Perkin Elmer 801 Gas Chromatograph

LKB 9000 Combination GC-MS (for Mass Spectrometry)


100
0
2K
8K
1K
A (16:0)
B (18:0)
C (18:1)
D (18:2)
E (18:3)
0
3
6
9
12
15
18
21
24
Recorder Response
Retention Time in Minutes

final result of this would be inaccurate data. The initial modification of this procedure included 0.1 ml of dimethyl sulfoxide (DMSO) as a polymerization inhibitor; but when it was used in the reaction mixture, the lines to the detector of the gas chromatograph became periodically blocked necessitating disassembly of the detector for cleaning. After DMSO was eliminated from the reaction mixture, this problem was corrected. The current method gave satisfactory results on standards and was the one that was used for all of the fatty acid analyses.

Tocopherol Analyses

Tocopherols in the pecan samples were determined by the methods of Nelson, et al. (80), and Slover (81), and will be discussed in the Procedures section of Chapter VI.

Oil Determination

A modification of the official A.O.A.C. method for fat determination was used (82).

Finely ground pecan samples (approximately 1 gm) were weighed and extracted with peroxide-free ether for 24 hours in a Goldfisch extraction apparatus. The ether was then removed using a hot plate and the samples were cooled in a dessicator and subsequently weighed.

Protein Determination

The Kjeldahl method was used for protein determination (83).

Results and Discussion

The oxidation studies on pecan oils consisted of a series of seven

experiments designed to establish factors involved in the stability of pecan oils and components of the oil which contribute to oxidative stability or instability.

The first experiment was merely an orientation experiment designed to show what was happening to the oil during the autoxidation process. In this experiment, 30 gm of pecan oil (Texas Prolific variety, 1968 crop) were weighed into a petri dish and placed in an oven at 70° C. Each day four samples were taken from the petri dish: (1) 1 gm for the TBA test, (2) 0.2 gm for tocopherol analysis, (3) 0.04 gm (two drops) for fatty acid analysis, and (4) a sufficient sample for spectrophotometric analysis at 452 mμ . The fourth sample was returned to the dish after use. This fourth sample was a measure of pigmentation since pecans contain carotenoids as well as other yellow pigments that absorb in the region around 450 mμ . The results of this experiment are shown in Figure 21.

The oxidation of pecan oils is similar to the oxidation of other oils. It is characterized by a lengthy induction period during which peroxides are formed, followed by a sudden increase in low molecular weight aldehydes which give a color with the TBA test. As seen in the figure, this break point is very sharp, and as mentioned before, it will be designated the keeping time of the oil.

During the course of this oxidation, little change was noted through day 4. The fatty acid concentrations remained constant; the TBA values underwent only minor changes; the pigment concentration (A_{452}) decreased about 30%; and the tocopherol concentration, which remained constant for two days, also decreased by about 30%. The results agreed with the predicted outcome. It was assumed that the tocopherol concen-

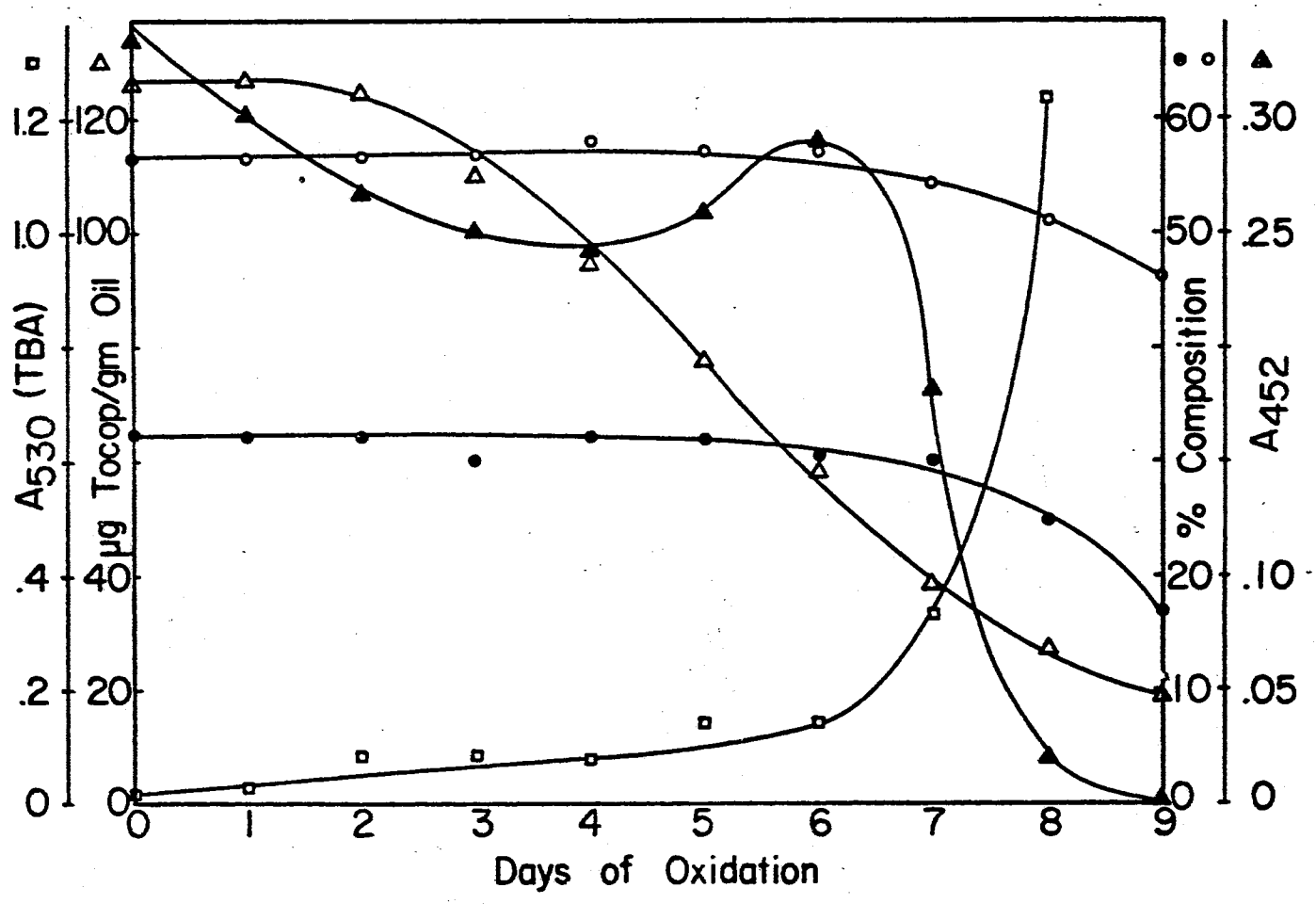


Figure 21. Changes in Tocopherol (Δ) Concentration, Oleic (o) and Linoleic (●) Acid Concentrations, Yellow Pigmentation (▲), and the TBA Value (▫) During the Oxidation of a Pecan Oil

tration would remain constant until peroxides were formed and would then be used in their antioxidant capacity until they were consumed. It was also assumed that β-carotene, which is more susceptible to oxidation than the fatty acids, would be the first component to decrease; finally when the tocopherol and pigments had disappeared, the fatty acids would start to oxidize and the TBA value would increase. At day 4, the system diverted from the expected pathway, and the absorbance at 452 mμ increased. Even though the absorbance was increasing, it was a secondary absorbance because the color was not a pure yellow, but appeared to have a reddish character. Apparently what was produced was a red pigment with a maximum absorbance at a longer wavelength which still exhibited a marked absorption at 452 mμ . Aside from this red pigment, the components of the oil continued to respond as anticipated. This absorption increased through day 6 and then sharply decreased, such that by day 8, the oil was colorless. After this sudden loss of pigmentation, rancid components began to form and the linoleic acid (and to a lesser extent, oleic acid) began to disappear.

Having observed the actions of tocopherols and carotenes (and other pigments) during the course of the first experiment, a second was planned to determine if added tocopherol and β-carotene would increase the keeping time of the oils. Five oil samples (Texas Prolific, 1968 crop) were used in this experiment: (1) control, un-adulterated pecan oil; (2) stripped oil (see procedures section); (3) stripped oil with 500 μg of γ-tocopherol/gm of oil added; (4) stripped oil with enough β-carotene added to bring the absorbance at 452 mμ (A_{452}) to about 0.075; (5) stripped oil with both β-carotene and 400 μg of γ -tocopherol/gm of oil. The results of this experiment are similar to

the first, but each variable (X-axis) for all five oils is graphed separately. The first of these to be examined was the rancidity development, Figure 22. The stripped oil was the most susceptible to oxidation. The β-carotene enriched oil had a keeping time about one-half day longer than the stripped oil, but lagged one and one-half days behind the control. Thus, β-carotene confers some stability on the oil through a co-oxidative action, but the keeping qualities do not equal those of the original oil. On the other hand, addition of γ-tocopherol to the stripped oil in an approximately two-fold excess over the control increased the keeping time by one day (or 10%). However the oil that was the most resistant to oxidation was the one containing both tocopherol (400 μg/gm oil) and β-carotene. Apparently there is a synergistic action involved as the sample containing both γ-tocopherol and β-carotene was deliberately prepared with 20% less tocopherol. Figure 23 shows the decrease in tocopherol during the course of the oxidation. It appears that if excessive amounts of tocopherol were added, the rate of disappearance of the tocopherol also increased. The net result was that after about eight days, all of the samples were void of tocopherol, regardless of the original amount present. From Figure 24, some conclusions can be drawn concerning the fate of the tocopherols. In the two tocopherol enriched oils, the A_{452} reached a maximum absorbance of about 0.400. The control peaked at about 0.300, whereas the stripped and carotene enriched oils peak at an absorbance of just over 0.100. A literature search (84) indicated that γ-tocopherol oxidized to toco-red, an ortho-quinone similar to tocopherol. This is in part responsible for the increase in A_{452}. The stripped oil also increased in A_{452} during the course of the oxidation, so it appeared that the stripped oil was

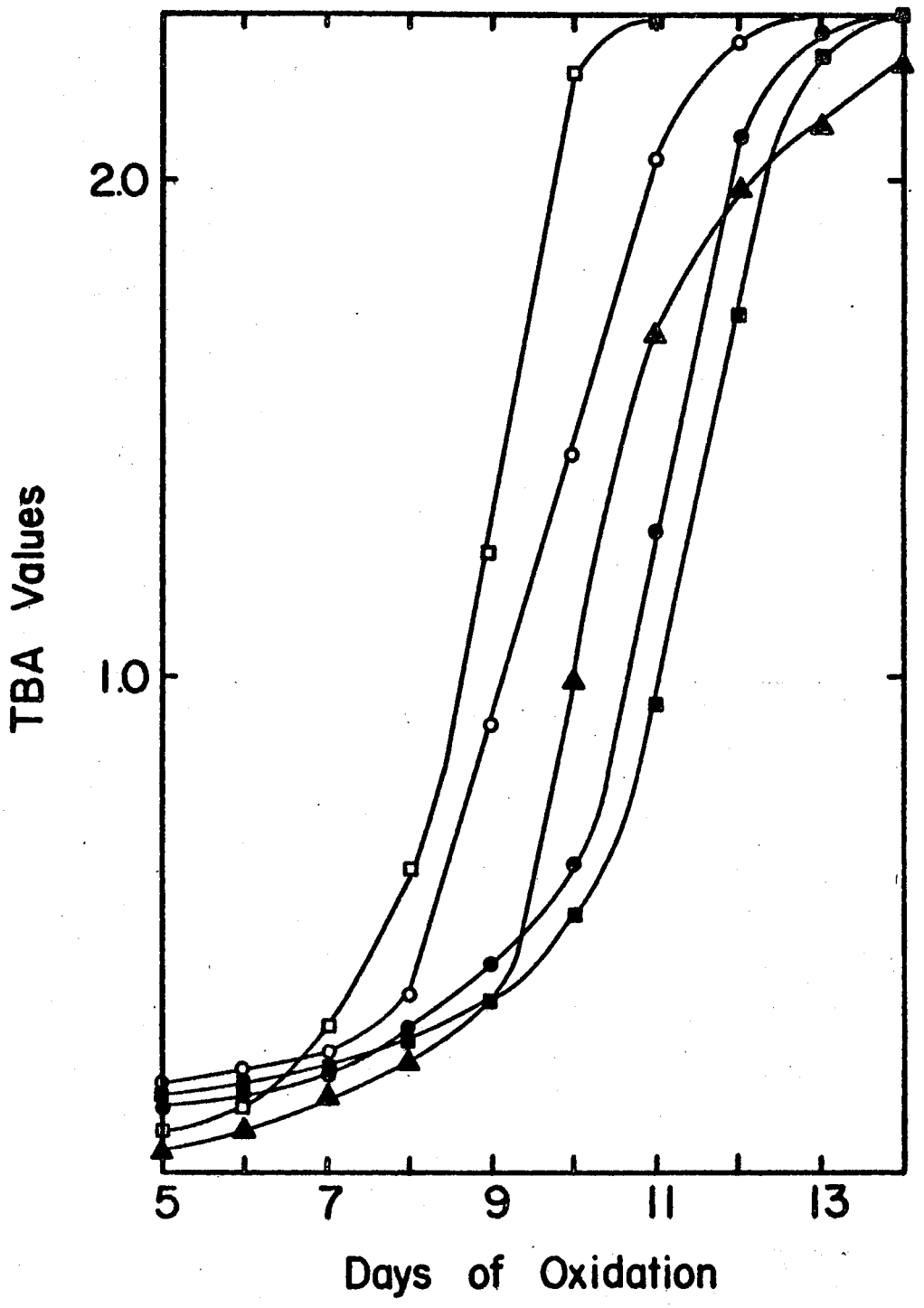


Figure 22. Comparison of the Oxidative Stabilities (TBA Values) of Stripped Oil (□), Stripped Oil Enriched With: (1) β-carotene (○); (2) γ-tocopherol (500 μg/gm Oil) (●); and (3) β-carotene plus γ-tocopherol (400 μg/gm Oil) (■), and the Untreated Oil (▲)

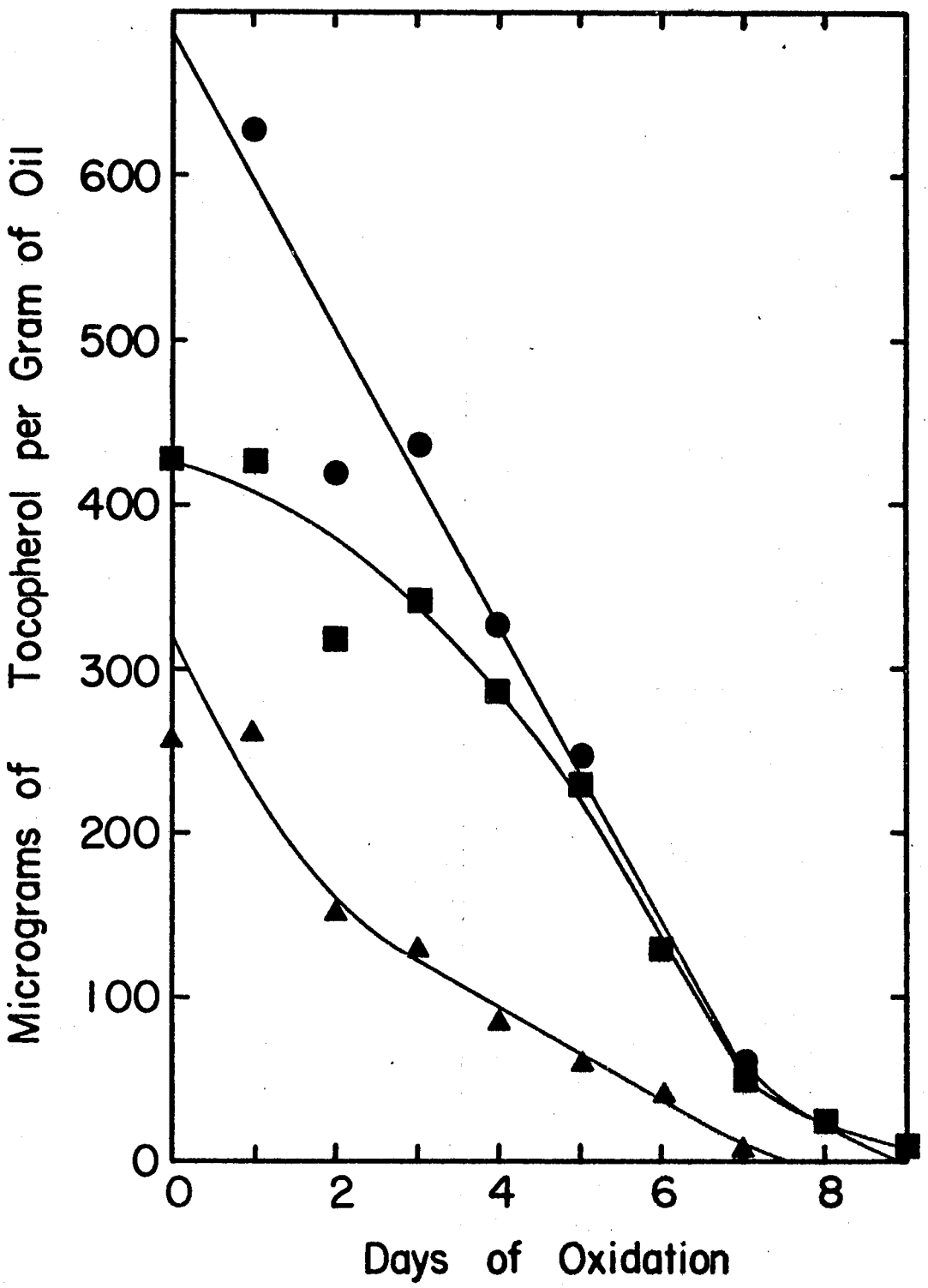


Figure 23. Changes in the Tocopherol Concentration of Stripped Oil Enriched With (1) γ-tocopherol (500 μg/gm Oil) (●) and (2) β-carotene and γ-tocopherol (500 μg/gm Oil) (■) and the Untreated Oil (▲) During Oxidation

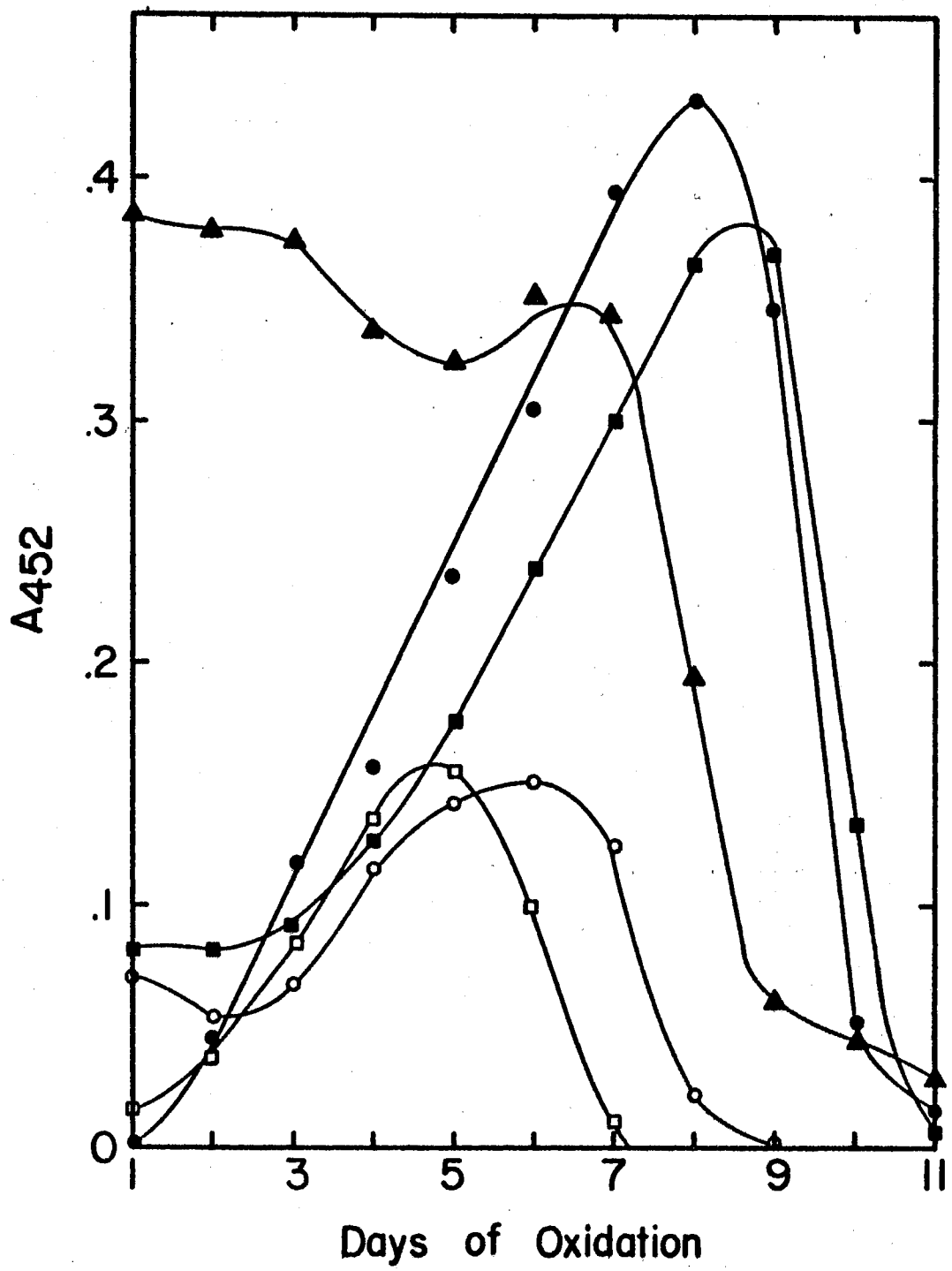


Figure 24. Changes in the Pigmentation (A_{452}) During the Oxidation of Stripped Pecan Oil (□), Stripped Oil Enriched With: (1) β-carotene (○), (2) γ-tocopherol (500 μg/gm Oil) (●), (3) β-carotene plus γ-tocopherol (400 μg/gm Oil), (■) and Untreated Oil (▲)

not completely void of tocopherol. Subsequent analysis of this oil showed however, that it contained absolutely no tocopherol. It might be noted at this point that the stripped oil had a faint yellow tint that even a second charcoal treatment could not remove. This pigment may be oxidizing to a more highly colored product or perhaps some colorless component yields this yellow color upon oxidation. Because of these colored components, β-carotene was not determined by direct A_{452} readings on the oils. The last graph in this series, Figure 25, is a plot of oxidation time vs. oleic/linoleic (O/L) ratio. The significance of these data is that since linoleate oxidizes both sooner than and at a more rapid rate than oleate; an increase in the O/L ratio signifies a decrease in the linoleic content of the oil. The order, as might be expected, parallels the TBA values with the stripped oil losing linoleate most rapidly. After 17 days of oxidation, there was essentially no linoleate left in the stripped oil.

During the fatty acid analyses, an interesting observation was made in all of the oxidation experiments, even those using charcoal treated oils. In all the oils the yellow pigment that appeared during the oxidation disappeared during the transesterification; whereas the oils that had oxidized 11 days or more and had been colorless turned yellow upon transesterification. This phenomenon was not investigated further.

In previous experiments, only one variety of oil had been used. In the third oxidation experiment, two oils were chosen: Barton, O/L = 3.29, and Western, O/L = 1.53. Three oil samples of each variety were oxidized: (1) stripped oil, (2) stripped oil and 500 μg γ-tocopherol/gm oil, and (3) control, un-adulterated oil. A sample of the nuts of each variety was also used. The objectives of this experiment were as

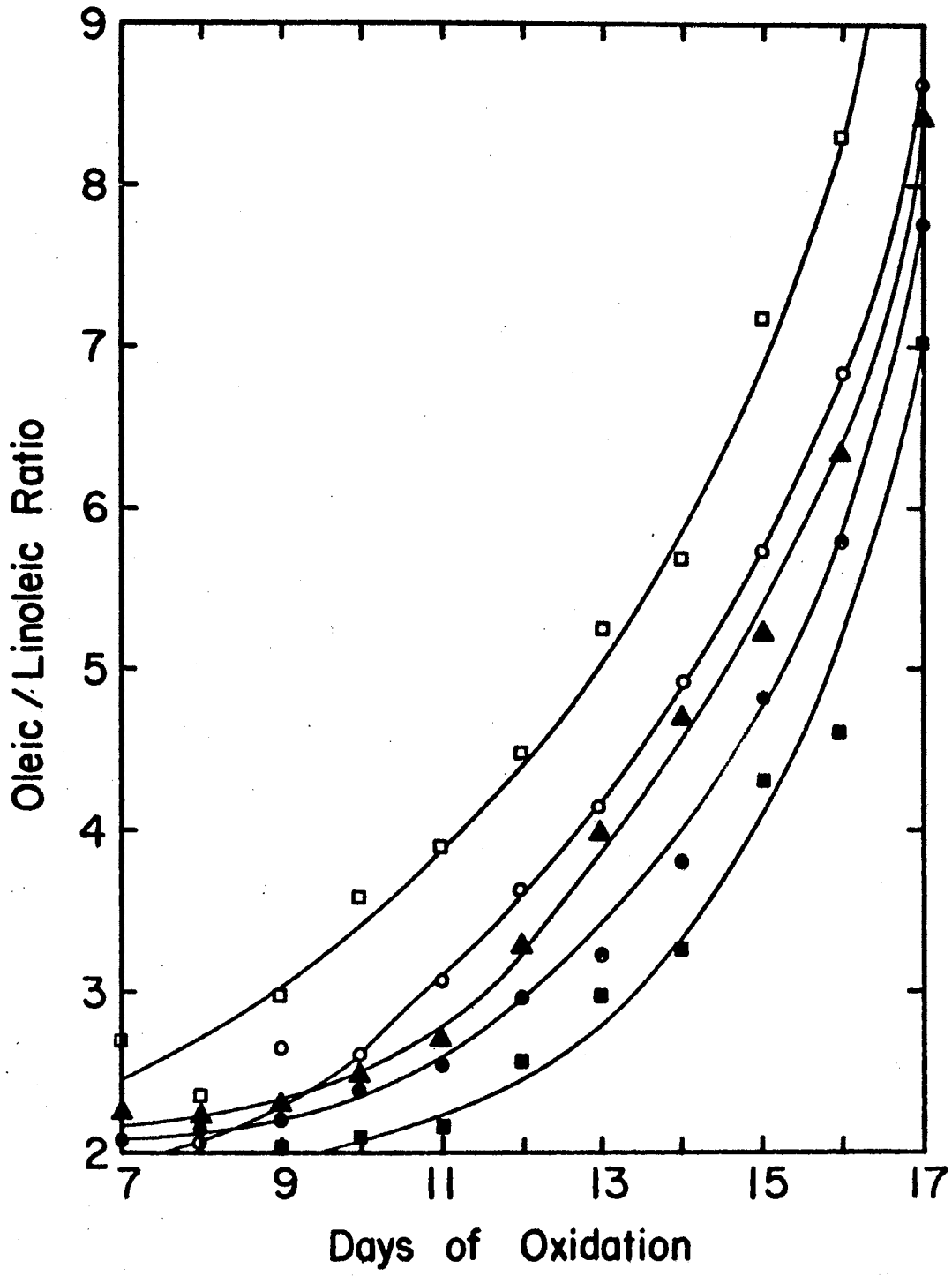


Figure 25. Changes in the Oleic/Linoleic Ratio During the Oxidation of Stripped Pecan Oil (□), Stripped Oil Enriched With: (1) β-carotene (○), (2) γ-tocopherol (500 μg/gm Oil) (●), (3) β-carotene plus γ-tocopherol (400 μg/gm Oil) (■), and the Untreated Oil (▲)

follows: (1) to compare the stripped and control oils of both varieties and determine if there was a difference in the keeping times; (2) to see if added tocopherol has a more protective effect on oils with a higher or lower O/L (i.e. a given amount of tocopherol might be expected to increase stability to a greater extent in an oil with less linoleate); (3) to see if measuring rancidity development in the oil is a true measure of rancidity in the nuts. These oils were oxidized at 75° C. Figure 26 illustrates the results of this experiment (Western--solid line; Barton--dotted line). The addition of tocopherol increased the keeping time by about one day in each oil over the stripped oil, so the tocopherol was no better an antioxidant in the oil with higher O/L than in the one with a lower O/L. In this experiment, the control in each case proved to be more resistant to oxidation than the tocopherol enriched stripped oils, contrary to the earlier observation with the Texas Prolific oil. Also, the Barton control had a keeping time four days longer than the stripped oil whereas the Western control oil had a keeping time only two days longer than its respective stripped oil. Perhaps there is another antioxidant present in the oils which is more efficient in stabilizing oils with a lower linoleate content.

Each day a sample of the pecans were removed from the oven, pressed, and analyzed by the TBA method. The absorbance at 530 mμ never passed 0.07, indicating that although the nuts may have been oxidizing (organoleptically), the malonaldehyde and other aldehydes formed were apparently reacting with the free amino acids and proteins of the nut. Hence, it was not determined whether rancidity development in the oil is truly representative of the nut. Peroxide value data would have been of no greater value, as sulfur containing amino acids act as antioxidants (61)

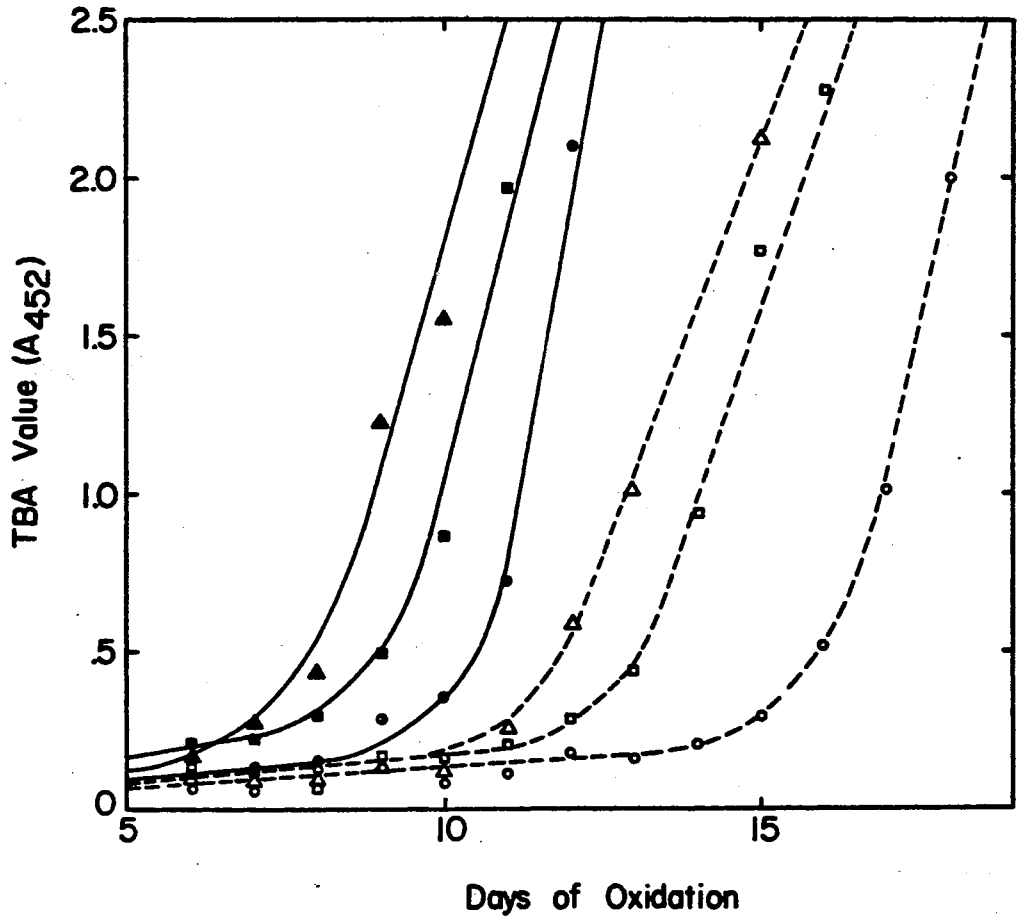


Figure 26. Effect of γ-tocopherol on Oils with Relatively High (———) and Low (----) Concentrations of Linoleic Acid. Stripped Oil (△, ▲), Tocopherol Enriched Oil (500 μg/gm Oil) (□, ■), and the Control Oil (○, ●)

or substrates for oxidation as could other cellular components.

In the fourth oxidation experiment, 60 ml of oil was pressed from 70 varieties of pecans. Thirty grams of this oil was used in the oxidation study and the remainder was saved for analytic purposes. Fatty acid, protein and tocopherol analyses were performed on the nuts. This data is summarized in Table IV. The fatty acid compositions are listed in Appendix A. After oxidizing these 70 varieties of nuts, the keeping times were correlated with various components in the oil. Figure 27 is a plot of keeping time vs. % linoleic acid in the oils. The points form a straight line with a standard deviation of 1.1 days. The straight line for this and the next two figures was calculated by Program 1 in Appendix B. It is essentially a linear least squares fit, and the line is statistically the best straight line through the points. Figure 28 shows an inverse correlation between % oleic acid and keeping time. This is explained by Figure 29, in which % oleic acid plotted against % linoleic acid for the 70 varieties of pecans is a straight line. The results show that the % oleic acid plus the % linoleic acid in a pecan equals approximately 90 $\pm$ 1%.

When keeping time was plotted against tocopherol concentration (Figure 30) no correlation was observed, indicating that tocopherols are not of prime importance in determining the oxidative stability of the oil.

Since biological systems usually tend towards maintaining functional ability, Harris (85) suggests that if a nut has a high concentration of linoleic acid, it might synthesize additional tocopherol to account for the additional poly-unsaturation. When tocopherol concentration is plotted against the % linoleic acid in the oil (Figure 31), no correla-

TABLE IV

KEEPING TIME (DAYS), % OLEIC ACID, % LINOLEIC ACID, TOCOPHEROL CONCENTRATION (μ G/GM OIL), AND % PROTEIN IN 70 VARIETIES OF PECANS

NO.	VARIETY	KEEPINGTIME	%OLEIC	%LINOLEIC	TOCOPHEROL	PROTEIN
1	WESTERN	11.35	54.26	35.39	490.	10.82
2	BARTON	18.70	70.03	21.28	375.	9.12
3	STUART	18.60	68.93	21.89	393.	8.08
4	DELMAS	11.15	54.10	36.21	287.	12.64
5	BURKETT	11.75	58.59	32.53	237.	13.03
6	SUCESS	15.70	64.84	26.04	376.	9.50
7	SAN SABA	14.15	62.61	28.52	413.	10.52
8	MONEYMAKER	14.40	64.53	26.93	290.	10.87
9	OKLAHOMA	14.70	66.64	24.06	248.	15.02
10	SQUIRELL	13.50	59.01	31.02	242.	11.68
11	MAJOR	12.85	68.84	22.25	290.	10.63
12	SCHLEY	14.40	63.48	27.50	309.	10.71
13	CADDO (BW)	15.70	67.71	22.93	246.	10.38
14	CHOCTAW (BW)	18.75	73.99	18.03	221.	10.21
15	CADDO	16.25	64.40	25.64	379.	9.50
16	WICHITA (BW)	19.75	71.49	18.36	325.	9.46
17	COMANCHE (BW)	16.15	64.52	26.05	323.	12.78
18	SHAWNEE (BW)	14.65	64.72	25.07	369.	10.68
19	MOHAWK (BW)	16.20	64.45	26.50	353.	9.79
20	SIOUX (BW)	15.85	65.99	25.14	291.	10.44
21	GREEN RIVER	18.55	71.87	19.60	253.	9.44
22	COWLEY	12.75	61.24	28.91	233.	8.88
23	MOUNT	16.65	67.81	23.33	263.	10.50
24	#-513	18.90	74.09	17.77	306.	10.54
25	#-511	19.85	71.28	19.24	456.	7.31
26	#-551	23.20	73.96	16.91	362.	9.18
27	GORMELY	20.20	72.11	18.74	266.	9.35
28	JACK BALLARD	15.35	63.73	26.75	353.	7.22
29	TEXAS PROLIFIC	15.10	63.65	26.89	324.	9.78
30	BARTON (BW)	14.00	58.58	31.72	410.	11.19
31	#-572	20.95	73.77	17.29	238.	10.13
32	#-542	14.20	65.01	25.35	277.	10.00
33	STUART SEEDLING	14.55	64.43	26.70	323.	8.13
34	#-600	20.95	68.81	21.75	131.	14.00
35	APACHE (BW)	18.80	71.83	19.27	253.	9.16
36	#-2412	17.35	70.58	20.59	464.	8.35
37	#-593	16.85	70.52	19.75	215.	13.16
38	#-507	13.10	62.61	27.13	213.	11.47
39	#-465	13.85	65.63	25.75	225.	14.16
40	SCHLEY SPARKS	17.55	68.65	22.75	309.	9.38
41	AGGIE	15.30	64.76	26.68	327.	10.35
42	#-502	12.15	57.09	32.16	412.	10.00
43	BUTTERICK	16.00	69.27	21.06	207.	9.28
44	#-495	16.20	69.46	22.13	285.	10.07
45	#-481	18.75	70.69	20.84	454.	12.07
46	PERUQUE	17.20	69.07	21.42	294.	8.28
47	GOLDEN	20.00	72.61	18.71	405.	7.85
48	#-4610	19.25	70.58	20.69	308.	8.69
49	TEXHAN	13.30	61.87	27.44	305.	10.88
50	#-472	15.60	69.93	21.39	231.	9.88
51	#-591	15.75	66.55	24.09	285.	8.57
52	#-571	16.45	67.02	23.83	282.	9.91
53	KENTUCKY	19.90	73.28	17.00	234.	10.06
54	#-519	20.15	72.22	17.32	388.	7.41
55	#-552	14.75	66.04	25.06	426.	10.69
56	#-493	17.75	70.34	20.51	246.	9.91
57	#-467	13.55	63.68	27.65	326.	11.41
58	#-581	19.75	73.27	17.81	323.	10.32
59	#-497	18.80	73.47	17.68	354.	8.07
60	PATRICK	12.45	59.58	31.15	337.	15.47
61	#-464	18.30	71.12	19.85	240.	9.82
62	#461	17.45	66.63	23.39	493.	7.91
63	#-503	15.55	64.73	26.23	333.	9.29
64	#-518	13.45	59.60	30.81	370.	10.22
65	#-61H	16.75	71.15	19.37	226.	12.38
66	#-583	15.60	68.07	22.78	255.	10.00
67	HAYES	10.45	53.42	37.36	324.	9.28
68	ELLIOT	16.55	67.00	24.44	457.	10.53
69	#-504	18.60	71.59	19.45	298.	16.88
70	DESIREABLE	15.60	68.40	23.27	313.	11.02

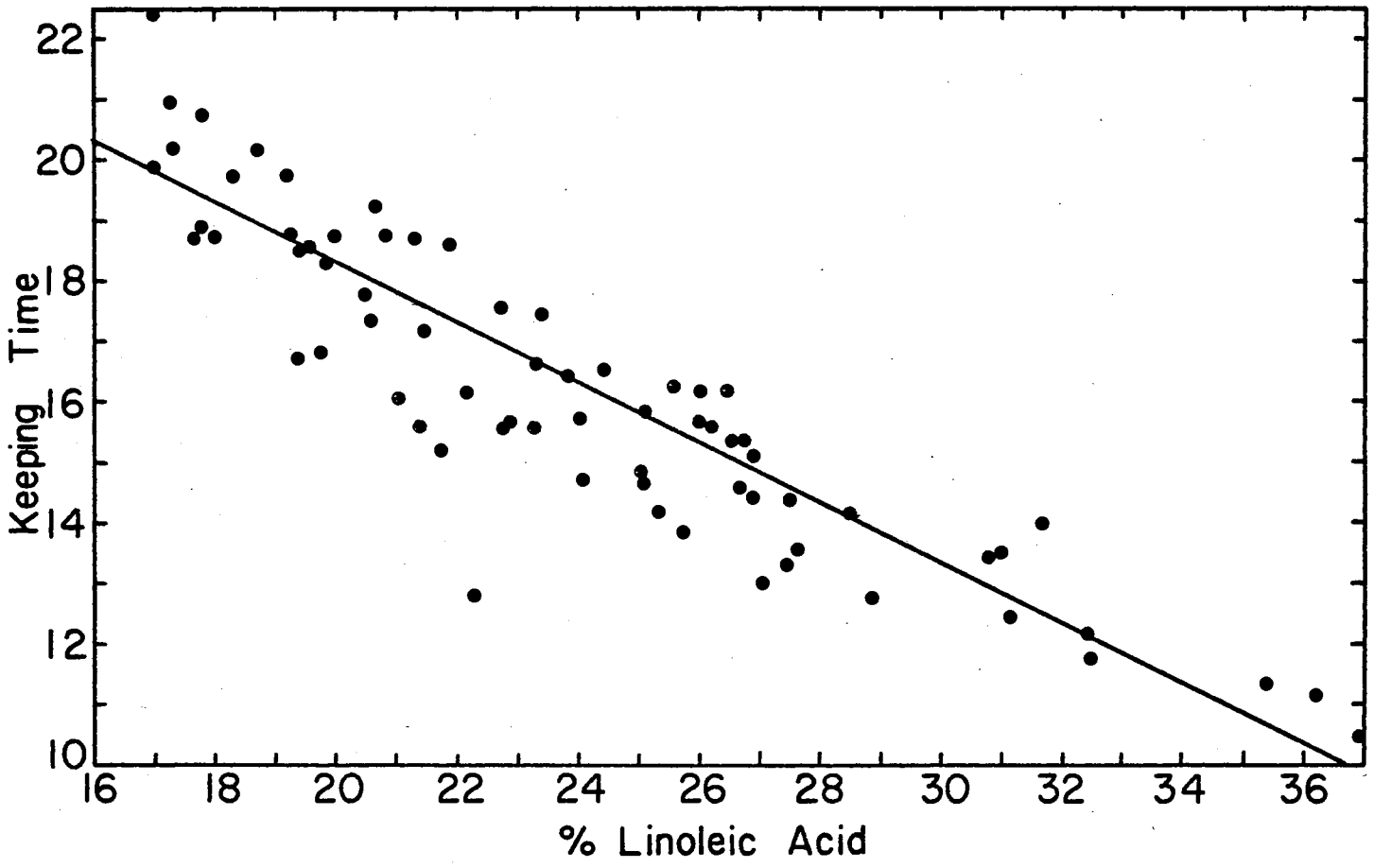


Figure 27. Variation of Keeping Time of the Oil (in Days) with the % Linoleic Acid From Oil of 70 Varieties of Pecans

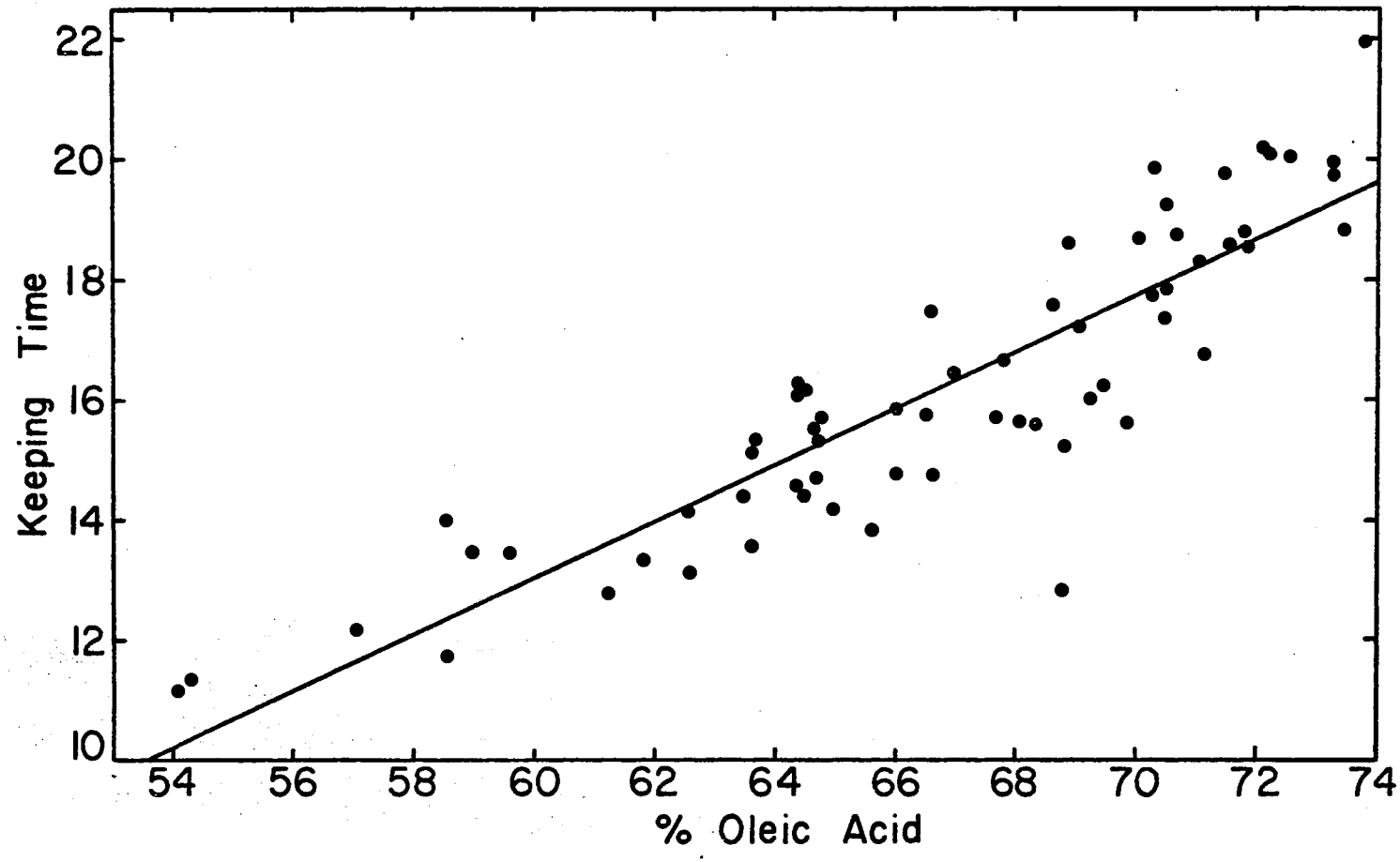


Figure 28. Variation in Keeping Time of the Oil (in Days) With % Oleic Acid From 70 Varieties of Pecans

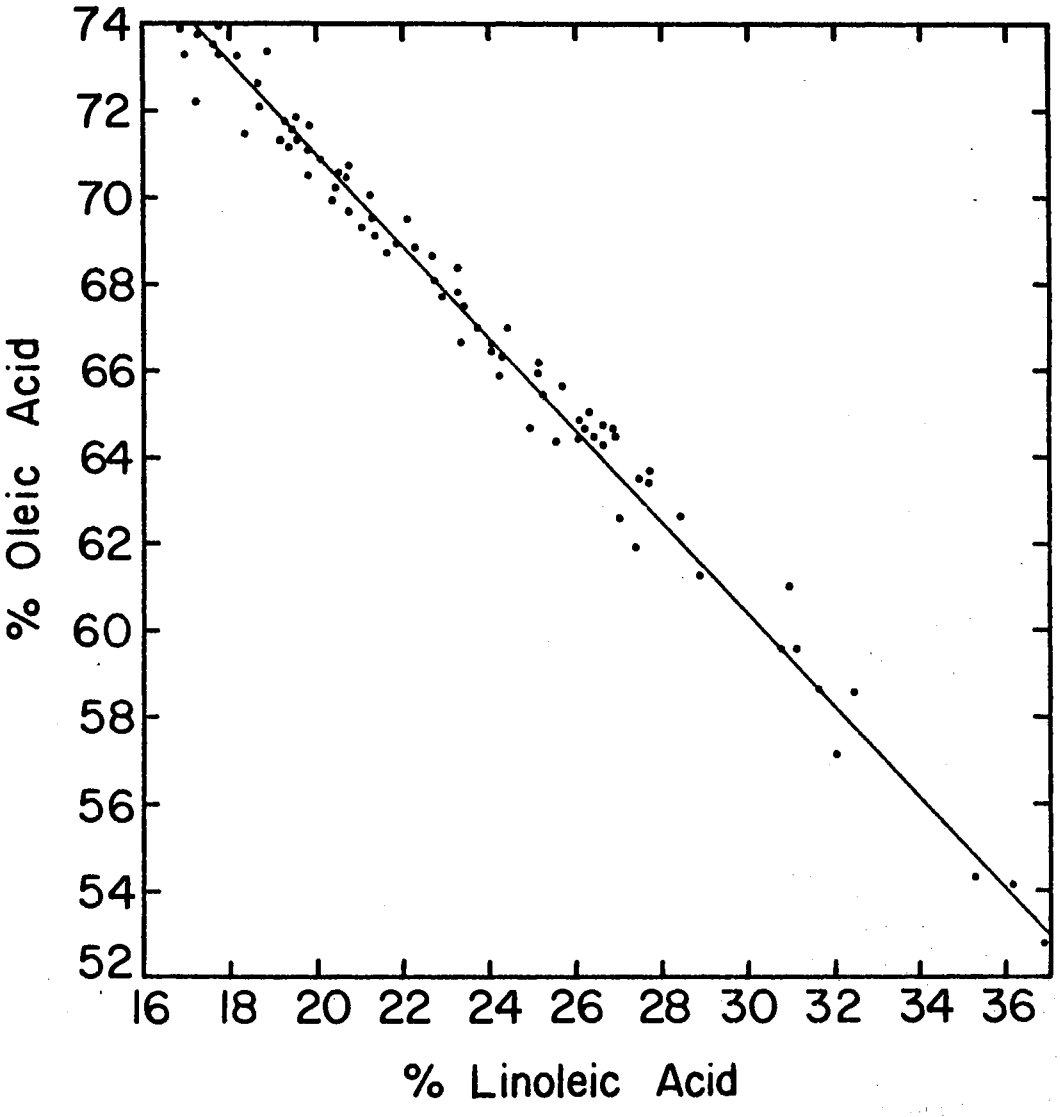


Figure 29. Complementary Relationship Between Oleic and Linoleic Acid Compositions of the Oils From 83 Varieties of Pecans

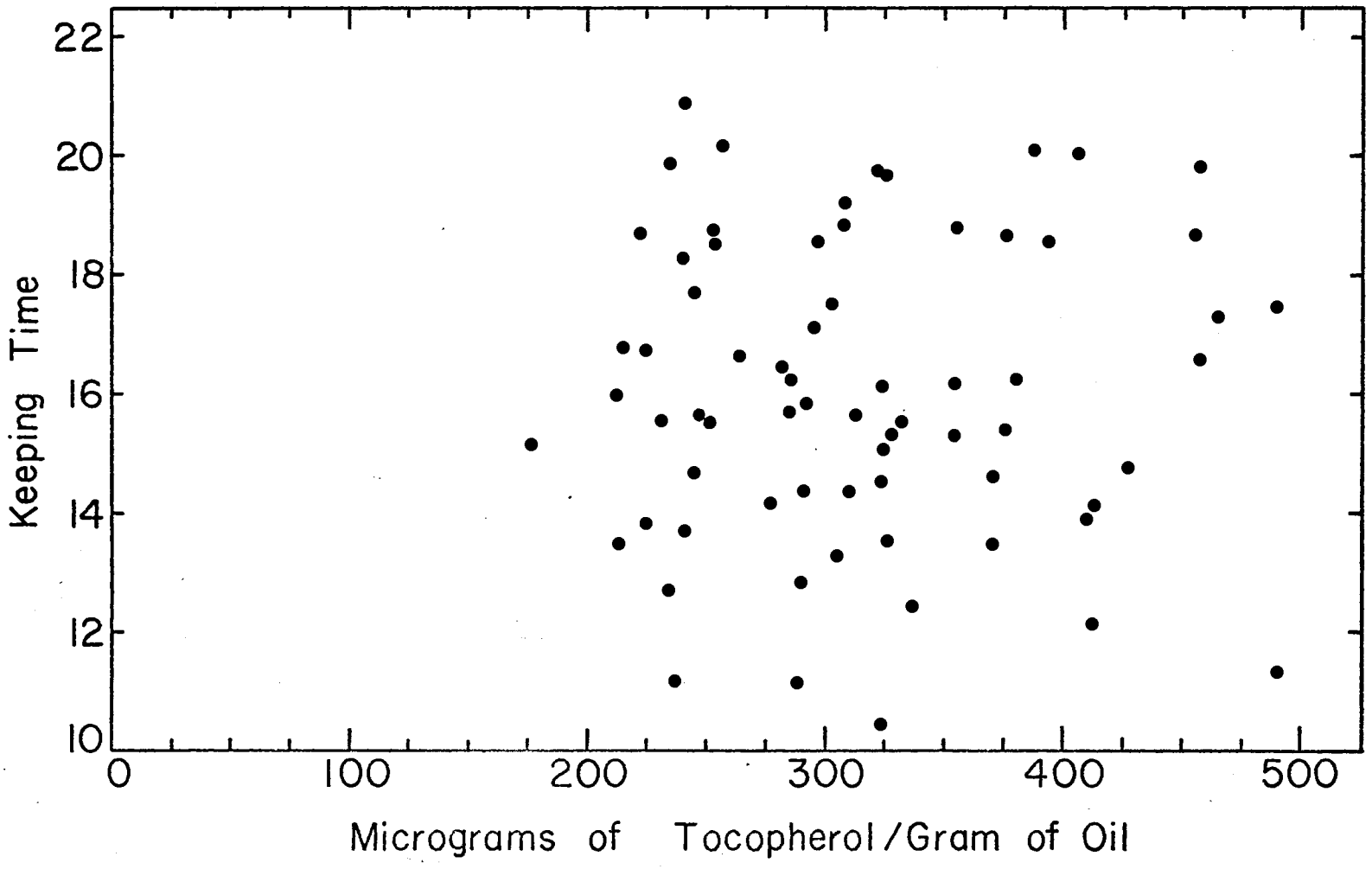


Figure 30. Relationship Between Tocopherol Content and the Keeping Time (in Days) of 70 Varieties of Pecans

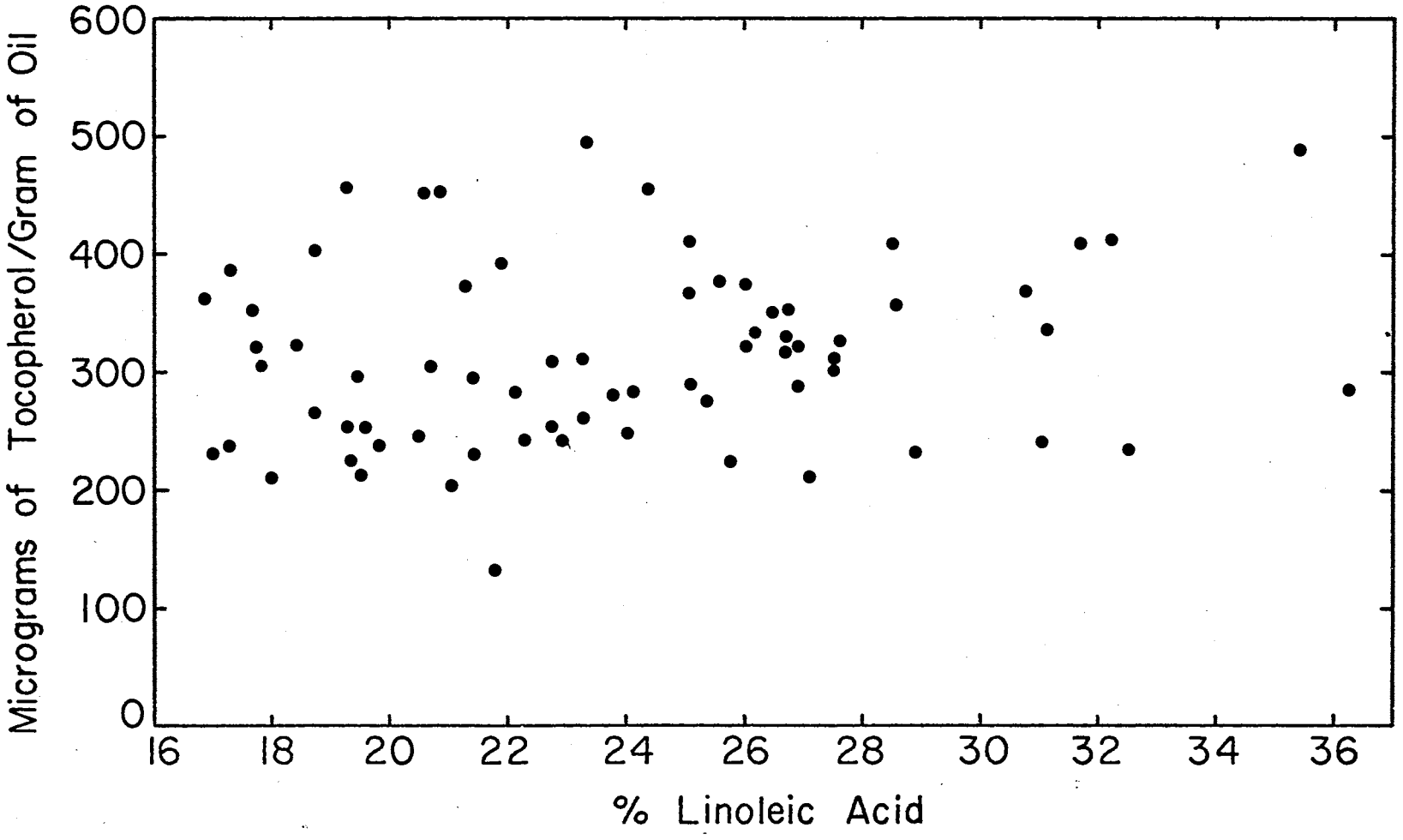


Figure 31. Relationship Between Tocopherol Content and Linoleic Acid Concentrations in 70 Varieties of Pecans

tion is observed.

It has been shown in previous experiments that tocopherols do have a function in controlling rancidity. Program 1 (Appendix B), in addition to calculating the linear least squares fit, covered the following: The program calculated the standard deviation of all points along the y-axis (i.e. the deviation of the keeping time). For the plot of keeping time vs. oleic acid, the standard deviation was 1.23 days; for the plot of keeping time vs. linoleic acid concentration, the standard deviation was 1.19 days. The computer made two other calculations with keeping time. Using an equation derived for peanuts (86)

$$kt = 31.41 - 0.396 \times (\% L) - 0.075 \times (\% \text{ protein}) - 0.063 \times (\% O)$$

where,

kt = keeping time

% O = % oleic acid in oil

% L = % linoleic acid in oil

% protein = % protein in nut,

the computer calculated a theoretical keeping time for the oils. Using the equation for the slope of a straight line, the "calculated keeping time" was determined. This is the value of the keeping time predicted for a nut with a given % linoleic acid. Of course the actual keeping time is the experimentally determined value. Also calculated was the difference between the calculated and actual keeping times or the deviation along the y-axis of each point from the line. A minus sign indicates the experimental point was below the calculated point. The data for the plot of keeping time vs. linoleic acid is given in Table V.

TABLE V

KEEPING TIMES, THEORETICAL, ACTUAL, AND CALCULATED, AND THE DIFFERENCE BETWEEN ACTUAL AND CALCULATED KEEPING TIMES FOR 70 VARIETIES

NO.	VARIETY	THEORETICAL KEEPING TIME	ACTUAL KEEPING TIME	CALCULATED KEEPING TIME	DIFFERENCE
1	WESTERN	13.17	11.35	10.57	0.78
2	BARTON	17.89	18.70	17.62	1.08
3	STUART	17.79	18.60	17.31	1.29
4	DELMAS	12.71	11.15	10.16	0.99
5	BURKETT	13.86	11.75	12.00	-0.25
6	SUCESS	16.30	15.70	15.24	0.46
7	SAN SABA	15.38	14.15	14.00	0.15
8	MONEYMAKER	15.87	14.40	14.80	-0.40
9	OKLAHOMA	16.56	14.70	16.23	-1.53
10	SQUIRELL	14.53	13.50	12.76	0.74
11	MAJOR	17.46	12.85	17.13	-4.28
12	SCHLEY	15.72	14.40	14.51	-0.11
13	CADDO (BW)	17.29	15.70	16.79	-1.09
14	CHOCTAW (BW)	18.84	18.75	19.24	-0.49
15	CADDO	16.49	16.25	15.44	0.81
16	WICHITA (BW)	18.93	19.75	19.07	0.68
17	COMANCHE (BW)	16.07	16.15	15.24	0.91
18	SHAWNEE (BW)	16.60	14.65	15.73	-1.08
19	MOHAWK (BW)	16.12	16.20	15.01	1.19
20	SIOUX (BW)	16.51	15.85	15.69	0.16
21	GREEN RIVER	18.41	18.55	18.46	0.09
22	COWLEY	15.44	12.75	13.81	-1.06
23	MOUNT	17.11	16.65	16.59	0.06
24	#-513	18.91	18.90	19.37	-0.47
25	#-511	18.75	19.85	18.64	1.21
26	#-551	19.37	23.20	19.80	3.40
27	GORMELY	18.74	20.20	18.88	1.32
28	JACK BALLARD	16.26	15.35	14.89	0.46
29	TEXAS PROLIFIC	16.02	15.10	14.82	0.28
30	BARTON (BW)	14.32	14.00	12.41	1.59
31	#-572	19.16	20.95	19.61	1.34
32	#-542	16.53	14.20	15.59	-1.39
33	STUART SEEDLING	16.17	14.55	14.91	-0.36
34	#-600	17.41	20.95	17.38	3.57
35	APACHE (BW)	18.57	18.80	18.62	0.18
36	#-2412	18.18	17.35	17.96	-0.61
37	#-593	18.16	16.85	18.38	-1.53
38	#-507	15.86	13.10	14.70	-1.60
39	#-465	16.02	13.85	15.39	-1.54
40	SCHLEY SPARKS	17.37	17.55	16.88	0.67
41	AGGIE	15.99	15.30	14.92	0.38
42	#-502	14.33	12.15	12.19	-0.04
43	BUTTERICK	18.01	16.00	17.73	-1.73
44	#-495	17.52	16.20	17.19	-0.99
45	#-481	17.80	18.75	17.84	0.91
46	PERUQUE	17.96	17.20	17.55	-0.35
47	GOLDEN	18.84	20.00	18.90	1.10
48	#-4610	18.12	19.25	17.91	1.34
49	TEXHAN	15.83	13.30	14.54	-1.24
50	#-472	17.79	15.60	17.56	-1.96
51	#-591	17.03	15.75	16.21	-0.46
52	#-571	17.01	16.45	16.34	0.11
53	KENTUCKY	19.31	19.90	19.75	0.15
54	#-519	19.45	20.15	19.59	0.56
55	#-552	16.52	14.75	15.73	-0.98
56	#-493	18.11	17.75	18.00	-0.25
57	#-467	15.59	13.55	14.44	-0.89
58	#-581	18.97	19.75	19.35	0.40
59	#-497	19.17	18.80	19.41	-0.61
60	PATRICK	14.16	12.45	12.69	-0.24
61	#-464	18.33	18.30	18.33	-0.03
62	#461	17.36	17.45	16.56	0.89
63	#-503	16.25	15.55	15.15	0.40
64	#-518	14.69	13.45	12.86	0.59
65	#-61H	18.33	16.75	18.57	-1.82
66	#-583	17.35	15.60	16.87	-1.27
67	HAYES	12.55	10.45	9.59	0.86
68	ELLIOT	16.72	16.55	16.04	0.51
69	#-504	17.93	18.60	18.53	0.07
70	DESIREABLE	17.14	15.60	16.62	-1.02

This difference was then plotted vs. tocopherol concentration (Figure 32), since the relative abundance or lack of tocopherols might be a significant factor in this deviation. Although the line is relatively level, the best straight line through these points has a slope of 0.6202 units/100 μg of tocopherol, indicating that there is some correlation between this difference and the tocopherol concentration. Thus, while the concentration of tocopherols does not correlate with keeping time, their presence certainly influences the oxidative stability of the nut.

The qualitative effects of tocopherols on oil stability have been described in the preceding paragraph, but no mention has been made of any quantitative effect. It seems reasonable to expect that the more tocopherol present, the greater will be the stability of a given oil. Swift (87) obtained evidence that at high concentrations (no values given) tocopherols may have a pro-oxidative effect, and Dugan (88) has shown that maximal anti-oxidant activity is attained with a tocopherol concentration of 100 μg/gm oil in lard.

The next experiment was designed to show the quantitative effects of tocopherol concentration on the stability of pecan oil. Pecan oil, Stuart variety, 1969 crop, was enriched with tocopherol at six different concentrations: 100, 200, 400, 600, 800, and 1000 μg γ-tocopherol/gm oil. The oils were then oxidized at 68° C. until they became rancid (as determined by the TBA test). Figure 33 is a plot of keeping time of the oils against micrograms of tocopherol/gram of oil. The general trend shows that the keeping time increased from 9.2 days in the control to 13.8 days in the oil enriched with 800 μg of tocopherol/gm oil. This is an increase of 50% in the keeping time of the oil. Most pecans have between 250 and 400 μg tocopherol/gm oil which would mean an increase

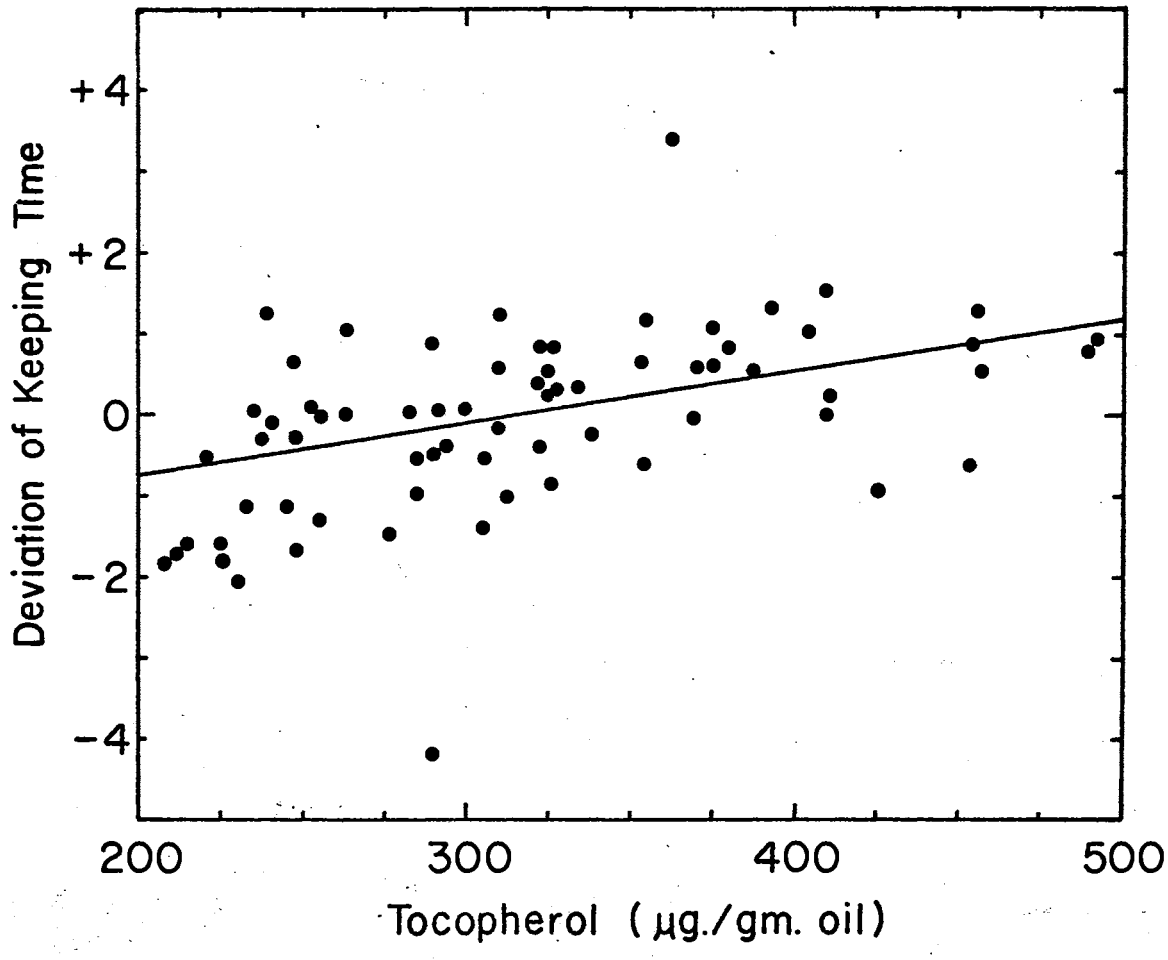


Figure 32. Relationship Between the Deviation of the Actual Keeping Time From the Calculated Keeping Time and Tocopherol Concentration

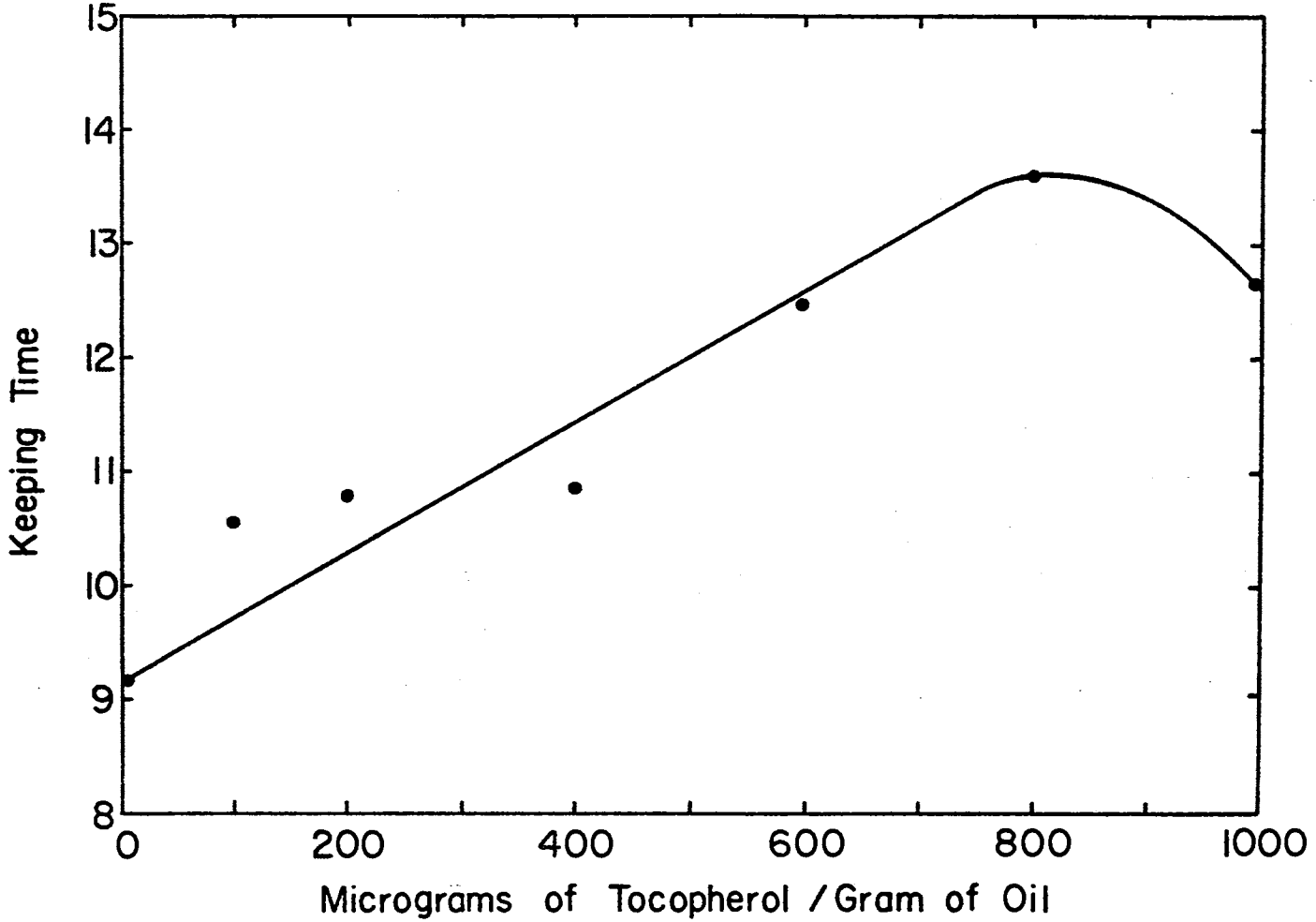


Figure 33. The Effect of Varying the Tocopherol Concentration of Pecan Oil, Stuart Variety, on the Keeping Time (in Days)

in keeping time of between 16 and 25% over an oil void of tocopherol. The slight dip in the graph could be significant. It would appear that the oil has reached a plateau of maximal anti-oxidant activity or that there is a loss of anti-oxidative ability due to pro-oxidative effects.

The final experiment of this series was designed to determine the keeping time of pecan oils with a given linoleic and tocopherol content at 20° C., 0° C., and -20° C. To accomplish this, five varieties with linoleic acid contents between 17 and 31% were oxidized at 67°, 76°, 85°, 95°, and 105° C. By application of the Arrhenius Equation, the keeping times would be extrapolated at 20°, 0°, and -20° C., common pecan storage temperatures. Finally, % L (as determined for each variety) would be plotted against keeping time for each of the storage temperatures. With the addition of the tocopherol factor, a minimal keeping time for a given variety could be chemically determined. When oils were oxidized at the higher temperatures (95° and 105° C.), the keeping times were less than two days, making the data insignificant so the results are not presented.

It should be noted that pecans and pecan oils are equated in this study, and anti-oxidant factors present in pecan meal could be involved in oxidative storage problems.

Summary

Chemical changes that occur during oxidation of pecan oils were investigated. During the course of oil oxidation the tocopherol concentration decreases; the yellow oil takes on a reddish quality then becomes colorless. This is followed by a rapid increase in rancidity products and a corresponding decrease in linoleate concentration. If

β-carotene is added to decolorized pecan oil, an increased oxidative stability is observed. The effect of added tocopherol is several orders of magnitude greater than β -carotene in these respects.

Linoleate appears to be the primary factor responsible for the stability of pecan oils. Tocopherols are of secondary importance as their concentration is not directly correlated with keeping time. The oxidative stability of the pecan nut may be proportional to the % linoleate in the oil, but no equating factor was established. The keeping time, % oleate, % linoleate, tocopherol content and % protein for 70 varieties of pecans are listed in tabular form.

A linear increase in the keeping time of pecan oils was observed by adding γ-tocopherol in a concentration up to 800 μg./gm. of oil. At this level, a 50% greater keeping time was observed.

CHAPTER VI

TOCOPHEROLS

Introduction

Occurrence

Tocopherols were discovered quite by accident, as were many other compounds with biological activity. In 1920, Matill and Young (89) showed that rats placed on a diet of cow's milk were unable to bear young. Several years later, it was discovered that this deficiency could be overcome by the inclusion of wheat germ oil in the diet. Although still uncharacterized, the active component was designated Vitamin E. Vitamin E was discovered independently by Evans and co-workers (90) some years later when oxidized lard was accidentally used in a feeding experiment and symptoms of vitamin E deficiency resulted. Evans *et al.* isolated this active component and gave it the generic name tocopherol (from the Greek meaning an alcohol, to bear, and childbirth). The structure of vitamin E (91, 92) was subsequently elucidated through the efforts of several laboratories.

Soon after their discovery, tocopherols were shown to be ubiquitous throughout the plant kingdom and primarily associated with those materials rich in oils and fats (93). By far the richest source of tocopherols is wheat germ oil. Lange (94) performed an extensive literature review on the tocopherol composition of over twenty-five oilseeds and oil-rich plants. In this review, he lists the total

tocopherol content along with % α-, β-, γ-, and δ- forms in tabular form. Several references list the tocopherol content of peanuts (95) and certain vegetable oils (96, 97, 98, 99, 100). Pecan oil is a particularly abundant source of tocopherols. Bailey (101) reported 450 μg of tocopherol per gram of crude pecan oil. Fisher (102) reported 420 μg of tocopherol per gram of crude pecan oil; 220 μg were of the gamma (γ) isomer and 200 μg were of the alpha (α) isomer.

Lambertson et al. (103) reported the α- and γ- tocopherol contents of eight different nuts. Filberts and almonds contain primarily α-tocopherol; walnuts, chestnuts, and pecans contain primarily γ-tocopherol. In peanuts and brazil nuts, there was no predominance of either isomer. Specifically, pecans were shown to contain: 72% oil, 15 μg α-tocopherol/gram of nuts, 170 μg γ-tocopherol/gram of nuts, and a trace of β-tocopherol.

The fatty acid composition has also been studied because Green (104) theorized that nuts with a high oleic acid content would contain predominately α-tocopherol; likewise, those nuts with a high linoleic acid content would contain predominately γ-tocopherol; and those with an oleic/linoleic ratio of close to 1 would have an α/γ ratio of close to 1. This pattern was observed in all of the nuts investigated, with the exception of the pecan.

Pyriadi (45) has reported the tocopherol content of oils from eight varieties of pecans. Alpha and non-α tocopherols are reported with the α-isomer predominating in all of the cases. No work was done to further characterize the non-α fraction.

Analysis

There are many methods available for the separation and quantitation of tocopherols. The classical method for detection of tocopherols is that of Emmerie and Engle (105), in which tocopherols reduce ferric chloride to the ferrous state which then reacts with α, α -dipyridyl. The resulting red color is measured spectrophotometrically. Nearly all of the available methods involve saponification of the oil with alcoholic KOH, followed by extraction and chromatography of the non-saponifiable lipid fraction. Column chromatography using a $MgHPO_4$ adsorbent (106, 107) and paper chromatography (108, 109) have been successfully employed for tocopherol analyses. Paper chromatography is the more sensitive of the two methods but is less adaptable for quantitation. Thin layer chromatography (TLC) is the most widely used method (110, 95, 96, 99, 103) as it combines the sensitivity of paper chromatography with the quantitative aspects of column chromatography. With TLC, it is possible to recover essentially all of the material from the plate.

The method used initially in this laboratory was the gas chromatographic method that Nelson (80) used for soybean sludge, using squalene as an internal standard. An improved method by Slover (111) is similar to the above procedure except that a weighed amount of 5,7-dimethyltocol, a non-naturally occurring tocopherol, is added to the original oil and is carried through the entire procedure. This latter method has been used successfully for tocopherol analyses in this lab. The dimethyltocol reference compound has the advantage of serving as an internal standard; added at the beginning of the experiment, the internal standard is saponified and extracted along with the unknown tocopherols

in the sample.

A method which is specific for γ-tocopherol was developed by Fisher (102). In this method, γ-tocopherol is oxidized to "toco-red," an ortho-quinone, and the absorbance is read at 470 mμ . This procedure worked well for pecans, as α-tocopherol does not interfere with the test. (β-tocopherol, not present in pecans, does interfere, however; therefore this method cannot be used for oils containing significant amounts of β-tocopherol.) Recently, an automated method for tocopherol analysis has been developed, utilizing a Technicon Auto-Analyzer and the Emmerie-Engle color reaction (112). This greatly increases the efficiency of tocopherol analyses.

In summary, the modified Slover method appeared to be the best choice: (1) the internal standard was added at the beginning of the analysis and carried through the entire procedure; (2) trimethylsilyl (TMS) ethers were made, so oxidation was not of concern; (3) GLC was used for the final quantitation; (4) the TLC step was removed eliminating the possibility of oxidation on the silica surface.

Antioxidant Activity

The antioxidant activity of α-, β-, and γ-tocopherols was demonstrated by Olcott and Emerson (113). Criewahn and Daubert (114) investigated the antioxidant properties of α-, β-, γ-, and δ-tocopherols on lard. Their results show that in order of descending antioxidative capability, the tocopherols are: δ-, γ-, β-, and α-. This is inversely related to the order in which the tocopherols overcome vitamin E deficiency (in which case α- is the most active isomer followed by β-, γ-, and δ- (88).

There is much concern at the present time over man's requirement for vitamin E in relation to his dietary intake of unsaturated fat. There appears to be an increased need for vitamin E when the consumption of polyunsaturated fat is increased (115). When considering this relationship, it appears that of the major commercial edible oils (cottonseed, soybean, safflower, and corn), only cottonseed oil has enough tocopherol to overcome the effect of the polyunsaturated fatty acids (99).

Tocopherols appear to function well at low concentrations (0.005%) and an increase in this level (to 0.01%) does not significantly increase the stability of lard (99). Swift et al. (87), working with γ-tocopherol, showed that an optimum amount exists for providing maximum stability of oils. If tocopherol is added in excess of this amount, the tocopherol acts as a pro-oxidant, resulting in decreased stability.

The autoxidation of butter fats has been shown to be inversely proportional to the tocopherol content of butter and directly proportional to the unsaturated fatty acid and soluble copper content (116).

The effect of tocopherol content on the oxidation of peanut oil and soybean oil at higher temperatures has been demonstrated by Schmidt (97). His data indicate that the α-isomer is the most effective antioxidant at 70°C. in peanut oil.

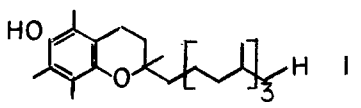
Kovats and others have conducted research on tocopherols and oxidation products in certain vegetable oils (98). Their data suggest that α-tocopherol is the major tocopherol having antioxidant activity in the presence of ultra-violet light, whereas γ-tocopherol is the principal antioxidant in the active oxygen reaction.

When oils are stored under oxidative conditions, there is a rapid

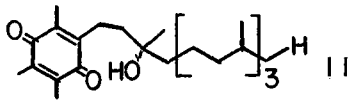
initial decrease in tocopherol content with an insignificant increase in peroxide number (117). Peroxides do not increase until some time after the tocopherol disappears due to the formation of compounds with anti-oxidant activity.

Harris (85) has investigated the linoleic acid-tocopherol relationships in fats and oils. After analyzing twenty-two fats and oils for tocopherol content and fatty acid composition, he observed a highly significant correlation (r=+0.79) for these two components.

The oxidation of α-tocopherol has been studied quite extensively (118). Upon mild oxidation with ferric chloride, α-tocopherol (I) is converted to α-tocopherolquinone (II) (119).

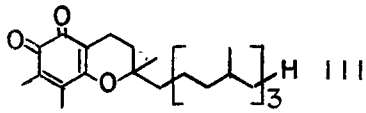


I



II

Oxidation of α-tocopherol under more vigorous conditions or oxidation of γ-tocopherol with ferric chloride produces a red pigment, toco-red (III), which is an ortho-quinone (120).



III

Toco-red also has antioxidant capabilities.

Dimerization appears to be another oxidative reaction undergone by tocopherols. Nilsson et al. (121) have isolated four dimers of γ- and δ-tocopherols from freshly prepared corn oils. They proceeded to synthesize and characterize nine tocopherol dimers and two tocopherol

trimers (122).

Spectral Studies

The infra-red spectra of α-tocopherol and its trifluoroacetate ester has been reported by Nair (123). Lambertsen reported the ultraviolet spectra of γ-tocopherol in ethanol ($E^{1\%}_{1cm}$ at 297 mμ = 93.53) and in cyclohexane ($E^{1\%}_{1cm}$ at 300.5 mμ = 97.57) (103).

A surprising lack of data and reported research on the mass spectra of tocopherols exists. Nair (123) has identified α-tocopherol from animal tissues using combination GC-MS. He presents the mass spectra of α-tocopherol and α-tocopheryl trifluoroacetate, showing only the origin of the major peaks in the spectra. Nilsson (124) has analyzed the mass spectra of some tocopherol models (i.e. chromanols) in which there are gem-dimethyls at position 2 of the typical tocopherol nucleus, thus simplifying the spectra. Wilham (125) has described the mass spectra of several cyclic aromatic ethers including chroman, 6-chromanol, and 2,2-dimethylchroman.. Nilsson (122) has reported some details on the mass spectra of dimers of α-, β-, γ-, and δ-tocopherols, but makes no mention of the spectra of α-, β-, or γ-tocopherols. The mass spectrum of γ-tocotrienol has been published by Mayer et al. (126).

Reagents and Apparatus

Reagents

β-Tocopherol, α-Tocopherol Acetate, and 5,7-Dimethyltocol, Practical Grade, Purity Checked by GLC. Pierce Chemical Co., Rockford, Illinois.

α-Tocopherol, N.F. Calbiochem, Los Angeles, Calif.

γ-Tocopherol, N.F. Distillation Products Industries, Division, Eastman Organic Chemicals, Rochester, N.Y.

OV-1, OV-17, Gas Chrom Q. Trimethylchlorosilane, Hexamethyldisilazane, Applied Science Laboratories, P.O. Box 440, State College, Pa.

Absolute, Pure Ethyl Alcohol, N.F. U.S. Industrials Chemicals, Division of National Distillers and Chemical Corp., New York, N.Y.

Pyrogallol, Potassium Hydroxide, Benzene, and Pyridine, Certified. Fisher Scientific Co., Fair Lawn, N.J.

Ethyl Ether, Anhydrous. J.T. Baker Chemical Co., Phillipsburg, N. J.

Squalene, K and K Laboratories, Plainview, N.Y.

Apparatus

Gas chromatographic tocopherol analyses on the 1968 pecan crop were performed on a modified Barber Coleman 5000, equipped with a hydrogen flame detector. The analyses on the 1969 crop were performed on a Perkin Elmer 801 Gas Chromatograph, equipped with a hydrogen flame detector, glass columns, and an all-glass injection system.

Low resolution mass spectral analyses were performed on an LKB-9000 previously described in Chapter III.

High resolution mass spectral data on the tocopherol standards were obtained on a CEC 21-110 B High Resolution Mass Spectrometer at the Mass Spectrometry Center, Chemistry Department, Purdue University, Lafayette, Indiana.

Procedures

In the tocopherol analyses a gas chromatographic analysis was used instead of Strum's method (95) in which the tocopherols are separated

using quantitative TLC and determined colorimetrically by their reaction with 9,10-phenanthroline and ferric chloride (105). The main reason for this change was the results of Pyriadi (45), who reported α-tocopherol as the major isomer present in pecans while using this method. However, a closer examination of Pyriadi's results indicated that the compound previously thought to be the α-isomer was actually an Emmerie-Engle positive compound (possibly a dimeric oxidation product of a tocopherol) that had an R_f similar to that of α-tocopherol. Thus, the gas chromatographic method of Nelson (80) was modified for use in this laboratory. The major modification was the implementing of a 4% OV-1 instead of a 4% SE-30 on Gas Chrom Q column. When a fluidized bed apparatus (16) was used for preparing the column packing, satisfactory results were obtained. Column conditions used and a typical separation are shown in Figure 34. Vertical slash marks indicate the points at which mass spectra were taken. The separation of squalene and three isomers of tocopherol, α-, β-, and γ-, is given in Figure 35. This method was very sensitive but satisfactory only when the column was stabilized by holding at an isothermal temperature (225°C.) for several hours before use. The column would deteriorate after a very small number of analyses (20-30) using the neat non-saponifiable lipid portion. Hence the procedure of Slover (81) was used for the 1969 crop due to the large numbers of tocopherol analyses required.

In Slover's procedure, he combines preparative thin layer chromatography (TLC) and analytical gas chromatography. The TLC cleans up the crude saponification extract, allowing the GLC columns to be unaffected by other compounds in the non-saponifiable lipid fraction. In this method, didecyl pimelate was used as an internal standard. It was added

Figure 34. Gas Chromatographic Analysis of the Non-Saponifiable Lipid Fraction of Pecans

Column - 6' x $\frac{1}{4}$" Glass, Silanized

Column Packing - 4% OV-1 on Gas Chrom Q

Column Temperature - 225° C.

Injection Port Temperature - 275° C.

Carrier Gas - Helium

Flow Rate - 130 ml./min. (30 ml./min. forms work for MS)

Detector - Hydrogen Flame (or MS)

Instrument - Modified Barber-Coleman 5000

- LKB 9000, Combination GC-MS

Mass Spectrometry Log Number - 1379, Code 26

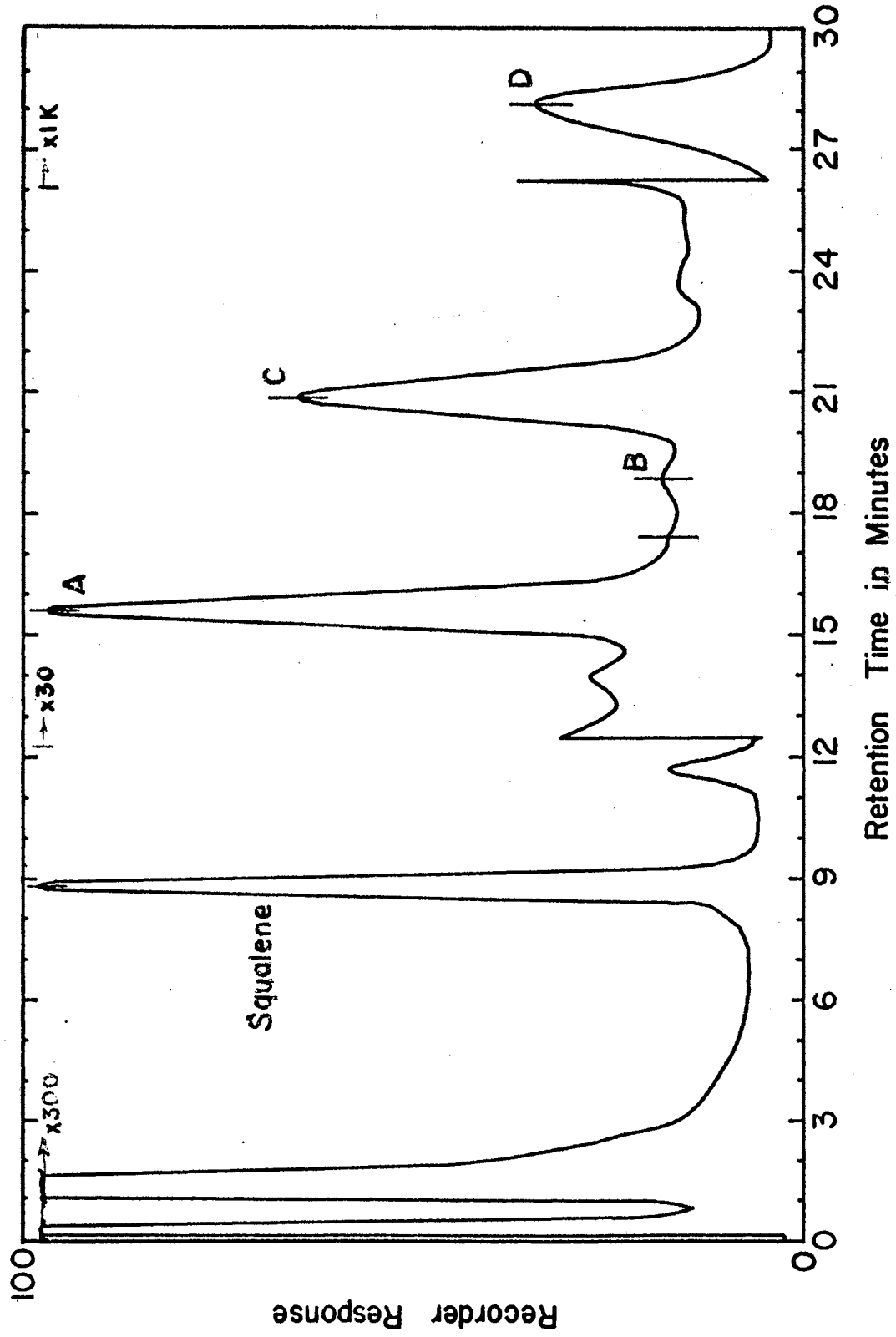

Recorder Response
100
0
0
3
6
9
12
15
18
21
24
27
30
Retention Time in Minutes
x300
x30
x1K
Squalene
A
B
C
D

Figure 35. GLC Tracing of α-, β-, and γ-Tocopherols and the Squalene Internal Standard

Column - 6' x $\frac{1}{4}$" Glass, Silanized

Column Packing - 4% OV-1 on Gas Chrom Q

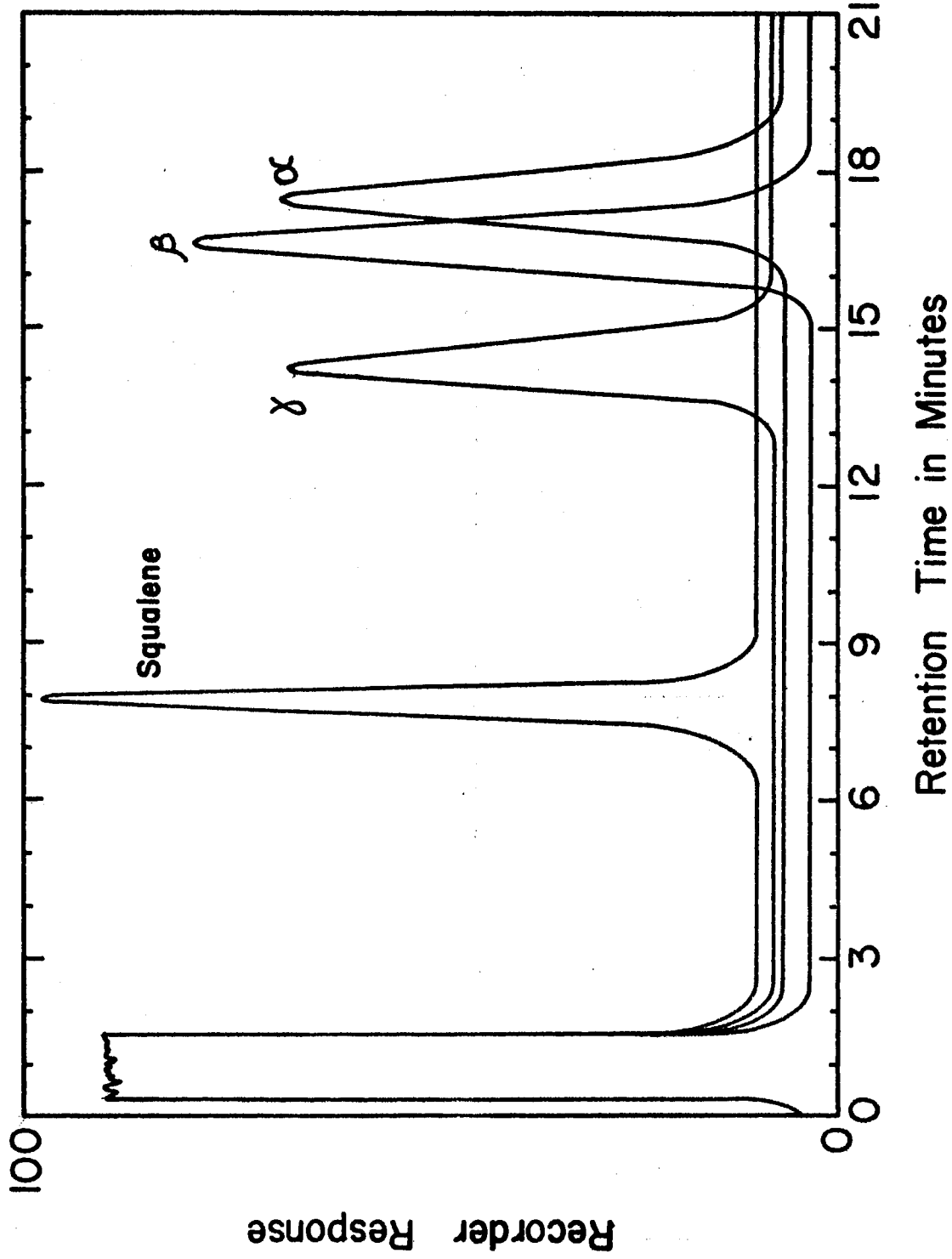
Column Temperature - 225° C.

Injection Port Temperature - 275° C.

Carrier Gas - Helium

Flow Rate - 130 ml./min.

Detector - Hydrogen Flame


Recorder Response
100
0
0
3
6
9
12
15
18
21
Retention Time in Minutes
Squalene
α
β
γ

after washing the non-saponifiable lipid portion (in ether) and was carried through the rest of the procedure. It has the ideal quality of eluting between α- and γ-tocopherol on both TLC and GLC. But in private communication, Slover (111) revealed that 5,7-dimethyltocol, a tocopherol that does not occur naturally, could be used as an internal standard instead. This tocol could be quantitatively added at the beginning of the experiment and would be treated in the same manner as the tocopherols present. Like didecyl pimelate, 5,7-dimethyltocol has a TLC R_f and a GLC retention time between the α- and γ-isomers. The TLC solvent used was benzene: methanol, 98:2. After separation and elution from the TLC plate, the tocopherols were silylated and the TMS ethers were separated and quantitated by GLC. This method was modified further by elimination of the TLC step. Injection of the crude mixture caused deterioration of the column at a faster rate but the cost was made up in time saved and the TLC solvents no longer used. Figure 36 shows a typical chromatogram and the column conditions used. Identifications are by GLC retention times. Component a was γ-tocopherol; component b was 5,7-dimethyltocol, the internal standard; the small peak component c was α-tocopherol, but there was not enough present to quantitate; component d was probably β-sitosterol, the major component of the non-saponifiable lipid portion as previously determined.

Results and Discussion

Since there is overlap between chapters on the tocopherol research, the data relating tocopherols with maturity and oxidative stability have been included in other chapters. This chapter deals primarily with identification and mass spectral analysis of the tocopherols.

Figure 36. Gas - Chromatographic Analysis of the Non-Saponifiable Lipid Portion of Pecans, Trimethylsilyl Ethers

Column - 15' x 2.8 mm., I.D.

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

Column Temperature - 245° C.

Injection Port Temperature - 265° C.

Detector Temperature - 220° C.

Carrier Gas - Nitrogen

Flow Rate - 60 ml./min.

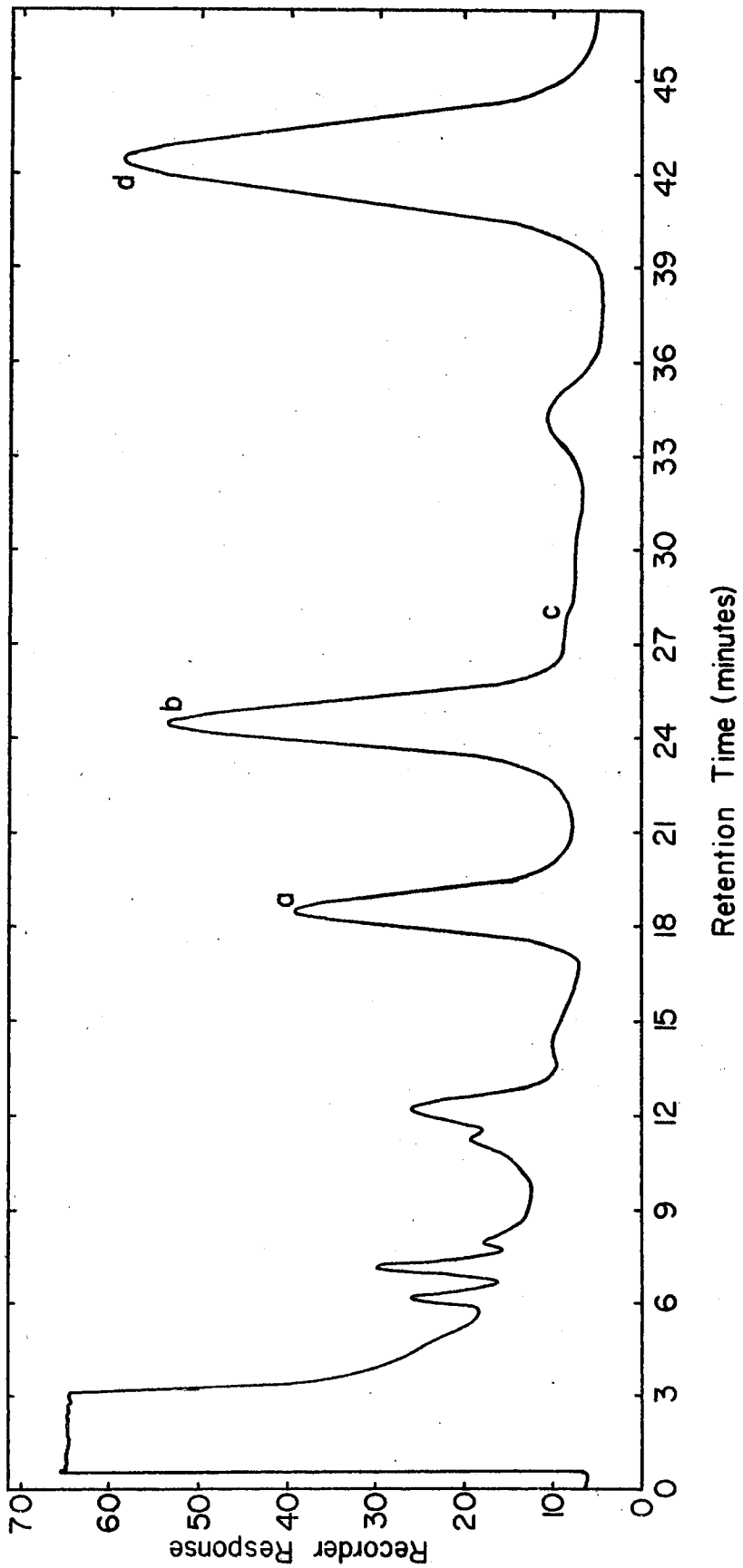
Detector - Hydrogen flame

Hydrogen Pressure - 20 lbs.

Air Pressure - 40 lbs.

Nitrogen Pressure - 75 lbs.

Instrument - Perkin Elmer 801


Recorder Response
70
60
50
40
30
20
10
0
3
6
9
12
15
18
21
24
27
30
33
36
39
42
45
Retention Time (minutes)
a
b
c
d

Tocopherol Analysis and Identification

In the tocopherol analyses of the 1968 pecan crop, the method of Nelson (80) was used. Standards of α-, β-, and γ-tocopherols were obtained. The GLC tracing of these standards and squalene was given in Figure 35. The non-saponifiable lipids were separated by GLC; this tracing was shown in Figure 34. Upon comparison of the unknown peaks with the standards, it appeared that the actual tocopherol present was the γ-isomer. The non-saponifiable lipids were then analyzed on the combination GC-MS instrument. Although the spectra were reduced in intensity due to a combination of column bleed at the high operating temperature and other material in the fraction, it was possible to discern molecular ions in most cases. Table VI lists the molecular ions, relative retention times of the tocopherol standards, and the unknown components in the non-saponifiable lipid portion. Peak D was the major component of the fraction, but it was not a tocopherol. The mass spectrum of this compound (Figure 37) suggested it may be β-sitosterol, molecular weight 414. Comparison of this spectrum with a reference spectrum (22) (Figure 38) indicated that this was probably the case. β-Sitosterol has been reported in walnuts (103), which are phylogenetically related to pecans, but no reference to its presence in pecans has been uncovered. As a standard was not available, the GLC retention time was not determined.

Peak A had a molecular weight of 416 and a relative retention time of 1.79, both values paralleling those of γ-tocopherol. The mass spectrum is given in Figure 39. A detailed survey of the literature indicated that γ-tocopherol was the major isomer present in pecans (102, 103). Thus the fact that γ-tocopherol is the primary isomer

TABLE VI

RELATIVE RETENTION TIMES AND MOLECULAR IONS OF TOCOPHEROLS AND UNKNOWNS IN THE NON-SAPONAFIABLE LIPID FRACTION OF PECAN OIL

Component	M^+	Relative T_r
Squalene, Internal Standard	410	1.00
α-Tocopherol, Standard	430	2.20
β-Tocopherol, Standard	416	2.07
γ-Tocopherol, Standard	416	1.80
α-Tocopheryl Acetate, Standard	472	-
Component A	416	1.79
Component B	430	2.19
Component C	?	2.48
Component D	414	3.08

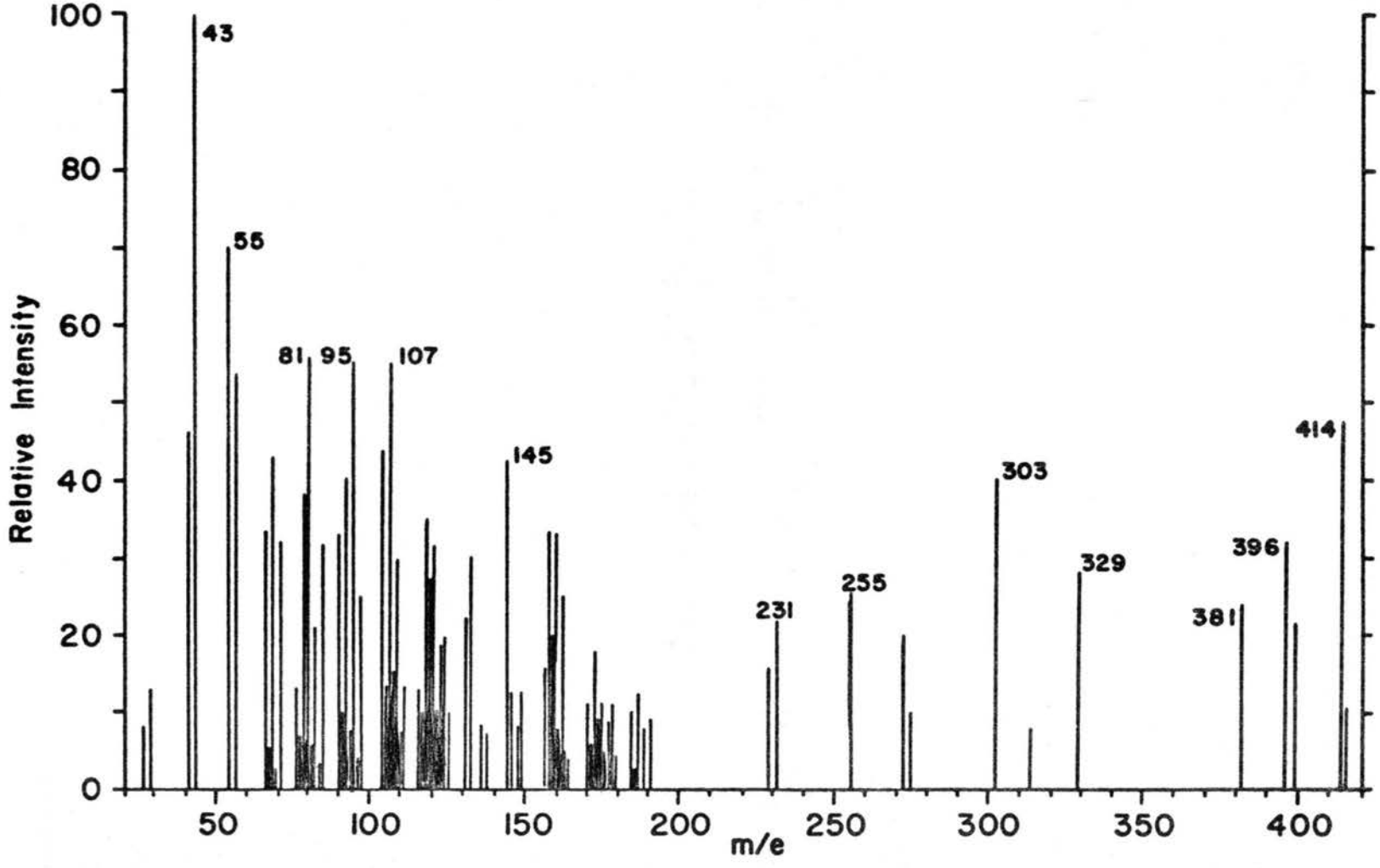


Figure 37. Mass Spectrum of Component D From the Non-Saponifiable Lipid Fraction

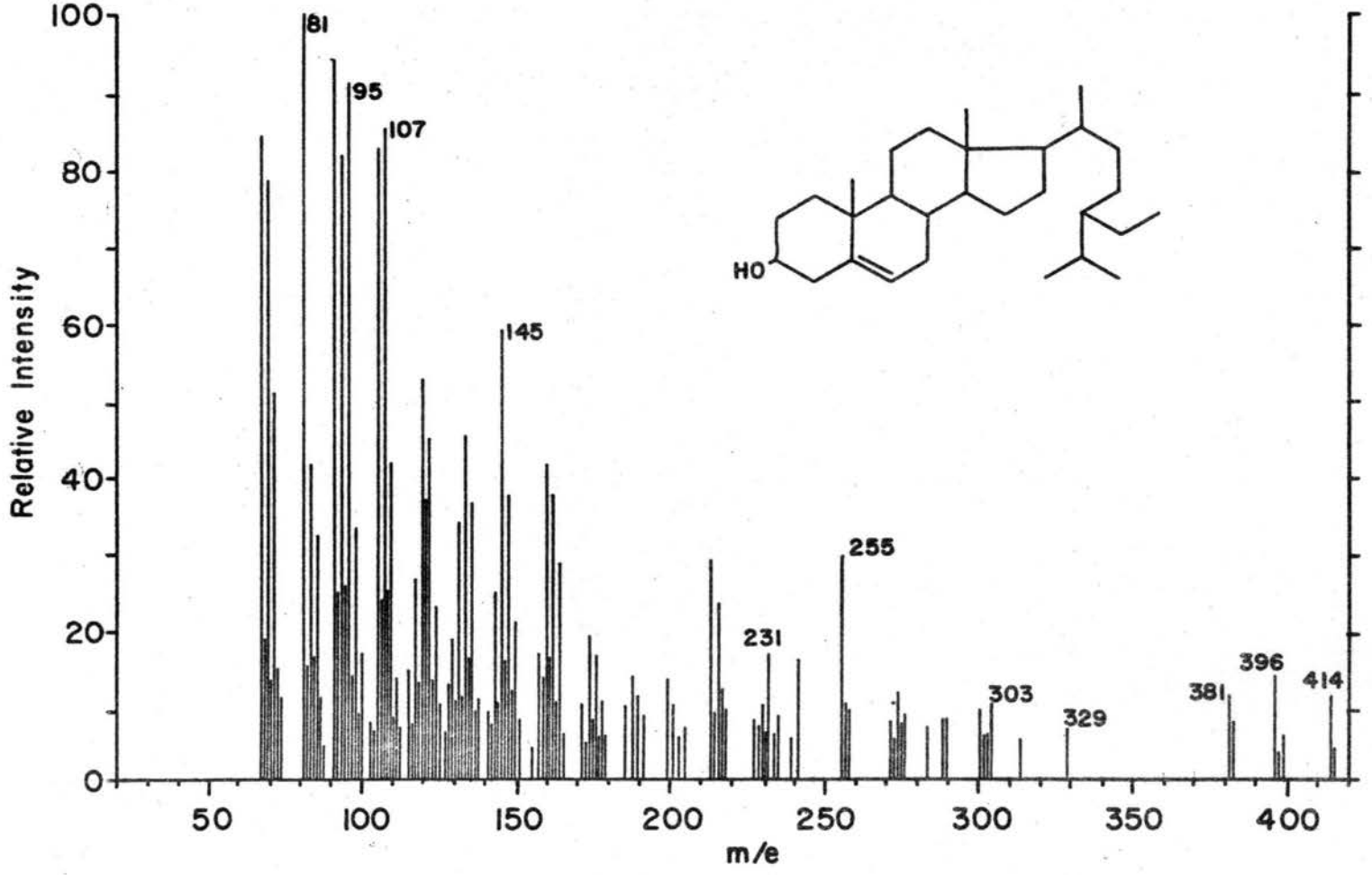


Figure 38. Mass Spectrum of β-Sitosterol, Reference Standard

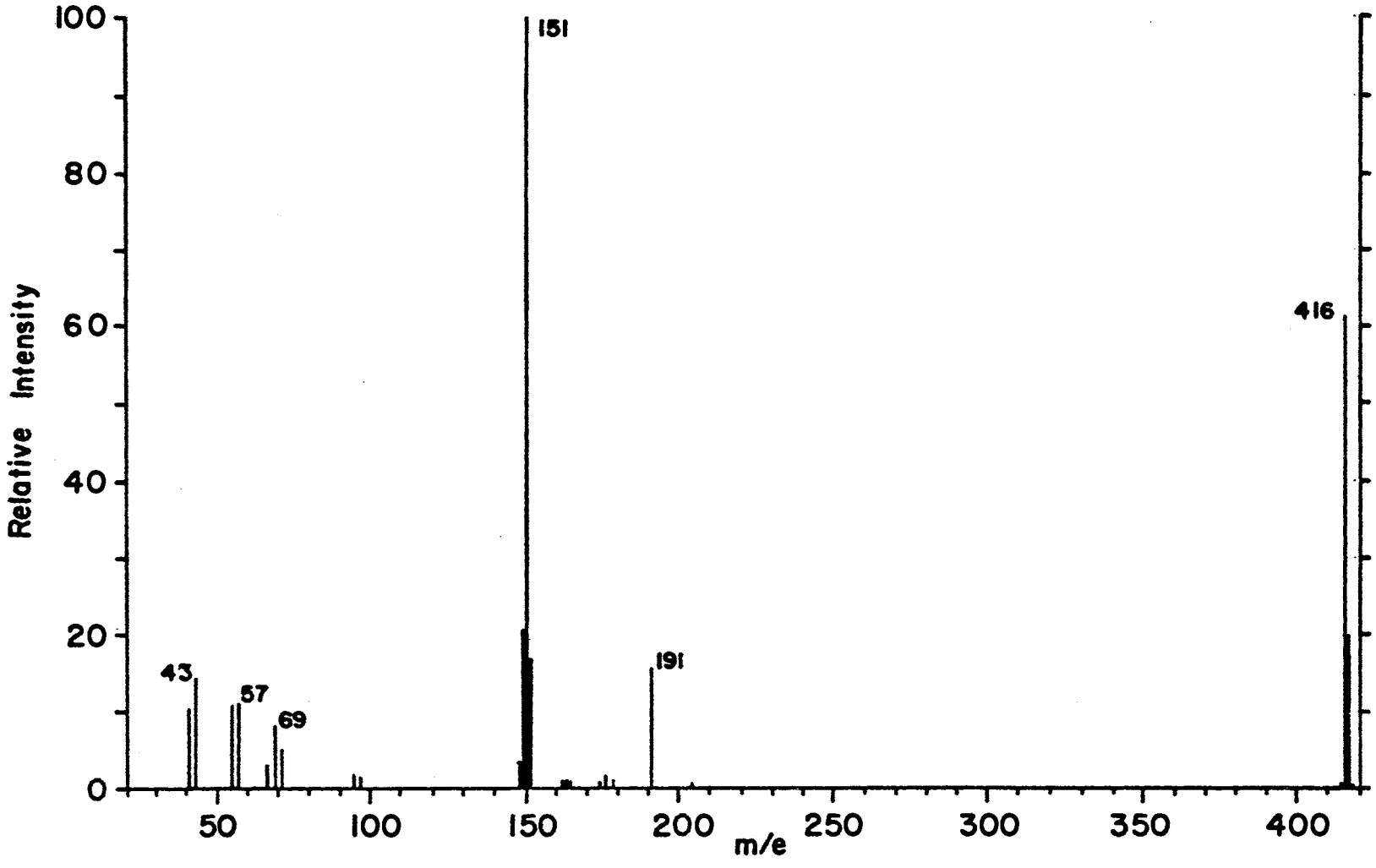


Figure 39. Mass Spectrum of Component A, Non-Saponifiable Lipid Fraction

present in pecans has been confirmed in this laboratory using combination GC-MS techniques.

Peak C was thought to be the α-isomer, but closer examination of the mass spectrum revealed that it was not. The identity of this compound has not been determined. The α-tocopherol (peak B) was a very minor component, representing less than 1% of the total tocopherol in the pecan. The spectrum of component B is given in Figure 40. After analyzing over thirty varieties of pecan oils, it was found that all contained 99+% of the γ-isomer. The other peaks present are probably steroids, but were not present in sufficient quantities for identification by GC-MS.

Mass Spectral Analysis--General Considerations

The mass spectra of α-, β- and γ-tocopherol and α-tocopheryl acetate are given in Figures 41, 42, 43, and 44, respectively. Upon initial examination of the spectra, the first thing observed was the lack of fragmentation and the presence of intense molecular ions. In each spectrum, the molecular ion was no less than 40% RI and varied in total ionization from 10-20%. Lack of fragmentation may be due to the absorption of the electron impact energy by the chromanol ring system.

The most intense fragment ion in the spectra of the β- and γ-isomers was at m/e 151. (The corresponding peak in the spectrum of the α-isomer was at m/e 165.) The formation of these ions from the molecular ion was predicted by metastable transitions at m* 54.8 for the β- and γ-isomers and at m* 63.3 for the α-isomer. Ions at m/e 151 and 165 were base peaks for the γ- and α-isomers, respectively. In the spectrum of the β-isomer, the molecular ion was the base peak and

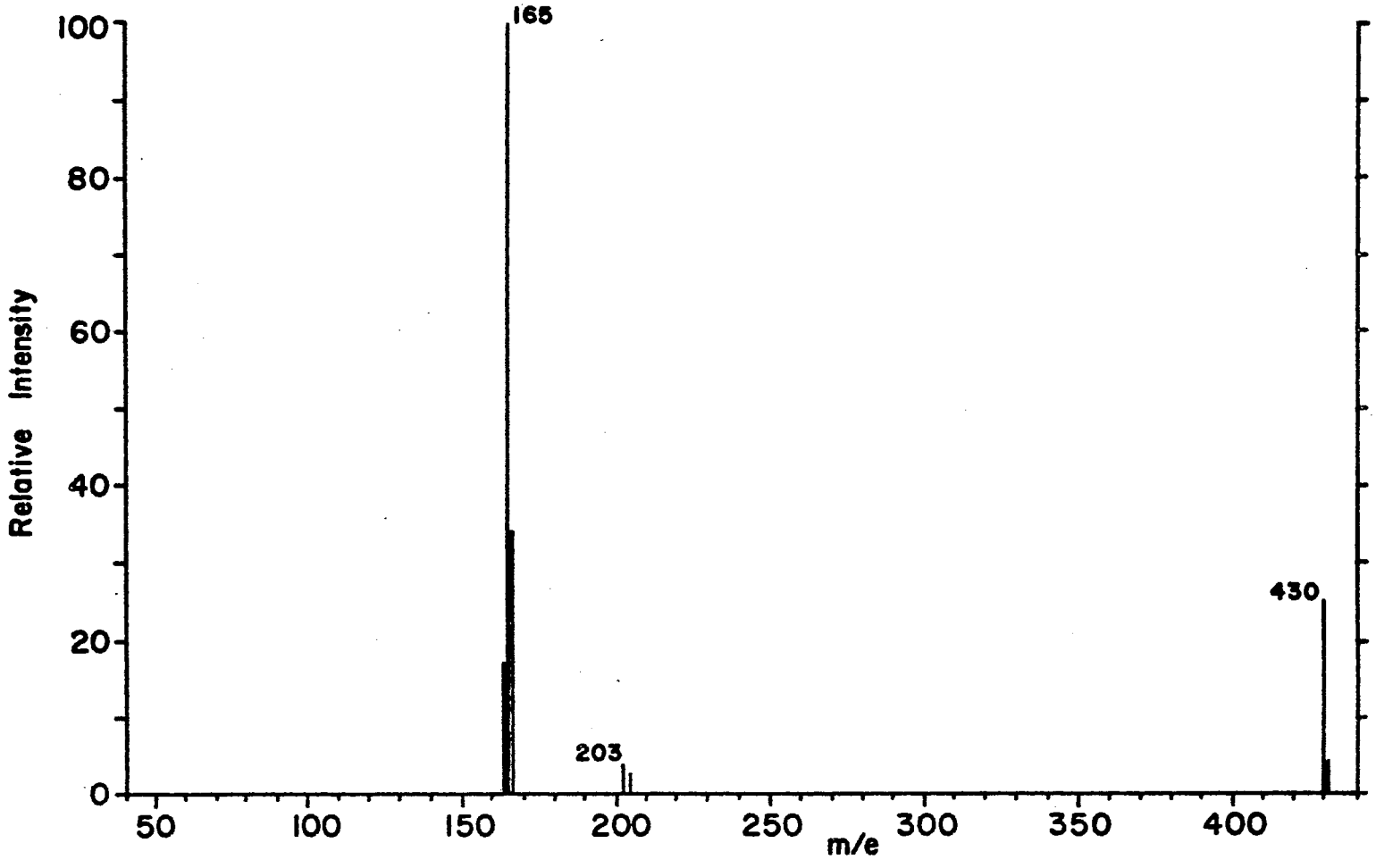


Figure 40. Mass Spectrum of Component B, Non-Saponifiable Lipid Fraction

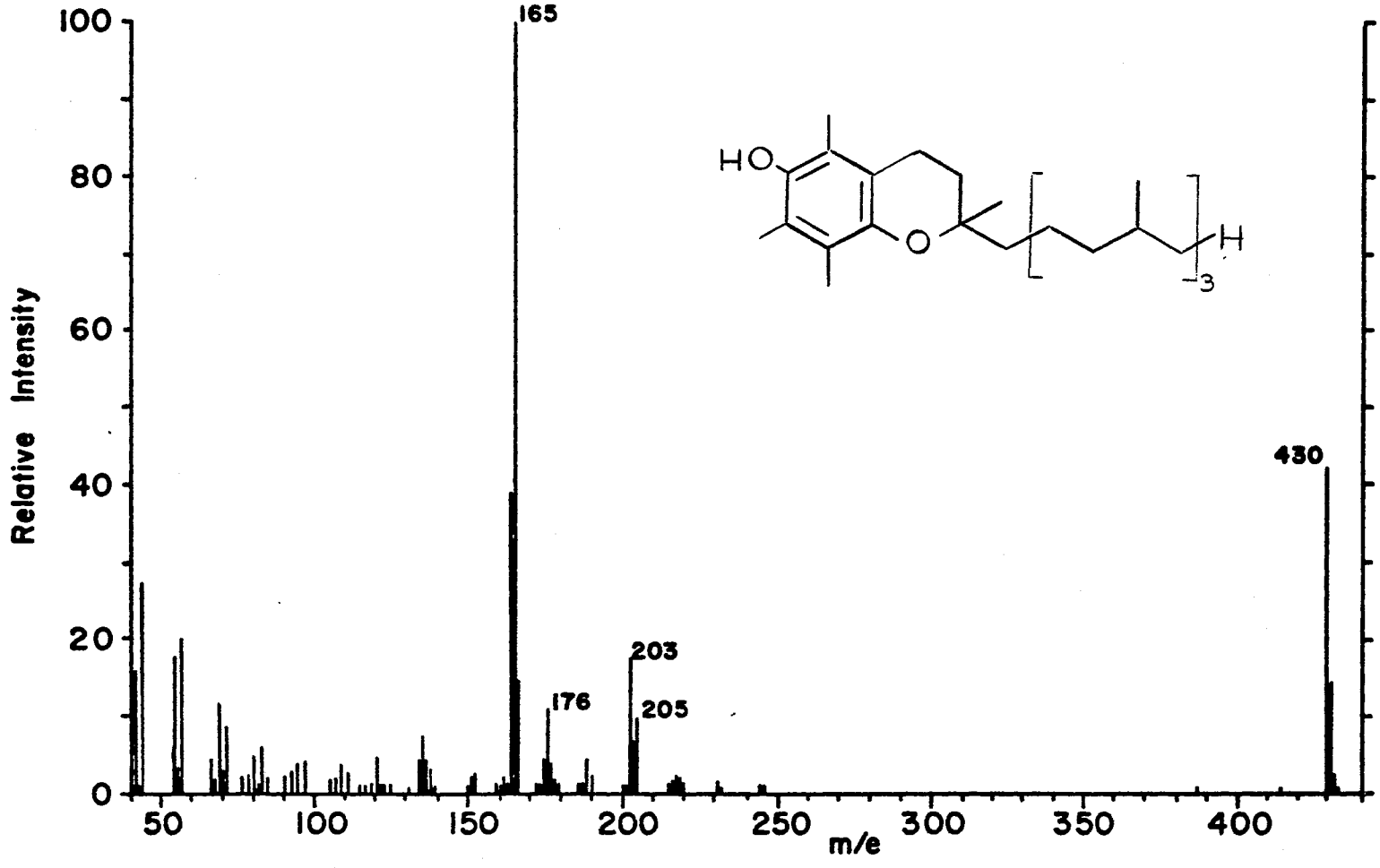


Figure 41. Mass Spectrum of α-tocopherol, Reference Standard

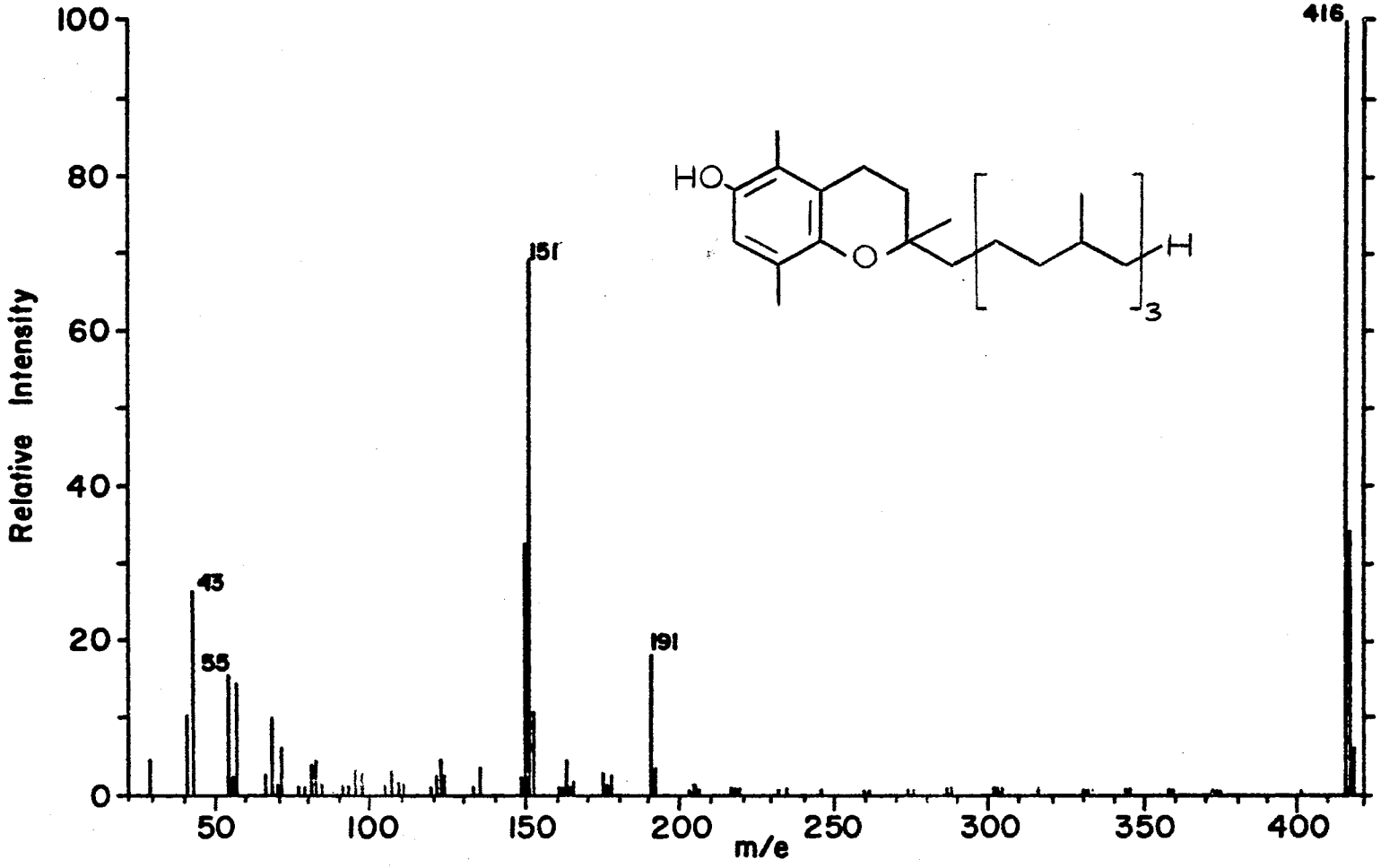


Figure 42. Mass Spectrum of β -tocopherol, Reference Standard

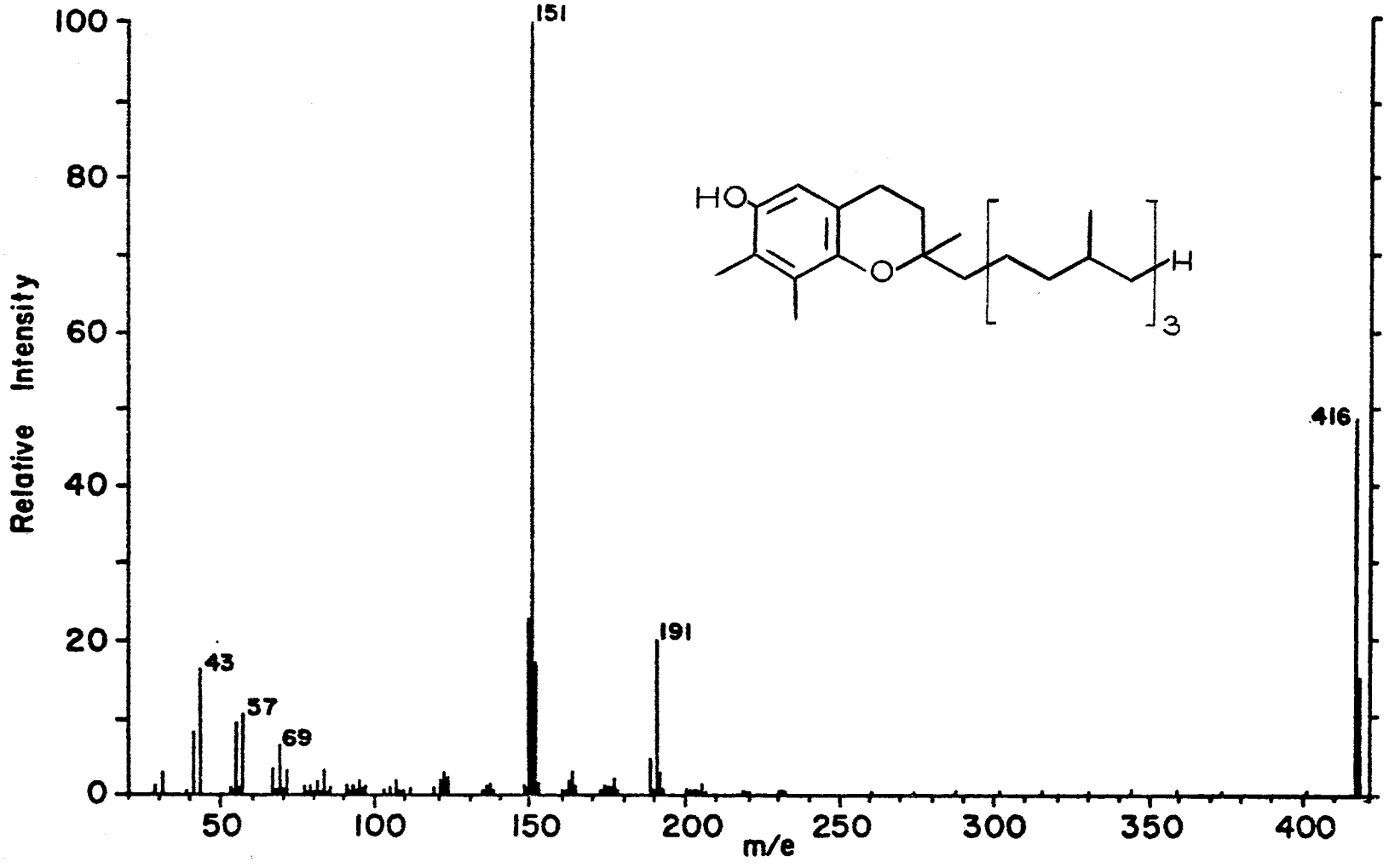


Figure 43. Mass Spectrum of γ-tocopherol, Reference Standard

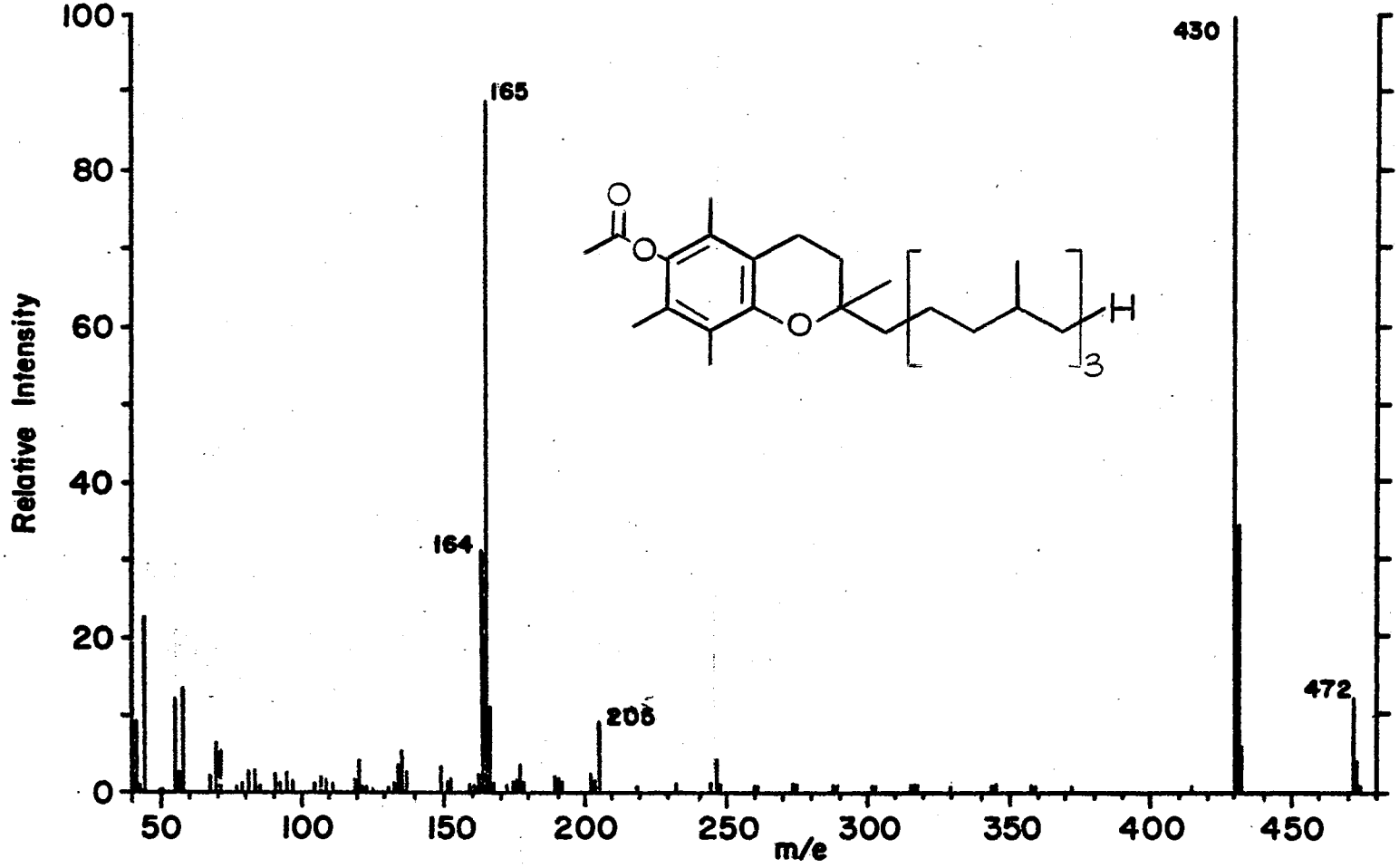
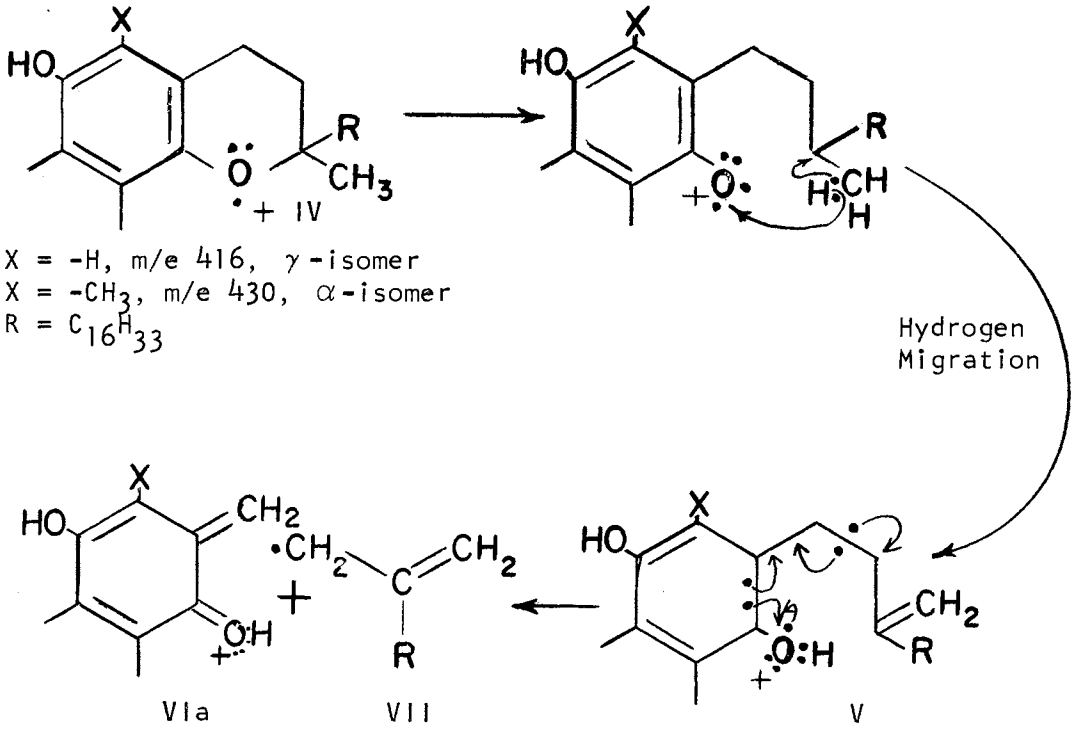


Figure 44. Mass Spectrum of α-tocopheryl Acetate, Reference Standard

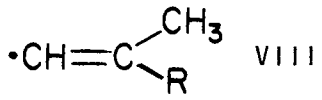
the peak at m/e 151 had an RI of 75%. Formation of this ion resulted from cleavage through the non-aromatic portion of the chromanol ring and a reasonable mechanism for this transition (α- and γ-isomers illustrated) might be envisioned as follows:

Scheme I



The bond most thermodynamically unstable in the molecule lies between the oxygen of the chromanol ring system and the tertiary atom at position 2. After bond fission a hydrogen atom could migrate from the methyl group to the oxygen atom. This yields a radical (V) which decomposes into the ion, m/e 151 (or 165) (VIa) and a resonantly stabilized allylic radical (VII). It seems more likely for the hydrogen to

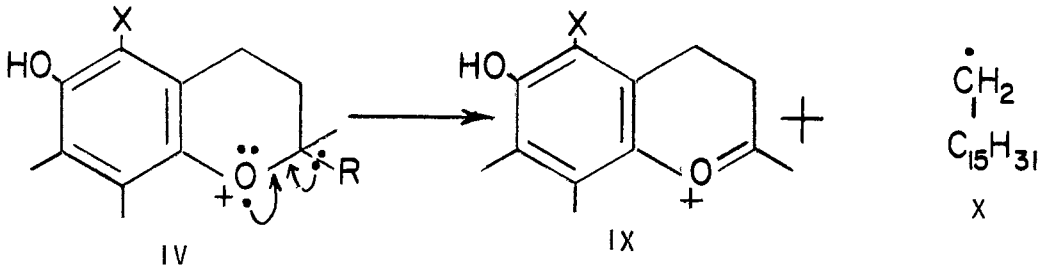
migrate from the methyl group since migration from position 3 in the chromanol ring system would result in the following ion being produced:



VIII

The only other obviously intense peak in the spectra resulted from cleavage of the $C_{16}H_{33}$ side chain from the molecule, producing ions at m/e 191 (β- and γ-isomers) and 205 (α-isomer). The formation of this ion from the molecular ion was confirmed by metastable transitions at m* 87.7 (β- and γ-isomers) and 97.7 (α-isomer). This mechanism might be envisioned as follows:

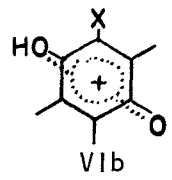
Scheme 2



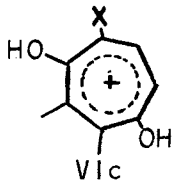
IV IX X

This reaction might be expected to be the predominant one as an intermediate would be a stable tertiary carbonium ion at position 2, but other factors need to be considered. In the first mechanism, the uncharged species was an allylic radical (VII) whereas the radical X is a less stable primary radical. This favors scheme 1. Furthermore, at least two additional structures for VIa are possible. Either a hydroxyl hydrogen transfer from the methylene group in VIa or a transfer of methyl hydrogen, scheme 1 structure IVa, to the benzylic would produce

resonantly stabilized VIb. Alternately, VIa or its immediate precursor could undergo ring expansion to form a stable substituted tropylium ion, IVc.



VIb

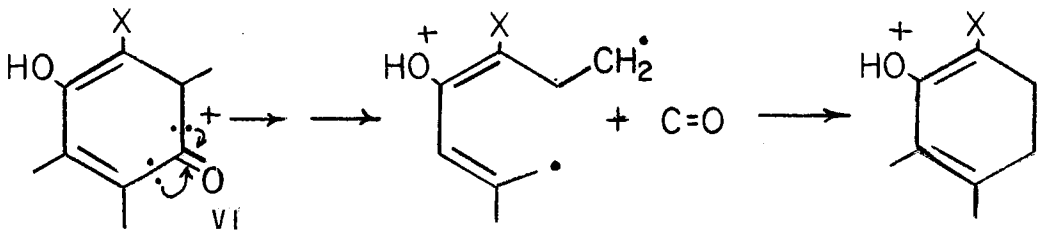


VIc

Strong supporting evidence for VIc is afforded by the observed loss of carbon monoxide from the dihydroxytropylium ion in the mass spectrum of m- and p- hydroxybenzyl alcohols, forming a hydroxybenzenium ion (39). A similar resonance for the ion at m/e 191 (205) would be depicted as follows:

This resonance structure (XI) would be unlikely due to valence shell expansion of the oxygen atom. Therefore, electron delocalization in the fragment ions as well as the stability of the ejected radicals favors formation of ions at m/e 151(165) over 191(205) or ring cleavage versus simple loss of side chain.

Another characteristic fragmentation present in each of the spectra was loss of carbon monoxide from ion m/e 151(165) (VI). This transition was confirmed by metastable transitions at m* 100.2 (β- and γ-) and 113.8 (α-). This transition could be envisioned as follows:



Superficially this fragmentation supports structure VIb or m/e 151(165), however the observed loss of carbon monoxide from the dihydroxytropylium ion formed from m-hydroxybenzyl alcohol can be taken as strong supporting evidence for structure VIc (39). The minor fragmentations for each tocopherol will be described below. The proposed structures using elemental composition data obtained from high resolution mass spectrometry conform to the elemental compositions and are postulated in the most reasonable manner possible. In any case, pathways are speculative.

Mass Spectral Fragmentation Pattern of β-Tocopherol

One aspect of the β-tocopherol spectrum which was not common to the other spectra was the presence of a very intense metastable at M^+ 378 that was ten times the intensity of the other metastables in the spectrum. The most reasonable transition is loss of water by the molecular ion, but there was no ion present from high resolution data with an m/e 398.

There were many small (less than 0.4% RI) peaks occurring between m/e 200 and the molecular ion that represented cleavage of the aliphatic side chain. They were of little significance to the fragmentation pattern. This description should be followed by referring to Figure 45.

The molecular ion lost a $C_{16}H_{33}$ fragment, as described previously, producing an ion, m/e 191, $C_{12}H_{15}O_2$. This ion then fragmented to lose

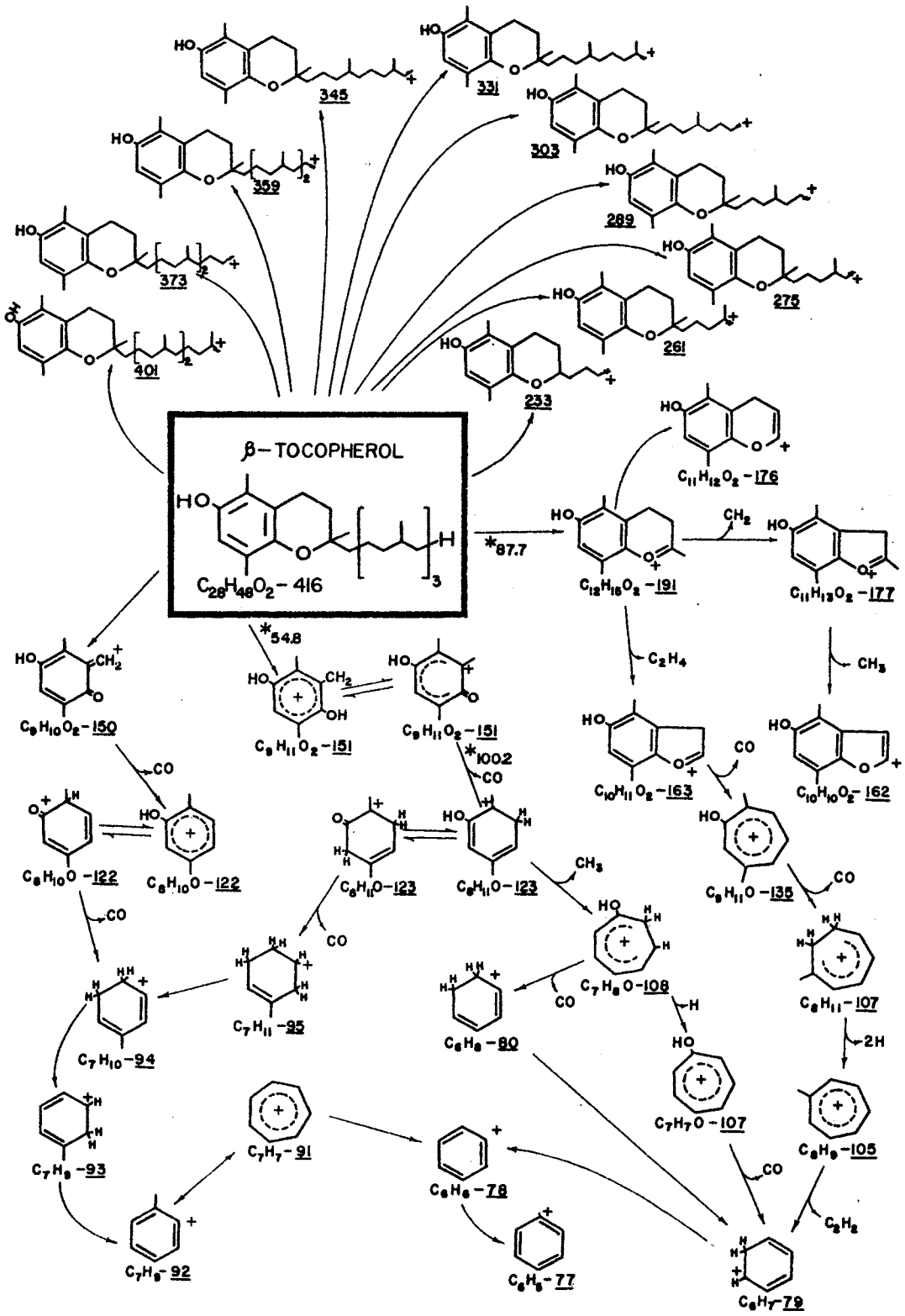


Figure 45. Proposed Mass Spectral Fragmentation Pattern of β-Tocopherol

C_2H_4 from the non-aromatic ring producing an ion, m/e 163, $C_{10}H_{11}O_2$ with a five-membered ring. This fragmentation scheme is characteristic of chromanols (124). The ion m/e 163 may lose carbon monoxide (CO) with the formation of m/e 135, $C_9H_{11}O$. The ion m/e 135 may then lose another molecule of CO followed by incorporation of a methyl into the ring forming m/e 107, C_8H_{11} which subsequently loses two hydrogens to form C_8H_7, m/e 105. This ion may lose acetylene and rearrange to give m/e 79, C_6H_7. A hydrogen is then lost with the formation of m/e 78, C_6H_6. According to high resolution data there were two doublets in the spectrum; the first occurred at m/e 107 with ions of the formulae C_7H_7O and C_8H_{11}; the second occurred at m/e 123 with ions $C_8H_{11}O$ and C_9H_{15}. Concerning the ions at m/e 107, C_8H_{11} was located in the pathway just discussed whereas C_7H_7O was in another major fragmentation route. Concerning the doublet at m/e 123, the ion $C_8H_{11}O$ was on the major fragmentation pathway, and its existence was substantiated by two metastable ion transitions (see Table VII). The origin of m/e 123, C_9H_{15} is uncertain.

The other major pathway in the fragmentation scheme of β-tocopherol began with a transition between the two most intense ions of the spectrum, m/e 416 and m/e 151, as denoted by a metastable ion at m* 54.8. The m/e 151 ion decomposes into m/e 123, $C_8H_{11}O$ with loss of CO. There was a metastable ion at m* 100.2 that confirmed this transition. The ion m/e 123, $C_8H_{11}O$ could decompose by two different pathways to yield benzene. The first of these sub-pathways involved initially the loss of CO to give m/e 95, C_7H_{11}, an ion structurally similar to toluene but with three additional hydrogen atoms. This ion lost hydrogens successively and rearranged finally to give the tropylium ion, m/e 91, C_7H_7. The ion m/e 123, $C_8H_{11}O$ could lose a ring methyl group before

TABLE VII

METASTABLE TRANSITIONS IN TOCOPHEROL MASS SPECTRA

Tocopherol	m^* Calculated	m^* Observed	Parent	Daughter	Neutral
	63.3	63.3	430^+	165^+	265
	97.7	97.9	430^+	205^+	225
α-Tocopherol	113.8	113.8	165^+	137^+	28
	348.3	348.4	430^+	387^+	43
	54.8	54.7	416^+	151^+	265
	87.7	87.8	416^+	191^+	225
	100.2	100.2	151^+	123^+	28
β-Tocopherol	334.4	334.0	416^+	373^+	43
	380.7	378.0	416^+	398^+	18
	386.6	387.0	416^+	401^+	15
	54.8	54.8	416^+	151^+	265
	87.7	87.8	416^+	191^+	225
γ-Tocopherol	100.2	100.2	151^+	123^+	28
	334.4	334.6	416^+	373^+	43
	386.6	387.3	416^+	401^+	15
	63.3	63.2	430^+	165^+	265
	113.8	113.8	165^+	137^+	28
α-Tocopheryl Acetate	131.5	131.6	207^+	165^+	42
	348.3	348.4	430^+	387^+	43
	393.4	392.1	472^+	430^+	42

losing CO, followed by rearrangement to give an ion m/e 108, C_7H_8O. This ion then can lose CO giving m/e 80, C_6H_8 which successively loses hydrogen to yield m/e 78, C_6H_6.

A third minor pathway serves to explain the ions at m/e 150 and 122, both of which may be produced simply by loss of a hydrogen atom from m/e 151 and m/e 123, respectively. Several ions were observed for which no further fragmentation could be detected. These were ions m/e 176, $C_{11}H_{12}O_2$, and m/e 162, $C_{10}H_{10}O_2$.

Mass Spectral Fragmentation Pattern of γ-Tocopherol

The mass spectrum of γ-tocopherol (see Figure 46) was very similar to that of β-tocopherol. Some difficulty was encountered at the mass spectrometry center, Purdue University, when the γ-isomer was analyzed. A set of filaments was burned out on each of the first two attempts at spectral analysis. On the third attempt apparently a much smaller quantity of sample was used, as the spectrum is not very intense.

There were some peaks present that were not observed in the spectrum of β-tocopherol. The first of these was m/e 152, $C_9H_{12}O_2$. It was similar to the base peak (m/e 151) but one mass unit higher. It was thought to be solely an isotope peak, but the high resolution mass spectrum indicated a doublet at m/e 152. Thus m/e 152, $C_9H_{12}O_2$ probably results from a McLafferty-like rearrangement (127) of a proton. This ion might then decompose to m/e 138, $C_8H_{10}O_2$. And may be more correctly represented by the following structure than the 7-membered ring depicted in Figure 46.

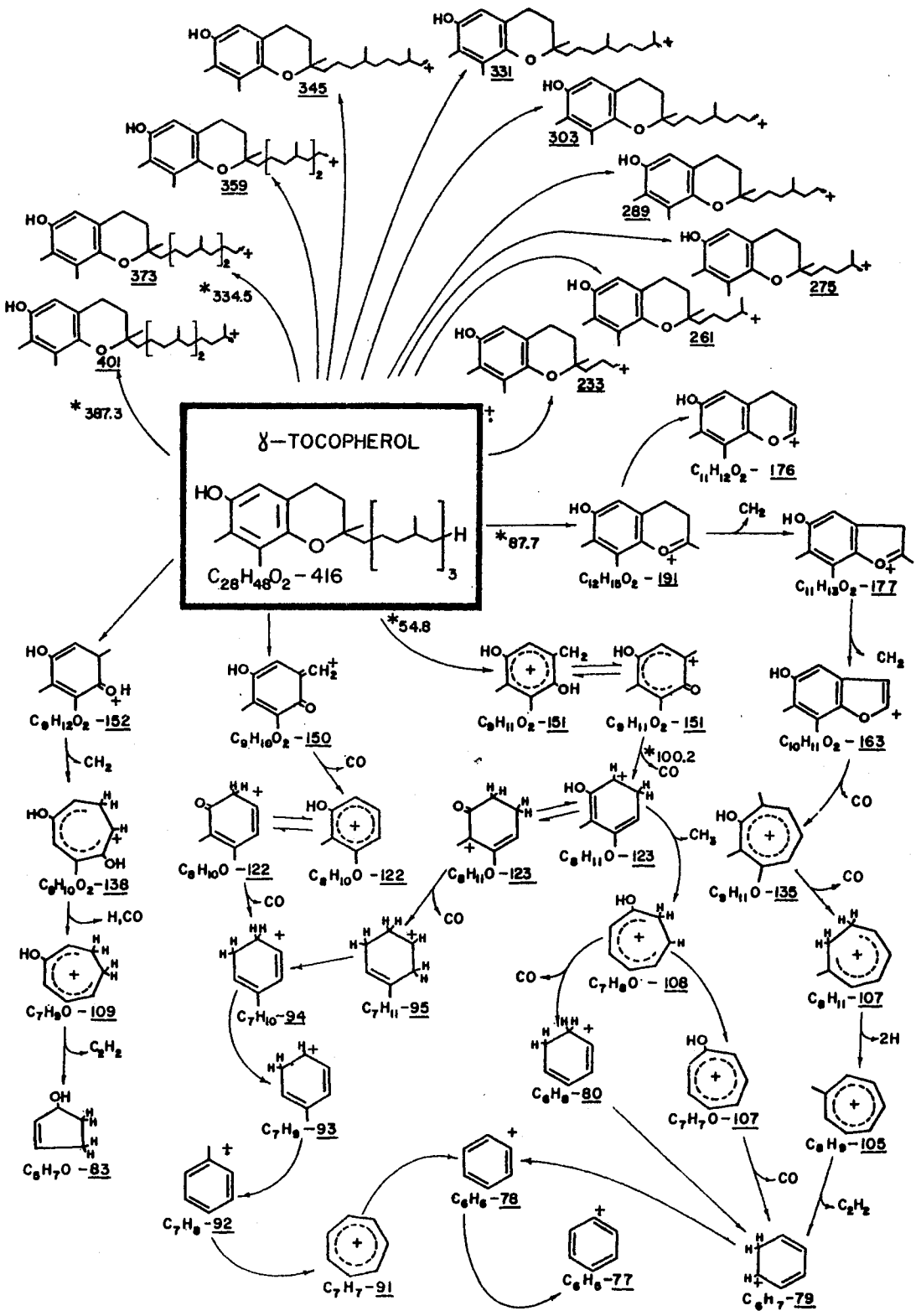
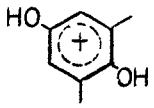


Figure 46. Proposed Mass Spectral Fragmentation Pattern of γ -Tocopherol



This molecule could then lose CO followed by hydrogen to give m/e 109, C_7H_9O. The ion, m/e 109 could fragment by one of two routes: first, it could lose one or two hydrogen atoms and be funneled into one of the major pathways at m/e 108, C_7H_8O; or second, lose C_2H_2. It could give rise to a five-membered non-aromatic structure, m/e 83, C_5H_7O. There was an intense peak at m/e 55, in the low resolution spectrum which may be the loss of CO from C_5H_7O, m/e 83 to yield C_4H_7.

Mass Spectral Fragmentation Pattern of α-Tocopherol

The mass spectral fragmentation pathways of the α- isomer were similar to those of the β- and γ-isomers with 14 mass units added to most of the ions.

The two major schemes were represented by metastable ions at m* 63.3 and 97.7. The parent ion can lose the $C_{16}H_{33}$ aliphatic side chain resulting in the methylated chromanol, m/e 205, $C_{13}H_{17}O_2$ (see Figure 47). This ion could then lose ethylene with the resultant reduction in size of the non-aromatic ring to five members to yield $C_{11}H_{13}O_2$, m/e 177. The ion then lost CO and rearranged to a delocalized ion, m/e 149, $C_{10}H_{13}O$. The loss of another molecule of CO followed by the incorporation of a methyl group into the ring produced an ion m/e 121, C_9H_{13}, which lost two hydrogen atoms to become fully aromatic, giving m/e 119, C_9H_{11}. This ion lost acetylene and, after two successive losses of hydrogen, was transformed into a tropylium ion, m/e 91, C_7H_7.

The parent molecule was also cleaved through the non-aromatic ring,

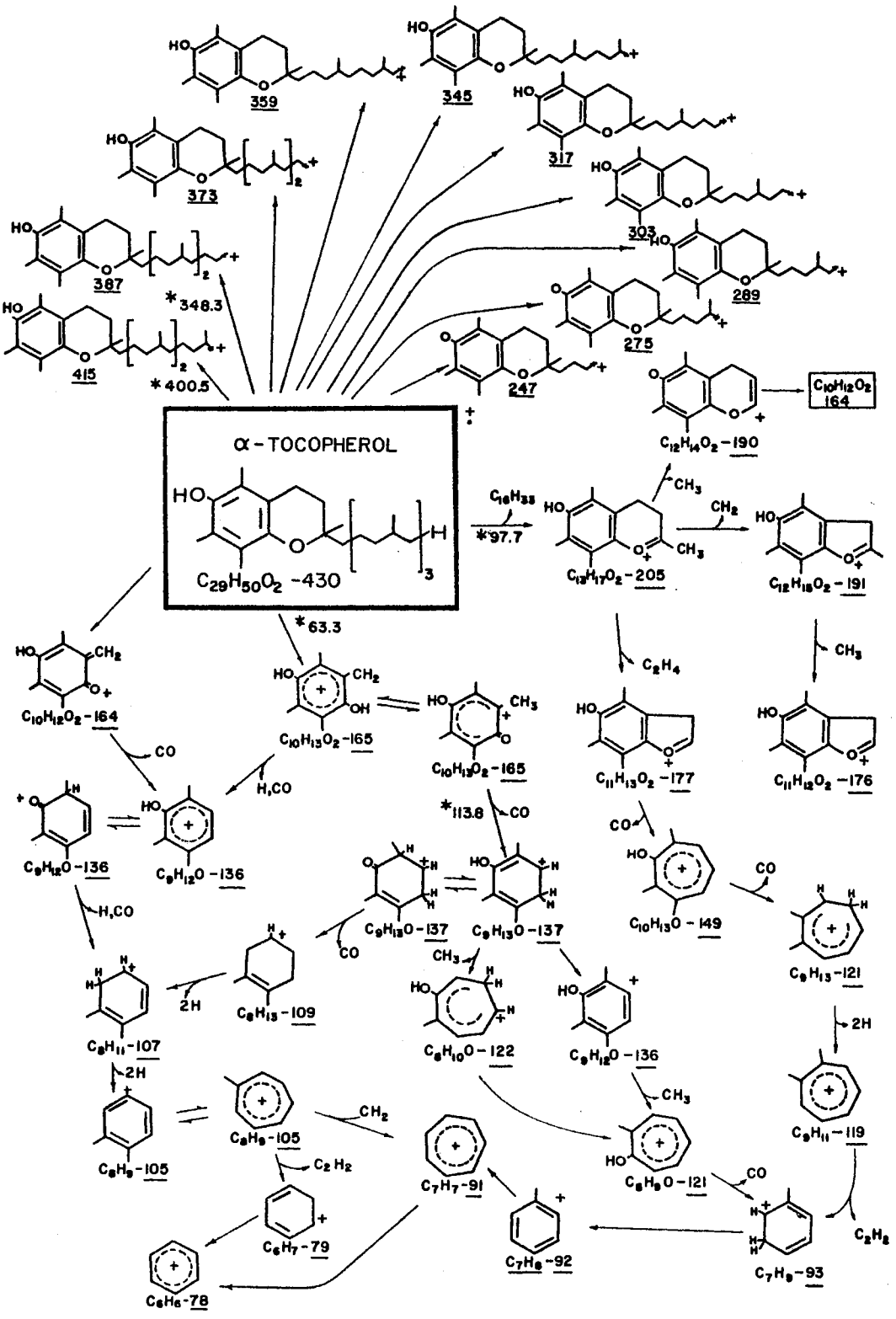


Figure 47. Proposed Mass Spectral Fragmentation Pattern of α-Tocopherol

producing the ion m/e 165, $C_{10}H_{13}O_2$. The metastable m* 63.3 confirmed this transition. This ion lost CO giving m/e 137, $C_9H_{13}O$, a transition predicted by a metastable at m*113.8. This ion can now follow two routes: (1) loss of CO and then acetylene or (2) lose a methyl group, then CO. Upon loss of CO from $C_9H_{13}O$ the ion m/e 109, C_8H_{13} was produced. This ion was highly saturated and underwent several successive dehydrogenations yielding a fully delocalized ion, m/e 105, C_8H_9. This ion then loses acetylene giving a benzene moiety. Proceeding by subpathway 2, m/e 137, $C_9H_{13}O$ lost a methyl group then a hydrogen atom, giving m/e 121, C_8H_9O, which then lost CO and joined the first major pathway at m/e 93, C_7H_9.

The other two metastable ions present represent loss of a methyl group (m* 400.5) and an isopropyl group (m* 348.3) from the aliphatic side chain.

Mass Spectral Fragmentation Pattern of α-Tocopheryl Acetate

The best high resolution mass spectrum was obtained from α-tocopheryl acetate. Thus, many small peaks were apparent which were not seen in the other spectra, and a more complete interpretation was possible. The proposed fragmentation pattern is presented in Figure 48.

α-Tocopheryl acetate can decompose without loss of the acetyl group, or it can lose the acetyl group giving α-tocopherol (and the major fragmentation pattern will be due to α-tocopherol). Since the acetate linkage is not very stable under electron impact, the latter situation was most predominant. There were, however, two ions present in the spectrum that contained the acetate linkage, those being m/e 207, $C_{12}H_{15}O_3$, and m/e 247, $C_{15}H_{19}O_3$. These ions were formed by cleavage of

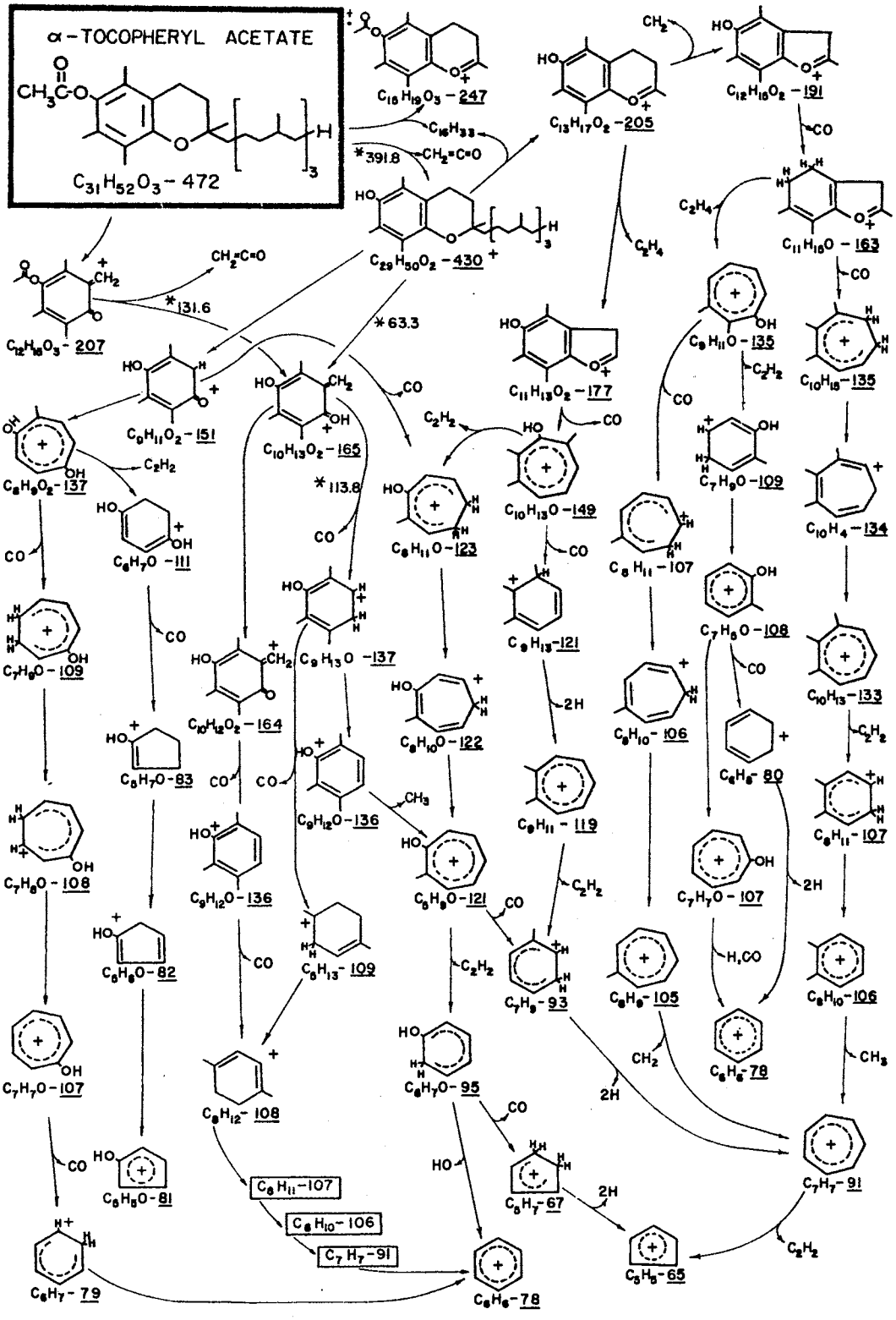
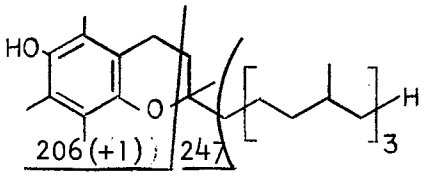


Figure 48. Proposed Mass Spectral Fragmentation Pattern of α-Tocopheryl Acetate

the parent compound (the acetate) in a manner analogous to the major fragmentation pathways of the other tocopherols, i.e.,



Both of these ions then lose ketene yielding m/e 165, $C_{10}H_{13}O_2$ (from $C_{12}H_{15}O_3$) and m/e 205, $C_{13}H_{17}O_2$ (from $C_{15}H_{19}O_3$). For these four reactions one would expect four metastables at m* 90.8 (m/e 472 ⟶ 207), 129.3 (m/e 472 ⟶ 247), 131.6 (m/e 207 ⟶ 165), and 170.2 (m/e 247 ⟶ 205). The only metastable observed was at 131.6 for the decomposition of m/e 207 to m/e 165. Two other metastables present denoted the decomposition of α-tocopheryl acetate to α-tocopherol (m* 391.8) and further decomposition of α-tocopherol to m/e 165 (m* 63.3), the second most intense peak in the spectrum. A metastable at m* 113.8 indicated the loss of CO from m/e 165 giving m/e 137, $C_9H_{13}O$. No metastable ion was observed for loss of the $C_{16}H_{33}$ side chain (i.e. at M* 97.7) from the α-tocopherol ion.

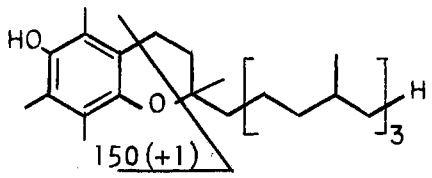
In proceeding further with an analysis of the fragmentation pattern, the pathways discussed in the section on α-tocopherol will not be mentioned. The two modes of decomposition of the ion m/e 205, $C_{13}H_{17}O_2$ involve loss of ethylene, yielding m/e 177, $C_{11}H_{13}O_2$, the major route, or loss of methylene, giving an ion m/e 191, $C_{12}H_{15}O_2$. In the spectrum of α-tocopherol, no ions resulting from the decomposition of m/e 191 could be found. In the more intense α-tocopheryl acetate spectrum, a secondary mode of fragmentation was observed. The ion $C_{12}H_{15}O_2$ lost

CO (from hydroxyl group) and one of the ring methyls was incorporated into the ring to give an ion m/e 163, $C_{11}H_{15}O$. This ion can either lose a second molecule of CO and rearrange to $C_{10}H_{15}$ or it can lose C_2H_4 and again rearrange to an ion, $C_9H_{11}O$ both having an m/e 135. If decomposition occurred by the second mode, further desaturation would likely occur, giving a fully aromatic system, m/e 133, $C_{10}H_{13}$. This ion could lose C_2H_2 and a methyl group could be incorporated into the ring. Successive loss of hydrogen would give ion m/e 106, C_8H_{10} which loses a methyl group enroute to forming the tropylium ion, C_7H_7, m/e 91. The first mode of decomposition is complicated by the fact that two pathways exist for the decomposition of m/e 135, $C_9H_{11}O$. The ion can lose CO and a hydrogen atom yielding m/e 106, C_8H_{10}, which may form the tropylium ion, m/e 91, C_7H_7 as just described; or the ion can also lose acetylene and rearrange with loss of a hydrogen atom to m/e 108, C_7H_8O, which subsequently loses CO to give an ion m/e 78, C_6H_6.

Another subpathway from the major pathway (through $C_{13}H_{17}O_2$, m/e 205) begins at m/e 149, $C_{10}H_{13}O$. The major mode of decomposition is loss of CO giving m/e 121, C_9H_{13}, which was described in the section on α-tocopherol. A secondary mode involved loss of acetylene from the $C_{10}H_{13}O$ giving an ion m/e 123, $C_8H_{11}O$, which loses two hydrogen atoms to yield a common intermediate in several of the pathways, m/e 121, C_8H_9O. The major mode of decomposition is loss of CO and has been described in an earlier section. This ion can also lose acetylene giving m/e 95, C_6H_7O, which can lose a hydroxyl group to give a cyclopentadiene ion, m/e 65, C_5H_5.

The last pathway involves the formation of an ion directly from α-tocopherol, not a secondary decomposition product of one of the two

major pathways. This ion, m/e 151, $C_9H_{11}O_2$ was first thought to be due to a β- or γ-tocopherol impurity. Subsequent GLC analysis showed neither to be present. The ion probably originated as follows:



This ion decomposed primarily through loss of CO to form m/e 123, $C_8H_{11}O$, which decomposed according to the scheme presented in the previous paragraph. A minor mode of decomposition involved conversion to an ion m/e 137, $C_8H_9O_2$. From this ion the pathway is split further into two sub-pathways. The first involves loss of CO, then dehydrogenation and subsequent rearrangement to an ion, m/e 107, C_7H_7O, which can lose a second molecule of CO and can be ultimately converted to a benzene ion m/e 78, C_6H_6. Ion m/e 137, $C_8H_9O_2$ can alternately lose an acetylene molecule to give an ion m/e 111, C_6H_7O. The most reasonable structure to explain this latter transition is through the formation of a hydroquinone molecule with an additional hydrogen atom. Such an ion can then lose CO, followed by desaturation to yield a five-membered hydroxylated ion, m/e 81, C_5H_5O.

An attempt has been made to thoroughly explain the high resolution data. While many of the minor fragmentation pathways have not been confirmed, evidence provided by high resolution mass spectra and metastable transitions point to their validity.

SUMMARY

The chemical constituency of the non-saponifiable fraction of pecan oil was partially characterized using combination GC-MS. The principal tocopherol present in this fraction was γ-tocopherol with a small amount (less than 1%) of α-tocopherol present. The most abundant component of this fraction was a sterol, tentatively identified as β-sitosterol from mass spectral data.

The mass spectral fragmentation patterns of four tocopherols, α-, β-, γ- and α-acetate were investigated. Mechanisms for principal ion formation including fragmentation of the chromanol ring (producing ions at m/e 151 and 165) and loss of the aliphatic side chain (producing ions at m/e 191 and 205) were investigated, and a general fragmentation pattern was proposed for each isomer.

CHAPTER VII

MATURITY

Introduction

General Studies of Pecans

During the maturation process, the cells and chemical constituents of an organism are in a dynamic metabolic state. In this chapter, the author is particularly concerned with lipid production, fatty acid synthesis, and tocopherol changes in pecans during maturation. The physiology of pecan-nut filling has been described by Finch and Van Horn (128). They report that various problems of pecan kernel filling and maturity are related to and influenced by the condition of vegetativeness of the tree, rather than by conditions such as soil moisture, soil nitrate and phosphate content, or any other single factor. They also found that the cotyledonary tissue was present first as a gel layer within the seed coat. This gel contains a high concentration of sugar and little fat. The conversion of the gel to solid tissue parallels the transformation of sugar to fat.

Later, Hammer and Hunter (129) studied some physical and chemical changes in the composition of pecan nuts during kernel filling. They noted that the most critical period in the filling of the kernel was from August 25 to September 15 (for Albany, Ga., 1946). During this period 63% of the dry weight, 64% of the oil, 43% of the ash, and 71% of

the protein that was ultimately to be found in the kernel were formed.

Maturity Studies on the Walnut

Although little else has been reported on changes in the pecan nut during maturation, a considerably more detailed study has been devoted to a close relative, the walnut. Speranski and Strakhova (130) examined changes in the composition of the walnut during ripening, maturity, and storage. They found that during the maturation process the concentrations of invert sugar, sucrose, starch, tannins, and protein decrease, while the lipids are accumulated. Lopushanski (131) found large quantities of vitamin C in the middle of the walnut and in the green nut covering. As fat accumulates, ascorbic acid declines. Miric and Damanski (132) noted that the non-saponifiable lipid portion decreases during the maturation process. Several authors have studied the fatty acid composition of the walnut (103, 133). Miric and Damanski followed changes in the fatty acid composition of the walnut during maturation (132). Palmitic acid levels were very high in the early stages of maturation (35%) and at maturity had decreased to 14% of the total fatty acid content. Linolenic acid likewise was relatively high (14%) and it decreased to 2% at maturity. On the other hand, the oleic and linoleic acids contents increased by 6 and 28% respectively.

Lipid Compositions of Several Species

Although no similar studies have been done on pecans, a number of researchers have reported on the fatty acid composition of mature pecans. Woodroof and Heaton (5) have reported the oleate and linoleate contents of over forty varieties of pecans. French (44) analyzed pecan

oils for oleate and linoleate by gas chromatography and ultraviolet spectroscopy, and found that the oleic acid content varied from 51 to 77%. Lambertsen (103) compared the % oleate and linoleate with the main tocopherol isomers present in filberts, walnuts, brazil nuts, pecans, almonds, peanuts and chestnuts. He reported that nuts in which linoleate is the predominant fatty acid usually contain principally the γ-tocopherol isomer; conversely, nuts in which oleate predominated, contain mainly the α-isomer. The only exception to this was the pecan containing over 50% oleate and greater than 95% γ-tocopherol.

Much research has been done with oil and fatty acid changes during maturation of nuts other than pecans, especially almonds and cashews. Galoppini (134) studied the fatty acid changes in almonds monthly from April to September. Palmitic acid content decreased from 13 to 5% during the five month period; linoleic acid also decreased from 35 to 14%; oleic acid increased from 50 to 79%; while stearic and palmitoleic acids remained relatively constant. This was later confirmed by Lotti (135). Several workers have reported the fatty acid composition of almonds (136, 137, 138, 139, 140, 141, 103). Popov (142) has reported that two varieties of almonds contain eleostearic acid.

Another nut whose oil and fatty acid composition has been widely investigated is the cashew nut (143, 144, 145, 146, 147, 148). This nut has been found to contain myristic acid (less than 1%), palmitic acid (11%), stearic acid (5%), arachidic acid (4%), oleic acid (61%), and linoleic acid (18%). Carotenoids were not detected. β-Sitosterol was the major constituent (over 80%) of the non-saponifiable lipid fraction, the rest being tocopherols (5%), hydrocarbons (11%), and other sterols (4%). Squalene was the major hydrocarbon. No research has been

reported on fatty acid variations during maturation in cashews.

Along with the normal saturated and unsaturated fatty acids present in oil seeds, hydroxylated moieties have also been found. Takeda (149) has written a review from 62 references on hydroxy fatty acids in oilseeds.

Dubinskaya (150) studied the fatty acids, carotenoids and tocopherols of sunflowers during maturation. She observed that as the seed ripens, tocopherol decreases from 76 to 14 mg%; carotenoids decrease from 0.092 to 0.060 mg%; and oleate and the saturated fatty acids decrease while linoleate increases. Rutowski (151) noted that the tocopherol content of rapeseed oil increases from 31.4 to 44.6 mg% in nine days before harvesting. Pattee and Purcell (152) reported that the oil of immature peanuts contains 60 μg of β-carotene and 138 μg of lutein per liter of oil, whereas the total carotenoid content of mature peanuts is less than 1 μg per liter of oil. Holley and Young have shown that this color reduction in peanuts is highly correlated with maturity (153). Edwards and Reuter (154) have found that lycopene is the dominant pigment in mature tomatoes while chlorophyll, lutein-5,6-epoxide and β-carotene are dominant in the immature fruit. Miric and Cupic (155) studied the fatty acid composition of tomato seed oil at various degrees of maturity and found it to remain constant regardless of the degree of maturity. Sims (156) followed the increase in erucic acid in several varieties of crambe (0-44%) and brassica (0-60%) from seven days after fertilization to the mature seed (6-8 weeks old). Miric (157) followed the fatty acid composition of apricot stones through development and ripening. Miric states that palmitic acid is initially at 38% and declines to 7%; stearic remains constant at 1-2%; oleic begins at

11%, remains constant for 5-7 weeks, then rises to 63% after 11 weeks; linoleic begins at 41%, increases to 50% after 7 weeks, and declines to 28.4%; linolenate begins at 8.3% rises to 14.8% at the fourth week, and disappears after nine weeks.

Maturation and Fatty Acid Synthetic Enzymes

Kartha (158) was one of the first to examine enzyme systems that might be responsible for the erratic changes in the fatty acid compositions of oils during the maturation process of mustard seed. His data suggest that in the early stages of development, only the C-16 and C-18 saturated fatty acids are formed and the production of enzyme systems for the synthesis of the specific acids (e.g. myristic, erucic, etc.) starts at a subsequent stage. The data further show that there is no increase in the higher saturated acids indicating that both the chain elongating and $\Delta^{13,14}$ desaturating enzymes are produced simultaneously. In an earlier paper Kartha (159) has shown that unsaturated fatty acids are produced by desaturation of the corresponding saturated acids and are not intermediates in their synthesis. In a third paper Kartha reported oil development during the maturation process. At all stages of development the oil present was in the form of triglycerides; no mono- or diglycerides were detected (160).

Hereditary and Environmental Effects

Uzzan and Arondel (143) have studied the effect of variety, growth, size, and culture conditions on fatty acid composition of peanuts, rapeseed, sunflower seeds, and soybeans, implying an influence by environment on fatty acid composition. Conversely, much work has been done

with peanuts, attempting to breed nuts with a high oleic/linoleic acid ratio (161).

Apparatus and Reagents

Fatty Acid Analyses

The fatty acid analyses reported in this chapter were performed on a Perkin Elmer 801 gas chromatograph. Details of the analysis are given in Chapter V. The reagents necessary for the transesterification are listed in the Reagents section of Chapter V.

Tocopherol Analyses

The reagents and apparatus used in the tocopherol analyses are given in that section of Chapter VI.

Procedures

Fatty Acid Analyses

Fatty acid analyses were performed according to our modification of Young's (79) method which was described in the Procedure section of Chapter V.

Tocopherol Analyses

Tocopherol analyses on the 1968 crop were performed according to the method of Nelson (80). The analyses on the 1969 crop utilized Slover's (81) method. Both are described under Procedures in Chapter VI.

Preparative Gas Chromatography of Fatty Acid Methyl Esters

The preparative work on pecan fatty acids was performed on a Varian Aerograph, Autoprep Model 711. The column used was a 17' x 10 mm O.D. glass column packed with diethylene glycol succinate, 20% on Gas Chrom Q. Column conditions were the same as for the analytical work described in Chapter III except for flow rate which was 150 ml./min. A 45:1 stream splitter was used.

Results and Discussion

During the maturation process, the pecan undergoes numerous chemical and physical changes. In the light of the previous six chapters, it is natural that this author should look at changes in oil, fatty acids, and tocopherols during maturation. Woodroof (5) suggests that during maturation the oil of the pecan becomes more saturated until finally at maturity the oil is in its most saturated state. Variations of specific fatty acids in the pecan during maturation have not been reported. In this chapter the author reports the results of studies of the changes in fats and oils during pecan nut maturation.

Fatty Acid Composition

The fatty acid compositions of many varieties of pecans have been tabulated and placed in Appendix A. Data are given for the 1967, 1968, and 1969 crops. The typical fatty acid composition of pecans (5) has been established to be: palmitic acid, 6%; stearic acid, 2%; oleic plus linoleic acids, 90%; and about 1% of a fifth fatty acid, probably linolenic acid. The GLC tracing for the fatty acids was reported in Figure 20, Chapter V; however, the only proof available concerning the

identity of these fatty acids was GLC retention times. In order to further confirm the identity of these components, combination GC-MS was used. The mass spectra of component A and methyl palmitate are shown in Figure 49; component B and methyl stearate in Figure 50; component C and methyl oleate in Figure 51; component D and methyl linoleate in Figure 52; and component E and methyl linolenate in Figure 53. From the mass spectra, components A through D matched the respective standards and their presence in pecans was confirmed. However, due to the small amount of component E present, the material was purified using preparative gas chromatography and concentrated for mass spectral analyses. The spectrum of component E reported is actually this purified product. When compared with reference methyl linolenate, component E had a qualitatively similar spectrum. The relative intensities of the standard were greater at higher mass units, though, while the relative intensities of the unknown were greater at lower mass numbers. This fact may be due to the position during the peak at which the spectrum was taken. Pyriadi (45) reported this to be a trienoic acid but could not discern whether it was linolenic or eleostearic, a fully conjugated trienoic acid whose presence has been reported in several varieties of almonds (142).

In examining the spectra of both standard methyl linolenate and the isolated trienoic acid, a peak at m/e 236 (M -56) is present in each. This ion results from fragmentation between carbon atoms 14 and 15 followed by a hydrogen migration to yield 1-butene and a charged ion (162). This fragmentation would not occur in eleostearic acid due to lack of a double bond in the $\Delta^{15,16}$ position. From these results, this compound must be linolenic acid.

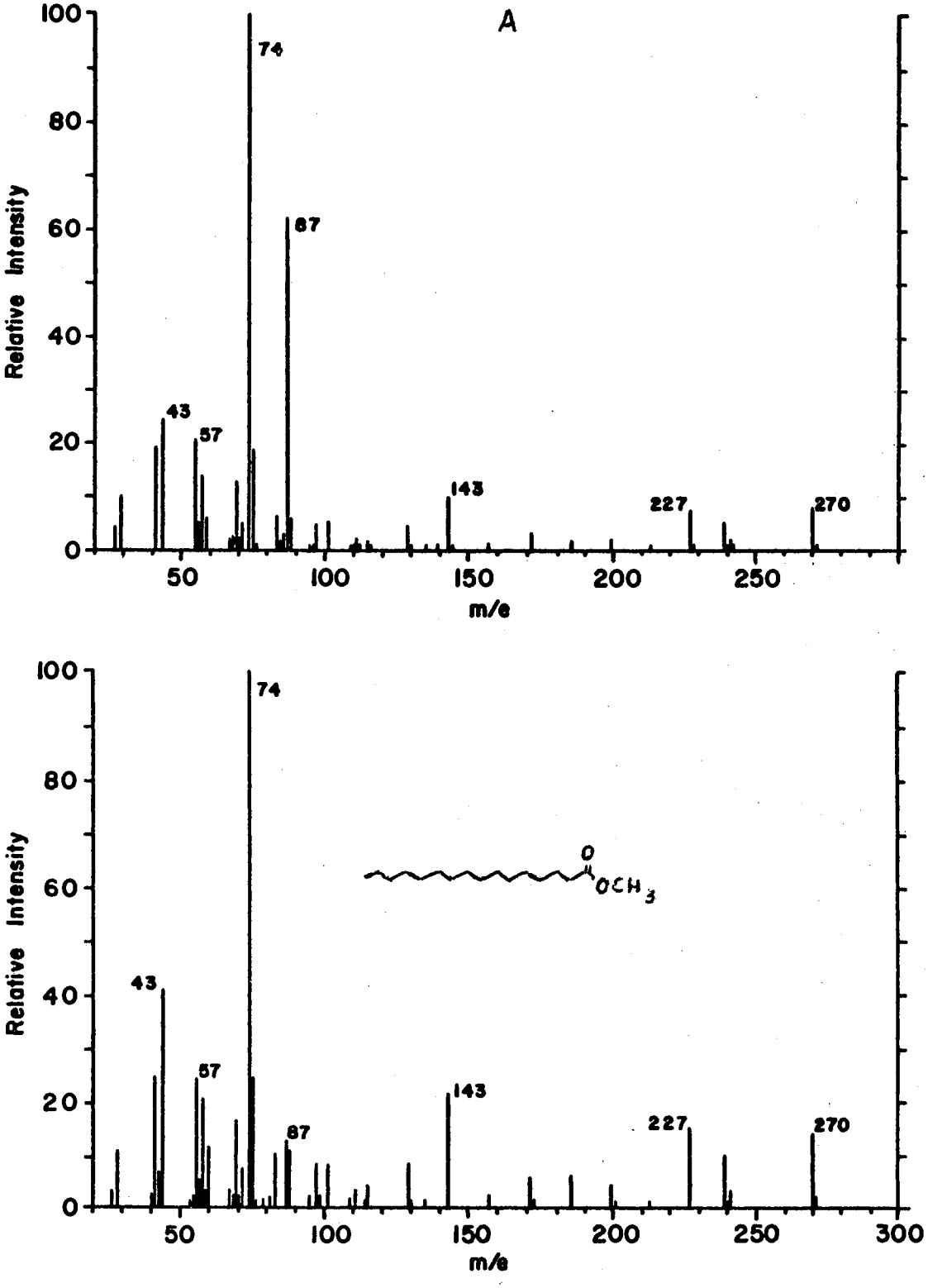


Figure 49. Mass Spectrum of Component A and Methyl Palmitate, Reference Standard

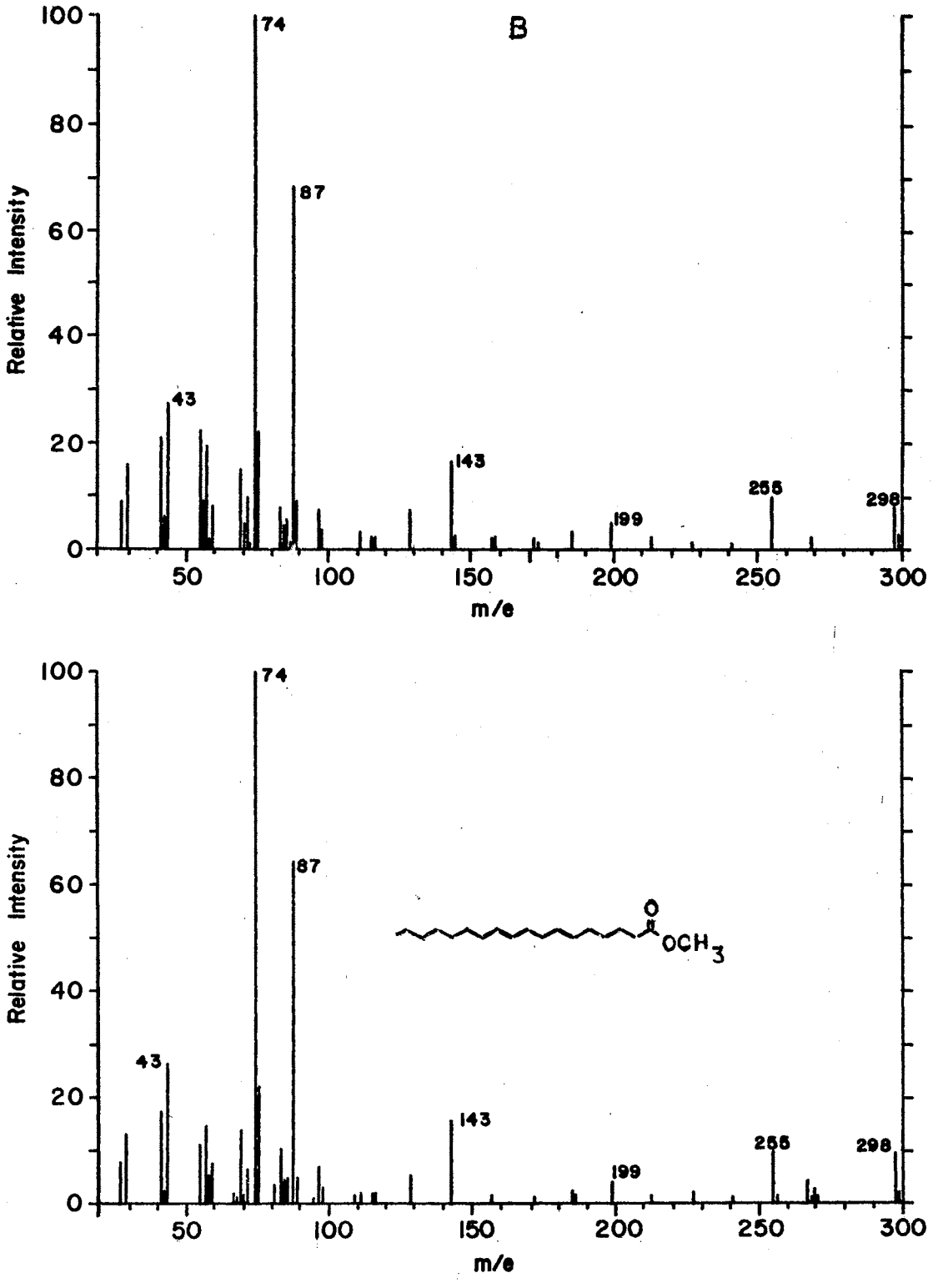


Figure 50. Mass Spectrum of Component B and Methyl Stearate, Reference Standard

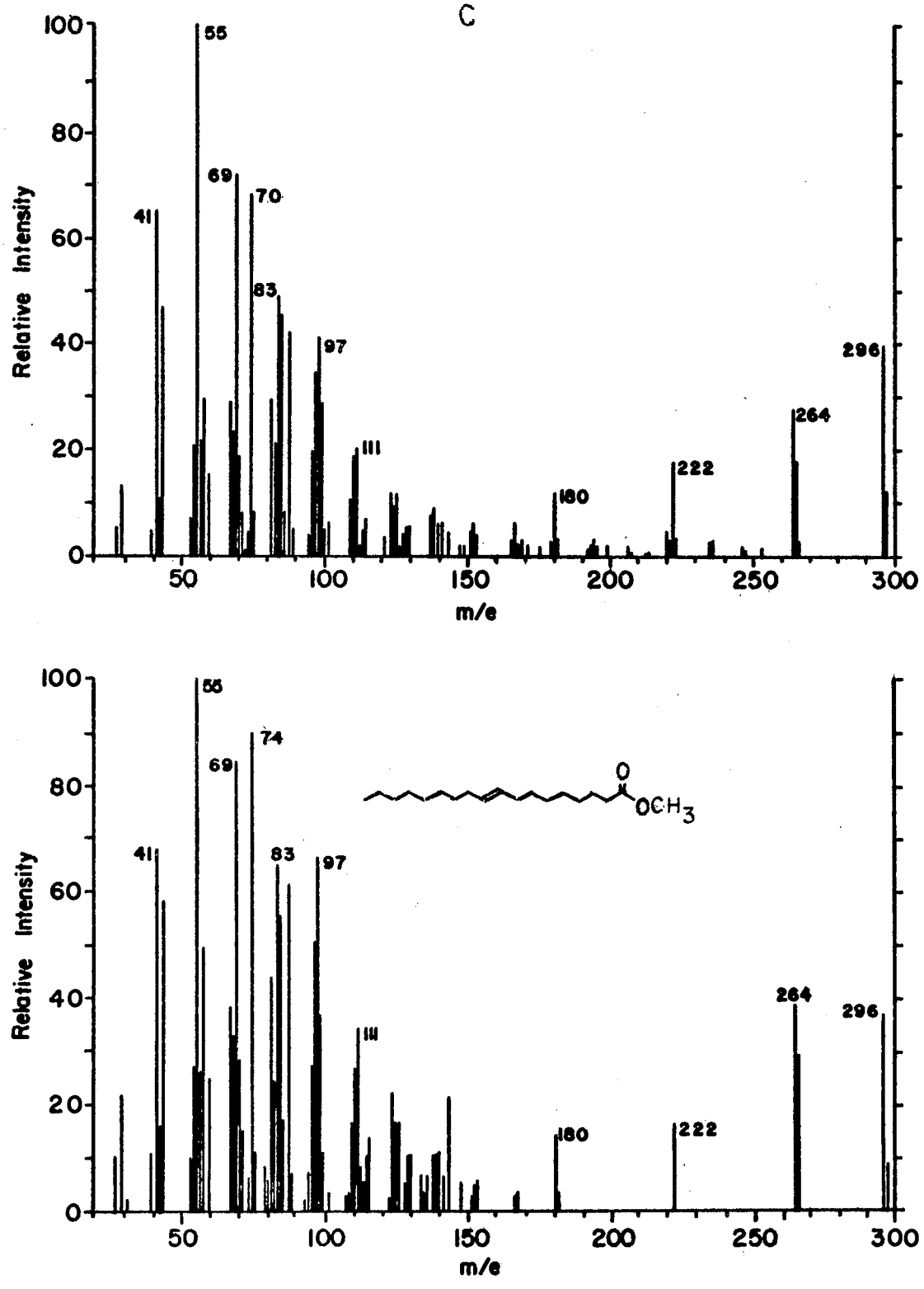


Figure 51. Mass Spectrum of Component C and Methyl Oleate, Reference Standard

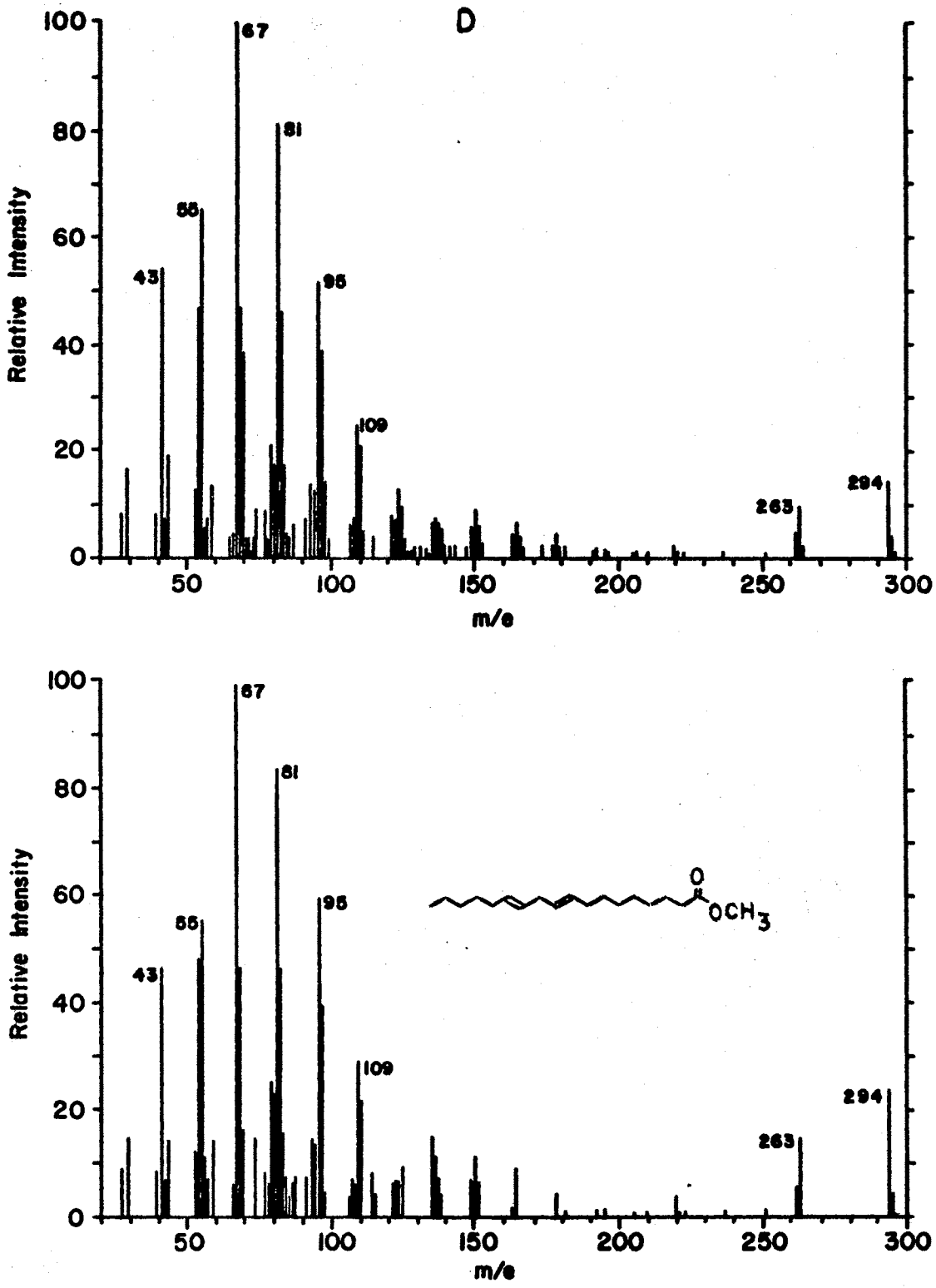


Figure 52. Mass Spectrum of Component D (top) and Methyl Linoleate, Reference Standard

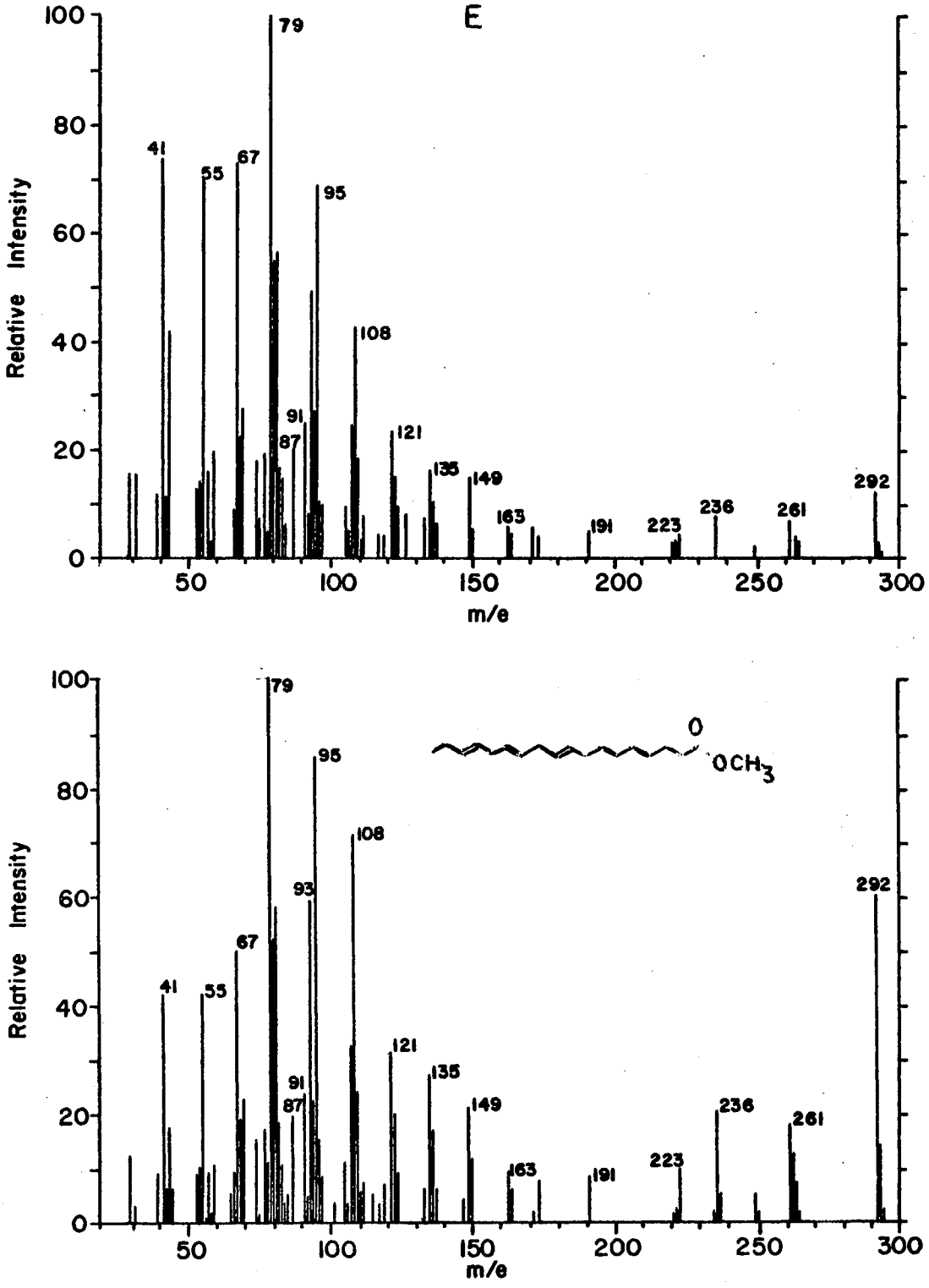


Figure 53. Mass Spectrum of Component E and Methyl Linolenate, Reference Standard

Fatty Acid Variations During Maturation

With safflower, geneticists and plant breeders have worked together trying to develop an oil with a high O/L ratio resulting in better keeping qualities (163). Similar work is currently being applied to peanuts in hopes of increasing the O/L ratio and still maintaining good peanut flavor (161). With this in mind, this author studied the fatty acid composition of nine varieties of pecans over a period of four years. The data on the 1966 crop were obtained from Pyriadi (45). This data is tabulated in Table VIII. When the variation of oleic acid for a single variety over the four year period is considered, Schley had the lowest range at 6% (from 56.7% in 1968 to 63.5% in 1969). Most of the varieties had a range of oleic acid composition from 12% to 23%. Hence, fatty acid distribution may not be influenced by genetic factors. Closer inspection of the data does not reveal any pattern to the fatty acid distribution. Nuts of the Barton variety, 1969 crop, obtained from the Oklahoma Agricultural Experiment Station, had an O/L ratio of 3.29. Another sample of Barton obtained from the USDA Field Station, Brownwood, Texas, was analyzed separately, and was found to have an O/L ratio of 1.36 (see Appendix A). This suggests that while genetic factors may have some role, environmental factors have a larger role. As a result of these findings, the author investigated fatty acid changes in maturing nuts.

For the 1968 study, three varieties from the Stillwater Experiment Station were used--Oklahoma, Delmas and Success. Figure 54 shows the variation in % oleate, linoleate, and oil during maturation of the Delmas variety. At six weeks before maturity this nut contained 6% more linoleate than oleate. Then saturation began, as related by

TABLE VIII

COMPARISON OF FATTY ACID DISTRIBUTIONS IN NINE VARIETIES OF PECANS

OVER A FOUR YEAR PERIOD

Variety	Year	O/L	% Palmitic	% Stearic	% Oleic	% Linoleic	% Linolenic
Barton	1966	1.34	11.3	2.7	48.7	36.2	1.1
	1967	5.23	7.4	2.3	73.4	14.1	1.9
	1968	4.01	5.4	1.8	73.1	18.2	1.5
	1969	3.29	6.0	1.6	70.0	21.3	1.2
Hayes	1966	1.79	10.0	1.3	56.9	31.8	.4
	1967	3.04	8.0	2.4	66.0	21.7	1.1
	1968	2.32	6.6	1.6	62.9	27.1	1.8
	1969	1.43	6.1	1.3	53.4	37.4	1.9
Patrick	1966	1.34	10.7	1.6	50.3	37.4	.3
	1967	3.13	7.4	2.2	66.5	21.2	1.9
	1968	3.04	5.5	2.0	68.0	23.0	1.6
	1969	1.91	5.9	1.5	59.6	31.2	1.8
San Saba Improved	1966	1.70	8.7	2.0	56.3	33.1	.3
	1967	4.35	6.1	2.9	71.7	16.5	2.0
	1968	1.82	5.9	1.7	58.5	32.1	1.8
	1969	2.20	5.6	1.8	62.6	28.5	1.4
Schley	1966	2.02	8.5	1.6	59.7	29.6	.6
	1967	1.96	8.3	2.2	57.7	29.5	1.7
	1968	1.78	7.4	2.1	56.7	31.9	1.9
	1969	2.31	6.4	1.3	63.5	27.5	1.3
Stuart	1966	1.65	8.5	1.6	55.3	33.6	1.0
	1967	2.56	8.1	2.4	62.6	24.4	2.0
	1968	1.82	6.0	1.8	58.3	22.1	1.8
	1969	3.15	6.3	1.6	68.9	21.9	1.3
Success	1966	1.60	7.7	1.5	55.9	34.9	1.0
	1967	3.55	6.8	2.5	69.5	19.6	1.4
	1968	1.47	6.9	1.9	53.0	36.2	2.0
	1969	2.49	6.0	1.8	64.8	26.0	1.4
Texas Prolific	1966	1.29	8.4	1.1	51.0	39.6	.4
	1967	1.60	7.9	3.0	53.6	33.6	1.7
	1968	1.72	5.9	1.9	57.4	33.3	1.6
	1969	2.37	6.2	1.8	63.7	26.9	1.6
Western	1966	1.25	10.6	1.3	48.8	39.2	.3
	1967	2.43	7.6	2.9	62.1	25.5	1.5
	1968	1.44	7.7	2.0	52.1	36.2	2.1
	1969	1.53	6.8	1.9	54.3	35.4	1.7

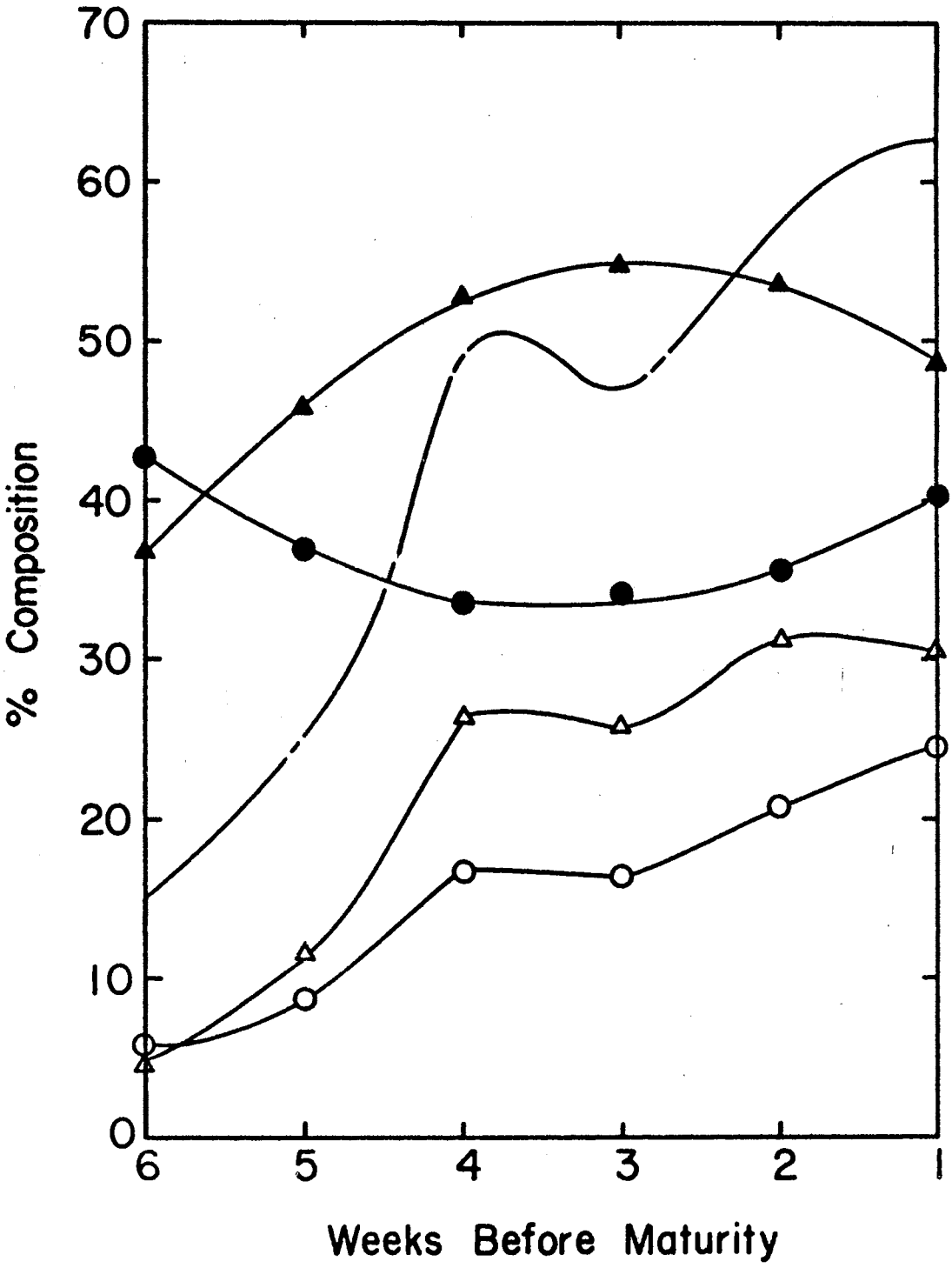


Figure 54. Variation in Oleate (▲ △), Linoleate (● ○), and Oil Content (—▪—) During the Maturation of Pecans, Delmas Variety. Fatty Acids are Shown Both as % of the Oil (▲ ●) and % of the Dry Weight of the Nut (△ ○), 1968 Crop

Woodroof (5), and at three weeks before maturity, the nuts contained 20% more oleate than linoleate. This increase in oleic acid was expected to level off or to increase at a slower rate as the nut approached maturity. But in the last two weeks before maturity, desaturation of the fatty acids occurred and the linoleic acid concentration increased, presumably at the expense of the oleic acid. This was contrary to Woodroof's results (5) which indicated that the nut becomes steadily more saturated during maturation. In the Oklahoma variety (Figure 55) the same trend is apparent--a uniform increase in oleate until four weeks before maturity at which time the % oleate decreased and the % linoleate increased. The data from the Success variety was very similar to that of the Oklahoma variety (Figure 56). From Figures 55 and 56, it is apparent that the periods during which oleic acid is increasing are coincidental with the periods of rapid oil synthesis. If the fatty acid composition was studied on a % of nut weight basis (see Oklahoma variety), it was found that during the period of rapid oil synthesis (6-4 weeks before maturity) the oleic acid composition increased whereas the linoleic acid remained constant. Linoleic acid synthesis did not resume until oil synthesis stopped. No conclusions can be made from this 1968 pilot study. Apparently this experiment was initiated too late in the season, as the earliest pecan samples had as much as 15% oil. This pilot experiment was to be repeated on the 1969 crops.

From the 1969 crop, six pecan varieties were selected: Oklahoma, Squirrel, Schley, Stuart, Major, and Delmas. Samples of the first three varieties were not obtained at an early enough stage, as the oil concentration was up to 5% of the dry weight. In the Oklahoma variety (Figure 57), the oil, oleate and linoleate paralleled the results ob-

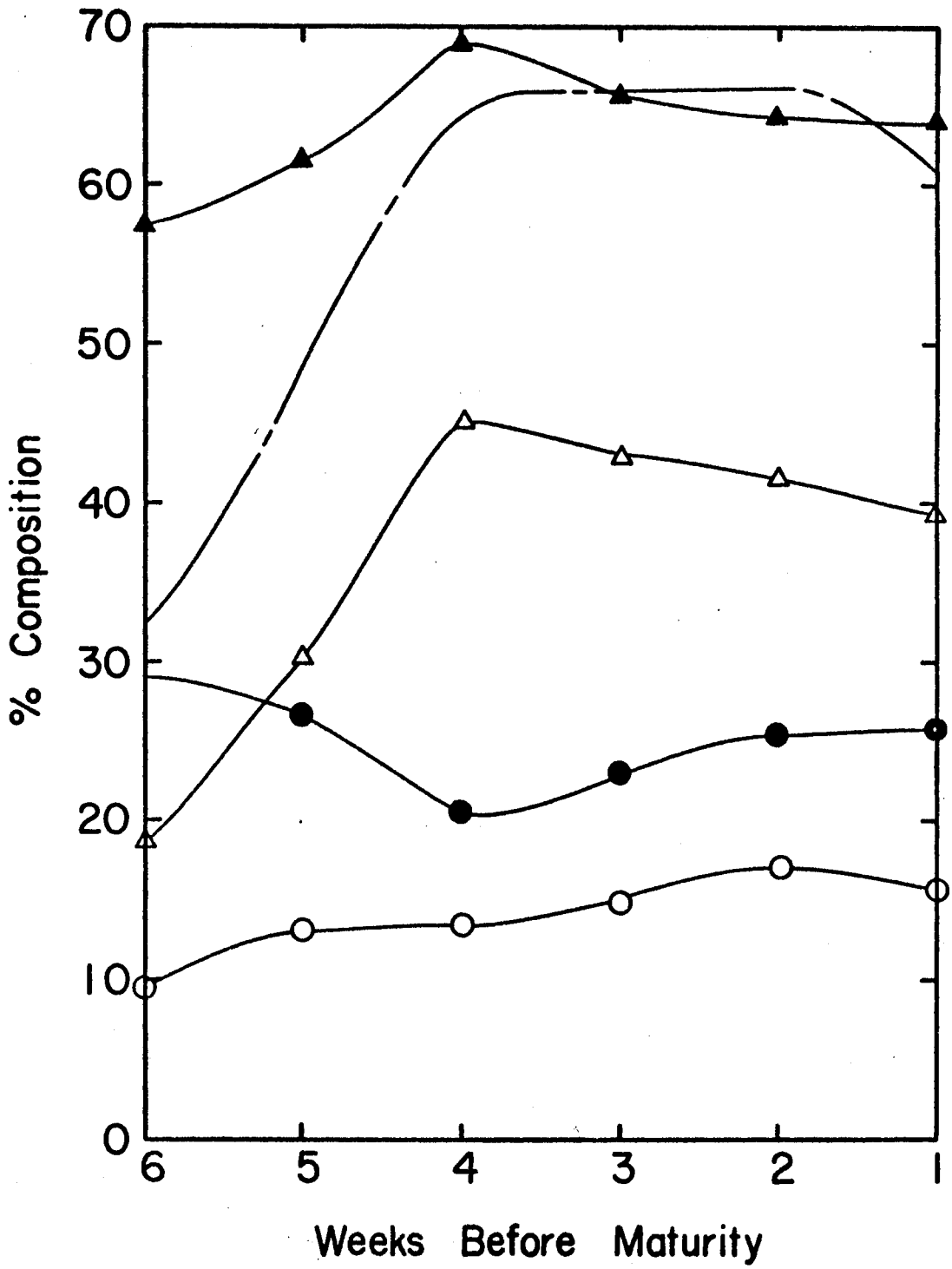


Figure 55. Variation in Oleate (▲ △), Linoleate (● o), and Oil (———) Content During the Maturation of Pecans, Oklahoma Variety, 1968 Crop. Fatty Acids are Shown Both as % of the Oil (▲ ●), and % of the Dry Weight of the Nut (△ o)

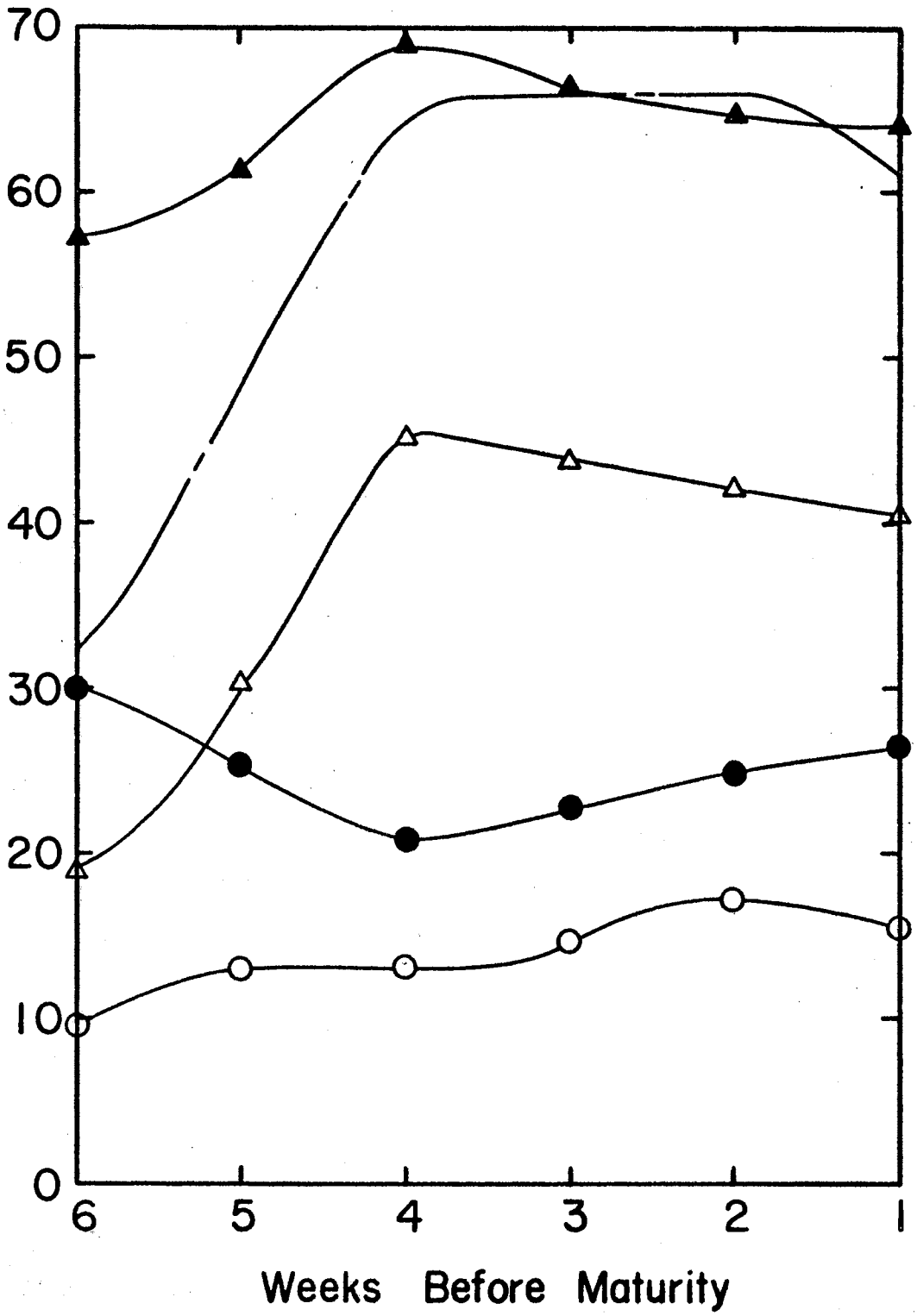


Figure 56. Variation in Oleate (▲ △), Linoleate (● o), and Oil (——) Content During the Maturation of Pecans, Success Variety, 1968 Crop. Fatty Acids are Shown Both as % of the Oil (▲ ●) and % of the Dry Weight of the Nut (△ o)

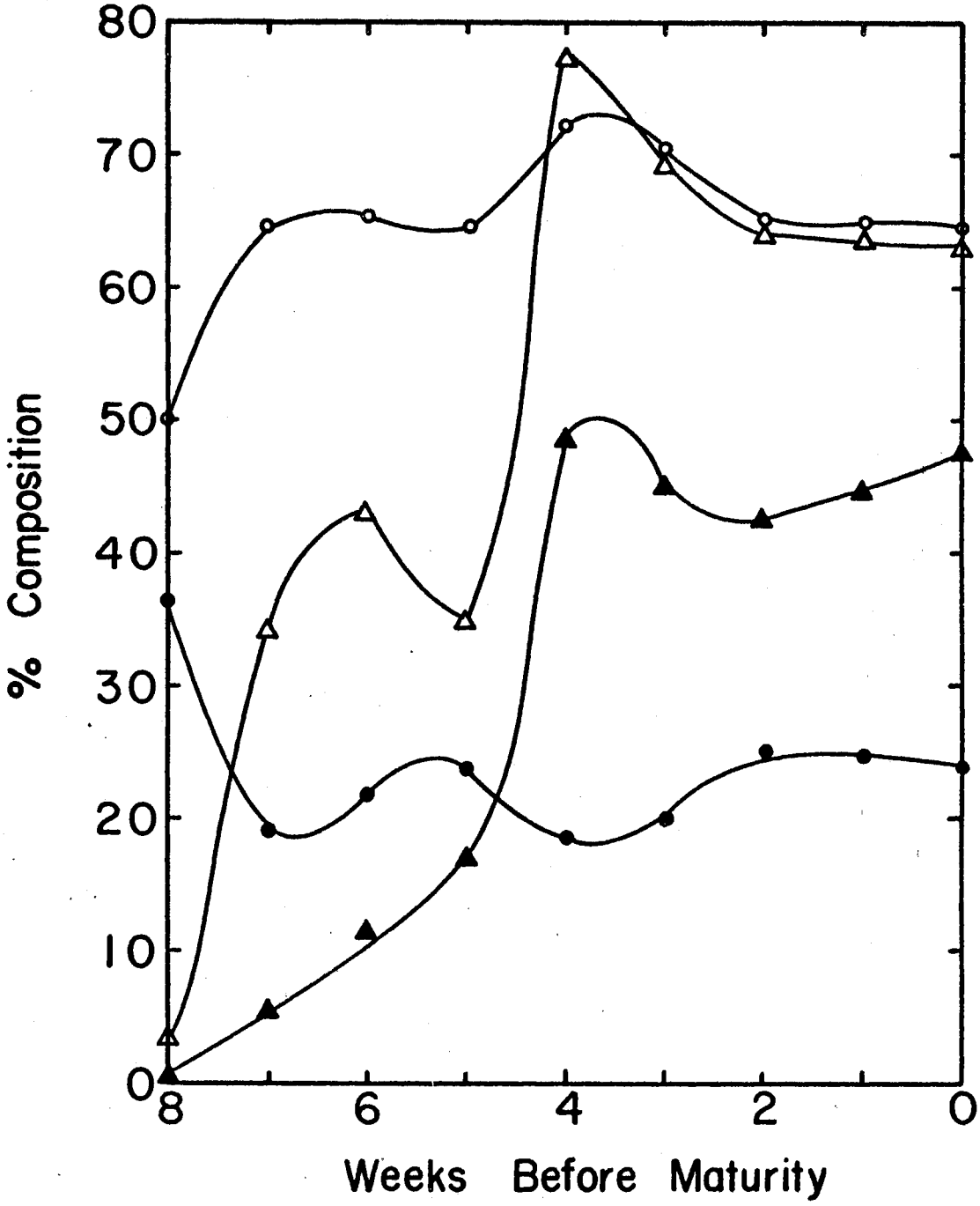


Figure 57. Variation of Oleate, o , and Linoleate, ● , (as % of Oil) and Variation of Oil (% Dry Wt., △ , and % Wet Wt., ▲) During the Maturation of Pecans, Oklahoma Variety, 1969 Crop.

tained for the 1968 crop, except that this year a double peak was present. In the period eight to six weeks before maturity the oil concentration increased from 4% to 43% of the dry weight of the nut; also, during this period, the concentration of oleate increased and linoleate decreased. At six weeks before maturity the oil concentration in the nut actually decreased to about 35% and the fatty acid synthesis pattern reversed. In the one week period between five and four weeks prior to maturity, oil synthesis was again activated and the oil concentration rose from 34% to 78%, a maximum point from which it slowly tapered to 66%, four weeks later at maturity. Likewise, at four weeks before maturity, oleic acid concentration reached a maximum of 73% while linoleic acid bottomed out at 18%. The concentrations of these two components at maturity were 64% and 24%, respectively. The nut at four weeks before maturity had just started to take on the characteristics of a pecan, but it was far from being horticulturally mature. It was fully enclosed by a shuck at this stage. Figure 58 (Squirrel) and Figure 59 (Schley) confirm this observation noted in the nuts of Oklahoma variety; but these too were early maturing nuts and samples were not collected soon enough.

The Stuart, Delmas and Major varieties were first sampled in the late water stage of development, just prior to gel formation. At this stage, the nuts contained less than 1% oil. Figure 60 (Stuart variety) is probably the best illustration of this point. At nine weeks before maturity the Stuart pecans contained 1-2% oil, dry weight basis. Over 50% of this was palmitic acid. Oleic and linoleic acids comprised about 18% each of the remaining 50%, leaving 14% unaccounted for. Although not illustrated for the sake of clarity, the stearate concentration at

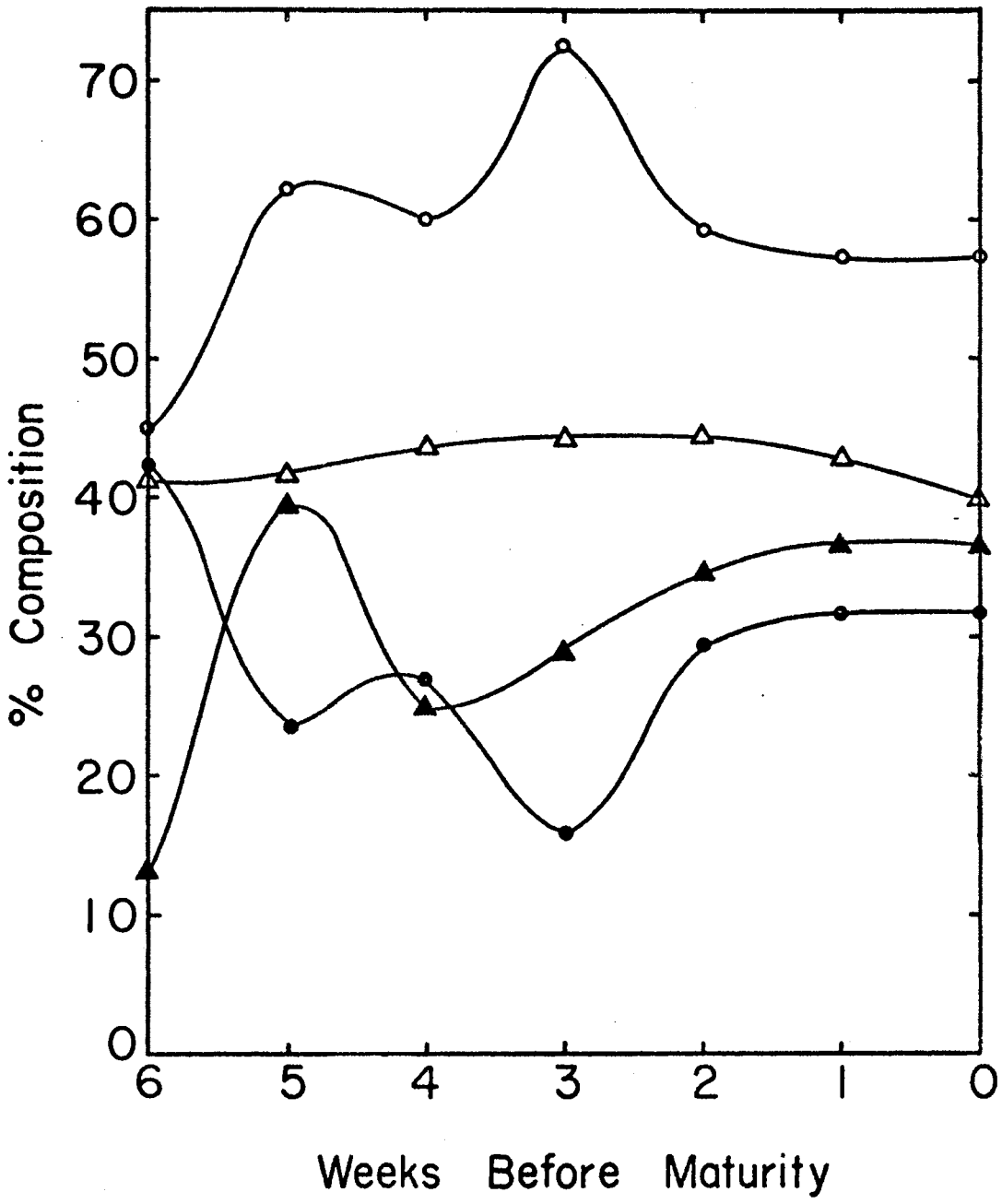


Figure 58. Variation of Oleate, o , and Linoleate, ● , (as % of Oil) and Variation of Oil Content (% Dry Wt., Δ , and % Wet Wt., ▲) During the Maturation of Pecans, Squirrel Variety, 1969 Crop

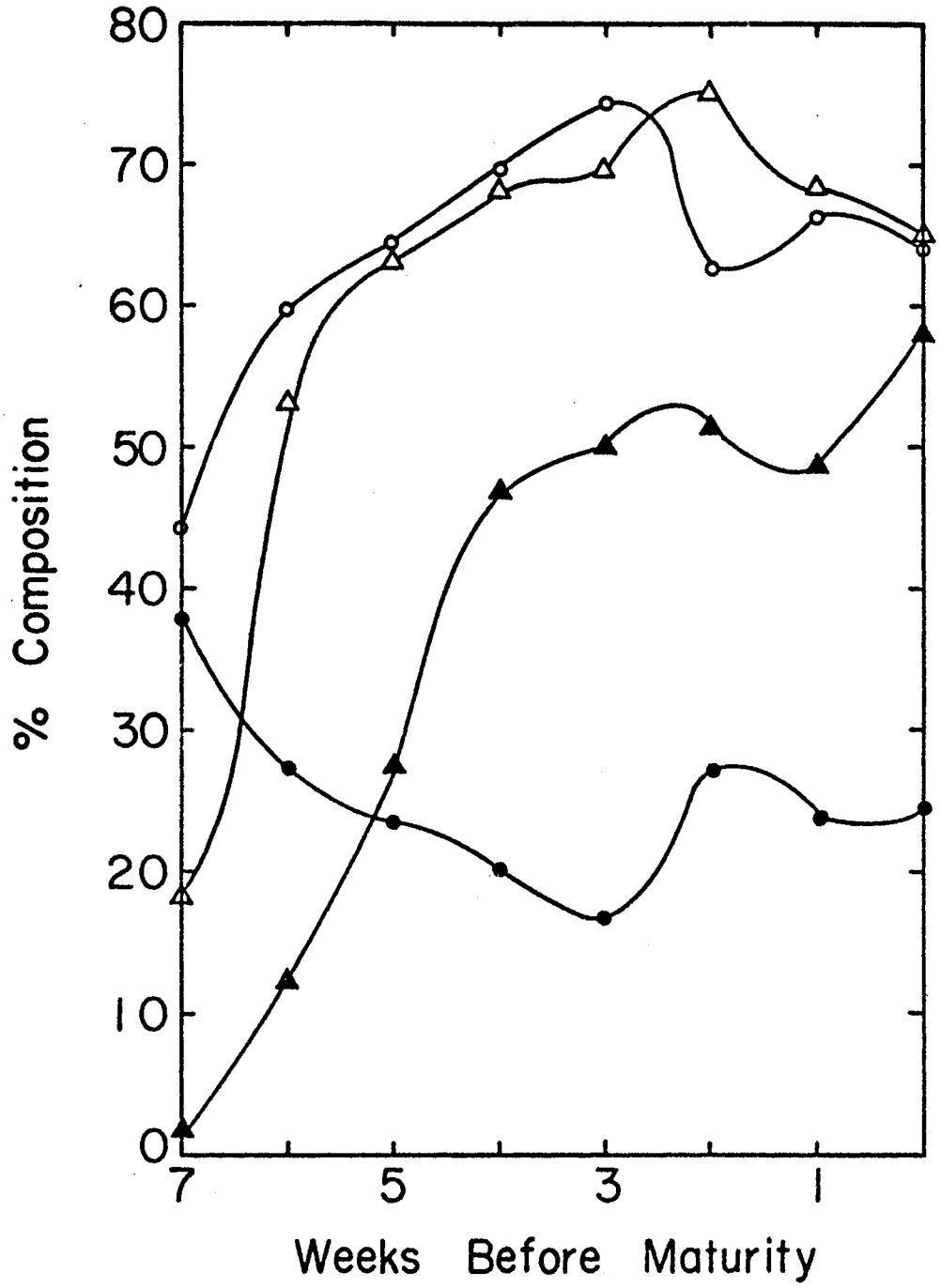


Figure 59. Variation in Oleate, o , and Linoleate, ● , (as % of Oil) and Variation of Oil Content (% Dry Wt., Δ, % Wet Wt., ▲) During the Maturation of Pecans, Schley Variety, 1969 Crop

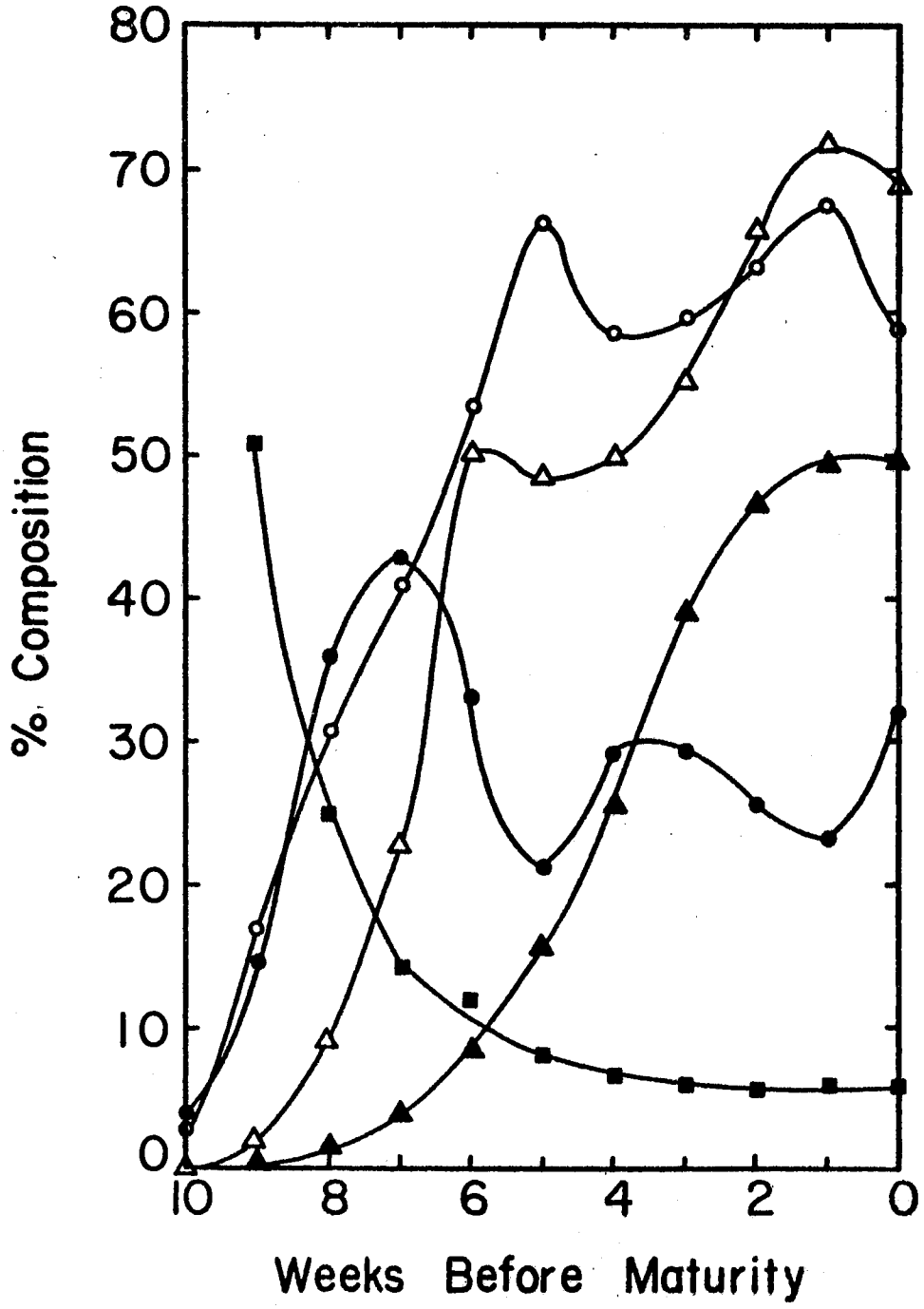


Figure 60. Variation in Palmitate, ■ , Oleate, ○ , and Linoleate, ● , (as % of Oil) and Variation of Oil Content (% Dry Wt., △ , % Wet Wt.,▲) During the Maturation of Pecans, Stuart Variety, 1969 Crop

nine weeks before maturity was 8% of the oil; by seven weeks before maturity it had been reduced to 2%, its level in the mature nut. Likewise, the linolenic concentration was up, but only to about 6%. By seven weeks before maturity it had decreased to its level in the mature nut of 1 to 2%. In the period from seven weeks prior to maturity through maturity, the nut's development was similar to the six previously described varieties. It is these earlier periods that will be dealt with in the discussion of the Stuart variety. During this early period, there was a sharp decline in the % palmitate from 51% to about 13%; at the same time, oleic and linoleic acids rose in a parallel manner until at seven weeks before maturity both acids represented 40% each of the total fatty acids. Then in the next two week period, the linoleic acid content decreased to 20% and the oleic acid content increased to 67%. This is the end of the first phase of oil synthesis and the rest of the plot is similar to that described for the Oklahoma variety. Figures 61 and 62 show a similar pattern for the Delmas and Major varieties, respectively.

% Oil and Maturity

Woodroof (5) states that the mature pecan has a maximal amount of oil and a minimal amount of linoleic acid. Since these data were available from the 1969 crop, they were plotted against each other. The result is shown in Figure 63. In general this correlation is supported, but the correlation is far from ideal.

Tocopherol Variations During Maturation

After following the fatty acid composition of several pecan oils

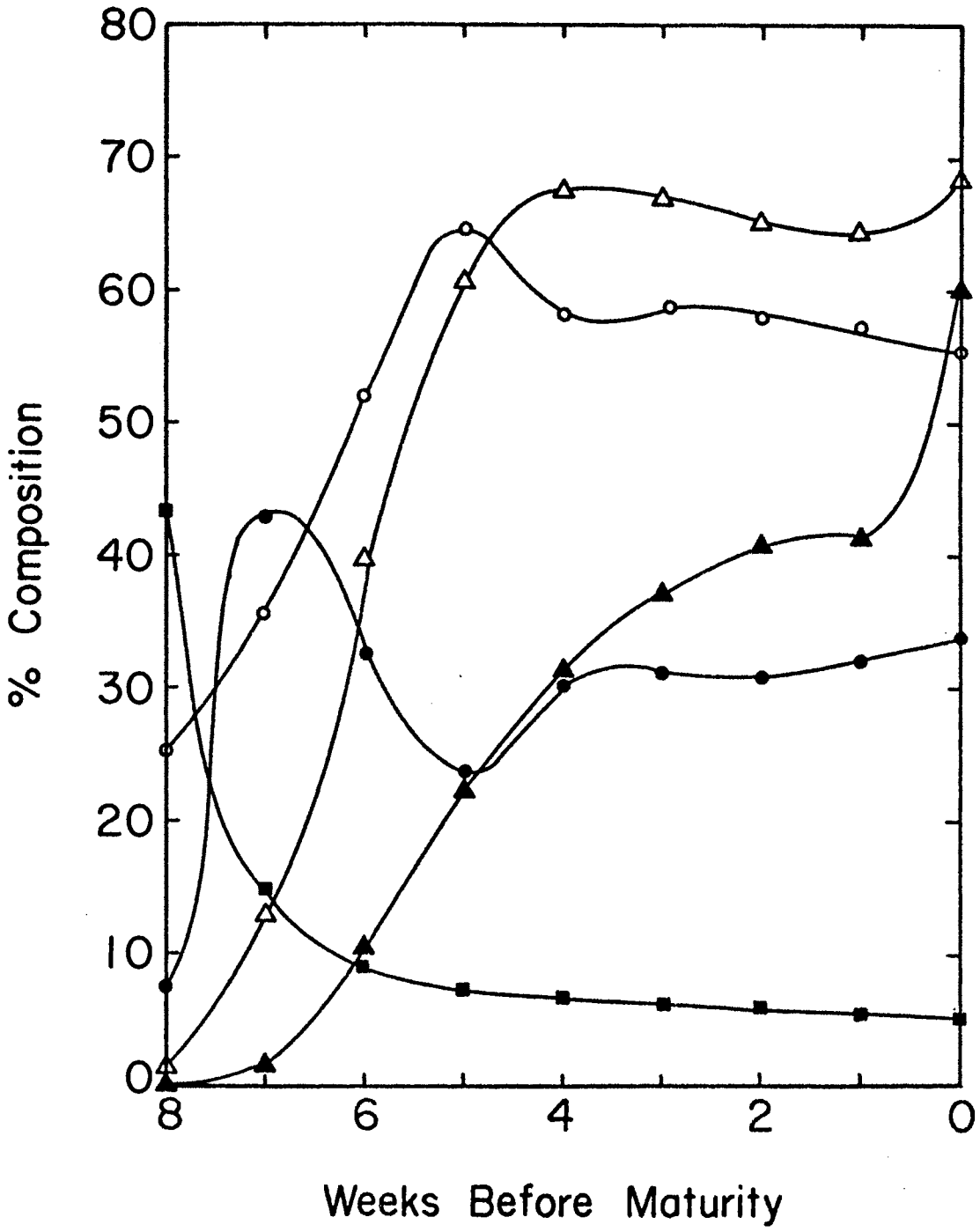


Figure 61. Variation in Palmitate, ■ , Oleate, ● , and Linoleate, ○ , (as % of Oil) and Variation of Oil Content (% Dry Wt., △ , % Wet Wt., ▲) During the Maturation of Pecans, Delmas Variety, 1969 Crop

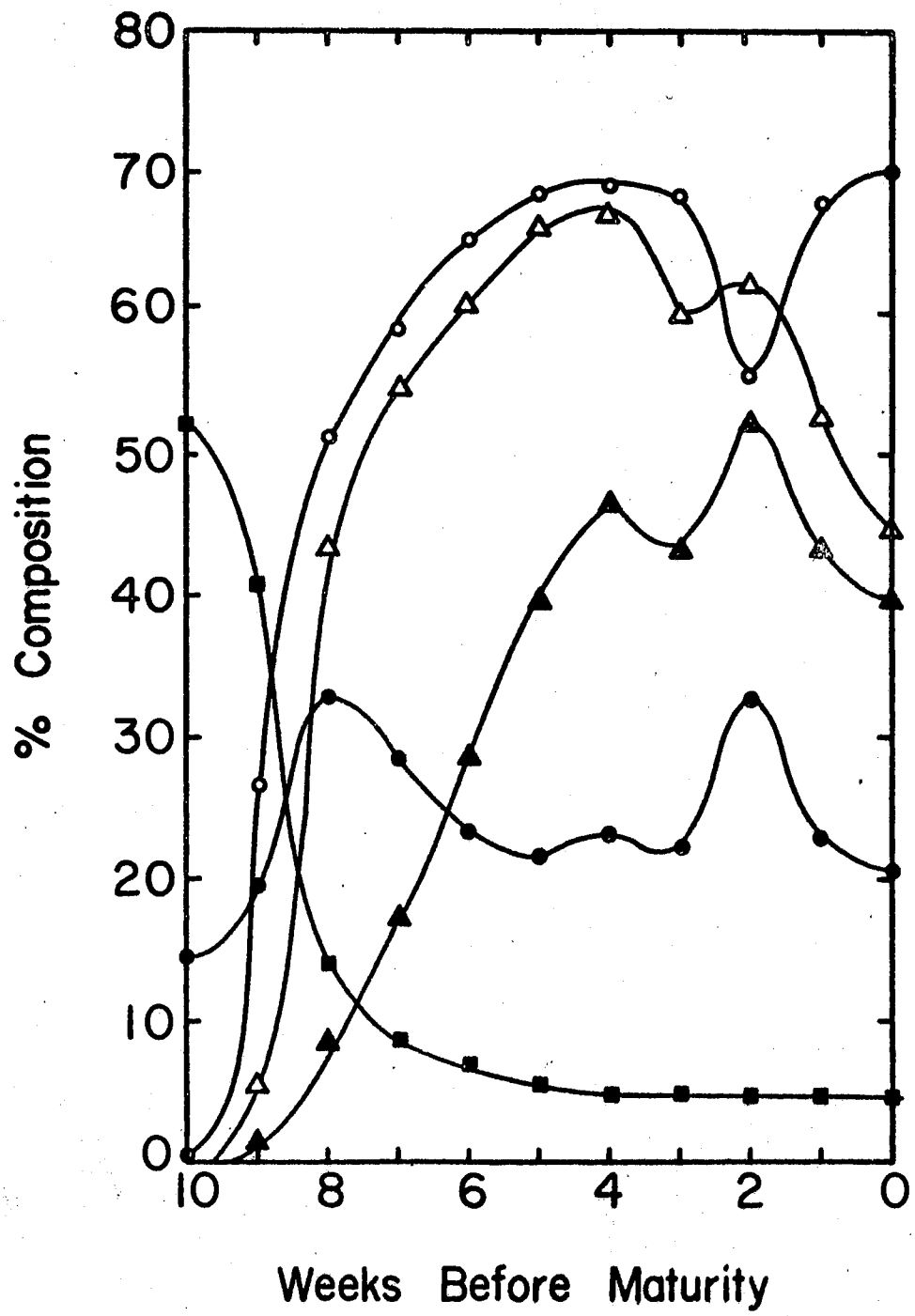


Figure 62. Variation in Palmitate, ■ , Oleate, o , and Linoleate, ● , (as % of Oil) and Variation of Oil Content (% Dry Wt., △ , % Wet Wt., ▲) During the Maturation of Pecans, Major Variety, 1969 Crop

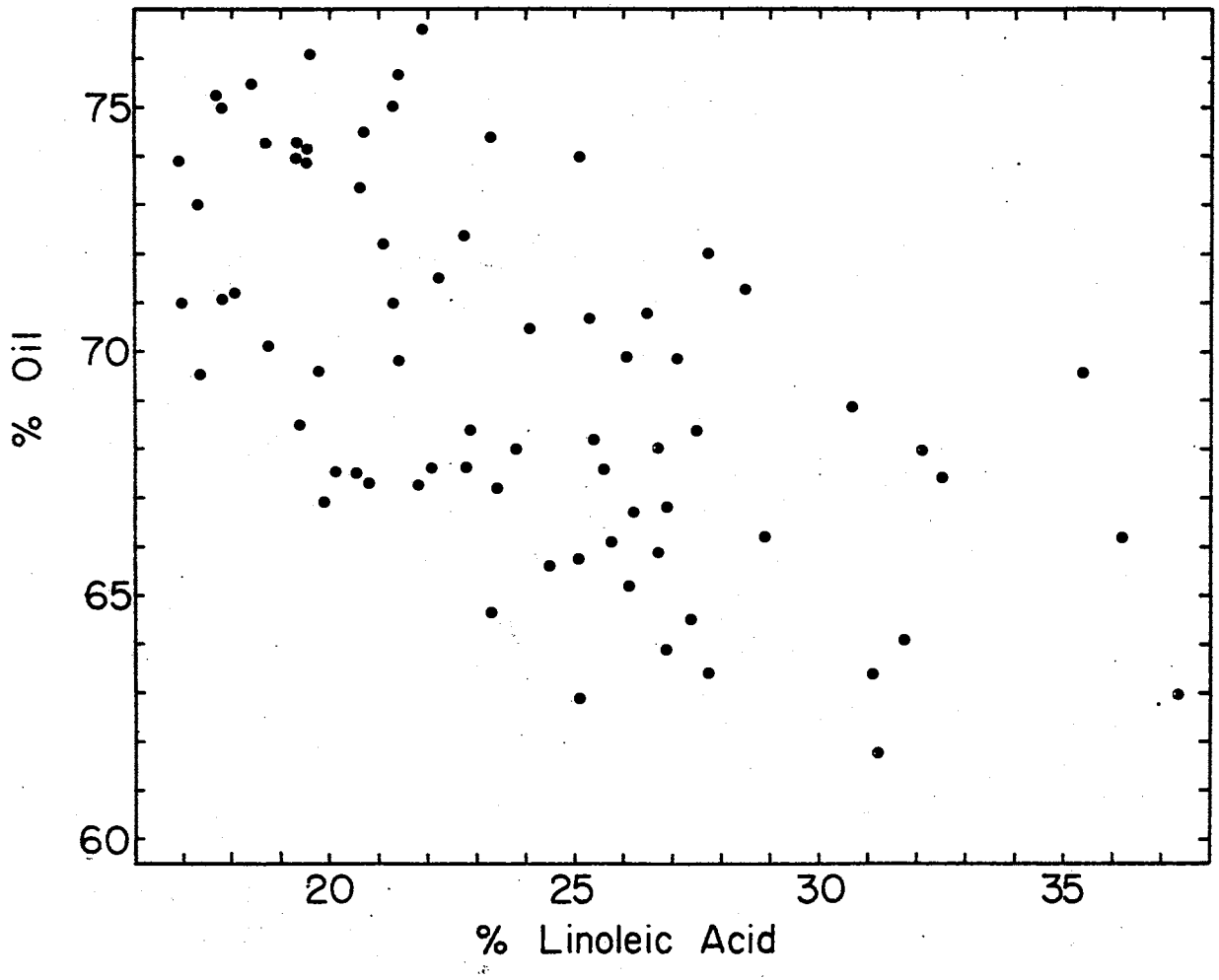


Figure 63. Plot of % Oil Against the % Linoleate for 83 Varieties of Pecans

during maturation, this author decided it would be a necessary corollary to this work to investigate changes in the tocopherols of pecan oils during maturation. Two samples were chosen for this work: Stuart, 1968 crop (Figure 64) and Schley, 1969 crop (Figure 65). In each sample there was a common trend apparent. In the more mature nuts, the tocopherol concentration was much higher (by a factor of two to five) than in the mature nut. Hence, either the tocopherol is synthesized very early and diluted out as oil was synthesized or the tocopherol is consumed in the process of triglyceride synthesis. It might be noted that these values are on a per gram of oil basis. If the y-axis was put on a per gram of nut basis, there would still be a higher tocopherol concentration in the immature nuts. This suggests a possible colormetric assay for maturity in pecans. By oxidizing the oil with nitric acid, tocored would be formed which can be determined spectrophotometrically.

Summary

A possible conclusion, considering Kartha's work on the enzymology of fatty acid synthesis, is that saturated fats are present in the pecan at a steady state level and are being continually desaturated as rapidly as they are formed. The enzyme systems for monodesaturation to oleic acid are very active, but the synthesis is stimulated only during periods of rapid oil synthesis. The enzyme responsible for desaturation of oleate to linoleate is always present and has a much lower turnover number. Hence, there is always some conversion of oleate to linoleate in maturing pecans, but this net synthesis is nullified by the very rapid synthesis of oleic acid which occurs during the periods of oil synthesis. The fact that linoleate concentration parallels the oleate concentration

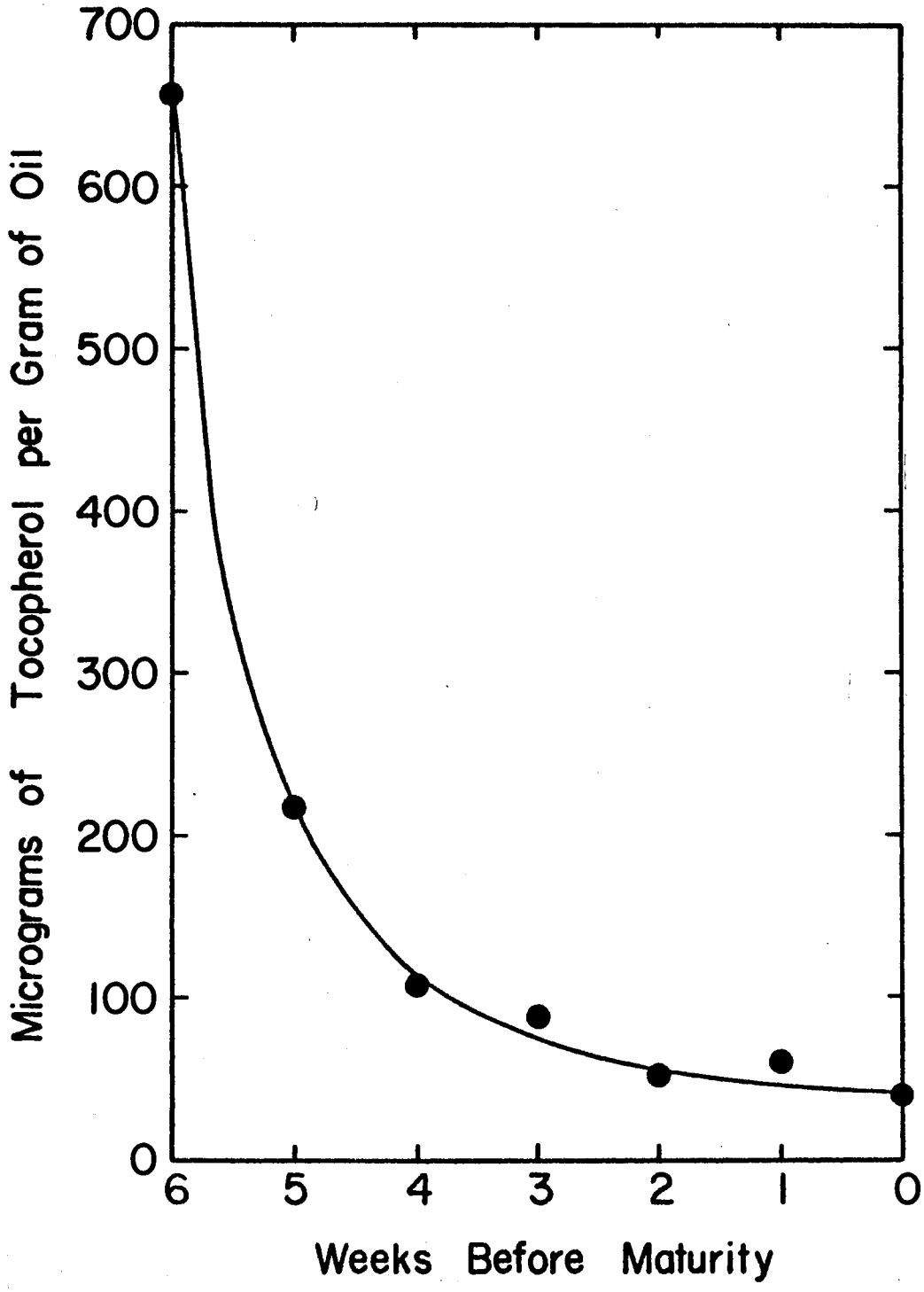


Figure 64. Variation in Tocopherol Content During the Maturation of Pecans, Stuart Variety, 1968 Crop

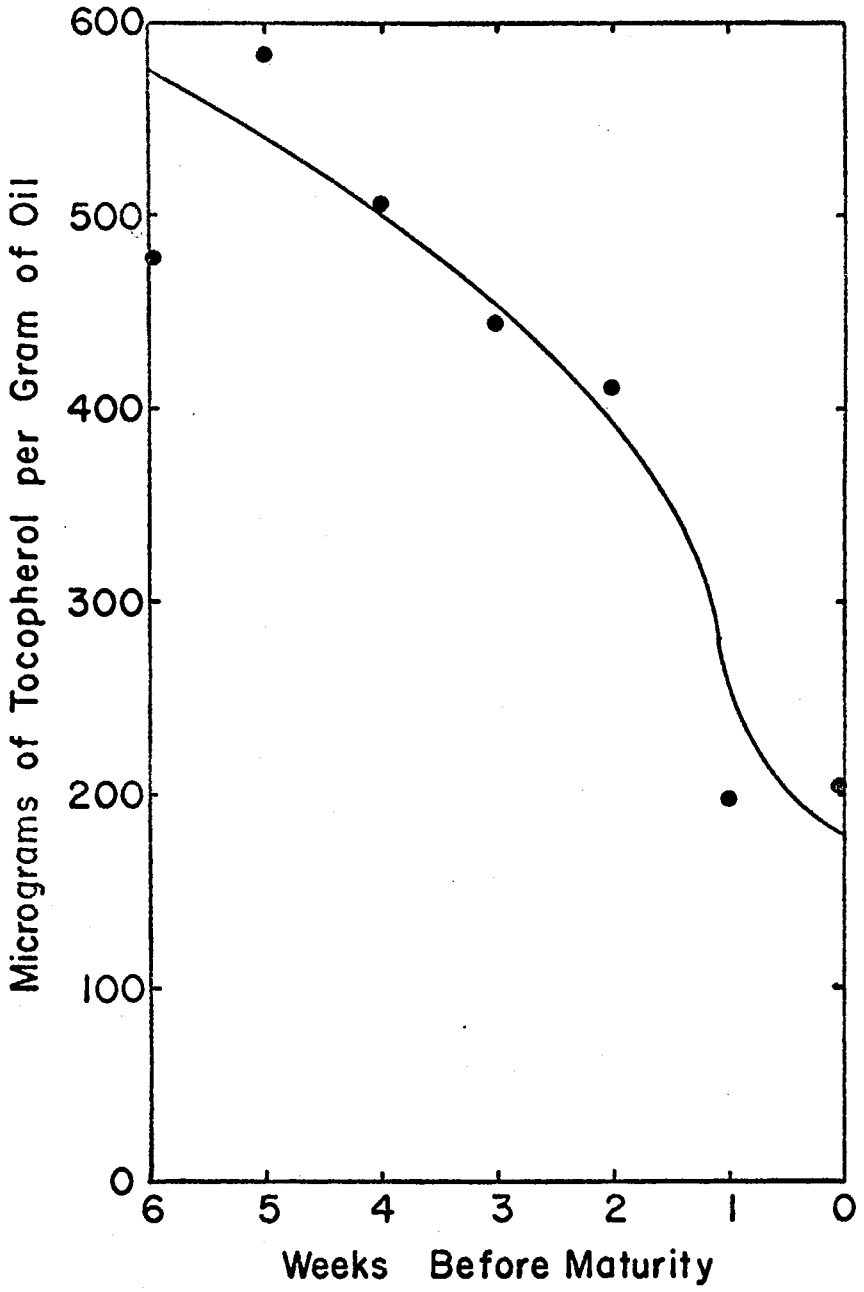


Figure 65. Variation in Tocopherol Content During the Maturation of Pecans, Schley Variety, 1969 Crop

during the early stages of maturity tends to support rather than to deny this hypothesis. Since logarithmic oleic acid synthesis has not begun at this stage (i.e. the oil concentration is not very high), the oleate to linoleate enzymes can keep up with the enzymes responsible for oleate synthesis from stearate.

Tocopherol concentration during maturation also appears quite variable as the nuts have as much as 600 μg/gm of oil 6 weeks before maturity. This concentration is reduced to 100-300 μg/gm oil at maturity.

CHAPTER VIII

SUMMARY

Volatile aroma constituents from pecans have been analyzed using primarily high vacuum techniques and combination gas chromatography-mass spectrometry. Compounds tentatively identified include four low molecular weight alcohols (C_5-C_8), four low molecular weight aldehydes (C_6-C_{10}) and a γ-lactone (C_5). These compounds, along with several unidentified compounds, may play a significant role in pecan flavor.

Two pyridines, ten pyrazines and a pyrole were tentatively identified from roasted pecans. When peanuts were roasted under a nitrogen atmosphere, pyrazines, furans, pyridines, pyrroles and possibly a dihydropyrazine were produced but no aldehydes, ketones or other products of fatty acid oxidation were observed. The nuts had typical peanut flavor.

The primary chemical component responsible for oxidative changes in pecan oils was the linoleic acid concentration. Tocopherol concentration did not correlate with any of the parameters investigated (i.e. % linoleate, keeping time, etc.), yet a unique correlation presented shows tocopherols to be of secondary importance in influencing the oxidative stability of pecan oils. For an oil with a constant % linoleate, keeping time increased in a linear fashion with tocopherol concentration up to 800 μg/gm of oil at which point a plateau was reached. γ-Tocopherol was the major tocopherol isomer present in the 70 varieties of pecans investigated varying between 150 and 500 μg/gm of oil.

β-Sitosterol was the major sterol present. The mass spectra of the tocopherols was characterized by very intense molecular ions and limited fragmentation. The major fragmentation involves cleavage of the chromanol ring to produce ions of m/e 151 (or 165). The only other intense diagnostic ion in the spectra was at m/e 191 (205) and was due to loss of the $C_{16}H_{33}$ aliphatic side chain.

Mono- and di-unsaturated fatty acid synthesis in the pecan appeared to be under the influence of environmental factors. There was no consistent year to year pattern in the oleate and linoleate composition on a varietal basis. There was also a large variation in the fatty acid composition of pecans during maturation. This variation was characterized by periods of rapid oil synthesis, during which oleic acid concentration usually reached a maximum followed by an increase in linoleate several weeks before maturity after oil synthesis was completed. Likewise, there was a two to five fold elevation in the tocopherol content in the pecan oils prior to maturity which gradually leveled off at maturity.

These data will be of value in the pecan breeding program, to processors and to researchers working with pecans and other oilseeds.

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APPENDIX A

FATTY ACID ANALYSES OF PECAN OILS

TABLE IX

FATTY ACID ANALYSES OF 12 VARIETIES OF PECANS
1967 CROP

Variety	% Palmitic	% Stearic	% Oleic	% Linoleic	% Linolenic
Barton	7.4	2.3	73.4	14.1	1.9
Desireable	6.7	2.5	66.2	21.9	2.0
Graking	6.7	3.0	68.5	19.3	1.7
Hayes	8.0	2.4	66.0	21.7	1.1
Nugget	6.7	2.3	67.9	20.6	1.9
Patrick	7.4	2.2	66.5	21.2	1.9
San Saba Improved	6.1	2.9	71.7	16.5	2.0
Schley	8.3	2.2	57.7	29.5	1.7
Stuart	8.1	2.4	62.6	24.4	2.0
Success	6.8	2.5	69.5	19.6	1.3
Texas Prolific	7.9	3.0	53.6	33.6	1.7
Western	7.6	2.9	62.1	25.5	1.5

TABLE X

FATTY ACID ANALYSES OF 63 VARIETIES OF PECANS
1968 CROP

Variety	% Palmitic	% Stearic	% Oleic	% Linoleic	% Linolenic
Apache	5.4	2.4	65.9	24.9	1.4
Barton	5.4	1.8	73.1	18.2	1.5
Buekett	5.2	1.9	58.3	32.9	1.8
Butterick	5.8	2.0	65.8	25.0	1.4
Busserson	10.7	3.5	50.0	33.4	2.5
Caddo	6.9	1.9	54.1	34.9	2.1
Choctaw	5.6	2.6	62.6	27.5	1.6
Coffman	6.4	3.0	61.6	27.3	1.7
Commanche	6.4	2.6	64.5	25.1	1.4
Commonwealth	5.2	2.3	67.5	23.6	1.5
Delmas	7.5	1.6	53.0	36.1	1.9
Desireable	5.3	1.5	57.4	34.4	1.5
Dodd	5.5	2.9	69.1	21.1	1.4
Dooley	5.6	1.4	53.2	37.6	2.1
Elliot	5.7	2.2	63.2	27.8	1.2
Farley	6.0	2.3	57.0	33.3	1.5
Garner	4.9	2.4	55.8	34.1	2.8
Giles	6.0	2.8	61.4	28.2	1.6
Gra-Tex	6.2	2.4	69.7	20.6	1.1
Green River	5.8	1.8	59.4	31.3	1.7
Halbert	7.0	1.3	39.4	49.4	2.9
Hayes	6.6	1.5	62.9	27.1	1.8
Indiana	7.2	2.1	59.7	29.7	1.4
Kentucky	5.9	2.6	67.8	22.6	1.2
Lewis	6.6	2.1	54.5	32.1	1.6
Louisiana Seedling	5.7	1.5	66.5	24.8	1.5
Major	5.1	1.4	62.1	29.1	1.8
Mantura	6.0	1.8	62.0	28.6	1.7
Mohan	7.2	2.0	46.7	42.5	1.7
Mohawk	5.4	2.2	69.2	21.7	1.4
Moneymaker	7.2	1.9	60.4	28.8	1.6
Moore	6.1	2.9	62.7	26.7	1.6
Mount	5.9	1.9	64.0	26.8	1.5
Nuggett	6.1	2.0	61.4	28.6	1.9
Oklahoma	5.1	1.9	64.8	26.4	1.9
Patrick	5.5	2.0	68.0	23.0	1.6
Paul Hudson S.S.	10.0	4.6	47.9	34.8	2.5
Robson 5006	7.4	1.8	56.8	32.6	1.5
San Saba Improved	5.9	1.7	58.5	32.1	1.8
Schley	7.4	2.1	56.7	31.9	1.9

S.S. = Seedling Selection

TABLE X (Continued)

Variety	% Palmitic	% Stearic	% Oleic	% Linoleic	% Linolenic
Seminole	6.1	2.0	56.7	32.3	2.0
Shawnee	7.2	2.1	58.2	30.5	2.0
Sioux	7.0	2.1	57.0	32.4	1.6
Squirrel	5.2	2.0	66.7	24.7	1.4
Stuart	6.0	1.8	58.3	22.1	1.8
Stuart S.S.	9.0	2.0	49.6	36.5	2.0
Success	6.9	1.9	53.0	36.2	2.1
Texas-60	6.5	2.2	53.7	35.6	2.1
Texas Prolific	5.9	1.9	57.4	33.3	1.6
Texhan	6.4	1.9	64.0	26.7	1.6
Tulley Seedling	4.9	2.1	61.4	29.8	1.8
Western	7.7	2.0	52.1	36.2	2.1
Wichita	6.6	2.4	61.2	27.9	1.9
4-6-96	4.9	1.6	70.3	21.7	1.6
46-6-96	6.3	1.9	67.3	23.1	1.5
476	9.1	2.3	66.4	21.1	1.2
502	7.7	1.8	61.8	27.2	1.5
503	9.1	2.7	48.5	37.8	1.8
504	6.1	4.3	66.5	21.6	1.5
507	7.5	2.0	58.6	30.1	1.7
572	4.8	3.1	71.0	19.8	1.3
581	5.8	3.1	72.0	18.1	.9
T-130	6.2	3.0	70.5	19.2	1.0

TABLE XI

FATTY ACID ANALYSES OF 83 VARIETIES OF PECANS
1969 CROP

Variety	% Palmitic	% Stearic	% Oleic	% Linoleic	% Linolenic
Aggie	5.6	1.6	64.8	26.7	1.4
Apache (BW)	5.4	2.2	71.8	19.3	1.3
Barton	6.0	1.6	70.0	19.3	1.3
Barton (BW)	6.1	1.9	58.6	31.7	1.7
Burkett	5.8	1.5	58.6	32.6	1.6
Butterick	6.3	1.9	69.3	21.1	1.5
Caddo	7.0	1.6	64.4	25.6	1.3
Caddo (BW)	5.8	2.3	67.7	22.9	1.4
Choctaw (BW)	5.7	1.6	74.0	18.0	1.1
Comanche (BW)	6.0	1.8	64.5	26.1	1.4
Cowley	5.4	2.6	61.2	28.9	1.7
Delmas	6.8	1.3	54.1	36.2	1.5
Desireable	5.8	1.4	68.4	23.3	1.2
Elliot	5.3	2.3	67.0	24.4	1.4
Golden	5.8	1.8	72.6	18.7	1.2
Gormely	5.6	2.3	72.1	18.7	1.3
Green River	5.5	1.9	71.9	19.6	1.4
Hardy Giant	6.0	2.1	71.3	19.5	1.2
Hayes	6.1	1.3	53.4	37.4	1.9
Jack Ballard	6.1	2.0	63.7	26.8	1.5
Kentucky	6.3	2.0	73.3	17.0	1.0
Major	6.5	1.2	68.8	22.3	1.3
Mohawk	5.8	1.7	64.5	26.5	1.8
Moneymaker	6.1	1.5	64.5	26.9	1.0
Mount	5.1	2.1	67.8	23.3	1.8
Nugget	5.7	1.7	73.3	18.3	1.2
Oakla	5.0	1.8	73.3	18.9	1.1
Oklahoma	5.9	1.6	66.6	24.1	1.8
Patrick	5.9	1.5	59.6	31.2	1.8
Peruque	6.4	2.0	69.1	21.4	1.1
San Saba Improved	5.6	1.8	62.6	28.5	1.4
Schley	6.4	1.3	63.5	27.5	1.3
Schley Sparks	5.8	1.4	68.6	22.8	1.4
Shawnee (BW)	6.3	2.1	64.7	25.1	1.7
Sioux	6.1	1.7	65.5	25.3	1.5
Sioux (BW)	6.0	1.7	66.0	25.1	1.3
Squirrel	6.6	1.9	59.0	31.0	1.5
Stuart	6.3	1.6	68.9	21.9	1.3

BW = U.S.D.A. Field Station, Brownwood, Texas

TABLE XI (Continued)

Variety	% Palmitic	% Stearic	% Oleic	% Linoleic	% Linolenic
Stuart Seedling	5.7	1.7	64.4	26.7	1.4
Success	6.0	1.8	64.8	26.0	1.4
Texas Prolific	6.2	1.8	63.7	26.9	1.6
Texhan	7.3	1.9	61.9	27.4	1.7
Western	6.8	1.9	54.3	35.4	1.7
Wichita (BW)	6.7	1.9	71.5	18.4	1.3
461	6.3	2.3	66.6	23.4	1.5
464	6.0	1.7	71.1	19.9	1.4
465	5.3	1.8	65.6	25.8	1.5
466	5.6	1.9	67.6	23.4	1.6
467	5.5	1.5	63.7	27.7	1.7
472	5.8	1.6	69.9	21.4	1.2
476	5.1	2.1	71.7	19.8	1.3
481	5.2	1.9	70.7	20.9	1.3
491	5.8	2.1	69.5	21.3	1.4
493	6.0	2.0	70.3	20.5	1.4
495	5.4	1.9	69.5	22.1	1.2
497	5.6	1.8	73.5	17.7	1.5
502	7.0	1.6	57.1	32.2	2.2
503	5.9	1.6	64.7	26.2	1.5
504	5.7	1.9	71.6	19.5	1.4
507	6.8	1.6	62.6	27.1	1.9
511	6.3	2.1	71.3	19.2	1.5
513	5.4	1.6	74.1	17.8	1.2
518	6.1	2.1	59.6	30.8	1.5
519	6.6	2.5	72.2	17.3	1.4
542	5.9	2.0	65.0	25.5	1.7
551	5.9	2.2	74.0	16.9	1.4
552	6.2	1.4	66.0	25.1	1.4
554-Choctaw	6.4	2.3	65.9	24.3	1.3
571	6.9	2.2	67.0	23.8	1.1
572	5.3	2.5	73.8	17.3	1.1
581	5.6	1.9	73.3	17.8	1.4
582	5.9	1.9	70.9	20.1	1.3
583	6.0	1.9	68.1	22.8	1.4
591	6.6	1.4	66.6	24.1	1.4
593	5.4	2.3	70.5	19.8	1.5
600	5.6	2.2	68.8	21.8	1.6
2412	5.9	1.9	70.6	20.6	1.1
4610	6.0	1.7	70.6	20.7	1.4
5110	5.7	2.5	69.7	20.8	1.3
61H	5.6	2.4	71.2	19.4	1.5
68L1	4.9	1.9	78.1	14.0	1.1
68L3	5.1	2.6	74.9	16.4	1.1
K-69	6.7	1.4	66.4	24.3	1.4

APPENDIX B

COMPUTER PROGRAMS

TABLE XII

COMPUTER PROGRAM I

```
C                    STUDIES ON THE 1969 PECAN CROP
      DIMENSION NO(80),AKT(80),OLE(80),ALINO(80),TOC(80),PROT(80),TITLE(
     *80,7),X(80),THKT(80),CORKT(80),DIFF(80)
    1 FORMAT (I2,1X,6A4,A3,F5.2,5X,F5.2,5X,F5.2,5X,F5.0,5X,F5.2)
    2 FORMAT (I2)
    3 FORMAT(5X,6HSLOPE=,F8.4)
    4 FORMAT(5X,'Y INTERCEPT=',F7.2,4HDAYS)
    5 FORMAT(5X,'NO.   VARIETY                KEEPINGTIME %OLEIC  %LINOLEI
     *C TOCOPHEROL  PROTEIN')
    7 FORMAT(5X,'STANDARD DEVIATION=',F12.6)
    8 FORMAT (5X,42H    THEORETICAL     ACTUAL      CALCULATED)
    9 FORMAT (5X,55HNO KEEPING TIME  KEEPING TIME  KEEPING TIME  DIFFERE
     *NCE)
   10 FORMAT (5X,I2,3X,2(F7.2,7X),F7.2,5X,F7.2)
  111 FORMAT (5X,I2,1X,6A4,A3,F5.2,5X,F5.2,5X,F5.2,5X,F5.0,5X,F5.2)
      READ (5,2) N
      DO 11 I=1, N
   11 READ(5,1) NO(I),(TITLE(I,J),J=1,7),AKT(I),OLE(I),ALINO(I),TOC(I),P
     *ROT(I)
      AN=N
      J=-2
      DO 59 I=1,N
   59 X(I)=OLE(I)
      GO TO 70
   60 DO 660 I=1,N
  660 X(I)=ALINO(I)
      GO TO 70
   61 DO 661 I=1,N
  661 X(I)=TOC(I)
   70 CONTINUE
      SX=0.0
      SY=0.0
      SX2=0.0
      SXY=0.0
      DO 50  I=1, N
      SX=SX + X(I)
      SY=SY + AKT(I)
      SX2=SX2 + X(I)*X(I)
   50 SXY=SXY + X(I)*AKT(I)
      SL=(AN*SXY-SY*SX)/(AN*SX2-SX*SX)
      YINT=(SY*SX2-SX*SXY)/(AN*SX2-SX*SX)
      WRITE (6,3) SL
      WRITE (6,4) YINT
      DO 51 I=1, N
   51 THKT(I)=31.41-0.396*ALINO(I)-0.075*PROT(I)-0.063*OLE(I)
      SUM=0.0
      DO 100 I=1,N
      CORKT(I)=SL*X(I)+YINT
  100 SUM=SUM+(AKT(I)-CORKT(I))**2
      RMSLN=SQRT (SUM/AN)
      WRITE (6,7) RMSLN
      DO 110 I=1, N
  110 DIFF(I)=AKT(I)-CORKT(I)
      WRITE (6,8)
      WRITE (6,9)
      DO 120 I=1, N
  120 WRITE (6,10) NO(I),THKT(I),AKT(I),CORKT(I),DIFF(I)
      J=J+1
      IF (J) 60,62,62
   62 CONTINUE
      WRITE (6,5)
      WRITE(6,111) (NO(I),(TITLE(I,J),J=1,7),AKT(I),OLE(I),ALINO(I),TOC(
     *I),PROT(I),I=1,N)
      STOP
      END
```

TABLE XIII

COMPUTER PROGRAM II

```
      DIMENSION NO(200),TOC(200),ATOC(200),ISWT(200),ARDMT(200),WS(200),
     *TITLE(200,7),DIFF(200)
    1 FORMAT (I2,F8.3,F10.2)
    2 FORMAT (I2,1X,6A4,A3,4X,F6.2,F10.2,F10.4,F10.2)
    3 FORMAT (5X,47H                                          TOCOPHEROL)
    4 FORMAT (5X,49HSAMPLE                                   CONCENTRATION)
    5 FORMAT (5X,51H  NO     VARIETY                       MICROGRAMS/GM.OIL)
    6 FORMAT (7X,I2,4X,6A4,A3,F9.0)
    8 FORMAT(5X,6HSLOPE=,F10.6)
    9 FORMAT(5X,'Y INTERCEPT=',F10.5,5HUNITS)
      READ (5,1)N,AKT,WTIS
      READ (5,2) (NO(I),(TITLE(I,J),J=1,7),ATOC(I),ARDMT(I),WS(I),DIFF(I
     *),I=1,N)
      DO 100 I=1,N
  100 TOC(I)=(AKT*ATOC(I)*WTIS)/(ARDMT(I)*WS(I))
      AN=N
      SX=0.0
      SY=0.0
      SX2=0.0
      SXY=0.0
      DO 50  I=1, N
      SX=SX + TOC(I)
      SY=SY + DIFF(I)
      SX2=SX2 + TOC(I)*TOC(I)
   50 SXY=SXY + TOC(I)*DIFF(I)
      SL=(AN*SXY-SY*SX)/(AN*SX2-SX*SX)
      YINT=(SY*SX2-SX*SXY)/(AN*SX2-SX*SX)
      WRITE (6,3)
      WRITE (6,4)
      WRITE (6,5)
      WRITE (6,6)(NO(I),(TITLE(I,J),J=1,7),TOC(I),I=1,N)
      WRITE (6,8) SL
      WRITE (6,9) YINT
      STOP
      END
```

APPENDIX C

MASS SPECTRA OF NITROGEN-CONTAINING COMPOUNDS REFERENCE STANDARDS

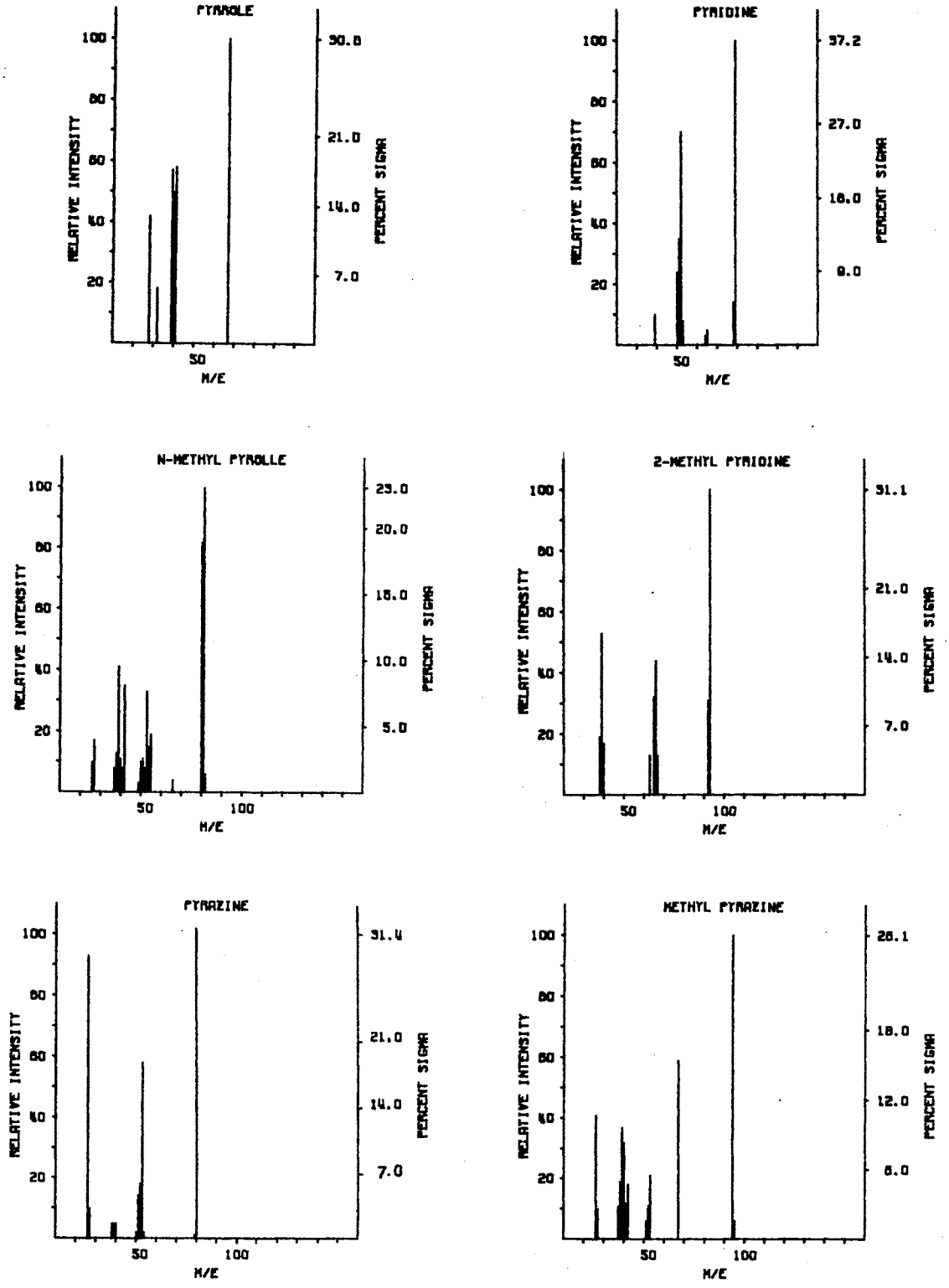

Figure 66. Mass Spectra of Nitrogenous Compounds, Reference Standards

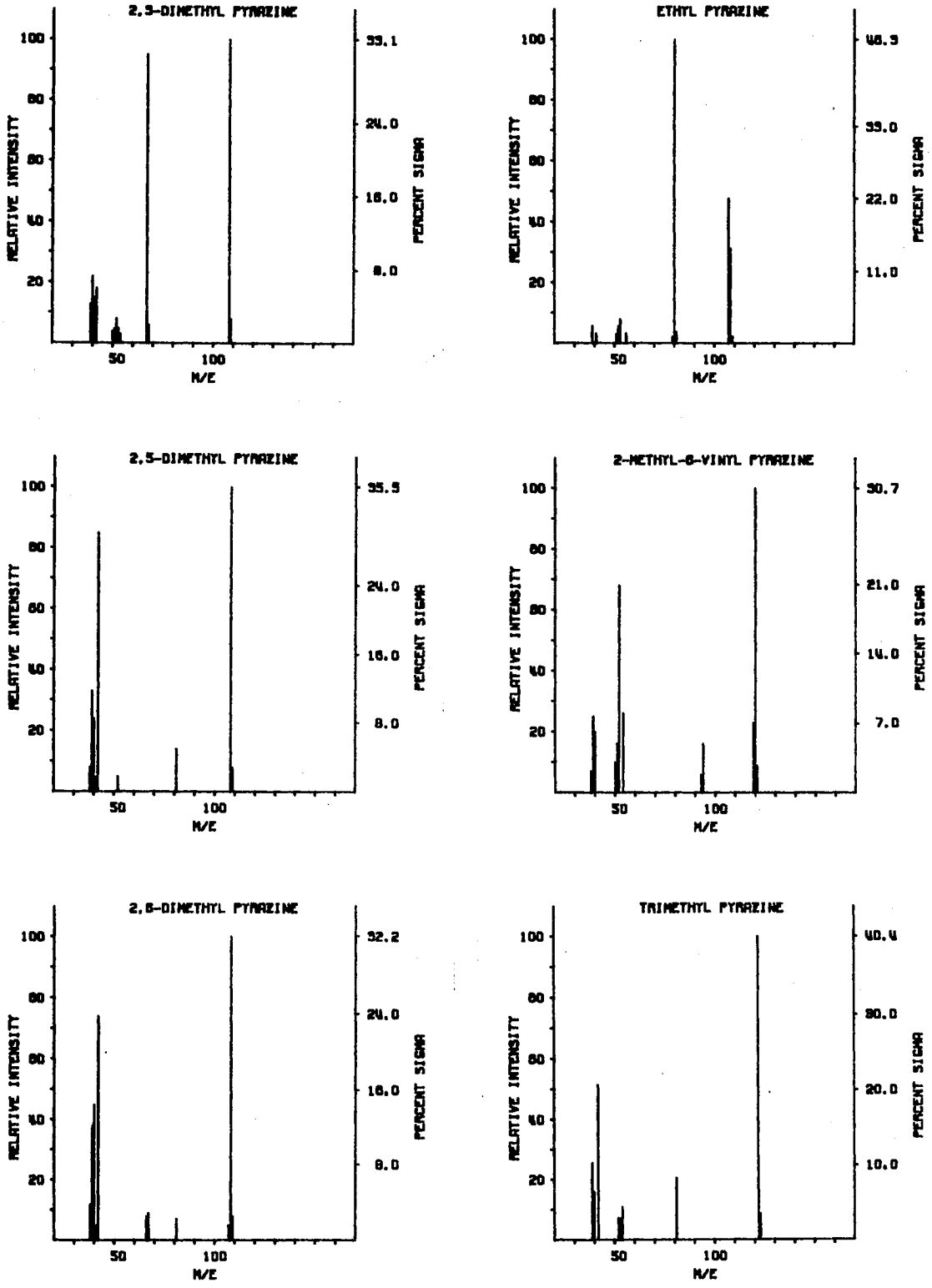

Figure 66 (Continued)

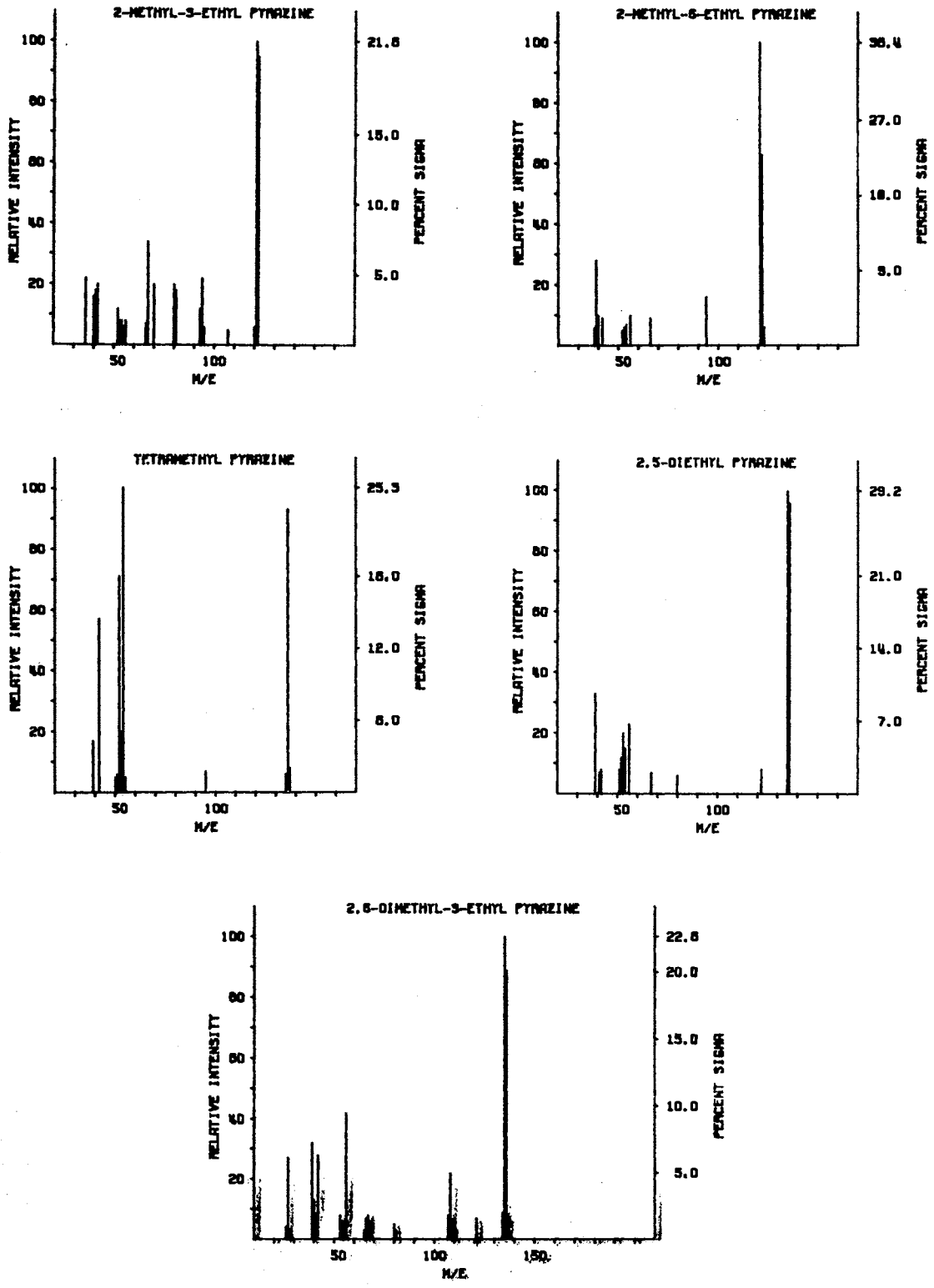

Figure 66 (Continued)

APPENDIX D

MASS SPECTRA OF OXYGEN-CONTAINING COMPOUNDS

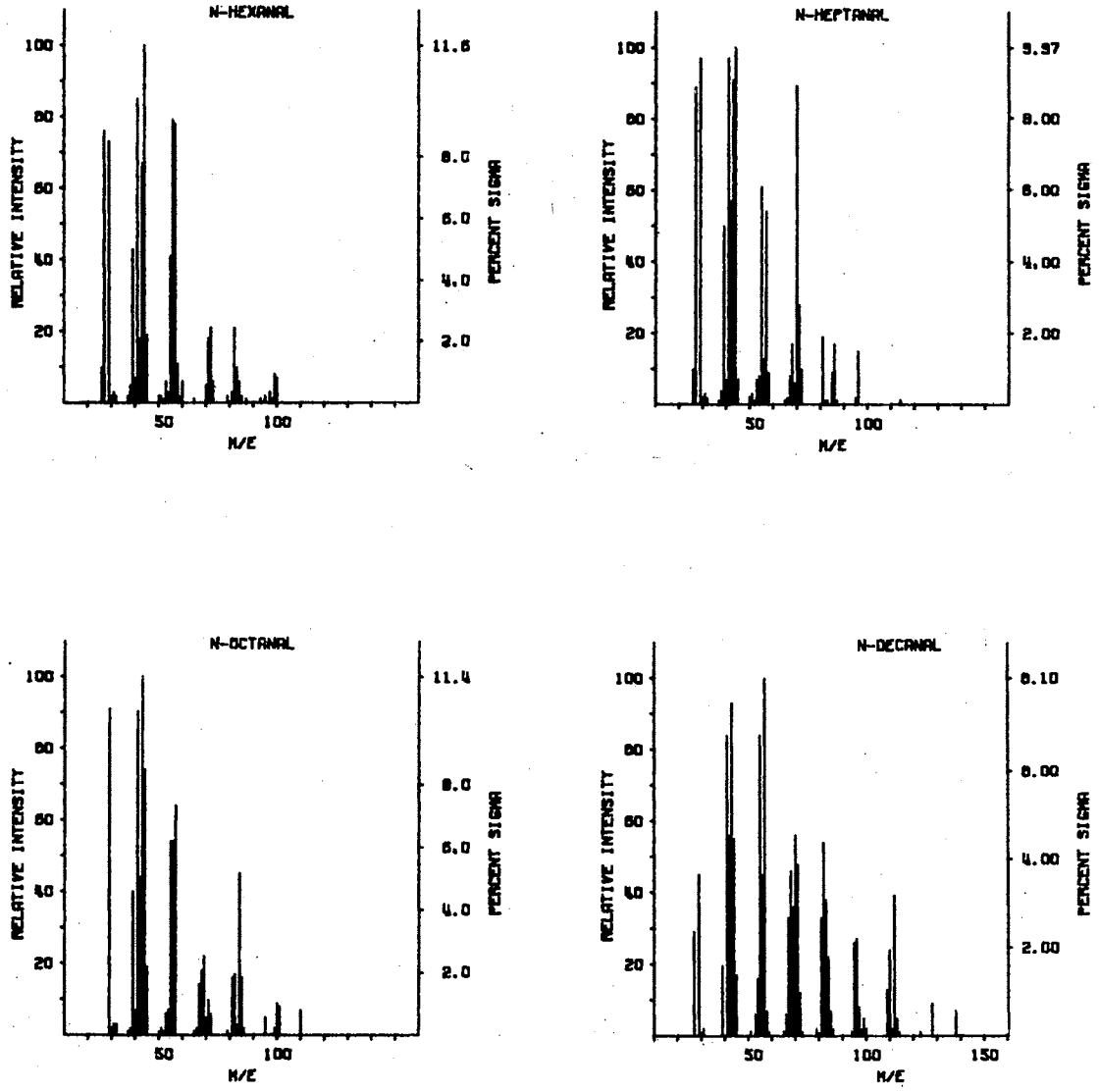

Figure 67. Mass Spectra of Oxygen-Containing Reference Standards (22)

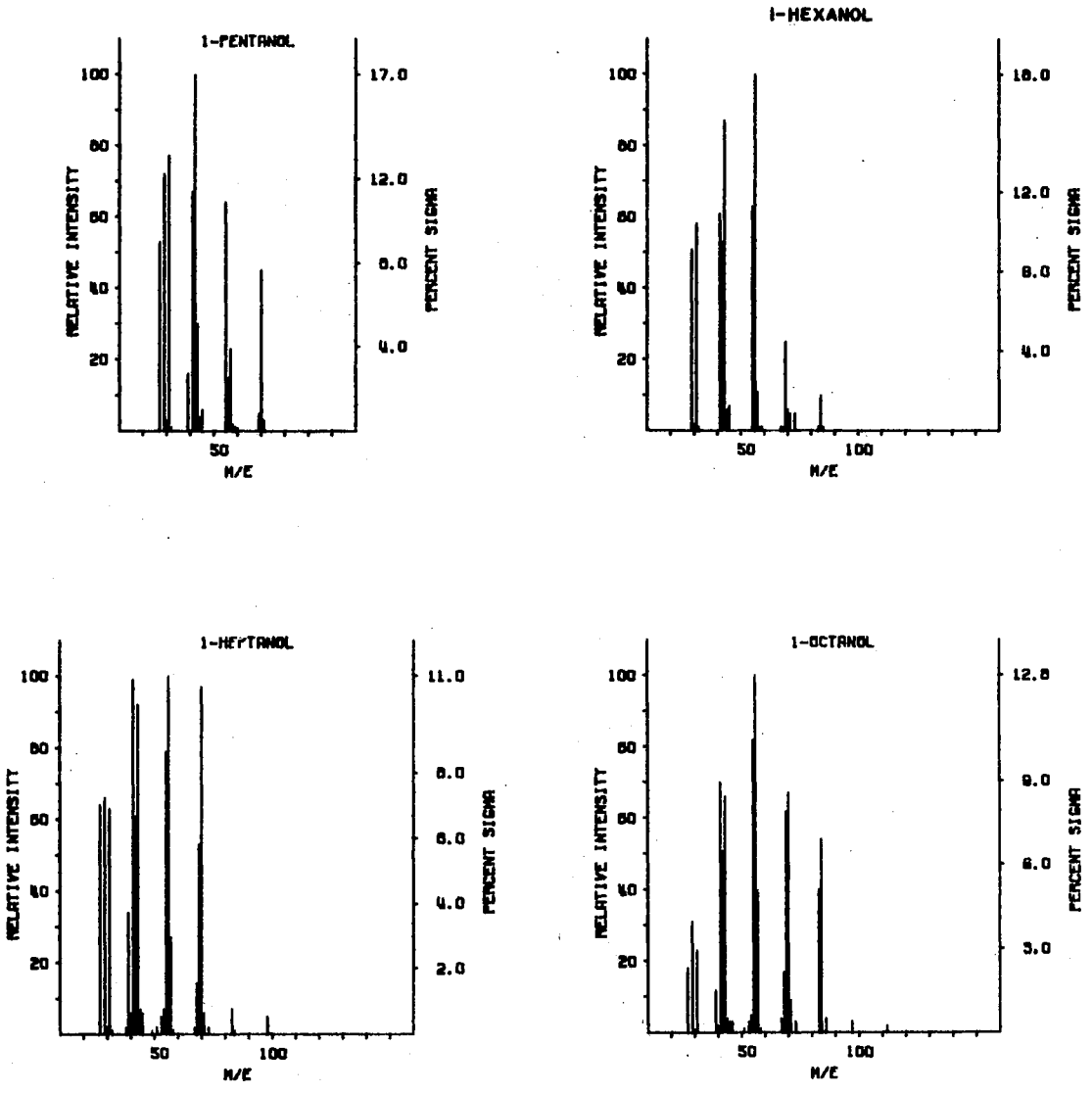

Figure 67 (Continued)

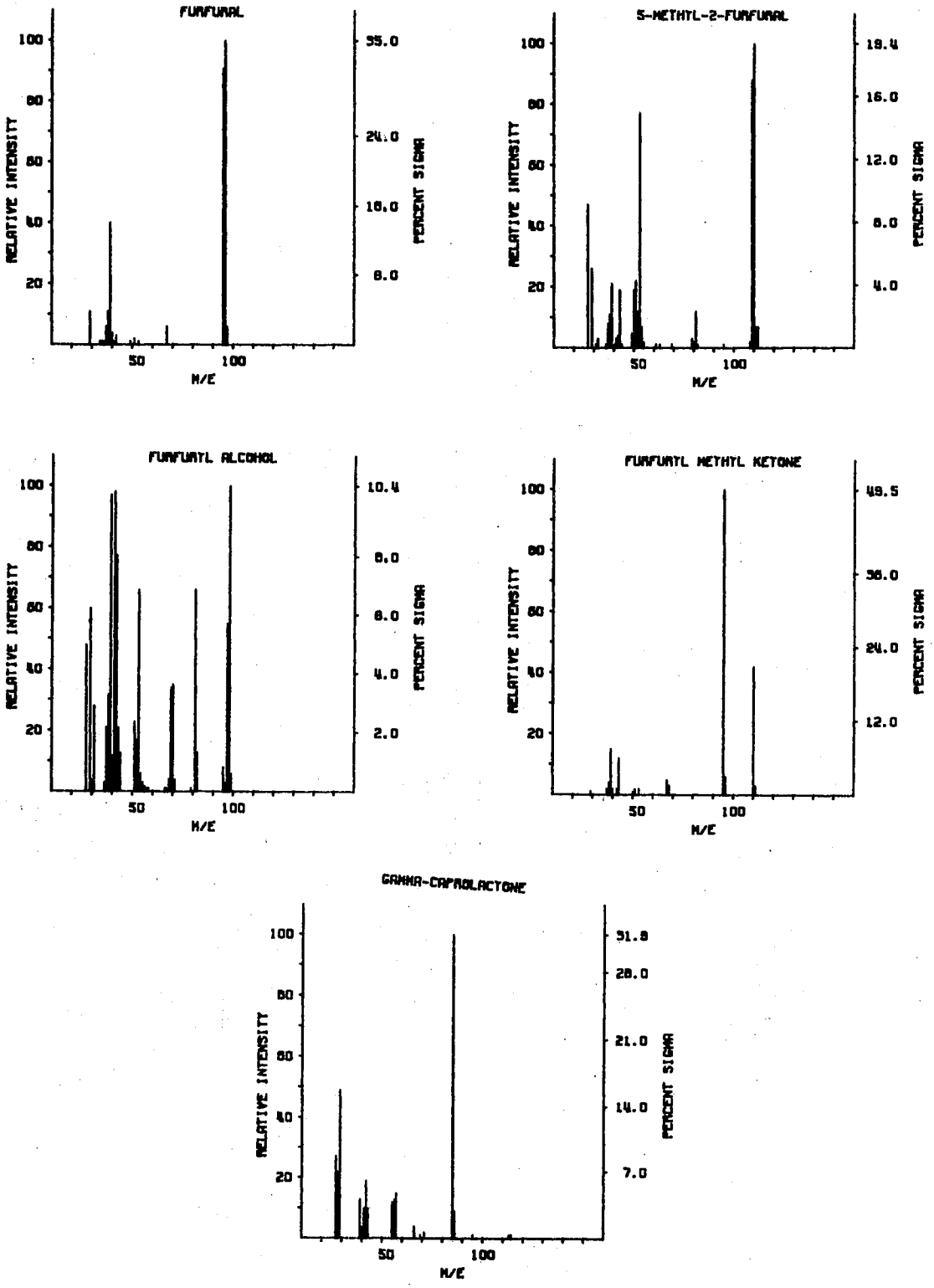

Figure 67 (Continued)

VITA

Charles John Rudolph, Jr.

Candidate for the Degree of

Doctor of Philosophy

Thesis: FACTORS RESPONSIBLE FOR FLAVOR AND OFF-FLAVOR DEVELOPMENT IN PECANS

Major Field: Biochemistry

Biographical:

Personal Data: Born in Tampa, Florida, August 22, 1945, the son of Charles J. and Marjorie Rudolph. Married Martha Diana Cox of Sherman, Texas, in Dallas, Texas, August 6, 1966.

Education: Attended the public schools of Philadelphia, Pennsylvania, and Dallas, Texas; graduated from Bryan Adams High School, Dallas, Texas, in May, 1963; received the Bachelor of Arts degree with a major in chemistry from Austin College, Sherman, Texas, in August, 1966; completed requirements for the Doctor of Philosophy degree in May, 1971.

Professional Experience: Summer High School Research Trainee, Wadley Institute of Molecular Medicine, Dallas, Texas, summer, 1963; Undergraduate Research Assistant, Department of Chemistry, Austin College, May, 1965 to May, 1966; Campbell Soup Fellow, August 1966, to August, 1970.

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