COMPOSITION AND STABILITY OF PECAN OILS

By

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COMPOSITION AND STABILITY OF PECAN OILS

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CHAPTER I

INTRODUCTION

Oils and fats are among the major dietary sources of energy and act as a source of many lipid-soluble vitamins such as vitamins A, D, E, and K. More important economically is the role of oils in industry. Detergents, glycerol, paints and many other products produced from fats and oils are too numerous to mention.

For a long time, people realized that oils and fats developed an off-flavor and become rancid when they were kept in contact with air and exposed to light, especially at higher temperatures.

The problem of instability of pecan oils was studied by the author for three reasons: (1. Pecan's flavors are notoriously unstable and change readily upon storage even at reduced temperatures; (2. Oklahoma is one of the important pecan producing states since pecans constitute about 30 percent of the horticultural output of the state; (3. Little, if any, work has been done to determine the reasons for the poor keeping quality of pecan oils.

In order to determine the cause of this flavor instability one must first know the chemical composition of the triglycerides and nonsaponifiable portion of the oil, since both can play a role in stability of oils (1,2). A correlation between composition and stability could reveal possible reasons for different rates of rancidity development among pecan fruit, and perhaps suggest a means by which an

unstable oil could be stabilized. The compositional information which correlated with instability could also be used in selecting parent material for a breeding program to produce highly stable fruit.

This work reports the analysis of the fatty acids and non-saponifiable portions of pecan oils. Correlations of stability of the oils with their degree of unsaturation, pigments, and antioxidants, especially tocopherols, were also investigated.

CHAPTER II

REVIEW OF LITERATURE

Very limited studies have been reported on the chemical composition of pecan oils; none of these reports establish the relation between the degree of unsaturation in the oils and their stability.

Deiler and Fraps (4) reported 70.4 percent oil in pecan kernels lecithin, 0.5 percent, and cholesterol, 0.28 percent. George and Gertler (5) mentioned the occurrence of only oleic and linoleic acids in pecan oil. Friedman (6), Whitehead (7), and Boone (8) reported some physical constants of pecan oil with their studies on carbohydrate and protein contents of the nuts.

Some of the work on the fatty acid contents prior to the advent of gas chromatography was not reliable. A recent report by French (9) has recorded the composition of several pecan oils using gas chromatography. He used a five foot column packed with 10 percent Lac-446, (a glycol adipate polymer), on 30-60 mesh firebrick. He also used ultraviolet spectrometry to identify the unsaturated acids.

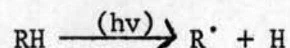
Josephin et al. (10) found that nuts heated to 80°C., then cooled were more rancid than the untreated pecans, as measured by palatability score, Kreis values and peroxide values.

The effect of addition of some antioxidants in retardation of rancidity in pecan kernels was studied by Godkin et al. (11). They found that coating the nuts with a solution of antioxidant like

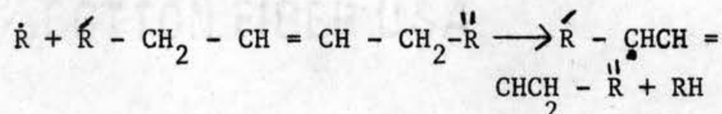
tocopherols or ascorbic acid delayed the development of rancidity. Woodroof and Heaton (12) reported the percentage of the two main unsaturated fatty acids, oleic and linoleic, in 46 species of pecans, using older methods. They also reported relative stability of pecan kernels and unshelled nuts. The data showed no direct relationship between stability of pecan kernels to oxidative rancidity and the degree of unsaturation of the oils.

Regarding the studies on stability of oils and fats, much work has been done (3, 10, 11, 12, 13, 14, 15, 16). Different proposals have been suggested to explain reactions taking place in the process of oxidative rancidity. An acceptable mechanism for the reversion (the initial step in the process of developing off-flavors in oils and fats) of an oil is the following free radical steps proposed by Kummerow (3):

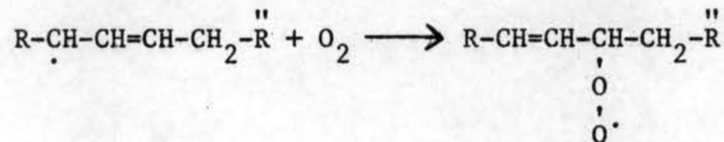
1. Free radical formation by light,



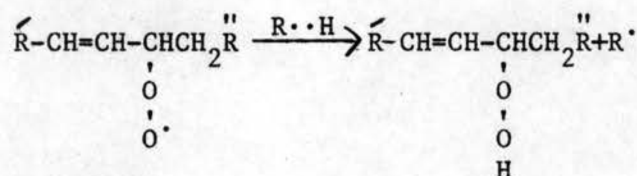
2. Abstraction of an H atom from an unsaturated fatty acid molecule,



3. Formation of peroxide radical,



4. Hydroperoxide production and the generation of a new free radical,

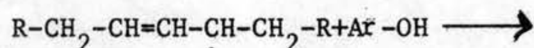


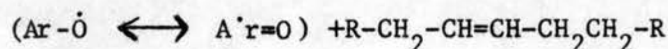
The hydroperoxides thus formed may cleave in one or two ways yielding alkanals, alkenals, alk-dienals, dicarboxyls, alcohols, and hydrocarbons depending on the fatty acids involved (13, 14, 15). Thermal decomposition of the hydroperoxides formed from unsaturated fatty acids yields about 90 percent by weight of dimeric polymers (16). These dimers can "revert" a freshly deoderized oil to one that has off-flavor and odors. Other mechanisms have been proposed to account for the formation of monocarbonyls produced from fatty acid hydroperoxides (15, 17).

The importance of fatty acids containing double bonds in this mechanism is implicitly suggested in the preceeding discussion and have also been discussed by Gunstone and Hilditch (18). Therefore, the knowledge of the unsaturated fatty acid content of oils is essential to understanding their relative stabilities. However, the difference in degree of unsaturation in fats and oils might not always be sufficient to explain their relative instabilities (1, 2). Other factors may be of considerable importance, such as the presence of antioxidants in the nonsaponifiable portion of oils (2, 19). An antioxidant such as vitamin E or phenolic compounds are easily oxidized, and hence, they prevent the polymerization and breakdown of the oil.

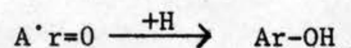
A mechanism of antioxidant action is proposed by Stuckey (20) as follows:

1. Re-formation of a fatty acid molecule and the conversion of the antioxidant molecule (Ar-OH) to a free radical,





2. Reduction of the oxidized antioxidant ($A^{\cdot}r{=}O$)



Tocopherols, vitamin E compounds, which are usually associated with oils and fats (21), are thought to be primarily responsible for the stability of vegetable oils (22). They are known to produce P-quinones on mild oxidation from which, by proper reduction, the corresponding P-hydroquinones are obtained (23); the latter can be recycled in acid solution to the original compound. Thus, the tocopherol, quinone, hydroquinone mixture represents a potential biological oxidation-reduction system.

Although the antioxidant function of tocopherols in stabilizing tissue lipids in vivo has been investigated (24, 25), the effects of naturally occuring tocopherols on the stabilities of edible fats and oils has not been extensively studied.

Many different methods are available for measuring rancidity (26-37). However, the history of these objective tests begins over 50 years ago with development of the Kreis test (27) which was based on the estimation of short chain aldehydes and ketones in rancid oils. This test produces a pink color with phloroglucinol reagent (38). The oxygen absorption method (31) which accelerates the time for a fat to become rancid by flushing air through the sample followed by peroxide determination, is considered acceptable for comparing the stability of one fat with another. This test may also be used to compare the relative effectiveness of antioxidants to improve oil stability.

The baking industry developed the "oven test" for rancidity, which became commonly known as the "Schaal oven test" (28), and depends on organoleptic evaluation of rancidity. The peroxide method developed

by Lea (29) and modified by Wheeler (30), which measures mainly peroxides titrimetrically, was followed by the "Swift test" or the active oxygen method (31) already mentioned.

Many early workers recognized the presence of aldehydes and/or ketones in autoxidized fats, and they attempted to develop objective tests based on their concentrations. It was only after the development of quantitative colorimetric tests for carbonyl compounds in low concentrations, that substantial agreement between carbonyl content and organoleptic evaluation of rancidity, was accomplished.

Sidwel et al. (26) studied the use of 2-thiobarbituric acid (TBA) as an agent for the measurement of fat oxidation in animal and vegetable fats. This test is based on the reaction of TBA with the oxidation products of unsaturated fatty acids to produce a red pigment. The spectrophotometric determination of this red pigment has been used to follow development of rancidity in a wide variety of food products (26, 39, 41-54). Several references (43, 56, 47, 52) review the earlier literature dealing with this reaction.

Traladgis et al. (55) studied the chemistry and side reactions of TBA test which measures rancidity more completely than other tests. The TBA reagent reacts with the three carbon fragments, malonaldehyde, derived from the oxidation of mono and polyenoic fatty acids, as well as with noncarbonyl compounds, known as TB-reactive substances (TBRS) which are responsible for off-flavor development in inverted oils and fats (35, 36). Furthermore, it was found that sensory evaluations by a taste panel showed a direct relationship between TBA values and off-flavor in stored butterfat (36).

The latter method and the peroxide method, which is an official method approved by the American Oil Chemist's Society, were used to

CHAPTER III

ANALYSIS OF PECAN OILS

Apparatus

A mechanical nut chopper was used to chop the pecan kernels, and a Soxhlet apparatus was used for oil extraction. Gas-liquid chromatograms were obtained with a Perkin-Elmer Model 800 gas chromatograph equipped with a dual hydrogen flame detector.

Mass spectral analyses were obtained with a combination gas chromatogram-mass spectrometer (GC-MS). The latter was a prototype of the LKB 9000 mass spectrometer.

Reagents

Optically pure hexane was prepared by distilling high purity n-hexane over KOH pellets onto a silica gel column. Benzene, reagent grade, was dried over sodium. 2,2- Dimethoxy propane was obtained from the Dow Chemical Company. Dry methanol was redistilled through a column of molecular seive 5A to remove traces of water. Methanolic HCl was prepared according to the procedure of Mason and Waller. (60)

Neutralizing agent: Sodium bicarbonate, sodium carbonate and sodium sulphate (anhydrous), were mixed in a 2:1:2 ratio by weight, dried overnight at 110°C., and stored in an air tight container. Sodium methoxide, approximately 0.5N, was made by reacting sodium metal with dry methanol.

Standard methyl esters (Applied Science Laboratories), chromatographically pure, were used without further purification. Isopropylidene glycerol (IPG), for determining retention time of IPG was synthesized by Mason and Waller (60). The oils were obtained from samples of thirty-four popular varieties of pecans grown near Stillwater on the Oklahoma State University Experiment Station.

Procedures: Extraction of Oils

About two grams each of thirty-four varieties of chopped nuts were weighed, dried in a vacuum overnight at 55°C. and twenty millimeters of mercury then the oils were extracted with pure hexane in a Soxhlet extractor. The hexane was evaporated and the oil cooled in a disiccator to room temperature before weighing. The dry meal was ground in a mortar, weighed and re-extracted overnight. After weighing, the oils from the second and first extraction, were combined and transferred to small vials which were kept in a desiccator under nitrogen at -20°C. The following equation was used to obtain the percentage of oil:

$$\% \text{ Oil} = \frac{x \left[(Y)(Z-A) \right] + A}{(Y)\ (Z)} 100$$

where, x = weight of oil from second extraction as decimal fraction, Y = wet weight of sample, Z = percent dry matter as decimal fraction, and A = weight of oil from first extraction.

Transesterification

Approximately 0.2 gram samples of pecan oils were accurately weighed and converted to methyl esters following the procedure of Mason *et al*. (61). Standard methyl esters and IPG were prepared by diluting known quantities of nine methyl esters and IPG to 25 ml. with

benzene in a volumetric flask.

Gas Chromatographic Analysis

A six foot x 1/8 inch o.d (0.062 inch i.d) stainless steel column packed with 14.5 percent ethylene glycol succinate (EGS) on Anakrom 100/110 mesh type A was used. Nitrogen was the carrier gas used at a flow rate of 60 ml. per minute. Column temperatures were linear programmed from 75°C. to 195°C. at 10° per minute, while the injector and detector temperatures were 220° and 230°C respectively.

Identification of peaks was achieved by comparing retention times of unknowns with reference standards. Quantitative estimations were accomplished by multiplying peak areas by appropriate micromole/area relationships calculated from gas chromatograms of reference standards.

Mass Spectrometry

One-half microliter quantities of the standard methyl esters and unknowns were injected into the combination GC-MS instrument to obtain separation of methyl esters and the mass spectra of each. A glass column 0.25 inch o.d x 6 foot long packed with 14.5 percent EGS on Anakrom 100/110 mesh was used. Helium was the carrier gas flowing at a rate of 35 ml. per minute and the column was maintained at 155°C.

Determination of the homogeneity or heterogeneity of the peaks was possible using the GC-MS instrument equipped with a rapid scanning device. A mass spectrum could be obtained every 1.6 seconds if needed which allowed spectra to be taken at successive points along each peak.

Results and Discussion

Table I lists oil and fatty acid contents of the thirty-four

varieties of pecans analyzed. Total oil contents ranged from 66.9 percent to 75.2 percent. It is interesting to note that the combined percentages of oleic and linoleic acids were remarkably constant, about 89 percent, in all samples. This phenomenon was also reported by Bailey et al. (62).

Comparison of retention times of the gas chromatographic peaks from unknowns with the reference standards revealed the presence of IPG, palmitate, stearate, oleate, linoleate, and linolenate. The latter ester might also be eicosenoic methyl ester since both linolenate and eicosenoate have the same retention time (63). The presence of any acid with eighteen carbon atoms and three double bonds, regardless of position would probably have nearly the same retention time.

Percentages of saturated fatty acids were relatively low, totaling from 8.1 percent to 12.9 percent. Oleic acid was the major fatty acid component while that which thought to be linolenic acid was the minor component.

Figures 1, 3, 5, 7, and 9 are mass spectra of standard reference compounds while Figures 2, 4, 6, 8, and 10 show spectra for respective unknown compounds. Unknown spectra were generally in agreement with reference spectra obtained in these laboratories as well as those appearing in the literature (64, 65, 66), with the following exceptions: In the spectrum of standard IPG, the 82 fragment was absent but appeared in the unknown spectrum at 10 percent relative intensity, while literature values show less than 1 percent relative intensity (64). Fragment ion 87 in methyl palmitate spectrum had a relative intensity of 45 percent while the corresponding standard had only 10 percent relative intensity.

TABLE I

PERCENT OILS (ON WET BASIS) AND FATTY ACID COMPOSITION (PERCENT OF OIL) OF THIRTY-FOUR VARIETIES OF PECANS

Variety	Percent Oil	Percent Palmitic	Percent Stearic	Percent Oleic	Percent Linoleic	Percent Linolenic
Barton	72.6	11.3	2.7	48.7	36.2	1.1
Burkett	67.6	7.2	0.9	54.3	37.6	T*
Busseron	73.6	8.3	1.6	63.9	24.7	1.5
Butterick	68.8	8.3	1.5	65.7	23.4	1.2
Commonwealth	70.6	6.2	2.8	63.9	24.7	2.4
Delmas	68.2	8.1	1.6	56.7	33.5	T
Desirable	71.0	8.1	1.7	61.6	28.6	T
Dooly	66.9	7.5	1.4	52.5	37.0	1.7
Green River	74.1	7.7	1.4	63.6	27.1	0.2
Graking	68.3	8.6	1.9	55.7	31.1	2.7
Halbert	71.0	8.9	1.7	50.5	39.0	T
Hays	73.0	10.0	1.3	56.9	31.8	T
Hirschi	70.9	7.0	2.1	61.7	28.7	0.5
Indiana	73.1	9.0	1.7	56.3	31.3	1.6
Kentucky	74.4	7.8	2.3	68.5	19.1	2.3
Mantura	73.3	8.3	3.0	56.9	31.8	T
Major	74.4	7.5	1.8	64.6	24.0	2.2
Mahan	67.9	10.0	2.9	53.8	32.0	1.4
Moneymaker	69.4	7.8	1.8	58.0	32.4	T
Niblack	74.9	9.1	1.8	68.1	19.0	2.0
Nugget	69.5	7.5	1.6	60.0	30.7	0.2
Oklahoma	67.7	4.9	5.8	56.1	33.3	T
Partick	68.3	10.7	1.6	50.3	37.4	T
Perque	73.1	10.2	1.7	53.8	34.3	T
Posey	73.7	8.5	1.7	59.3	28.4	2.1
San Saba Improved	71.5	8.7	2.0	56.3	33.1	T
Squirrel	74.8	9.1	3.1	56.6	31.1	0.1
Stark Hardy Giant	75.2	7.8	2.0	67.4	21.5	1.4
Success	65.5	7.7	1.5	55.9	34.9	T
Stuart	73.4	8.5	1.6	55.3	33.6	1.0
Schley	73.0	8.5	1.6	59.7	29.6	0.6
Texas 60	73.0	7.8	2.3	58.3	31.3	0.3
Texas Prolific	72.5	8.4	1.1	51.0	39.6	T
Western	74.8	10.6	1.3	48.9	39.2	T

*T = Trace

The two carbon fragment m/e 29 (65) was not in the unknown spectrum corresponding to methyl palmitate although it was present in the standard spectrum. A 101 fragment did not show up in the spectrum of standard methyl stearate, whereas it appeared at 7 percent relative intensity in the spectrum of the unknown corresponding to methyl stearate. This fragment has been reported previously in the mass spectrum of methyl stearate (65).

The methyl oleate spectrum from both the reference standard and the corresponding unknown showed m/e 55 as the base peak, while that reported in the literature was m/e 264. Fragment 228, which is reported in the spectrum of methyl oleate (65) at less than 1 percent relative intensity, appeared at relative intensity of 5.5% in the reference spectrum obtained in these laboratories. This fragment was absent in the unknown spectrum corresponding to authentic methyl oleate.

In the standard methyl linoleate spectrum, fragment 32, was present at 7.7 percent relative intensity, whereas it was absent in the corresponding unknown spectrum. This fragment was also absent in spectra reported in the literature (65).

Based on mass spectral data and gas chromatographic analysis, the conclusion was drawn that the compounds obtained from GLC separation were: IPG, methyl palmitate, methyl stearate, methyl oleate, and methyl linoleate. That which was thought to be methyl linolenate was present in such low concentration that backgroud from column bleed and neighboring methyl linoleate obliterated its spectrum. Thus, the identity of the compound, thought to be methyl linolenate, remains inconclusive.

The fact that some other fatty acid esters were not eluted unre-solved from the major peaks was indicated by obtaining successive

spectra as each peak was eluted. The data obtained in this manner indicated that all the gas chromatographic peaks, with the exception of the last peak, were homogeneous. The mass spectrum of the last peak (methyl linolenate) was not readable because the gas chromatographic peak was small.

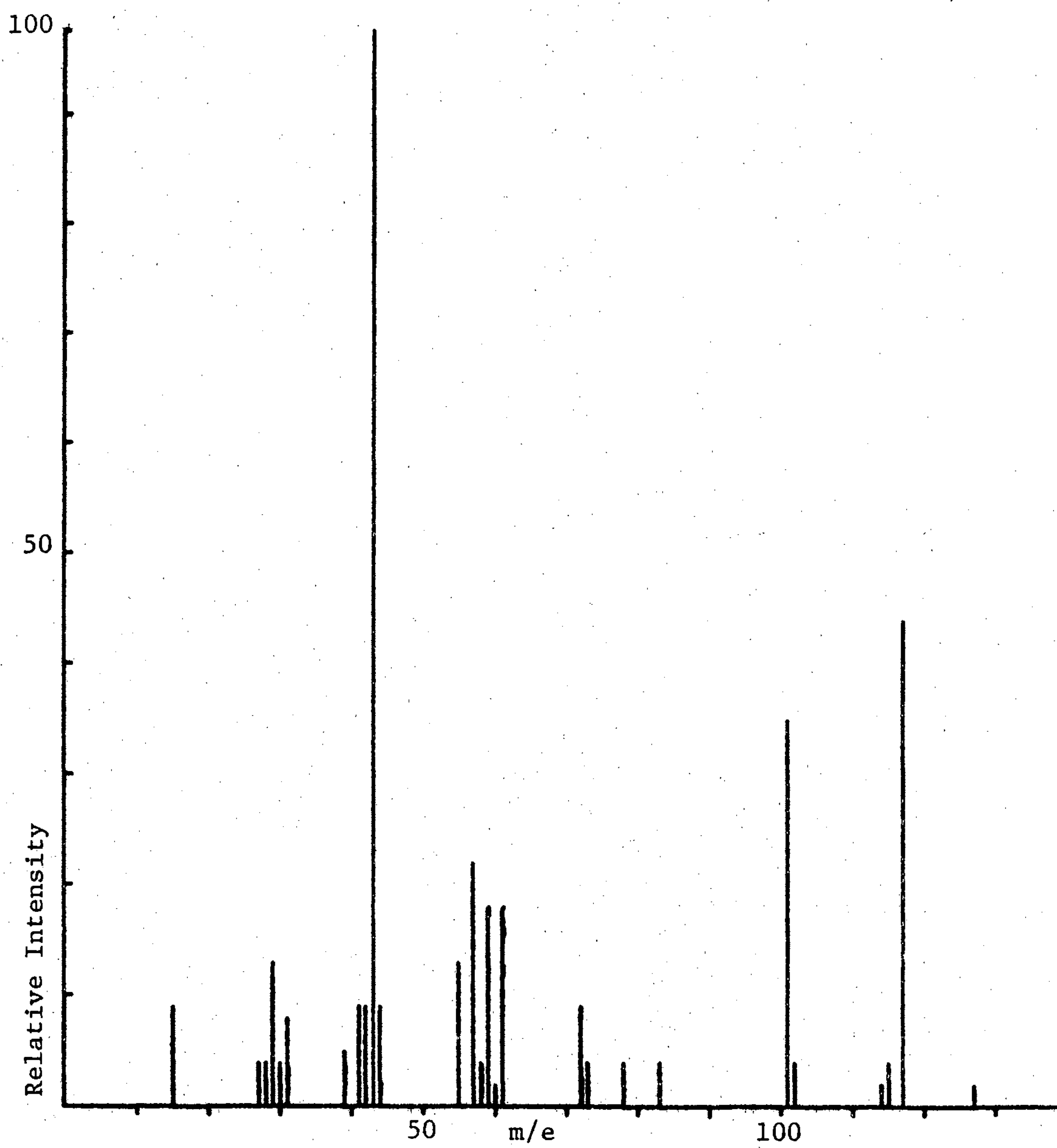


Figure 1. Mass Spectrum of Reference IPG.

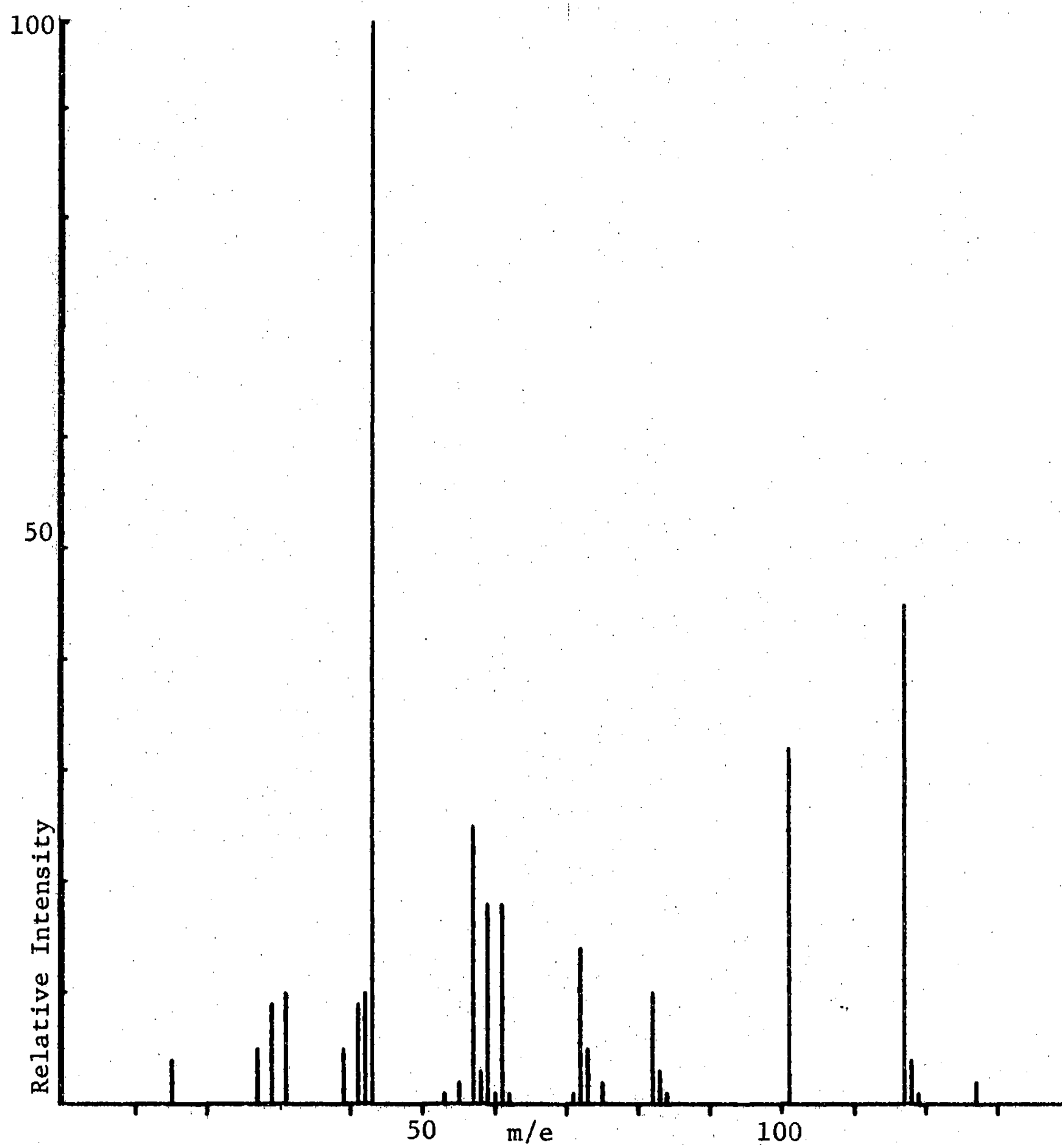


Figure 2. Mass Spectrum of IPG from Pecan Oil.

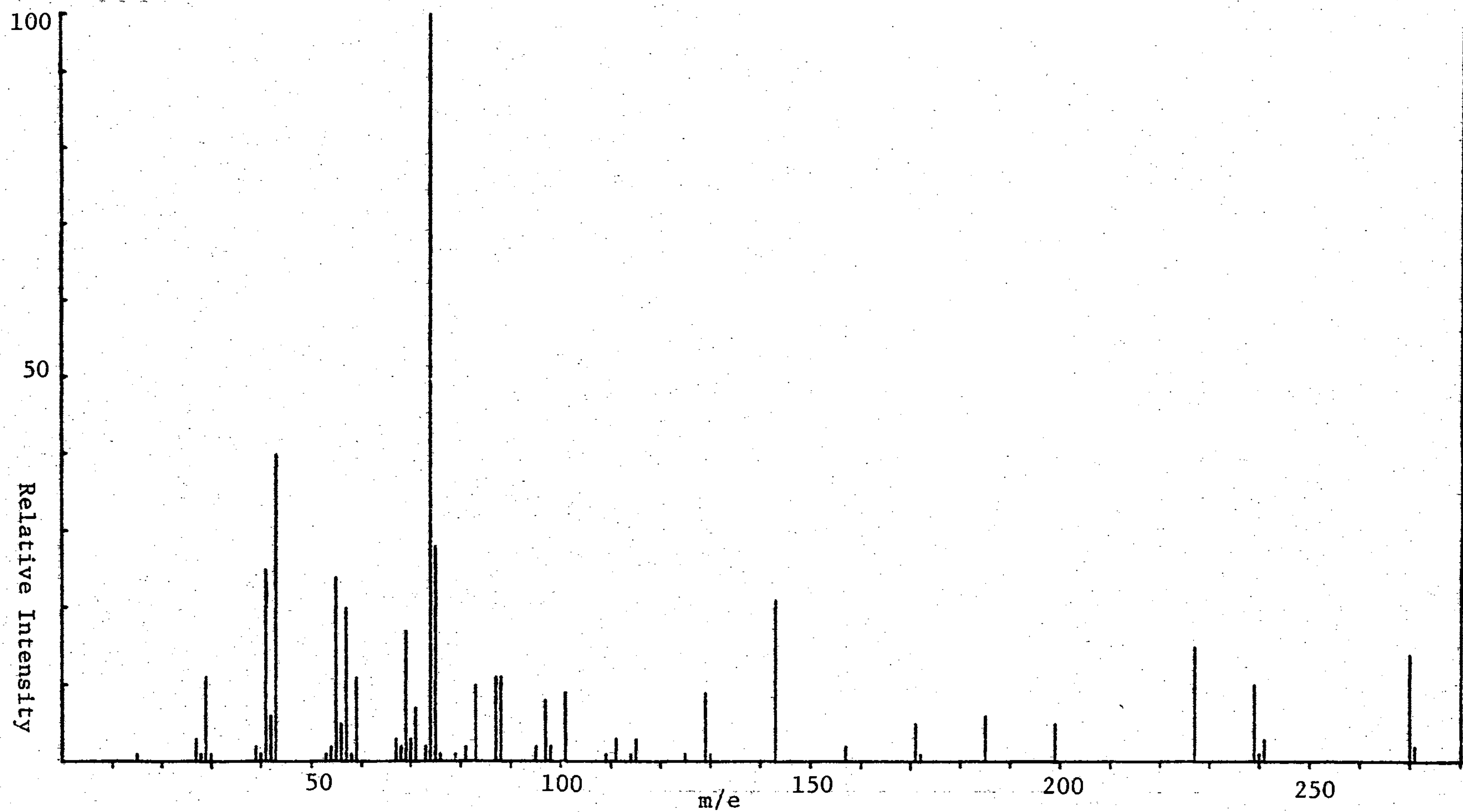


Figure 3. Mass Spectrum of Reference Methyl Palmitate.

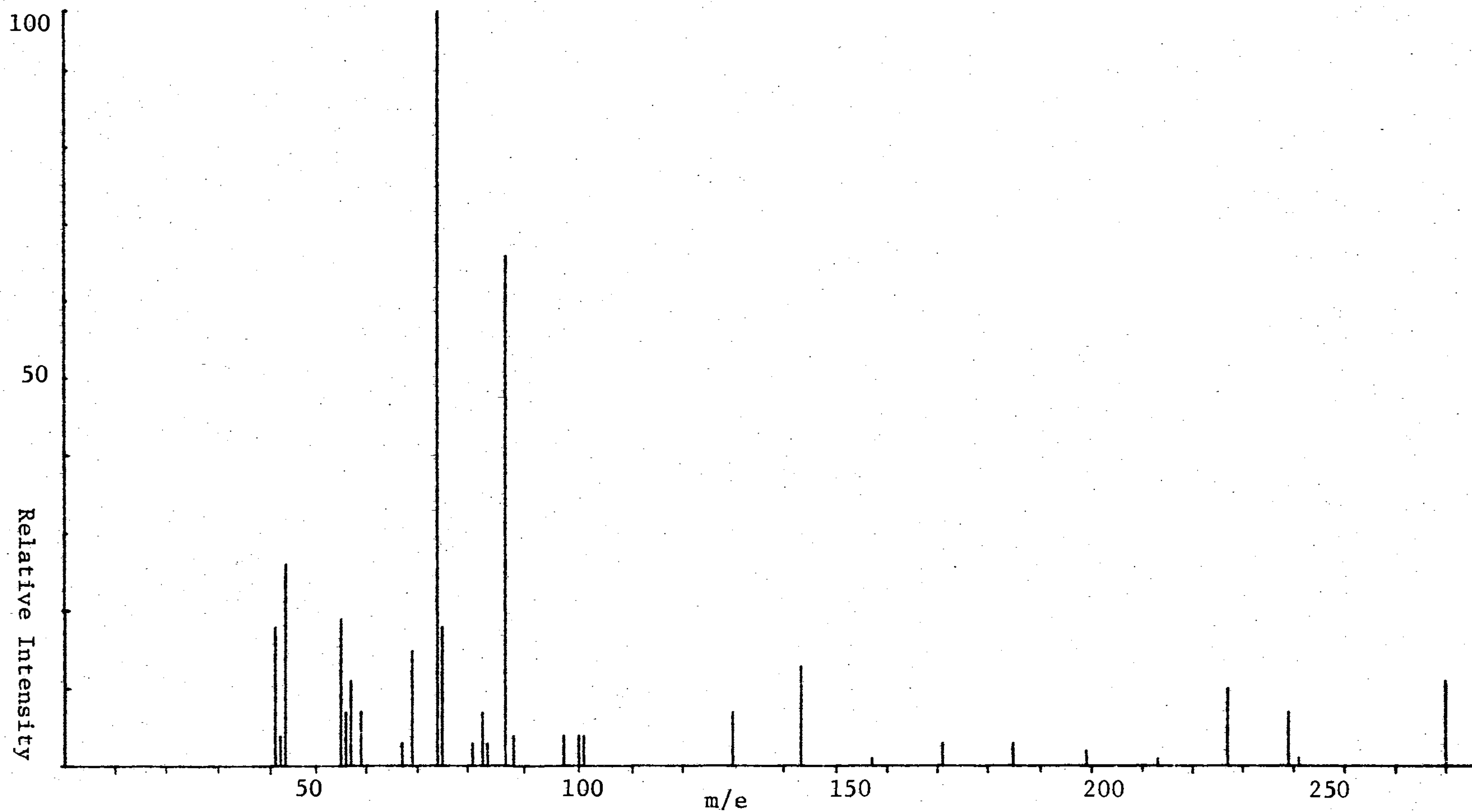


Figure 4. Mass Spectrum of Methyl Palmitate from Pecan Oil.

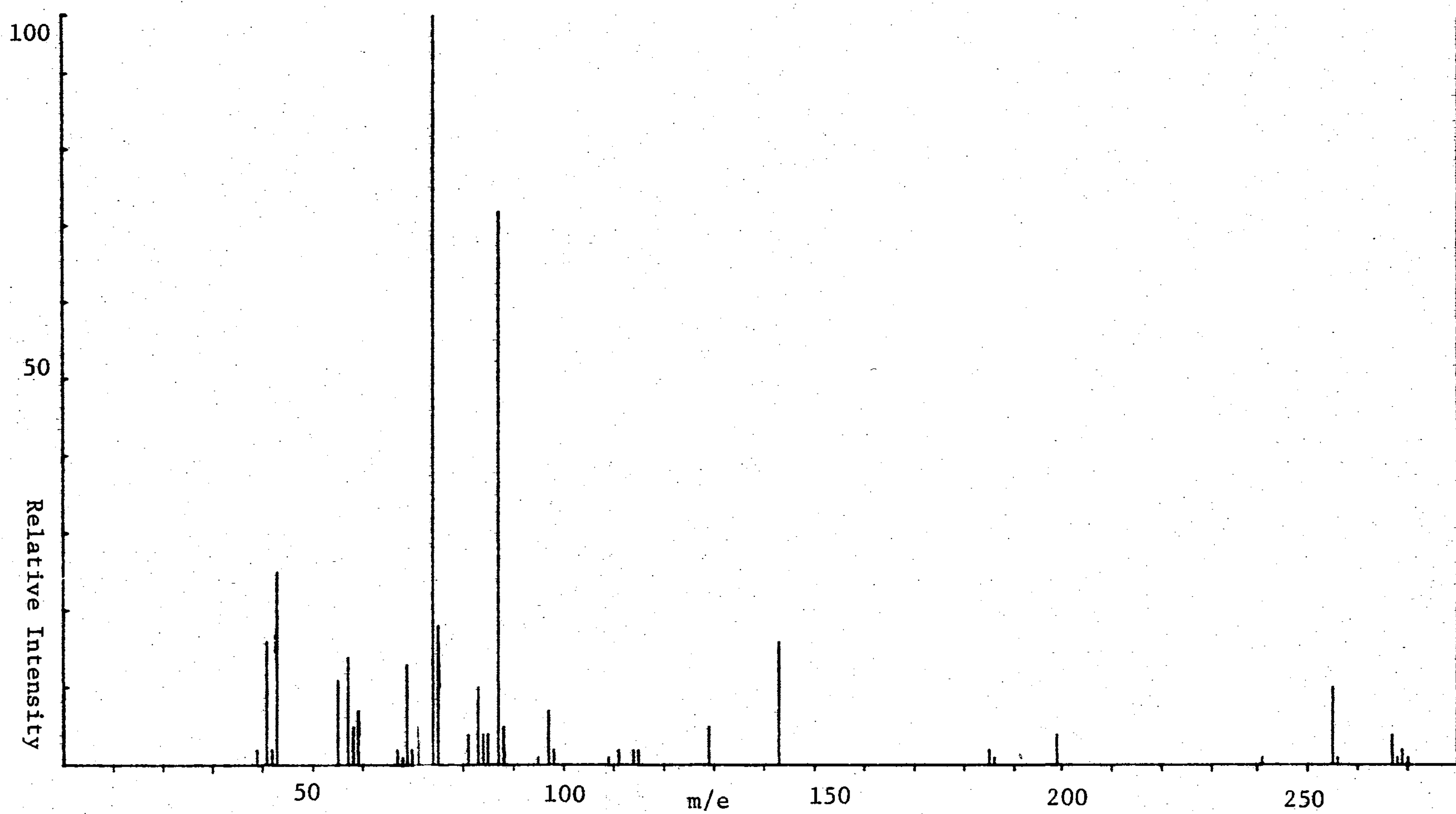


Figure 5. Mass Spectrum of Reference Methyl Stearate.

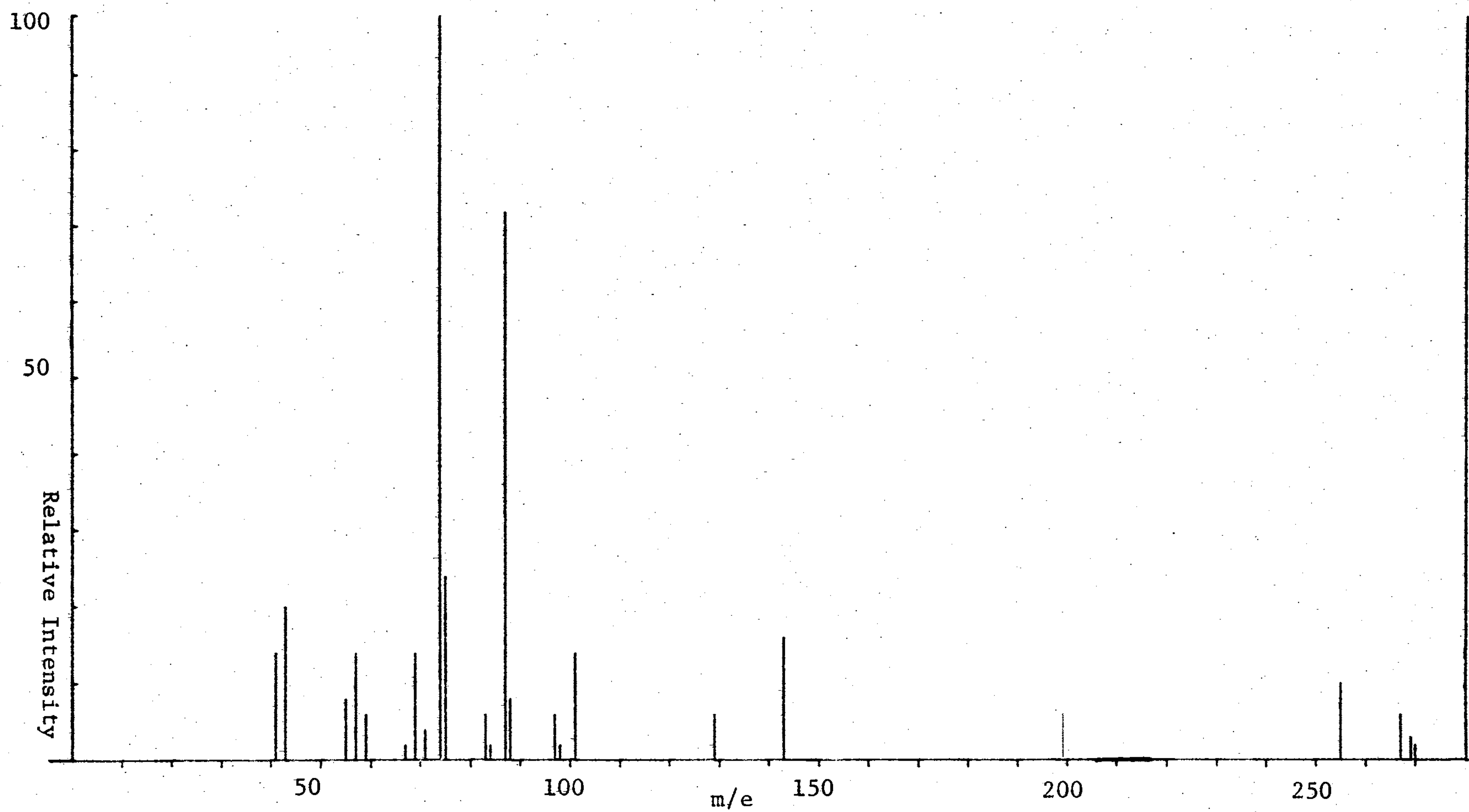


Figure 6. Mass Spectrum of Methyl Stearate from Pecan Oil.

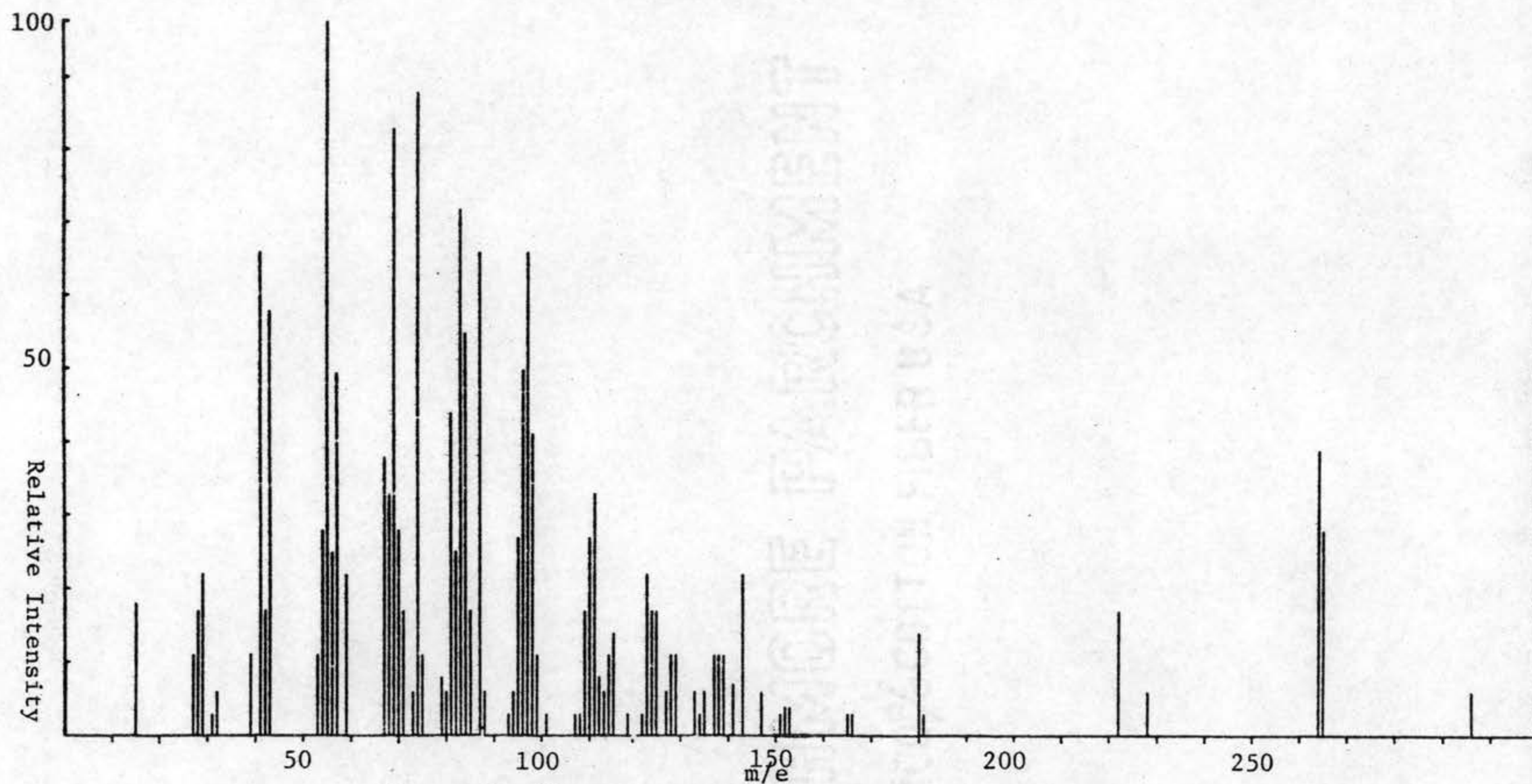


Figure 7. Mass Spectrum of Reference Methyl Oleate.

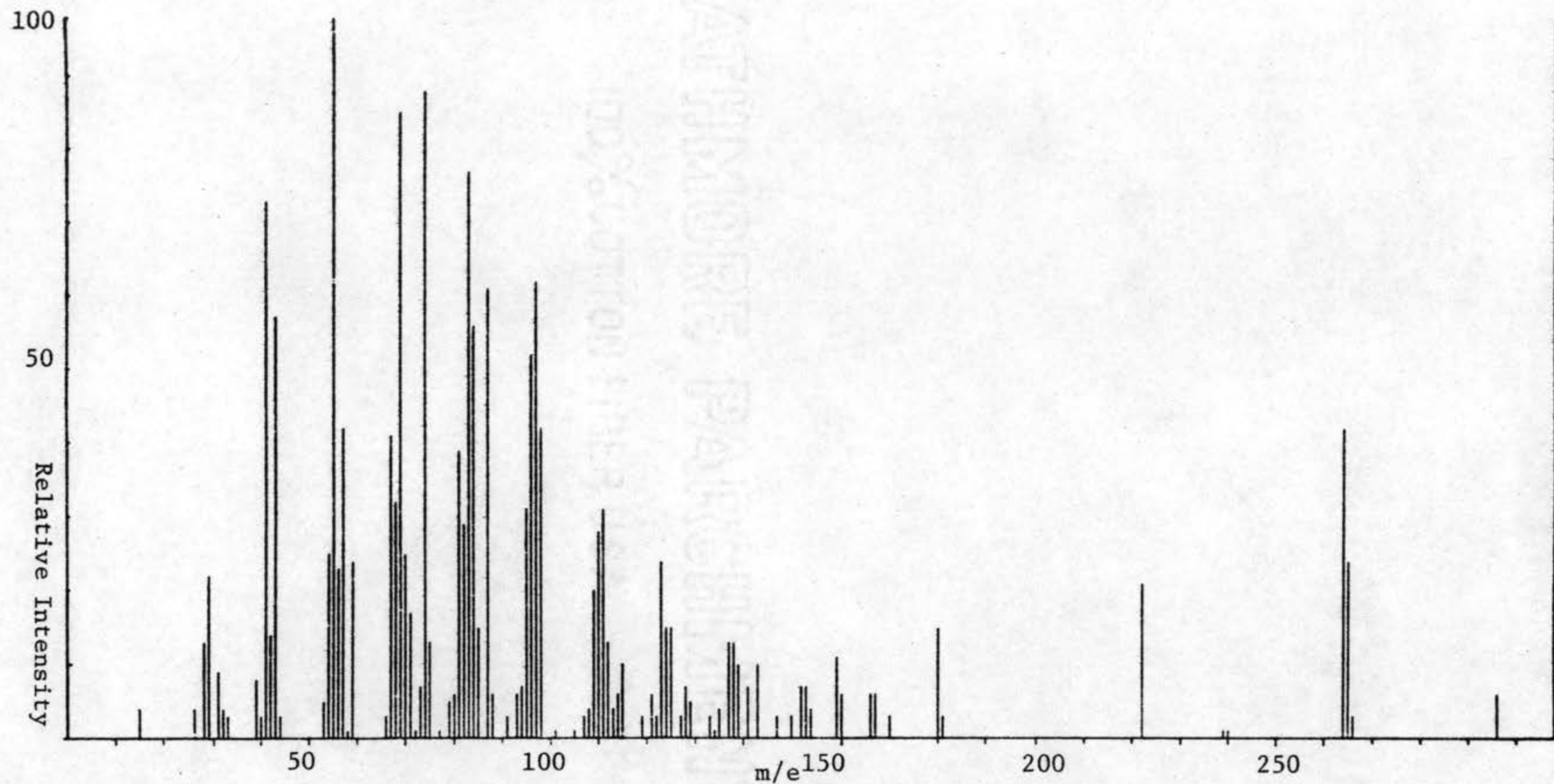


Figure 8. Mass Spectrum of Methyl Oleate from Pecan Oil.

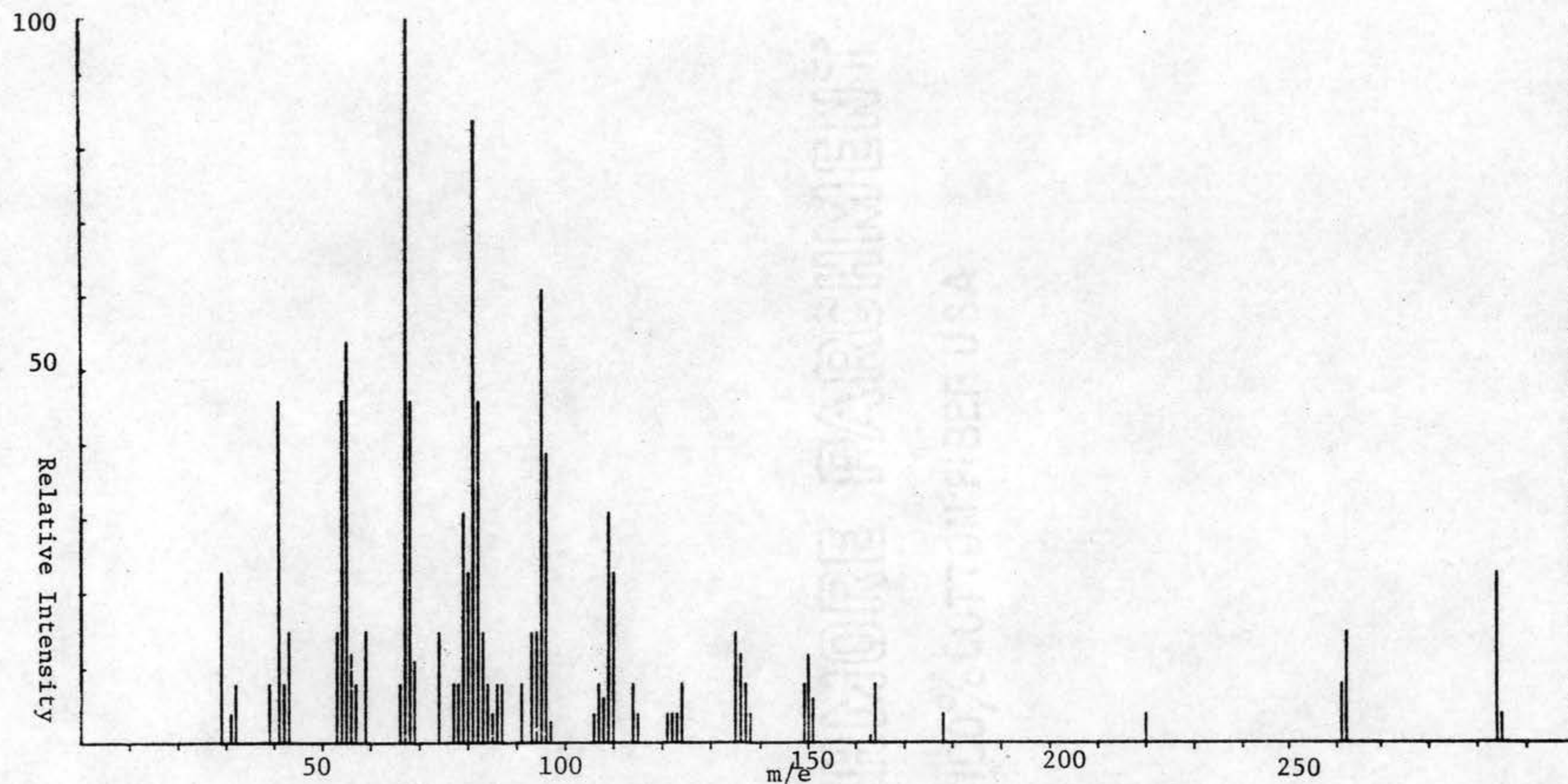


Figure 9. Mass Spectrum of Reference Methyl Linoleate.

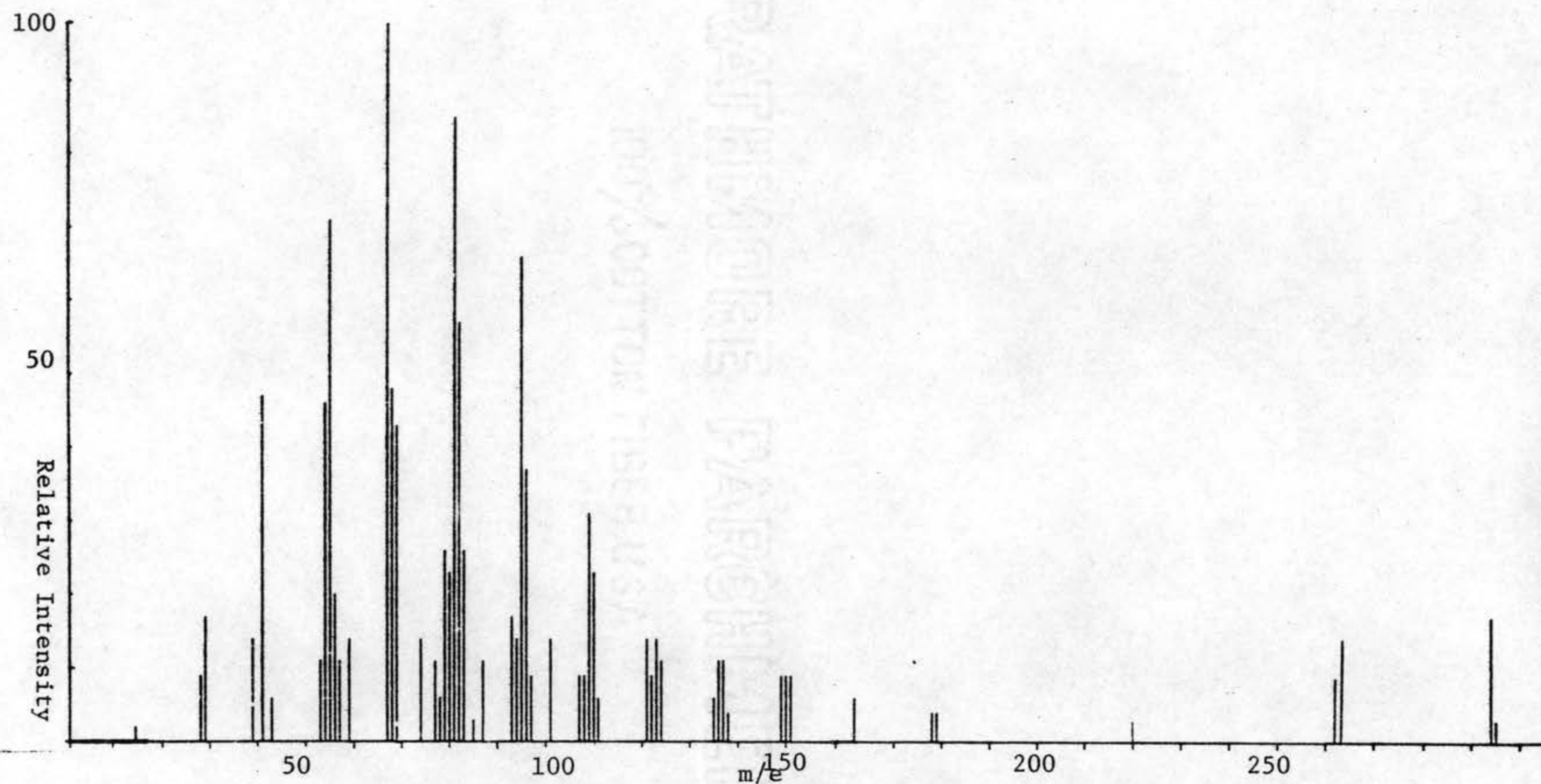


Figure 10. Mass Spectrum of Methyl Linoleate from Pecan Oil.

CHAPTER IV

STABILITY STUDIES

Apparatus and Reagents

Oils were obtained as described in Chapter III. A constant temperature oil bath was used to maintain constant temperature during oxidation of the oils. Ultraviolet spectra were obtained on a Cary model 14 recording spectrophotometer, and a Klett colorimeter was used to measure carotenoids.

2-Thiobarbituric acid (TBA) was obtained from Eastman Organic Chemicals. The TBA reagent was prepared according to the procedure of Sidwel *et al*. (26).

Procedures

Eight samples of pecan oils were chosen for stability studies on the basis of their degree of unsaturation. Oils representative of high, low, and intermediate unsaturation were chosen.

Ten milliliters of fresh oil from each sample were placed in Petri dishes and maintained at 60°C. in an oil bath. The oils were exposed to light and air for two weeks. Samples were taken from each dish at various times and absorbancy (carotenoids) was measured using a filter (approximately 452 millimicrons at which carotenoids exhibit maximum absorbancy) on Klett colorimeter, while visible and ultraviolet

spectra of the oils were obtained on the Cary model 14 recording spectrophotometer.

Two different methods of measuring oxidative rancidity, namely the TBA (26, 38, 39, 67) and peroxide tests (68), were used to evaluate the oxidative deterioration of the oils at different time intervals. The reasons for selecting these methods have been discussed in Chapter II.

Results and Discussion

Absorbancy at 452 millimicrons decreased as the yellow color disappeared. Apparently, carotenoids are largely responsible for the yellow color of pecan oil based on the fact that the color disappearance which accompanied oxidation of the oil was also accompanied by absorbancy decrease at 452 millimicrons and also upon TLC separation of the nonsaponifiable part which will be discussed in Chapter V. The rate of decrease at 452 millimicrons varied in pecan oils from different species as oxidation continued and the readings decreased as the deterioration progressed until the oils were colorless. Oxidation continued after the destruction of carotenoids was complete, but an increase in rate of deterioration at the point where all carotenoids were oxidized (Figure 11), indicates that oil oxidation was inhibited to some extent by carotenoids but not in a catalytic manner as suggested by Stuckey (20). Table II shows peroxide, TBA, and absorbancy values (blue filter - approximately 452 millimicrons) of the eight different oils for different times of oxidation.

The average values of TBA tests and color absorbancies were plotted versus time of oxidation (Figure 11). It was clear that at the early stages of deterioration, little oxidation was taking

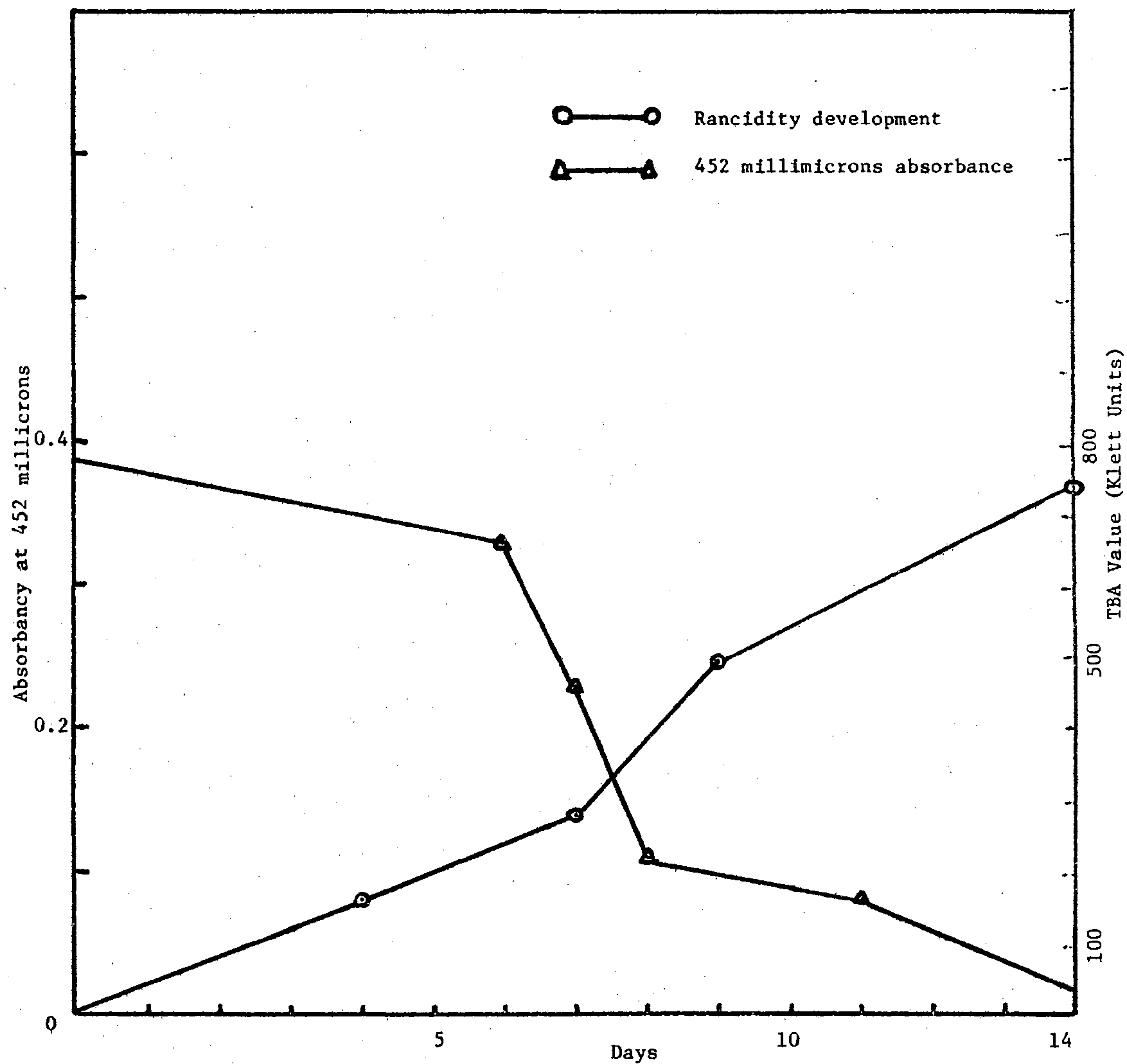


Figure 11. Variations in the Average TBA Values and Pigment Absorbancy Versus Time in Pecan Oils during Oxidative Deterioration.

TABLE II

ABSORBANCY, PEROXIDE, AND TBA VALUES OF PECAN OILS AT DIFFERENT STAGES OF OXIDATIVE DETERIORATION

Variety	Days					
	0	6	7	8	11	14
	Absorbancy 452 millicrons					
Commonwealth	0.32	0.27	0.23	0.09	0.06	0.01
Texas Prolific	0.30	0.20	0.16	0.09	0.08	0.00
Stuart	0.30	0.25	0.24	0.08	0.07	0.01
Texas 60	0.50	0.30	0.24	0.13	0.06	0.02
Kentucky	0.04	0.29	0.26	0.10	0.06	0.02
Barton	0.32	0.27	0.23	0.13	0.10	0.03
Major	0.46	0.28	0.21	0.10	0.07	0.04
Hays	0.50	0.30	0.28	0.16	0.15	0.02

Variety	Days				
	0	4	7	9	14
	Peroxide Value				
Commonwealth	0.00	38	152	302	501
Texas Prolific	0.00	122	207	457	647
Stuart	0.00	44	167	361	654
Texas 60	0.00	35	50	104	527
Kentucky	0.00	31	85	262	769
Barton	0.00	16	27	42	102
Major	0.00	32	140	315	650
Hays	0.00	28	72	140	555

Variety	Days				
	0	4	7	9	14
	TBA Value = Klett Reading				
Commonwealth	0.00	144	258	601	960
Texas Prolific	0.00	373	570	950	1400
Stuart	0.00	173	302	600	640
Texas 60	0.00	135	179	246	775
Kentucky	0.00	178	200	500	640
Barton	0.00	120	230	250	280
Major	0.00	200	300	570	700
Hays	0.00	156	182	230	700

place. This was true partly because of carotenoids, as mentioned above, and partly because this period represented the latter steps of the "Induction Period", where the initial events of reversion occurs, and oxidation products are low. Such behavior is typical of free radical reactions (19) where, during initiation, free radical concentration is minute, but increases rapidly when the reaction progresses to the propagation steps. Perkings (17) demonstrated this behavior in oils undergoing oxidation, as well as other properties such as viscosity increase and polymer formation.

From peroxide values of Table II the following order of decreasing relative stabilities were found after four days of oxidation: Barton (1.00), Hays (0.57), Kentucky (0.52), Major (0.51), Texas 60 (0.46), Commonwealth (0.42), Stuart (0.37), and Texas Prolific (0.13). The numbers between parenthesis are reciprocals of the normalized peroxide values (peroxide value of Barton = 1.00) after four days oxidation.

On the other hand, TBA values after four days gave the following order: Barton (1.00), Texas 60 (0.89), Commonwealth (0.83), Hays (0.77), Stuart (0.69), Kentucky (0.67), Major (0.60), and Texas Prolific (0.32).

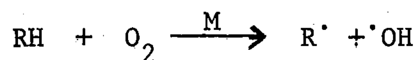
The different ranking order obtained for some of the species, which were close in stability rates, was expected since the TBA test measures terminal products of oxidation which are directly related to rancidity, while the peroxide method detects peroxides which are formed in the earlier stages of the oxidative process. This is explained in the data of Table II where the average TBA values at the fourth day of oxidation were relatively lower than the average peroxide values obtained at the same stage. However, after two weeks of

oxidation, the order was reversed, and the average peroxide values were less than the corresponding average TBA values. This is reasonable since after two weeks the concentrations of TBRS were relatively higher than that of peroxides.

It was interesting to note that although Stuart and Commonwealth pecan kernels are known to be relatively stable to oxidative deterioration, their oils did not respond accordingly (12).

The role of unsaturation in the stability of oils and fats was discussed in Chapters I and II. It was shown by Gunstone and Hilditch (18) that the relative rates of oxidation of a mixture of oleate, linoleate, and linolenate, are in the ratio of 1:12:25, respectively. Therefore, to obtain the total degree of unsaturation, the three unsaturated acid percentages for the varieties of pecan oils studied (Table I) were multiplied by the corresponding relative rate of oxidation. Total unsaturation values obtained in this manner are listed in Table V. Comparison of total unsaturation values with normalized relative stabilities (relative to Barton = 1.00) showed no apparent correlation. Similar results have been obtained by several other workers (1, 12, 69).

Degree of unsaturation cannot explain all the differences in stability of oils and fats (1,11). In fact, other factors are known to affect the process of oxidative rancidity (2), for instance trace metals (2, 19), which are usually present as complex ions or salts, act as catalysts in accelerating the first steps of free radical formation (19):



$$RH + O_2 \xrightarrow{M} R^{\cdot} + {}^{\cdot}OH$$

where, RH represents the fat, and M a metal catalyst. Metal catalysis

in fat oxidation has been extensively studied by Ingold (70), and Uri (71). A second factor is the presence of antioxidants (5, 19), which play an important role in stabilizing even a highly unsaturated oil (70, 72).

Another factor is enzymatic catalysis of lipid peroxidation in biological systems. Hematin compounds (73) and lipoxidase (73, 74) are two outstanding, well-defined peroxidation catalysts; both are proteins and possess greater catalytic activity than any other known catalysts in all reactions.

CHAPTER V

TOCOPHEROLS IN PECAN OIL

Apparatus and Reagents

A Carver laboratory press was used to obtain pecan oils, and a Cary model 14 recording spectrophotometer was used to confirm the presence of tocopherols. Estimation of tocopherols was achieved by reading color development using the Emmerie-Engle (E-E) procedure (58) and a Klett colorimeter.

Bathophenanthroline (4,7 -Diphenyl - 1,10 -Phenanthroline) and alpha-tocopherol were obtained from the Pierce Chemical Company, Rockford, Illinois. Ether (anhydrous), peroxide free, was obtained from Mallinckrodt Chemical Works, St. Louis, Missouri.

Procedures

Oils were obtained using a laboratory press and were then centrifuged. About 0.5 gram of the supernatant was accurately weighed, saponified, and the nonsaponifiable fraction was extracted with ether according to the procedure of Sturm *et al.* (57). The ether was evaporated under nitrogen, the residue dissolved in 3 ml. of a mixture of chloroform-methanol, and the spectra of the solution in the visible and ultraviolet regions were obtained. The nonsaponifiable materials from the eight varieties of pecan oils mentioned in Chapter IV were chromatographed on silica gel G coated plates using chloroform as the

developing solvent. Plates were sprayed to detect tocopherols. Reference alpha-tocopherol was also chromatographed with the unknowns to compare R_f values. Unsprayed zones corresponding to each spot were removed from TLC plates, extracted, and examined spectrophotometrically to confirm tocopherol bands. Zones corresponding to tocopherols were removed and estimated colorimetrically according to the method of (E-E) (58), using a green filter (approximately 534 millimicrons) in Klett colorimeter. A calibration standard was prepared using standard alpha-tocopherol. Thus, the total-, alpha-, and non-alpha-tocopherols in all samples were estimated.

Results and Discussion

TLC of the nonsaponifiable fraction of pecan oils showed four spots after spraying with potassium ferricyanide followed by ferric chloride reagents. Table III lists the average R_f values for reference alpha-tocopherol and unknown spots from all samples, while Figure 12 is a reproduction of a typical TLC plate after spraying.

In Figure 12, spot I, which remained close to the origin, was the yellow colored carotenoids as confirmed by visible spectra of the eluted band.

The nonsaponifiable material of pecan oil had a maximum at 452 millimicrons and another at 299 millimicrons, indicating the presence of carotenoids and tocopherols respectively. The evidence for the presence of carotenoids in the crude oil of pecans has already been discussed in Chapter IV.

Extracts from zones II and III showed a single maximum at 299 millimicrons showing that these bands were tocopherols. Zone IV (Emmerie-Engle positive) was eluted but no absorption maximum was

TABLE III

R_f VALUES FOR SPOTS ON PLATE FROM TLC OF THE NONSAPONIFIABLE PORTION OF PECAN OIL

Spot	Average R_f Values
Standard Alpha-tocopherol (V)	0.45
Spot (III)	0.45
Spot (II)	0.35
Spot (IV)	0.84

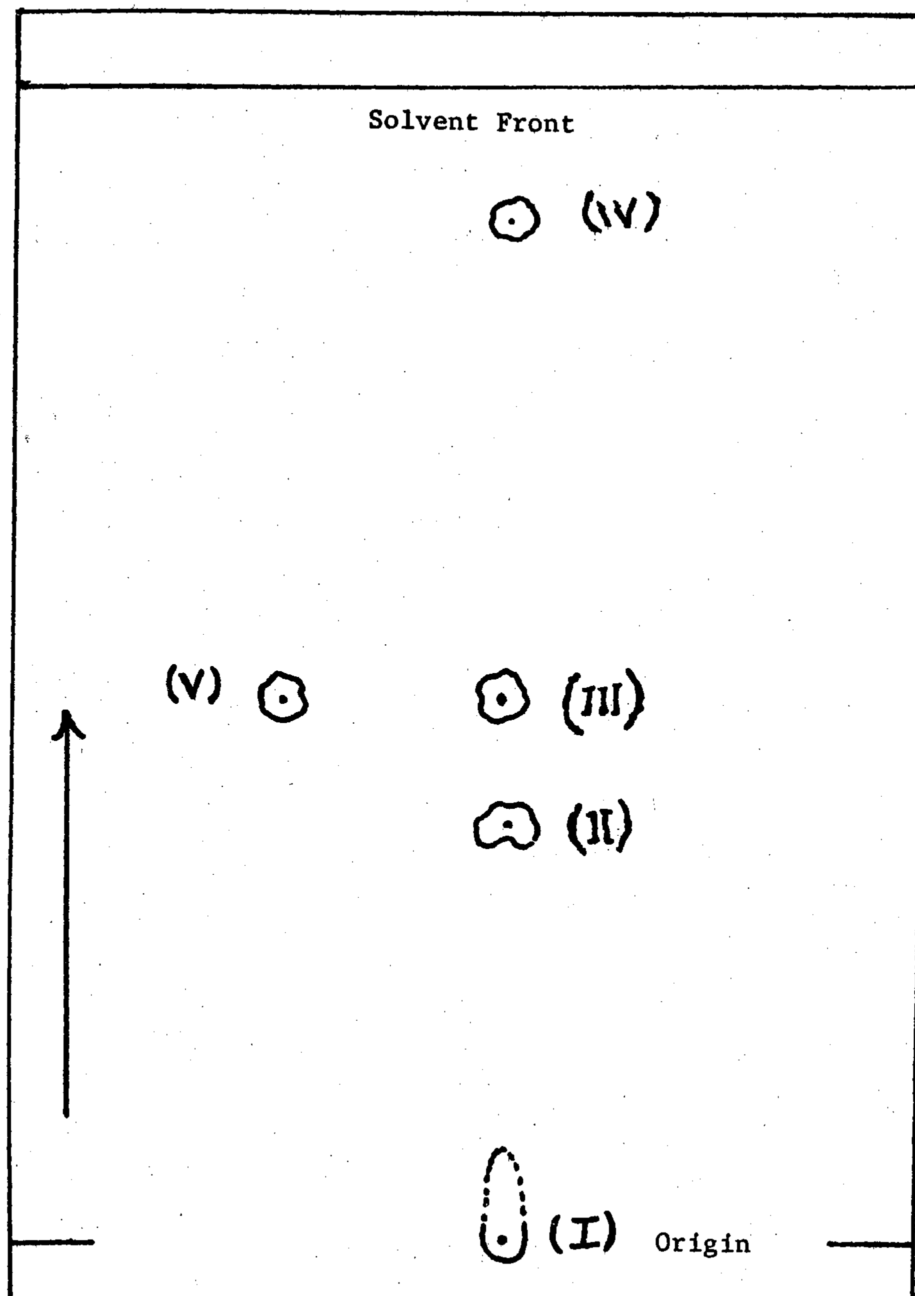


Figure 12. Typical Thin Layer Chromatogram of the Non-saponifiable Portion of Pecan Oils on Silica Gel G Coated Plates Developed in Chloroform. (Arrow shows direction of Solvent Movement.)

TABLE IV

CALIBRATION STANDARD, MICROGRAMS ALPHA-TOCOPHEROL VERSUS THE OPTICAL DENSITY OF THE COLOR OBTAINED USING E-E METHOD*

Microgram Alpha-tocopherol/ ml. Ethanol	Optical Density at 534 Millimicrons
1.05	0.19
2.10	0.29
3.15	0.41
4.20	0.52
5.25	0.58
6.30	0.70
7.35	0.75
8.40	0.92

*Reference (58)

observed in either the visible or ultraviolet spectra. The R_f value of spot III corresponds to the reference known alpha-tocopherol (Table III), while spot II had an R_f value of about 0.35 and corresponded to either beta-or gamma-tocopherol or a mixture of both (57).

Vitamin E content of zones II, III, and their sums are shown in Table V. Table V also contains the relative degrees of unsaturation of the oils calculated on the basis of Gunstone and Helditch's work (18) which was mentioned in Chapter IV.

Alpha-tocopherol was the predominant isomer in pecan oils (Table V). Hays variety had the highest relative amount while Commonwealth contained the lowest.

Total tocopherols recorded in Table V were plotted versus the reciprocal normalized (Barton = 1.00) peroxide values (Table II) after four days of oxidation and the plot appears in Figure 13. The best straight line was fitted using the method of least squares and an increase in stability of the oil was related to higher tocopherol content.

In Figure 14, total tocopherol values were plotted versus the reciprocal normalized (Barton = 1.00) TBA values after four days oxidation. This straight line had a smaller slope than the line in Figure 13. The difference in slope of the two lines may be explained by the different methods used in testing oxidative deterioration as was discussed in Chapter IV.

Figure 15 shows a plot of vitamin E content of pecan oils versus stability after correcting for unsaturation (Table V). To correct for unsaturation, deviations from the mean unsaturation were multiplied by the reciprocal normalized (Barton = 1.00) peroxide values after

TABLE V

TOTAL-, ALPHA-, NON-ALPHA-TOCOPHEROL CONTENTS
AND TOTAL UNSATURATION IN EIGHT
VARIETIES OF PECAN OILS

Variety	Total Unsaturation	Micrograms Tocopherol/Gram Oil		
		Alpha	Non-Alpha	Total
Commonwealth	420	20	14	34
Major	407	38	40	78
Texas 60	383	34	1	35
Kentucky	355	108	9	117
Hays	441	112	40	152
Texas Prolific	533	30	12	42
Stuart	484	33	16	49
Barton	511	82	60	142

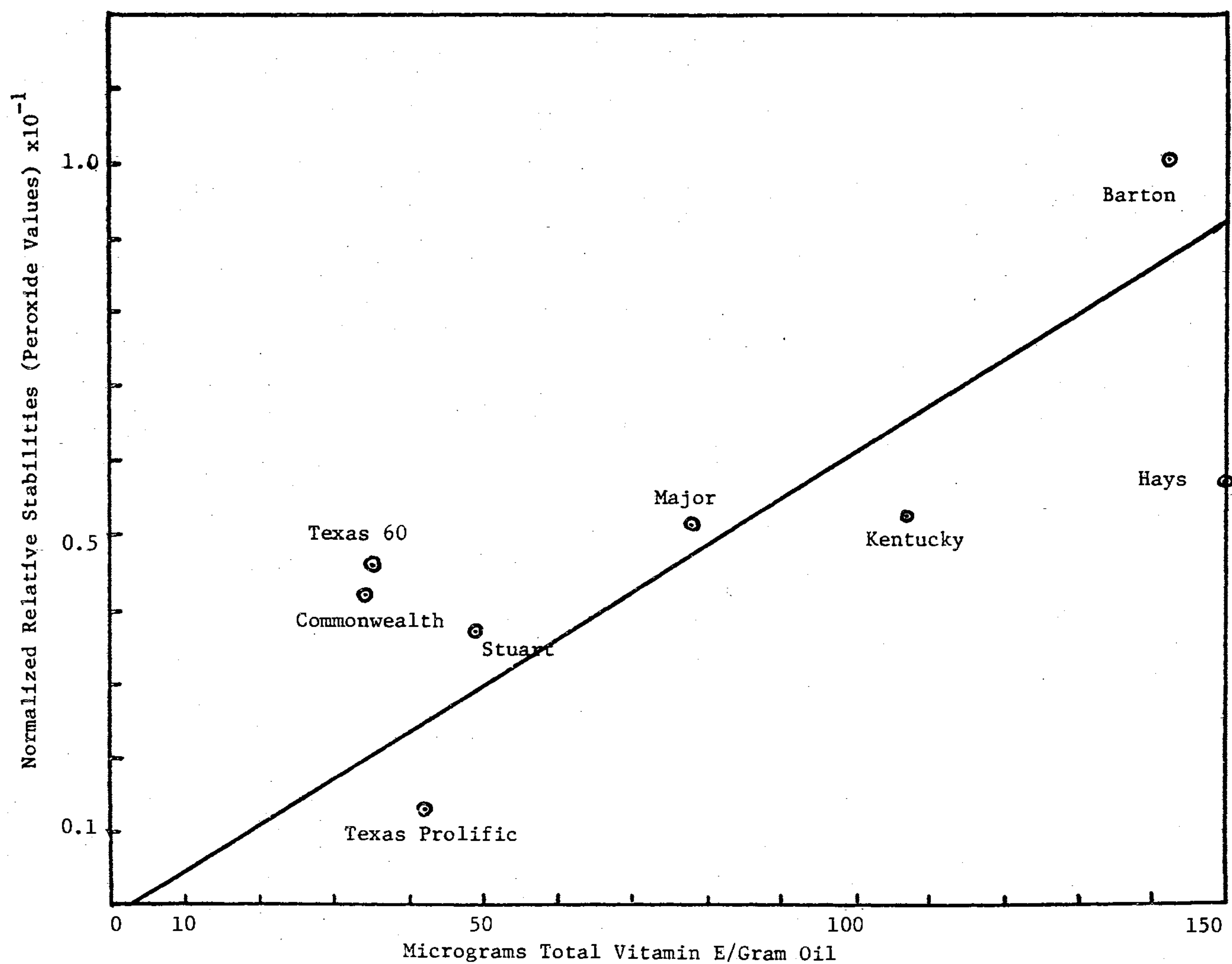


Figure 13. Plot of Total Tocopherols Versus Normalized Relative Stabilities (Normalized Peroxide Values, Setting Barton = 1.00) in Pecan Oils.

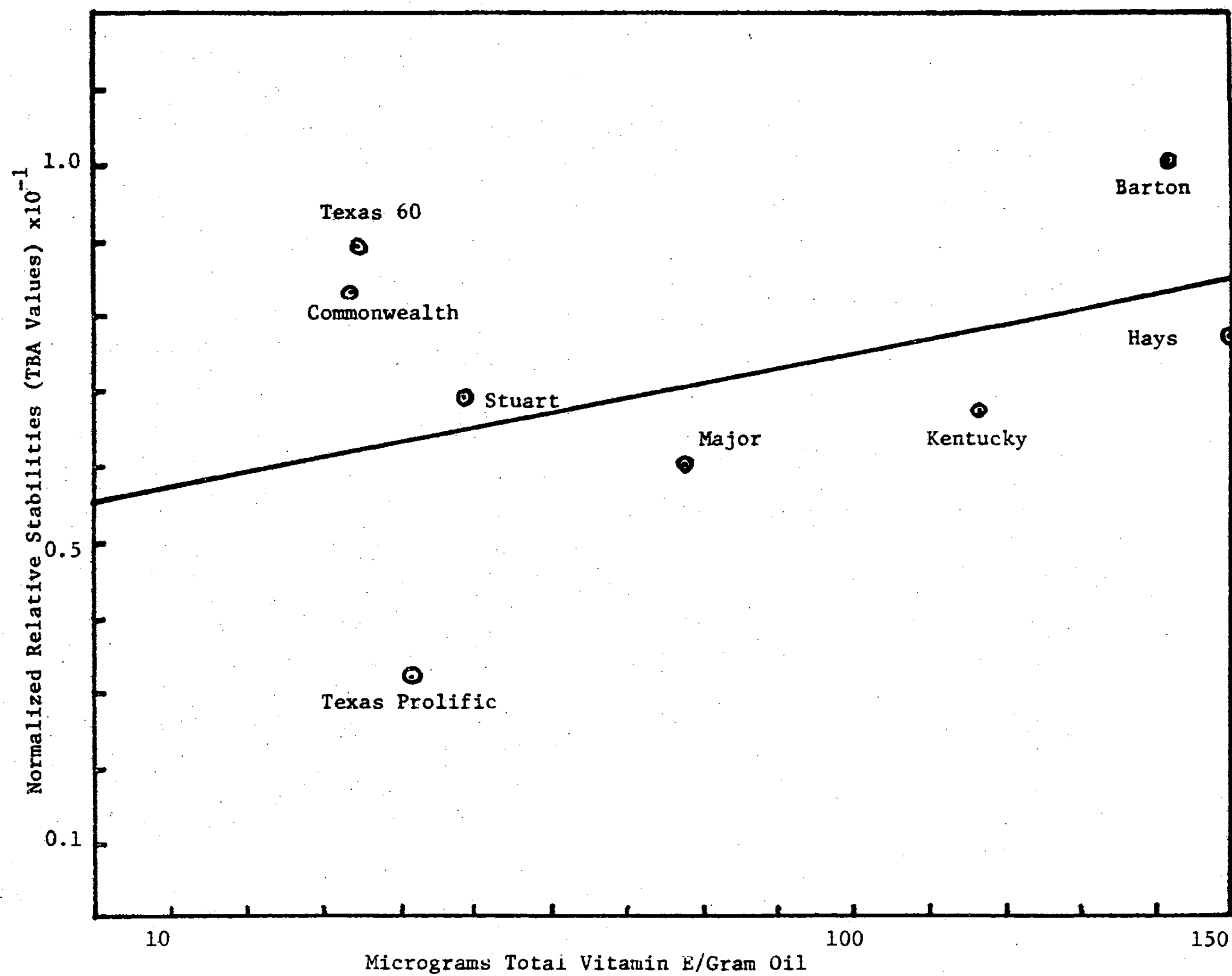


Figure 14. Plot of Total Tocopherols Versus Normalized Relative Stabilities (TBA Values, Setting Barton = 1.00) in Pecan Oils.

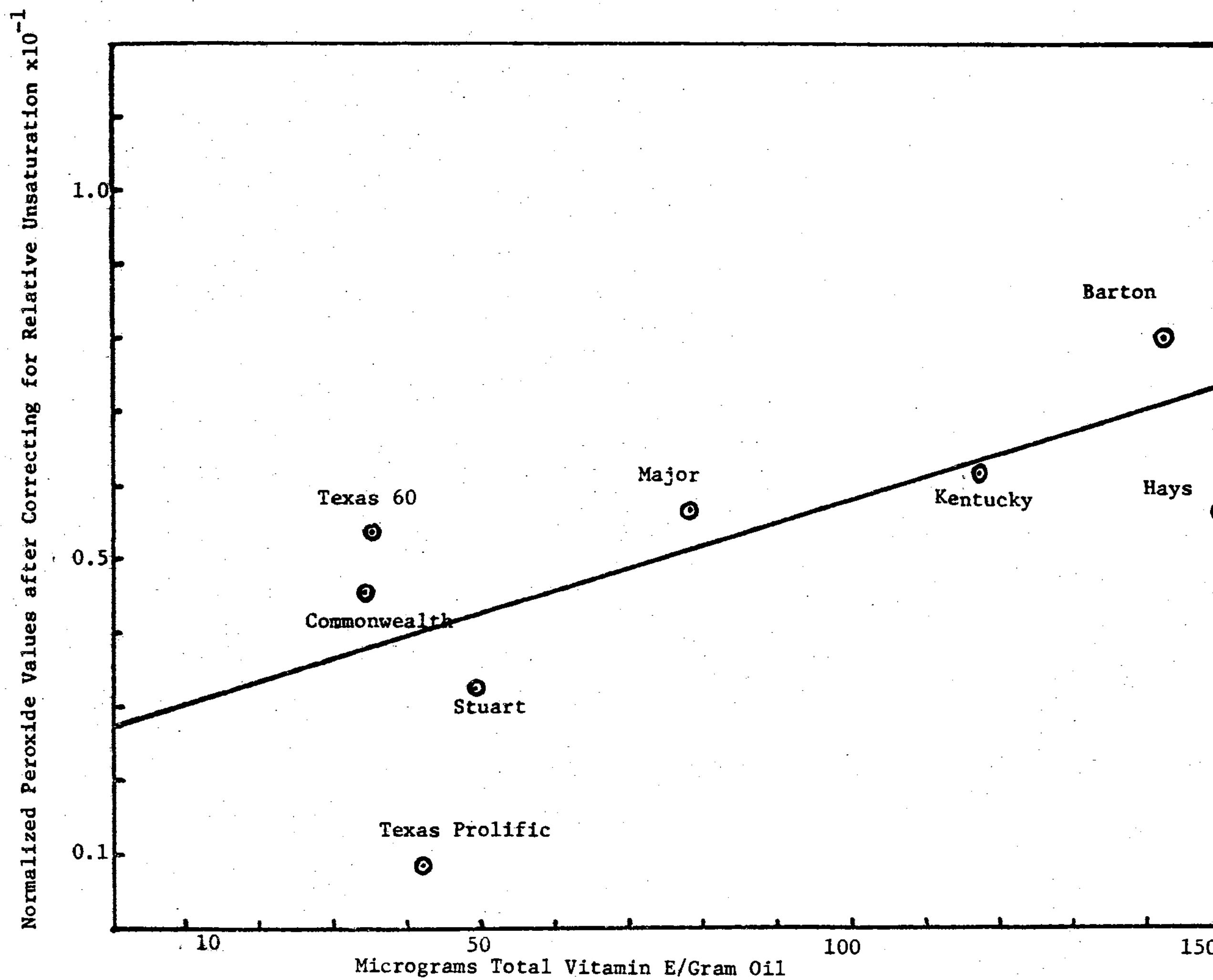


Figure 15. Relation of Total Tocopherols to Stability (Normalized Peroxide Values after 4 Days Oxidation, Setting Barton = 1.00) after Correcting for Relative Unsaturation in Pecan Oils.

four days oxidation and the results were plotted against total tocopherol contents. The best fitting straight line had a slope of 0.3.

The conclusion drawn from plots in Figures 13 and 14 was that the tocopherol content of pecan oils correlated to their stability, while Figure 15 reveals the interesting effect of unsaturation on the extent of the above correlation. Comparison of the relative positions of Texas 60, Commonwealth, Hays, Barton, Major, and Kentucky in Figure 13 with respective positions in Figure 15 shows the importance of unsaturation in these species, for their positions relative to Figure 13 were moved toward the straight line. However, the opposite was true with Texas Prolific and Stuart varieties where both moved away from the straight line of Figure 15. Thus, the majority of pecan oils, in our studies plotted much closer to a straight line when both tocopherol contents and unsaturation were taken into account.

Texas Prolific and Stuart varieties, although moved away from the straight line in Figure 15, both had high relative unsaturation (Table V), low relative stabilities (Chapter IV), and very low vitamin E content (Table V). Therefore, the importance of high unsaturation and low tocopherol content in Texas Prolific and Stuart pecan oils were also evident.

The fact that the straight line in Figure 13 passes almost through origin was not interpreted to mean that tocopherols were exclusively responsible for pecan oil stability, since the peroxide values of Figure 13 are not absolute quantitative parameters for stability (Chapter II).

Figure 14 shows a smaller slope and a larger (y) intercept which simply indicates that something other than tocopherols are involved in

stabilizing pecan oils. This was also in agreement with the fact that the TBA test was a better measurement for the determination of end-products of oil deterioration than peroxide method.

It was clear from the data that both unsaturation and tocopherols in pecan oils play an important role in resistance to oxidative deterioration. Both factors were considered important in all samples studied.

The scattered points in Figures 13, 14, and 15 indicates the need for using larger numbers of analyses and more varieties to minimize the experimental errors in the correlation curve.

Factors other than tocopherols and unsaturation are probably also involved in pecan oil stability to oxidative deterioration, but degree of unsaturation and tocopherols are good parameters for predicting the stability of pecan oils.

CHAPTER VI

SUMMARY

The triglycerides and nonsaponifiable portions of pecan oils were studied and a correlation of the degree of unsaturation and vitamin E content with oil stability was achieved.

Gas chromatographic analysis of the methyl esters and IPG from pecan oil showed the presence of palmitate, stearate, oleate, linoleate, and another unidentified fatty acid, present in relatively low concentration. The latter was thought to be linolenate, eicosenoate, or another fatty acid with 18 carbon atoms and three double bonds.

The presence of IPG, methyl palmitate, methyl stearate, methyl oleate, and methyl linoleate in the esterified pecan oil was confirmed by mass spectrometry.

Relative stabilities of eight varieties of pecan oils, selected on the basis of the differences in unsaturation, were obtained by exposing the oils to air and light at 60°C. and measuring oxidative deterioration using the peroxide and TBA methods.

The yellow color of pecan oil was shown to be largely due to carotenoids which were destroyed as oxidative rancidity progressed. They appeared to repress the rate of oxidation in a noncatalytic manner.

Thin layer chromatography of the nonsaponifiable portion of pecan oils, after spraying, produced four spots, one of which was alpha-tocopherol and the other beta- or gamma-tocopherol or their mixture.

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