Composition and Stability of Pecan Oils

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Abstract

Analyses of pecan oils by GLC and mass spectrometry showed the presence of palmitic, stearic, oleic, linoleic, and linolenic acids. The TLC of the nonsaponifiable materials of pecan oil, followed by identification of individual zones, revealed the presence of carotenoids, α-tocopherol, other tocopherols, and an Emmerie-Engel positive compound which was unknown. A quantitative estimation of tocopherols was achieved in pecan oils from eight varieties. Total tocopherol content was correlated with oil stability; however a better correlation with stability was observed when both tocopherols and the degree of unsaturation of the oils were taken into account.

Introduction

PECAN NUTS, which contain high levels of oil, show considerable flavor instability even at reduced temperatures. In this regard, flavor changes in foods containing fats and oils have been related to the oxidation of fats (1). Numerous reports have been published concerning the stability of oils and fats to oxidative rancidity, and much of this work has been reviewed recently (1,2). Woodroof and Heaton (3) reported relative stabilities of pecan kernels and unshelled nuts, stored at temperatures ranging from 10C to 20C and relative humidities from 90% to 20% for seven years. Evaluation of the deterioration of 46 varieties by a taste panel indicated stability rankings from very good to poor.

The effect of unsaturation of the oil on stability has been discussed by Gunstone and Hilditch (4), and the importance of double bonds in accelerating fat oxidation has been explained by the ease of free radical attack on allylic positions to form peroxide radicals (5–7).

Investigation of other factors, such as tocopherols (8–11), trace metals (12), and enzymatic oxidation of fats in biological systems (13), have indicated their importance in the stability of fats and oils.

French (14) determined the fatty acid composition and content of several pecan oils by using GLC analysis. However insufficient evidence was obtained adequately to identify all fatty acids, as evidenced by the incorrect assignment of linolenic acid to the component in peanut oils which was actually *cis*-11-eicosenoic acid (15).

The purpose of this work was two-fold: to determine whether or not the degree of unsaturation or carotenoid and tocopherol contents were related to the instability of pecan oils and to establish the identities of the fatty acids of pecan oil by a physical procedure other than GLC. Confirmation of the identities of the unsaturated acids seemed especially important in terms of their possible contribution to the instability of pecan oils.

Experimental

Apparatus

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 chromatograph-mass spectrometer (GC-MS). The latter was a prototype of the LKB-9000 mass spectrometer, 12221 Parklawn Drive, Rockville, Md. The UV spectra were obtained on a Cary Model 14 recording spectrophotometer, and a Klett colorimeter was used for colorimetric measurements of carotenoids, tocopherols, and TBA values. A Carver laboratory press was used to obtain fresh pecan oils for tocopherol estimation.

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. The 2,2-dimethoxypropane was obtained from the Dow Chemical Company. Dry methanol was redistilled through a column of molecular sieve 5A to remove traces of water. Methanolic HCl was prepared according to the procedure of Mason and Waller (16).

Neutralizing Agent. Sodium bicarbonate, sodium carbonate, and sodium sulfate (anhydrous) were mixed in a 2:1:2 ratio by weight, dried overnight at 110C, and stored in an air-tight container. Sodium methoxide, approximately 0.5N, was made by reacting sodium metal with dry methanol. The 2-thiobarbituric acid (TBA) was obtained from Eastman Organic Chemicals, Rochester, N. Y. The TBA reagent was prepared according to the procedure of Sidwel et al. (17). Bathophenanthroline (4,7-diphenyl-1,10-phenanthroline) and α-tocopherol were obtained from the Pierce Chemical Company, Rockford, Ill. (Ether (anhydrous), peroxide-free, was obtained from Mallinckrodt Chemical Works, St. Louis, Mo.

Standard methyl esters, chromatographically pure (Applied Science Laboratories), were used without further purification. Isopropylidene glycerol (IPG), for determining retention time of IPG, was synthesized by Mason and Waller (16). The oils were obtained from samples of 34 varieties of pecans grown near Stillwater, Okla.

Silica Gel G was obtained from Brinkmann Instruments Inc., New York, and 4,5-dibromofluorescein was obtained from K and K Laboratories, California.

Transesterification

Pecan oils were converted to methyl esters according to the procedure of Mason et al. (18). 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.

GLC Analysis

A 6-ft × ⅛-in. O.D. (0.062 in. I.D.) stainless-steel column, packed with 14.5% 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 linearly programmed from 75C to 195C at 10° per minute; the injector and detector temperatures were 220C and 230C respectively. Identification of peaks was achieved by comparing retention times of unknowns with reference standards. Quantitative estimations were accomplished by multiplying peak areas by

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appropriate μmole/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 in. in O.D. × 6-ft in length, packed with 14.5% EGS on Anakrom 100/110 mesh, was used. Helium was the carrier gas, flowing at a rate of 35 ml per minute. The column was maintained at 155C.

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

Argentation TLC

Methyl esters from pecan oil were separated according to the procedure of Morris (19). The band containing triunsaturated fatty acids, which was the least mobile and of lowest concentration, was eluted with a mixture of water-methanol (3:1) and centrifuged. The triunsaturated ester was then extracted with n-pentane. The ester was concentrated by evaporating most of the solvent before its mass spectrum was obtained on the GC-MS instrument.

Stability Studies

Eight of the oils from the 34 varieties were chosen for stability studies on the basis of their degree of unsaturation. Oils representative of relatively high, low, and intermediate unsaturation were selected.

Ten mililiters of fresh oil from each sample were placed in Petri dishes and maintained at 60C in an oil bath. The oils were exposed to laboratory light and air for two weeks. Samples were taken from each dish at various times, and carotenoids were measured by using the Klett colorimeter with the proper filter. Analysis of the oils on the Cary Model 14 spectrophotometer revealed only two areas of absorption: a broad band absorbing maximally at 452 mμ and a sharp stronger band absorbing maximally at 298–299 mμ. Thus the loss in the characteristic broad band during oxidation was followed by use of the Klett colorimeter.

Two different methods of measuring oxidative rancidity, namely, the TBA (17) and peroxide tests (20), were used to evaluate the oxidative deterioration of the oils at different intervals of time.

Tocopherol Determination

Oils obtained with a laboratory press had the same fatty acid content, determined by GLC, as those obtained by hexane extraction. Oils obtained by pressing were used for tocopherol studies because the method was rapid and did not require the use of the heated solvent. About 0.5 g of the clear oil was accurately weighed and saponified; the nonsaponifiable fraction was extracted with ether according to the procedure of Sturm et al. (21). The ether was evaporated under nitrogen, and the residue was dissolved in 3 ml of a mixture of chloroform-methanol (4:1). The spectra of the solution in the visible and UV regions, as seen with the Cary Model 14 spectrophotometer, revealed the presence of two regions of absorption: a broad band with λ_{max} at 452 mμ and a narrow sharper band with λ_{max} at 298 mμ.

The nonsaponifiable materials from the eight varieties of pecan oils were chromatographed on Silica Gel G coated plates with chloroform as the developing solvent. Plates were sprayed with potassium ferricyanide, followed by ferric chloride reagent, to detect tocopherols (21). Reference standard α-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 Emmerie and Engel (22) by using a green filter (approximately 534 mμ) in the Klett colorimeter. A calibration standard was prepared with standard α-tocopherol. Thus the total, α-, and non-α-tocopherols in all samples were estimated.

Results and Discussion

Table I lists mean and extreme values of oil and fatty acid contents of the 34 varieties of pecans. The combined percentages of oleic and linoleic acids were remarkably constant (approximately 89%) in all samples. This phenomenon was also reported by Bailey et al. (23). GLC analysis of the esterified oil showed the presence of IPG, palmitate, stearate, oleate, and linoleate, as well as a sixth peak that was neither eicosenoate or linolenate.

Mass spectra of methyl esters on the GC-MS instrument agreed qualitatively with published spectra (24–26). When these spectra were compared with reference standard spectra obtained in the same manner, excellent quantitative agreement was noted with one exception. The last peak to elute in the gas chromatograms represented such a small amount of material that the spectra represented tailing from the preceding peak, which proved to be linoleate.

This component, thought to be methyl linolenate, was isolated from the mixture of methyl esters by using argentation TLC to isolate the small amount of material which migrated in the position of a triunsaturated fatty acid. This material was eluted from the plates and chromatographed on the GC-MS instrument. The retention of this triunsaturated methyl ester was identical to that of the minor component observed previously, and successive mass spectra of this peak were all in excellent agreement with reference standard linolenate, indicating that this component was a homogeneous peak of methyl linolenate.

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, methyl linoleate, and methyl linolenate.

The fact that some other fatty acid esters were not eluted, unresolved from the major peaks, was indicated by obtaining successive spectra as each peak was eluted. The utility of this procedure for determining the number of components under a single peak was recently demonstrated by Ryhage et al. (27) in work with milk fat.

TABLE I

Mean and Extreme Values (%) for Fatty Acid and Oil Content of 34 Varieties of Pecans

	Oil	Palmitic	Stearic	Oleic	Linoleic	Linolenic
Mean	71.48	8.40	0.96	57.96	30.91	.....
Extremes	65.5—75.2	4.9—11.3	0.9—5.8	48.7—68.5	19.1—39.6	Trace—2.7

The conclusion that carotenoids were responsible for the yellow color of pecan oil was based on the fact that the color disappearance, measured on the Klett colorimeter, which accompanied oxidation of the oil was also accompanied by absorbancy decrease at 452 mμ as measured on the Cary Model 14 spectrophotometer. The nonmobile band in TLC separation of the nonsaponifiables was shown to be a yellow material with a broad band absorbing maximally at 452 mμ. The rate of decrease of absorbance at 452 mμ 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 at an increased rate of deterioration, as shown by the plot of mean absorption versus time of oxidation in Fig. 1. This indicated that carotenoids inhibited the oxidation of the fat by acting as substrates rather than as antioxidants for oxidative reactions. Plots of data for each variety revealed that oxidative deterioration was similar in all samples. Table II shows peroxide, TBA, and absorbancy values of the eight different oils over the oxidation period.

It was clear that, at the early stages of deterioration, little oxidation was taking place. This was, no doubt, partly because carotenoids were present and partly because such behavior is typical of free radical reactions (9) where, during initiation, free radical concentration is minute but increases rapidly when the reaction progresses to the propagation steps. Perkings (28) demonstrated this property in oils undergoing oxidation.

From peroxide values of Table II the following order of decreasing relative stabilities was found after four days of oxidation: Barton (1.00), Hays (0.57), Kentucky (0.52), Major (.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 of oxidation.

The 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 relative stability, could be expected since the TBA test measures terminal products of oxidation which are directly related to rancidity; the peroxide method detects peroxides which are formed in the earlier stages of the oxidative process. Table II indicates that 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.

It was shown by Gunstone and Hilditch (4) that the relative rates of oxidation of a mixture of oleate, linoleate, and linolenate were in the ratio of 1:12:25 respectively. Therefore, to obtain an estimate of effective unsaturation in these pecan oils, the three percentages for the unsaturated acids for each variety (Table I) were multiplied by the corresponding relative rate of oxidation and these products were summed. Comparison of these values with normalized relative stabilities (relative to Barton = 1.00) showed no apparent correlation. Similar results have been obtained in other oils by other workers (29,30).

The degree of unsaturation cannot explain all the differences in stability of oils and fats (29) since other factors are known to affect the process of oxidative rancidity (31).

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 mμ					
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	0.40	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	38	152	302	501
Texas Prolific	0	122	207	457	647
Stuart	0	44	167	361	654
Texas 60	0	35	50	104	527
Kentucky	0	31	85	262	769
Barton	0	16	27	42	102
Major	0	32	140	315	650
Hays	0	28	72	140	555

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

In looking for other causes of pecan oil instabilities, the nonsaponifiable fraction was investigated. The TLC of the nonsaponifiable fraction of pecan oils showed four spots after spraying (Fig. 2). Spot I, which remained close to the origin, contained the yellow carotenoids as confirmed by visible spectra of the eluted band.

Eluates from Zones II and III showed a single maximum at 299 mμ, indicating that these bands were tocopherols. Zone IV ($R_f = 0.84$) (Emmerie-Engel

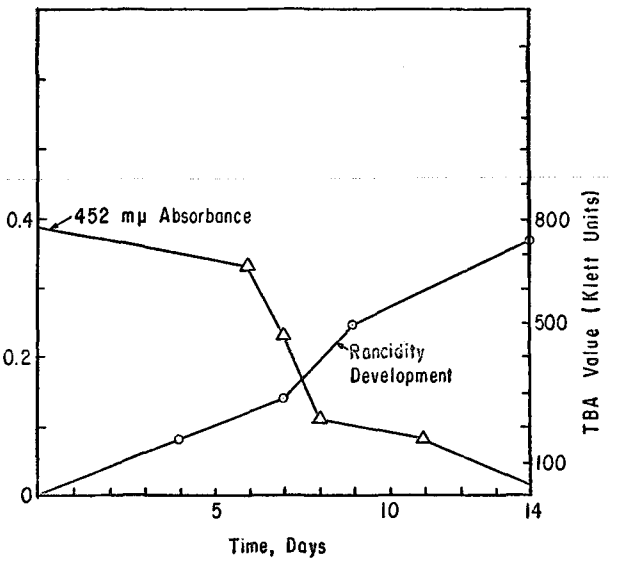

FIG. 1. Variations in the average TBA values and pigment absorbancy versus time in pecan oils during oxidative deterioration.

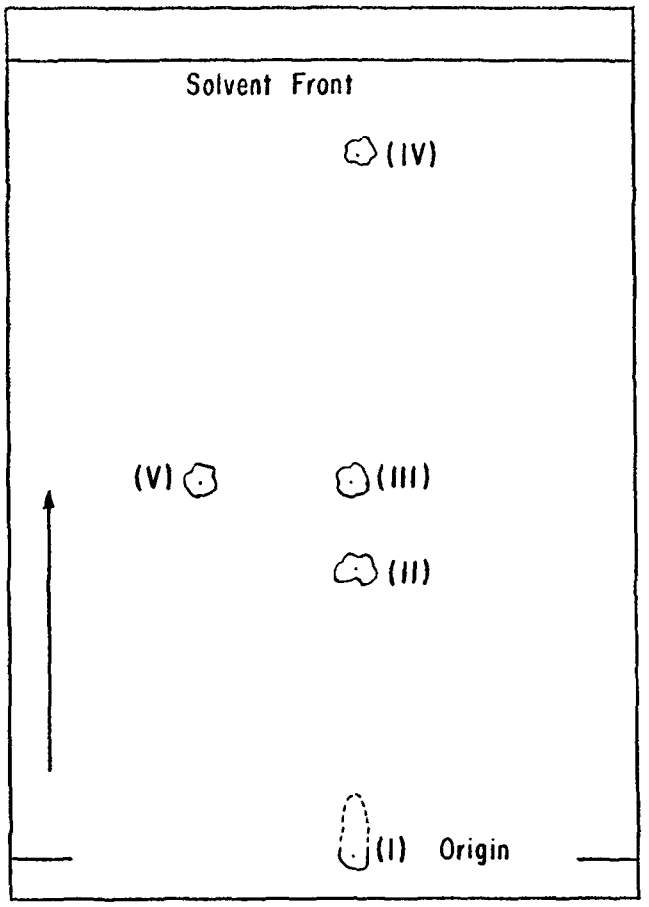

FIG. 2. Typical thin-layer chromatogram of the nonsaponifiable portion of pecan oils on Silica Gel G-coated plates, developed in chloroform. (Arrow shows direction of solvent movement).

positive) was eluted, but no absorption maximum was observed in either the visible or UV region. The R_f value of Spot III (0.45) corresponded to the reference known α-tocopherol; Spot II had an R_f value of about 0.35 and corresponded to β- or γ-tocopherol or a mixture of both (21).

Total tocopherols were plotted versus the reciprocal normalized (Barton = 1.00) peroxide values (Table II) after four days of oxidation. The best-fitting straight line obtained by the method of least squares had a slope of 0.63 and a (Y) intercept of 0.02. Although the points were somewhat scattered, the results indicated that the stability of the oil was related to higher tocopherol content since the stability of the oils increased as the total tocopherols increased. A similar treatment of the TBA values led to the same conclusion except that the closest-fitting straight line had a lower slope (0.20) and a higher (Y) intercept (0.56) than that which was obtained with peroxide values.

A plot of tocopherol content of pecan oils versus stability, after correcting for effective unsaturation, was also made. To correct for effective unsaturation, differences of each variety from the mean unsaturation for all varieties were multiplied by the reciprocal normalized peroxide values (Barton = 1.00) after four days of oxidation, and the new values were plotted against total tocopherol content. The best-fitting straight line had a slope of 0.31 and a (Y) intercept of 0.28; the points for six of the varieties fell considerably closer to the best-fitting straight line.

Thus the majority of pecan oils were plotted much closer to a straight line when both tocopherol content and effective unsaturation were taken into account; both factors have an important influence on pecan oil stability. However the deviations from the best-fitting straight line of two varieties were sufficiently great to suggest that still other factors may be involved. Further, the data indicated that the naturally occurring carotenoids have an effect on oil stability at a substrate level rather than a catalytic level.

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