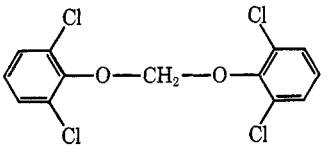
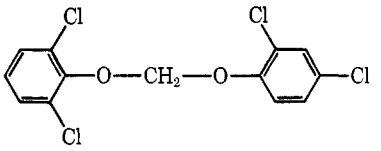
and **6**. Structures **5** and **6** were synthesized and shown to be identical to I and II, respectively, by comparison of glc retention time, on two columns and mass spectra (Table III).



5



6

Semiquantitative analysis of the commercial sample of 2,4-D used in this study indicated that compounds I, II, and III were present at levels of 1, 10, and 30 ppm, respectively. The toxicological significance of these three compounds as impurities in production grade 2,4-D is not known. However, from a study of the teratogenic effects of both production grade 2,4-D and purified 2,4-D (Khera, 1972), it appears that compounds I, II, and III have no adverse effects at the levels administered in his investigation.

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Varietal Differences and Seasonal Effects on Fatty Acid Composition and Stability of Oil from 82 Peanut Genotypes

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Eighty-two peanut genotypes of diverse genetic background were examined over a 3-year period for varietal differences and seasonal effects on fatty acid composition and oil stability (autoxidation induction period). The range in oil stability among genotypes was 11.6 to 18.5 days and the ranges in fatty acid values were: 7.4 to 12.9% palmitic; 1.6 to 5.3% stearic; 35.7 to 68.5% oleic; 14.1 to 40.3% linoleic; 0.9 to 2.2% arachidic; 0.6 to 2.0% eicosenoic; 1.3 to 5.1% behenic; and 0.6 to 2.0% lignoceric acid. Yearly mean fatty acid values for all varieties showed relatively small but significant ($p < 0.01$) yearly variations in fatty acid composition. Yearly variations in oil stability values were large and could not be accounted for by yearly variations in fatty acid composition. Simple regression of oil stability on various fatty acids or combinations thereof showed significant correlations within a given year but with wide variations in magnitude of r^2 and estimated regression coefficients among years.

It has been shown that oils obtained from different botanical types of peanuts (*Arachis hypogaea* L.) differ considerably in tendency to develop oxidative rancidity and that this tendency is related, at least in part, to the content of linoleic acid (Fore *et al.*, 1953; Higgins and Holley, 1951; Holley and Hammons, 1968). Crawford and Hilditch (1950) called attention to the differences in oleic and linoleic acid content of peanut oil from different sources and suggested that these differences should be reflected in oil stability. In consideration of these differences and their probable bearing on liability to oxidative rancidity, Crawford and Hilditch (1950) advocated the commercial production of varieties low in linoleic acid. In an investigation of the relationship between raw peanut oil composition and stability, Fore *et al.* (1953) found linoleic acid to be a factor in the development of oxidative rancidity but were unable to explain all observed differences in stability on the basis of either linoleic acid or tocopherol content. The stability values obtained by Fore *et al.* (1953) were approximately twice those reported earlier (Fisher *et al.*, 1947) for a freshly refined, bleached, and deodorized oil of similar linoleic acid and tocopherol content. As a result of these and other observations, Fore *et al.* (1953) suggested that the rate of autoxidation of crude peanut oil is influenced by antioxidants and/or synergists other than tocopherols and that these components are removed in refining.

Holley and Hammons (1968) reported a correlation of −0.92 between linoleic acid content and oil stability. This

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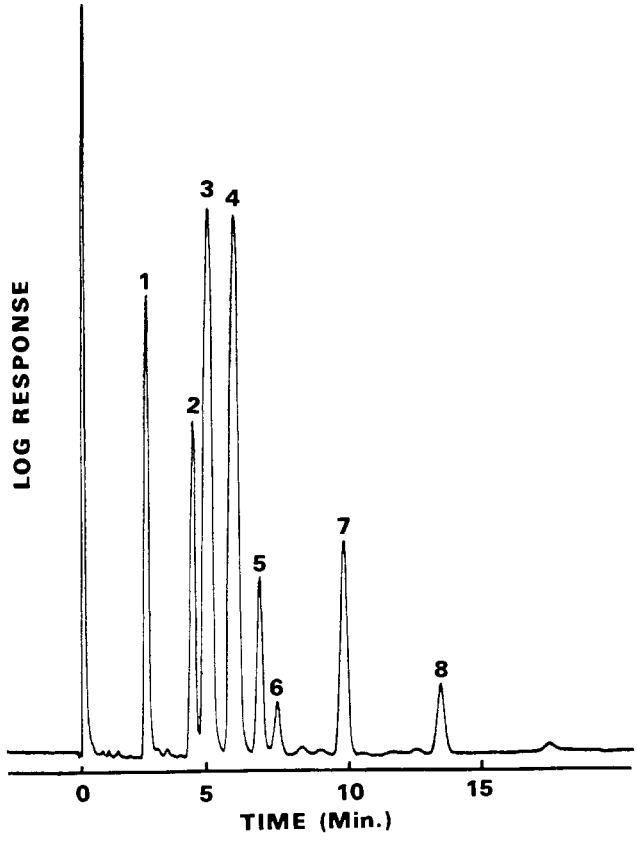

Figure 1. Typical gas–liquid chromatogram of peanut oil fatty acid methyl esters. Peak identifications: 1-methyl palmitate; 2-methyl stearate; 3-methyl oleate; 4-methyl linoleate; 5-methyl arachidate; 6-methyl eicosenoate; 7-methyl behenate; 8-methyl lignocerate

correlation was based on 66 varieties grown during one season and analyzed for linoleic acid content by the iodine–thiocyanogen procedure. These authors reported large yearly variations in the stability of oils from 26 varieties, but did not account for this variation.

The limited data available show considerable variation among peanut genotypes for both major and minor fatty acids (French, 1962; Worthington and Holley, 1967; Worthington and Hammons, 1971). However, extensive data of this nature are not available. Considerable attention has been given to the genetic variability in fatty acid composition of other seed oils (Kinman and Earle, 1964; Knowles, 1965; Yermanos *et al.*, 1966; Jellum, 1970) and information of this nature has been used to effect desirable changes in the fatty acid composition of new varieties of safflower (Knowles *et al.*, 1965) and rape (Stefansson and Hougen, 1964). Since fatty acid composition is important both from the standpoint of oil stability and possible physiological effects of some of the fatty acids (Grande *et al.*, 1970; Kritchevsky *et al.*, 1970; McGandy *et al.*, 1970), this factor should be taken into account in the future development of new varieties.

The present investigation was undertaken to determine the variability in major and minor fatty acid composition and in oil stability among peanut genotypes of diverse genetic origin. Eighty-two genotypes were examined during three growing seasons to determine yearly variation in oil stability and its relationship to yearly variation in fatty acid composition.

A statistical evaluation of the sampling and analytical procedures employed in this study was made to determine the magnitude and source of variance (experimental error).

EXPERIMENTAL PROCEDURES

Genotypes. Selection of the 82 genotypes was based either upon prior knowledge of chemical composition (Holley and Hammons, 1968) or upon possible use in the development of new commercial varieties. Virginia (*A. hypogaea* subsp. *hypogaea*) and Spanish (subsp. *fastigiata*) botanical types, varying widely in fatty acid composition, seed size, growth habit, duration, and other characteristics, were included in the group, as were several representative commercial cultivars. All genotypes were grown at the Coastal Plain Station, Tifton, Georgia, in 1965, 1967, and 1968, employing standard cultural practices (McGill and Samples, 1969). At harvest the plants were dug, allowed to dry in the windrow for 24 to 48 hr, stacked, and cured in the stack for 6 to 8 weeks prior to picking.

Sample Preparation and Analysis. Samples were hand-shelled and stored at 4–5°C until time of analysis. Since fatty acid composition is influenced by seed maturity (Worthington, 1969) only those seeds that appeared to be fully mature, as indicated by seed size, absence of wrinkles, and by pigmentation of seed coat and interior of pericarp, were retained for analysis. Oil was expelled with a silver-plated Carver press, filtered through Whatman paper, and the autoxidation induction period measured by the method of Olcott and Einset (1958) as modified by Young and Holley (1965). Oil samples (0.2 ml in 10-ml beakers) were heated in a forced draft oven at 60°C and weighed at daily intervals.

Fatty acid methyl esters (FAME) were prepared by transmethylation with a 3% solution of sulfuric acid in methanol, as previously described (Jellum and Worthington, 1966) and held at −20°C under nitrogen until time of analysis.

Analyses of FAME were made on either an F&M Model 700, or a MicroTek Model 220 gas chromatograph, equipped with an Infotronics electronic integrator. Glass columns, 1.85-m × 4.0-mm i.d., were packed with 70/80 mesh Chromosorb W (AW) (DMCS) coated with 10% (w/w) stabilized diethyleneglycolsuccinate (Analabs, Inc.). During the last 2 years of the study column packings were prepared by the filtration–fluidization technique (Kruppa *et al.*, 1967). Column efficiencies generally exceeded 400 theoretical plates per column foot, as measured with methyl stearate at a column temperature of 200°C and a helium flow of 100 ml/min.

Column temperature was generally programmed from 195 to 225°C at 3°C per min. However, these parameters were varied slightly, depending upon column characteristics, to maintain adequate resolution of peaks. The detector and injection port were maintained at 300 and 275°C, respectively, and the helium flow rate was held at 100 ml/min. On-column injections of approximately 0.1 μl of methyl esters were made with a Hamilton 7101N 1-μl syringe. Percentage fatty acid composition was determined by digital integration and normalization of peak areas; the values reported are therefore relative proportions of total fatty acids. A typical chromatogram of peanut oil is shown in Figure 1.

The accuracy of the system was monitored with either National Heart Institute type fatty acid standard KD (Applied Science Laboratories) or with the appropriate standard recommended in American Oil Chemists' Society tentative method Ce 1-62 (Analabs, Inc. Number 0013). Analysis of standards gave results that agreed with the stated composition with a relative error of less than 3% for major components (>5% of total mixture) and less than 7% for minor components (<5% of total mixture).

Iodine numbers were calculated from the fatty acid values and are therefore 6 to 7% higher than would have been ob-

Table I. Estimate of Components of Variance and Standard Deviation Associated with Sampling and Analytical Procedures

	Fatty acid							
	16:0	18:0	18:1	18:2	20:0	20:1	22:0	24:0
Components of variance[a]								
Sampling	−0.003	0.012[d]	0.099[d]	0.046	0.002	0.000	0.001	−0.001
Preparation of methyl esters	0.068[e]	0.018[e]	0.214[e]	0.119[e]	0.008[e]	0.000	0.057[e]	0.009[e]
Gas-liquid chromatography	0.026	0.015	0.061	0.048	0.006	0.004	0.014	0.002
Sample mean (%)[b]	11.49	3.97	43.72	33.30	1.90	0.89	3.46	1.26
Estimated standard deviation[c]	0.30	0.22	0.61	0.46	0.13	0.06	0.27	0.10

[a] Components of variance are based on four replications for each step. [b] Mean of the two varieties used in the determination of components of variance. [c] Estimate of the standard deviation expected with experimental design of one sample, one methyl ester subsample, and one gas–liquid chromatographic analysis. [d,e] Mean square significant at the 5% and 1% level, respectively.

tained by direct measurement. This difference is due to the fact that the eight fatty acids measured in this study (Figure 1) account for approximately 93 to 94% of crude peanut oil, with the remaining 6 to 7% composed of other constituents, including the glycerol portion of triglycerides.

Evaluation of Sampling and Analytical Procedures. Estimates of the reliability of the actual laboratory procedure were provided by a repeated subsampling or nested sampling technique (Anderson and Bancroft, 1952). This procedure was used to estimate the components of variance associated with sampling and preparation of oil samples, preparation of methyl ester subsamples, and analysis by gas–liquid chromatography (glc). Two varieties were chosen for this part of the study. Four oil samples were prepared from each variety, four methyl ester subsamples were prepared from each oil sample, and each methyl ester subsample was analyzed by glc on four different days. In addition, oil samples were tested in duplicate for oil stability and the data were subjected to an analysis of variance.

Analysis of Genotype Data. In the study of the 82 genotypes, one gas–liquid chromatographic analysis was obtained from a single methyl ester subsample prepared from each genotype each of the 3 years. Sample material was maintained in cold storage during the course of the study and those genotypes showing unusual year to year variations in fatty acid composition were reexamined for possible errors in glc or preparation of methyl esters.

The data were subjected to analysis of variance to determine the importance of year effects and of genotype on fatty acid composition and oil stability. Simple regression of oil stability on iodine number, linoleic acid, and two combinations of linoleic and oleic acid were made with data obtained each year to determine the probable significance of these factors on oil stability. These regressions were tested for statistical differences of constants and coefficients over years to determine if one set of equations could be estimated for all years.

RESULTS AND DISCUSSION

Evaluation of Sampling and Analytical Procedures. Estimates of the components of variance associated with the various steps in experimental procedure and of the standard deviation to be expected from an experimental design of one sample, one methyl ester subsample, and one glc are presented in Table I. Although variance ratios indicated highly significant differences among methyl ester subsamples for all fatty acids except 20:1, the magnitude of differences was small. The level of variance associated with the subsample was due largely to one subsample which fell well outside the normal distribution pattern and was apparently the result of an error in preparation and extraction of methyl esters. At the glc level the variance was uniformly low. Differences among oil samples were quite small, though significant ($p < 0.05$) for two fatty acids, 18:0 and 18:1. Oil stability values did not vary significantly among samples. An average induction period of 12.37 ±0.65 days was obtained.

The low level of variance estimated for one sample, one methyl ester subsample, and one glc indicates a high degree of reliability for the procedure.

Table II. Yearly Mean Values and Standard Deviations for Oil Stability Values, Iodine Numbers, and Fatty Acid Percentages for 82 Peanut Genotypes

	1965		1967		1968	
Variate	Mean	±	Mean	±	Mean	±
Oil stability	15.07	2.19	16.62	2.62	10.70	1.44
Iodine no.	97.46	6.06	99.60	5.31	101.55	6.31
Palmitic	10.73	1.79	10.88	1.80	10.52	1.69
Stearic	3.61	0.83	3.38	0.69	3.23	0.85
Oleic	50.12	10.05	49.12	9.03	50.34	10.09
Linoleic	28.48	8.20	30.13	7.21	30.64	8.33
Arachidic	1.52	0.31	1.65	0.26	1.36	0.26
Eicosenoic	1.05	0.28	1.10	0.29	1.00	0.27
Behenic	3.08	0.79	2.67	0.79	2.10	0.51
Lignoceric	1.37	0.46	0.98	0.40	0.68	0.22

Genotypes. The eight fatty acids measured in this study account for approximately 98% of the total fatty acids of peanut oil. Traces of several additional fatty acids (Iverson *et al.*, 1963) were frequently observed. A number of genotypes were examined for linolenic acid, and in those samples examined the level of this fatty acid did not exceed the level previously reported for peanut oil (Worthington and Holley, 1967).

Differences in fatty acid composition among the 82 genotypes (and consequently iodine number) were quite large. The ranges in fatty acid composition of oil (percent of total fatty acids) among genotypes (3-year averages) were as follows: palmitic 7.4–12.9; stearic 1.6–5.3; oleic 35.7–68.5; linoleic 14.1–40.3; arachidic 0.9–2.2; eicosenoic 0.6–2.0; behenic 1.3–5.1; and lignoceric 0.6–2.0. Gentoype differences in length of oil autoxidation induction period were also large and ranged in value from 11.6 to 18.5 days. Yearly mean values and standard deviations for all genotypes are given in Table II. Although the yearly variations in average fatty acid values were small, analysis of variance showed both year and genotype effects to be highly significant for all fatty acids and for oil stability. The most striking result was the year effect on oil stability. In general the Virginia types, particularly the late maturing large seeded varieties, were higher in oleic and lower in linoleic acid than the earlier maturing Spanish types. Yearly fatty acid, oil stability, and cal-

Table III. Regression of Oil Stability on Iodine Number and Various Fatty Acids by Years

Independent variables		Year[a] 1965	1967	1968
Iodine no.	Regression constant	30.50	56.44	26.53
	Regression coefficient	−0.158	−0.400	−0.156
	t (regression coefficients)	−4.35	−12.37	−8.41
	r^2	0.192	0.657	0.469
Linoleic acid	Regression constant	18.56	26.07	14.29
	Regression coefficient	−0.122	−0.313	−0.127
	t (regression coefficients)	−4.61	−15.26	−8.30
	r^2	0.210	0.744	0.463
Oleic acid/linoleic acid	Regression constant	14.01	12.45	9.01
	Regression coefficient	0.492	2.236	0.859
	t (regression coefficients)	3.01	14.80	9.49
	r^2	0.102	0.733	0.529
Oleic acid + 10× linoleic acid	Regression constant	19.74	29.13	15.45
	Regression coefficient	−0.0139	−0.0357	−0.0133
	t (regression coefficients)	−4.61	−15.17	−8.33
	r^2	0.210	0.742	0.464

[a] The differences in regression coefficients and intercepts among years were significant at the 1% level for all independent variables.

culated iodine values for each genotype will be published elsewhere.

The regression of oil stability on iodine number and various combinations of fatty acids are given in Table III. The intercepts and coefficients obtained with each year's data were significantly different and for this reason the data were not pooled to obtain regression equations based on the 3 years' data. The size of the respective *t* values for the regression coefficients indicates that all independent variables are useful as indicators of oil stability. Iodine number does not take into account the much greater susceptibility of polyenes to autoxidation. However, only in 1967 was it the poorest indicator of oil stability. The reaction rate of linoleic acid in autoxidation has been reported to be approximately one order of magnitude greater than oleic acid (Gunstone and Hilditch, 1945); this fact was taken into account in the independent variable, percent oleic acid + 10× percent linoleic acid. Nevertheless, this variable proved to be no better than percent linoleic acid alone as an indicator of oil stability. The remaining variable, percent oleic acid ÷ percent linoleic acid, was the poorest indicator in 1965 and the best indicator in 1968. Of greater significance is the yearly or seasonal variation in the magnitude of r^2. For example, regression of oil stability on percent linoleic acid indicates that 21% of variation in oil stability in 1965, 74% in 1967, and 46% in 1968 was correlated with variation in linoleic acid content. All variables proved to be relatively poor indicators in 1965.

The data obtained in this study indicate that the seasonal or yearly effect on oil stability is very pronounced. This yearly variation, reported earlier by Holley and Hammons (1968), has been shown in this study to be largely unrelated to levels of linoleic acid. The source of this variation is unknown, but appears to be related to factors which are seasonal in nature—*i.e.*, yearly variations in environmental conditions prevailing during seed formation. Yearly differences in seed treatment during curing and handling are an additional possible source of variation in oil stability; however, the contribution from this source is believed to be small.

Of considerable importance is the wide genetic variability, both in fatty acid composition and oil stability. The magnitude of this diversity should permit the development of new commercial varieties with desired levels of unsaturation and improved stability characteristics.

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