Chemical Studies on Oil Bearing Seeds. I. Okraseed*

J. R. CLOPTON, AMMARETTE ROBERTS, and H. A. JESKEY†, Texas Research Foundation, Renner, Texas

A HEAVY demand for fats and oils during the war and the period following, together with decreased production at home and seriously reduced imports, has stimulated unusual interest in the development of new sources of vegetable oils. Among the various oilseed yielding plants investigated okra poses itself as one of the potential sources of valuable edible oils.

Okra, known botanically as *Hibiscus esculentus L.*, is commonly called "gumbo" and is a well known tropical and subtropical garden plant which has been cultivated as a food for approximately eight centuries (2). Botanically it is closely related to cotton and is somewhat similar to cotton in appearance although its leaves and stems are larger and coarser than those of cotton. Like cotton, okra is subject to root rot and cotton wilt but is otherwise relatively resistant to disease.

The okra plant is native to Africa. However, it is grown extensively in the southern states as a vegetable crop. The edible portion consists of the pods and their contents, which must be gathered while still green and tender. When cultivated for seed purposes or as an oilseed crop, the pods and seeds are allowed to mature fully before harvest.

Until recently it has been impractical to cultivate okra as an oil seed crop because of the loss of seed incurred by shattering of the pods during maturation and harvest. However, several new non-shattering varieties have been developed including the Dwarf Louisiana Green Velvet and the Louisiana Green Velvet, the latter giving best yields and recommended as most suitable for oilseed production, especially where harvesting is done by combine. The present yields of seed obtainable from okra, the ease of harvesting and processing, and the high edible quality of the oil focuses more than usual attention on its potentialities as an oilseed crop. Yields of 1,200 to 2,000 pounds of seed per acre have been reported on the rich delta soils of Louisiana. Field plantings in 1945 and 1946 on the blackland prairie soils of the Texas Research Foundation near Dallas, Texas, gave yields of 600 to 1,000 pounds per acre when combined. However, under more favorable cultural conditions small plots on the same soil, a 1947 crop of Louisiana Green Velvet gave an extraordinary yield of 2,800 pounds of seed per acre when hand harvested.

Much credit is due Julian C. Miller and his associates (3) of the Louisiana State University for the development of the new round-podded, non-shattering varieties of okra mentioned above. The cultivation and harvest of okraseed have been discussed elsewhere (1, 3, 7, 8). It grows best on sandy loam or loamy clay which drains well and does not become waterlogged.

The primary purpose of this report is to present the results of investigations on the characteristics and chemical composition of okraseed and okraseed oil which have been conducted in the laboratories of the Texas Research Foundation during the past two years. The work has been prompted by the growing importance of okra as a potential oilseed crop. Very little recent data on the composition of the seed and oil has been presented, and the newer techniques of oil and fatty acid analysis have not heretofore been utilized in okraseed oil studies.

A review of the literature indicates that until recently the chemical composition of okraseed oil has been accepted as substantially that which was reported by Jamieson and Baughman (5) in 1920. Analyses reported in recent papers (3, 4, 7) either quote previously published figures (5) or report values differing insignificantly from them (7). Among other things, evidence is presented herein to indicate that the fatty acid composition of okraseed oil differs in several respects from that reported in the literature.

Composition of the Okraseed

All okra seeds examined were taken from those harvested from experimental plots of the Texas Research Foundation. Analyses were performed on seeds which were first ground to pass a 40-mesh sieve. Moisture determinations were made so that results of the analyses could be calculated back to the moisture-free basis. The procedures used for the seed analyses were those listed as official by the Association of Official Agricultural Chemists Manual (1945).

The composition of okraseed is given in Table I. For purposes of comparison similar analyses were performed on cottonseed and the data, along with corresponding data from the literature, are also included in the table.

TABLE I

Chemical Composition of Okraseed and Cottonseed

	Okraseed		Cottonseed	
	Literature %	This Lab. %	Literature %	This Lab. %
Moisture	5.7-9.1	13.2	.....	9.9
Oil	14.7-22.0	14.5-16.0	20.21	21.6
Protein (N % x 6.25)	19.8-26.0	23.5-25.1	20.74	21.5
Crude Fiber	19.7-27.6	26.3	24.0	25.1
N-Free Extract	35.0	24.1	35.0	31.8
Ash	2.9-4.9	3.9	4.9	5.2

The above data show that the oil percentages of okraseed is somewhat lower than that of cottonseed. However, it compares favorably with that of the soybean. The protein content of okraseed is slightly greater than that of cottonseed and, so far as it is known, it probably does not contain gossypol or other toxic substances.

A rather hard and brittle hull encloses the kernel of the okraseed. With the development of a means of dehulling, less useful portions of the seed could be removed before processing. Much higher oil and protein content would be available from the kernels than from the seed as a whole.

Although Table II shows the percentage of protein in dehulled okraseed as 45.4 after oil extraction, the

* Presented at the 39th Annual Meeting of the American Oil Chemists' Society, New Orleans, La., May 4, 1948.

† Associate professor of chemistry, Southern Methodist University, Dallas, Texas.

TABLE II

Composition of Okraseed Components

	Whole Seed	Meat	Hull
Oil	14.46	29.47	0.66
Protein	24.86	45.40	6.26
Crude Fiber	29.19	4.9	47.03
Total Sugars (as glucose)	2.43	.....	.....
Pentosans	21.33	4.8	37.1

oil-free meal would actually contain about 64.5% protein, which compares with that of nut meats and meat scraps. The dehulled and extracted okraseed meal would prove valuable as a protein supplement in feeds and may prove valuable in the manufacture of plastics, fibres, protective coatings, and adhesives.

Characteristics of Okraseed Oil

The okraseed oil used in the study of its characteristics and fatty acid analyses was obtained from the ground seeds by solvent extraction with petroleum ether. Characteristics of the okraseed oil and those of cottonseed oil as shown in Table III were determined by American Oil Chemists Society Official Methods (1946). The data also contain values obtained from the literature. It may be observed that okraseed oil and cottonseed oil are somewhat similar in properties. The principal difference between the two oils is in the iodine value. The lower value in okraseed oil is accounted for by a higher percentage of saturated fatty acids (mainly palmitic acid) and a lower percentage of linoleic acid. Values obtained in this laboratory agree for the most part with those already reported in the literature (4, 5, 6). Okraseed oil obtained by solvent extraction is reddish brown in color while the cold pressed oil is green, presumably due to the presence of chlorophyl.

Okraseed oil is classified, on the basis of its iodine value, as a non-drying oil, and in this respect it may be considered better suited for edible purposes than cottonseed oil. Edwards and Miller (3) report that refined okraseed oil resembles a good grade of peanut oil and that hydrogenated okraseed oil has a flavor keeping quality favoring its unlimited use in shortenings. However, the flavor stability of unhydrogenated okraseed oil is questionable.

Fatty Acid Composition of Okraseed Oil Experimental

As a preliminary step in determining the fatty acid composition of okraseed oil it was necessary to first convert the glycerides into methyl esters. This was accomplished as follows: the oil was saponified with alcoholic potassium hydroxide. The soaps were washed with ether to remove unsaponifiable matter and then hydrolyzed with sulfuric acid (50%). The liberated fatty acids were extracted with water until free of sulfuric acid and finally dried with anhydrous sodium sulfate. The ether was stripped off under reduced pressure and the mixture of free fatty acids was esterified with a large excess of absolute methanol using sulfuric acid as a catalyst. After stripping off about two-thirds of the excess alcohol, the remainder was washed out with water which also removed the sulfuric acid. The methyl esters were then dried with anhydrous sodium sulfate.

The methyl esters of the mixed fatty acids of okraseed oil were distilled through a 48-inch glass helice packed column with a partial take-off distilling head of the Penn State type. Distillations were performed at 1 mm. Hg pressure and reflux ratio of approximately 10:1. Out of an original charge of 330 g., 31 fractions were obtained. The refractive index, iodine value, and saponification value determinations were made on each fraction. Also ultra-violet absorption measurements were made on each fraction at 234, 268, and 310 millimicrons, using a Beckmann DU Spectrophotometer, to determine the amounts of linoleic, linolenic, and arachidonic acids respectively following alkali isomerization of each sample according to the method of Mitchell and Kraybill (8). From these data the fatty acid composition of okraseed was obtained. The results are shown in the following table along with data taken from the literature (4) on okraseed oil and cottonseed oil.

TABLE IV

Fatty Acid Composition of Okraseed Oil and Cottonseed Oil

Acid	Okraseed Oil		Cottonseed Oil
	Literature	This Lab.	Literature
Myristic	.....	3.8	1.0
Palmitic	26.0	33.1	20.0
Stearic	3.0	0.5	2.0
Arachidic	0.1	7.9 ?	1.0
Oleic	42.0	41.8	33.0
Linoleic	28.0	13.2*?	43.0

*Determined by summation of the data obtained by spectrophotometric analysis of each fraction resulting from the distillation of the methyl esters of okraseed oil fatty acids.

Discussion of Results

There are several significant differences in the results obtained in this laboratory and those reported

TABLE III

Comparison of the Characteristics of Okraseed Oil and Cottonseed Oil

Determination	Okraseed Oil		Cottonseed Oil	
	Literature (5)	This Lab.	Literature	This Lab.
Specific Gravity, 25°/25°	0.9160-0.9187	0.9170	0.9170-0.9180	0.9161
Titer, C	.....	37.5	32.0-38.0	31.2
Refractive Index (n_d^{25})	1.4692-1.4702	1.46885	1.4743-1.4752	1.46928
Iodine Value (Wijs)		90.7	100.00-115.0	103.5
(Hanus)	93.2-100.3	89.2	.....	.....
Thiocyanogen Value	.....	59.20	61.0-65.0	62.3
Saponification Value	195.5-	199.3	192-200	192.5
Acetyl Value (Roberts & Schuette)	11.5-24.0	8.7	21-25	12.2
Reichert Meissl Value	0.09-0.14	.....	0.95	0.25
Polenske Value	.....	0.32	.....	0.20
Free Fatty Acids (Oleic) %	0.34-1.42	3.09	0.6-0.9	0.04
Unsaponifiable Matter %	0.37	1.03	0.6-2.0	0.45
Saturated Fatty Acids,				
(Bertram) %		31.6		
(Pb-salt) %	29.22	32.94		
Ash		0.256		
Diene Value		1.56		
Hexabromide Test		Negative		
Halphen Test		Positive		

previously in the literature. We obtained 3.8% of myristic acid whereas it has not been reported previously as a constituent of okraseed oil even in small amounts.

Fractional distillation of the methyl esters produced nearly 8% of an ester derived from a 20 carbon acid which may be arachidic, gadoleic, or gadolinic acid; or a combination of two or all three of these. The value of 7.9% for the C_{20} fractions is assumed to be somewhat high because of some polymerization of linolenate during the later stages of the distillation process. The percentage oleic acid we obtained agrees closely with that reported by other investigators. No evidence of linolenic acid could be found either spectrophotometrically (Figure I) or by hexabromide number determination.

With regard to the linoleic acid content of okraseed oil, determinations were made in this laboratory by three different methods, namely 1. thiocyanogen and iodine values, 2. calculations based on iodine and saponification values of the fractions obtained by distillation of the prepared methyl esters of the okraseed oil fatty acids and, 3. ultraviolet spectrophotometric measurements on the individual methyl ester fractions and on the whole oil. Results obtained by the first two methods agree fairly well with one another and also with the value of Jamieson and Baughman derived from the tetrabromide value (5). They are 27.1, 26.1 and 26.6% respectively.

When a summation was made, however, of the values obtained by spectrophotometric measurements on the series of fractions resulting from distillation of the methyl esters of okraseed oil fatty acids a total of only 13.2% linoleic acid was obtained. A similar spectrophotometric measurement made on a fresh sample of raw okraseed oil showed 13.1% linoleic acid.

At first it was suspected that there was some error in application of the spectrophotometric method. In order to rule out this possibility several known mixtures, made up from purified samples of linoleic acid and stearic acid were analyzed by the spectrophotometric procedure. The results, as shown in Table V, show that our procedure was accurate to within 1 or 2%.

TABLE V

Spectrophotometric Analysis of Linoleic-Stearic Acid Mixtures

	Linoleic Acid %			Stearic Acid %		
Theoretical	12.6	26.3	48.6	87.4	73.7	51.4
Found	12.9	25.9	49.4	87.1	74.1	50.6

We therefore were confronted with the conclusion that either okraseed oil contains an isomer of linoleic acid which absorbs iodine in a normal manner but which is not isomerized to the conjugated position by heating 30 minutes at 180 C. in an alkaline ethylene glycol solution according to the method commonly used (8) or that previous reports on the linoleic acid content of okraseed oil are in error and that palmitoleic, and possibly gadoleic acid, are present in sufficient amounts to account for the actual iodine absorbed and previously attributed to linoleic acid.

A second batch of methyl esters were prepared, fractionally distilled, and analyzed as before. The results obtained were essentially the same, with the spectrophotometric data showing the same low linoleic acid content as before.

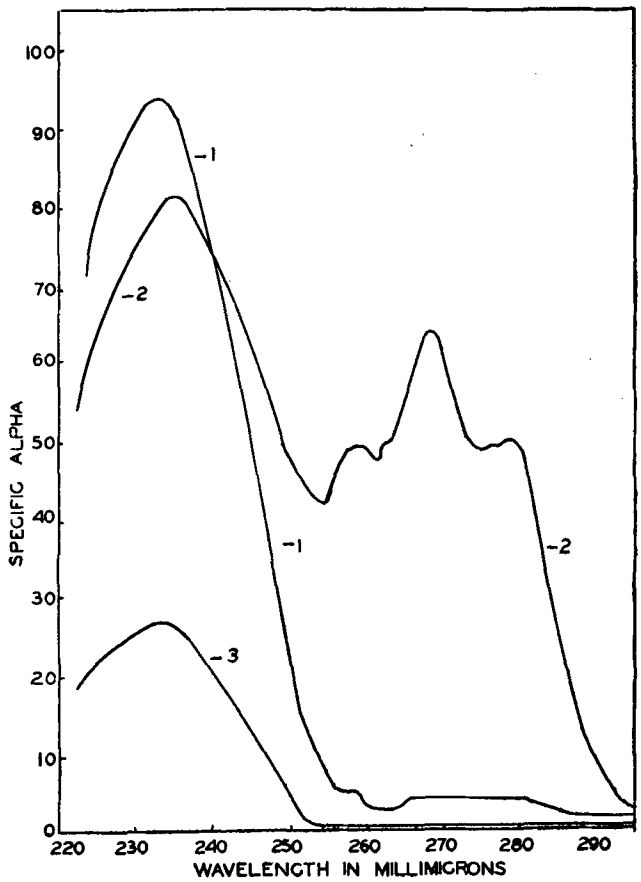

FIG. 1. Comparison of Ultraviolet Absorption Spectra of Methyl Ester Fraction of Okraseed Oil Fatty Acids with Samples of Linoleic and Linolenic Acids.

1. Linoleic Acid, 2. Linolenic Acid, 3. Methyl Ester Fraction of Okraseed Oil.

It is of interest to note that analyses of the methyl ester fractions indicate the presence of 44.8% of C_{14}, C_{16} and C_{20} fatty acids, whereas the total saturated fatty acids obtained by the lead salt ether method was 32.9%. This difference may indicate the presence of one or more unsaturated fatty acids containing C_{14}, C_{16} or C_{20} chains. This is further evidenced by the fact that the methyl ester fractions containing these acids showed iodine values which exceeded that expected from mixtures containing them and the unsaturated C_{18} components.

Similar high iodine values on the methyl ester fractions of C_{18}-C_{20} fatty acids suggest the occurrence of some gadoleic or gadolenic acid in okraseed oil.

However, the general agreement of other methods of analysis showing the percentage of linoleic acid as about 27 instead of 13.2 strongly suggest the presence of a linoleic acid isomer which does not respond in the usual manner toward the alkali isomerization treatment prior to spectrophotometric analysis. It may be postulated that an isomer of linoleic acid whose double bonds are separated by two or more methylene groups would not be conjugated by the above mentioned alkali isomerization treatment and, therefore, would escape detection during spectrophotometric analysis. Also the view may be taken that cis-trans isomerization may account for the apparent low linoleic content by reason of the fact that the cis-trans isomers of linoleic acid have different extinction coefficients and that the isomer having the highest extinction coeffi-

cient predominates in okra seed oil, thus giving a lower calculated value for linoleic acid. At present it is impossible to say which is the correct assumption. However, the evidence seems to favor the belief that a position isomerism, instead of a cis-trans type, exists in the linoleic acid of okraseed oil.

So far as is known the natural occurrence of an isomer of linoleic acid which will not isomerize to the conjugated position when heated with alkali in the manner described above has not been reported before. Edwards and Miller (3) have mentioned a similar discrepancy between the values obtained on okraseed oil by the spectrophotometric method and those obtained by other accepted methods of analysis, but no data are given. Similar discrepancies have been obtained by other investigators during spectrophotometric studies on sesameseed oil. Such an isomer, 9,15-linoleic acid, has been postulated as being present in reverted hydrogenated soybean and linseed oils and is believed to arise from the partial hydrogenation of the linolinate fractions of these oils. The natural occurrence of this, or a similar isomer with isolated double bonds, is entirely a reasonable possibility and is strongly indicated by our observations on okraseed oil.

We are continuing our investigations on the fatty acid composition of okraseed oil for the purpose of proving or disproving the presence of such a linoleic acid isomer in this oil and to positively identify the other unsaturated acids accounting for the amount of iodine absorbed.

If such an isomer occurs naturally in oils, it may place limits on the applicability of the spectrophotometric method of fat analysis or may necessitate modifications in the presently accepted technique.

Summary and Conclusions

Okraseed have been analyzed for their principal constituents. The characteristics of the oil have been determined, and it has been analyzed to determine its fatty acid composition. Characteristics of the oil as determined in this laboratory agree well with values previously reported by other investigators. However, our study of the oil components shows a considerably different fatty acid composition from that recorded in the literature. Myristic acid is reported present for the first time, and the possible presence of palmitoleic acid is indicated. The amount of linoleic acid determined spectrophotometrically does not agree with the percentage arrived at by other accepted methods of analysis. Evidence exists which suggests the presence of an isolated double bond isomer of linoleic acid in okraseed oil. Further work is in progress.

Okraseed can be produced in sufficient yields to make it a profitable oilseed crop (3). The oil is suitable for edible purposes, particularly when hydrogenated and used in shortening or margarine manufacture (3). Extracted okraseed meal should be a desirable protein rich food, being somewhat richer in protein than cottonseed and is not known to be toxic.

REFERENCES

1. Beattie, W. R., Culture and Uses of Okra, U. S. Dept. of Agri., Farmer's Bull. No. 232, 11 pp. (1905) (1940 revision).

2. Culpepper, C. W. and Moon, H. N., The Growth and Composition of the Fruit of Okra in Relation to Its Eating Quality, U. S. Dept. of Agri. Circ. No. 595, 17 pp. (1941).

3. Edwards, W. R. and Miller, J. C., Okraseed Oil. The Chemurgic Digest *6*, 29, 31-3. (Jan. 31, 1947).

4. Jamieson, G. S., Vegetable Fats and Oils, Ind. Ed., Reinhold Publishing Co., p 195 (1943).

5. Jamieson, G. S., and Baughmann, W. F., Okraseed Oil, J. Am. Chem. Soc. *42*, 166-70 (1920).

6. Halverson, J. O. and Naiman, B., Chemical Composition of Okraseed. J. Oil and Fat Ind. *3*, 386 (1926).

7. Markeley, K. S. and Dollear, F. G., Okra As a Potential Oilseed Crop, Chemurgic Paper No. 576 (1947 Series, No. 3).

8. Mitchell, J. H., and Kraybill, H. R. and Scheile, F. P., Quantitative Spectral Analysis of Fats, Ind. Eng. Chem., Anal. Ed. *15*, 1-3 (1943).

9. Woodroof, J. G., Okra. Georgia Expt. Sta. Bull. No. 145, 164-85 (1927).

Micro-Methods in Lipide Chemistry

NORMAN KRETCHMER,* Department of Physiological Chemistry, University of Minnesota, Minneapolis, Minnesota. Departments of Biochemistry and Pathology, University of Vermont, Burlington, Vermont

MOST of the methods of lipide chemistry have generally been applied to large samples of material and very little attention has been centered on micro-analysis. Techniques for the analysis of small samples of lipides have distinct and definite purposes in biology. In the analysis of small biological samples it is impossible to use classical lipide methods since these determinations require much more material than can often be obtained from a biological sample. Recently there has been considerable literature stressing the micro-analysis of lipides and their component parts. Due to the fact that this is the case and that the literature concerning these micro-analyses is widely scattered, it was felt that this is an opportune time to review the present status of the literature and to point out the way in which some of these analyses can be applied to biology.

This discussion will include the micro-analysis of main categories of lipide substances, the analysis of their component parts, and finally the analysis and determination of fatty acids.

Reviews which take up this work in part have been presented by Piskur (1) and Thannhauser (2).

Extraction

Stetten (3) has devised a liquid-liquid extractor which can be used for samples of about 10 mgms. in size and utilizes the principles employed in the Soxhlet extractor. Stetten employed this micro-extractor for various fatty acids and obtained complete extraction, in most cases, in three hours. The apparatus was applied, in this laboratory, to re-extract an alcohol-ether extract of lipide with petroleum ether (boiling point 30°-60°C.). Complete extraction was obtained in approximately two hours.

Bloor (4) has suggested a procedure for the extraction of approximately 50 mgms. of sample as follows

* Present address: Department of Pathology, Long Island College of Medicine, Brooklyn, New York.

The author would like to acknowledge the assistance extended by W. O. Lundberg and C. P. Barnum in the preparation of this manuscript.