The Analysis and Characterization of the Oil from the Seed of *Citrullus colocynthis*[1]

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CITRULLUS COLOCYNTHIS, commonly known as Colocynth, is a species belonging to the botanical family of *Cucurbitaceae*. The plant is perennial, and it has a dry fruit which contains seed representing 75% of its weight (9). It grows in Morocco, Algeria, Sahara Desert, India, and elsewhere (7, 3).

Power and Moore (9) identified palmitic, stearic, oleic, and linoleic acids in the mixed acids obtained from oil from the fruit. They determined also some of the characteristics of the oil from the seed, and they were able to isolate and identify a phytosterol in the unsaponifiable matter of that oil. Jamieson (7) cited three references to work on the oil and gave average values for some of its characteristics. Curtis (3) presented data on analysis of the seed for the ash, fat, protein, etc., and compared them to those of soybean.

The purpose of this investigation was to determine the characteristics of the oil from the seed and to make a quantitative estimation of its component fatty acids.

Characteristics of the Oil

The seed used in this work were obtained from the Sahara Desert, 300 miles south of Algiers, Algeria (Africa) in December, 1944, by L. C. Curtis, formerly with the National Cottonseed Products Association Inc. The oil was obtained from the seed by extracting the finely ground meal with Skellysolve F in a percolator and then distilling off the solvent under reduced pressure. The oil had a light yellow color and a faint agreeable odor. The seed contained about 18% oil.

The physical and chemical characteristics of the oil were determined according to the methods of the Association of Official Agricultural Chemists (1) except for the unsaponifiable matter for which the method described by Jamieson (8) was used. These characteristics are shown in Table I.

TABLE I

Characteristics of the Oil

Specific gravity d_{25}^{25}	0.9200
Refractive index n_D^{25}	1.4741
Iodine value (Wijs)	129.1
Saponification value	191.6
Acetyl value	15.05
Acid value	1.77
Unsaponifiable matter %	1.01
Saturated acids %	16.6
Oil content of seed %	18.1
Tetrabromide m. p.	114.0°

Determination of the Fatty Acids

This has been attempted by three methods for a comparison of the different techniques.

The first method was the method of Hilditch, in which the oil was saponified with KOH and the mixed acids were separated into practically saturated and unsaturated fractions by the lead salt-alcohol method. The free acids from the saturated fraction had an iodine value of 11.4, and they represented 16.6% of the total acids. The free fatty acids from each fraction were converted into the methyl esters by refluxing for 2.5 hours with 4 times their weight of methanol containing 1% sulfuric acid. The methyl esters were fractionally distilled on a Todd column.

These data are reported in Tables III, IV, and V.

The method of calculation of the constituent methyl esters in the different fractions obtained from fractional distillation is essentially the method used by Hilditch and his coworkers (6).

In the case of unsaturated or liquid fractions (denoted by L in the tables) the following assumptions were made:

1. For ester fractions of equivalent below 270 it was assumed that their saturated and unsaturated parts have the same equivalent.
2. For fractions containing methyl oleate, methyl linoleate, and esters of saturated acids the assumption was made that the oleate and linoleate distill in the same ratio as in the first pure C_{18} unsaturated esters.
3. For fractions consisting only of pure C_{18} unsaturated esters the calculations were based on the iodine values alone.

The second method employed low temperature crystallization as a means of a preliminary separation, and this separation was applied to the mixed methyl esters rather than to the mixed fatty acids. The methyl esters were prepared directly from the oil by alkali-catalyzed methanolysis.

Preliminary resolution of the methyl esters to practically saturated and unsaturated fractions was accomplished by using a procedure similar to that of De la Mare and Shorland (4). This consists in allowing a solution of the methyl esters in acetone (15 ml. acetone per gram of esters) to cool down to —35° in a glass cylindrical vessel supported in a cooling bath containing acetone and dry ice. The solution was kept at —35° for 3 hours while mechanically stirred. The stirrer was stopped, and half an hour was allowed for settling. To avoid disturbance of the equilibrium attained, filtration was performed directly from the controlled temperature confines of the crystallizer. For this purpose a precooled fine grade porous steel filter, connected to a vacuum pump through a thick-walled Erlenmeyer flask, was carefully lowered into the solution. The precipitate was filtered dry and packed around the filter. This precipitate was recrystallized from a volume of acetone equal to that used in the first crystallization. The filtrates from both crystallizations were combined, and the acetone was distilled off. The solid fraction of the methyl esters had an iodine value of 7.2, and it represented 14.95% of the total mixed methyl esters. Both fractions were then subjected to fractional crystallization.

The composition of the several fractions obtained by distillation was calculated after determination of the weight, iodine value, and saponification values of

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[1] From the thesis of A. M. Abu-Nasr, submitted in partial fulfillment of the requirements for the M.S. degree at the Agricultural and Mechanical College of Texas.

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each fraction. The method of calculation is essentially the same as used by Hilditch and coworkers (6).

The third method is based on the measurement of the ultraviolet absorption of the oil both before and after alkali-isomerization, using a Beckman DU spectrophotometer. The procedure of Brice and Swain (2) was followed, using 11% KOH-glycerol solution with a cooking period extending 30 minutes. The percentage of linoleic acid calculated from the absorption coefficient at 2,320 Å equal to 55.14 (Fig. 1) is 62.03% (oil basis) equivalent to 64.90% (fatty acid basis). The monounsaturated acids were calculated as oleic acid, and the percentage of saturated acids was obtained by difference. Results of calculations are shown with those from the other two methods in Table II.

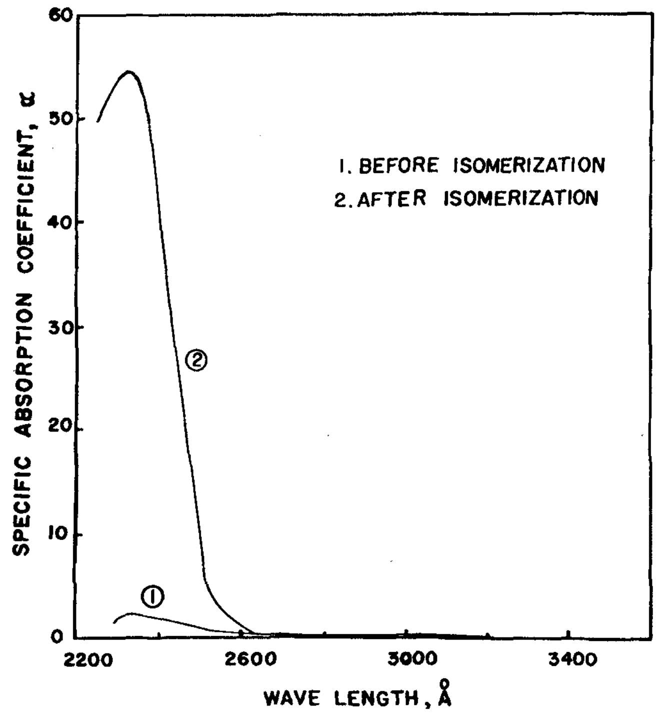

FIG. 1. Ultraviolet absorption spectra of the oil.

TABLE II

Percentages of Constituent Fatty Acids[a]

Acid	First method	Second method	Third method
Myristic	1.2	1.5	15.6
Palmitic	8.9	9.1	
Stearic	5.6	5.5	
Tetradecenoic	0.9	0.2	19.5
Hexadecenoic	1.2	0.3	
Oleic	17.2	13.9	
Linoleic	65.0	67.0	64.9
Unanalyzed	......	2.5[b]	......
Total	100.0	100.0	100.0

[a] Mixed fatty acid basis.
[b] Residue left after distillation of the unsaturated fraction.

Discussion

The seed of *Citrullus colocynthis* yield a linoleic rich, semidrying oil which has a light yellow color and a faint agreeable odor. The mixed fatty acids consist of myristic, palmitic, stearic, myristoleic, palmitoleic, oleic, and linoleic acids, the last two being major constituents.

In connection with the two methods applied for the preliminary resolution it seems that the technique of low temperature crystallization is advantageous not only by reason of its simplicity and directness but also due to its efficiency as evidenced by an iodine value of 7.2 for the saturated fraction.

It is interesting to note that the three methods employed in this investigation gave results which agree fairly well with each other, keeping in mind that the spectrophotometric method is limited in application to the determination of polyunsaturated fatty acids. The ester fractionation, although long and tedious, might well continue to be an important tool in fat analysis.

TABLE III

Fractional Analysis of Methyl Esters of the Solid Acids
(Lead salt method)
Solid acids 16.6% of total mixed acids

Fraction	Wgt. g.	Boiling pt. °C. at about 2mm. Hg.	Sapon. equiv.	Iodine value	Saturated acids			Oleate
					C_{14}	C_{16}	C_{18}	
S_1	3.95	125-143	267	0.70	0.47	3.45	......	0.03
S_2	4.97	143-167	285	21.36	......	2.16	1.57	1.24
S_3	2.24	167-168	291	9.01	......	0.54	1.46	0.24
S_r	1.50	......	291	9.01	......	0.15	1.19	0.16
Wt. ester	12.66	......	......	......	0.47	6.30	4.22	1.67
Wt. acids	12.04	......	......	......	0.44	5.98	4.03	1.59
% acid in fraction	100.0	......	......	......	3.7	49.6	33.5	13.2
% acid in total mixed acids	16.6	......	......	......	0.6	8.2	5.6	2.2

TABLE IV

Fractional Analysis of Methyl Esters of the Liquid Acids
(Lead salt method)
Liquid acids 83.4% of total mixed acids

Fraction	Wt., g.	Boiling pt. °C. at about 3 mm. Hg.	Sapon. equiv.	Iodine value	Saturated			Unsaturated		C^{2u}_{18}
					C_{14}	C_{16}	C^{u}_{14}	C^{u}_{16}	C^{u}_{18}	
L_1	2.89	135-160	257.0	60.96	0.50	0.61	0.76	1.02	......	......
L_2	35.40	160-173	293.4	158.0	......	0.06	......	......	5.07	30.27
L_3	6.77	173-174	294.9	160.1	......	......	......	......	0.99	5.78
L_4	23.16	174-175	295.8	158.8	......	......	......	......	3.64	19.52
L_5	2.46	175-179	296.0	116.9	......	......	......	......	1.59	0.87
L_R	3.21	......	296.0	116.9	......	......	......	......	2.02	1.19
Wt. esters	73.89	......	......	......	0.50	0.67	0.76	1.02	13.3	57.63
Wt. acids	70.29	......	......	......	0.47	0.64	0.72	0.97	12.7	54.80
% acid in fraction	100.0	......	......	......	0.67	0.90	1.02	1.37	18.1	78.00
% acid in total mixed acids	83.4	......	......	......	0.6	0.7	0.9	1.2	15.0	65.00

TABLE V

Fractional Analysis Summary
(Lead salt method)

Acid	Solid fraction	Liquid fraction	Total
Myristic	0.6	0.6	1.2
Palmitic	8.2	0.7	8.9
Stearic	5.6	......	5.6
Tetradecenoic	......	0.9	0.9
Hexadecenoic	......	1.2	1.2
Oleic	2.2	15.0	17.2
Linoleic	......	65.0	65.0
Total	16.6	83.4	100.0

The fact that the *Citrullus colocynthis* seed contains about 18% oil and that the acre yields about 6,000 lb. of seed (3) focuses attention on the potentialities of the seed as a source of oil.

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Influence of Variety and Environment on the Iodine Value of Cottonseed Oil[1]

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EACH species of plants elaborates its own specific mixture of fatty acids in its seed fat. The fatty acid composition, and consequently the iodine value, of the oil from the seed of a given species varies and is influenced by the genetical characteristics of the variety and the climatic environment under which the oil is synthesized. It has been demonstrated for a number of oils, including soybean (2), linseed (9), sunflower (4), and others (3, 6), that the mean temperature prevailing during development of the seed is the predominating factor influencing the iodine value of the oil. High temperatures contribute to the production of oils having low iodine values. Some workers have associated the degree of unsaturation with the geographic source of the seed (7, 8) but failed to recognize that the variability of the climate at a given location can and does cause variations, in some instances as large as that associated with widely separated locations.

It has been shown that cottonseed oils obtained from seed of different varieties grown under different environments vary widely in iodine value and that the percentages of linoleic, oleic, and saturated acids are very highly correlated with the iodine value (10).

Although the iodine value is widely used in characterizing cottonseed oils and knowledge of it is considered important in commercial processing of these oils, no systematic investigation of the influence of environment and variety on the iodine value of the oil has heretofore been reported. The present communication is a part of a study, of the influence of variety and environment on the chemical composition and physical properties of cottonseed and seed cotton.

Samples and Methods of Analysis

Samples of cottonseed of eight commercial varieties of cotton were obtained from experimental growths at 13 locations during 1947, 1948, and 1949 through the cooperation of the Division of Cotton and Other Fiber Crops and Diseases of the Bureau of Plant Industry, Soils, and Agricultural Engineering. The varieties and locations are listed in Table I. The plantings were irrigated at State College, New Mexico; Sacaton, Arizona; and Shafter, California.

The seed cottons were picked from recently opened bolls and air-dried under cover. The ginned seed was stored in sealed containers at 0°F. as previous work had proved that under such storage there is no significant change in chemical composition (11). The low free fatty acid contents of the oils demonstrated that the seed had not suffered field deterioration.

The oils were extracted from freshly separated and ground cottonseed meats by use of A.O.C.S. Official Method Ba 3-38 (1). Wijs iodine values of the oils were determined according to the specifications of A.O.C.S. Official Method Cd 1-25 (1).

Discussion of Results

The iodine values of the oils of the 312 samples of seed are tabulated and summarized in Table I. The missing iodine value for the oil of the seed of Acala 1517W from Jackson, Tennessee, for 1949 has been calculated by an approved method. The data show that with but few exceptions the oils from Stoneville 2B seed are high and those from Coker Wilds seed are low in iodine values. It is also noted that the iodine values of the oils from seed grown at individual stations vary for different years and that the varieties tend to rank themselves with respect to the iodine value of the oil.

It is observed from the individual results and the variety and location averages of the data in Table I that the ranges for influence of environment are approximately 50% greater than those for the influence of variety alone. With greater differences in either or both environmental and varietal characteristics more divergent results may be anticipated.

The analysis of variance of the iodine values (Table II) indicates that the effects of both variety and environment are highly significant statistically.

In relating the variations in the iodine values to temperature and rainfall factors of environment,

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