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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TABLE I

Iodine Value of the Oil in Cottonseed of Eight Varieties Grown at 13 Locations During Three Years

Year and location	Iodine value of oil from cottonseed of varieties indicated								
	Acala 4-42	Acala 1517W	Rowden 41B	Mebane	Stoneville 2B	Deltapine 15	Coker 100W	Coker Wilds	Mean
1947									
Statesville, N. C.	106.0	105.7	106.0	110.0	108.3	104.2	107.0	103.3	106.3
Florence, S. C.	107.3	106.5	108.3	110.0	114.1	106.8	109.9	105.6	108.6
Tifton, Ga.	105.7	106.5	107.1	109.0	112.2	104.6	108.7	103.6	107.2
Auburn, Ala.	106.1	105.5	105.8	108.0	111.0	103.6	107.9	102.5	106.3
Jackson, Tenn.	103.2	103.4	103.2	105.2	108.7	101.5	106.3	99.1	103.8
Stoneville, Miss.	101.9	101.7	102.1	104.5	109.7	101.0	105.1	96.9	102.9
St. Joseph, La.	104.6	104.3	104.2	106.1	111.1	102.4	107.3	101.0	105.1
Chickasha, Okla.	100.9	99.7	97.1	98.5	103.5	95.7	101.9	90.9	98.5
Greenville, Tex.	94.0	96.9	96.7	98.5	106.2	96.4	100.8	90.7	97.5
College Station, Tex.	105.1	104.6	104.6	106.4	111.9	103.3	107.9	101.4	105.7
State College, N. M.	112.6	111.3	112.7	112.9	114.5	108.0	113.5	106.8	111.5
Sacaton, Ariz.	101.2	100.3	100.6	102.3	108.1	100.4	102.7	96.6	101.5
Shafter, Calif.	109.0	108.8	107.0	109.9	113.6	105.3	110.3	105.2	108.6
Mean	104.4	104.2	104.3	106.3	110.2	102.6	106.9	100.3	104.9
1948									
Statesville, N. C.	107.7	107.6	107.4	109.8	112.4	105.6	109.2	106.6	108.3
Florence, S. C.	110.0	108.7	108.3	109.6	114.3	105.7	111.2	106.7	109.3
Tifton, Ga.	106.0	105.8	106.4	107.2	110.6	103.8	108.1	105.3	106.7
Auburn, Ala.	112.2	108.6	108.6	110.8	114.7	107.6	110.4	106.8	110.0
Jackson, Tenn.	106.3	105.9	105.8	108.8	111.1	103.7	107.7	104.9	106.8
Stoneville, Miss.	107.5	106.5	106.6	108.4	113.1	105.4	109.6	102.5	107.5
St. Joseph, La.	111.1	109.3	111.4	112.0	115.6	108.5	111.9	106.4	110.8
Chickasha, Okla.	101.4	101.7	103.1	104.6	107.8	100.5	104.5	99.7	102.9
Greenville, Tex.	96.7	96.9	98.8	98.7	104.9	97.0	99.9	91.3	98.0
College Station, Tex.	91.0	92.2	95.9	95.1	102.3	94.0	97.0	88.1	94.5
State College, N. M.	108.4	109.4	110.4	111.6	112.9	107.4	111.0	106.3	109.7
Sacaton, Ariz.	99.7	98.7	101.8	103.0	107.1	99.4	103.1	95.7	101.1
Shafter, Calif.	110.4	110.0	111.7	112.7	115.8	109.0	113.5	107.5	111.3
Mean	105.3	104.7	105.9	107.1	111.0	103.7	107.5	102.1	105.9
1949									
Statesville, N. C.	113.6	112.3	111.9	113.2	116.8	110.5	114.4	109.6	112.8
Florence, S. C.	107.9	107.0	108.2	108.4	113.7	106.8	110.7	104.9	108.5
Tifton, Ga.	108.1	107.8	107.8	109.9	113.8	107.2	108.4	105.3	108.5
Auburn, Ala.	108.4	106.8	108.5	109.7	112.2	105.7	109.4	105.1	108.2
Jackson, Tenn.	112.1	109.5	111.9	113.0	109.8	108.4	113.2	108.0	110.7
Stoneville, Miss.	105.9	105.4	106.0	107.9	112.0	105.0	108.7	104.4	106.9
St. Joseph, La.	110.2	108.5	110.8	109.9	114.9	109.6	111.3	107.3	110.3
Chickasha, Okla.	112.9	111.4	111.9	112.9	116.1	109.6	113.3	109.2	112.2
Greenville, Tex.	103.8	103.7	106.2	105.2	111.6	104.0	107.9	100.0	105.3
College Station, Tex.	103.0	102.3	103.4	107.0	110.4	101.9	105.6	100.0	104.2
State College, N. M.	111.8	112.6	113.2	114.1	115.6	110.1	112.7	107.5	112.2
Sacaton, Ariz.	102.2	100.3	102.7	105.5	109.1	101.6	105.5	99.6	103.3
Shafter, Calif.	107.2	109.2	110.3	112.2	113.3	105.9	110.7	105.7	109.3
Mean	108.2	107.4	108.7	109.9	113.0	106.6	110.1	105.1	108.6
3-year mean									
Statesville, N. C.	109.1	108.5	108.4	111.0	112.5	106.8	110.2	106.5	109.1
Florence, S. C.	108.4	107.4	108.3	109.3	114.0	106.4	110.6	105.7	108.8
Tifton, Ga.	106.6	106.7	107.1	108.7	112.2	105.2	108.4	104.7	107.5
Auburn, Ala.	108.9	107.0	107.6	109.5	112.6	105.6	109.2	104.8	108.2
Jackson, Tenn.	107.2	106.3	107.0	109.0	109.9	104.5	109.1	104.0	107.1
Stoneville, Miss.	105.1	104.5	104.9	106.9	111.6	103.8	107.8	101.3	105.7
St. Joseph, La.	108.6	107.4	108.8	109.3	113.9	106.8	110.2	104.9	108.7
Chickasha, Okla.	105.1	104.3	104.0	105.3	109.1	101.9	106.6	99.9	104.5
Greenville, Tex.	98.2	99.2	100.6	100.8	107.6	99.1	102.9	94.0	100.3
College Station, Tex.	99.7	99.7	101.3	102.8	108.2	99.7	103.5	96.5	101.4
State College, N. M.	110.9	111.1	112.1	112.9	114.3	108.5	112.4	106.9	111.1
Sacaton, Ariz.	101.0	99.8	101.7	103.6	108.1	100.5	103.8	97.3	102.0
Shafter, Calif.	108.9	109.3	109.7	111.6	114.2	106.7	111.5	106.1	109.8
Mean	106.0	105.5	106.3	107.8	111.4	104.3	108.2	102.5	106.5

TABLE II

Analysis of Variance of Iodine Values of Cottonseed Oil

Source	D.F.	Mean square	F value
Varieties	7	281.82	211.89[a]
Station-years	38	154.59	116.23[a]
Error	265	1.33	....
Total	310	....	....

[a] Highly significant. F values required for 1% level are 2.73 and 1.69, respectively.

three periods in the development and maturation of the seed and three combinations of them were considered significant for study. They are:

Period 1. Squaring—21 days prior to average blooming date, which is considered as 10 days after first bloom.

Period 2. Fiber length development—17 days after average blooming date.

Period 3. Maturation—35 days following period 2 to average boll opening.

Period 4. Combination of periods 1 and 2.

Period 5. Combination of periods 2 and 3.

Period 6. Combination of periods 1, 2, and 3.

Simple correlation coefficients for the over-all relations between the iodine values and the mean maximum, minimum, and mean temperatures for each of the six periods (Table III), demonstrate that the iodine values are negatively correlated with temperature. In considering the first three periods, it is seen that the temperature plays a more important role during period 3, judged by the levels of the correlation coefficients. This was anticipated from the observation by Grindley (5) that the oil is elaborated in the seed almost entirely during the last 20 days before the bolls open at maturation. Higher

TABLE III

Correlation Coefficients of Relations Between Iodine Values of Cottonseed Oil and Temperatures

Period	Range in mean temperature			Correlation coefficients		
	Maximum	Minimum	Mean	Mean maximum temperature	Mean minimum temperature	Mean temperature
	°F.	°F.	°F.			
1	85.2- 99.8	60.0-71.9	75.4-84.4	−0.23	−0.09	−0.29
2	85.3-107.2	62.2-80.0	75.2-91.6	−0.42[b]	−0.43[b]	−0.54[b]
3	83.6-107.6	60.2-75.4	71.9-91.4	−0.59[b]	−0.60[b]	−0.69[b]
4	86.0-101.9	61.0-73.1	75.6-84.2	−0.38[a]	−0.28	−0.52[b]
5	86.1-106.0	61.6-76.8	74.6-89.1	−0.57[b]	−0.53[b]	−0.71[b]
6	85.9-103.8	61.5-73.9	76.9-86.6	−0.52[b]	−0.52[b]	−0.72[b]

N = 312. Range in iodine value was 88.1-116.8.
[a] Significant—r value required at 5% level is 0.317.
[b] Highly significant—r value required at 1% level is 0.408.

coefficients were obtained for the relations with mean than with either maximum or minimum temperatures.

The iodine values were found to be positively correlated with rainfall (Table IV), with the coefficient being higher for the maturation period 3 when the oil is being synthesized than for the earlier periods. The high coefficients for periods 4, 5, and 6 seem to reflect some benefit of rainfall during periods 1 and 2 or earlier in contributing greater plant vigor. When the data for the irrigated stations are included in the statistical calculations, coefficients indicate much less significance for the influence of soil moisture.

TABLE IV

Correlation Coefficients of Relations Between Iodine Values of Cottonseed Oil and Rainfall

Period	Range in rainfall		Correlation coefficients	
	Rain-grown stations	Irrigated stations[1]	Rain-grown cotton	Rain-grown and irrigated cotton
	inches	*inches*		
1	0.00- 8.81	4.00- 8.10	+0.41[a]	+0.29
2	0.00-11.12	4.00- 8.05	+0.27	+0.30
3	0.00- 8.38	8.46-16.00	+0.48[b]	+0.30
4	0.33-13.58	8.00-12.35	+0.46[b]	+0.39[a]
5	0.86-17.30	13.85-20.01	+0.47[b]	+0.35[a]
6	1.63-19.76	18.00-28.01	+0.55[b]	+0.37[a]

[1] Includes irrigation as synthetic rainfall.
N = 312. Iodine value ranged from 88.1 to 116.8.
[a] Significant—r value required at 5% level is 0.361 for rain-grown and 0.317 for rain-grown and irrigated cotton.
[b] Highly significant—r value required at 1% level is 0.463 for rain-grown and 0.408 for rain-grown and irrigated cotton.

In the foregoing correlation analyses varietal effects were eliminated. As it has been cited above that the influence of variety approaches that of the temperature, simple correlations were calculated for the relationships between the iodine values of the oils from seed of each variety and the mean temperatures for periods 3 and 6. The results are tabulated in Table V. The correlation coefficients for all varieties and both periods are all negative and significant at the 1% level. Although the oil is synthesized during the last 20 days of period 3 (5), there was a significant reduction in the iodine value with increase in mean temperature for the combined three periods of seed development and maturation (i.e. period 6) as well as for period 3. The regression coefficients of the equations indicate an average reduction in iodine value of the oil per °F. rise in average mean temperature of 0.760 unit during period 3 and 1.172 units during period 6. However the standard errors of estimate (S_{yx}) are rather large for the equations to be used for prediction purposes.

Of the varieties used in the investigation Stoneville 2B and Coker Wilds exhibited the widest differences with respect to the average iodine value (Table I) and the influence of temperature on the iodine value of the oil (Table V). The oils from the seed of the Stoneville variety averaged the highest in iodine value and the lowest in correlation coefficients, regression coefficients, and standard error of estimate for the relations between iodine value and mean temperature. The least squares regression equations for the relations for Stoneville 2B and Coker Wilds varieties, as well as those for the other six varieties, for the maturation period 3 are shown graphically in Figure 1. Scatter diagrams of the individual values of each variety would show considerable overlapping.

It is expected that differences in the iodine value of commercial oils will not be so large as found for

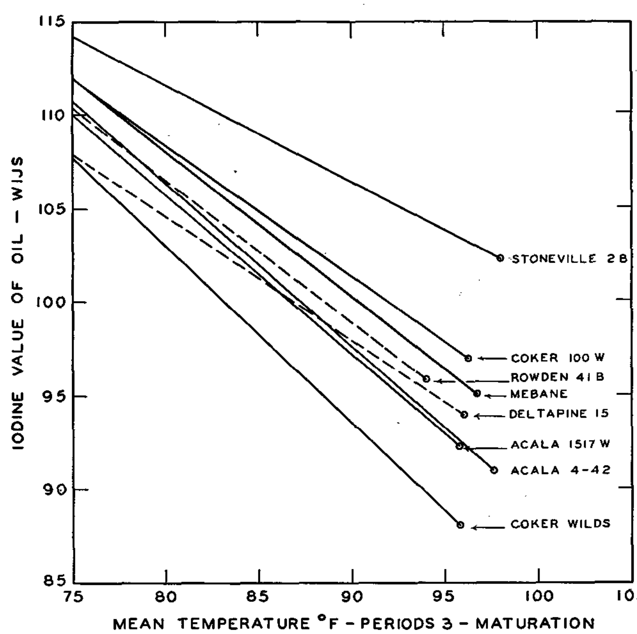

FIG. 1. Least squares regression equations for the relations of iodine values of oils of eight varieties of cottonseed to mean temperatures prevailing during the period of seed maturation.

TABLE V

Relations Between Iodine Values of Cottonseed Oils from Seed of Eight Varieties and Mean Temperatures During Two Periods of Boll and Seed Development

Period and variety	r_{xy}	Regression equation $y = I_2$ value x = mean temperature	S_{yx}
			±
Period 3—Maturation			
Acala 4.42	−0.68[b]	$y = 176.3 - 0.874x$	3.80
Acala 1517W	−0.74[b]	$y = 173.7 - 0.849x$	3.15
Rowden 41B	−0.68[b]	$y = 168.0 - 0.767x$	3.36
Mebane, Watson's	−0.68[b]	$y = 170.1 - 0.775x$	3.38
Stoneville 2B	−0.58[b]	$y = 152.6 - 0.513x$	2.90
Deltapine 15	−0.65[b]	$y = 157.6 - 0.662x$	3.16
Coker 100W	−0.69[b]	$y = 164.5 - 0.701x$	2.97
Coker Wilds	−0.69[b]	$y = 178.0 - 0.938x$	4.00
Period 6—Squaring, fiber elongation, and maturation			
Acala 4-42	−0.70[b]	$y = 213.2 - 1.332x$	3.70
Acala 1517W	−0.75[b]	$y = 209.1 - 1.286x$	3.09
Rowden 41B	−0.69[b]	$y = 200.1 - 1.165x$	3.32
Mebane, Watson's	−0.73[b]	$y = 207.0 - 1.232x$	3.15
Stoneville 2B	−0.61[b]	$y = 175.5 - 0.796x$	2.82
Deltapine 15	−0.68[b]	$y = 187.3 - 1.031x$	3.04
Coker 100W	−0.70[b]	$y = 192.7 - 1.050x$	2.93
Coker Wilds	−0.73[b]	$y = 222.1 - 1.485x$	3.78

N = 39 in all instances.
[b] Highly significant—r value required at 1% level is 0.408.

these experimental samples since there is always some blending of seed of different production lots at the gin and mill and later of oils. However the results should interest oil processors desiring oils of certain iodine values for the production of special products as they offer a basis of selection of source areas, knowing the temperatures prevailing during the development of the cottonseed.

Summary

Data are reported on the variation of the iodine value of the oil from the seed of eight varieties of cotton grown at 13 locations during three years. Analysis of variance showed the influence of variety and of station-years to be highly significant statistically. Iodine value was found to be negatively correlated with the temperatures. The highest correlation was obtained for the period of maturation (35 days before the bolls open) when the oil is being synthesized. The coefficients for the relations with mean temperatures were higher than those for maximum and minimum temperatures.

Simple correlations for the relations between iodine value of the oils from seed of individual varieties and mean temperatures during two periods of boll and seed development were negative and highly significant. For the maturation period (35 days before boll opening) and the combined periods for squaring, fiber elongation, and maturation (73 days before boll opening) the average reductions in iodine value per °F. increase in temperature were found to be 0.760 and 1.172 units, respectively. Of the eight varieties investigated, temperature influenced the iodine value of the oil least for Stoneville 2B and most for Coker Wilds.

Acknowledgment

This paper is one of a series reporting the results of a Regional Cottonseed Study, 1947-1949. Acknowledgment is made to H. D. Barker and his associates of the Division of Cotton and Other Fiber Crops and Diseases of the Bureau of Plant Industry, Soils, and Agricultural Engineering. Dr. Barker assisted with the over-all plans and developed coordination among the field locations. Gerald T. Den Hartog developed certain of the statistical procedures and assisted in interpreting the results. W. H. Tharp assisted in planning the influence of environment. Individuals responsible for growing the cotton varieties at the field locations and the observation of temperature and rainfall included R. H. Tilly, P. M. Kime, W. H. Jenkins, S. A. Parham, H. B. Tisdale, J. M. Epps, N. I. Hancock, J. B. Dick, C. B. Haddon, I. M. Parrott, M. G. Keathley, D. R. Hooton, T. R. Richmond, A. R. Leding, R. H. Peebles, G. J. Harrison, and E. G. Smith.

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Determination of the Methoxyl Content of Fats

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THE use of sodium methoxide as a catalyst for the rearrangement of lard, according to a recent patent (1), produces some methyl esters. Although the deodorization procedure removes practically all of these esters, it is necessary to measure the methoxyl content of the final product.

Chromotropic acid (1,8-dihydroxynaphthalene-3,6-disulfonic acid) has been used as a specific reagent for the determination of formaldehyde (2) and methanol (3). As the methyl esters present in the fat may be saponified and the methanol removed by distillation, a procedure was developed that will measure less than 0.0005% methoxyl in fat.

Experimental

A 100 g. sample of the fat is saponified in a mixture of 30 g. potassium hydroxide, 25 ml. of water, and 75 ml. of diethylene glycol. The saponification is carried out in a one-liter, one neck, round bottom flask, which is equipped with a six-inch distillation column and a condenser leading to the receiver. A dropping funnel at the top of the column is used to add acid and water to the saponification mixture.

A hemispherical Glass-Col heater is used, and agitation is produced by a magnetic stirrer. The mixture is heated and stirred until saponification is complete, as evidenced by a homogenous solution. It is then acidified by the addition of 18 ml. concentrated sulfuric acid in 50 ml. of water, and a slow distillation is carried out until about 50 ml. of distillate have been collected. The column is washed down several times during the distillation with small portions of water added through the dropping funnel. The distillate obtained in this manner consists of a very dilute solution containing not only methanol but also steam volatile fatty acids, some aldehydes, and other materials. To remove the fatty acids and the aldehydes the solution is redistilled from a small distillation flask after adding about ½ g. of lime and about ⅒ g. of metaphenylene diamine hydrochloride. A 100-ml. volumetric flask is used as a receiver. The distillation is carried almost to dryness and then water added and distilled until almost 100 ml. have been collected. The receiver is filled to the mark with water.

The oxidation and color-forming reactions are carried out in 25-ml. volumetric flasks. Aliquots of the distillate, which contain less than 0.2 mg. of methanol, are pipetted into the flask, diluted to approximately 10 ml., and oxidized by 1 ml. of a potassium permanganate solution containing 6 g. $KMnO_4$, 200 ml. of