Molecular Distillation and Low Temperature Crystallization of Cottonseed Oil and the Stability of the Molecularly Distilled Fractions[†]

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THE results of investigations in recent years on vitamins, essential fatty acids and antioxidants emphasize the importance of fundamental research on the composition of oils and fats.

Satisfactory methods of separating or isolating the constituents of oils are lacking. The process of molecular distillation has been shown to be effective in concentrating certain vitamins (1) and some of the minor constituents. Fawcett (2) distilled crude cottonseed oil and by measurements of oxygen absorption of the distilled fractions concluded that the total antioxidants were effectively concentrated into the most and least volatile fractions. Rawlings (3) in a recent publication reported analytical results of the molecular distillation of soybean and corn oils. No great fractionation of the glycerides was found but nearly all of the unsaponifiable material was concentrated into the first two fractions.

Crystallization procedures have also been used to effect at least a partial separation of the glycerides, as well as some of the minor constituents of oils and fats. The recent book by Schönfeld and Hefter (4) contains a summary of most of the published results in this connection. In general, crystallization procedures were found to be effective chiefly in the separation and purification of some of the simple saturated glycerides such as tripalmitin and tristearin.

The present investigation was undertaken to obtain more information as to the extent of separation of the constituents of cottonseed oil that may be effected by molecular distillation and by crystallization. In this connection it was desirable to determine the comparative stability of the molecularly distilled fractions. Cottonseed oil, alkali-refined from prime crude oil, was distilled into a series of fractions. Analytical data were obtained on the distilled samples which give some indication of the extent of separation of the oil constituents. The distilled portions were subjected to uniform air-blowing conditions and the deteriorative changes followed by means of peroxide and iodine number determinations.

A series of fractions was also obtained by crystallization of cottonseed oil from acetone at various temperatures. The composition of these separated portions, calculated from the iodine numbers and thiocyanogen numbers, indicates appreciable fractionation of the glycerides.

EXPERIMENTAL

The cottonseed oil used throughout these experiments was refined in the Barrow-Agee Laboratory at Memphis, Tennessee, by caustic soda treatment of prime crude oil. The color of the refined oil correspond to 5.2 Red and 35 Yellow in Lovibond units.

Molecular Distillation—A cyclic molecular still of the type described by Hickman (5) was used for the distillation of the cottonseed oil. A preliminary test-run was made to determine what spread in iodine numbers may be effected by distillation of the oil into 15 fractions and by a redistillation of the lowest- and highest-iodine number portions thus obtained. The second fraction of the series of 15 had the lowest iodine number, 97.7; fraction 15 had the highest, 120.8. The redistilled portions from fraction 2 had iodine numbers ranging from 86.1 to 109.8; from fraction 15, 116.3 to 123.8.

In order to get more complete analytical data and also larger fractions for stability comparisons, 1400 gms. of cottonseed oil were distilled into 15 fractions and a residue. The first fraction which contained the bulk of the more volatile constituents, distilled at the rate of about 10 cc. per hour at 150 to 220°C. under a pressure of 0.004 to 0.006 mm. of mercury; the remainder of the oil distilled from 220 to 250°C. at an average rate of 25 cc. per hour under an average pressure of 0.002 mm. The color of the distilled fractions varied from orange in fraction 1 to pale lemon-yellow in the later fractions. The residue was yellowish green by visual inspection. The analytical values are given in Table I.

ANALYSIS OF DISTILLED FRACTIONS OF COTTONSEED OIL

FRACTION NO.	WEIGHT GMS.	SAP. EQ.*	F. ACID AS OLEIC PERCENT	REF. INDEX 40°	UNSAP. PERCENT	IODINE NO. (WIJS)	SCN. NO. (4 HOUR)	COMPOSITION		
								LINOLEIC PERCENT	OLEIC PERCENT	SATURATED PERCENT
1	88.0	280.2	0.38	1.4660	5.83	100.6	57.3	50.0	16.2	33.8
2	93.7	284.0	0.14	1.4634	0.60	100.0	58.4	48.0	19.5	32.5
3	93.0	284.3	0.13	1.4635	0.34	101.2	59.3	48.3	20.1	31.6
4	89.0	285.2	0.11	1.4638	0.25	103.0	60.3	49.3	20.4	30.3
5	87.7	285.4	0.09	1.4639	0.23	103.4	60.6	49.4	20.7	29.9
6	93.5	284.1	0.07	1.4639	0.16	103.9	60.7	49.8	20.4	29.8
7	90.5	284.6	0.06	1.4641	0.16	106.1	61.3	51.7	19.1	29.2
8	89.5	285.0	0.05	1.4642	0.13	106.1	61.9	51.0	20.6	28.4
9	90.0	284.7	0.05	1.4644	0.14	107.9	62.2	52.7	19.2	28.1
10	93.0	284.0	0.06	1.4645	0.17	109.3	63.5	52.8	20.6	26.6
11	91.0	286.3	0.06	1.4646	0.13	110.3	63.9	53.5	20.4	26.1
12	92.0	286.6	0.05	1.4648	0.09	111.9	65.2	53.9	21.5	24.6
13	89.0	286.6	0.05	1.4650	0.07	114.4	66.3	55.5	21.2	23.3
14	87.5	287.3	0.06	1.4654	0.11	116.6	67.9	56.2	22.3	21.5
15	85.5	290.1	0.06	1.4661	0.13	120.9	70.2	58.5	22.7	18.8
RESIDUE	43.5	290.0	0.09	1.4706	0.68	118.9	67.2	59.6	18.0	22.4
ORIGINAL OIL	1417.0	285.8	0.10	1.4645	0.49	108.3	62.3	53.1	18.9	28.0

*CORRECTED FOR UNSAPONIFIABLE MATERIAL

Table I. The analysis of fractions obtained by molecular distillation of cottonseed oil.

From the fatty acid composition calculated from the iodine and thiocyanogen numbers and other analytical data it is apparent that some, although slight, fractionation of the glycerides occurred. The bulk of the unsaponifiable material, however, was effectively concentrated into the first fraction. The calculated fatty acid composition of this fraction is somewhat in error due to the relatively large amount of unsaponifiable material.

Stability of the Distilled Fractions—To determine whether any difference in stability of the fractions existed, samples were subjected to uniform air-blowing conditions similar to those used in the Swift Stability

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Test, except that the samples were aerated in a water bath maintained at 60°C. Care was taken to exclude light from the samples by placing a covering of several thicknesses of black cloth above and around the sides of the water bath. Air inlet and outlet tubes were made of 3 mm.-inside diameter tubing. The rate of air flow was 13cc. per minute. Preliminary test runs on samples of cottonseed oil were carried out to establish that aeration conditions were uniform and the results duplicable. By the use of pilot tubes, it was found possible to determine the approximate curve of peroxide formation. Then the most essential points of the curve could be accurately determined with only few additional sample tubes.

From the peroxide-formation curves in Figure 1, and the curves showing decrease in iodine numbers in Figure 2, it is apparent that fraction 1 exhibits unusual differences from the other fractions.

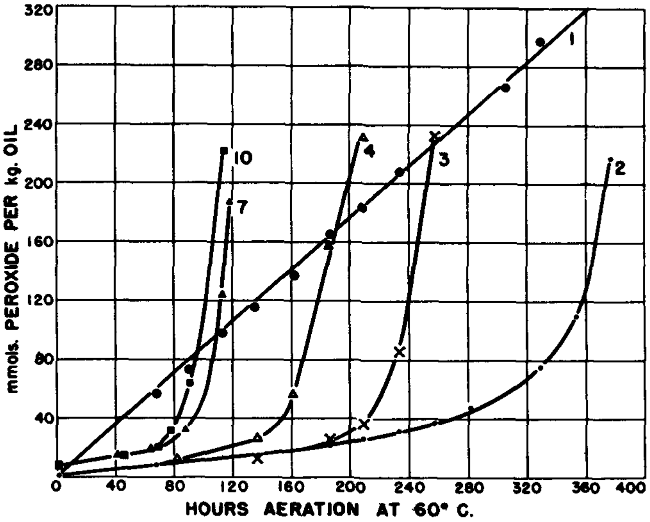

Fig 1. Peroxide formation by aeration of distilled fractions of cottonseed oil at 60°C. The curve numbers 1, 2, 3, etc , correspond to the fraction numbers of the distilled portions.

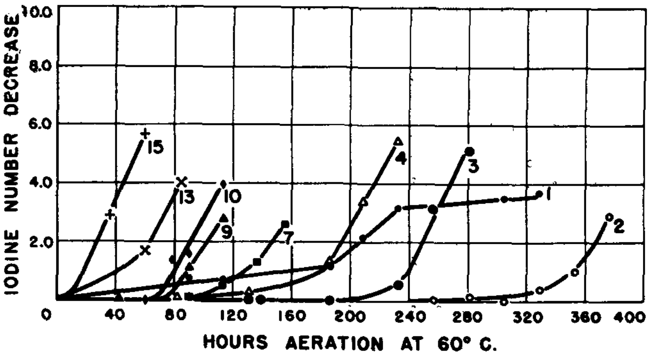

Fig. 2. Iodine number decrease by aeration of distilled fractions of cottonseed oil. The curve number corresponds to the fraction number.

The fact that it has a higher initial rate of peroxide formation than the others and that this rate is almost constant for 400 hours might at first thought to be interpreted as indicating the absence of protecting substances. However, the typical rancid odor was not detectable in this sample until after 250 hours of aeration and even then the odor was only slight, nor was the yellow color entirely bleached after 400 hours' aeration. In contrast with these observations, the strong rancid odor and also distinct bleaching of the other fractions of the series coincided with the sharp upward break in the peroxide and iodine number curves. Presumably, this behavior of fraction 1 is in some way associated with the presence of the unsaponifiable material.

The curves for fractions 5 and 6 were almost identical with those for fraction 4 and for this reason are not included in the graph. Likewise, the curves for fractions 8 and 9 were almost the same as those for fraction 10. It is noted that the sharp decrease in iodine numbers of the fractions during the aeration occurs at about the same points as the sharp increase in peroxide values. In order to ascertain whether antioxidants were present, a number of mixtures were made and subjected to the aeration procedure. These mixtures were made as follows:

M 1—10 per cent fraction 1, 90 per cent fraction 13.
M 2—10 per cent fraction 2, 90 per cent fraction 13.
M 3—10 per cent residue, 90 per cent fraction 15.

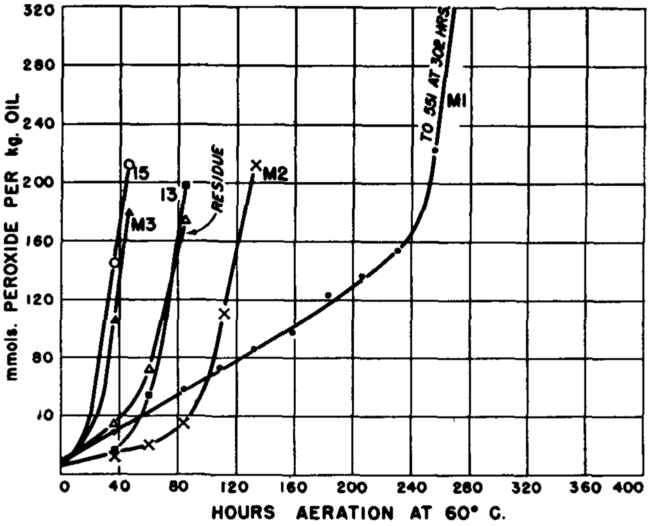

Fig 3. Comparison of peroxide formation of mixtures of distilled fractions of cottonseed oil. Mixture of Fraction 1 and Fraction 13 is represented by M 1; Mixture of Fraction 2 and Fraction 13, by M 2; Mixture of Residue and Fraction 15, by M 3.

The curves showing the peroxide values of these mixtures and of fractions 13, 15 and the residue are shown in Figure 3.

The curve for M 1 in comparison with that for fraction 13 shows a definite protective influence of fraction 1. The initial rate of peroxide formation, though somewhat higher than in fraction 13, did not exhibit the sharp upturn until after 250 hours' aeration. The yellow color imparted to M 1 by the addition of 10 per cent of fraction 1 was not bleached until after 250 hours' aeration. The organoleptic rancid stage was also closely correlated to the steep portion of the curve.

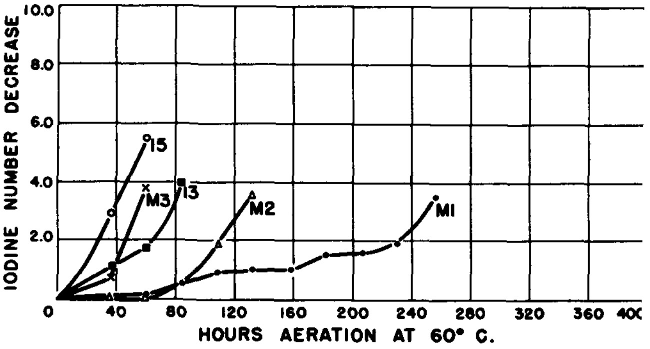

Fig. 4. Curves showing decrease in iodine number of mixture of distilled fractions of cottonseed oil. The curves are numbered the sam as in Fig. 3.

The curve M 2 shows only slight antioxidant activity due to fraction 2, whereas curve M 3 indicates practically no antioxidant effect of the residue.

Corroborative evidence is supplied by the curves of the iodine number decrease (Figure 4).

To ascertain whether molecular distillation had destroyed or appreciably changed the antioxidants or glycerides of the oil, aeration experiments were carried out on a composite of all the distilled fractions, excluding the residue. The composite contained approximately the same proportions of these fractions as were represented in the original oil. In addition, a sample of undistilled cottonseed oil from which a portion comparable to fraction 1 (Table I) was removed by distillation was also aerated to determine the effect of the absence of fraction 1. In this series there was included also a sample of cottonseed oil which had been degassed at 30-35° in vacuo until a constant pressure of .001 mm. of mercury was attained. This was done to determine, by comparison with the untreated oil, whether the rate of peroxide formation in the early stages may be affected by removal of the dissolved gases. The curves for this series of aeration experiments are shown in Figure 5.

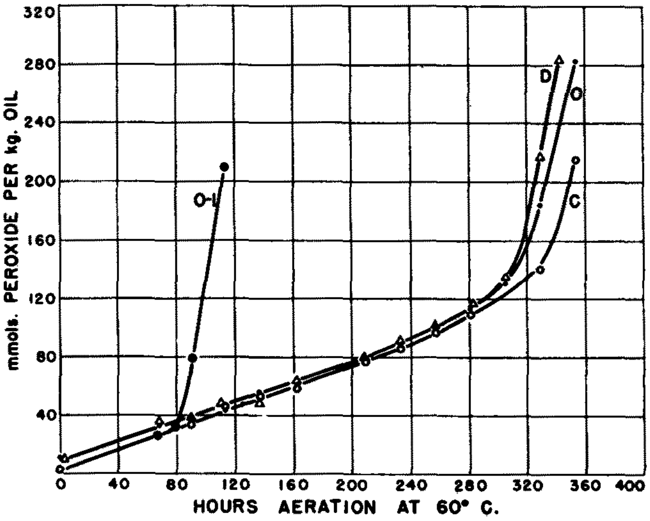

Fig. 5. Comparison of peroxide formation of cottonseed oil with that of degassed cottonseed oil, that of a composite of the distilled fractions of the oil, and that of oil from which Fraction 1 had been removed by molecular distillation. Curve O, original oil; Curve C, composite; Curve D, degassed cottonseed oil; Curve O-1, original oil minus Fraction 1.

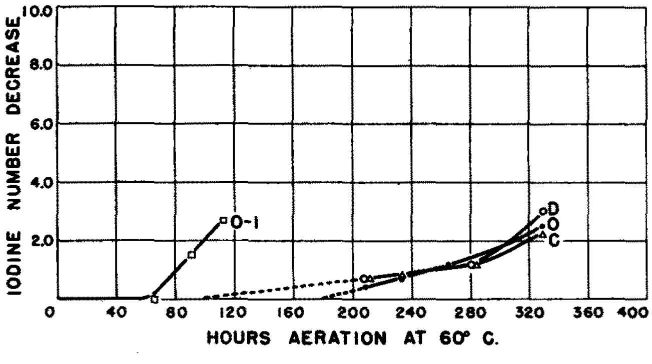

Fig. 6. Comparison of decrease in iodine number of cottonseed oil with that of degassed cottonseed oil, that of a composite of the distilled fractions of the oil, and that of oil from which Fraction 1 had been removed by molecular distillation. The curves are numbered the same as in Fig. 5.

From inspection of the curves O, C and D in Figure 5, it is concluded that molecular distillation or degassing caused no appreciable change in the behavior of the oil toward peroxide formation. Curve O-1 shows clearly the effect of removal of fraction 1 on the balance of the oil and furnishes indirect evidence that fraction 1 contained the bulk of the antioxidants or protective substances. The curves showing decrease in iodine number of the original oil (Figure 6) do not exhibit the sharp upturn characteristic of the distilled fractions (Figure 2).

The results shown in Figures 1 to 6 confirm the opinion held by others that the selection of a definite peroxide value or iodine number-decrease as the "rancid point" is not valid. However, upon consideration of the entire series of curves, it appears safe to say that the stability of the fractions decreases from fraction 1 to fraction 15. Further investigation of the composition and nature of the constituents of fraction 1 is necessary before adequate explanation of its unusual behavior can be made. It is possible that the antioxidant activity of fraction 1 may be masked by the presence of easily oxidized substances, such as the carotenoids and their degradation products.

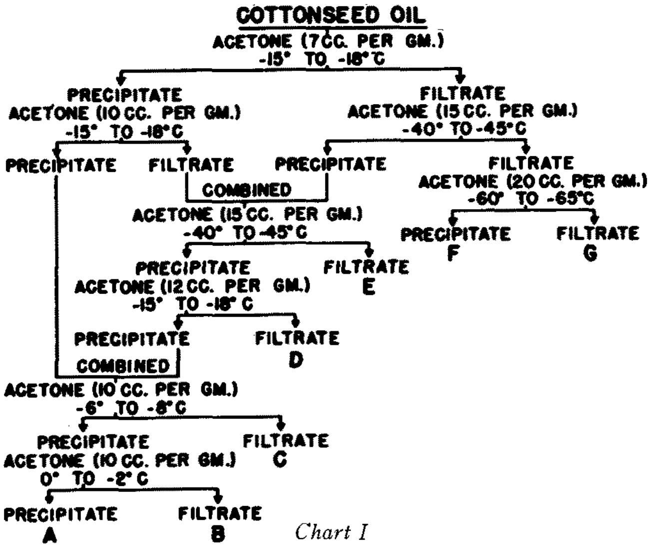

Chart I

ANALYSIS OF FRACTIONS OBTAINED BY CRYSTALLIZATION OF COTTONSEED OIL						
FRACTION	WEIGHT GMS.	IODINE NO. WIJS	SCN. NO. 4 HOUR	FATTY ACID COMPOSITION		
				LINOLEIC PERCENT	OLEIC PERCENT	SATURATED PERCENT
A	149	52.5	30.8	25.0	10.6	64.4
B	11	58.2	33.5	28.5	10.2	61.3
C	18	66.3	38.3	32.3	12.0	55.7
D	451	107.1	62.1	51.9	19.9	28.2
E	154	133.4	74.8	67.6	18.8	13.6
F	145	140.6	78.8	71.4	19.7	8.9
G	48	148.3	79.9	79.0	13.3	7.7

Table II. Analysis of fractions obtained by crystallization of cottonseed oil.

Crystallization of Cottonseed Oil—A kg. of cottonseed oil was subjected to a series of crystallizations from acetone at various temperatures. An outline of the steps is given in Chart 1.

The analyses of the final fractions obtained by this procedure and the fatty acid composition, calculated from the iodine numbers and 4-hour thiocyanogen numbers (6) are given in Table II.

Molecular distillation of these fractions effected further slight fractionation comparable to that shown in Table I.

The data in Table II suggest that crystallization methods may be used to advantage in connection with other physical and chemical methods for the separation of the oil components. The high percentage of linoleic acid glycerides present in Fraction G indicates the presence of trilinolein, whereas the high saturated-acid content (combined as glycerides) of Fraction A suggets a concentration of oleo- and linoleo-dipalmitins.

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Report Smalley Foundation Committee

WE ARE presenting herewith the 22nd report of the Smalley Foundation Committee of the American Oil Chemists' Society. During these past twenty-two years considerable progress has been made in the accuracy of the determination of oil and nitrogen on cottonseed meal. While the results obtained in the determination of nitrogen were slightly lower than last year, this is the first time, to our knowledge, in which any collaborator went through the season without the loss of any point in the determination of oil. This record was made by Mr. A. G. Thompson, Jr., of the Southern Cotton Oil Company Laboratory at Columbia, S. C. It must be understood, in gauging the accuracy of the results a difference of two points in either direction from the average is permitted without a deduction from the grade. It is also interesting to note that six of the collaborators were tied for first place in the determination of nitrogen.

As usual, thirty samples of cottonseed meal were distributed to the collaborators.

There are attached to this report four tables indicating the standing in percentage of the members taking part. Table No. I gives the standing of 60 collaborators who reported oil determinations on all samples. Table No. II gives the standing of 68 collaborators who reported nitrogen results on all samples. Table No. III gives the standing of 60 collaborators who reported oil and nitrogen on all samples. In these tables we have taken into consideration the results of those reports which were received within the time specified in our original announcement of the Smalley Foundation work. In table No. IV we have given the standing of those collaborators who reported on all samples, but some of whose reports were received too late to be included under the rules.

The winning collaborators are as follows:

The "American Oil Chemists' Society Cup" for the highest efficiency in the determination of both oil and nitrogen on all samples is awarded to Analyst No. 55, A. G. Thompson, Jr., Southern Cotton Oil Company, Columbia, S. C., with an average of 99.993 per cent. The average efficiency is higher than that of last year, which was 99.964 per cent. The certificate for second place goes to Analyst No. 79, Chas. W. Rice and Company, Columbia, S. C., who had an efficiency of 99.987 per cent, as compared with 99.942 per cent for last year.

The certificate for the highest efficiency in the determination of oil only is awarded to Analyst No. 55, A. G. Thompson, Jr., Southern Cotton Oil Company, Columbia, S. C., with an average of 100.00 per cent, as compared with 99.947 per cent for last year. The certificate for second place goes to Analyst No. 79, Chas. W. Rice and Company, Columbia, S. C., with an efficiency of 99.989 as compared with 99.943 per cent for last year.

The certificate for the highest efficiency in the determination of nitrogen is awarded to Analysts Nos. 13, 28, 51, 55, 64 and 79, Barrow-Agee Laboratories, Memphis, Tenn.; F. F. Hasbrouck, Allied Mills, Inc., Peoria, Ill.; E. H. Tenent, Woodson-Tenent Company, Memphis, Tenn.; A. G. Thompson, Jr., Southern Cotton Oil Company, Columbia, S. C.; T. L. Rettger, Buckeye Cotton Oil Company, Memphis, Tenn.; Chas. W. Rice and Company, Columbia, S. C., with an average of 99.985 per cent, as compared with 99.996 for last year. The certificate for second place goes to Analysts Nos. 8 and 85, The Battle Laboratories, Montgomery, Ala., and Armour and Company, Chicago, Ill., the analytical work having been done by L. E. Norem, with an average of 99.975 per cent, as compared with 99.990 per cent for last year.

We thought it might be well to include in this report a list of the previous winners of the highest award for both oil and nitrogen. They are as follows:

1918-1919	G. C. Hulbert, Southern C. O. Co., Augusta, Ga.
1919-1920	G. C. Hulbert, Southern C. O. Co., Augusta, Ga.
1920-1921	C. H. Cox, Barrow-Agee Lab's, Memphis, Tenn.
1921-1922	Battle Lab's, Montgomery, Ala.
1922-1923	Battle Lab's, Montgomery, Ala.
1923-1924	L. H. Forbes, Memphis, Tenn.
1924-1925	E. H. Tenent, International Sugar Feed Co. No. 2, Memphis, Tenn.
1925-1926	Battle Lab's, Montgomery, Ala.
1926-1927	W. F. Hand, Miss. State College, State College, Miss.
1927-1928	E. H. Tenent, International Sugar Feed Co., Memphis, Tenn.
1928-1929	Geo. W. Gooch Lab's, Los Angeles, Calif.
1929-1930	Southwestern Lab's, Dallas, Texas.
1930-1931	W. F. Hand, Miss. State College, State College, Miss.
1931-1932	J. N. Pless, Royal Stafolife Mills, Memphis, Tenn.
1932-1933	J. B. McIsaac, International Veg. Oil Co., Savannah, Ga.
1933-1934	W. F. Hand, Miss. State College, State College, Miss.