and for the same reason it should be more adaptable to micro quantities of sample. Work on this adaptation is under way.

An interesting observation was made on the composition of lard. The two concentrates of polyunsaturated constituents of lard methyl esters (Table II) exhibited a definite peak in the pentaene region. The position of the peak and the magnitude of the absorption strongly indicate the presence of pentaene acids in the original lard. This is believed to be the first report that lard contains acids of greater unsaturation than arachidonic.

TABLE II

Spectrophotometric Analyses of Various Samples of Fats and Oils Isomerized by Standard Methods and by the 21 % KOH Glycol Method

Sample	Component acid	Method[1]	
		21% KOH glycol	Standard[2]
		%	%
Cottonseed oil 1	Linoleic	51.6	51.5
Cottonseed oil 2	Linoleic	49.1	49.4
Soybean oil 1	Linoleic	50.8	52.6
	Linolenic	7.7	8.3
Soybean oil 2	Linoleic	50.9	52.2
	Linolenic	7.7	8.3
Methyl esters[3] from cottonseed oil	Linoleic	73.3	71.9
Methyl esters[3] from soybean oil	Linoleic	62.8	62.6
	Linolenic	9.0	9.5
Methyl esters[3] from linseed oil	Linoleic	18.1	16.6
	Linolenic	49.0	49.9
Methyl esters[4] from lard 1 fraction 7	Linoleic	23.8	25.2
	Linolenic	6.8	7.9
	Arachidonic	4.4	4.8
	Pentaenoic (50% C_{20}-50% C_{22})	1.0	1.8[6]
Methyl esters[4] from lard 1 fraction 8	Linoleic	21.2	21.8
	Linolenic	10.6	11.3
	Arachidonic	7.4	8.7
	Pentaenoic (50% C_{20}-50% C_{22})	3.5	5.3[6]
Methyl esters[5] from adrenal lipids	Linoleic	24.1	22.9
	Linolenic	4.9	4.1
	Arachidonic	15.9	16.5

[1] All results are reported as percentage of acid in sample.
[2] Standard method may be either 6.6% KOH glycol or 11% KOH glycerol; where data were available by both methods the average values are given.
[3] Saturated esters removed by low temperature crystallization.
[4] Concentrate of polyunsaturated components obtained by low temperature crystallization and high vacuum distillation.
[5] Fraction obtained by adsorption separation on silicic acid.
[6] Calculated from coefficients given in Table I.

Summary

Optimum conditions for production of maximum conjugation of methyl arachidonate were determined. These comprise heating the sample in 21% KOH glycol for 15 minutes at 180°C. The optimum conditions of isomerization have also been applied to methyl linoleate, methyl linolenate, methyl eicosapentaenoate, and docosapentaenoate prepared by physical methods. These conditions greatly increased the sensitivity of the spectrophotometric method for all the polyunsaturated acids except linoleic, for which the sensitivity was unchanged.

Analyses of a series of fats and oils isomerized under optimum conditions and also under standard conditions were in good agreement. Constants are given for use when pentaene acids are present as well as for acids of less unsaturation.

Spectroscopic evidence strongly indicates that pentaene acids are present in lard.

REFERENCES

1. Baldwin, A. R., and Longenecker, H. E., Oil and Soap, *22*, 151-153 (1945).
2. Beadle, B. W., and Kraybill, H. R., J. Am. Chem. Soc., *66*, 1232 (1944).
3. Berk, L. C., Kretchmer, N., Holman, R. T., and Burr, G. O., Anal. Chem., *22*, 718-720 (1950).
4. Brice, B. A., and Swain, M. L., J. Opt. Soc. Am., *35*, 532-544 (1945).
5. Brice, B. A., Swain, M. L., Schaeffer, B. B., and Ault, W. C., Oil and Soap, *22*, 219-224 (1945).
6. Brice, B. A., Swain, M. L., Herb, S. F., Nichols, P. L. Jr., and Riemenschneider, R. W., J. Am. Oil Chem. Soc., *29*, 279-287 (1952).
7. Herb, S. F., Riemenschneider, R. W., and Donaldson, J., J. Am. Oil Chem. Soc., *28*, 55-58 (1951).
8. Herb, S. F., Witnauer, L. P., Riemenschneider, R. W., J. Am. Oil Chem. Soc., *28*, 505-507 (1951).
9. Hilditch, T. P., Morton, R. A., and Riley, J. P., The Analyst, *70*, 68-74 (1945).
10. Holman, R. T., and Burr, G. O., Arch. Biochem., *19*, 474-482 (1948).
11. Matthews, N. L., Brode, W. R., and Brown, J. B., J. Am. Chem. Soc., *63*, 1064-1067 (1941).
12. Mitchell, J. H. Jr., Kraybill, H. R., and Zscheile, F. P., Ind. Eng. Chem., Anal. Ed., *15*, 1-3 (1943).
13. Nichols, P. L. Jr., Riemenschneider, R. W., and Herb, S. F., J. Am. Oil Chem. Soc., *27*, 329-336 (1950).
14. O'Connor, R. T., Heinzelman, D. C., and Dollear, F. G., Oil and Soap, *22*, 257-263 (1945).
15. Report of the Spectroscopy Committee, J. Am. Oil Chem. Soc., *26*, 399-404 (1949); *28*, 331-335 (1951).
16. Riemenschneider, R. W., Herb, S. F., and Nichols, P. L. Jr., J. Am. Oil Chem. Soc., *26*, 371-374 (1949).
17. Swain, M. L., and Brice, B. A., J. Am. Oil Chem. Soc., *26*, 272-277 (1949).

[Received March 18, 1952]

A Study of the Spectrophotometric Method for Polyunsaturated Fatty Acids in Cottonseed Oils and A Comparison with Chemical Methods[1]

R. T. O'CONNOR, M. F. STANSBURY, H. G. DAMARE',[2] and S. M. STARK JR., Southern Regional Research Laboratory,[3] New Orleans, Louisiana

IN the evolution of the American Oil Chemists' Society's spectrophotometric method, Cd 7-48, for determining polyunsaturated acids (1) in fats and oils attention was given to the development of a general method. The purpose of the present investigation was to determine how much simplification could be made in the complicated equation specified for the calculation of linoleic acid content in the case of cottonseed oils without adversely affecting the precision and the accuracy of the results. In addition, an attempt has been made to evaluate the use of the spectrophotometric method for the analysis of cottonseed oils by a comparison of the results obtained for unsatu-

[1] Report of a study made under the Research and Marketing Act of 1946.
[2] Present address: 116 Elm drive, Naval Base Subdivision, Charleston, S. C.
[3] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

rated fatty acids with those calculated from iodine and thiocyanogen values. As a further test, the total saturated fatty acid content of cottonseed oils, calculated from spectrophotometric data, was compared with the total saturated fatty acid content determined by the Pelikan and von Mikusch (9) modification of the Bertram oxidation method, further modified by use of sintered glass filter sticks for filtration (3).

The cottonseed oils used in this investigation were obtained from 48 lots of cottonseed selected from 312 samples to give an even distribution in iodine number over a range from 89.8 to 117.0. These selected samples represented a random distribution with respect to 8 varieties, 13 stations, and 3 years. Their fatty acid compositions were calculated from their iodine and thiocyanogen values by use of the equations specified in the American Oil Chemists' Society method Cd 2-38 (1). A more complete description of the samples and details of the analysis of the oils are given in an earlier paper by Stansbury and Hoffpauir (14).

The Ultraviolet Spectrophotometric Method for Linoleic Acid

The American Oil Chemists' Society ultraviolet spectrophotometric method for polyunsaturated acids, tentative method Cd 7-48 (1), with certain modifications in the constants and equations for the calculations as recommended by the 1951 report of the Spectroscopy Committee of the American Oil Chemists' Society (10), was used for all spectrophotometric measurements. This tentative method specifies the use of the following equation for the calculation of linoleic acid content:

(I) Percentage of linoleic acid $= 1.16\ (K'_{233} - K_{233}) - 1.33\ (4.1\ [K'_{268} - \frac{1}{2}\ (K'_{262} + K'_{274})] - 2.8\ [K_{268} - \frac{1}{2}\ (K_{262} + K_{274})]) + 0.09\ (2.5\ [K'_{316} - \frac{1}{2}\ (K'_{310} + K'_{322})] - 2.5\ [K_{316} - \frac{1}{2}\ (K_{310} + K_{322})])$

Consideration of the generally accepted knowledge that normal vegetable oils, such as cottonseed oil, contain no arachidonic acid led the Spectroscopy Committee to recommend simplification of this equation by elimination of all measurements in the tetraenoic region (310-322 mμ) (10). The same committee report recommended the use also of revised constants based on extinction coefficients obtained by measurement of pure polyunsaturated fatty acids prepared by physical methods which were shown to yield pure all cis-isomers, more comparable to the fatty acids as they occur in normal vegetable oils (2, 6, 7, 11). The simplified equation with the revised constants is:

(II) Percentage of linoleic acid $= 1.073\ [K'_{233} - K_{233}] - 1.271\ (4.1\ [K'_{268} - \frac{1}{2}\ (K'_{262} + K'_{274})] - 2.8\ [K_{268} - \frac{1}{2}\ (K_{262} + K_{274})])$

The committee reported that while "it feels that the use of background corrections in the calculation of substantial amounts of arachidonic, linolenic, and linoleic acids is generally unnecessary," collaborative work definitely to determine those cases where background corrections may safely be eliminated was considered necessary before any recommendation could be made. However in a very recent paper (2) the authors of the background-correction method have said that the corrections for extraneous background were introduced for use in the spectrophotometric determinations of small proportions of polyunsaturated fatty acids in various materials and that experience has shown that their application to the analysis of common vegetable oils is not only unnecessarily laborious but can lead to erroneous results. Elimination of background corrections would simplify equation II:

(III) Percentage of linoleic acid $= 1.073\ (K'_{233} - K_{233}) - 1.271\ (K'_{268} - K_{268})$

Equation III can be simplified further in the analysis of cottonseed oils and similar vegetable oils by the following considerations. None of the 48 oils measured in the present work has shown any trace of linolenic acid when the trienoic absorption has been properly corrected for preformed conjugation, which is now considered to arise from oxidized linoleic or oleic acids (5, 8, 15). When no arachidonic acid is present in the sample to be analyzed, it has been recommended that measurements in the tetraenoic region be eliminated (10). Similarly when a sample has been shown to contain no linolenic acid, measurements in the trienoic region (262-274 mμ) can be eliminated. Equation III then becomes:

(IV) Percentage of linoleic acid $= 1.073\ (K'_{233} - K_{233})$

The Spectroscopy Committee considered one additional modification, the use of 45-minute isomerization time instead of 25 minutes. From collaborative data no basis for the selection of one period of isomerization over another could be made, and the question of isomerization is to receive further collaborative study. The present authors feel that the advantages of the longer time of isomerization will be manifested, if at all, only by such collaborative comparisons. Within a single laboratory, with the same apparatus, and the same chemists making the determinations, selection of the time of isomerization is not considered very critical. For this reason no attempts have been made during these studies to determine the more suitable time. A period of 45 minutes for isomerization was used throughout this work, and the equations II through IV have been based on the revised constants for this time of isomerization as published in the recent report of the Spectroscopy Committee (10).

The data obtained from measurement of the 48 cottonseed oils have been calculated by use of equations II, III, and IV to determine what effect, if any, the proposed simplifications have on the precision and accuracy of the method.

Results

Diene-conjugated constituents were calculated from spectrophotometric measurements of the non-isomerized cottonseed oils, using the simplified equation recommended in the 1951 report of the Spectroscopy Committee (10):

(V) Percentage of diene constituents $= 0.87\ (K_{233} - 0.07)$.

Triene-conjugated constituents were obtained by use of the equation recommended in the American Oil Chemists' Society Tentative Method Cd 7-48 (1):

(VI) Percentage of triene constituents $= 0.47\ (2.8\ [K_{268} - \frac{1}{2}\ (K_{262} + K_{274})])$

As cottonseed oils are generally acknowledged to contain no arachidonic acid, no measurements were

TABLE I
Fatty Acid Composition of Cottonseed Oils

Sample. no.	I_2 value Wijs	T. C. value	Conjugated dienoic acids[1] %	Linoleic acid		Oleic acid		Saturated acids		
				I_2–T. C.[2] %	Spec.[1] %	I_2–T. C.[2] %	Spec.[1] %	I_2–T. C.[2] %	Spec.[1] %	Bertram[3] %
1	89.8	62.2	0.16	34.0	36.8	36.0	30.0	29.2	32.2	30.0
2	90.6	62.6	0.18	34.4	36.1	35.9	32.0	28.9	30.8	29.4
3	91.1	62 5	0.15	35.2	37.3	35.0	30.3	29.1	31.5	29.9
4	93.7	62.3	0.23	38.7	42.7	31.0	22.5	29.6	33.9	31.2
5	94.6	62.8	0.21	39.2	41.8	31.0	25.2	29.1	32.1	29.7
6	95.0	63.5	0.51	38.8	42.0	32.3	24.9	28.3	32.0	29.8
7	95.9	63.1	0.48	40.4	42.2	30.1	25.6	28.9	31.1	30.0
8	96.4	63.6	0.24	40.4	42.5	30.6	25.8	28.4	30.8	28.8
9	96.7	63.3	0.23	41.2	41.8	29.5	27.6	28.6	29.6	29.4
10	97.4	64.0	0.44	41.2	45.4	30.3	20.9	27.9	32.6	29.4
11	98.7	63.2	0.27	43.8	44.4	26.5	24.5	29.1	30.2	29.8
12	98.7	64.3	0.40	42.4	44.7	29.3	23.8	27.7	30.5	27.9
13	98.8	65.0	0.31	41.7	43.5	30.9	26.6	26.8	29.0	27.5
14	100.2	64.5	0.39	44.0	46.3	27.8	22.4	27.6	30.3	28.3
15	100.5	64.1	0.35	44.9	45.6	26.4	24.2	28.0	29.1	29.1
16	100.8	63.8	0.38	45.7	46.4	25.2	22.8	28.5	29.8	29.2
17	100.9	63.5	0.45	46.2	47.6	24.3	20.5	28.9	30.8	29.5
18	100.9	63.6	0.36	46.0	48.1	24.6	19.5	28.8	29.4	29.3
19	101.0	64.5	0.36	45.0	47.7	26.7	20.6	27.6	30.6	28.9
20	101.3	64.4	0.31	45.5	48.5	26.1	19.4	27.7	30.2	27.3
21	101.7	65.0	0.52	45.3	48.1	27.0	20.2	27.1	30.5	27.8
22	102.2	64.9	0.63	46.0	49.1	26.1	18.8	27.3	30.9	28.4
23	102.7	65.6	0.42	45.8	49.0	27.2	19.7	26.3	30.1	27.1
24	104.8	66.9	0.59	46.8	50.7	27.7	18.6	24.9	29.5	26.7
25	105.1	66.3	0.51	47.9	49.7	25.7	20.7	25.8	28.5	27.0
26	105.5	66.0	0.43	48.8	50.6	24.4	19.7	26.1	28.6	27.2
27	105.7	65.7	0.44	49.4	50.9	23.4	19.4	26.6	28.7	27.0
28	105.8	66.4	0.21	48.6	51.6	25.0	18.5	25.8	29.0	26.6
29	106.9	67.8	0.71	48.2	53.5	27.1	15.3	24.1	30.0	26.2
30	107.3	66.8	0.52	50.0	52.6	24.0	17.7	25.3	28.4	26.6
31	108.1	68.5	0.50	48.9	52.6	27.2	18.5	23.3	27.7	24.9
32	108.2	67.0	0.38	50.9	53.6	23.3	17.2	25.2	28.2	26.0
33	109.2	68.5	0.42	50.2	52.8	25.8	15.7	23.3	26.3	25.2
34	109.8	68.2	0.58	51.4	54.7	24.2	16.3	23.8	27.8	26.3
35	110.5	67.8	0.26	52.7	54.3	22.2	18.4	24.4	26.3	24.9
36	110.6	67.7	0.42	53.0	54.7	21.9	17.4	24.4	26.7	25.7
37	110.8	68.7	0.50	52.0	54.5	24.1	18.2	23.3	26.3	24.5
38	111.1	70.5	0.75	50.1	54.7	28.2	17.2	21.1	26.7	24.3
39	111.9	69.2	0.55	52.7	53.8	23.9	20.6	22.8	24.5	24.3
40	112.2	68.6	0.36	53.8	56.3	22.0	16.3	23.6	26.5	23.4
41	113.0	69.8	0.46	53.3	55.5	23.9	18.7	22.1	24.6	24.1
42	113.2	69.7	0.17	53.7	56.4	23.4	17.6	22.3	25.2	23.6
43	113.7	70.1	0.27	53.8	55.0	23.7	20.9	21.8	23.1	23.2
44	114.6	69.9	0.48	55.2	56.5	22.0	18.3	22.1	24.0	23.7
45	116.0	71.1	0.28	55.4	57.3	23.2	18.7	20.7	23.0	21.8
46	116.2	71.1	0.31	55.7	57.1	22.9	18.9	20.7	22.6	22.4
47	116.8	71.1	0.40	56.4	57.5	22.1	18.8	20.8	22.6	22.1
48	117.0	71.1	0.44	56.7	59.7	21.8	14.9	20.9	24.3	22.1
Highest value	117.0	71.1	0.75	56.7	59.7	36.0	32.0	29.6	33.9	31.2
Lowest value	89.8	62.2	0.15	34.0	36.1	21.8	14.9	20.7	22.6	21.8
Range	27.2	8.9	0.60	22.7	23.6	14.2	17.1	8.9	11.3	9.4
Average	104.5	66.3	0.39	47.1	49.5	26.5	20.9	25.7	28.5	26.8

Summary deviation of spectrochemical from I_2—T. C. and Bertram:

Maximum	+5.3	−11.8	+5.9	+3.8
Minimum	+0.6	− 1.9	+0.6	0.0
Mean	+2.35	− 5.60	+2.76	+1.66
Standard deviation	±1.06	± 2.06	±1.14	±0.98

[1] Calculated as percent acid in total fatty acids. [Saturated fatty acid contents corrected by subtraction of percent unsaponifiables—see reference (14).]

[2] Calculated as percent triglyceride in oil. [Saturated fatty acid contents corrected by subtraction of percent unsaponifiables—see reference (14).] This method of expressing results is comparable to that in footnote 1.

[3] Calculated as percent triglyceride in oil from Bertram oxidation values, using the factor 1.04946.

made in the tetraene region and no calculations of conjugated tetraenoic constituents were made. In all of the cottonseed oils measured the concentration of triene-conjugated constituents was zero. The percentage of diene-conjugated constituents in each of the 48 oils is given in Table I. The values range from 0.15% to 0.75% with an average of 0.39%. These low values for conjugated dienoic acids indicate that the oils had undergone no appreciable oxidation.

Using either the longer equation with background corrections, but without corrections for arachidonic acid, as recommended in the 1951 report of the Spectroscopy Committee (10), for isomerization time of 45 minutes and with the revised constants:

(VII) Percentage of linolenic acid $= 2.028\ (4.1\ [K'_{268} - \frac{1}{2}\ (K'_{262} + K'_{274})] - 2.8\ [K_{268} - \frac{1}{2}\ (K_{262} + K_{274})])$

or a simplified equation for the same conditions but eliminating all corrections for background

(VIII) Percentage of linolenic acid $= 2.028\ (K'_{268} - K_{268})$

the percentage of linolenic acid, calculated from the data on the cottonseed oils was zero for all samples.

Linoleic acid was calculated by use of equations II, III, and IV. Results by use of equations II and IV were identical for all 48 cottonseed oils. Equation III gave values slightly lower than equations II or IV. Evidently the factor for correction for linolenic acid (in samples which contain no linolenic acid) results in an overcorrection (equation III). However when this factor is itself corrected, probably overcorrected, for background (equation II), the over-all correction is negligible. Results by use of equations II and IV are therefore identical within the numerical significance of the values found. A comparison of actual values obtained for nine of the cottonseed oils, selected to represent the entire range of iodine values, calculated from equation II (or IV) and from equation III, are given in Table II. In this table are listed each of the duplicate results for these cottonseed oils. Using either equation the average deviation between the duplicate determinations is 0.4% with the greatest difference being 0.7% and the least 0.2% of the actual values.

TABLE II

Effect of Choice of Equations on the Spectrophotometric Determination of Linoleic Acid

Sample no.	A.O.C.S. method as revised (equation II) or with simplified equation IV		Simplified equation III	
	A[1]	B[1]	A[1]	B[1]
	%	%	%	%
1	37.0	36.6	36.8	36.5
4	42.6	42.9	42.5	42.8
22	49.4	48.7	49.4	48.7
24	50.8	50.6	50.8	50.5
25	49.3	50.0	49.2	49.9
37	54.3	54.6	54.2	54.6
39	53.6	54.0	53.5	53.9
43	54.9	55.2	54.8	55.1
48	59.6	59.8	59.5	59.7

[1]A and B are duplicate determinations on the same oil.

(For all 48 oils the average deviation was 0.4%, greatest was 0.9%, and the least was 0.0%.) As might be expected from data obtained from a single laboratory, duplicate determinations are considerably closer in agreement than results obtained between laboratories, as given in the Spectroscopy Committee report for 1951. These values may be considered as a measure of the precision with which linoleic acid can be measured by the spectrophotometric method. The differences between the values obtained by using equation II (or IV) and equation III are: greatest 0.32%, least 0.00%, with an average of 0.10%. This average difference is only one quarter as great as that found for duplicate analyses. In other words, they are well within the experimental precision of the method. Simplicity and convenience are the only factors to be considered in the selection of the equation to be used for these calculations.

Use of the simplified equation IV is suggested in analyzing all normal vegetable oils known to contain no linolenic acid, or where equations VII or VIII have shown no linolenic acid to be present. The simplified equation IV is applicable to cottonseed oils, peanut oils, sesame oils, etc. The simplified equation is highly desirable in the use of the spectrophotometric method for polyunsaturated acids as a routine analytical tool. Calculation of linolenic acid first from equation VIII will establish the criterion for safe use of the simplified equation IV if the complete absence of linolenic acid from the sample has not been previously established. For vegetable oils which are shown to contain linolenic acid, the simplified equation III is recommended for calculation of the linoleic acid content.

Comparisons with Previously Reported Chemical Values

The averages of duplicate determinations of linoleic, oleic, and total saturated fatty acids in the 48 cottonseed oils by the spectrophotometric method are given in Table I. Values on these same oils previously obtained by calculation from iodine and thiocyanogen values (14) are given for comparison. In addition, total saturated fatty acid values as determined by a modified Bertram oxidation method (9) are included.

A comparison of the spectrophotometrically and chemically determined values for linoleic acid shows that the former are consistently higher than the latter. The average difference is 2.4, the greatest 5.3, and the least 0.6 percentage units. The consistent differences between the two sets become even more apparent if the spectrophotometric values are multiplied by the constant 0.95138, the average ratio of chemical to spectrophotometric values. The spectrophotometric values thus obtained agree much more closely with the chemical values. The average difference is 0.8 with only 5 cottonseed oils showing a difference of more than 1.6 percentage unit. This indicates that there is some consistent error in either the spectrophotometric or the chemical method, or in both.

The values for oleic acid calculated from the spectrophotometric data are consistently lower than those calculated from iodine-thiocyanogen values. The differences between the two sets of oleic acid values are considerably greater than in the case of linoleic acid. The average difference is 5.6, the greatest difference 11.8, and the least 1.9 percentage units. It must be remembered however that an error of 1 percentage unit in the determination of linoleic acid will result in an error of about 2 percentage units in the opposite direction in the calculated value for oleic acid. The oleic acid values calculated from the spectrophotometric data and from iodine-thiocyanogen values would thus be expected to disagree by approximately twice the difference found when linoleic acid values by the two methods were compared. Results given in Table I confirm this conclusion.

Since total saturated fatty acids are calculated by difference, a positive error of 1 percentage unit in the value for linoleic acid, resulting in a negative error of 2 percentage units in the oleic acid value, will cause a net positive error of 1 percentage unit in the saturated fatty acid value. Consequently comparisons of saturated fatty acid contents calculated from spectrophotometric data with those calculated from iodine-thiocyanogen values would be expected to show differences in the same direction and of about the same magnitude as linoleic acid differences. Inspection of the data in Table I shows that this is the case. The total saturated fatty acid values from calculations from spectrophotometric data are uniformly higher than the corresponding values calculated from the iodine-thiocyanogen method, the average difference being 2.8 percentage units. It can be concluded therefore that the differences between the spectrophotometric and the iodine-thiocyanogen calculated values for oleic and saturated fatty acids are largely a reflection of the differences in the linoleic acid content upon which they depend.

Consideration of the values obtained directly by a modified Bertram oxidation does not offer much assistance in choosing between the spectrophotometric and the iodine-thiocyanogen calculated values for total saturated fatty acids. In all cases the values obtained by direct oxidation are intermediate between those obtained by the other two methods. Although the oxidation values agree somewhat better with the chemically determined ones, the poor precision with which the total saturated fatty acids can be determined by Bertram oxidation does not permit a conclusive choice between the spectrophotometric and iodine-thiocyanogen results.

Factors Affecting Spectrophotometric Accuracy

As the deviations in results obtained for oleic and for total saturated fatty acid content by the two methods appear to be due principally to the deviations in the determined values for linoleic acid, some consideration of the factors which may affect the accuracy of the spectrophotometric determination of this acid seems indicated.

Three factors which might affect the accuracy of the spectrophotometrically determined values for linoleic acid are: a) spectrophotometric slit width changes; b) cis-trans isomeric changes of the fatty acids in the oil samples; and c) absolute accuracy of the extinction coefficients used in establishing the equations.

The effect that changes in slit widths might introduce are apparent. If the measurements of the cottonseed oils, after isomerization, are made with slit widths appreciably narrower than those used in the original determination of the extinction coefficients from measurements on pure acids or esters, values somewhat higher for the extinction coefficients of the oil, and consequently correspondingly higher values for calculated linoleic acid content, might be expected. Brice *et al.* (2) comment on the effect of slit width changes on the measurements of the very sharp maxima and minima encountered in the background correction in the triene region 262-274 mμ. Measurements in the diene region, at 233 mμ, are made where the absorption band is quite broad, and the effect of slit width changes would not be expected to be very critical. However, to test the possibility of this explanation for higher values obtained from spectrophotometric data, measurements of some of the alkali isomerized oils were made at various slit widths (by control of the sensitivity knob of the Beckman Model DU quartz spectrophotometer).[4] These experiments demonstrated that, within reasonable limits, slit width changes do not affect the value of the extinction coefficient as measured at 233 mμ. Slit width changes cannot account for the observed deviations between spectrophotometric and iodine-thiocyanogen results.

Several investigators have shown that upon alkali isomerization under given conditions the pure all-cis isomer of the fatty acids isomerizes to the conjugated form at a rate considerably more rapid than the all-trans or the cis-trans or trans-cis modifications (2, 4, 11). The constants of equation IV are based on the all-cis isomer as this is the modification found in normal vegetable oils. If however extraction or any subsequent handling produced any isomeric change to trans modifications, the constants of the equation would not be strictly applicable. The postulation of trans-isomer formation in the cottonseed oils is however a weak argument to account for the differences observed in the results of the spectrophotometric determinations as compared to those calculated from iodine-thiocyanogen data. Appearance of trans modifications would mean that the alkali isomerization to conjugated forms would not proceed as far as during the alkali isomerization and erroneously low values for the extinction coefficients of the oils would be obtained with resulting lower values from calculations from the spectrophotometric data. Actually the values are too high when compared to iodine-thiocyanogen values. The recent papers of Shreve *et al.* (13) and Swern *et al.* (16) describe a convenient method for detecting the presence of trans isomers by means of infrared absorption measurements. In order to test the possibility of trans-isomer formation, six cottonseed oils were so selected as to cover the range of iodine values with some consideration for those samples having the higher diene conjugation values. If diene conjugation is a measure of the degree of oxidation of the oil, it might be argued that the factors which promoted the greatest degree of oxidation would likewise promote the greatest amount of trans-isomer formation. The infrared absorption spectra of the six selected cottonseed oils was measured through the region 9-11 microns. The spectra of all six were identical with no indication of a maximum at 10.3 microns; the position of an absorption band maximum was attributed to bending vibrations of the C-H bond about a trans C=C group (12). The six oils, selected at random, did not contain any trans isomers. Isomeric changes occurring within the oils cannot therefore account for the deviation between results calculated for spectrophotometric and from iodine-thiocyanogen methods.

The absolute accuracy with which the extinction coefficient for the pure linoleic acid used to obtain the constants in equation IV was determined is of paramount importance to the accuracy of the spectrophotometric procedure. Obviously if a higher value of this extinction coefficient were more accurate, the value of the first constant in equation IV (which is 100/extinction coefficient) would be smaller, and the resulting determined linoleic acid would be lower. Values in closer agreement with those from iodine-thiocyanogen data would therefore be obtained by use of an extinction coefficient somewhat greater than that used to derive equation IV. This conclusion becomes of interest when considered with two more recent evaluations of this constant both of which report somewhat higher values. Brice *et al.* (2) report an average value of 93.9 and Jackson *et al.* (4) a value of 94.2. Both of these values are for the measured extinction of the methyl ester of linoleic acid, calculated to the acid basis. Isomerization was in ethylene glycol under nitrogen for 45 minutes. Use of these extinction coefficients in deriving equation IV would result in constants of 1.065 and 1.062, respectively. Ten of the cottonseed oils were recalculated using this latter value. The results are summarized in Table III. From these results it is obvious that while these increases in the value of the extinction coefficient of alkali isomerized linoleic acid do decrease the calculated percent of this acid in the cottonseed oils, the decrease is too small to account for the deviations between the spectrochemical and iodine-thiocyanogen results. Decrease in the constant of equation IV from 1.073 to 1.062 resulted in a decrease in the average difference between the values obtained from spectrophotometric and from iodine-thiocyanogen data from 2.19 (the average difference between the two methods

TABLE III

Effect of Magnitude of Extinction Coefficient on Calculated Value of Linoleic Acid

Sample no.	Equation IV using 1.073 constant	I_2–T.C.	Equation IV using 1.062 constant
	%	%	%
1	36.8	34.0	36.4
2	36.1	34.4	35.7
3	37.3	35.2	36.9
4	42.7	38.7	42.3
5	41.8	39.2	41.4
44	56.5	55.2	55.9
45	57.3	55.4	56.7
46	57.1	55.7	56.5
47	57.5	56.4	56.9
48	59.7	56.7	59.1
Average deviation	2.19		1.69

[4] The mention of names of firms or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.

for these 10 cottonseed samples using 1.073) to 1.69 (the average difference using 1.062). This is a 22.8% decrease in the difference. To obtain nearly perfect agreement a constant of 1.021, corresponding to an extinction coefficient of 97.9, would be required. Such a value for the extinction coefficient of pure linoleic acid after alkali isomerization seems improbable. The absolute accuracy of the extinction coefficient, upon which equation IV for linoleic acid is based, is of utmost importance to the accuracy with which this acid can be determined by spectrophotometric methods. Further attempts to establish this value most accurately are desirable. In the meantime the results of this study indicate that adoption of the newer constant as reported by either Brice *et al.* (2) or by Jackson *et al.* (4) into the equations for the A.O.C.S. official method seems to be desirable. It is a final observation however that future modifications of this constant do not seem to be very likely to afford complete agreement between the results of spectrophotometric and iodine-thiocyanogen methods. Careful measurement of the thiocyanogen values of natural acids and comparison of the results with values from acids obtained by bromination and debromination might be an approach toward better agreement between the two methods.

Summary

Forty-eight cottonseed oils, selected to represent a random distribution with respect to variety, station, year of growth, and iodine value over the wide range of 89.8 to 117.0 have been analyzed for fatty acid content by the spectrophotometric method.

Equations for calculating the linoleic acid content from spectrophotometric data have been examined, and considerable simplification has been found possible without affecting the final values. A simplified equation has been recommended for the calculation of this acid in cottonseed oils and other vegetable oils containing no linolenic acid. A procedure, whereby the linolenic acid content is first calculated from the spectrophotometric data, has been suggested as a criterion for use of the simplified equation for linoleic acid.

The precision with which linoleic acid can be determined spectrophotometrically in cottonseed oils has been indicated, by the average differences between duplicate determinations on the 48 oils, as 0.4%.

The spectrophotometrically determined values for linoleic acid have been compared with values previously reported which were obtained by calculation from iodine and thiocyanogen values. The average difference in percentage of linoleic acid by the two procedures was 2.4, with the spectrophotometric values being uniformly higher. It is concluded that there is some systematic error in the spectrophotometric method, the chemical method, or in both.

Consideration of the values obtained for oleic and total saturated fatty acid contents by both methods indicates that these differences can be attributed mainly to differences in the linoleic acid determinations upon which they depend.

Evaluation of the relative accuracy of the spectrophotometric and the iodine-thiocyanogen methods, by comparison with the total saturated fatty acid content determined independently by a direct Bertram oxidation procedure, is not very satisfactory. The oxidation values are all intermediate between those calculated from spectrophotometric data and from iodine-thiocyanogen data.

Three factors have been studied which might affect the accuracy of the spectrophotometric determinations: slit width changes; cis-trans isomeric changes; and absolute accuracy of the extinction coefficient of alkali-isomerized pure cis-cis-linoleic acid. These studies show that the first two factors do not influence the accuracy of the linoleic acid determinations in the 48 cottonseed oils. Use of the rather improbable value of 97.9 for the extinction coefficient of alkali-isomerized pure cis-cis linoleic acid would be required for most perfect agreement between the two methods. Use of the more recently reported higher values for the extinction coefficient of pure cis-cis linoleic acid does result in somewhat better agreement between the two methods.

Acknowledgment

The authors gratefully acknowledge the assistance of Henry M. Pearce Jr. in completing many of the calculations involved in these investigations and of Mrs. Elsie F. DuPré for the infrared absorption data.

REFERENCES

1. American Oil Chemists' Society, Official and Tentative Methods, 2nd Ed., edited by V. C. Mehlenbacher, Chicago, 1946, rev. to 1950.
2. Brice, B. A., Swain, M. L., Herb, S. F., Nichols, P. L. Jr., and Riemenschneider, R. W., J. Am. Oil Chem. Soc., *29*, 279-287 (1952).
3. Fisher, G. S., O'Connor, R. T., and Dollear, F. G., J. Am. Oil Chem. Soc., *24*, 382-387 (1947).
4. Jackson, J. E., Paschke, R. F., Tolberg, W. Boyd, H. M., and Wheeler, D. H. Presented before the 25th Annual Fall Meeting of the American Oil Chemists' Society, Chicago, Ill., Oct. 8-12, 1951.
5. Jefferson, M. E., "Physical Properties of Soybean Oil" in "Soybean and Soybean Products," edited by K. S. Markley, Interscience Publishers Inc., New York, 1950, Vol. I, Chapter 7.
6. Nichols, P. L. Jr., Herb, S. F., and Riemenschneider, R. W., J. Am. Chem. Soc., *73*, 247-252 (1951).
7. Nichols, P. L. Jr., Riemenschneider, R. W., and Herb, S. F., J. Am. Oil Chem. Soc., *27*, 329-336 (1950).
8. O'Connor, R. T., Field, E. T., Jefferson, M. E., and Dollear, F. G., J. Am. Oil Chem. Soc., *26*, 710-718 (1949).
9. Pelikan, K. A., and von Mikusch, J. D., Oil & Soap, *15*, 149-150 (1938).
10. Report of the Spectroscopy Committee of the American Oil Chemists' Society 1951, R. C. Stillman, chairman, J. Am. Oil Chem. Soc., *28*, 331-335 (1951).
11. Riemenschneider, R. W., Herb, S. F., and Nichols, P. L. Jr., J. Am. Oil Chem. Soc., *26*, 371-374 (1949).
12. Sheppard, N., and Sutherland, G. B., Proc. Roy. Soc., *A196*, 195 (1949).
13. Shreve, O. D., Heether, M. R., Knight, H. B., and Swern, Daniel, Anal. Chem., *22*, 1261-1264 (1950).
14. Stansbury, M. F., and Hoffpauir, C. L., J. Am. Oil Chem. Soc., *29*, 53-55 (1952).
15. Swain, M. L., and Brice, B. A., J. Am. Oil Chem. Soc., *26*, 272-277 (1949).
16. Swern, Daniel, Knight, H. B., Shreve, O. D., and Heether, M. R., J. Am. Oil Chem. Soc., *27*, 17-21 (1950).

[Received April 2, 1952]