for valuable advice and assistance with the desalting of the hydrolysate of the solid pigment fraction.

REFERENCES

1. Boatner, Charlotte H., Hall, C. M., O'Connor, R. T., Castillion, Leah E., and Curet, M. C., J. Am. Oil Chemists' Soc., *24*, 97–106, 276–283 (1947).
2. Dechary, J. M., Kupperman, R. P., Thurber, F. H., and O'Connor, R. T., J. Am. Oil Chemists' Soc., *31*, 420–424 (1954).
3. Frampton, V. L., and Webber, H. H., Oil and Soap, *23*, 318–323 (1946).
4. Hardy, T. L., Holland, D. O., and Nayler, J. H. C., Anal. Chem., *27*, 971–974 (1955).
5. King, W. H., and Thurber, F. H., J. Am. Oil Chemists' Soc., *33*, 169–171 (1956).
6. Mueller, G. C., Bowman, G., and Herranen, A., Anal. Chem., *27*, 1357–1358 (1955).
7. Pons, W. A. Jr., Hoffpauir, C. L., and O'Connor, R. T., J. Am. Oil Chemists' Soc., *28*, 8–12 (1951).
8. Pons, W. A. Jr., Stansbury, M. F., and Hoffpauir, C. L., Assoc. Off. Agr. Chemists' J., *36*, 492–504 (1953).
9. Shirley, D. A., and Sheehan, W. C., J. Org. Chem., *21*, 1069–1070 (1951).
10. Singleton, W. S., and Bailey, A. E., Oil and Soap, *21*, 224–226 (1944).
11. Winkler, L. W., Z. Angew. Chem., *26*, 231–232 (1913).

[Received June 10, 1957]

Report of the Spectroscopy Committee, 1956-57

DURING THE YEAR ending with the 48th Annual Meeting of the American Oil Chemists' Society in New Orleans the Spectroscopy Committee met in two committee sessions. The first of these was held during the annual fall meeting on September 25 in the Hotel Sherman, Chicago, Ill. Six of the 10-member committee were present: N. D. Fulton, W. E. Link, Robert D. Mair, B. N. Rockwood, Donald H. Wheeler, and Robert T. O'Connor. The second meeting was held at the Roosevelt hotel in New Orleans, La., April 29, 1957, during the 48th annual meeting. Seven members, or their duly authorized alternates, were present: Mr. Fulton, S. F. Herb, K. E. Holt (representing Mr. Link), C. E. Ireland (representing Hans Wolff), Mr. Mair, Dr. Wheeler, and Mr. O'Connor. Joseph McLaughlin Jr., arrived at the 48th Annual Meeting too late to attend the Spectroscopy Committee meeting but met with the chairman at a later date.

During the past few years the committee has been considering three principal problems:

a) modification of the present A.O.C.S. Tentative Method Cd 7-48 for polyunsaturated fatty acids to simplify it and to extend its scope.

b) establishment of a method for the determination of polyunsaturated fatty acids in the presence of large quantities of conjugated constituents, *i.e.*, the determination of linoleic acid in tung oil (containing *ca.* 80% conjugated eleostearic acid).

c) investigation of the infrared absorption method for the determination of *trans* acids in the presence of nonconjugated *cis*-unsaturated and of saturated constituents.

These three problems were discussed at both meetings, and the committee has been actively engaged in collaborative work regarding them during the periods preceding each of these meetings.

Modification of A.O.C.S. Tentative Method Cd 7-48

Based on collaborative study, a completely revised edition of A.O.C.S. Tentative Method Cd 7-48 was approved by the Spectroscopy Committee and accepted by the Uniform Methods Committee. The revised procedure, which both simplifies and extends the scope of the method, is included in the Official and Tentative Methods of the A.O.C.S. as Cd 7-48 (rev. April 1956).

During the year two additional minor revisions have been suggested by members of the committee. The first is the adoption of a more accurate value for the absorptivity for computing preformed conjugated diene. The value, 119, used in the method, is based on original measurements of *trans*-10, *trans*-12-linoleic acid. Advances, particularly in infrared absorption spectroscopy, have shown that preformed conjugation in natural products is not entirely the *trans, trans*-acid, that probably little, if any, *cis, cis*-acid is present, and that very probably a mixture of *cis, trans*- and *trans, trans*-acids accounts for the measured dienoic conjugation. Recent work has shown that upon iodine treatment an equilibrium mixture consisting of 68% *trans, trans*-conjugation and 32% *cis, trans*-conjugation is obtained. Using absorptivities of 119 and 94 for these components, respectively, the absorptivity for the equilibrium mixture is 110. Following discussions of this problem, both in Chicago and in New Orleans, and with correspondence between the two meetings, it was finally agreed that the absorptivity of 110 is the most probable value and the one to be substituted for the obviously too high value of 119. This change has been recommended to the Uniform Methods Committee for adoption.

The second minor modification involves the mathematical expressions for the percentage of linolenic and arachidonic acids in the simplified procedure Cd 7-48 (rev. April 1956). Adhering strictly to the rule that "background" correction should not be made when the absorptivity is greater than 1 and should be applied when it is less than 1 results in an equation having background corrections for the alkali-isomerized portion (where the absorptivities are rather large) but not for the preformed conjugation (where they are small). Subtracting the absorption before isomerization from the value after isomerization is, in itself, a very suitable "background" correction. However, when one of these values has been "corrected," the absorptivity after isomerization is undercorrected. At the New Orleans meeting it was decided that these equations should be modified. The necessary minor changes have been recommended to the Uniform Methods Committee for adoption.

Determination of Polyunsaturated Fatty Acids in the Presence of Large Quantities of Conjugated Constituents

During the first half of the past year the committee undertook the collaborative investigation of a method for the determination of linoleic acid in the presence of large quantities of preformed conjugation, a determination outside the scope of Cd 7-48 (rev. April 1956). Five samples, three mixtures of *alpha*- and *beta*-tung oils and samples of pure *alpha*- and *beta*-eleostearic acids, were sent to each of the 10 members of the Spectroscopy Committee. Results were obtained from eight. Collaborators were asked to determine conjugated diene, *alpha*-eleostearic acid, *beta*-eleostearic acid, and linoleic acid content. Average values reported by the collaborators are given in Table I. From the data furnished the values were recalculated, Table II, by the chairman on the basis of new and improved formulas for *alpha*- and *beta*-eleostearic acids. The tabulated results show very unsatisfactory agreement, caused mainly by isomerizations of the *alpha*-eleostearic acid in the samples to the *beta*-isomer. After considerable discussion during the Chicago meeting it was decided that the committee should first investigate the method for the determination of *alpha*- and *beta*-eleostearic acids as no official procedure for this determination has ever been established. At the New Orleans meeting this discussion was continued. The committee decided to examine, in a collaborative study, the determination of *alpha*- and *beta*-acids in prepared mixtures of these two isomers as a preliminary step to their determination in natural commodities such as tung oil. Collaborative work on this project will be initiated early during the coming year as soon as the pure acids can be obtained in sufficient quantity.

TABLE I

A.O.C.S. Collaborative Tung Oil Samples

Collaborator	Conjugated diene	Conjugated triene		Linoleic acid
		α-Eleo. acid	β-Eleo. acid	
Sample 1				
(1)	1.0	72.6	10.0	4.6
(2)	0.87	73.45	3.07	0.50
(3)	1.00	75.7	0.0	5.68
(4)	0	76.3	0	8.98
(5)	1.55	74.2	7.0	5.8
(6)	1.18	61.3	8.70	8.11
(7)	0.60	75.0	0	8.26
(8)	0.75	76.4	3.95	5.02
Sample 2				
(1)	0	65.1	13.9	7.3
(2)	0	77.47	0.04	3.00
(3)	0.84	73.3	0.0	6.85
(4)	0	70.4	0	16.2
(5)	0.44	65.5	16.5	5.8
(6)	1.55	60.0	4.15	7.38
(7)	1.32	70.8	0	8.01
(8)	0.12	79.1	0	6.29
Sample 3				
(1)	1.6	15.1	58.8	4.7
(2)	1.35	15.62	55.61	0.00
(3)	1.91	17.7	53.0	4.25
(4)	0	0	67.5	16.29
(5)	2.35	14.2	62.9	3.1
(6)	5.18	2.80	48.8	2.83
(7)	1.08	32.1	45.5	5.94
(8)	1.36	9.9	64.1	4.04
Sample 4				
(1)	0	15.8	8.7	0
(2)	0.26	11.09	14.21	0.00
(3)	0.25	23.5	0.00	0.24
(4)	0	23.8	1.8	2.91
(5)	0	0.04	0.02	0.94
(6)	0.44	10.16	14.84	0
(7)	[1.65]	[96.1]	[0]	[—]
(8)	0.41	25.1	0	0
Sample 5				
(1)	0.4	0.5	22.6	0
(2)	1.24	0	23.81	0.00
(3)	0.65	0.0	20.9	0.00
(4)	0	0	24.2	5.6
(5)	0.01	0.0	0.09	0.50
(6)	1.50	0	23.40	0
(7)	[1.68]	[20.3]	[77.6]	[—]
(8)	0.72	0	24.4	0

Determination of Trans Acids as Elaidic by Means of Infrared Absorption

During the second half of the past year the committee has been engaged on the first collaborative test of published methods for the determination of *trans* acids in the presence of nonconjugated *cis*-unsaturated and of saturated constituents. The committee measured as "elaidic acid" or as "methyl elaidate" the *trans*-acid content of six samples: hydrogenated vegetable shortening, animal shortening, *trans* olefin, partially hydrogenated cottonseed oil—normal conditions, partially hydrogenated cottonsed oil—highly selective conditions, and partially hydrogenated cottonseed oil—nonselective conditions. Results reported by eight collaborators are given in Tables III and IV. The collaborators were furnished, along with the samples for analysis, standard samples of elaidic, stearic and oleic acids, copies of two published methods (J. Am. Oil Chemists' Society, *27*, 17–21 [1950] and *28*, 61–5 [1951]) and laboratory "working procedures" submitted by the two laboratories responsible for the published methods. Table III shows the results calculated from measurements on the samples as furnished with either elaidic acid used as the instrument calibration or after conversion of this standard to the methyl elaidate. Table IV gives the results after conversion of the samples to the methyl esters before attempting the analyses.

From an analysis of these results the committee, after a general discussion at their New Orleans meeting, reached the following general conclusions.

a) If all collaborators were to follow in strictest detail a very specific set of experimental procedures, good collaborative agreement might be expected.

b) Each infrared spectrophotometer to be used for the quantitative determination of *trans* acids must be calibrated individually with a suitable standard or standards.

TABLE II

Recalculations on Collaborative Tung Oil Samples, Using New Formulas for α- and β-Eleostearic Acid

Collaborator	Conjugated diene	Conjugated triene		Linoleic acid
		α-Eleo. acid	β-Eleo. acid	
Sample 1				
(1)	1.40	72.9	7.0	4.57
(2)	1.20	72.6	1.3	1.08
(3)	1.40	72.2	0	5.88
(4)	0.09	72.8	0	9.00
(5)	1.88	74.0	4.6	3.92
(6)	1.50	61.8	6.1	8.14
(7)	......	......	......	......
(8)	1.20	75.4	1.8	5.12
Sample 2				
(1)	.25	66.6	10.4	7.33
(2)	.49	74.0	0	3.30
(3)	1.10	69.9	0	7.00
(4)	0	67.2	0	10.3
(5)	.84	67.1	12.5	5.70
(6)	1.78	59.5	2.3	7.12
(7)	......	......	......	......
(8)	.50	76.2	0	6.50
Sample 3				
(1)	1.55	27.9	48.5	4.08
(2)	1.63	27.7	45.7	0
(3)	1.96	29.0	43.7	3.70
(4)	0	13.1	57.8	9.96
(5)	2.38	27.9	51.9	2.21
(6)	2.88	16.9	53.2	3.36
(7)	......	......	......	......
(8)	1.36	24.0	53.0	3.42
Sample 4				
(1)	.10	17.4	6.91	0
(2)	.21	14.0	11.6	0
(3)	.38	22.3	0	.32
(4)	.44	23.7	1.07	0
(5)	0	.05	.02	1.40
(6)	.46	13.4	12.2	0
(7)	......	......	......	......
(8)	0.25	26.4	0	0
Sample 5				
(1)	.34	5.52	18.7	0
(2)	1.18	0	30.1	0
(3)	.62	2.73	18.9	0
(4)	.40	2.54	22.3	.30
(5)	0	.03	.07	.50
(6)	1.50	0	23.5	0
(7)	......	......	......	......
(8)	0.56	0	25.4	0

(7) Insufficient data furnished for these calculations.

TABLE III

Percentage of *Trans* Acids as "Elaidic Acid" from Analyses on Samples "as received" (Glycerides)

Collaborator	No. 1[a]		No. 2	No. 3[d]		No. 4[a]		No. 5[e]		No. 6[d]					No. 7	No. 8				Average all results[f]	S.D.[g]	C.V.[h] %
Method[b]	J&C	J&C	J&C	J&C	J&C	Swern	J&C	Swern	J&C	Swern					Swern	Swern	Swern	J&C	J&C			
Instrument[c]	PE-21	IR-4	PE-21	IR-3	IR-3	PE-21	PE-21	PE-21	PE-21	PE-21	PE-21	IR-3	IR-3	IR-3	PE-21	PE-21	IR-2T	PE-21	IR-2T			
	Av. 2 detn.		Av. 2 detn.			Av. 2 detn.		Av. 3 detn.		Av. 2 detn.					Av. 2 detn.	Av. 2 detn.						
No. 1—Hydrogenated vegetable shortening	39.4	39.9	47.0	42.1	42.5	42.1	39.2	37.9	37.5	35.8	38.5	39.9	39.9	41.1	42.0	36.8	38.3	38.0	41.5	40.0	2.5	6.27
No. 2—Animal shortening	—	—	0.0	4.1	4.0	8.1	3.5	3.3	3.2	3.3	3.5	3.2	3.4	3.7	1.0	1.4	2.9	1.4	3.1	3.1	1.7	53.87
No. 3—*Trans* olefin	61.7	60.8	77.4	69.0	69.3	59.7	66.0	65.3	64.5	58.9	61.9	65.5	65.4	66.4	71.6	62.4	61.6	64.5	66.7	65.2	4.4	6.67
No. 4—Hydrogenated cottonseed oil (normal)	32.8	33.0	40.6	35.3	36.3	36.2	33.1	33.9	33.5	31.5	32.6	34.1	34.2	35.1	34.3	31.2	32.1	32.2	34.8	34.0	2.1	6.18
No. 5—Hydrogenated cottonseed oil (selective)	39.9	39.9	50.1	43.0	43.7	43.3	40.4	41.8	41.3	37.9	40.0	41.9	41.7	43.3	43.6	37.7	40.1	39.0	43.5	41.7	2.7	6.50
No. 6—Hydrogenated cottonseed oil (nonselective)	23.3	24.1	30.4	26.0	26.4	27.3	23.7	24.9	24.6	22.6	24.2	24.7	24.6	25.6	24.0	21.9	23.4	22.6	25.4	24.7	1.9	7.65

[a] Results reported as "methyl elaidate" converted to "elaidic acid" by chairman for comparison.

[b] Letters in this row indicate method used for analysis:

J&C = Jackson, Frank L., and Callen, Joseph E., J. Am. Oil Chemists' Soc., *28*, 61–65 (1951), and laboratory variations thereof involving baseline technique background correction.

Swern = Swern, Daniel, Knight, H. B., Shreve, O. D., and Heether, M. R., J. Am. Oil Chemists' Soc., *27*, 17–21 (1950), and laboratory variations thereof, involving background corrections from measurements of stearic and oleic acids or derivatives.

[c] Letters and numbers in this row indicate instrument used for analysis:

PE-21 = Perkin-Elmer Corp. Spectrophotometer Model 21.
IR-3 = Beckman Instruments Inc. Spectrophotometer Model IR-3.
IR-4 = Beckman Instruments Inc. Spectrophotometer Model IR-4.
IR-2T = Beckman Instruments Inc. Spectrophotometer Model IR-2T.

[d] Collaborator reported analyses at two (or more) instrumental slit widths.

[e] Values computed by chairman from absorptivities reported.

[f] Eight laboratories using 11 different spectrophotometers including four different models.

[g] S.D. = Standard deviation.

[h] C.V. = Coefficient of variation.

TABLE IV

Trans Acids as "Methyl Elaidate" from Analyses of Samples After Their Conversion to Methyl Esters

Collaborator	No. 1				No. 2[a]	No. 5[d]	No. 6			Average all results[e]	S.D.[g]	C.V.[h] %	No. 7[f]	
Method[b]	Swern	Swern	J&C	J&C	Swern	J&C	Swern	J&C[a]	J&C[a]				J&C	J&C
Instrument[c]	PE-21	IR-4	PE-21	IR-4	PE-21	PE-21	PE-21	IR-3	IR-3				PE-12C	PE-112
	Av. 2 detn.				Av. 2 detn.	Av. 3 detn.	Av. 2 detn.						Av. 2 detn.	Av. 4 detn.
No. 1—Hydrogenated vegetable shortening	33.8	33.6	33.7	34.5	31.1	36.8	41.5	36.9	39.0	35.7	3.0	8.43	36.6	37.4
No. 2—Animal shortening	—	—	—	—	0.0	0.3	0.0	0.1	0.1	—	—	—	0.4	0.4
No. 3—*Trans* olefin	—	—	—	—	60.1	—	—	—	72.2	66.2	6.1	9.14	61.7	—
No. 4—Hydrogenated cottonseed oil (normal)	27.0	27.7	27.6	28.2	25.3	29.8	34.2	30.4	31.6	29.1	2.5	8.73	31.9	31.2
No. 5—Hydrogenated cottonseed oil (selective)	34.6	34.4	36.1	36.6	32.6	38.2	42.1	39.1	40.4	37.1	2.9	7.84	39.1	27.9
No. 6—Hydrogenated cottonseed oil (nonselective)	18.4	17.6	18.3	19.9	17.5	20.7	22.2	21.0	21.0	19.6	1.6	8.27	22.0	21.7

[a] Values reported as "Elaidic Acid" converted by chairman for comparison.

[b] Letters in this row indicate method of analysis:

Swern = Swern, Daniel, Knight, H. B., Shreve, O. D., and Heether, M. R., J. Am. Oil Chemists' Soc., *27*, 17–21 (1950), and laboratory variations thereof, involving background corrections from measurements of stearic and oleic acids or derivatives.

J&C = Jackson, Frank L., and Callen, Joseph E., J. Am. Oil Chemists' Soc. *28*, 61–5 (1951), and laboratory variations thereof involving baseline technique background correction.

[c] Letters and numbers in this row indicate instrument used

PE-12C = Perkin Elmer Corp. Spectrophotometer Model 12C.
PE-21 = Perkin Elmer Corp. Spectrophotometer Model 21.
PE-112 = Perkin Elmer Corp. Spectrophotometer Model 112.
IR-3 = Beckman Instruments Inc. Spectrophotometer Model IR-3.
IR-4 = Beckman Instruments Inc. Spectrophotometer Model IR-4.

[d] Values computed by Chairman from absorptivities reported.

[e] Average of results from four collaborators, using six different instruments, including three different models.

[f] Collaborator No. 7 reported directly triglyceride (trielaidin) content from measurements on Perkin Elmer Models PE-12C and PE-112.

[g] S.D. = Standard deviation.

[h] C.V. = Coefficient of variation.

c) Ideally, if acids are to be analyzed, the standard should be elaidic acid; if esters, elaidates; and if triglycerides, trielaidin.

d) The procedure whereby each and every sample is converted to some other form, in order to complete the analysis on the same basis as the standardization, *i.e.*, converting triglycerides to methyl esters to agree with a methyl elaidate standard, is not to be recommended.

e) Considerably more collaborative work is required before a specific procedure can be selected for recommendation to the Society as a tentative A.O.C.S. method.

It was agreed at the New Orleans meeting that the immediate activities should consist of a) composition of a specific procedure by the chairman, b) examination of this procedure by each committee member and revision in accordance with collected comments and criticisms, and c) collaborative test of the agreed specific procedure, probably with simple mixtures of acids, esters, or triglycerides, using the corresponding standard elaidic acid, elaidate, or trielaidin for instrument calibration. Action on these proposals is to be initiated immediately, and a second round of collaborative testing is planned later during the coming year.

Acknowledgment

The committee gratefully acknowledges the cooperation and assistance of several individuals in the completion and interpretation of the collaborative work. Thanks go particularly to those individuals in infrared spectroscopy laboratories which are apart from the actual committee representative, who participated in the determination of *trans* acids, and to several individuals who assisted in computing and compiling the various sets of data. The chairman in particular wishes to acknowledge the able assistance of Miss Dorothy Heinzelman for compilation of the collaborative work on the tung oil samples and Miss Elizabeth R. McCall and of Mrs. Elsie F. DuPre for compilation of the infrared absorption data included in this report.

Robert T. O'Connor, chairman
N. D. Fulton
Seymore Goldwasser
Samuel F. Herb
William E. Link
Joseph McLaughlin Jr.
Robert D. Mair
B. N. Rockwood
Donald H. Wheeler
Hans Wolff

Thermal-Diffusion Fractionation: Oils, Fats, and Some Derivatives[1]

C. W. Seelbach[2] and F. W. Quackenbush, Department of Biochemistry, Purdue University, Lafayette, Indiana

Recent reports of the effectiveness of thermal diffusion in concentrating certain components of petroleum oils (5, 7) or crude natural oils (3, 4)[3] have suggested the possible usefulness of this principle in separating biochemical substances, especially the lipides and their derivatives. In this laboratory studies have included the fractionation of mixtures containing acids, esters, alcohols (12), and natural isoprenoid compounds such as carotenoids, squalene, phytol, or terpene oils (13). This paper reports experiments with fat and oil components and derivatives: mono-, di-, and triglycerides, fatty acids, methyl esters of fatty acids from soybean oil, polymeric triglycerides, and certain trace components, particularly pigments, in crude vegetable or marine oils.

Experimental

Apparatus. A thermal-diffusion column used in this work is described elsewhere (12). It was constructed with parallel, stainless-steel plates, heated with condensing steam (atmospheric pressure) and cooled with tap water. Oils placed in the column were allowed to diffuse for 72 hrs.; then the contents were removed in 10 fractions of 2 ml. each.

Analytical Methods. Refractive index values were determined with an Abbé refractometer thermostated at 40 ± 0.2°C. Acid values and saponification values were determined by standard methods. Monoglyceride and glycerol values were obtained by the method of Pohle and Mehlenbacher (9).

Spectrophotometric measurements were obtained with a Beckman instrument, Model D. U. Absorbance values were determined in the usual manner for the oils dissolved in commercial hexane (Skellysolve B), then the absorptivity values of the oil were calculated after the manner of O'Connor *et al.* (8). This permitted the direct comparison of the color of fractions when absorbance was of necessity determined at different solute (oil) concentrations.

Materials. Glyceryl trioleate (Emery 2230) was obtained from Emery Industries Inc., Cincinnati, O., and glyceryl (mono) oleate (S-1097) was obtained from Glyco Products Company, Brooklyn, N. Y.

Crude methyl esters were prepared from soybean oil by methanolysis at room temperature for 24 hrs. with 1.6 equivalents of methanol and 0.5% potassium hydroxide as catalyst. The glycerol layer that separated was drawn off, then the crude methyl ester preparation was washed three times with warm water and dried over anhydrous sodium sulfate.

Mixed oleic and linoleic acids (Neofat 3R)—Armour Chemical Division, Chicago, Ill.

Dehydrated castor oil (Castung 103GH) and thermally polymerized, dehydrated castor oil (Castung 403UV)—The Baker Castor Oil Company, New York, N. Y.

Cod-liver oil refined, and vitamins A and D (Nopco XX)—Nopco Chemical Company, Harrison, N. J.

Crude vegetable and marine oils as follows:

Wheat germ oil—Viobin Corporation, Monticello, Ill.

Sesame and safflower oils—Pacific Vegetable Oil Corporation, San Francisco, Calif.

Tung, palm, and refined rapeseed oils—Balfour, Guthrie and Company, New York, N. Y.

---

[1] Published as Journal Paper No. 842, Purdue University Agricultural Experiment Station.

[2] Present address: Esso Research and Engineering Company, Esso Research Center, Linden, N. J.

[3] The concentration of carotenoids in palm oil, of tocopherol in acidulated deodorizer sludge, and of steroids in soya acid oil have also been accomplished at the Chemical and Physical Research Division Laboratory of the Standard Oil Company, Ohio (14).