Spectrophotometric Determination of Small Proportions of Polyunsaturated Constituents in Fatty Materials

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Introdution

THE thiocyanometric method for determining fatty acid compositions, even when carried out under carefully controlled conditions, is subject to considerable uncertainty when applied to substances having relatively small proportions of polyunsaturated constituents. Deficiency of the method has been recognized by the frequent supplementary use of the insoluble bromide test, particularly for such substances as arachidonic acid. The difficulties and inherent errors of the latter method of analysis need no elaboration.

Recently developed spectrophotometric methods (4, 9, 2, 7a, 5) offer the possibility of obtaining more detailed information regarding polyunsaturated constituents of fats and oils than can possibly be gained by use of the thiocyanometric method. Specific examples of conditions under which spectrophotometric methods are likely to prove exceptionally valuable for analysis of fats are: (a) cases in which the proportions of individual polyunsaturated constituents are relatively small, as in animal fats and oils, partially hydrogenated fats and oils, specially purified fats, fatty acids, and esters; (b) cases in which conjugated fatty acids as well as nonconjugated fatty acids occur.

It is difficult to estimate the significance of having more definite information regarding the nature and concentration of individual polyunsaturated constituents in fats and fatty materials. It seems entirely reasonable, however, to expect that ready means for obtaining such information will lead to increased knowledge regarding such important reactions as oxidation, hydrogenation, polymerization, and isomerization.

A recent research program at this laboratory required the accurate analysis of animal fats and their soaps, including some partially hydrogenated products, for polyunsaturated constituents. Difficulties were encountered in attempting to apply the spectrophotometric method of Mitchell, Kraybill, and Zscheile (9) and its extension by Beadle and Kraybill (2), to these materials. These difficulties have been largely overcome in a modified method proposed by Brice and Swain (5). This method involves measurement of the ultraviolet absorption of a sample; correction of data for absorption by extraneous constituents; conversion of the nonconjugated fatty acid constituents to their absorbing conjugated forms by an alkali isomerization treatment, the latter in glycerol instead of ethylene glycol in order to obtain a more transparent and reproducible "blank"; measurement of the ultraviolet absorption after isomerization; correction of data for absorption by extraneous compounds and by conjugated fatty acid constituents originally present and remaining undestroyed by the isomerization treatment; and calculation of the proportions of conjugated and nonconjugated diene, triene, and tetraene fatty acid constituents present in the sample.

The purpose of the present paper is to present and discuss the results of analysis, by this modified method, for a number of oils, fats, soaps, and purified acids and esters. Details regarding the development and standardization of the method will be published elsewhere (5). The complete procedure used in the analysis, however, will be presented here also.

Apparatus and Materials

ALL ultraviolet absorption measurements were made with a Beckman ultraviolet photoelectric spectrophotometer equipped with hydrogen lamp and an absorption cell compartment adapted to hold cells up to 10 cm. long. Demountable absorption cells were used, consisting of Pyrex glass bodies (Aminco Style DX) 22 mm. in diameter and 1, 2.5, and 5 cm. in length, equipped with threaded metal ends, metal screw caps, cork gaskets, and crystalline quartz windows.

The constant temperature bath used for the isomerization reaction comprised a large Pyrex glass jar mounted in an insulated wooden box fitted with a transite cover drilled to hold accessories and six reaction tubes. The bath was equipped with a 500-watt stainless steel immersion heater, a 12-inch mercury thermoregulator and controls, stirrer, and Fisher bathwax as a medium. Bath temperature was maintained at 180° C. ±0.1°.[2] The reaction tubes were 6 x 1 inch Pyrex glass test tubes, each suspended by a bent steel wire attached to a cork fitting a 1¾ inch hole in the transite bath cover. With this arrangement, the reaction tube was immersed to a depth of 4½ inches in the bath liquid and the top of the tube was beneath the bath cover. Crucible covers No. 00 were used as covers for the reaction tubes.

The isomerization reagent solution was prepared by adding 17.5 grams of KOH (A. C. S. standard grade) per 100 ml. of glycerol (U. S. P. XII), and heating the mixture with constant stirring to 200° C. to dissolve the KOH and drive off excess water. The resulting reagent should have a KOH concentration of 10.9 to 11.0% by weight and should be checked by titration. A stock solution can be kept at least one week without significant change.

Ethyl alcohol (95%)[3] was used as a solvent in the analysis for conjugated constituents of soaps, fatty acids, and esters. Neohexane (technical, Phillips Petroleum Company) was used as a solvent for oils and fats. Isooctane (2,2,4-trimethyl pentane, Rohm and Haas) is also satisfactory. The hydrocarbon solvents were rendered sufficiently transparent for

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[2] A constancy of ±0.5°C. has been found sufficient.

[3] Absolute methyl alcohol, synthetic, has also been found to be suitable. We are indebted to E. L. Borg of the U. S. Rubber Company for suggesting this solvent.

use by passage through a column of silica gel (7). Absolute ethyl alcohol[3] was used as a solvent for the isomerized product in the analysis for nonconjugated constituents.

Small Pyrex glass cups 10 mm. in diameter and 15 mm. high were used as sample containers, both for weighing and for introducing samples into the reaction tubes.

Purified Fatty Acid Standards

PURIFIED 10,12-linoleic acid was prepared from dehydrated castor oil by alkali isomerization of commercial dehydrated castor oil fatty acids followed by repeated fractional crystallization from a petroleum solvent. It melted at 57.0° C. Specific extinction coefficients[4] of 119 in neohexane and 117 in ethyl alcohol were obtained for this acid, the absorption maximum being at 231 mμ in both cases.

Purified beta-eleostearic acid was used as a standard in the analysis for conjugated triene fatty acids. This acid was prepared from tung oil by converting it to tung butter with a trace of sulfur as catalyst. The beta-eleostearic acid was then purified by repeated fractional crystallization of the tung butter fatty acids first from a petroleum solvent and later from acetone. It melted at 72.0° C. Specific extinction coefficients of 214 at 267 mμ in neohexane and 209 at 268 mμ in ethyl alcohol were obtained for this acid.

A fraction rich in linoleic acid was prepared by low temperature crystallization of corn oil fatty acids from acetone. Linoleic acid was then prepared from this material by the method of Rollet (12) and subjected to two further crystallizations from petroleum ether at —65° C. The high purity of the acid was indicated by the iodine value 181.9 (theory 180.3) and acid value 198.9 (theory 200.2). Spectrophotometric examination of the final product indicated approximately 0.3% linolenic, 1.7% conjugated dienoic, and 0.02% conjugated trienoic acids, and consequently a purity of not greater than 98.0%. After isomerization for 30 minutes at 180° C. in 11% KOH-glycerol, by the procedure prescribed below, the specific extinction coefficient at 232 mμ, adjusted to 100% linoleic acid, was 88.9.

A linolenic acid concentrate was prepared by removal of the more saturated components from perilla oil fatty acids at —49° C. Linolenic acid was then prepared by isolation and reduction of the hexabromides to give a product having the following constants: iodine value 273.5 (theory, 273.6); acid value, 200.6 (theory, 201.7). Spectrophotometric examination indicated approximately 0.1% tetraenoic and 1.5% conjugated dienoic acids. Calculations based on these data indicate a purity of not more than 98.4% for the final product. After isomerization by the standard procedure, specific extinction coefficients (adjusted to 100% linolenic acid) were 60.0 at 232 mμ and 53.4 at 268 mμ.

No pure arachidonic acid was available for standardization tests in glycerol. It was assumed that the specific extinction coefficients of Beadle and Kraybill (2) for this acid apply with sufficient accuracy to isomerization for 30 minutes in glycerol.

[4]Specific extinction coefficient as used here is the spectral density of a 1 cm. layer of solution having a concentration of 1 gram per liter, compared with an equal layer of solvent.

Procedure

Conjugated Constituents: Weigh out, to the nearest milligram, a sample approximately 200 mg. in size. Dissolve it in about 75 ml. of neohexane, 95% ethyl alcohol, or synthetic methyl alcohol. Transfer to a 100 ml. volumetric flask and make up to volume. Solutions of soaps containing added silicate should be filtered before spectrophotometric measurements are made.

Measure spectral densities of the solution, compared with solvent, preferably at 2 mμ intervals between wave lengths 324-306 mμ (tetraene region), 282-260 mμ (triene region), and 240-226 mμ (diene region). Adjust dilutions and cell lengths so that whenever possible observed densities lie between 0.2 and 0.8.

Nonconjugated Constituents: Weigh out in small Pyrex glass vessels, to the nearest half milligram, two samples approximately 100 mg. in size.[5] Weigh out 11.0 gram portions of the KOH-glycerol reagent solution into three or more reaction tubes (one for the blank and two for each sample). Cover the tubes and suspend them in the bath to a depth of 4½ inches.

After 5 to 10 minutes of heating, remove the covers and insert a thermometer into one of the tubes. When a temperature of 180° is indicated, drop the Pyrex glass vessel containing a weighed sample into the tube. Remove the tube from the bath and swirl it vigorously for one minute. Return it to the bath and replace the cover. At the end of one minute heating in the bath, remove the tube and inspect it. If the solution is clear, return the tube to the bath; if saponification or solution is not complete, again swirl the tube a few times, replace the cover and return the tube to the bath. At intervals of three minutes, other samples can be introduced in other tubes in the bath, and the same procedure repeated. Drop an empty sample container in the tube reserved for the KOH-glycerol blank.

The agitation by swirling is essential for prompt saponification of fats, acids, and esters, and prompt solution of soaps. A higher KOH concentration is required with glycerol than with ethylene glycol to effect ready saponification. Soap samples must be wetted with three drops of water or glycerol before dropping them into the reaction tube; otherwise they will not readily dissolve. Add the glycerol or water to the vessel containing the weighed soap sample and mix by stirring with a small glass rod until a homogeneous pasty mass is obtained. Drop the stirring rod into the reaction tube along with the sample. Treat the blank in a similar way. Monohydric esters saponify less readily than acids or glycerides. When analyzing such esters add about 0.1% of pure palmitic or stearic acid to the KOH-glycerol reagent after the treatment to remove excess water. This acts as an emulsifier and effects prompt saponification.

At the end of exactly 30 minutes after dropping the sample container into the reaction tube, remove the tube, cool it under tap water, add about 20 ml. of absolute ethyl alcohol, and stir. Place the tube in a beaker of hot water over a low flame and continue stirring until the viscous product dissolves completely. Transfer the solution quantitatively to a 100 ml. volumetric flask and make up to volume with

[5]The actual weight used should in general be within 10% of the standardized 100 mg. size for the nonconjugated analysis.

absolute ethyl alcohol or synthetic methyl alcohol. No settling or filtering is required.

Make spectral density measurements at the wave lengths specified in the analysis for conjugated constituents. When further dilutions of the sample solution are required for making density measurements, make similar dilutions of the blank solution.

Calculations

Conjugated Constituents: Plot spectral densities vs. wave length in order to inspect curve shapes. Calculate specific extinction coefficients at 232, 262, 268, 274, 310, 316, and 322 mμ. In the equations which follow,[6] subscripts 2, 3, and 4 refer to the number of double bonds; subscripts 232, 268, etc., refer to the wave length.

The specific extinction coefficient at 232 mμ due to conjugated diene components, corrected for absorption by COOR groups, is approximately:

$$k_2 = k_{232} - 0.07 \quad \text{for fats and esters,} \qquad (1)$$

$$k_2 = k_{232} - 0.03 \quad \text{for soaps and acids,} \qquad (1a)$$

where k_{232} is the observed coefficient for the sample. This equation applies principally to fatty materials having small proportions of nonconjugated polyunsaturated constituents.

The concentration of conjugated diene fatty acid components, expressed as percent acid in the sample, is:

$$C_2 = 100\, k_2/119 \qquad (2)$$

If substantial amounts of linoleic and linolenic acids are present in the sample, this result will be somewhat high, since absorption by these acids at 232 mμ was not taken into account in equation (1).

The specific extinction coefficient at 268 mμ due to conjugated triene components, corrected for linear absorption by extraneous compounds, is approximately:

$$k_3 = 2.8\, [k_{268} - \tfrac{1}{2}(k_{262} + k_{274})] \qquad (3)$$

where k_{262}, k_{268}, and k_{274} are observed coefficients for the sample. The concentration of conjugated triene components, expressed as percent acid in the sample, is:

$$C_3 = 100\, k_3/214 \qquad (4)$$

The constants 214 and 2.8 apply to beta-eleostearic acid in neohexane or alcohol (5). Slight differences in these values for different solvents are not important. If the calculated value for k_3 is zero or negative, or if there is no evidence for absorption maxima simultaneously near 268 and 278 mμ, conjugated triene acids are reported as absent.

Similarly, the corrected specific extinction coefficient at 316 mμ due to conjugated tetraene fatty acid components, is approximately:

$$k_4 = 2.5\, [k_{316} - \tfrac{1}{2}(k_{310} + k_{322})] \qquad (5)$$

and the percent of conjugated tetraene acids present in the sample is:

$$C_4 = 100\, k_4/220 \qquad (6)$$

If the value calculated for k_4 is zero or negative, conjugated tetraene acids are reported as absent.

Nonconjugated Constituents: Plot spectral densities vs. wave length in order to inspect curve shapes. Calculate specific extinction coefficients k′ at wave lengths 232, 262, 268, 274, 310, 316, and 322 mμ. In the equations which follow, primed coefficients refer to the isomerized product in absolute alcohol, unprimed coefficients to the original material in alcohol or neohexane.

The specific extinction coefficient at 268 mμ due to conjugated triene constituents in the isomerized mixture, corrected for linear background absorption, is approximately:

$$k'_3 = 4.1\, [k'_{268} - \tfrac{1}{2}(k'_{262} + k'_{274})] \qquad (7)$$

The constant 4.1 was evaluated from isomerized pure linolenic acid. If the quantity within the brackets is zero or negative, no linolenic acid is present. A further criterion must be used with materials low in linolenic acid when positive values are obtained for k'_3: if the plotted curve of density vs. wave length shows no evidence of simultaneous absorption maxima or inflections near wave lengths 268 and 278 mμ, no linolenic acid is present.

The specific extinction coefficient at 316 mμ due to conjugated tetraene acids in the isomerized mixture, corrected for linear background absorption, is approximately:

$$k'_4 = 2.5\, [k'_{316} - \tfrac{1}{2}(k'_{310} + k'_{322})] \qquad (8)$$

where k'_{310}, k'_{316}, and k'_{322} are the observed coefficients and the constant 2.5 was evaluated from isomerized arachidonic acid.[7] If the quantity within the brackets is zero or negative, no arachidonic acid is present.

Correction of data is next made for conjugated acids originally present and remaining essentially undestroyed by the isomerization treatment (5). The specific extinction coefficient at 232 mμ corrected for original conjugated diene acids is approximately:

$$k'_2 = k'_{232} - k_{232} + 0.04 \quad \text{for fat and ester sample,} \qquad (9)$$

$$k'_2 = k'_{232} - k_{232} \quad \text{for soap and acid samples,} \qquad (9a)$$

where k'_{232} and k_{232} are the observed coefficients after and before isomerization.

The specific extinction coefficient at 268 mμ corrected for undestroyed conjugated triene acids is approximately:

$$k''_3 = k'_3 - k_3 \qquad (10)$$

where k'_3 and k_3 are taken from equations (7) and (3). This correction is usually small. Similarly, for tetraene acids at 316 mμ

$$k''_4 = k'_4 - k_4 \qquad (11)$$

where k'_4 and k_4 are taken from equations (8) and (5). This correction is usually negligible.

Using the standardized data obtained for pure linoleic and linolenic acids, and the data of Beadle and Kraybill (2) for arachidonic acid, the final equations are:

$$x = 1.125\, k'_2 - 1.27\, k''_3 + 0.04\, k''_4 \qquad (12)$$

$$y = 1.87\, k''_3 - 4.43\, k''_4 \qquad (13)$$

$$z = 4.43\, k''_4 \qquad (14)$$

where x, y, and z are the percentages of linoleic, linolenic, and arachidonic acids, respectively, in the mixture, and the corrected specific extinction coefficients k'_2, k''_3, and k''_4 are defined by equations (9), (10),

[6] Reference (5) should be consulted for derivation of these equations.

[7] We are indebted to Dr. B. W. Beadle for supplying us with data for calculating the value of this constant.

TABLE I.

Thiocyanometric and Spectrophotometric Analysis of Miscellaneous Samples for Polyunsaturated Fat Acids.

Sample	Number	Thiocyanometric Analysis			Spectrophotometric Analysis						Total Polyunsaturated Acids as % of Total Sample
					Non-Conjugated Acids			Conjugated Acids			
		Iodine Number	Thiocyanogen Number	Linoleic Acid** As % of Total Fat Acids	Linoleic	Linolenic	Arachidonic	Diene	Triene	Tetraene	
					%	%	%	%	%	%	
TALLOWS AND GREASES, Crude	1	49.8	45.0	5.3	2.4	0.50	0.20	0.89	0.016	0.0007	4.0
	2	56.7	48.8	8.7	5.0	.48	.30	.79	.013	.001	6.6
	3*	60.4	50.1	11.4	7.5	.49	.20	.34	.022	.0002	8.5
TALLOWS AND GREASES, refined, bleached	1RB	50.2	45.9	4.8	2.0	.39	.13	.97	.009	.0003	3.5
	2RB	56.7	48.7	8.8	4.8	.52	.23	.82	.013	.0005	6.4
TALLOWS AND GREASES, partially hydrogenated	1RBH1	40.5	.....	.....	0.11	.00	.00	.08	.000	.000	0.19
	1RBH2	30.9	.....	.....	0.06	.00	.00	.04	.000	.000	0.10
	1RBH3	21.1	.....	.....	0.04	.00	.00	.04	.000	.000	0.08
	3H1	47.8	47.0	0.9	0.06	.00	.00	.15	.000	.000	0.21
SOAPS from crude tallows and greases	S1	50.5	46.7	4.2	2.5	.43	.20	.83	.011	.001	4.0
	S2	58.3	50.4	8.7	4.9	.50	.28	.71	.009	.0009	6.4
	S3	62.6	52.3	11.4	7.0	.56	.42	.30	.000	.0005	8.3
SOAPS from refined, bleached tallows and greases	S1RB	51.5	46.8	5.2	2.7	.50	.16	.79	.010	.0007	4.2
	S2RB	57.1	50.5	7.3	4.9	.52	.25	.70	.007	.0003	6.4
SOAPS from partially hydrogenated tallows and greases	S1RBH1	41.1	38.4	3.0	0.36	.07	.00	.06	.000	.000	0.46
					0.31	.07	.00				
	S1RBH2	32.6	30.9	1.9	0.11	.05	.00	.06	.000	.000	0.22
	S1RBH3	20.8	19.2	2.9	0.12	.03	.00	.03	.000	.000	0.18
	S3H1	49.4	48.3	1.2	0.14	.03	.00	.14	.000	.000	0.31
LARD, prime steam	4	68.0	56.0	14.2	11.7	.62	.41	.23	.001	.000	13.0
LARD, prime steam	5	68.6	56,0	14.9	11.2	.65	.33	.18	.001	.000	12.5
					11.4	.68	.33				
LARD, drip rendered	6	68.0	56.7	13.4	10.7	.47	.40	.22	.001	.000	11.8
LARD, hydrogenated	7	.....	.....	.....	.04	.02	.00	.03	.000	.000	.09
TALLOW, edible	8	41.0	37.9	3.4	.96	.42	.06	.55	.02	.003	2.0
					.87	.42	.06				
RED OIL	9	90.4	77.7	14.0	8.0	.75	.21	2.2	.000	.000	11.2
RED OIL	10	93.7	76.9	18.6	10.5	.87	.53	1.4	.03	.000	13.3
OLEIC ACID, highly purified	11	.....	.....	.....	0.10	.01	.00	.15	.000	.000	0.26
OLEIC ACID, purified	12	91.1	87.2	4.3	1.1	.04	.00	.52	.000	.000	1.7
METHYL OLEATE, highly purified	13	85.5	.....	.....	.05	.00	.00	.006	.000	.000	.06
STEARIC ACID, single pressed	14	.....	.....	.....	.97	.13	.04	.31	.01	.002	1.5
					.97	.13	.04				
PERILLA OIL, commercial	15	207.3	125.5	.....	13.0	55.6	1.3	2.2	.07	.02	72.2
					13.1	55.6	1.3	2.2	.07	.02	
PERILLA OIL, cold extracted	16*	203.7	.....	.....	17.2	53.9	.13	.19	.001	.002	71.1
					16.6	53.9	.13				
TOBACCO SEED OIL	17	140	.....	.....	70.1	.54	.02	.17	.005	.000	70.8
TOBACCO SEED OIL, from N. rustica	18*	141.3	80.52	73.0	75.0	.39	.00	.20	.006	.000	75.6
TOBACCO SEED OIL, from flue-cured	19*	142.6	80.42	74.8	74.4	.19	.00	.09	.004	.0005	74.7
TALL OIL FATTY ACIDS	20*	.....	.....	.....	27.6	.27	.00	10.7	.000	.000	38.6
TALL OIL FATTY ACIDS	21	.....	.....	.....	33.2	.30	.00	7.9	.000	.000	41.4

*Isomerized in 1.3 N KOH in Ethylene Glycol.
**Calculated assuming the presence of saturated, oleic, and linoleic acids only.

and (11), respectively. For animal fats and hydrogenated fats, and their soaps, the last term of equation (12) is usually negligible.

Analysis of Miscellaneous Samples

RESULTS on the spectrophotometric analysis of miscellaneous fatty materials for polyunsaturated constituents is shown in Table I. Iodine values, thiocyanogen values, and linoleic acid calculated therefrom, are shown also where data were available.

The spectrophotometric analyses are reported as percent acid in the total sample, whether the sample is a fat, ester, or soap. The thiocyanometric analyses are reported as percent acid in the total fatty acids. This difference is of no importance except for samples high in linoleic and linolenic acids, i.e., the oil samples in the table. For these samples the appropriate correction factor should be applied to the spectrophotometric values in order to compare them with the thiocyanometric data.

Analyses for iodine values and thiocyanogen values were obtained by standard procedures. For iodine values, the one-half hour Wijs method was used; for most thiocyanogen values, 0.1 N thiocyanogen solution was used in 150% excess, the absorption period being 3 hours for acids and 4 hours for glycerides. Where appreciable amounts of linolenic acid were present, as in the purified linolenic acid and perilla oil, the absorption period was 24 hours. A slightly different method was used for determining the thiocyanogen numbers for the soaps and hydrogenated tallows,[8] involving use of a 0.2 N thiocyanogen solution in 100 to 150% excess, and an absorption period of 24 hours.

Calculated values for oleic and saturated acids are not included in the table. These acids of course can be calculated from the proportions of polyunsaturated constituents indicated by the spectrophotometric data, their theoretical iodine numbers, and the iodine number of the sample.

The results shown definitely establish for the first time the detailed composition of animal fats and their soaps with respect to their polyunsaturated constituents. No consistent changes in composition by refining and bleaching are indicated by the results in Table I, although a more closely controlled experiment would be required in order to give a definite answer on this

[8]The authors are indebted to W. J. Patterson of the Lever Brothers Company and to Victor Mills of the Procter and Gamble Company, for supplying the crude, refined bleached, and partly hydrogenated tallows and greases, and the soaps made from these fats, and for permission to include their iodine and thiocyanogen values for the soaps and hydrogenated tallows.

point. Although apparently no consistent changes in composition by saponification are indicated in the data for the ordinary tallows and soaps, the hydrogenated tallows are appreciably lower in polyunsaturated acids than the corresponding soaps. No significance is attached to this observation in connection with saponification, since the samples stood in the laboratory several weeks before analysis, and details regarding their previous history were not available. Separate tests on a sample of tallow indicated a substantial loss of polyunsaturated components on allowing the sample to stand in the laboratory for three months.

Mild selective hydrogenation of tallow and lard is seen to reduce the linoleic and linolenic acid contents to extremely low levels and to reduce beyond the limit of detection the tetraene and conjugated triene constituents. Although previous methods have indicated this to be the case, the spectrophotometric method furnishes more direct quantitative evidence. The possibility exists that the hydrogenation of tallow produces from linolenic acid a small proportion of an isomer of linoleic acid which does not produce diene conjugation by the hot alkali treatment (8). Since the proportion of linolenic acid in tallow is small, however, any errors due to this cause are probably not important.

Appreciable quantities of linolenic and arachidonic acids are shown to be present in lard. Beadle *et al* (3) have reported 0.2 to 0.6% arachidonic acid in lard by the spectrophotometric method. Relatively high amounts of polyunsaturated components are shown to be present in commercial oleic acid (red oil).

The usefulness of the spectrophotometric method is further illustrated by analyses of purified acids and esters, Samples No. 11, 12, and 13. The purified oleic acid samples were prepared from different lots of commercial oleic acid by two crystallizations from acetone. The methyl oleate was prepared from lard by methanolysis, fractionation by seven crystallizations from acetone, and four vacuum distillations. This ester has exceptionally low amounts of polyunsaturated material. The application of sensitive and precise spectrophotometric methods of analysis to purified compounds which are to be used in further research is obviously important.

The applicability of the spectrophotometric method to some samples having polyunsaturated acids as major constituents is illustrated in Table I. It is of interest to note that tobacco seed oil is very rich in linoleic acid and contains practically no linolenic acid. The analyses are in fair agreement with the thiocyanometric analyses. These samples will be discussed in more detail elsewhere (10).

DATA on the composition of perilla oil present some anomalies. Riemenschneider, Swift, and Sando (11) have reported, from evidence based on thiocyanometric analysis and examination of the hexabromide, that linoleic acid was not present in appreciable amount in a sample of perilla oil they examined. As shown in Table I, the spectrophotometric analysis of two other oils, one a commercial sample (No. 15), and one (No. 16) prepared in this laboratory by cold ether extraction of perilla seed (obtained from Prof. W. L. Burlison of the University of Illinois), indicates the presence of appreciable amounts of linoleic acid. The thiocyanometric analysis of oil No. 15 showed 65.2% linolenic acid, 14.7% linoleic acid, 6.6% saturated acids (Bertram method), and 13.5% oleic acid (as glycerides). The spectrophotometric value for linolenic acid is appreciably lower than the thiocyanometric value. Moreover, the iodine number cannot be accounted for in full by the amount of unsaturated acids found spectrophotometrically. The reason for this discrepancy is not clear; however, such a result would obtain if a polyunsaturated component were present with double bonds isolated in such a manner that the alkali isomerization process is ineffective in producing conjugation. The presence of small amounts of tetraenoic components in these oils is of interest.

One of the outstanding illustrations of the value of spectrophotometric methods of analysis is furnished by the data on tall oil fatty acids. The presence of about 10% conjugated diene compounds is established. Anderson and Wheeler (1) have recently reported 9 to 13% conjugated linoleic acid in the fatty acids of tall oil by spectrophotometric analysis. Chapman, Hastings, and Pollak (6) have shown that considerable care must be used in selecting a method for the determination of the iodine number of tall oil. They infer that the difficulties were due chiefly to the presence of resin acids. In view of our findings it would appear that only those iodine number methods applicable to substances containing conjugated acids should be used (13). Furthermore, there is no published information regarding the thiocyanogen values of the conjugated acids. Hence, composition calculations based upon iodine and thiocyanogen values of tall oil are open to serious question. The very small amount of linolenic acid found in these tall oil fatty acids is of some interest. It should be pointed out that the values given for linoleic acid in these cases is in some doubt because of the presence of such a high proportion of conjugated diene acids.

The accuracy of the spectrophotometric method, at least as applied to the determination of small proportions of polyunsaturated compounds in fatty materials, is difficult to evaluate until further work is done. Many factors contribute to the uncertainty of results. The most important are: the imperfections in the corrections made for interfering constituents and overlapping absorption, the difficulty in preparing acids sufficiently pure for use as standards, and the possibility that in some samples isomers may be present having rates of isomerization different from those of the standards. It is estimated roughly that the probable error of the spectrophotometric results is ±10% of the quantity actually present when that quantity is about 10%; about ±25% when the quantity present is around 1%; and probably correct only in order of magnitude when the quantity present is 0.1% or less. The reproducibility of results is fortunately much higher than the probable accuracy. Typical duplicate analyses are shown in Table I for Samples S1RBH1, 5, 8, 14, and 15. Throughout the table the number of significant figures retained is consistent with reproducibility of results rather than accuracy. Additional data are shown elsewhere to illustrate the reproducibility (5) of the method.

Agreement between the thiocyanometric method and the spectrophotometric method, for the samples examined in this investigation, has been best in cases where the sample contained linoleic acid as a major

constituent (e.g., tobacco seed oil and linoleic acid concentrates). Agreement for lards and tallows falls within the limits of error in the two methods. The precision, sensitivity, and scope of the spectrophotometric method is, however, far superior to that of the thiocyanometric method in detecting and estimating low proportions of polyunsaturated constituents in fatty materials, and furnishes a valuable means of following changes in these constituents.

Summary

Existing ultraviolet spectrophotometric methods have been modified for application primarily to the detection and estimation of low proportions of conjugated and nonconjugated unsaturated constituents in fats, oils, and soaps. The method is applicable also to fatty materials having high proportions of these constituents.

Modifications include corrections for absorption by interfering substances, use of alkaline glycerol as an isomerization medium in the analytical procedure, and correction of absorption data on the isomerized product for absorption by conjugated constituents in the material before isomerization.

The presence of small proportions of highly unsaturated conjugated and nonconjugated compounds is established in lards, tallows, tallow soaps, and highly purified esters and acids. Tall oil fatty acids are shown to contain approximately 10% of conjugated diene acids and a small amount of linolenic acid.

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