under various conditions and the changes in characteristics compared by the removal of small samples at selected intervals of time. The results indicated that during the initial stages of autoxidation of $\Delta^{9, 12}$ methyl linoleate at 30°C. all of the oxygen could be demonstrated as peroxide oxygen. On the other hand, no peroxide oxygen was former until the $\Delta^{10, 12}$ methyl linoleate had been oxidized for more than 100 hours. Furthermore, it was suggested that oxygen at room temperature was as effective in rearranging the CH:CH CH_2 CH:CH system as alkali hydroxide and high temperature.

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Report of the Spectroscopy Committee

November 15, 1948

AT the special meeting of the Spectroscopy Committee, held in Chicago during the 1947 Fall Meeting of the American Oil Chemists' Society, the spectrophotometric method for the analysis of fats and oils was discussed in detail. A few minor revisions were made. It was decided to analyze four oil samples (linseed, soybean, cottonseed, and lard) by the revised method before submitting it to the Uniform Methods Committee for action by the Society. In order to limit the amount of work necessary a simplified set of calculations was attached, in which the background corrections were eliminated. The calculations are as follows:

Absorption coefficient is defined as $k = D/bc$ where D is the observed spectral density of a solution of thickness b cm. (compared with solvent of the same thickness) and of concentration of c grams per liter, the concentration of c is equal to W/v, where W is the weight of sample in grams, and v is the total volume of solution in liters (0.1 the initial volume used times dilution factor). In the equations which follow subscripts 233, 268, etc., refer to wave length.

k = absorption coefficient before isomerization.
k' = absorption coefficient of isomerized materials.
$C_2 = (k_{233})\ 0.8403$ = % conjugated diene.
X = % arachidonic acid $= (k'_{316})\ 4.424$.
Y = % linolenic acid $= (k'_{268} - 0.534\ X)\ 1.880$.
Z = % linoleic acid $= (k'_{233} - k_{233} - 0.593\ X - 0.60\ Y)\ 1.124$.

Eight collaborators analyzed the four oils following the details of the method as closely as it was possible, in the individual laboratories. The variations were minor. The data obtained are shown in Tables I and II.

TABLE I

Collaborator	% Conj. Diene	% Acid Arach.	% Acid Lino-lenic	% Acid Linoleic
Soybean Oil				
1	.19	.23	8.17	53.2
2	.20	.18	8.20	53.0
3	.19	.16	7.87	53.8
4	.20	.19	8.36	55.4
5	.19	.22	8.42	53.7
6	.20	.27	9.15	55.8
7	.20	....	8.93	55.2
8	.20	.29	8.06	49.7
Average	.20	.22	8.40	53.7
Linseed Oil				
1	.26	.42	48.5	16.1
2	.27	.35	48.0	16.8
3	.25	1.21*	44.9	17.2
4	.26	.41	48.0	18.0
5	.25	.50	49.5	16.0
6	.26	.49	53.2	17.4
7	.26	....	50.4	17.6
8	.28	.31	44.8	15.0
Average	.26	.41	48.4	16.8
Cottonseed Oil				
1	.14	.13	.15	51.1
2	.15	....	.29	51.0
3	.14	.09	.13	49.5
4	.16	.05	.16	52.0
5	.15	.09	.15	51.5
6	.16	.13	.12	54.4
7	.15	....	.26	52.3
8	.15	.20	.14	45.9
Average	.15	.12	.18	51.0
Lard				
1	.23	.52	.77	12.1
2	.23	.51	.71	12.2
4	.23	.55	.69	12.2
4	.25	.40	.78	11.0
5	.23	.51	.73	12.2
6	.26	.50	.81	12.2
7	.24	.52	.82	13.2
8	.25	.41	.94	10.8
Average	.24	.49	.78	12.0

* Not in average.

An examination of the data in Table I shows that exceptionally good checks were obtained by all collaborators, except No. 8, which appears to be low in all cases for linoleic acid. This would appear to be a consistent error which is occurring in the laboratory of that collaborator rather than a fault in the method of analysis itself. The percentages of arachidonic acid found by the short calculations are without question in error, since it is very doubtful if arachidonic acid occurs in soybean, linseed, or cottonseed oil. In Table II is shown a comparison of the average results in Table I, with the same samples analyzed in one of the laboratories using the long calculations. It should also be noted that the long calculations show a higher percentage of linoleic acid and a somewhat lower percentage of linolenic acid. While it cannot be stated with certainty that the differences in linolenic and linoleic acid are significant, the lower values for arachidonic obtained by the long calculations are certainly more correct. Hence, in the method which becomes a part of this report, the long calculations

are included. For many purposes, the short calculations may be used with sufficient accuracy.

Although the oils analyzed by the Committee during 1948 were not the same oils as those in the October 20, 1947 report of the Committee, comparison of the data show that almost identical results were obtained on the four oils.

TABLE II

Sample	% Conjugated Diene C_2	% Arachidonic Acid X	% Linolenic Acid Y	% Linoleic Acid Z
Short Calculation				
Soybean Oil	.20	.22	8.4	53.7
Cottonseed Oil	.15	.12	.18	51.0
Linseed Oil	.26	.41	48.4	16.8
Lard	.24	.49	.78	12.0
Long Calculation				
Soybean Oil	.15	.05	7.5	55.4
Cottonseed Oil	.11	.00	.00	52.8
Linseed Oil	.24	.04	47.1	17.4
Lard	.20	.38	.51	12.8

The Committee is well satisfied that a method has been set up which enables various laboratories to check the analysis of fats and oils. Accordingly, the method of analysis is included in this report and recommended to the Uniform Methods Committee for adoption. The Committee feels that the next step is to investigate the accuracy of some of the constants involved in the calculations, together with reasons for the differences between the spectrophotometric method and the thiocyanogen method of analysis.

B. W. BEADLE — R. T. MILNER
B. F. DAUBERT — R. T. O'CONNOR
R. H. FERGUSON — R. C. STILLMAN, chairman

Detailed Method for the Spectrophotometric Analysis of Fats and Oils

Outline of Method

The method is based on the measurement of the ultraviolet absorption of an oil or fat sample, both before and after conjugation of the poly-unsaturated constituents in the sample. The conjugated constituents are determined by measuring the absorption in purified iso-octane. The non-conjugated poly-unsaturated constituents are conjugated by heating in a glycol-KOH medium. The sample is protected from oxidation during the isomerization period by blanketing with nitrogen. The poly-unsaturated constituents are calculated from the absorption, after isomerization, using constants obtained by isomerization of pure acids and their mixtures. Reference is made to various articles which have appeared in the scientific literature (see footnotes).

Apparatus

Ultraviolet Photoelectric Spectrophotometer

Beckman (8) Model DU (or other suitable spectrophotometer) equipped with absorption cell compartment assembly for cells up to 10 cm. long. Adjust the focus of the hydrogen lamp so that, with the slit open to maximum width (2.0) and with the sensitivity knob at the counter-clockwise limit, and with no absorption cell in the beam, the meter balances at the lowest possible wave length (usually 211 mμ. or lower). Thereafter leave the sensitivity knob at about 3 counter-clockwise turns and use the slit width adjustment for balancing the instrument.

Extra Hydrogen Lamp (9)

Absorption Cells (10)

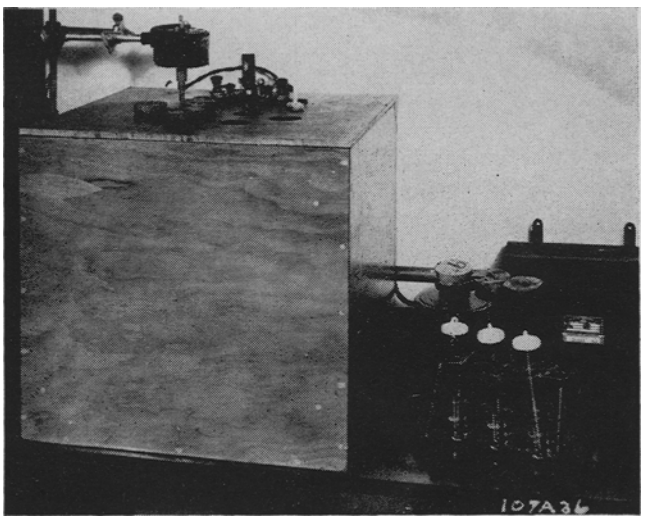
The demountable type with an outside diameter of 28 mm. is preferable. Each should consist of Pyrex glass cell body of outside diameter of approximately 22 mm. with centered ground glass stopper, threaded metal jackets or ends with threaded metal cans, polished crystalline quartz windows, and cork gaskets; bodies to be in matched pairs of lengths 1.000, 2.500, and 5.000 cm., ± 0.005 cm. Non-demountable cells may be used if more expedient. Matched cells should show the same optical density to 0.01 unit when filled with a solvent such as water or iso-octane.

Constant Temperature Bath

Although there are many types of isomerization baths that may be used, two are described below. Regardless of the type used, nitrogen blanketing must be provided.

1. *Rubber Reserve Model*

Constant temperature bath (11) operated at 180°C. ± 0.5°C.; Pyrex glass jar 12" in diameter and 12" high, mounted in an insulated 16" x 16" x 16" wooden box, the latter fitted with a transite cover drilled to hold accessories and six reaction



tubes; stainless steel immersion heater, 500 watt; 12" mercury thermo-regulator and control box; motor stirrer; standardized thermometer reading in tenths of a degree to 200°C.; and bath wax (12). A picture of this set-up is attached.

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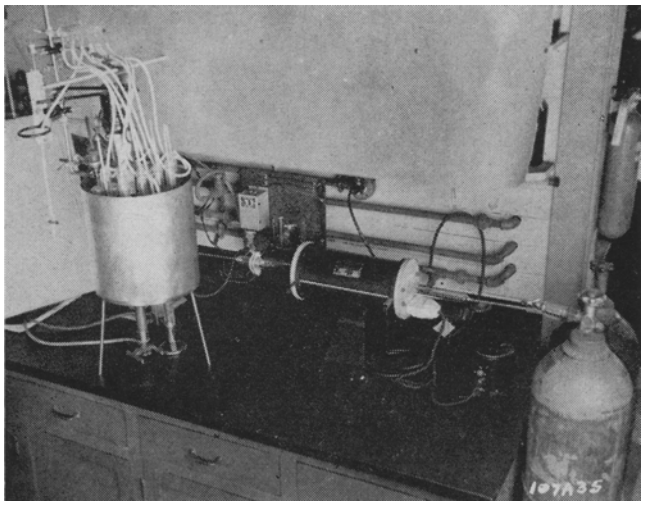
9. National Technical Labs., Hydrogen Discharge Lamp No. 2230 in Lamp Housing No. 2240.
10. Available from American Instrument Co., Silver Spring, Md., from Nat'l Tech. Labs. and from Fischer & Porter Co., Hatboro, Pa.
11. American Instrument Co. No. 4-01E constant temperature assembly with 12" mercury thermo-regulator No. 4-202 is satisfactory when mounted in an insulated box with wooden cross pieces in the bottom 2" high to hold the glass jar.
12. Fisher Scientific Co., Pittsburgh, Pa., No. 15-532 is satisfactory.

2. *Alternate Set-up Showing Nitrogen Blanketing*

A cylindrical bath 12″ in diameter, 12″ high, of stainless steel, is equipped with an Aminco mercury thermoregulator, No. 4—202, with No. 4—210 protective case; supersensitive mercury relay, Aminco No. 4—291 and Aminco motor stirrer. The bath is heated to just below 180°C. with a Meker burner, with the burner so regulated, the additional and controlled heat is provided by a 250-watt bayonet heater. The bath is equipped with a sample rack with about 20 holes. Usually a maximum of 8 samples are handled at one time. The balance of the holes aid in securing good circulation. The assembled bath with sample tubes in place is illustrated in the photograph.

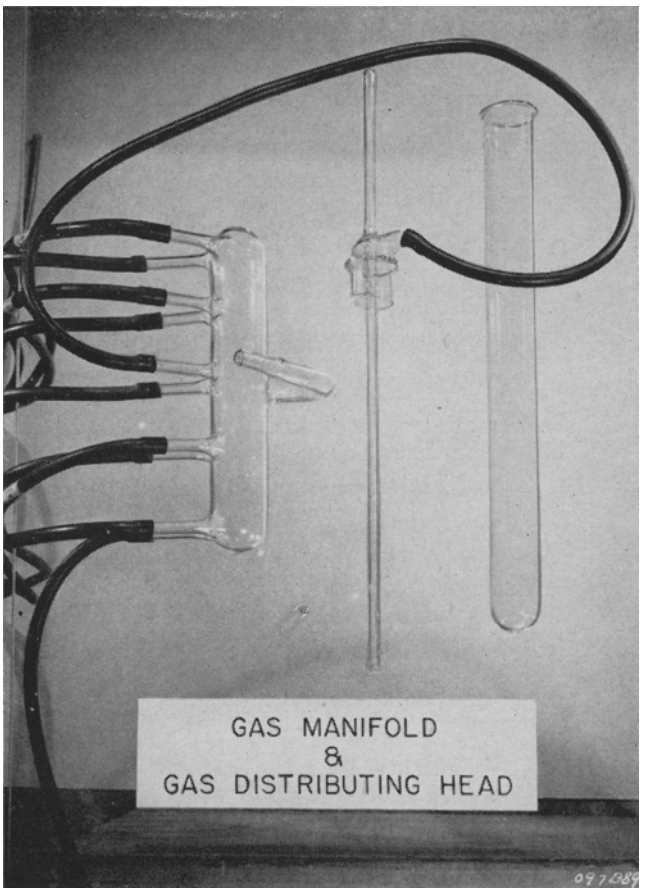


Sample Tubes

These are 10″ x 1″ diameter Pyrex test tubes. They are provided with a special distributing head shown in the attached photograph. The nitrogen is passed into a manifold which is also shown in the photograph. From the manifold, gas may be passed to any number of isomerization tubes. The gas passes from one of the manifold outlets through a rubber tube connection to the top of the gas distributing head. The rubber tubing is constricted at the point where it leaves the gas manifold by a small piece of capillary tubing which serves to insure a uniform flow of gas to each of the distributing heads. In the distributing head, the nitrogen enters at the top of the test tube, flows downward through the space above the sample, and leaves the test tube through the 7-mm. tubing, which forms the center part of the gas distributing head. This 7-mm. tubing is open at the bottom and has two small holes approximately 1″ and 1½″ from the bottom. The lower end of the 7-mm. tube is open to allow any glycol which passes into the tube to condense and return back to the reaction mixture. A uniform flow of gas to each of the tubes is obtained by maintaining a constant pressure of nitrogen to the manifold.

Furnace and Nitrogen Purification

Nitrogen used for purification must be low in oxygen. Nitrogen in cylinders containing less than 0.01% O_2 may be used directly without further purification. If purification is necessary, the apparatus, which is described below, has been found to be satisfactory.



Electric Multiple Unit Furnace, 12″ long, made by Hevi-Duty Electric Co., Milwaukee, Wis., 110 (or 200) volt is suitable. The temperature is controlled by a Variac, Type 200 GM made by General Radio, Cambridge, Mass. Setting 88 has been found to give satisfactory temperature (about 300°C.). Any other furnace that will give controlled temperatures around 300°C. would be satisfactory.

The Pyrex combustion tube is of special design. It contains a copper gauze coil to remove any oxygen present in cylinder nitrogen which is used to blanket the samples while in the isomerization bath. After use, the coil is reduced to metallic copper by cylinder hydrogen. The excess gas is conducted from the muffle tube through a by-pass.

The purified nitrogen passes from the furnace through a train of two wash bottles, the first containing glass beads only and the second containing concentrated sulfuric acid and glass beads. From wash bottles, the nitrogen passes to the distributing manifold. The rate of flow is controlled by a manometer, which contains H_2O, methyl orange, and a trace of H_2SO_4.

Reagents and Materials, Suitable Grades and Suppliers

a) Ethylene glycol, Eastman No. 133.
b) Methanol, absolute synthetic (13).
c) Ethanol, (purify, if necessary, following procedure for methanol).

13. Methanol suitable for use without purification has been obtained from E. I. du Pont de Nemours and Co., Wilmington, Del., and from B. R. Elk & Co., Inc., Garfield, N. J. The spectral density of a 1-cm. layer, compared with distilled water, should be less than 0.4 set at 220 mμ.

d) Iso-octane (2,2,4-trimethyl pentane), Nat'l Bureau of Standards certified grade (14).
e) Hexane (must be purified).
f) Cyclohexane (Phillips, Dow, du Pont).
g) Potassium hydroxide, A.C.S. standard (15).
h) Silica gel, 40 to 60 mesh (16).
i) Column for purifying solvents.
j) Test tubes, Pyrex, 6 x 1" or 10 x 1", for reaction vessels.
k) Pyrex glass cups (17) 1 ml., 10 x 14 mm., as sample containers, for weighing out samples and dropping into reaction vessel.
l) Glass stoppered 100-ml. volumetric flask.

Purification of Reagents

If the alcohol has an optical density of less than 0.4 at 220 mμ., it need not be purified.

a) PURIFYING ABSOLUTE METHANOL

Apparatus

3-liter flask. Standard taper connections.
Erlenmeyer flask.
Glass stopper and reflux tube for 3-liter flask.
Funnel.
Condenser—ground glass connections.
Trap—ground glass connections.
Filter paper.
Metal pot.
Meker burner.

Material

Absolute methanol—*order in new drums or in glass bottles.*
85% C. P. KOH.
Zn dust.

Method

Place 2,000 ml. of methanol in the flask. Add one heaping teaspoonful of 85% KOH and one heaping teaspoonful of Zn dust. Place glass stopper in one outlet of flask and reflux tube in other. Place on the steam bath—allow to remain for 3 hours.

Remove from steam bath and distill in hot water bath. Catch distillate in flask. Store in stoppered glass bottle.

Check transmission of methanol against distilled water through range of wave lengths used on conjugated and non-conjugated analysis. (See reference 13 under "Reagents" for limits.)

b) PURIFYING ISO-OCTANE

Apparatus

Glass filter tube approximately 32" x 1¾" or other suitable size.*
Cork stopper covered with aluminum foil.
Erlenmeyer flask.

Material

Silica gel, Davison Chemical Co., Code 11-08.
Glass wool.
Iso-octane.

Method

Place approximately 3½" of glass wool in filter tube above stop cock. Add about 12" of silica gel. Attach filter tube to ring stand to hold upright. Position Erlenmeyer flask under filter tube. Pour iso-octane into tube slowly, filling approximately ¾ full. Loosely place aluminum covered cork stopper in top of tube and wait for iso-octane to filter.

A uniform rate of flow and column action may be obtained without trouble by using a column nearly filled with silica, and supplying the iso-octane to the column by means of an inverted volumetric flask (about 1 liter) in a ring stand. A constant head is thus maintained without attention, by immersing the mouth of the flask in the iso-octane in the column.

Check transmission of filtered iso-octane against distilled water through range of wave lengths used on conjugated and non-conjugated analyses. The resultant curve should be smooth and the transmission above 85% at all points.

Preparation of Glycol-KOH and Dry Glycol

Prepare a solution of KOH in glycol.† This solution should be 1.3 N with respect to KOH.

a) Weigh approximately 750.0 grams of dry glycol into 1-liter round bottom glass stoppered Pyrex flask. In place of the solid glass stopper, insert a glass stopper containing two glass openings, one of which reaches to the bottom of the flask and through which nitrogen may be passed. The other opening serves as an exit for the nitrogen. Connect to an oxygen-free nitrogen supply and bubble sufficient nitrogen through the glycol to exclude all air and to agitate the sample slightly.

b) Raise a heated oil bath (100 to 150°C.) around the flask and apply heat. Raise the bath temperature to 190°C., and hold at 190°C. for 10 minutes. Remove the bath and allow the temperature to drop to 150°C. At 150°C. add, with care, 60 grams of potassium hydroxide (85%), keeping the sample under nitrogen. Again raise the oil bath around the flask and reheat to 190°C. Hold at 190°C. for 10 minutes. Remove the bath and allow to cool. Keep under nitrogen throughout the preparation and during storage.

c) Check the concentration of KOH in the cooled mix by dissolving a weighed sample in alcohol and titrating to phenolphthalein with standard HCl.

$$\% \text{ KOH} = \frac{(\text{titration}) \times N \times 5.61}{\text{wt. of sample}}$$

d) Dry some straight glycol by the same procedure as for glycol-KOH above.

If the % KOH is not 6.5 to 6.6 in "c" above, adjust to this concentration with the pure dried glycol.

Sample Preparation

Melt the sample carefully on the steam bath. Stir thoroughly to insure a representative sample. If the sample is not clear, or if it contains water, it should be filtered. Weigh out in 1-ml. Pyrex glass cups, one sample of approximately 200 mg. in size, weighed to the nearest mg., and two samples of approximately

14. Rohm and Haas Co., 222 W. Washington Sq., Philadelphia 5, Pa.; Phillips Petroleum.
15. Mallinckrodt Chemical Works, A. R. Pellets No. 6984.
16. Davison Chemical Co., Baltimore, Md.
17. Fisher "Petticups" are satisfactory.
* See Graff, O'Connor, & Skau, Ind. Eng. Chem., Anal Ed., *16*, 556-557 (1944).

† Smaller amounts of glycol-KOH solution may be prepared where usage is low.

100 mg., weighed to the nearest 0.5 mg. The 200-mg. sample is for analysis of conjugated constituents and the 100-mg. samples are for analyses in duplicate on non-conjugated constituents. The 200-mg. sample may be weighed out on a watch glass if more convenient. More than 200 mg. may be used if necessary to bring the density up to 0.2.

Procedure

Analysis for Conjugated Constituents

a) Drop the Pyrex cup containing the 200-mg. sample into a beaker holding about 75 ml. of purified solvent.‡ Dissolve the sample by warming it, if necessary. Transfer it quantitatively to a 100-ml. glass stoppered volumetric flask and make it up to volume. If the sample is weighed on a watch glass, it should be transferred quantitatively to the 100-ml. volumetric flask with approximately 75 ml. of the purified solvent and made to volume.

b) Measure spectral densities of the solutions, using matched cells in the Beckman spectrophotometer at 233, 262, 268, 274, 310, 316, and 322 mμ. Use solvent only in the blank cell. Start with the solution as prepared in the paragraph above, in a 5-cm. cell, adjusting subsequent dilutions and cell lengths so that, whenever possible, observed densities lie between 0.2 and 0.8. Tabulate wave lengths, densities, dilutions, cell lengths, and weight of sample.

Analysis for Non-conjugated Constituents

a) Weigh out 11.0-gram portions of the KOH-glycol solution into 10″ x 1″ Pyrex test tubes. Suspend the tubes at a constant depth of 4½″ in a constant temperature bath operated at 180°C. ± 0.5°C. Place the nitrogen protection covers in place and start the nitrogen through the tubes. The flow of nitrogen should be controlled by a manometer. A minimum of 50 to 100 ml. of N_2 should be passed through each tube per minute. If blanks do not check, it may be necessary to increase the volume of N_2 used.

b) After 20 minutes of heating, remove the N_2 distributing head and drop the Pyrex vessel containing the weighed sample into a reaction tube. Then replace the N_2 head. Remove the tube from the bath and swirl it vigorously, for a few seconds. Return it to the bath. At the end of one minute of heating in the bath, remove and inspect the tube. If the solution is clear, return the tube to the bath. If saponification or solution is not complete, again swirl the tube two or three times, and then return the tube to the bath. Keep the N_2 head in place at all times.

c) At intervals of three minutes, introduce other samples in other tubes into the bath, repeating the same procedure. Drop an empty sample container into the tube containing the potassium hydroxide-glycol "blank."

d) Follow the swirling steps carefully to assure complete saponification of fat samples.

‡ Iso-octane, hexane, cyclohexane.

e) Exactly 25 minutes after dropping the sample into the tube, remove sample tube from bath, wipe clean, and place in a 3,000-ml. beaker to cool. Continue to blow nitrogen over the sample during cooling. Add cold water to the beaker to shorten the cooling period. When cool, wash the cover with approximately 20 ml. of purified methanol or ethanol. (The alcohol should be poured from a beaker. Do not use a wash bottle.) Catch the alcohol in the sample tube. Insert a long stirring rod with curved end into the tube and work the sample cup up and down until the KOH-glycol and alcohol are completely mixed. Transfer the contents quantitatively to 100-ml. glass stoppered volumetric flask. Make to volume with purified alcohol and mix thoroughly.

f) Make spectral density measurements at wave lengths and under the conditions specified in the analysis for conjugated constituents. When further dilutions of sample are required for making density measurements, be sure to make similar dilutions of the "blank" solution. Tabulate wave lengths, densities, dilutions, cell lengths, and weight of sample.

Notes and Precautions

Satisfactory results with this method of analysis require extreme attention to details. Particular attension must be paid to the following:

1. The concentration of KOH in the glycol isomerization mixture.
2. The isomerization time—the factors used in the calculation are based on an exact isomerization time of 25 minutes.
3. Isomerization temperature.
4. Purity of reagents at the time of use—This applies to the glycol-KOH mixture, as well as the solvents used in making the density measurements.

Calculations

Absorption coefficient is defined as $k = D/bc$ where D is the observed spectral density of a solution of thickness b cm. (compared with solvent of the same thickness) and of concentration c grams per liter, the concentration c is equal to W/v, where W is the weight of sample in grams, and v is the total volume of solution in liters (0.1 the initial volume used times dilution factor). In the equations which follow, subscripts 2, 3, and 4 refer to the number of double bonds; subscripts 233, 268, etc., refer to wave length.

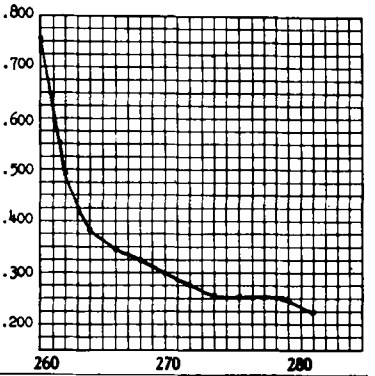
Conjugated Constituents

1. Specific extinction coefficient at 233 mμ. corrected for COOR and C = C Groups; P_1 is the estimated proportion of oleic acid.
$$k_2 = k_{233} - 0.029 - 0.052\,P_1$$
2. Specific extinction coefficient at 268 mμ. corrected for background absorption.
$$k_3 = 2.8\,[k_{268} - \tfrac{1}{2}(k_{262} + k_{274})]$$
3. Specific extinction coefficient at 316 mμ. corrected for background absorption.
$$k_4 = 2.5\,[k_{316} - \tfrac{1}{2}(k_{310} + k_{322})]$$
4. % Conjugated diene $= C_2 = 0.87\,k_2$.
5. % Conjugated triene $= C_3 = 0.47\,k_3$.
6. % Conjugated tetraene $= C_4 = 0.49\,k_4$.

SPECTROPHOTOMETRIC ANALYSES

Sample No. XOS 100 Date September 21, 1948
Material Cottonseed Oil
For

#	Non Conjugated	W.L.	D	b	c	W.L.	D	b	c				
	S/100→10/100→10/100	238	.452	1.000	0.0100	260	.757	2.500	1.000	k'_2 = 45.0180	k'_{238} = 45.2000	k'_{266} = .1300	k'_{316} = .0236
	S/100	262	.496	2.500	1.0000	264	.382	"	"	k'_3 = .0000	.1820	k'_{262} = .1980	k'_{310} = .0284
	"	268	.325	"	"	266	.342	"	"	k''_3 = .0000	45.0180	k'_{274} = .1028	k'_{322} = .0186
	"	274	.257	"	"	270	.303	"	"	k'_4 = .0003		2 | .3008	2 | .0470
	"	310	.142	5.000	"	272	.275	"	"	k''_4 = .0003		.1504	.0235
	"	316	.118	"	"	276	.254	"	"			.1300	.0236
	"	322	.093	"	"	278	.257	"	"			.1504	.0235
						280	.249	"	"			xxxx	.0001
	Sample Wt.					282	.223	"	"				
	2.7476	% Linoleic acid = 1.16 (45.0180) - 1.33 (.0000) + 0.09 (.0003) = 52.2											
	2.6476	% Linolenic acid = 1.88 (.0000) - 4.43 (.0003) = 0.00											
	.1000	% Arachidonic acid = 4.43 (.0003) = 0.00											
		% Saturated Acid = 29.5 I.V. = 107.2											
	Treatment:	% Oleic Acid = 13.8 T.V. = 62.8											
	Glycol - KOH	% Linoleic Acid = 52.2 % Composition from I.V.&T.V.											
	Isomerized	% Linolenic Acid = 0.00 % Linoleic Acid = 52.3											
	25 min. at 180°C.	% Arachidonic Acid = 0.00 % Oleic Acid = 14.9											
	Methyl Alcohol Solvent	Polymerization Index = % Saturated Acids = 28.4											
		Assuming 95.6 % T.F.A. 95.6% T.F.A. Basis											
	Conjugated	W.L.	D	b	c	Bl. 6 Vs. 8				k_3 = .1452			
	S/100	233	.364	1.000	2.0000	W.L.	%T			k_5 = .0025			
	"	262	.244	5.000	"	232	97.			k_4 = .0000			
	"	268	.244	"	"	262	97.				k_{238} = .1820	k_{266} = .0244	k_{316} = .0068
	"	274	.226	"	"	268	97.				.0290	k_{262} = .0244	k_{310} = .0067
	"	310	.057	"	"	274	98.				.1530	k_{274} = .0226	k_{322} = .0043
	"	316	.048	"	"	310	99.				.0078	2 | .0470	2 | .0110
	"	322	.043	"	"	316	99.				.1452	.0235	.0055
	Sample Wt.					322	98.					.0244	.0048
	.2000 gm.											.0235	.0055
												.0009	xxxx
	In purified neohexane	% Conjugated-diene = 0.13											
		" -triene = 0.00								$k_2 = k_{233} - 0.029 - 0.050\ k_1$			
		" -tetraene = 0.00											
										$k_2 = \frac{36.}{1x2} - 0.029 - 0.050\ (15) = .1450$			



If the quantities within the brackets are zero or negative, no characteristic absorption maxima are present and the corresponding constituent is reported as absent.

Non-conjugated Constituents

7. Specific extinction coefficient at 233 mμ. corrected for conjugated diene acids originally present. k_{233} and k'_{233} are observed specific extinction coefficients before and after isomerization (diene region).

$$k'_2 = k'_{233} - k_{233}$$

8. Specific extinction coefficient at 268 mμ. corrected for background absorption (triene region).

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

9. Specific extinction coefficient at 268 mμ. corrected for undestroyed conjugated triene (the value k_3 is taken from the conjugated analyses data, equation 2).

$$k''_3 = k'_3 - k_3$$

10. Specific extinction coefficient at 316 mμ. corrected for background absorption (tetraene region).

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

11. Specific extinction coefficient at 316 mμ. corrected for undestroyed conjugated tetraene (the value k_4 is taken from equation 3).

$$k''_4 = k'_4 - k_4$$

12. % Linoleic acid = X = $1.16\ k'_2 - 1.33\ k''_3 + 0.09\ k''_4$

13. % Linolenic acid = Y = $1.88\ k''_3 - 4.43\ k''_4$

14. % Arachidonic acid = Z = $4.43\ k''_4$

Total Composition

15. % Conjugated and non-conjugated poly-unsaturated acids are calculated as the above.

16. % Oleic acid =

$$\frac{\text{I.V. of sample} - [1.811(C_2+X) + 2.737(C_3+Y) + 3.337(C_4+Z)]}{.899}$$

17. % Saturated acids = % total fatty acid* - (% oleic + % conjugated + % unconjugated poly-unsaturated acids)

18. To calculate to a fatty acid basis, multiply all values above by 100, divided by total fatty acid.**

* 95.6 for most naturally occurring oils.
** 1.046 for most naturally occurring oils.