

Fig. 7. Gelatinous precipitate of lead oleate in medicinal white oil (B). 150,000 \times .

tion, long-range attractive forces exist, the potential minimum thus arising is deeper parallel to the linear aggregate than at the ends. This would lead to a loose lateral association of the linear aggregates. Behavior of this type could apparently account for most of the phenomena observed in the electron micrographs presented in this paper if the rodlike micelles are assumed to be linear aggregates of spherical micelles.

Long-range attractive and short-range repulsive forces may exist in hydrocarbon solutions of detergents and soaps. It is difficult to believe however that such forces could arise from an electrokinetic potential in view of the extremely low electrical conductivity of such solutions. (The specific conductance of such oil solutions is of the order of 10^{-10} mho/cm. at 100°C.) The possible effects of electron bombardment in the electron microscope create some doubt however.

An obvious alternative explanation for the apparent short-range repulsion is that the micelles are solvated with oil. The layer of bound oil increases the effective particle radii by up to 25 Å. Such solvated micelles might be stabilized against aggregation in much the same manner as if an electrokinetic potential were present.

Further evidence is clearly necessary to permit a definite explanation of the spacings evident in the electron micrographs presented.

The limitations of the electron microscope are well known. The specimens viewed are always in vacuum and at a poorly defined temperature. The electron

bombardment to which a specimen must be subjected introduces additional uncertainty. Surface-tension effects during the evaporation of the solvent may markedly alter the appearance of the specimen. Making due allowance for such complicating factors, it appears that the electron microscope is capable of yielding useful information about colloidal solutions of detergents in oil.

Summary

1. Electron micrographs of certain detergent and soap micelles presumably existing in oil solution are presented.

2. Both "spherical" and rodlike micelles appear to exist in oil. These micelles are apparently two molecular lengths in diameter. The rodlike micelles are of variable length, ranging up to over 300 Å. It seems likely that in some instances these rods are small linear aggregates of "spherical" micelles.

3. Association of rodlike micelles to form paracrystalline sheaves of long fibers is indicated. These structures apparently maintain fairly uniform spacings of from 80 Å to 100 Å between centers of the rods or fibers although the micelles appear to be only about 50 Å in diameter.

4. Aggregation of spherical micelles to form small colloid crystalline regions in large agglomerates is demonstrated.

Acknowledgment

The author wishes to acknowledge the valuable advice of D. H. Birdsall regarding the preparation of slides and the operation of the electron microscope.

REFERENCES

1. Arkin, L., and Singleterry, C. R., *J. Am. Chem. Soc.*, **70**, 3965 (1948); *J. Colloid Sci.*, **4**, 537 (1949).
2. Dervichian, D. G., *J. Chim. Phys.*, **38**, 59 (1941).
3. Doscher, T. M., and Vold, R. D., *J. Phys. and Colloid Chem.*, **52**, 97 (1948); Vold, R. D., and Vold, M. J., *ibid.*, **52**, 1424 (1948).
4. Etzler, D. H., and Farrington, B. B. (to Standard Oil Company of California), U. S. Patent 2,360,302.
5. Farrington, B. B., and Birdsall, D. H., *Inst. Spokesman (Nat'l Lubricating Grease Inst.)* **11**:4 (1947); Birdsall, D. H., and Farrington, B. B., *J. Phys. and Colloid Chem.*, **52**, 1415 (1948); Farrington, B. B., *Ann. N. Y. Acad. Sci.*, **52**, 979 (1951); Hotten, B. W., and Birdsall, D. H., *J. Colloid Sci.*, **7**, 284 (1952).
6. Harkins, W. D., *J. Chem. Phys.*, **16**, 156 (1948).
7. Klevens, H. B., *Chem. Revs.*, **47**, 16 (1950).
8. Lawrence, A. S. C., *Trans. Faraday Soc.*, **34**, 660 (1938); *ibid.*, **35**, 702 (1939); *J. Phys. and Colloid Chem.*, **52**, 1504 (1948).
9. McBain, J. W., "Colloid Science," chapter 19, D. C. Heath and Company (1950).
10. McBain, J. W., Mysels, K. J., and Smith, G. H., *Trans. Faraday Soc.*, **42B**, 173 (1946); Marsden, S. S., Mysels, K. J., and Smith, G. H., *J. Colloid Sci.*, **2**, 265 (1947).
11. Marsden, S. S., Jr., *J. Am. Oil Chemists' Soc.*, **26**, 57 (1949).
12. Neely, G. L., and Gardiner, E. W. (to Standard Oil Company of California), U. S. Patent 2,228,671 (January 14, 1941).
13. Philippoff, W., *Kolloid Z.*, **96**, 255 (1941).
14. Pink, R. C., *Trans. Faraday Soc.*, **37**, 180 (1941); Martin, E. P., and Pink, R. C., *J. Chem. Soc.*, 1948, 1750.
15. Rees, A. L. G., *J. Phys. and Colloid Chem.*, **55**, 1340 (1951).
16. Singleterry, C. R., and Weinberger, L. A., *J. Am. Chem. Soc.*, **73**, 4574 (1951).
17. Usher, F. L., *Proc. Roy. Soc. (London)*, **A125**, 143 (1929).
18. Van der Waarden, M., *J. Colloid Sci.*, **5**, 448 (1950).

[Received April 8, 1957]

Direct Determination of Saturated Fatty Acids in Fats, Oils, and Methyl Esters¹

D. F. KUEMMEL, The Procter and Gamble Company, Miami Valley Laboratories, Cincinnati, Ohio

ALTHOUGH much has been published in recent years on the determination of polyunsaturated acids in fats and oils, there has been relatively little work done to improve or develop procedures for the

determination of the saturated acids present. In 1925 Bertram described one of the first attempts to determine saturated acids directly (1). This method, which has become known as the Bertram Oxidation Method (4), involves the oxidation of the unsaturated linkages with permanganate, followed by a tedious pre-

¹ Presented at the spring meeting, American Oil Chemists' Society, New Orleans, La., April 28 to May 1, 1957.

precipitation and filtration of magnesium soaps. This technique has a poor reputation as to accuracy and precision and does not appear to be widely used at present. A crystallization method for saturated acids based on the insolubility of solid acids in acetone at low temperature has also been proposed (2).

The introduction of the ultraviolet method for polyunsaturated acids in the early 1940's (3, 5) afforded a method of calculating the saturated acid content, assuming the iodine value and polyunsaturates have been accurately determined. In this method the polyunsaturates are determined directly by their ultraviolet absorption after alkali isomerization. Oleic acid is determined by an iodine value balance while the saturated acids are assumed to make up the difference between the total unsaturated acids present and the total fatty acid content of the sample. This indirect, difference calculation is inherently subject to considerable error. Also the ultraviolet method is usually not applicable to partially hydrogenated materials because of the isomers of polyunsaturated acids which are formed during the hydrogenation. These acids do not isomerize or isomerize at a much slower rate than the natural polyunsaturated materials. More recently, in 1951, Schuette and Nogare (6) proposed a combination of permanganate oxidation and chromatography for the determination of the saturated components in binary mixtures of methyl esters.

An accurate and direct determination of saturated acids suitable for routine use was desired. It was therefore decided to set up the method on a triglyceride basis, starting with a small sample of the fat or oil, rather than require methyl esters or fatty acids as the starting material and leave their preparation up to the person submitting or analyzing the sample. Accordingly three commonly used techniques, that is, methanolysis, permanganate oxidation, and alkaline washing, were combined into a single gravimetric procedure for determining saturated acids in fats and oils. The method, of course, can also be applied to samples of methyl esters since these are intermediates in the procedure.

Procedure

Special Apparatus. Magnetic stirrer-hot plate combination: "Thermo-Stir," available from Scientific Glass Apparatus Company, Bloomfield, N. J., or equivalent.

Magnetic stirring bars: Kel-F coated, $\frac{7}{8}$ -in. No. 9235-U7, available from Arthur H. Thomas Company, Philadelphia, Pa., or equivalent.

Condensers: Liebig, sealed, straight inner tube, 300-mm. jacket, F joint 24/40 and 200-mm. jacket, F joint 19/38.

Separatory funnels: Ultramax, 250-ml., Squibb, or conventional type equipped with Teflon stopcocks.

Drying flasks: modified Erlenmeyer, 125-ml., with pouring spout (Figure 1).

Apparatus for removing residual solvent: see Figure 1.

Filter paper: glass fiber, 9-cm., No. X-934-AH, available from H. Reeve Angel and Company, New York, N. Y.

Reagents. Dry, alkaline methanol: dissolve a single, weighed pellet of sodium hydroxide in sufficient methanol (300–600 ml.) to give a solution containing 2.5 mg. of sodium hydroxide per 10 ml. Dry portions of this solution, as needed, over anhydrous, granular sodium sulfate.

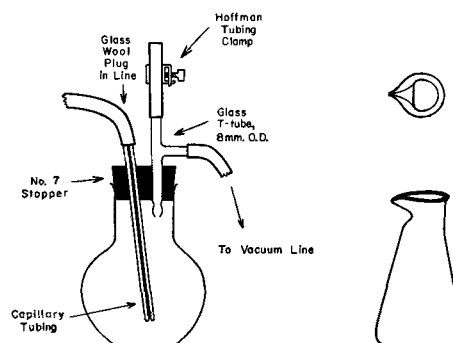


FIG. 1. Flasks for removing residual solvent and for drying extracts.

Dry acetone: dry over anhydrous, granular sodium sulfate.

Potassium permanganate: crush small portions of the reagent in a mortar into finely divided, minute crystals.

Sodium sulfate: a granular form is much more convenient to use than the powdered reagent and is recommended.

NOTE: All inorganic chemicals and organic solvents used were reagent grade. All met A.C.S. specifications, with the exception of sodium bisulfite, for which specifications have not yet been written.

Methanolysis of Fat or Oil. (If the original sample is in the form of methyl esters of fatty acids, omit this step and proceed directly to the oxidation step.) Melt the sample, if it is a solid, on the steam bath and thoroughly mix. Weigh a 0.5 ± 0.02 -g. sample into a 50-ml., F 19/38 Erlenmeyer flask. Add 10 ml. of the dry alkaline methanol and reflux gently with stirring for 1.5 hrs.

After cooling, neutralize the mixture with 1 N hydrochloric acid to the pink end-point of methyl orange. Add 10 ml. of chloroform to the solution, and transfer the contents of the flask to a 250-ml. separatory funnel with the aid of a small, short stem funnel placed on top of the separatory funnel. Rinse the flask with 15 ml. of additional chloroform and 70 ml. of distilled water, adding rinses to the separatory funnel. Extract the methyl esters into the chloroform layer by inverting the separatory funnel about 25–30 times in 25 seconds (avoid vigorous shaking). Allow the mixture to stand 2 min. before drawing off the lower chloroform layer into a second separatory funnel. Repeat the extraction of the water-methanol mixture with three 25-ml. portions of chloroform, rinsing the original flask with each portion before transferring to the separatory funnel. Wash the combined chloroform extracts in succession with 40 ml. of distilled water, 40 ml. of 4% sodium carbonate solution, and another 40 ml. of water. Allow the mixture to stand 5 min. after the alkaline wash and about 2 min. after the water washes before drawing off the chloroform layer. After the final water wash, draw off the chloroform layer into a 125-ml. Erlenmeyer flask equipped with pouring spout and containing 20–25 g. of anhydrous, granular sodium sulfate. Swirl the flask until the emulsion breaks. If the chloroform layer contains appreciable emulsion (high turbidity), it is a help to transfer the chloroform layer to the Erlenmeyer flask in three or four portions, swirling after each addition until the emulsion breaks and the solution is clear.

Filter the chloroform extracts through a 9-cm.,

glass fiber paper into a 125 ml., F 24/40 Erlenmeyer flask, being careful to keep as much of the sodium sulfate as possible in the original flask. Wash the sodium sulfate in the flask and the filter paper with three 10-ml. portions of chloroform. The pouring spout of the drying flasks facilitates the dropwise washing of the filter paper with the 10-ml. portions of chloroform.

Evaporate off the chloroform on a steam bath under a nitrogen or (less preferably) air stream, using a glass wool plug in the nitrogen or air line to prevent contamination. Stop heating the residue of methyl esters after it is apparent that no more solvent is coming off. Remove residual chloroform by passing nitrogen or air over the residue under reduced pressure for a few minutes, using the apparatus described in Figure 1.

Yields of the methyl esters ranged from 93 to 97% when several 0.5-g. synthetic mixtures of glycerides were carried through the procedure described above. The lower yields were generally associated with the mixtures of low saturates content.

Oxidation of Unsaturated Esters. (If the original sample is in the form of solid methyl esters of fatty acids, melt the sample on the steam bath and thoroughly mix. Weigh a 0.5 ± 0.02 -g. sample of the esters into a F 24/40, 125-ml. Erlenmeyer flask.) Add 15 ml. of dry acetone to the flask containing the methyl esters, and bring to a gentle reflux while stirring on the magnetic stirrer-hot plate combination. Add small portions of the finely divided potassium permanganate through the top of the condenser, according to the schedule given in the table below. After the permanganate has been added, pour an additional 5 ml. of dry acetone down the condenser. If this rinse plugs up the inner condenser tube, remove the flask and condenser from the hot plate momentarily, and swirl gently until the contents of the tube fall into the flask. Reflux for the prescribed interval (see table below). Although the mixture is stirred constantly, an occasional vigorous swirl of the reaction flask during the oxidation is of help in dispersing the permanganate which accumulates on the bottom of the flask. Cool the mixture to room temperature after the reflux period.

Dissolve the amount of sodium bisulfite indicated in the table in 40 ml. of distilled water. Add this solution slowly, in small portions, to the oxidation mixture in the flask, avoiding excessive frothing. Then add 1:4 sulfuric acid, a few drops at a time, with frequent but gentle swirling of the flask, until effervescence ceases. When the mixture clears sufficiently to see an indicator, add three drops of Congo red indicator and continue adding 1:4 sulfuric acid until the indicator is a definite blue. Since the indicator changes slowly near the end point, add the acid slowly when the intermediate purple color appears. Stir the mixture on a magnetic stirrer during the pH adjustment, until most of the brownish-black residue has dissolved. A few small black particles may remain after this treatment and will not interfere in the subsequent extraction.

Estimated saturates content	Total g. KMnO_4 added	KMnO_4 added in first	Total reflux period	G. NaHSO_3 added after oxidation
80-100%	2.0	20 min.	60 min.	5.0
40-79 %	3.5	30 min.	90 min.	6.0
0-39 %	5.0	45 min.	120 min.	7.0

Isolation of Saturated Esters. After cooling, add 10 ml. of chloroform to the oxidation mixture, and transfer the contents of the flask to a 250-ml. separatory funnel with the aid of a small funnel, as before. Rinse the flask with 15 ml. of chloroform and 10 ml. of distilled water, adding rinses to the separatory funnel. Extract by inverting separatory funnel 25-30 times in 25 seconds. Allow the layers to separate for about 2 min. before drawing off the chloroform layer. Extract three more times with 25-ml. portions of chloroform, rinsing out the original flask with each portion before transferring to the separatory funnel. Large amounts of chloroform-soluble oxidation products accompany the unattacked, saturated esters into the chloroform layer, and further treatment of these extracts to isolate the saturated components is therefore necessary.

Wash the combined chloroform extracts with two 40-ml. portions of 4% sodium carbonate solution, followed by a 40-ml., distilled-water wash. Allow the mixture to stand 5 min. after the alkaline washes and 2 min. after the water wash before drawing off the chloroform layer. After the final water wash, draw off the chloroform layer as before into a 125-ml. Erlenmeyer flask with pouring spout and containing 15-20 g. of sodium sulfate. Swirl the flask to break the emulsion.

Filter through 9-cm., glass fiber filter paper into a tared, 150-ml. extraction flask. Wash the sodium sulfate and filter paper with three 10-ml. portions of chloroform as before. Evaporate off the chloroform on a steam bath under a nitrogen or air stream. Stop heating the residue of methyl esters after it is apparent that no more solvent is coming off. Remove residual chloroform by passing nitrogen or air over the residue under reduced pressure for about 10 min. Adjust the gas supply and vacuum so that the stream just breaks the surface of the liquid in the flask. If the residue of saturated methyl esters is a solid, warm (avoid strong heating) the bottom of the Soxhlet flask with a small hot plate to melt the residue and keep it in a liquid phase while removing the residual solvent. Cool and place in a desiccator for about 10 min. Weigh the flask and residue. Repeat the passing of gas over the sample under vacuum for 10 min. until a constant weight (within 0.0005 g.) is obtained.

Calculations. For fats and oils, from equation:

$$\% \text{ saturated acids} = 102 (\text{grams of methyl ester residue}) / \text{g. sample.}$$

For methyl ester mixtures, from equation:

$$\% \text{ saturated acids} = [100 (\text{grams of methyl ester residue}) - 1] / \text{g. sample.}$$

General Comments. The analysis may be interrupted and the samples allowed to stand at any point in the procedure in which the sample is in an organic solvent. Thus samples may stand after the methanolysis (before acidifying) or oxidation (before adding sodium bisulfite), or after either extraction where the esters are in chloroform solution.

The precaution of removing the flasks containing the esters (particularly the final residue of saturated esters) from the steam bath as soon as the solvent has been evaporated cannot be too strongly emphasized. The saturated esters can be volatilized at the temperature of the steam bath, giving low results.

TABLE I
 Saturated Acids in Synthetic Mixtures of Glycerides

Sample No.	Composition of mixtures ^a					% Saturated acids		
	% TP	% TS	% TO	% ML	% Others	Calculated	Found ^b	Difference
1.....	4.1	31.7	64.2	3.9	4.4	+0.5
2.....	8.3	8.3	83.4	8.0	7.4	-0.6
3.....	8.7	18.7	28.4	44.2	26.2	24.9	-1.3
4.....	16.8	27.5	18.3	37.4	42.4	40.7	-1.7
5.....	40.1	17.7	42.0	0.2	55.2	55.5	+0.3
6.....	21.9	50.5	10.1	17.5	69.2	68.8	-0.4
7.....	14.9	67.0	9.6	8.5	78.4	78.3	-0.1
8.....	94.5	0.1	5.4	90.3	90.5	+0.2
9.....	12.6	77.6	9.8	12.1	12.8	+0.7
10.....	11.8	73.3	14.9	11.3	10.0	-1.3
11.....	7.9	9.5	25.0	57.6	16.7	16.5	-0.2
12.....	24.9	24.5	24.9	25.7	47.2	46.5	-0.7
13.....	3.5	11.6	66.0	18.9	14.5	14.6	+0.1
14.....	2.3	6.1	59.2	32.4	8.0	9.1	+1.1
15.....	1.7	6.9	58.1	33.3	8.2	8.8	+0.6
16.....	19.0	43.8	36.8 MO, 0.4 MS	18.5	20.6	+2.1
17.....	9.1	37.8	52.6 MO, 0.5 MS	9.1	10.8	+1.7
18.....	21.1	6.2	45.7	23.6	3.4 TM	29.3	28.1	-1.2
19.....	8.9	31.0	49.6	10.5 TM	8.5 ^c	8.7	+0.2
								Avg. 0.8

^a TP = tripalmitin, TS = tristearin, TO = triolein, ML = monolinolein, MO = monoolein, TM = trimyristin, MS = monostearin.

^b % Saturated acids = 102 (weight of saturated esters)/sample weight.

^c Total saturated acids present = 18.4%, C₁₆ + C₁₈ saturated acids = 8.5%.

Results and Discussion

In the course of developing the method numerous known mixtures containing both saturated and unsaturated esters were carried through the oxidation and washing steps. The recovery of the saturated components, although very reproducible, was less than theoretical for mixtures containing high percentages of saturated esters. This is believed to be caused by the various manipulative losses in the procedure. The weight of the final residue obtained from mixtures of high unsaturates content was, in turn, always greater than theoretical, probably because of incomplete oxidation of the unsaturated esters. The 93 to 97% efficiency of the methanolysis step, mentioned previously, must also be taken into account. Although the deviations from the theoretical were small, it was apparent that some correction factor would be necessary to compensate for the trends noted above. The possible alternate solution of increasing the oxidation period and the number of extractions and washes was not investigated. It seemed more feasible to develop a reproducible procedure involving small correction factors than to attempt to reach 100% efficiency on all of the steps involved.

The experimental factors correcting for these effects were determined using 0.5-g. synthetic mixtures of known saturates content carried through the entire procedure. The composition of the mixtures varied over a wide range in stearic, palmitic, oleic, and linoleic acid concentration. Two different factors were needed, depending upon whether the original sample is a triglyceride mixture (fat or oil) or methyl ester mixture since the procedure for the latter involves less manipulation. The weight of the saturated esters recovered (Y) from these known samples was plotted *versus* the theoretical weight of saturated esters taken (X) or *versus* the weight of esters equivalent to the original saturated glycerides taken. The equations of the straight lines, which were obtained upon plotting these data, were used to establish the recovery factors. The derivation of the equations are given below. The final calculations for the % saturated acids take into account the experimental factors and an average gravimetric factor (0.95) for converting esters to acids.

For triglyceride mixtures:

$$\text{from plot: slope} = 0.93, \text{ Y intercept} = 0 \\ Y = 0.93 X$$

$$\text{wt. sat. acids} = 0.95 X = 0.95 (Y/0.93) = 1.02 Y \\ \% \text{ sat. acids} = (102 Y)/\text{sample wt.}$$

For methyl ester mixtures:

$$\text{from plot: slope} = 0.95, \text{ Y intercept} = 0.01 \\ \text{wt. sat. acids} = 0.95 X = 0.95 [(Y - 0.01)/ \\ 0.95] = Y - 0.01 \\ \% \text{ sat. acids} = (100 Y - 1)/\text{sample wt.}$$

Tables I and II list the results obtained on synthetic mixtures of glycerides and on methyl ester mixtures, respectively. The composition of the mixtures used to determine the recovery factors, and the results obtained when the factors are applied to these mixtures, are given as the first set of data in Tables I and II. The average difference from the calculated value was 0.8% for the glyceride mixtures and 0.7% for the methyl ester mixtures. Trimyristin was added to the last two glyceride mixtures (numbers 18 and 19) in addition to the other saturated components. The result obtained on mixture number 18 indicates that a small amount of myristic acid, present with relatively large amounts of higher saturated acids, causes no difficulty. A large amount of myristic acid however will not be recovered by the method, as is indicated by the result on the last mixture, which approximates only the C₁₆ and C₁₈ content of the sample. The method is therefore not applicable to such materials as coconut and palm kernel oils, which contain large amounts of C₁₂ and C₁₄ saturated acids. The C₁₂ and C₁₄ esters are probably attacked somewhat by the permanganate and, in addition, can be removed from the other saturated esters by vacuum at room temperature. The purest glyceride containing linoleic acid available at the time the method was developed was a monolinolein. This was used in the experimental work although a trilinolein or mixed glyceride of oleic and linoleic acid would have been preferred.

Table III is a comparison of results obtained on various materials by both the ultraviolet spectrophotometric method and the present method. The first four samples are part of a hydrogenated series, in which the original cottonseed oil was hardened to various iodine values. The next four samples of soybean oil also are members of a similar series. The present direct method gives the expected trend in the saturates content. The anomalous decrease in

TABLE II
Saturated Acids in Synthetic Mixture of Methyl Esters

Sample No.	Composition of mixture ^a				% Saturated acids		
	% MS	% MO	% EL	% Others	Calculated	Found ^b	Difference
1.....	0.3	58.7	41.0	0.3	0.0	-0.3
2.....	0.4	99.6	0.4	0.9	+0.5
3.....	2.1	82.8	15.1	2.0	2.3	+0.3
4.....	8.6	91.4	8.3	10.5	+2.2
5.....	9.6	90.4	9.3	8.7	-0.6
6.....	19.5	80.5	18.6	19.6	+1.0
7.....	39.1	10.5	50.4	37.3	36.8	-0.5
8.....	56.5	43.5	53.9	53.9	0.0
9.....	70.4	13.8	15.8	67.1	66.8	-0.3
10.....	72.8	27.2	69.4	71.3	+1.9
11.....	76.3	14.0	13.7	72.7	72.4	-0.3
12.....	38.4	61.6	36.6	37.1	+0.5
13.....	78.4	21.6	74.7	74.5	-0.2
14.....	55.4	44.6	52.8	51.6	-1.2
15.....	7.1	74.2	18.7	6.7	9.2	+2.5
16.....	21.0	54.4	24.6	20.0	20.5	+0.5
17.....	53.7	32.3	14.0	51.2	50.8	-0.4
18.....	99.6	0.4	94.9	95.4	+0.5
19.....	99.6	0.4	94.9	94.5	-0.4
20.....	0.4	99.6	0.4	0.6	+0.2
21.....	99.6	0.4	94.9	95.6	+0.7
22.....	1.2	13.0	85.8	1.1	0.3	-0.8
23.....	2.7	97.3	2.6	3.0	+0.4
24.....	88.7	11.3	84.9	85.3	+0.4
25.....	0.2	42.0	57.8 MP	55.0	56.0	+1.0
							Avg. 0.7

^a MS = methyl stearate, MO = methyl oleate, EL = ethyl linoleate, MP = methyl palmitate.
^b % Saturated acids = [100 (weight of saturated esters) - 1]/sample weight.

saturated acids indicated by the spectrophotometric method for these two series emphasizes the inapplicability of this method to samples which have been subject to hydrogenation. This situation is also apparent in the results obtained on sample number 9. The two methods are in reasonable agreement for a majority of the other samples. Most of the samples listed in Table III were good-quality, refined materials.

Table IV gives an idea of the reproducibility of the method. Duplicate and triplicate determinations on numerous other samples have also indicated this order of precision. The method has been in routine use in a service laboratory for almost a year. Results obtained on the sample by different analysts have generally been in good agreement.

Summary

A direct gravimetric method has been developed for the determination of saturated fatty acids in fats, oils,

TABLE III
Comparison of Results Obtained by UV Spectro and Direct Methods

Sample No.	Material	% Saturated acids	
		UV spectro	Direct method
1	Cottonseed oil (I.V. = 113)	25.3	25.9
2	No. 1 hydrogenated to I.V. of 92	23.2	27.3, 26.8
3	No. 1 hydrogenated to I.V. of 73	22.3	27.5, 26.8
4	No. 1 hydrogenated to I.V. of 58	31.5	35.9
5	Soybean oil (I.V. = 128)	15.2	15.7
6	No. 5 hydrogenated to I.V. of 98	11.9	18.0
7	No. 5 hydrogenated to I.V. of 81	11.0	19.4, 18.9
8	No. 5 hydrogenated to I.V. of 63	25.8	30.7
9	Hydrogenated soybean oil ^a	0.0	16.7
10	Cottonseed oil	27.8, 26.2	27.2, 27.2
11	Cottonseed oil	24.9	25.6
12	Cottonseed oil	22.9	25.5
13	Soybean oil	15.7	17.4
14	Soybean oil	17.6	16.7
15	Soybean oil	16.5	15.7
16	Corn oil	15.2	15.5, 15.2
17	Corn oil	14.5	16.9, 15.2
18	Corn oil	15.1	15.0
19	Corn oil	15.8	15.6
20	Corn oil	11.5	14.0
21	Safflower oil	11.0	11.7
22	Lard	35.5	36.4
23	Lard	35.8	36.2
24	Lard	34.9	37.9
25	Lard	35.5	35.5
26	Cocoa butter	57.1	54.6

^a I.V. = 89, % trans (as trielaidin) = 68%.

TABLE IV
Precision of Direct Saturates Method

Sample No.	Material	% Saturated acids			
		Number of determ.	Range	Mean	Standard deviation
1	Corn oil	5	17.9-18.7	18.2	0.31
2	Triolein	5	11.3-12.7	12.0	0.49
3	Olive oil	6	16.4-17.4	16.9	0.31
4	Cottonseed oil	5	27.9-29.0	28.4	0.36

and methyl esters. The procedure involves methanolysis of the triglycerides to produce methyl esters, followed by oxidation of the unsaturated methyl esters by potassium permanganate. The undesired, acidic oxidation products are removed by alkaline washing and the saturated methyl esters thus isolated are weighed directly.

The method is intended for the determination of saturated fatty acids having C₁₆ or longer carbon chains. Small quantities of C₁₄ saturated acids will be included in the determination if present with other higher saturated acids. The method is applicable to both natural and hydrogenated vegetable oils. It is not applicable to oils containing large amounts of C₁₄ and lower saturated acids.

Concentrations of saturated acids ranging from 3 to 90% in known glyceride mixtures and from 0.3 to 95% in mixtures of methyl esters were determined with an average difference from the calculated value of 0.8%. Replicate determinations on samples in the 10 to 30% saturates range gave a standard deviation of 0.3 to 0.4%.

REFERENCES

- Bertram, S. H., *Z. deut. Oel-Fett-Ind.*, **45**, 733-6 (1925); *C. A.* **20**, 1145 (1926); also *C. A.*, **21**, 2391 (1927).
- Earle, F. R., and Milner, R. T., *Oil & Soap*, **17**, 106-8 (1940).
- Kass, J. P., Miller, E. S., Hendrickson, M., and Burr, G. O., abstracts of papers, 99th meeting, American Chemical Society, Cincinnati, O., April 1940, p. 11A.
- Markley, K. S., "Fatty Acids," pp. 401-6, New York, Interscience Publishers Inc., 1947.
- Mitchell, J. H. Jr., Kraybill, H. R., and Zscheile, F. P., *Ind. Eng. Chem., Anal. Ed.*, **15**, 1-3 (1943).
- Schuette, H. A., and Nogare, S. D., *J. Am. Oil Chem. Soc.*, **28**, 229-31 (1951).

[Received May 21, 1957]