Discussion of the Determination of Glycerin in Fats and Oils

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DURING 1937 the Glycerin Analysis Committee of the American Oil Chemists' Society carried out a number of tests with a dichromate method of analysis. (1) Although the results obtained by the cooperating laboratories were in generally good agreement for the high-grade stocks tested, it was not proved that a satisfactory degree of absolute accuracy had been attained. Furthermore, certain laboratories reported difficulty in saponifying some of the stocks tested and the Committee did not recommend adoption of the method.

Accordingly, a careful study of the accuracy of the proposed procedure was undertaken with a view also to possibly improving the saponification method.

Although it was obvious that the saponification step in the procedure was open to considerable question, it was believed that a better assay of the various saponification methods could be made if it were possible to determine, with certainty, the glycerin content of an aqueous solution.

Oxidation with Dichromate

In working with solutions of chemically pure glycerin, it was found that satisfactory results could be obtained only when a close control of acid and dichromate concentrations was maintained. (2,3) At relatively low dichromate concentration and high acid concentration or relatively high dichromate and relatively low acid concentration satisfactory results were obtained. The latter set of conditions was the same as employed in the tentative A. O. C. S. method.

All concentration conditions satisfactory for pure glycerin solutions were not equivalent, however, when oxidizing impure solutions resulting from saponification of fats and oils. In this latter case, high results were invariably obtained when using the higher acid concentration. It was evident that, under these conditions, the reaction was sufficiently vigorous to oxidize all or a part of the soluble fatty acids or other impurities present.

To confirm this observation, saturated solutions of caproic, caprylic, and capric acid were prepared and oxidized by the tentative A. O. C. S. procedure and also at high acid and low dichromate concentration. In all instances, a smaller proportion of the acids present was oxidized when the A. O. C. S. method was used. Since this method was not completely selective, it appeared that some form of purification should be employed.

Purification

Saturated solutions of caproic and caprylic acid were treated with aluminum sulfate, copper sulfate, silver carbonate, basic lead acetate, and a combination of silver carbonate and basic lead acetate (2), after which the solutions were oxidized by the tentative A. O. C. S. method. In the case of caproic acid, none of the treatments were effective, but with caprylic acid both the silver carbonate and the copper sulfate treatments materially reduced the amount of oxidizable material.

In order to determine whether or not these treatments might result in loss of glycerin through adsorption, tests were made on solutions of pure glycerin of known strength. In no case, however, was any loss observed. Since the copper sulfate and silver carbonate treatments were about equally effective in removing soluble fatty acids, the former is recommended as simpler to use.

Saponification

With the assurance that a reliable means of determining the glycerin content of aqueous solutions resulting from the saponification of fats and oils was available, we were in a position to assay the merits of the various saponification methods. Extensive tests on some of these methods have recently been reported from the laboratories of Lever Brothers Company. (4)

Since the glycerin content of refined stocks may be safely calculated by the ester value method, it was desirable, for check purposes, to be able to easily and completely saponify such stocks. Of all the methods which have been suggested, the use of alcoholic potash seemed most suitable for this purpose, but it became necessary to determine whether or not all the alcohol could be removed without loss of glycerin. To clarify this point, a series of experiments was conducted with 7-gram samples of refined coconut oil saponified with 70 ml. of alcoholic potash by boiling for 20 minutes on the hot plate, followed by evaporation to approximately 20 ml. volume and addition of water to 150 ml. The water was then evaporated to the extent shown in Table I and glycerin was determined in the manner described.

TABLE I

Extent of Evaporation	Approximate Time Required (minutes)	% Glycerin Found
150 ml. to 100 ml.	30	14.97
150 ml. to 50 ml.	90	14.10
150 ml. to 50 ml.) plus 150 ml. to 100 ml.)	150	14.02
150 ml. to 50 ml.) plus 150 ml. to 50 ml.)	180	13.98
Theoretical glycerin from ester value		13.94

These experiments indicate that satisfactory results may be obtained by twice evaporating with water to one-third the original volume.

As a further test of the method of alcoholic saponification, a number of determinations were made with four neutral stocks. For comparison, saponification was also carried out by a slight modification of the tentative A. O. C. S. method. The results are given in Table II.

TABLE II

	Vegetable Shortening	Cottonseed Oil	Coconut Oil	Tallow
% Glycerin by Alcoholic Saponification	10.63	10.55	13.99	10.74
	10.61	10.51	13.98	10.73
	10.56	10.51	13.94	10.65
				10.69
				10.71
				10.70
Average	10.60	10.52	13.97	10.70
% Glycerin by Aqueous KOH Saponification	10.43	10.47	13.78	10.68
	10.40	10.44	13.76	10.55
				10.61
				10.58
Average	10.42	10.46	13.77	10.61
% Glycerin from ester value	10.65	10.57	13.94	10.72

The results with alcoholic saponification are in excellent agreement with the theoretical values while those obtained by aqueous saponification are uniformly low, perhaps because of incomplete saponification.

Although we prefer alcoholic saponification for all fats and oils, we recognize the fact that satisfactory results may be obtained for acid stocks by the tentative A. O. C. S. or other aqueous potash methods.

Glycerin Content of Acid Stocks

In order to calibrate our proposed procedure of analysis, it was desirable to devise some independent method of checking the true glycerin content of acid fats and oils.

Such stocks may be assumed to contain only triglycerides, diglycerides (with possibly some monoglycerides), free fatty acids, mosture, and unsaponifiable matter. Since the total and free fatty acids, moisture, and unsaponifiable matter may be determined with considerable accuracy, it should be possible, with a few additional assumptions, to calculate the glycerin content by difference. The calculation was carried out as follows:

The sum of the per cent fatty acid anhydride calculated from the per cent combined fatty acids, plus the per cent glycerin anhydride calculated from the former figure, plus the per cent free fatty acids, plus the per cent moisture, subtracted from 100 gives the per cent glycerin present in the sample in excess of the amount which would be present were all the combined fatty acids in the form of triglycerides. This excess was added to the per cent glycerin represented by the glycerin anhydride, giving the total theoretical glycerin. When the determination of total fatty acids is carried out in the usual manner by saponification, acidulation and extraction, the unsaponifiable matter is included and no separate estimate of this quantity is required. The glycerin anhydride equivalent is calculated from the saponification value of the fatty acids and is uneffected by the presence of unsaponifiable matter.

Table III contains a comparison of the results calculated in this manner for a number of representative stocks with those calculated from the ester value and determined by the proposed analytical procedure. In spite of the excellent agreement of the calculated and experimental values, we do not recommend substitution of the method of calculation for the method of direct analysis.

TABLE III

Stock	% Glycerin Calculated	% Glycerin from Ester Value	Difference	% Glycerin by Proposed Method	Difference
Bleached Palm Oil No. 1	10.41	10.24	—0.17	10.47	+0.06
Bleached Palm Oil No. 2	10.35	10.20	—0.15	10.54	+0.19
Bleached Palm Oil No. 3	9.78	9.27	—0.51	9.67	—0.11
Bleached Palm Oil No. 4	6.84	5.98	—0.86	7.01	+0.17
Bleached Palm Oil No. 5	7.32	5.99	—1.33	7.13	—0.19
Bleached Palm Oil No. 6	7.43	6.37	—1.06	7.40	—0.03
Tallow No. 1	9.48	8.74	—0.74	9.37	—0.11
Tallow No. 2	9.21	8.64	—0.57	9.22	+0.01
Tallow No. 3	9.26	8.77	—0.49	9.24	—0.02
Recovered Tallow No. 1	6.15	5.78	—0.37	6.31	+0.16
Recovered Tallow No. 2	6.21	5.37	—0.84	5.91	—0.30
Recovered Tallow No. 3	8.27	7.85	—0.42	8.14	—0.13
Average Difference			0.63		0.12

As a final overall check of the proposed method, a sample of tallow and one of palm oil and a copy of the method were supplied to each of four analysts. Of these men, two had had no previous experience with the method and a third had used only a few steps of the complete procedure. Working entirely independently and without any outside comment or assistance, each man prepared and standardized the necessary solutions and then ran duplicate determinations on the two samples. The results of this test are given in Table IV.

TABLE IV

Analyst		% Glycerin Tallow	% Glycerin Palm Oil
A	Determination No. 1	9.86	7.15
	Determination No. 2	9.90	7.18
	Average	9.88	7.17
B	Determination No. 1	9.91	7.22
	Determination No. 2	9.88	7.23
	Average	9.90	7.23
C	Determination No. 1	9.81	7.23
	Determination No. 2	9.81	7.09
	Average	9.81	7.16
D	Determination No. 1	9.87	7.27
	Determination No. 2	9.83	7.21
	Average	9.85	7.24
		Tallow	Palm Oil
Maximum Deviation of Individual Analyses		0.10	0.18
Maximum Deviation of Average Analyses		0.09	0.08

These results appear to indicate that the proposed method of analysis is capable of giving reliable results in the hands of the average analyst.

The Complete Method

Following are the complete details of the proposed method.

PRINCIPLE:

The glycerin, resulting from the saponification of the fat or oil, is oxidized by potassium dichromate under certain definite conditions, to carbon dioxide and water. The potassium dichromate thus consumed is equivalent to glycerin.

APPARATUS:

Pipette, 50 ml.

Beakers, 300 ml., the inner, bottom surfaces of which have been scratched with a diamond pencil.

Volumetric flasks, 250 ml., calibrated to contain five deliveries from the 50 ml. pipette which is to be used for subsequent aliquoting during the procedure (temperature of calibration — 25°C.).

Erlenmeyer flasks, 500 ml., with 29/42 standard tapered ground glass joints to which are fitted 30-inch air reflux condensers.

A Fisher 9-312 All-Purpose Electrometric Titrimeter (quantitative unit is not necessary).

Hot plate.

Steam bath.

Cooling bath.

REAGENTS

Alcoholic Potassium Hydroxide. Dissolve 40 grams of C. P. KOH pellets in one liter of U.S.P. 95% grain alcohol and filter through a rapid filter. Preserve in a rubber-stoppered bottle.

Sulfuric Acid, 1:1. Use C. P. 36 N H_2SO_4 and dilute with an equal volume of distilled water.

Methyl Orange Indicator Solution. 0.1% aqueous, filtered solution.

Copper Sulfate Solution. Dissolve 20 grams of C. P. $CuSO_4.5H_2O$ in 100 ml. of distilled water.

Sodium Hydroxide, 10% solution. Dissolve 100 grams of C. P. NaOH pellets in one liter of distilled water.

Potassium Dichromate Solution. Dissolve 76 grams of Merck's powdered reagent grade $K_2Cr_2O_7$ in about 500 ml. of warm, distilled water. Filter through a rapid filter paper (similar in grade to No. 13255, 24 cm. Cenco) and do not wash the filter paper. Cool the filtrate to 25°C. and make up to one liter. Standardize against iron wire (see standardization).

Ferrous Ammonium Sulfate Solution. Add 100 ml of concentrated C. P. H_2SO_4 to 750 ml. of distilled water. Mix and cool to room temperature, then

add 300 grams of C. P. $FeSO_4(NH_4)_2SO_4.6H_2O$. Warm the solution to 25°C. and stir frequently until dissolved. Make up to one liter and mix. Filter through a rapid filter paper (similar in grade to No. 13255, 24 cm. Cenco) into a brown, glass bottle. Do not wash the filter paper.

Standardization of the Potassium Dichromate Solution

REAGENTS

Iron Wire. Iron wire of known purity (Cenco Cat. No. 89650-B is satisfactory) is thoroughly cleaned with fine sand paper No. 0 and the dust is removed by drawing the wire through a folded filter paper. Remove tarnished ends of the wire by means of wire cutters.

Stannous Chloride Solution. Dissolve 3 grams of crystalline $SnCl_2.2H_2O$ in 30 ml. of 12 N HCl by warming in a beaker on the hot plate. Remove the beaker from the hot plate and add 20 ml. of distilled water. This solution must be freshly prepared before each standardization.

Mercuric Chloride Solution. Dissolve 6 grams of $HgCl_2$ in 100 ml. of distilled water by stirring frequently and vigorously.

PROCEDURE

Weigh 2 to 2.5 grams of the clean iron wire on the analytical balance and place in a 300 ml. beaker. Add 30 ml. of concentrated HCl., cover the beaker with a watch glass, and place on a steam bath[1] until the iron is completely dissolved[2]. Do not put over uncovered hole. Wash down the watch glass and the walls of the beaker with a small volume of distilled water from a wash bottle and remove the watch glass. Add the solution of stannous chloride dropwise to the hot iron solution[3] until the yellow color no longer fades. The yellow color will not completely disappear. Do not add more than two drops of the stannous chloride in excess. Dilute the solution with cold distilled water to about 150 ml. volume and quickly cool to room temperature[4] by placing the beaker in an ice bath or a water bath. Rapidly add 30 ml. of the mercuric chloride solution and insert the beaker under the monometallic electrode-stirrer of the Fisher Titrimeter and stir the solution for three minutes[5]. Then immediately titrate the ferrous solution by rapidly adding the potassium dichromate solution[6] from a burette to within 2 ml. of the calculated amount[7], titrate slowly until the "magic eye," which was almost wide open at the start of the titration and which is kept adjusted to this same position as the titration progresses, suddenly closes and instantly reopens. (This point is only a drop or two from the end point). The titration is immediately stopped and then continued dropwise[8] until the eye closes permanently. The burette reading is noted and then the eye control knob on the Qualitative Unit is adjusted to its original open position. One small drop of the dichromate solution is added, and if the eye completely closes, the burette reading is noted, the eye opened again, and another drop of dichromate is added. The burette reading preceding the addition of the drop of dichromate that only partially closes the eye is taken as the last reading. The average of the first and last burette readings is recorded as the end point.

$$\frac{(\text{grams iron wire})\ (\%\ \text{Fe in wire})\ (.17780)^9}{\text{ml. } K_2Cr_2O_7 \text{ solution used}} = G$$

G = Grams of glycerin per one ml. of the $K_2Cr_2O_7$ solution.

Notes on the Standardization of the Dichromate Solution

(1) The iron wire should be dissolved on the steam bath and the beaker covered with a watch glass to avoid loss of ferrous chloride due to spattering.

(2) While the wire is being dissolved, some of the iron will be oxidized to ferric chloride by the oxygen of the atmosphere, causing the solution to turn yellow.

$$2\ Fe^{++} + 2\ H^+ + \tfrac{1}{2}\ O_2 \rightarrow 2\ Fe^{+++} + H_2O$$

The time of contact of the ferrous chloride solution with the atmosphere should be as short as conveniently possible in order to avoid the formation of a large amount of ferric chloride. Therefore, the procedure should be continued as soon as the iron wire is dissolved.

(3) Stannous chloride reduces ferric iron much faster in hot, concentrated solution than in cold, dilute solution. A large excess of stannous chloride is undesirable because metallic mercury may be formed upon addition of the mercuric chloride solution. Metallic mercury is oxidized by dichromate.

(4) The ferrous solution should be cooled to room temperature before the addition of mercuric chloride to avoid formation of metallic mercury. The mercuric chloride solution should be added rapidly for the same reason. The ferrous solution should be cooled rapidly so that the oxygen in the atmosphere will not have time to reoxidize the ferrous chloride.

(5) After the addition of mercuric chloride, the solution should remain for three minutes before titrating with the potassium dichromate solution. This is to allow the mercuric chloride to oxidize the excess stannous chloride. A silky white precipitate of mercurous chloride should form in the solution. If the solution becomes grey or drops of metallic mercury are visible, the solution should be discarded and the determination started afresh.

(6) The reaction that takes place during the titration is as follows:

$$6\ Fe^{++} + Cr_2O_7^{--} + 14\ H^+ \rightarrow 6\ Fe^{+++} + 2\ Cr^{+++} + 7\ H_2O$$

(7) The approximate amount of $K_2Cr_2O_7$ necessary to titrate the iron solution can be calculated by means of the formula, assuming the grams of glycerol to be 0.010 per ml. $K_2Cr_2O_7$ solution.

(8) Split drops of titrating solution can be obtained by removing part of a drop with a clean stirring rod and washing it from the stirring rod into the beaker.

(9) The factor 0.11780 is the glycerin factor of iron and is obtained in the following manner:

$$2\ C_3H_5(OH)_3 + 7\ O_2 \rightarrow 6\ CO_2 + 8\ H_2O$$

$$28\ Fe^{++} + 7\ O_2 + 28\ H^+ \rightarrow 28\ Fe^{+++} + 14\ H_2O$$

That is, $2\ C_3H_5(OH)_3$ are equivalent to $28\ Fe^{++}$

$$\frac{2\ C_3H_5(OH)_3}{28\ Fe^{++}} = \frac{184.19}{1563.52} = 0.11780$$

Glycerin Determination

PROCEDURE

Weigh ten grams (± .2 g.) of the sample of fat into a 300 ml. beaker on the analytical balance. For coco-

nut or palm kernel oil take only 7 grams.) Add 70 ml. of approximately 0.6 N alcoholic KOH (a 15% excess), cover the beaker with a watch glass and bring the alcohol to a boil on the hot plate. Place the beaker on an asbestos pad on the hot plate and continue to boil gently for 20 minutes. Remove the watch glass, insert a glass stirring rod, and evaporate the alcohol until the volume in the beaker approximates 20 ml. Foaming may be expected at this point. This must be minimized and loss of solution prevented by stirring, or cooling or both. Remove from the hot plate and allow to cool for about one minute, then wash down the inside of the beaker with hot, distilled water until the beaker is one-half full (150 ml.) and replace on the hot plate. Evaporate the water by gently boiling until the volume approximates 50 ml. Observe previously mentioned precautions to prevent foaming. Then add 100 ml. more of hot, distilled water and again evaporate to approximately 50 ml. volume.

Add sufficient hot water to dissolve all of the soap, but be careful not to exceed 150 ml. total volume. When the soap has dissolved, remove the beaker from the hot plate, add two drops of methyl orange indicator solution, and cautiously acidify with 1:1 H_2SO_4, avoiding a large excess of acid. Immerse the beaker in a steam bath and stir the fatty acids occasionally until they melt into a clear, oily layer. Remove from the steam bath and place in an ice bath. Do not disturb until the fatty acid layer has completely solidified; then remove from the ice bath, and, if possible, remove the cake of fatty acids from the beaker by withdrawing the stirring rod. Wash off the cake of fatty acids with a stream of cold, distilled water from a wash bottle, catching the washings in the same 300 ml. beaker. If the cake of fatty acids cannot be removed as a unit, break the cake in halves or quarters by pressing gently with the end of the stirring rod.

Immediately filter the aqueous solution through a wet 12.5 cm. No. 40 Whatman filter paper (or similar grade) into a 250 ml. volumetric flask, retaining the fatty acids in the beaker. Wash the broken cake of fatty acids in the beaker twice, by additions of about 15 ml. of cold, distilled water and filter the washings through the same filter paper into the volumetric flask. Up to this point do not allow the solution to drain completely through the filter paper. Now, allow the solution to completely drain, then wash the filter paper once with cold water and, after the water has drained through, immediately remove funnel and filter paper from the flask.

Adjust the temperature of the solution in the volumetric flask to 25°C. and then neutralize the solution by addition of 10% NaOH solution. Add 6 ml. of N/2 NaOH and then 3 ml. of 20% $CuSO_4.5H_2O$ solution. Make the solution up to volume with distilled water. Stopper the flask, thoroughly mix the contents, and allow to stand for about five minutes before filtering through a dry No. 13255, 24 cm. Cenco filter paper (or similar grade) into a dry flask.

Pipette a 50 ml. aliquot of the purified filtrate into a 500 ml. Erlenmeyer flask that can be fitted with a 30 inch air reflux condenser. Add exactly 25 ml. of standardized $K_2Cr_2O_7$ solution, followed by 20 ml. of concentrated H_2SO_4 and gently rotate the flask in order to thoroughly mix the solutions. Attach the air condenser to the flask and immerse in a steam bath (90-100°C.) so that the water in the bath is at a higher level than the solution in the flask. Leave the flask in the steam bath for exactly two hours, remove, cool in a water bath to room temperature, wash down the condenser with a little distilled water, remove the condenser, and transfer the contents of the flask quantitatively into a 300 ml. beaker.

Determine the excess $K_2Cr_2O_7$ present in the solution by titrating with approximately 0.75 N ferrous ammonium sulfate solution using the Fisher Titrimeter with the monometallic (Platinum-platinum) electrode-stirrer. The eye must be almost closed at the start of, and during the titration, since the titration is in the opposite direction to that used in the standardization. Run two blank determinations along with the sample, using 50 ml. of distilled water in place of the 50 ml. aliquot of glycerin solution. 25 ml. $K_2Cr_2O_7$ will take about 50 ml. or over of ferrous solution.

A blank must be run on each new lot of alcoholic KOH used. Place 70 ml. of the alcoholic KOH in a 300 ml. beaker. Evaporate the alcohol on an asbestos pad on the hot plate until salts begin to crystallize. Add 150 ml. of distilled water and gently evaporate to 50 ml. Add 100 ml. of distilled water and again evaporate to 50 ml. Add two drops of methyl orange indicator and acidify very slightly with 1:1 H_2SO_4. Cool, neutralize with 10% NaOH, add 6 ml. of N/2 NaOH and 3 ml. of $CuSO_4$ solution. Let stand for 5 minutes and filter into a 500 ml. Erlenmeyer flask. Oxidize as later described. If any appreciable amount of oxidizable is found, this must be corrected for in the final result.

CALCULATION

From the blank determination, the ratio of ferrous ammonium sulfate solution to the dichromate solution is calculated.

$$\frac{\text{ml. dichromate solution}}{\text{ml. ferrous solution}} = \text{Ratio} = R$$

The difference between the dichromate originally added and that titrated with the ferrous solution is equivalent to the glycerin. Therefore —

$$\frac{(25 - T\,R)\,(G) \times 100 \times 5}{\text{Wt. of Sample}} = \%\ \text{Glycerin}$$

Where T = volume of ferrous ammonium sulfate solution (ml.).
R = ratio of dichromate solution to ferrous solution.
G = glycerin factor of dichromate solution.

In conclusion, the authors wish to express their appreciation of the valuable criticism of Dr. L. B. Parsons and the experimental assistance of Mr. F. C. Duemmling.

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