hydroperoxides, some of which contain *trans* double bonds.

4. Under all of the conditions employed in the present investigation, the oxidation of methyl oleate and linoleate led primarily to the formation of monomeric peroxides which retained most of the unsaturation of the parent compound.

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[Received April 13, 1954]

The Determination of Monoglycerides and Glycerin in Mixtures

MARGARET KRUTY, J. B. SEGUR, and C. S. MINER JR.,
Miner Laboratories, Chicago, Illinois

THE most satisfactory methods of determining monoglycerides and glycerin are based upon their quantitative oxidation with periodic acid according to the following equations:

$$CH_2OH - CHOH - CH_2OR + H_5IO_6 \longrightarrow CH_2O + CHO - CH_2OR + HIO_3 + 3H_2O$$

Monoglyceride

$$CH_2OH - CHOH - CH_2OH + 2H_5IO_6 \longrightarrow 2CH_2O + HCO_2H + 2HIO_3 + 5H_2O$$

Glycerin

In each reaction, quantitative oxidation can be obtained if an excess of periodic acid is used. The amount of monoglyceride or glycerin is determined by measuring the amount of periodic acid consumed. Glycerin can also be determined by titration of the formic acid that is produced (6).

Several variations in procedure have been proposed (1, 2, 3, 4, 5). Each of the methods has one or more of the following disadvantages: a) high-melting samples must be warmed, resulting in side reactions; b) the sample must be extracted with water or a salt solution to remove free glycerin, if any is present; c) the sample and oxidizing reagent are in separate liquid phases which must be kept mixed by constant stirring; and d) when the excess of periodic acid is determined (addition of potassium iodide and titration with sodium thiosulfate), the precision of the titration is limited because the sample titration must be equal to at least 80% of the blank titration.

In the methods in current use the sample of monoglyceride, dry or dissolved in chloroform or ethyl acetate, is added to a measured amount of a solution of periodic acid dissolved in 80% or 95% acetic acid. The excess periodate remaining after the reaction is complete is reduced to iodate, and all of the iodate (including that formed during the oxidation reaction) is reduced to iodine by the addition of potassium iodide to the acid solution. The amount of iodine formed is measured by titration with sodium thiosulfate.

$$H_5IO_6 + 2HI \longrightarrow HIO_3 + I_2 + 3H_2O$$

$$HIO_3 + 5HI \longrightarrow 3I_2 + 3H_2O$$

$$4I_2 + 8Na_2S_2O_3 \longrightarrow 8NaI + 4Na_2S_4O_6$$

The difference between the blank and sample titrations therefore represents the amount of periodate which had been reduced to iodate by the monoglyceride. By this method three-fourths of the oxidizing power is spent in the reduction of iodate to iodine. If stoichiometric amounts of monoglyceride and periodate were used, the sample titration would be 75% of the blank. Since a 20% excess of periodic acid is advised, the sample titration is at least 80% of the blank. Therefore, in order to obtain a reasonable difference in the two titrations, it is necessary to know in advance (or by preliminary analysis) the approximate percentage of monoglyceride.

The characteristic feature of this proposed method is the determination of periodate using conditions

under which the iodate is not reduced. It has been shown that in neutral or slightly alkaline solution, iodate is not reduced to iodine by potassium iodide. Therefore, in the proposed method, the periodic acid is dissolved in a neutral solvent—95% methanol—instead of in acetic acid. A measured portion of this reagent is added to a sample of monoglyceride dissolved in chloroform. When the oxidation is complete, the reaction mixture is neutralized and buffered with sodium bicarbonate. The excess periodate is reduced to iodate by the addition of potassium iodide; but no further reduction of the iodate will occur. The released iodine is determined by titration with standard sodium arsenite reagent. The sodium arsenite is used because titration of iodine with sodium thiosulfate in neutral solution leads to erroneous results.

$$H_5IO_6 + 2KI \longrightarrow HIO_3 + 2KOH + I_2 + H_2O$$

$$I_2 + 1/2As_2O_3 + H_2O \longrightarrow 1/2As_2O_5 + 2HI$$

Thus the blank titration represents only the oxidizing power of the periodate actually available for the oxidation of monoglyceride, and the sample titration represents only the unused portion of this. That is, if stoichiometric amounts of monoglyceride and periodic acid were to be used, the sample titration would be zero, and the maximum difference desired between blank and sample titrations could be obtained. However 20% excess periodate is necessary to insure complete reaction; a 33% excess of periodic acid is suggested to give a more rapid reaction and to permit analysis of samples of less accurately known monoglyceride content. Even with the 33% excess periodate, the sample titration is only 25% of the blank.

A second feature of the proposed method provides for analysis of a glycerin-containing monoglyceride without separating the glycerin and monoglyceride by an initial extraction as is done in current methods. The analysis is made using conditions under which, in one case, both glycerin and monoglyceride are oxidized, and in the other case, only glycerin is oxidized. The difference in the amount of periodate consumed by both glycerin and monoglyceride and that consumed by the glycerin alone corresponds to the amount of monoglyceride present. Thus the time-consuming extraction period is eliminated.

Method of Analysis

Discussion of Method. The sample to be analyzed is dissolved in a mixed solvent of 5% dimethylformamide in chloroform. The presence of dimethylformamide is necessary to insure solubility of samples containing 1% or more glycerin. It does not interfere with either analysis and does not change the values of the blanks.

The total amount of material containing vicinal hydroxy groups is determined by oxidation in chloroform-methanol solution. Methanol was chosen because it is neutral, is a good solvent for periodic acid, and yields a homogeneous oxidation mixture with the chloroform solution. It is not however an ideal solvent since it is very slowly oxidized by periodate. This slow deterioration of the methanol-periodic acid reagent requires that the solution be prepared frequently and that a blank determination be made with each set of samples.

The rate of oxidation is slower in chloroform-methanol solution than in chloroform-acetic acid solution. In order to obtain complete oxidation in 30 minutes, it is necessary to heat the reaction mixture to boiling (50-55°C.).

When the oxidation is complete, the reaction mixture is diluted with water and a solution of sodium bicarbonate and potassium iodide is added. The sodium bicarbonate is necessary to neutralize the acidity due to periodic and iodic acids to provide a buffered solution. Also carbon dioxide must be present at the end-point of the iodine-arsenious oxide titration. Best results are obtained when the sodium bicarbonate and potassium iodide are added simultaneously, as a single solution. (Lower results are obtained when the addition of potassium iodide is made even one minute after the sodium bicarbonate.) The released iodine is measured by titration with standard sodium arsenite solution, using a starch indicator.

The determination of glycerin in the presence of monoglyceride is accomplished by oxidation in a two-phase system of chloroform and water. An aliquot of the sample in chloroform is added to 100 ml. of distilled water and swirled to extract the glycerin into the aqueous phase. When a measured portion of periodic acid in water is added, glycerin only is oxidized. The solubility characteristics are such that the glycerin and periodic acid are exceedingly water-soluble whereas the monoglyceride is chloroform-soluble. Essentially the extraction is made during the analysis instead of before it, as in other methods. The excess periodate remaining after the oxidation is complete is determined as before by addition of a solution of sodium bicarbonate and potassium iodide, followed by titration with standard sodium arsenite reagent.

Some prior knowledge of the approximate glycerin content is helpful in determining the proper sample size since it is dependent upon both monoglyceride and glycerin content. However wide limits of excess periodate are permissible in this method.

The method determines glycerin and α-monoglycerides in the presence of β-monoglycerides, diglycerides, and triglycerides. Little, if any, of the β-isomer is normally present, and it is disregarded. The method is applicable to commercial monoglycerides.

Apparatus

five-ml. pipette
25-ml. pipettes
100-ml. glass-stoppered volumetric flasks
1000-ml. volumetric flask
50-ml. burette
500-ml. Erlenmeyer flasks
glass-stoppered brown bottles for periodate solutions.

Reagents

1. Periodic acid in 95% methanol. Stock solution: dissolve 12.0 g. of periodic acid in water and dilute to 100 ml. Reagent: dilute five ml. of the stock solution to 100 ml. with anhydrous methanol. The stock solution is stable. The reagent solution should not be kept more than four or five days, and a blank determination must be made with each group of analyses. Store the solutions in glass-stoppered brown bottles.
2. Periodic acid in water: dissolve 3.0 g. of periodic acid in 500 ml. of distilled water. Store in a glass-stoppered brown bottle.
3. Sodium bicarbonate-potassium iodide solution: dissolve 75 g. of potassium iodide (A.C.S. grade) and 50 g. of sodium bicarbonate (A.C.S. grade) in distilled water and dilute to 1 liter. Store in a brown bottle.
4. Sodium arsenite, 0.05 *N*: arsenious oxide is a primary standard and may be obtained from the National Bureau

of Standards. To make a 0.0500 *N* solution, weight out 2.4728 g. of a standard sample into a 1-liter volumetric flask. Moisten the sample with distilled water, add 7.5 g. of sodium hydroxide (A.C.S. grade) and 100 ml. of distilled water. Swirl the contents of the flask gently until the arsenious oxide is in solution. Dilute to 250 ml. with distilled water and saturate the solution with carbon dioxide, thus converting all of the sodium hydroxide to sodium bicarbonate. This neutralization will require 45 to 60 minutes and is considered complete when the addition of phenolphthalein to a drop of the solution in distilled water produces only a very faint pink color or no color. Make up to volume and mix thoroughly. A solution thus prepared will preserve its titre almost indefinitely.

5. One per cent starch indicator solution: make a homogeneous paste of 10 g. of soluble starch and 1.25 g. of salicylic acid (preservative) in 10 ml. of cold distilled water. Add to this 1 liter of boiling distilled water, stir rapidly, and cool.
6. Chloroform (A.C.S. grade).
7. Methanol, C.P. grade or equivalent, anhydrous.
8. Dimethylformamide (manufactured by du Pont): to be used as a 5% solution in chloroform. Mix one volume of dimethylformamide with 19 volumes of chloroform.

Procedure

1. Accurately weigh duplicate samples into 100-ml., glass-stoppered volumetric flasks (see note 1).

2. Add about 40 ml. of a solution of 5% dimethylformamide in chloroform. Swirl until sample dissolves (see note 2).

3. Dilute to 100 ml. with 5%-dimethylformamide-in-chloroform solution. Mix well (see note 3).

4. Pipet two 25-ml. aliquots from each sample into 500-ml. Erlenmeyer flasks. (Glycerin content will be determined on one aliquot, and glycerin-plus-monoglyceride content will be determined on the other.) Prepare four blanks containing 25 ml. of 5%-dimethylformamide-in-chloroform.

Analysis for monoglyceride-plus-glycerin:

5. Pipet a 25-ml. portion of periodic-acid-in-methanol reagent into each sample for glycerin-plus-monoglyceride analysis, and into two of the blanks.

6. Add a boiling chip to each sample, and heat on a hot plate until the solution begins to boil (50° to 55°C.). Swirl several times while heating. Do not heat the blanks.

7. Set the flasks aside to cool for 30 minutes.

8. Add 200 ml. of distilled water to each blank and sample and swirl several times.

9. Allow samples to stand for five minutes (see note 4). The blanks do not need to stand after the addition of water and should be analyzed while the samples are standing.

10. Stir well and add 40 ml. of the sodium bicarbonate-potassium iodide reagent to the reaction mixture. Allow to stand for one minute. Resume stirring and titrate with 0.05 *N* sodium arsenite. As the end point is approached, *i.e.*, the red iodine color fades to a yellow, add 2-3 ml. of the starch indicator solution and continue titrating to the disappearance of all starch-iodine color.

It is important to have adequate stirring during the titration. A mechanical stirrer may be used. For convenience a magnetic stirrer is recommended (see note 5).

Analysis for glycerin:

11. Add 100 ml. of distilled water to each sample for glycerin analysis and to two of the blanks. Swirl four or five times to extract glycerin.

12. Pipet a 25-ml. portion of periodic-acid-in-water reagent into each blank and sample.

13. Allow flasks to stand for 30 minutes. Swirl four or five times during the reaction period (see note 6).

14. The directions for determining the excess periodate are the same for all samples and blanks. Proceed as in step 10 above.

Calculations

Glycerin: $$\% = \frac{(B_1 - S_1) \times N \times 2.302}{W}$$

Monoglyceride: $$\% = \frac{[(B_2 - S_2) - (B_1 - S_1)] \times N \times M/20}{W}$$

B_1 = Titration of H_5IO_6 in water blank.
S_1 = Titration of sample for glycerin analysis.
B_2 = Titration of H_5IO_6 in methanol blank.
S_2 = Titration of sample for monoglyceride and glycerin analysis.
N = Normality of As_2O_3 solution.
2.302 = Molecular weight of glycerin divided by 40.
W = Weight of sample in 25-ml. aliquot.
M = Molecular weight of monoglyceride.

Notes:

1. Solid samples in flake form should be mixed without melting before taking a portion for analysis.

Solid samples not in flake form, semi-solid samples, and liquid samples should be liquefied at not more than 10° above their melting point and mixed thoroughly before taking a portion for analysis.

The determination of proper sample size is dependent upon both monoglyceride and glycerin contents. The reagent concentrations are calculated to analyze a total of 1.0 milliequivalent of vicinal hydroxy groups, leaving a 33% excess of periodic reagent. If the approximate composition is known, the sample size can be determined from the following equation:

$$S = \frac{0.4}{\%\ M/e.w.M + \%\ G/23}$$

S = Sample size to be taken for 100-ml. volumetric flask.
% M = % Monoglyceride.
e.w.M = Equivalent weight of monoglyceride, (½ of mol. wt.).
% G = % Glycerin.
23 = Equivalent weight of glycerin.

In general, it is better to overestimate the glycerin and monoglyceride contents. Only general approximations are necessary since wide limits of excess periodate—20-100%—are permissible. There is less freedom in choice of sample size with monoglycerides containing relatively large amounts of glycerin.

The following table is given for convenience in determining the proper sample size of monostearin:

% Monostearin	% Glycerin	Sample size
50	1	1.2 g.
50	3	1.0 g.
50	5	0.8 g.
50	7	0.7 g.
50	10	0.56 g.
40	1	1.5 g.
40	3	1.1 g.
40	5	0.9 g.
40	7	0.8 g.
40	10	0.61 g.

A 20% excess of periodic acid is necessary to insure complete reaction. Therefore the ratio $\frac{S_2}{B_2 - S_2} \times 100$ must be greater than 20, but not more than 100.

If this is not the case, the analysis must be repeated using a smaller sample.

2. If the sample is not soluble in a solution of 5% dimethylformamide in chloroform, dissolve in a solution of 25-50% concentration and dilute with chloroform to 5% concentration.

3. With a few monoglyceride samples a slight gelatinous precipitate was observed. This did not seem to interfere with the analysis.

4. These solutions may be allowed to stand as long as 45 minutes.

5. A magnetic stirrer allows the use of an Erlenmeyer flask rather than an open beaker, is convenient to use, and gives good mixing without splashing.

6. Complete oxidation of glycerin can be obtained in five to ten minutes stirring with a magnetic stirrer. This technique is preferable if a magnetic stirrer is to be used for the titration.

Verification of Method. A sample of glyceryl monostearate was analyzed by eight analysts in six labora-

TABLE I
Comparison of Analytical Results

	New Method		P. and M. Method		T.G.A. Method	
	Mono-glyceride	Glyc-erin	Mono-glyceride	Glyc-erin	Mono-glyceride	Glyc-erin
No. of observations..	25	25	14	13	16	16
Minimum	42.5%	4.20%	42.7%	3.60%	41.7%	4.23%
Maximum	44.6%	4.50%	44.6%	4.47%	47.8%	4.37%
Average	43.4%	4.33%	43.8%	4.22%	45.1%	4.31%
Standard deviation.	0.70	0.065	0.51	0.27	1.77	0.038

tories,[1] using the new method reported here, the Pohle and Mehlenbacher method (3) and the Toilet Goods Association Method (5). In the Pohle and Mehlenbacher method the sample is dissolved in chloroform, free glycerol is extracted with water, the two solutions are separated, and each oxidized with a solution of periodic acid in 95% acetic acid. In the T.G.A. method the sample is dissolved in ethyl acetate, free glycerol extracted with 10% sodium sulfate solution, the two solutions are separated, and each is oxidized with a solution of periodic acid in 80% acetic acid. In both methods the excess of periodic acid is determined by adding potassium iodide and titrating liberated iodine with sodium thiosulfate. Monoglyceride and glycerin contents are calculated from the amounts of periodic acid consumed. Results of all the analyses are summarized in Table I.

[1] The laboratories of the following companies collaborated with the authors' laboratory: Colgate-Palmolive Company, Emery Industries Inc., Lever Brothers Company, Procter and Gamble Company, and Swift and Company.

Summary

A new method of analyzing mixtures containing monoglyceride and glycerin is presented. It is based upon oxidation of the sample with periodic acid. The new method is more rapid than the older methods because it is not necessary to separate the two layers when the glycerin is extracted from the solution of the sample. Precision of the titrations is improved because in the older methods the sample titration must equal at least 80% of the blank titration, but in the new method the sample titration can be very small and a correspondingly greater difference represents the monoglyceride or glycerin in the sample.

Acknowledgment

This work was sponsored by the Glycerine Division, Association of American Soap and Glycerine Producers, New York, N. Y.

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[Received April 13, 1954]

The Determination of Chlorophyll in Oil[1]

R. C. STILLMAN, Procter and Gamble Company, Cincinnati, Ohio

THE green coloring matter in plants, leaves, seeds, etc., is called chlorophyll. The classical work of Willstatter and Stoll (1) has adequately dealt with the structure and properties of the material. The present-day popularity of chlorophyllin preparations has lent glamor to the green stuff and made chlorophyll a household word. To the oil chemist and to the oil processor green in an oil is just another color pigment requiring time and material to remove and adding to the cost of the resulting chlorophyll-free products. Green may be excellent in many preparations, but in a shortening or other edible oil green lends no glamor and must be removed by bleaching. Pritchett, Taylor, and Carroll (2) have discussed "Chlorophyll Removal During Earth Bleaching of Soybean Oil." In this paper the need for chlorophyll removal is adequately covered.

In 1934 Zscheile (3) discussed the preparation and purification of chlorophyll A and B and made quantitative measurements of their absorption spectra. In the same year Long and Stevenson (4) delivered a paper on "A Simple Test to Detect Chlorophyll in Tallow." While no quantitative measurements were made, a simple test for chlorophyll detection was revealed and has been extensively used. In 1941 Zscheile and Comar (5) reported in detail on the absorption spectra of the various chlorophylls and the influence of preparative procedures on their purity. Shortly after Comar (6) published a paper on the analysis of plant extracts for chlorophyll A and B, using a commercial spectrophotometer. Chlorophyll concentrations in extracts were expressed in milligrams of chlorophyll per liter and could be translated into other appropriate terms by simple calculations if desired.

It is interesting to note that extracts of chlorophyll A analyzed by Zscheile and Comar (5) and by Comar (6) show a principal absorption at 660 millimicrons while a tremendous amount of recent work carried out directly on oils shows the absorption peak at 670 millimicrons. It is not within the scope of this paper to discuss why this discrepancy occurs. That the principal absorption of chlorophyll does occur at 670 millimicrons or close to that wavelength when absorption measurements are made directly on green oils is substantiated in a report by Melvin, MacMillan, and Senti (7). If one assumes that chlorophyll B absorbs strongly at about 17.5 millimicrons below chlorophyll A, there is little or no indication of the presence of chlorophyll B in refined or refined and bleached cottonseed or soybean oils. The reason for no apparent chlorophyll B can only be due to its absence in the seeds processed or to its insolubility in expressed or solvent extracted oil. For the purposes of this paper we shall call the green pigment in oil which absorbs at about 670 millimicrons, chlorophyll, and confine ourselves to its

[1] Presented at the annual meeting, American Oil Chemists' Society, San Antonio, Tex., April 12-14, 1954.