# A Modified Method for the Determination of Monoglyceride in Fats and Oils by Oxidation With Periodic Acid*

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A RAPID, precise, and easily-reproducible method for the determination of monoglycerides in fats became an essential requirement from a quality control standpoint when the use of monoglycerides in shortening blends became widespread. Hydroxyl-number determinations have been used on concentrates, but the method is not particularly suitable for routine quality control purposes. On blended shortenings, interfacial tension measurements as well as alcohol extractions have been made, but these are neither specific nor sensitive enough. The periodic acid procedure described by Pohle, Mehlenbacher, and Cook in Oil & Soap *22* (115-119) 1945 fitted the requirements very well, but, as specified, it was not found to be rapid and convenient enough for our routine control purposes.

For several years iodine value and Kaufmann-number determinations have been carried out in our laboratories in sixteen-ounce rubber-stoppered gas bottles instead of the usual glass-stoppered iodine flasks. This variation permitted agitation with a mechanical stirrer during titration which increases the titration speed and, at the same time, eliminates the tedious hand-shaking portion of the procedure. The gas bottles are also more economical to use because their breakage rate and replacement cost is much lower than for the rather fragile iodine-value flasks. As a result of this development the number of samples analyzed by a single operator per day was increased considerably and fortunately no significant sacrifice in precision or reproducibility resulted.

It seemed reasonable to expect that the similar titration required by the periodic acid method could be carried out in the same manner; however, when the procedure, as described by Pohle *et al.*, was thus adapted in our laboratories, unsatisfactory agreement among operators and laboratories was obtained, and when special molecularly-distilled fractions were assayed, more often than not, estimates significantly higher than 100% were reported.

A study of the original paper coupled with our experience led us to suspect that secondary oxidation reactions induced by the heat necessary to insure good contact between the solid fat and the periodic acid reagent may have been responsible for the higher-than-theoretical estimates and that the relatively slow, indistinct, reappearing titration endpoint might be largely responsible for the poor correlation and reproducibility.

Since the main purpose of heating the sample—reagent mixture was to bring about contact between the monoglyceride and the periodic acid, it was reasoned that if such contact could be induced by other means, such as with a suitable inert solvent at room temperature, the secondary oxidation reactions which were indicated to be negligible at temperatures around 25°C. would be inhibited. Furthermore, if good contact could be obtained by means of a solvent, it seemed likely that the reaction might be speeded up sufficiently to eliminate the need for the prescribed thirty-minute standing time, and very likely the endpoint would also be improved.

In contemplating the problem at hand it was reasoned that a mixed solvent, which would tend to keep the fat and the aqueous acetic acid solution of periodic acid in one phase, would best fulfill our requirements. The most likely solvent was considered to be a 2:1 glacial acetic acid-chloroform mixture commonly used in the determination of peroxide values.

## Experimental

With these thoughts in mind a few simple experiments were set up to determine if the original method could be altered to meet our needs. A few preliminary runs were sufficient to indicate that our reasoning had practical possibilities because the use of a fat solvent so greatly improved the sharpness of the titration endpoint that a fairly high degree of reproducibility was obtained.

In the first experiment the effect of solvent amount and shaking time was investigated. Three solvent amounts, namely: 5, 10, and 15 c.c., and three shaking times (mechanical shaker) 2, 5, and 10 minutes were arbitrarily selected and three different monoglyceride concentrates were used to round out the experiment which was set up as a Latin square (2). Each determination was made in duplicate and the average of the two taken as the estimate for subsequent analysis. The data obtained as well as the analysis of variance performed on it are shown in Tables I and II.

TABLE I

Solvent Amount and Shaking Time

| | 2-Min. | 5-Min. | 10-Min. |
|---|---|---|---|
| 5 c.c. | Sample M6 45.28% | Sample M1 43.74% | Sample MP 95.06% |
| 10 c.c. | Sample M1 44.22% | Sample MP 94.07% | Sample M6 45.28% |
| 15 c.c. | Sample MP 94.54% | Sample M6 44.95% | Sample M1 43.34% |

TABLE II

Analysis of Variance (2)

| Source of Variation | Degrees of Freedom | Sum of Squares | Variance | F |
|---|---|---|---|---|
| Mono Lots | 2 | 5021.1902 | 2510.5951 | ..... |
| Solvent Amount | 2 | .2634 | .1317 | .66 |
| Shaking Time | 2 | .2905 | .1452 | .73 |
| Error | 2 | .3975 | .1987 | ..... |
| | 8 | 5022.1416 | | |

It is clear that within the reasonably wide arbitrarily adopted ranges, neither solvent amount or the

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shaking time were significant causes of monoglyceride estimate variability.

A mechanical shaker was used to agitate the samples with the reagent in this experiment, and the samples were titrated as soon as possible at the end of the specified shaking time. It is obvious that even two minutes of agitation by hand is apt to be tedious so an experiment was set up to ascertain the optimum standing time required if the samples were gently shaken by hand for one minute after the addition of the periodic acid reagent. This variation was investigated to extend the scope of the method to laboratories not having a mechanical shaker. Some preliminary tests indicated that low estimates were often obtained when the samples were titrated directly after but one minute of hand-shaking.

An experiment employing the same three mono-concentrates and designed in a very similar manner to the one just described was set up. All samples were shaken one minute by hand after addition of the periodic acid reagent, and then sets of three were allowed to stand 2, 5, and 10 minutes each. To complete the Latin square the previous solvent amounts of 5, 10, and 15 c.c., respectively, were again included in the design of the experiment. The data and appropriate analysis of variance are shown in Tables III and IV.

TABLE III

Effect of Standing Time and Amount of Solvent

| | 5 c.c. | 10 c.c. | 15 c.c. |
|---|---|---|---|
| Sample M1 | 5-Min. 43.77% | 2-Min. 44.24% | 10-Min. 43.34% |
| Sample M6 | 2-Min. 45.28% | 10-Min. 45.28% | 5-Min. 44.95% |
| Sample MP | 10-Min. 95.06% | 5-Min. 94.07% | 2-Min. 94.54% |

TABLE IV

Analysis of Variance (2)

| Source of Variation | Degrees of Freedom | Sum of Squares | Variance | F |
|---|---|---|---|---|
| Mono Lots | 2 | 5018.8917 | 2509.4458 | .... |
| Solvent Amount | 2 | .2762 | .1381 | .67 |
| Standing Time | 2 | .2832 | .1416 | .68 |
| Error | 2 | .4143 | .2071 | .... |
| Total | 8 | 5019.8654 | | |

After one minute of hand-shaking no significant variability in monoglyceride estimate was introduced by varying the standing time between two and ten minutes. The insignificance of solvent amount in this experiment checks with the observation of the first test.

The next step in our investigation was to determine if known amounts of various monoglyceride concentrates when added to triglyceride fats in about the customary concentrations commercially used, could be recovered with reasonable exactitude. In Table V some data collected for this purpose is listed.

The apparent tendency for the actual assays to run lower than the theoretical was not significant statistically, and in any event the average difference of minus .0067% was too small to be of any practical importance. In order to determine if extending the standing time beyond the ten minutes previously investigated had any influence upon monoglyceride estimates by the revised procedure, approximately 1, 2, and 3% blends of the same three samples of mono-

TABLE V

Comparison of Theoretical Monoglyceride Estimate With Actual Assay Results

| Theoretical Assay | Actual Assay | Difference (Theoretical-Actual) |
|---|---|---|
| 1.21% | 1.21% | 0 % |
| 2.07% | 2.09% | +.02% |
| 2.98% | 3.01% | +.03% |
| 1.26% | 1.25% | −.01% |
| 2.16% | 2.10% | −.06% |
| 3.04% | 2.95% | −.09% |
| 1.29% | 1.25% | −.01% |
| 2.26% | 2.24% | +.02% |
| 3.19% | 3.23% | +.04% |

Average difference = −.0067%; s = .043%; "t" = −.47; − not significant (3).

glyceride concentrates used in the other experiments were handled in the following manner:

Samples of suitable size weighed into gas bottles were melted and dissolved in 15 c.c. of solvent and cooled to room temperature, whereupon 25 c.c. of periodic acid solution was added by pipette and the mixture agitated for one minute by hand. The samples were allowed to stand in the dark at room temperature for intervals of 10, 20, and 30 minutes before being titrated. The familiar Latin square arrangement was again used to restrict the number of analyses required. The data and the analysis of it are contained in Tables VI and VII.

TABLE VI

Effect of Standing Time

| | 1% | 2% | 3% |
|---|---|---|---|
| Sample M1 | 10-Min. 1.17% | 20-Min. 2.06% | 30-Min. 3.01% |
| Sample M6 | 20-Min. 1.15% | 30-Min. 2.12% | 10-Min. 2.97% |
| Sample MP | 30-Min. 1.31% | 10-Min. 2.22% | 20-Min. 3.18% |

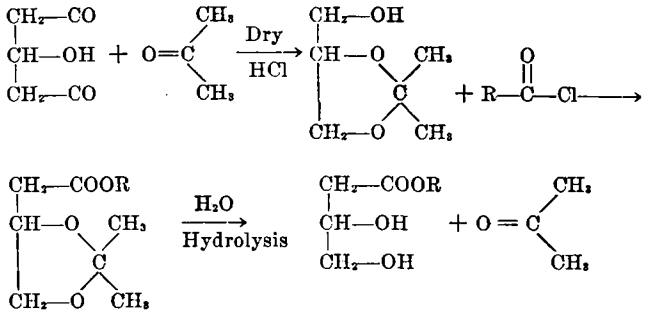
TABLE VII

Analysis of Variance (2)

| Source of Variation | Degrees of Freedom | Sum of Squares | Variance | F |
|---|---|---|---|---|
| Mono Variety | 2 | 0.0491 | 0.0246 | 16.93 |
| Mono Concentration | 2 | 5.0969 | 2.5484 | ..... |
| Standing Time | 2 | 0.0011 | 0.0006 | 0.38 |
| Error | 2 | 0.0029 | 0.0014 | ..... |
| Total | 8 | 5.1500 | | |

At room temperature and in the dark, reacted samples may stand in stoppered bottles for 10 to 30 minutes before titration without significantly effecting the estimation of monoglycerides by the revised procedure.

In order to check the reliability of this method for estimating monoglyceride a few rather pure fractions prepared by high-vacuum distillation of commercial monoglyceride-diglyceride mixtures were assayed. A sample of pure monomyristin was also prepared in accordance with the following synthesis:



The twice-recrystallized product of this synthesis was analyzed by the revised procedure and by the original adaptation of the method of Pohle *et al.* Its monoglyceride content was also estimated from the hydroxyl value. The saponification equivalent of the purified monomyristin was found to be 186.1 as compared with the theoretical value of 185.7. The monoglyceride estimates of the several distilled fractions as well as of the synthesized monomyristin, arrived at by the three indicated methods, are compared with one another in Table VIII.

TABLE VIII

Comparison of Monoglyceride Estimation by Old Method, Revised Method, and Theoretical Calc. From Hydroxyl Value

| Sample | Adaptation of Original Method | New Revised Method | Hydroxyl Value | Approximate Theoretical* Estimate |
|---|---|---|---|---|
| Mono-oleate | 100.8 | 93.8 | 308 | 97.5 |
| Mixed Fatty Acid Mono | 107.0 | 97.8 | 309 | 96.9 |
| Mixed Fatty Acid Mono | 101.0 | 94.3 | 305 | 96.0 |
| Monopalmitin | 93.0 | 94.7 | 331 | 96.5 |
| Monomyristin** | 102.6 | 99.9 | 368 | 99.2 |

* Based upon (pyridine) hydroxyl value and the relatively safe assumption that no triglycerides were present in the concentrate (4).

** Synthesized.

It is clear that monoglyceride estimates arrived at by the original adaptation of the method of Pohle *et al.* tend to run high, particularly on the solid-at-room-temperature $C_{18}$ monoglycerides, and that the estimates arrived at with the revised procedure are in better agreement with the theoretical potencies calculated from the hydroxyl values. Monoglyceride concentrates most commonly used in the shortening industry are made up largely from fat mixtures containing mostly $C_{18}$ fatty acid glycerides. As a result of this investigation the method of Pohle, Mehlenbacher, and Cook was modified for routine use in our laboratories and the recommended procedure reads as follows:

## Method

*Reagents:*

0.1 *N* sodium thiosulfate, standardized against potassium dichromate.

Oxidizing reagent: dissolve 5 gms. periodic acid in 200 ml. of water and then add 800 ml. of glacial acetic acid. Store solution in glass-stoppered bottle.

Potassium iodide solution: 150 gms. per liter.

Soluble starch solution: 1 gm. per 100 ml.

Solvent: mix 2 parts of glacial acetic acid with one part chloroform.

*Apparatus:*

Burette: 50-ml. accurately calibrated.

Pipettes: 25-, 15-, 5-ml.

Bottles: 16-oz., wide-mouth gas bottles.

Stoppers: No. 8 rubber.

Mechanical stirrer: tantalum or glass.

*International* bottle shaker.

*Procedure:*

Weigh duplicate samples (± 0.1 mg. for high concentrates and ± 5 mg. for low percentages) into 16-oz. wide-mouth bottles. Add 15 ml. of acetic acid chloroform (2:1) solvent to the weighed samples. If necessary, heat samples and solvent carefully on a steam bath (75-80°C.) until the samples are completely dissolved. *Cool bottles and dissolved samples to room temperature.* Pipette 25 ml. of periodic acid reagent into each. Stopper and place four bottles in the International bottle-shaker and agitate at a slow speed for two minutes. Wash down the sides of the bottles by pipetting 5 ml. glacial acetic acid into each sample. Add 15 ml. potassium iodide solution; shake and then dilute with 100 ml. water. Titrate with standard 0.1 *N* sodium thiosulfate solution to an almost complete disappearance of the iodine color. Add one to two ml. starch indicator and finish titration to disappearance of blue color. Read burette to 0.01 ml. The titration volume for any sample should be greater than 80% of the blank. Blanks should be run using similar volumes of solvent, reagent, and acetic acid.

Approximate Sample Weights

| % Monoglyceride | Sample Size | Required Weighing Accuracy |
|---|---|---|
| 100 | 0.15 | 0.1 mg. |
| 50 | 0.30 | 0.1 mg. |
| 25 | 0.60 | 0.1 mg. |
| 10 | 1.50 | 1 mg. |
| 5 | 3.00 | 1 mg. |
| 2 | 5.00 | 5 mg. |
| 1 or less | 10.00 | 0.1 gm. |

*Calculation:* Per cent Monoglyceride =

$$\frac{(\text{Vol Thio})\ (N\ \text{Thio})\ (\text{Factor})\ 100}{2{,}000\ \text{Wt. Sample}}$$

*Note:* If a mechanical shaker is not available, a one-minute hand-shaking with about ten minutes' standing time is recommended.

The data shown in Table IX will serve to demonstrate that satisfactory correlation can be obtained with this modified method when used by different operators independently in different laboratories.

TABLE IX

Correlation Between Operators and Laboratories With the Revised Periodic Acid Method

| Sample Designation | Expected Potency Blank +Wt. Added | Laboratory I | Laboratory II | Difference Lab II-Lab I |
|---|---|---|---|---|
| | % | % | % | % |
| MC1 | ..... | 43.2 | 44.0 | .80 |
| MC6 | ..... | 44.9 | 44.7 | —.20 |
| MCP | ..... | 94.7 | 92.4 | —2.30 |
| MCO | ..... | 93.5 | 94.0 | .50 |
| 1% MC1 | 1.21 | 1.21 | 1.23 | .02 |
| 2% MC1 | 2.07 | 2.09 | 2.09 | .00 |
| 3% MC1 | 2.98 | 3.00 | 3.10 | .10 |
| 1% MC6 | 1.26 | 1.25 | 1.24 | —.01 |
| 2% MC6 | 2.16 | 2.10 | 2.09 | —.01 |
| 3% MC6 | 3.04 | 2.95 | 3.00 | .05 |
| 1% MCP | 1.29 | 1.25 | 1.26 | .01 |
| 2% MCP | 2.26 | 2.24 | 2.24 | .00 |
| 3% MCP | 3.19 | 3.23 | 3.23 | .00 |
| Substrate (Blank) | ..... | 0.33 | 0.33 | .00 |
| | | | | =—1.04 |

Average difference = —.074%; s = .688%;
"t" = —.40; — not significant (3).

## Summary

The original periodic acid method of Pohle *et al.* has been modified to render it more suitable for routine control purposes. An attempt was made to minimize the danger of obtaining high estimates when assaying commercial concentrates prepared from mixed glycerides containing $C_{18}$ saturated and unsaturated fatty acids. The sharpness of the titration endpoint was markedly improved.

## Acknowledgment

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