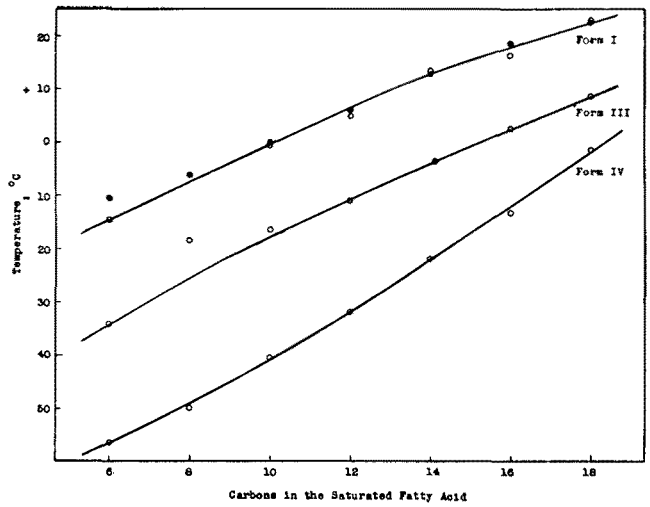

FIG. 4. Melting Points for the Polymorphic Forms of the 1-Acyl-2,3-dioleins.

● Capillary melting points
○ Transition points obtained from warming curves

It is assumed that the transition points intermediate between the highest and lowest melting forms, i.e. Form I and Form IV, correspond to the transition points of Form III. It is not likely this form corresponds to the Form II typical of the symmetrical 2-oleyl-1,3-disaturated triglycerides. While complete knowledge is lacking in the absence of x-ray diffraction data, the magnitude of the difference in temperature between the transition points of Form I and the transition points of the intermediate form (Form III) does not support an assumption that the intermediate form is Form II.

A warming curve for 1-capryl-2,3-diolein and typical for the series of the unsymmetrical monosaturated-dioleins is shown in Figure 3.

Since several highly purified saturated 2-monoglycerides were available in our laboratory, it was thought it would be highly desirable to determine whether these 2-monoglycerides exhibited the same kind of abnormal melting behavior as the isomeric 1-monoglycerides. The polymorphic nature of the 1-monoglycerides had been established by Malkin and associates (2) and later verified by others (5). An homologous series of 2-monoglycerides, containing capric, lauric, myristic, palmitic and stearic acids respectively, was subjected to thermometric measurement under the same variations of cooling and warming conditions as the previously mentioned unsaturated triglycerides. No evidence for polymorphism was found either by thermometric measurements or by capillary tube methods. Regardless of the rapidity with which a 2-monoglyceride was cooled and subsequently warmed either in bulk sample or in thin-walled capillary tubes the melting point was consistently identical with the melting point of the solvent crystallized (Form I) compound. X-Ray diffraction studies of the 2-monoglycerides cooled under varying conditions invariably gave a diffraction pattern identical with the solvent crystallized compound. While the x-ray diffraction data in themselves because of the long exposure time does not preclude the possibility of transition of other forms to Form I, the supporting evidence of thermometric measurement seems to indicate that 2-monoglycerides of saturated fatty acids do not exhibit polymorphism.

Summary

Transition point data are reported for a series of unsymmetrical monoöleyl-disaturated triglycerides and a series of unsymmetrical monosaturated-dioleins.

The results of thermometric measurements on a series of saturated 2-monoglycerides are also reported.

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Determination of Monoglyceride in Fats and Oils by Oxidation With Periodic Acid[1]

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SINCE Malaprade's (14) original work on the oxidation of polyalcohols with periodic acid and periodates, numerous studies and applications have been made of this reaction. Fleury *et al.* studied the action of periodic acid on hydroxy acids and sugars (3,4,5), glycerol phosphoric acid (6), glycerol in the presence of sugar (7), lactic acid and pyruvic acid (8) and tartaric acid (9). Hudson and co-workers (10, 12, 13) have used the reaction in their studies of sugars, and Nicolet and Shinn (15) showed that periodic acid reacted rapidly and quantitatively with alpha-amino alcohols. The kinetics of the periodic acid oxidation of 1, 2 glycols have been investigated by Price and associates (16,17). Oxidation with periodic acid has been applied by Bradford, Pohle, Gunther and Mehlenbacher (2) to the determination of glycerol, by Allen, Charbonnier and Coleman (1) to the determination of glycerol, ethylene glycol and diethylene glycol in the presence of each other and by Hoepe and Treadwell (11) to the determination of glycerol, ethylene glycol and 1, 2 propylene glycol in the presence of one another.

Fleury and Paris (6) showed that periodic acid oxidized alpha-glycerol phosphoric acid in a few minutes at ordinary temperatures. These results indicated that the fatty acid monoglycerides, which exist almost exclusively in the alpha form, might be determined directly by an oxidation with periodic acid. Di- and tri-glycerides are not oxidized by periodic acid at room temperature because it oxidizes poly-

[1] This paper was presented at the Chicago meeting of the American Oil Chemists' Society, October 25-27, 1944.

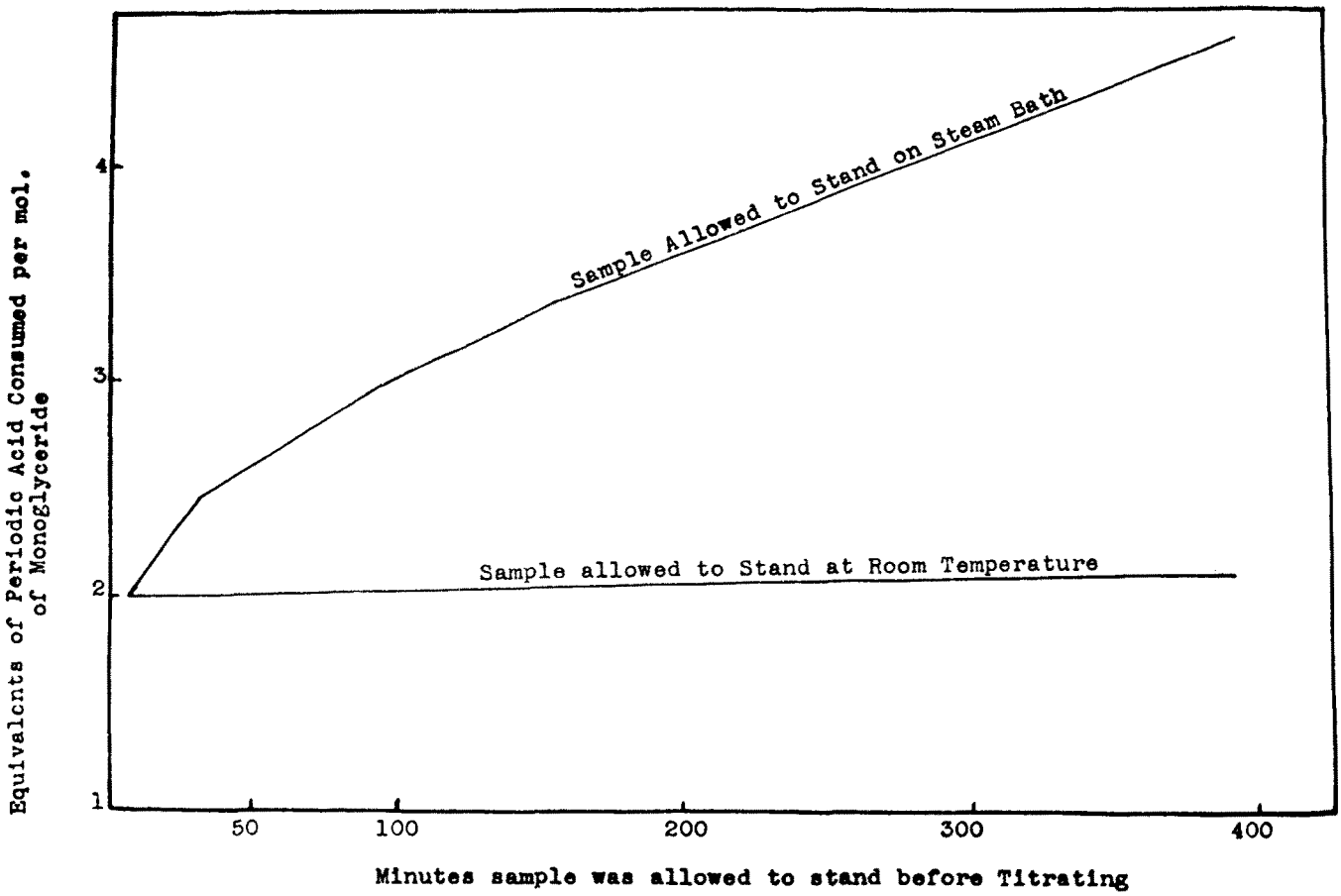
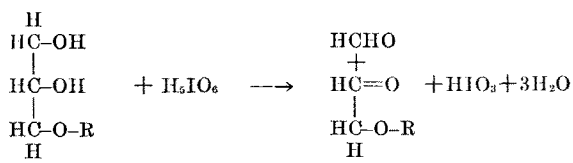


FIG. 1. Effect of temperature on oxidation of monoglyceride by periodic acid.

alcohols containing two or more adjacent hydroxyl groups.

The reaction of periodic acid with the monoglyceride at room temperature is as follows:



When the monoglyceride is oxidized as indicated in this equation, one mol. of periodic acid reacts with one mol. of monoglyceride to produce one mol. of iodic acid. Therefore, the amount of monoglyceride in a sample can be calculated from the difference between titrations of the oxidizing agent before and after the reaction with the sample. The reaction between periodic acid and monoglyceride consumes two equivalents or one-fourth the total oxidizing power of the periodic acid as measured by an iodometric titration. Thus, the maximum difference between the titration of the blank and the titration of the sample will be only one-fourth that of the blank. Since an excess of periodic acid is necessary for quantitative oxidation of the monoglyceride the titration of the sample will be more than 0.75 that of the blank.

The temperature of the solution during and after the reaction must be kept below the point at which secondary reactions occur. Secondary reactions involve oxidation beyond the primary reaction given in the equation and are common at elevated temperatures (8).

Experimental

PURE monostearin, monopalmitin, monolaurin and a commercial product (containing approximately 50% monoglyceride) were used in studying the reaction and conditions for analysis. The oxidizing solution was periodic acid in acetic acid solution (4 volumes glacial acetic acid and one volume water). Since these monoglycerides were solid at room temperature the reagent and monoglyceride had to be heated above the melting point of the monoglyceride in order to obtain sufficient contact for complete reaction. Monolaurin, at its melting point, appeared to be completely soluble in the oxidizing reagent.

The effect of temperatures on the reaction was determined by heating samples with the oxidizing agent for two minutes on a steam bath to liquefy the monoglyceride. These were then shaken and allowed to stand at different temperatures and for different periods before titrating. The reaction given by the equation was complete in a few minutes and secondary reactions were not appreciable when the sample was allowed to stand at room temperature. However, continued heating on the steam bath produced secondary reactions which continued as long as the sample remained on the bath. The results are presented graphically in Figure 1. These tests showed that the oxidation must be carried out at approximately room temperature after the sample has been liquefied.

To evaluate more accurately the effect of temperature in accelerating secondary reactions, additional tests were made in which the monoglyceride and the

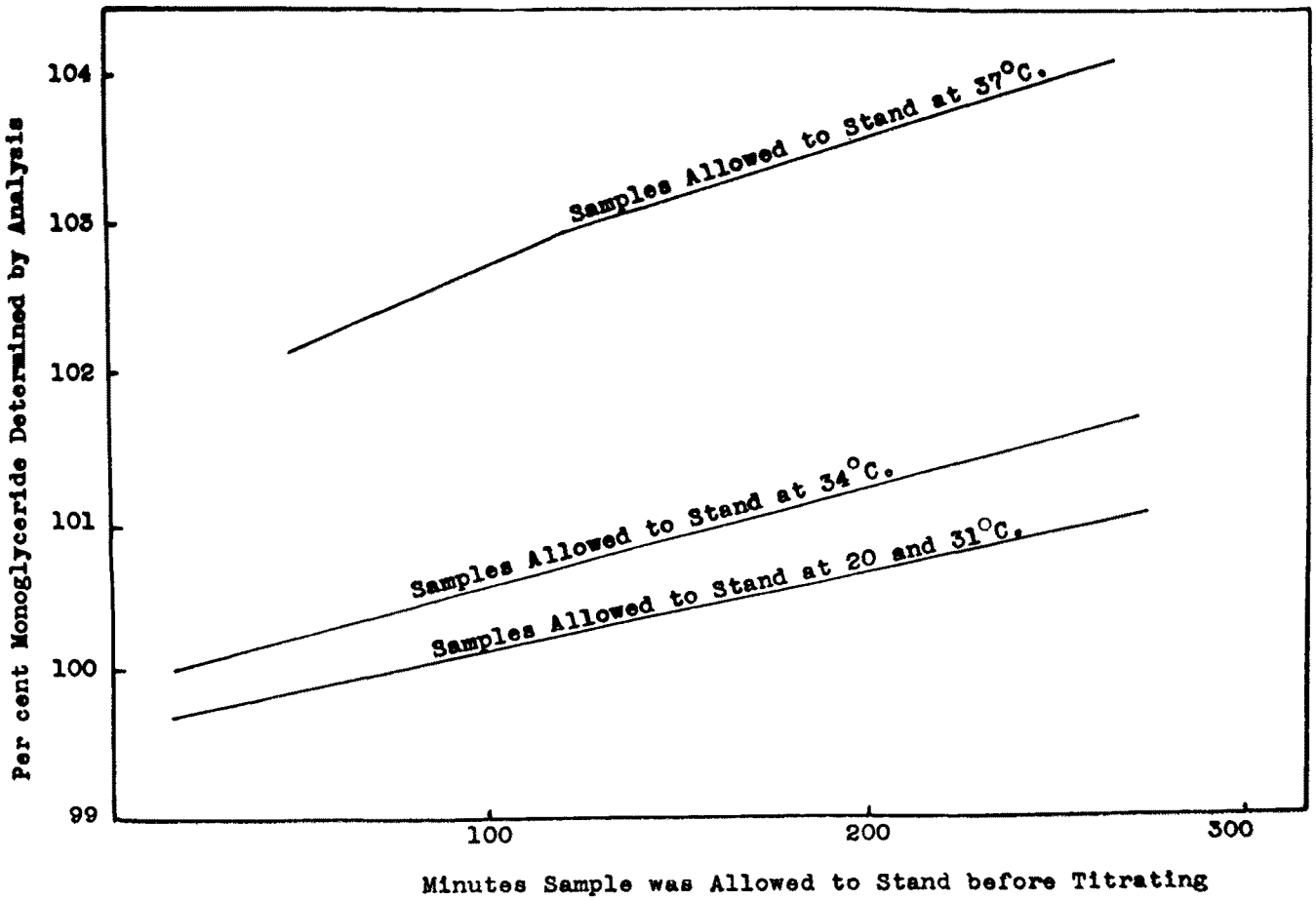


FIG. 2. Effect of temperature on monoglyceride analysis—using periodic acid.

oxidizing reagent were warmed two minutes on the steam bath, shaken, and then allowed to stand at 20°, 31°, 34°, and 37°C. for up to 270 minutes before titrating. The results are shown graphically in Figure R. These data indicated that the reagent and reaction product should be titrated within approximately 120 minutes when allowed to stand at 20° to 31°C. and within 60 minutes when allowed to stand at 34°C. A temperature of 37°C. was too high for quantitative results. If the room temperature rises above 34°C., the samples should be cooled after liquefying and shaking, by placing the flask in a water bath maintained at approximately 30°C.

TABLE I

Relation Between Size of Sample and Reduction of Oxidizing Reagent

Sample in. grams	Monoglyceride determined	Periodic acid reduced to iodic acid	100 (Titration of sample) / Titration of blank
Pure Monostearin	*per cent*	*per cent*	
0.0500	100.3	27.6	93.1
0.1000	100.4	55.3	86.2
0.1200	100.1	66.1	83.6
0.1400	100.3	77.2	80.6
0.1600	100.1	88.2	77.9
0.1800	98.1	97.3	75.8
0.2000	88.7	97.6	75.6
0.3000	59.2	97.7	75.6
Commercial Product			
0.1000	54.6	30.0	92.5
0.2000	55.0	60.5	84.9
0.2400	54.8	72.3	81.9
0.2800	54.8	84.6	78.8
0.3200	54.2	95.6	76.1
0.3600	49.4	97.3	75.7
0.4000	44.5	98.1	75.5
0.5000	35.9	98.6	75.3

The excess oxidizing reagent required for quantitative oxidation of the monoglyceride was determined by analyzing increasing amounts of monoglyceride while keeping the quantity of oxidizing reagent constant. The results of these tests are given in Table I and are shown graphically in Figure 3. The reduction of the periodic acid by reaction with the monoglyceride has been expressed as per cent periodic acid reduced to iodic acid and also as per cent reduction of the total oxidizing power of the periodic acid. The titration of the sample has been expressed as per cent of the titration of the blank. The above comparisons were made in order to illustrate more clearly the relation between the total oxidizing power of the solution and the amount of periodic acid available for

TABLE II

Analyses of Mono- and Tri-Glycerides

Composition of sample	Per cent monoglyce ide	
	Present	Found by analysis
Monolaurin	100	100.2, 99.8
Monopalmitin	100	100.3, 99.8
Monostearin	100	100.1, 99.2
Trilaurin	0	0
Tripalmitin	0	0
Tristearin	0	0
Ethylene glycol dipalmitate	0	0
Ethylene glycol monopalmitate	0	0
Tristearin + monostearin	50.0	49.8
Tripalmitin + monostearin	50.0	50.1
Trilaurin + monostearin	50.0	50.0
Ethylene glycol monopalmitate + monostearin	50.0	49.7
Ethylene glycol dipalmitate + monostearin	50.0	50.2

oxidizing monoglyceride. The reaction is quantitative up to about 93% conversion of periodic acid to iodic acid or a titration for the sample that is about 77% of that for the blank.

To prove conclusively that monoglycerides can be determined quantitatively in the presence of triglycerides, pure monoglycerides and pure triglycerides were analyzed separately and together. Typical data are given in Table II.

The results reported as zero actually varied from +0.08 to —0.02. However, these results are within the limits of experimental error.

THE results show that triglycerides do not react under the conditions of the analysis and that monoglycerides can be determined in the presence of triglycerides. Ethylene glycol monopalmitate showed a greater tendency for secondary reactions than the other compounds.

Products both liquid and solid at room temperature have been analyzed for monoglyceride content. The solid substances were liquefied to obtain good contact and complete reaction with the periodic acid solution. Products which were liquid at room temperature did not require heating for good contact and complete reaction. Heating the oxidizing solution and sample for two minutes on a steam bath was found sufficient to liquefy all of the solid samples tested and in many cases one minute was adequate. Heating two minutes on the steam bath raised the temperature of the contents of the flask to approximately 60°C. and heating for four minutes raised the temperature of the solution to approximately 75°C. To determine the effect of heating beyond that needed to liquefy the product, samples were heated for 2 and 4 minutes after the products were liquefied. The results are given in Table III. These data showed that heating beyond that needed for liquefaction of the sample caused, in some cases, secondary reactions that made the results high. Products liquid at room temperature reacted quantitatively with periodic acid without being heated and solid products reacted quantitatively when liquefied without excessive heating.

The effect of shaking the sample and oxidizing reagent for different periods of time was studied. When no secondary reactions occurred, shaking 30 and 120 seconds gave the same results. Thorough shaking for 30 to 60 seconds was found adequate for complete reaction when the sample was liquid throughout the period.

Samples which contain cellulose material, protein, or glycerol must be freed of these substances before analysis as they react with periodic acid and thus cause high results. Filtration is usually sufficient to remove the cellulose and protein materials encountered in ordinary fats and oils. A sample may be freed from glycerol by washing it with a salt solution (20 gm. of sodium chloride in 100 ml. of solution).

Method

Reagents:

0.1 N sodium thiosulfate, standardized against potassium dichromate.

Oxidizing Reagent: Dissolve 5 gm. periodic acid in 200 ml. of water and then add 800 ml. of glacial acetic acid. Store the solution in a dark, glass stoppered bottle.

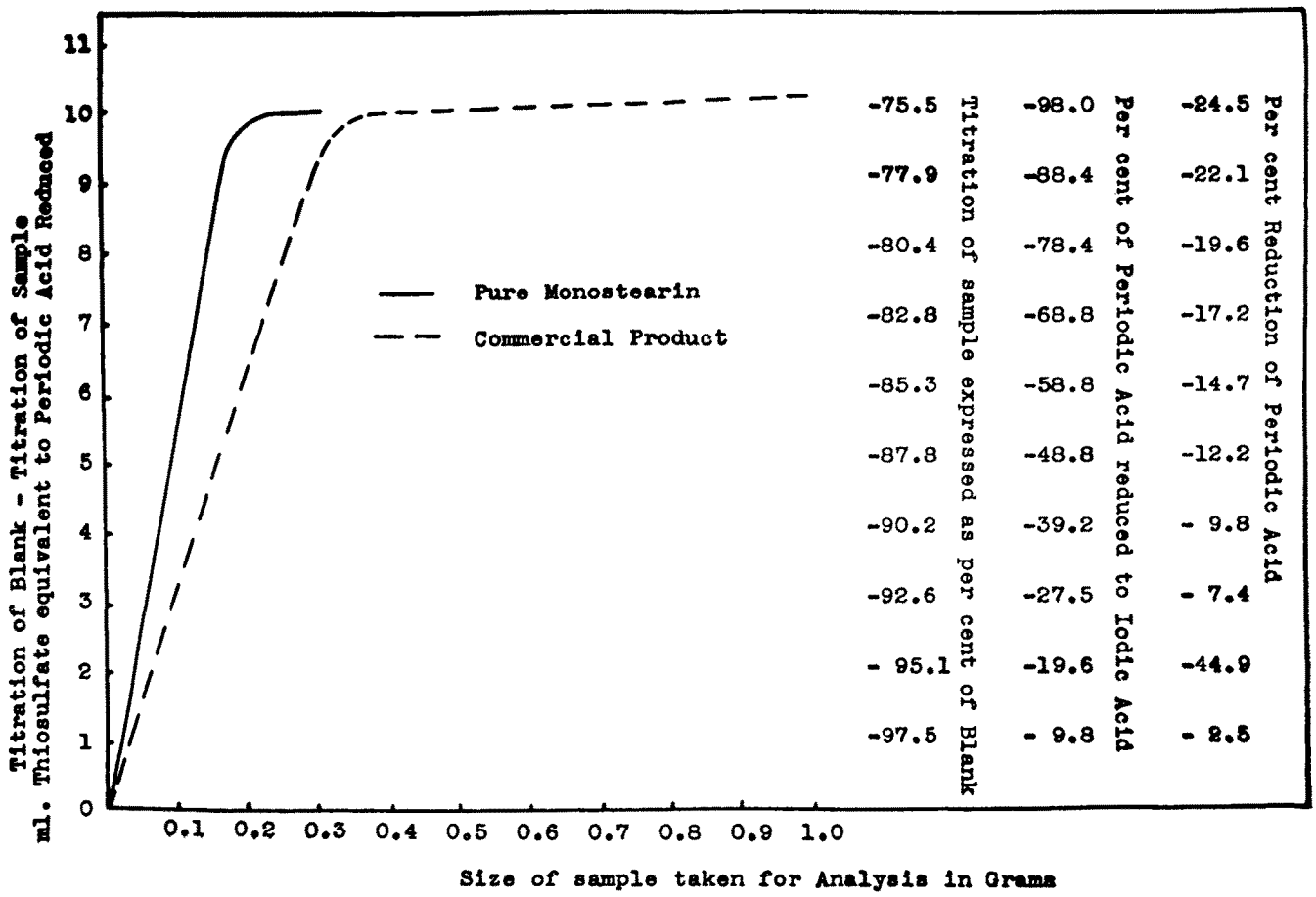


FIG. 3. Effect of size of sample on reduction of periodic acid.

TABLE III

Determination of Monoglyceride in Fats and Oils

Sample	Physical state at room temp.	Minutes on steam bath	Monoglyceride by analysis per cent	Monoglyceride present per cent
Commercial Product I	Solid	2	60.3	.....
Commercial Product I	Solid	4	60.5	.....
Commercial Product II	Very soft at room temp.	2	67.2	.....
Commercial Product II	Very soft at room temp.	4	67.4	.....
Shortening	Solid	2	0.17	.....
Shortening	Solid	4	0.17	.....
Shortening and Product II	Solid	2	1.58	1.62
Shortening and Product II	Solid	4	1.39	1.36
Shortening and Monostearin	Solid	2	1.22	1.18
Shortening and Monostearin	Solid	4	1.30	1.18
Cottonseed Oil	Liquid	0	0.21	.....
Cottonseed Oil	Liquid	1	0.22	.....
Cottonseed Oil	Liquid	2	0.23	.....
Cottonseed Oil	Liquid	4	0.38	.....
Cottonseed Oil and Product I	Liquid	0	1.30	1.37
Cottonseed Oil and Product I	Liquid	4	1.87	1.37
Cottonseed Oil and Product II	Liquid	0	1.61	1.67
Cottonseed Oil and Product II	Liquid	1	1.09	0.96
Cottonseed Oil and Product II	Liquid	2	1.37	0.95
Cottonseed Oil and Product II	Liquid	3	1.60	1.13
Cottonseed Oil and Product II	Liquid	4	1.54	0.91
Coconut Oil	Liquid	0	0.3	.....
Coconut Oil	Liquid	4	0.4	.....
Peanut Oil	Liquid	0	0.4	.....
Peanut Oil	Liquid	4	0.8	.....
Soybean	Liquid	0	0.2	.....
Soybean	Liquid	4	0.3	.....
Hardened Cottonseed	Liquid	0	0.6	.....
Hardened Cottonseed	Liquid	4	0.7	.....
Tallow (Refined)	Liquid	0	0.3	.....
Tallow (Refined)	Liquid	4	0.6	.....
Hardened Peanut	Solid	2	0.2	.....
Hardened Peanut	Solid	6	0.3	.....
Coconut Oil and Monoglyceride	Liquid	0	1.4	1.3
Peanut Oil and Monoglyceride	Liquid	0	1.4	1.4
Soybean Oil and Monoglyceride	Liquid	0	1.3	1.2
Hardened Cottonseed Oil and Monoglyceride	Liquid	0	1.7	1.6
Tallow (Refined) and Monoglyceride	Liquid	0	1.5	1.3
Hardened Peanut Oil and Monoglyceride	Solid	2	1.3	1.2

TABLE IV

Approximate Size of Sample to be Taken for Analysis

Per cent of monoglyceride in sample	Approximate size of sample to be weighed for analysis, in grams
100	0.15
50	0.30
25	0.60
15	1.00
10	1.50
5	3.00
1 or less	10.00

Potassium Iodide Solution. 200 gm. per liter.
Soluble Starch Solution. 1 gm. per 100 ml.

Apparatus:

Burette, 50-ml. accurately calibrated.
Pipette, 25-ml.
Pipette, 10-ml.
Glass Stoppered Flasks, 150- to 200-ml. capacity.

Procedure: Weigh the sample accurately into a 150-ml. glass stoppered flask (see Table IV). Pipette 25 ml. of the oxidizing reagent into the flask. Run a blank on the oxidizing reagent along with the sample.

If the sample is liquid at room temperature do not heat, but if the sample is solid at room temperature heat the flask containing the sample and reagent on a steam bath until the sample is just liquefied. One to two minutes is usually sufficient. Do not allow the temperature of the solution in the flask to rise above 60°C.

After liquefying the sample, shake for 30 to 60 seconds, wash down the stopper and walls of the flask with a few ml. of glacial acetic acid, and allow to stand for 30 minutes at room temperature (34°C. or less). Then add 10 ml. of the potassium iodide solution and titrate with 0.1 N sodium thiosulfate to the disappearance of the brown iodine color. Add 1 ml. of soluble starch solution and continue the titration to the disappearance of the blue iodo-starch color. Read the burette to hundredths of a ml. Titration of the sample should be more than 80 per cent of the blank.

Calculations:

$$\text{Per cent of monoglyceride in sample} = \frac{(x-y)\times N\times C\times 100}{W\times 1000}$$

x = ml. sodium thiosulfate solution required to titrate blank
y = ml. sodium thiosulfate solution required to titrate sample
N = Normality of sodium thiosulfate solution
C = Molecular weight of monoglyceride divided by 2
W = Weight of sample in grams.

Since the molecular weight of the monoglyceride is required for the calculation, the molecular weight of some pure monoglycerides and the average molecular weight of fatty acids from several fats and oils are given below:

Pure monoglyceride	Molecular weight
monolaurin	274.24
monopalmitin	330.29
monostearin	358.33
monoolein	356.31

Fat or oil	Saponification number of fatty acids	Average molecular weight of fatty acids	Average molecular weight of monoglyceride of the fatty acids
Coconut oil	270	207.8	281.8
Av. figures for cottonseed oil, tallow, palm oil, and soybean oil may be taken as	200	280.5	354.5

The molecular weight used in the calculation may be selected in accordance with the type of material being analyzed. In many cases, calculating the monoglyceride as monostearin or monoolein is sufficiently accurate. Individual analyses of pure repeatedly recrystallized monoglycerides were generally within ±0.6% of 100%. Individual analyses of commercial products were within ±0.6% of the average of all tests. Individual analyses of products containing 2% or less, usually were within ±0.1% of the average.

Summary

A method based upon oxidation of monoglyceride by periodic acid has been developed for the determination of monoglyceride in fats and oils. The reaction and conditions that influence the determination have been described and discussed.

Acknowledgment

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