APPENDIX I

Cotton Detergency Test

White Oxford cloth[1] is desized and conditioned for use by:

a) Washing in a Bendix washer, using 0.1 wt. % Rhozyme DM No. 731[2] based on the weight of the fabric (about 5 lb.).

b) Repeating the wash, using 0.2 wt. % solution of Ultrawet K followed by a complete rinsing cycle.

c) Washing in 0.2 wt. % solution of a built fatty acid soap and thoroughly rinsing (until no suds remain in the water).

d) Cutting the cloth, while still damp, into four-inch (4") strips and ironing dry.

e) Drying strips in an oven for 2 hours at 150°F.

f) Storing in a dessicator until used.

The soiling is accomplished by dipping five successive times, without pause between dippings, in No. 1 AX soil prepared as follows:

Weigh—0.9 g. Crisco,
3.1 g. Atreol 34[3] and
1.0 g. Lampblack into sufficient carbon tetrachloride

from a 500-ml. portion to just dissolve the oil and fat. Pass this concentrated soil slurry through a small hand operated homogenizer to obtain good dispersion of the carbon black and then add the balance of the 500 ml. of carbon tetrachloride.

The soiled strips are immediately hung from one end to dry (do *not* put them through a wringer) at room temperature. When dry, the strips are cut into 2" x 4" swatches, and the soiled reflectance is read (once on each side) on the photometer.[4] The soiled swatches are now ready for testing and are to be used within 48 hours or discarded.

Detergents are usually tested at 0.1, 0.15, 0.3, and 0.5 wt. % concentration, using 100 cc. of test solution, one soiled swatch, and 10 three-eighth inch hard rubber balls in each pint test jar. Duplicate tests are normally run with occasional resort to quadruple testing. The jars are sealed, preheated, and transferred from the constant temperature preheat bath to the launderometer, which is run (at 40-42 r.p.m.) for 20 minutes at 120°F.

The jars are then removed from the launderometer, the height of foam above the detergent solution in each jar is noted (following one quick inversion of each jar), the swatches removed and thoroughly rinsed in 120°F. tap water, and then air dried. The reflectance of the air dried swatches is again measured and the detergency values calculated:

$$\text{Detergency} = \% \text{ reflectance regained} = 100 \times \frac{(\text{Reflectance, washed swatch} - \text{reflectance, soiled swatch})}{(\text{Reflectance, original swatch} - \text{reflectance, soiled swatch})}$$

[1] Manufactured by Everfast Mills Inc., Eddystone, Pa.
[2] An enzyme preparation used to hydrolyze starch and thus facilitate its solution. Manufactured by Rohm and Haas, 222 W. Washington Square, Philadelphia, Pa.
[3] Manufactured by The Atlantic Refining Company, Philadelphia, Pa.
[4] Manufactured by the Photovolt Corporation, 95 Madison avenue, New York 16, N. Y.

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Limitations of the Periodate Oxidation Method for the Determination of Monoglycerides in Fats and Oils

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THE periodic acid oxidation method for the determination of monoglycerides in fats and oils originally reported by Pohle, Mehlenbacher, and Cooke (1) and later modified by Handschumaker and Linteris (2) is apparently based upon the supposition that the periodic acid consumed in the method is attributable to monoglyceride only. Since it is possible, and very likely, however that small quantities of materials, other than monoglycerides, are present in oils that may react with periodic acid, calculated values for the monoglyceride content of naturally occurring fats and oils may be in error.

It is quite well known that other vicinal dihydroxy or ketohydroxy fatty acids or triglycerides thereof will react with periodic acid. These compounds may either exist naturally or may be formed during oxidation of unsaturated fatty acids. In the course of the present investigation the possibility of periodic acid reaction with such materials was considered. If a fat or oil which had a periodate value is saponified, the fatty acids isolated and washed thoroughly to remove the glycerol formed on hydrolysis, and the fatty acids treated with periodic acid by the accepted method, four possibilities may occur: a) The fatty acid fraction should have zero periodate value if all the periodate value is attributable to monoglyceride; b) the periodate value of the fatty acid fraction should be less than that of the unsaponified fat if only part of the periodate value is due to monoglyceride; c) if the periodate value of the fat is due to unsaponifiable material, then the periodate value of the fatty acid fraction should be the same as that for the fat; d) if there are substances present in the fat which contain vicinal or amino and hydroxy or ketohydroxy groups in which at least one of those groups is combined with some other material so as to prevent the periodic acid reaction, then on saponification those vicinal groups would be liberated and the periodate value of the fatty acid fraction should be greater than that of the original fat.

The purpose of the present investigation, in view of the above possibilities, was to assess the reliability of the periodic acid method as an indication of pres-

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TABLE I

Periodate Values of Fats Before and After Saponification

Fat Sample	Periodate Value me/100 g. Before Saponification			Average	Periodate Value me/100 g. After Saponification			Average	Periodate Values Calculated as % Monoglyceride *	
									Before	After
Cottonseed Oil (Salad)	1.9	1.7	1.1	1.6	2.5	2.4	2.2	2.4	0.29	0.43
Corn Oil (Hexane Extracted)	4.2	4.2	4.4	4.3	4.1	4.0	4.2	4.1	0.77	0.73
Refined Soybean Oil	2.8	2.6	2.5	2.6	2.4	2.5	2.2	2.4	0.46	0.43
Raw Linseed Oil	2.5	2.3	2.3	2.4	2.7	2.9	3.3	3.0	0.43	0.53
Refined Peanut Oil	0.67	0.67	0.89	0.74	1.0	1.2	1.4	1.2	0.13	0.21
Refined Cottonseed Oil	2.8	2.7	2.6	2.7	2.9	3.1	2.7	2.9	0.48	0.52
Hydrogenated Soybean Oil (I.V. 80)	1.7	1.5	1.4	1.5	1.8	1.9	1.8	1.8	0.27	0.32
Lard No. 1 (Stored at 5° for 6 months)	3.4	3.3	3.6	3.4	3.7	3.4	3.9	3.7	0.61	0.66
Lard No. 2 (Prime Steam)	0.22	0.17	0.22	0.2	0.33	0.28	0.44	0.35	0.36	0.62
Refined Coconut Oil	0.67	0.78	0.50	0.65	1.4	0.9	1.0	1.1	0.12	0.20
Partially Hydrogenated Coconut Oil	0.78	0.78	0.67	0.74	0.9	0.95	0.78	0.88	0.13	0.16
Cottonseed Margarine Oil	1.4	1.6	1.6	1.5	1.3	1.5	1.3	1.4	0.27	0.25
Partially Hydrogenated Soybean Oil	1.4	1.4	1.7	1.5	2.3	2.2	2.0	2.2	0.27	0.39
Fully Hydrogenated Soybean Oil	1.6	1.5	1.5	1.5	1.8	1.9	1.8	1.8	0.27	0.32
Hydrogenated Cottonseed Oil Flakes	1.6	1.6	1.7	1.6	1.8	1.9	2.1	1.9	0.28	0.34

* Monoglyceride factor 356.41.

ence of small amounts of monoglycerides in fats and oils.

Experimental

Method. The modified periodate method of Handschumaker and Linteris (2) was used in all determinations. Approximately 10 g. of fat or oil was weighed carefully into iodine flasks and 15 ml. of acetic acid-chloroform (2:1) and 25 ml. of periodic acid reagent added. The flask was shaken one minute and allowed to stand 10 minutes and the solution titrated with 0.1 N sodium thiosulfate after the addition of 15 ml. of potassium iodide and 100 ml. of water. In the saponified samples all of the fatty acid fraction from 25 g. of fat was used for the periodate determination. The number of milliequivalents of periodate consumed for various fat samples is given in Table I.

Saponification of Fat or Oil. A 25-g. sample of fat was weighed into a 250-ml. centrifuge flask, 50 ml. of 15% alcoholic potassium hydroxide added, and the mixture refluxed for one hour. The soaps were cooled and the fatty acids liberated from their potassium salts by acidifying with 1:1 hydrochloric acid. The fatty acids were then washed 10 times with hot water and dissolved in ethyl ether (purified to zero periodate value) and the ether solution washed an additional five times with water. The final wash water which was shaken with the ether solution was treated with periodic acid reagent and showed no appreciable reaction. The data on the saponified fats are listed in Table I.

Preparation of Monoglyceride. In order to determine whether all fatty fractions obtained by the saponification of fat as described were glycerol free, preparations were made containing monoglyceride by the direct reaction of fatty acid with excess glycerol.

a) Sixty grams of fatty acid (obtained by cooling an acetone solution of corn oil to —20°) and 15 g. of glycerol were weighed into a 300-ml. flask. The flask was warmed on a steam bath and then heated on an oil bath at 250°C. for four hours under nitrogen with constant stirring. The flask was cooled to 60°C., its contents poured into a separatory funnel diluted with 1 liter of distilled water and 2 liters of Skellysolve B and shaken gently. The Skellysolve layer was washed free of glycerol with water and dried over anhydrous sodium sulfate. A portion of the Skellysolve was removed by distillation under reduced pressure and the remaining solution cooled to —20°C. The resulting crystalline precipitate was removed by filtration and dried in a vacuum desiccator.

The periodate values for the fatty acid and the monoglyceride (A) before and after saponification are given in Table II.

TABLE II

Periodate Values of Monoglyceride Preparation Before and After Saponification

Sample	Periodate Value me/100 g. Before Saponification				Periodate Value me/100 g. After Saponification			Periodate Values Calculated as % Monoglyceride
Lauric Acid	0.01	0.00	0.01					
Solid Fatty Acids (Corn Oil)	0.96	0.86	0.96					
Monoglyceride				(av.)				
B	25.2	25.2	25.3	25.2	0.01	0.015	0.01	4.5
A	90.1	89.7	91.4	90.4	1.00	0.90	1.00	16.1

b) Lauric acid (50 g.) was heated with glycerol for five hours in an atmosphere of nitrogen at a temperature of 200°C. The lauric acid used was prepared from Neo-fat No. 11 by fractional distillation of the methyl ester and fractional crystallization of the free acid. The periodate value of the final crystallized lauric acid was essentially zero. The periodate values of the monoglyceride preparation (B) expressed as milliequivalents per 100 g. before and after saponification are given in Table II.

Discussion

Periodate values of the various fats and oils listed in Table I were determined in triplicate and expressed in milliequivalents of periodate per 100 g. Although some variations are noted in individual determinations, the order of magnitude is the same. Average values of the three determinations of each fat were used for the purpose of calculating the milliequivalents of periodate per 100 g. as per cent monoglycerides. It will be noted that the calculated values for monoglyceride are all below 1%.

In no instance was a zero periodate obtained on the saponified fat. This would seem to indicate that the periodate value obtained on the original fat was not attributable solely to monoglyceride, if at all. Although no definite conclusions are apparent from the data in Table I relative to the actual amount of monoglyceride in a fat, they certainly demonstrate the non-specificity of the periodate method for monoglyceride determination, at least where the calculated

percentages are small.* As a control for determining percentages of monoglyceride in commercial monoglyceride preparations made from natural fats and oils, the periodate method is undoubtedly suitable because the error involved in the presence of small quantities of periodic acid reactive groups would be insignificant compared to the total calculated per cent of monoglyceride.

Laboratory synthesis of a monoglyceride preparation was performed for the purpose of determining whether the saponification of a fat and subsequent washing of the fatty acid fraction obtained resulted in a product free of glycerol. It might be expected that the presence of glycerol would have a considerable effect on the periodate value of the saponified product (1 mg. glycerol = 0.4 ml. of 0.1 N thiosulfate). The data in Table II indicates however that the washing process was effective in removing the glycerol. This conclusion arises from the fact that the periodate value of the fatty acid obtained by saponification of the monoglyceride preparation was essentially equivalent to that of the fatty acids used in the synthesis. This result reveals clearly that if the periodate value of the original fat is due to monoglyceride only, then the periodate value of the fatty acid fraction should be zero.

* The method of Pohle, Mehlenbacher, and Cooke was probably not intended to be applied to very small quantities of monoglycerides.

Summary

Periodate values were determined on a variety of fats and oils before and after saponification by the periodic acid oxidation method of Handschumaker and Linteris.

The values obtained indicate that the method is not specific for monoglycerides in natural fats and oils. Therefore previous reports of the natural occurrence of monoglycerides in fats and oils, where the periodate method has been used, may be open to question.

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Reactions of Tertiary Butyl Hypochlorite With Vegetable Oils and Their Derivatives. III. Chlorination of Soybean Oil in the Pilot Plant[1]

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A METHOD for the chlorination of soybean oil with *tert*-butyl hypochlorite and subsequent dehydrochlorination of the chlorinated oil has been reported by Teeter *et al.* (2). The preliminary evaluation of the drying properties of the dehydrochlorinated oil was sufficiently encouraging to indicate that an investigation of these reactions on a larger scale would be desirable. This paper describes the results of a study in which these reactions were successfully conducted on a small pilot-plant scale.

The chlorination with *tert*-butyl hypochlorite was conducted in conventional equipment in the pilot plant. However the preparation of the required hypochlorite and the dehydrochlorination of the chlorinated oil were conducted in the laboratory since suitable equipment for handling reactions involving large amounts of moist chlorine and anhydrous hydrogen chloride was not available in the pilot plant. Dehydrochlorination was conducted in an all-glass apparatus designed for continuous operation whereas this step was carried out previously (2) on small batches of oil in a current of steam.

No difficulty was experienced in handling the comparatively large quantities of *tert*-butyl hypochlorite required in the present work. It was of course necessary to avoid undue exposure to light and heat and to prevent contact with extraneous organic compounds which might react exothermically with this reagent. The chemical properties of *tert*-butyl hypochlorite have been described in detail by Teeter *et al.* (2), and by Chattaway and Backeberg (1).

Data obtained for the chlorinated oils are given in Table I which includes, for comparison, corresponding data for a sample prepared in the laboratory. These data show that the oil obtained in the pilot plant contained about 2% less chlorine than the oil prepared in the laboratory and was considerably darker in color. The laboratory and pilot plant oils had approximately the same total conjugation despite the differences in chlorine content. The varying distribution of conjugation among diene, triene, and tetraene forms, observed in the two samples of oil from the pilot plant, was not surprising in view of the results reported previously (2).

The lower chlorine content of the pilot-plant oil was caused primarily by evaporation of hypochlorite through the loading port during the reaction. For reasons described in the Experimental Part it was not convenient to close this port tightly.

The excessive darkening of the oil during chlorination was at first attributed to the rather high temperature (110°C.) reached during removal of by-product alcohol. In a second run this temperature was not permitted to rise above 80°. Nevertheless the product had a color of 15-16 as compared with a color of 18 in the previous run and a color of 6-7 for a laboratory product which had not been heated above 75°. These observations indicate that the darkening must have been due to local overheating because past experience has shown that darkening is caused only by exposure of the chlorinated oil to excessive heat.

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