and not the 2-monoesters. Accordingly the countercurrent distribution curve for oxidized 1-monostearin, for example, is void of any monoglyceride peak. A poorly defined peak appears however at a higher tube number. This peak is due to the hydroxyacetaldehyde ester which is the reaction product of the oxidation.

Oxidation of 2-monostearin, on the other hand, survives the periodic acid treatment and comes through the distribution in its proper place. Figures 2 and 4 are distribution curves for 2-monostearin and oxidized 2-monostearin. It will be noticed that the peak for the oxidized sample shifted slightly to a lower tube number. This, in addition to the appearance of the hydroxyacetaldehyde peak in Figure 4, clearly suggests that the original 2-monostearin was contaminated with some 1-monostearin. This was indeed the case as periodic acid analysis indicated the presence of this contaminant. The partition coefficient for pure 2-monostearin therefore is judged to be the 0.270 value.

The possibility of separating the 1- and 2-monoester has been mentioned. Calculations with Equation 2 and the partition coefficients for the 1- and 2-monostearins indicated that 150 transfers should be sufficient to resolve these substances into two separate peaks from a mixture of equal weights of the two. In addition, a yield of about 20% of each isomer should result in the tubes on the extremities of the curve. The experimental verification of these calculations was not successful, owing to the great tendency of the 2-isomer to revert readily to the 1-form. Apparently the additional time involved to carry out 150 transfers, even with the automatic equipment, over a 49-transfer distribution was sufficient for the isomerization to take place. The experimental effort expended in this direction was quite limited however.

Conclusions

1. The countercurrent distribution curves have been determined for eight pure and one commercial monoglyceride, one diglyceride, and one diglycerol monoester.
2. Certain generalities exist between the partition coefficients and the structures of the acid radicals of the monoglyceride.
   a. An increase in the chain length of the acid produces an increase in the partition coefficient.
   b. The 2-monoesters have lower partition coefficients than the corresponding 1-isomers.
   c. Unsaturation in the acid decreases the value of the partition coefficient.
3. The direct separation of mixtures of 1- and 2-monoglycerides by countercurrent distribution is thwarted by the rapid isomerization of the 2-isomer to the 1-form.

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2-Monoglycerides[1,2]

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IN THE YEAR 1920 Emil Fischer (1) established that all attempted syntheses of 2-monoglycerides up to that time had resulted in 1-monoglycerides. He pointed out the greater stability of the 1-isomer and developed an isomerization mechanism to explain this result. It was 1931 before Bergmann and Carter (2) synthesized the first 2-monoglyceride of a fatty acid (2-monopalmitin). Feuge and Bailey (3) postulated the presence of 2-isomers in mono-diglyceride interesterification mixtures but rejected them as the source of small discrepancies between theory and their observations. Mattson *et al.* (4) demonstrated the presence of unsaturated 2-monoglycerides in intestinal contents but did not isolate them. In fact, unsaturated 2-monoglycerides were not prepared until 1953 when Martin (5) used a boric acid hydrolysis rather than the classic hydrogenolysis of the intermediate. He also proposed a perchloric acid isomerization to aid in direct determination of 2-monoglycerides since periodic acid (or lead tetra-acetate) oxidation will not detect the 2-isomer (5, 6). Very recently Borgstrom (7) isolated 1- and 2-monoglycerides from *in vitro* hydrolyzed fat, using silicic acid adsorption of periodic acid oxidized products.

In a variety of publications (for example, 3) it has been established that reactions of fats or fatty acids with glycerol normally result in a randomized reaction product, that is, the composition of the product can be calculated, based on the input molar ratios. In mono-diglycerides the monoglyceride analysis by periodic acid oxidation agrees rather closely with the calculated value for total monoglyceride, assuming equal participation of all three positions possible in glycerin. This suggests that the 2-isomer played an important part in determining the composition of that equilibrium mixture. This 2-monoglyceride has been transient since complete or partial isomerization to 1-monoglyceride has occurred in the final product. It was our purpose to determine whether the final product contains a detectable quantity of 2-monoglycerides.

The work to be described shows that 2-monoglycerides do exist in commercial monoglycerides; they have been isolated; and they apparently behave as do the 1-isomers in at least one application. A rapid

[1] Communication No. 216 from the laboratories of Distillation Products Industries.
[2] Presented at the Minneapolis meeting of the American Oil Chemists' Society, Oct. 13, 1954.

analysis for total monoglyceride has been developed which incorporates Martin's isomerization procedure directly into the usual periodic acid analysis.

Isolation

In order to establish methods for investigation of commercial products, techniques were developed which permitted isolation of 2-monoglycerides from interesterification mixtures. Relatively pure 2-mono-olein and 2-monostearin were prepared from the reaction products of glycerin with recrystallized oleic acid and with high purity stearic acid. Partial isolation, along with isomerization analyses or countercurrent distributions, were carried out for commercial material. By these procedures all tested products (distilled monoglycerides and three mono-diglycerides) were found to contain 2-isomers.

2-MONOGLYCERIDE ISOLATION

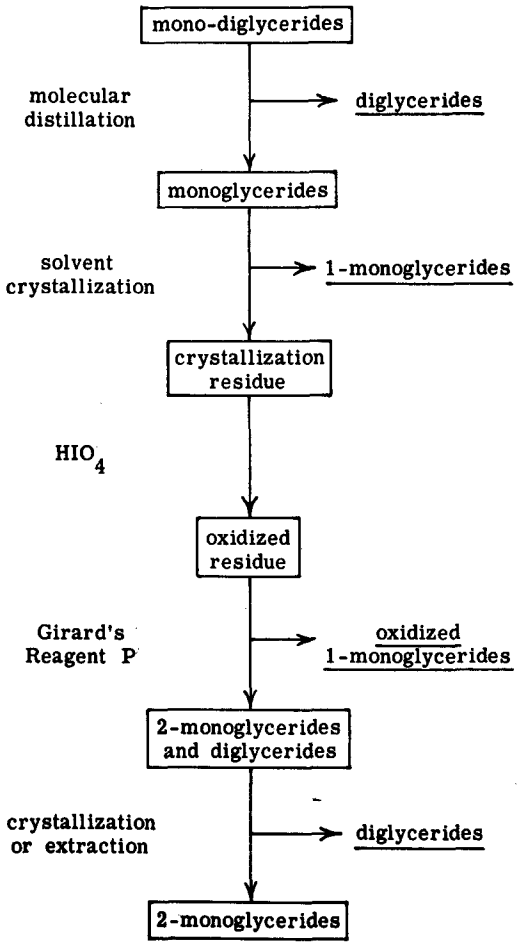

FIG. 1

An outline of the isolation procedure is shown in Figure 1. The monoglycerides were first separated by molecular distillation. After water-washing an ether solution to remove glycerin, the product was dissolved in methanol or ethyl ether and crystallized at successively lower temperatures until 85 to 90% of the distilled monoglyceride had been removed as crystals. The concentrations, temperatures, and results varied considerably, depending upon starting material, but, for distilled mono-olein, the filter cakes were removed from a 15% solution in ethyl ether at −10, −20, and, after removal of half the solvent, at −25°C. About 12% of the distilled monoglyceride remained in the filtrate. This crystallization residue contained 68.2% of 1-monoglyceride and an estimated 20 to 25% 2-monoglyceride along with some diglyceride.

An oxidation with periodic acid converted the remaining 1-monoglycerides to esters of hydroxyacetaldehyde. Borgstrom (7) showed subsequent separation by silicic acid adsorption.

We found that countercurrent distribution was practical, but aldehyde complexing was preferred for large quantities. The reaction conditions required for making phenylhydrazones or semicarbazones resulted in 2-monoglyceride destruction. However reaction with Girard's reagent P permitted removal of the aldehyde by simple water washing, without affecting the 2-isomer.

The remaining 2-monoglycerides and diglycerides were separated by crystallization from methanol or by solvent partition between Skellysolve B and 85% aqueous methanol. Some properties of the resultant 2-monostearin and 2-mono-olein are shown in Table I.

TABLE I

Analysis of 2-Monoglycerides

Property	2-Olein	2-Stearin	Theory (or lit.)	
			2-Olein	2-Stearin
OH value	311	.....	315	313
Periodic acid analysis	6.7	2	0	0
Isomerization analysis [a]	99.6%	98.0%	100	100
Melting point	.....	72.5-73.5°C.[b]	.....	74.5 (10)

[a] Using 70% perchloric acid with a factor of 1.15.
[b] No depression on admixture with an authentic 2-monostearin.

In addition to these properties, infrared absorptions were determined. The curve for this 2-monostearin was identical with that for material by classic synthesis. The curve for 2-mono-olein differed from the one for 1-mono-olein in the same features as noted for the stearate pair.

The 2-mono-olein was compared with 1-mono-olein in one cake baking test. A 140% sugar white cake formula with a batter weight of 340 g. was used. The volume of this test cake is critically dependent upon type and amount of monoglyceride added, with control volumes (0% addition) of about 1,040 ml./lb. of batter. The four test cakes (each isomer in duplicate at 1% of the shortening) had volumes ranging between 1,230 and 1,250 ml./lb. of batter. For this test the isomers may be considered of identical utility.

Countercurrent Distribution

Perry *et al.* (8) characterized pure monoglycerides by countercurrent distribution and showed the analytical utility of this technique for mixtures containing monoglycerides. The patterns for pure monoglycerides and for mixtures had shown the development of a single well-defined peak in the region between tubes 4 and 15, depending upon the predominant monoglycerides present.

When applied to distilled monoglycerides, including the commercial product, 95 to 100% of the recovered material was in this monoester peak. This was true

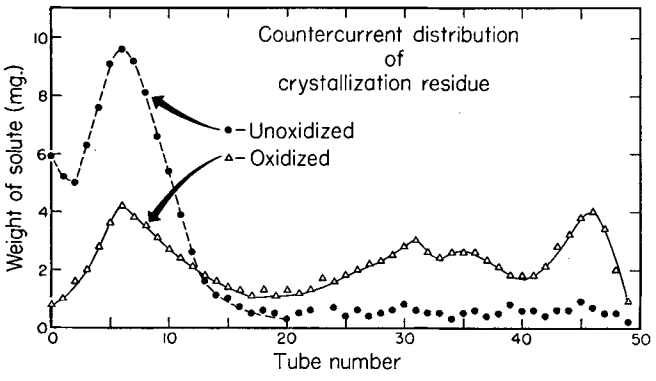

FIG. 2

even when periodic acid analyses for 1-monoglyceride were as low as 88 to 90%. In the crystallization residues (50 to 65% 1-monoglyceride) 75 to 80% of the weight was recovered as total monoglyceride by distribution (Figure 2). And in the isolated 2-monoglycerides, with periodic acid analyses of 0 to 10%, over 90% of the material occurred in the monoglyceride peak.

The material behaving as monoglyceride in all ways except by periodate analysis was considered to be 2-monoglycerides. By way of confirmation various products were oxidized and then distributed before or after complexing with Girard's reagent P. Oxidized distilled monoglycerides gave no monoglyceride peak, but the residue, after Girard's reagent complexing, did. When up to 8% of pure 2-monostearin was added to the distilled monoglyceride, oxidation and distribution without complexing still showed no remaining monoglyceride. Apparently the oxidation products interfere with normal distribution and have to be removed.

This interference was not complete in the case of the oxidized crystallization residues (Figure 2). Approximately 30% of these oxidized samples (0 to 5% 1-monoester by periodate analysis) was recoverable as total monoglyceride. If some interference existed, the actual content may have been higher.

Spontaneous Isomerization

Distilled monoglycerides from hydrogenated lard, when freshly distilled from a freshly prepared mono-diglyceride mixture, frequently would have a periodic acid analysis as low as 86%. Upon standing for 24 to 48 hrs., this figure increased to as much as 94%, with the average change being 4 to 6%. No other chemical change was observable. This change was not observed on freshly distilled product from aged mono-diglycerides. The unsaturated products were higher originally and did not change.

Crystallization residues from unsaturated monoglycerides always increased in analysis upon standing. One sample at an original 57.6% 1-monoglyceride content increased to 77.6% after 63 days at room temperature. Crystallized 2-monostearin did not undergo a change, but the solvent-partitioned 2-mono-olein did increase slowly in 1-monoglyceride content at room temperature.

Induced Isomerization

Stimmel and King (9) "isomerized" 2-monoglycerides by the addition of acid or base as catalyst. For example, they used N/20 alcoholic HCl and after 24 hrs. were able to crystallize pure 1-monostearin from the solution. During the current investigation it was noted that material recoveries were poor. If the HCl is neutralized and the solvent removed at room temperature under vacuum, glycerin and a mono-di-triglyceride is recovered. The product from this technique, or from water quenching followed by ether extraction of the isomerized mixture, shows complete reversion to the composition calculated from random distribution of a monoglyceride.

Martin (5), apparently recognizing the invalidity of earlier isomerization methods, proposed the use of perchloric acid. He showed that isomerization could be made essentially complete before reversion would seriously interfere. From this he developed an isomerization method, permitting determination of "total monoglyceride."

We had attempted adding various isomerization agents during analysis in hopes of obtaining simultaneous isomerization and oxidation. This should result in a total monoglyceride value. Adding sulfuric or hydrochloric acids, changing acetic acid concentration, and other such approaches were unsuccessful. If, during analysis, perchloric acid is added before periodic acid, the results are dependent upon the time interval and the isomerization-reversion characteristics of the system. Curves for 1- and 2-monopalmitin are shown in Figure 3.

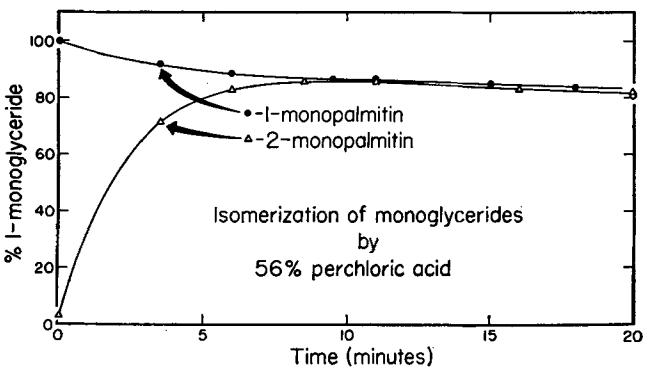

FIG. 3

Since the periodic acid is aqueous, it serves as the water quench for inactivating perchloric acid. Accordingly isomerization cannot be effected by adding perchloric acid simultaneously with or subsequent to the periodic acid addition. The use of 0.045 ml. of 56% perchloric acid and a 10-minute interval, as suggested by Martin, resulted in the same factor (1.15) that he reported.

The lack of effect of changed isomer ratio or of fat dilution is shown in Table II.

TABLE II
Perchloric Acid Isomerization Analysis for Total Monoglyceride

A		B	C
Composition of mixture		1-Mono found after isomerization	Total mono found (1.15B)
% 2-MP[a]	Other		
0	100% 1-MP[b]	86.9	100.0
50	50% 1-MP[b]	88.4	101.6
100		85.4	98.2
50	50% Fat A	43.2	49.6
10	90% Fat A	8.9	10.2

[a] 2-monopalmitin.
[b] 1-monopalmitin.

The above have been established as preferred conditions although preliminary work was done with 70% perchloric acid, which causes more rapid isomerization and introduces more reversion. With this strength of acid a 90-second interval with a factor of 1.15 can be used and was the method of analysis shown in Table I.

The precision and accuracy seem reasonable in the light of the limited work reported. The analytical method, which combines Martin's isomerization with the periodic acid oxidation procedure of Handschumaker and Linteris (11), consists of the following steps:

1. Dissolve a weighed sample (which contains approximately 100 mg. total monoester, glycerol-free by water washing in ether solution) in 15 ml. of 2:1 acetic acid:chloroform in a 250-ml. iodine flask.
2. Add 0.045 ml. of 56% $HClO_4$, shake for 60 seconds, and let stand for 9 minutes.
3. Add the 25 ml. periodic acid reagent and continue with the analysis as described by Handschumaker and Linteris (11).
4. Calculate the total monoglyceride content by multiplying the result of that analysis by 1.15.

Composition of Monoglycerides

Isomerization analyses indicated about 5 to 8% of 2-monoglyceride in distilled products; mono-diglycerides contained about half as much total monoglycerides with about 5% of this also being 2-isomer. For example, one product at 37.8% 1-monoglyceride analyzed for 39.6% total monoglyceride. Countercurrent distributions and chemical analyses were confirmatory. For example, hydroxyl values of distilled monoglycerides ranged from 316 to 322 in material where 322 was theoretical for 100% monoglyceride.

The crystallization residues contained, by a variety of techniques, about 25 to 30% 2-monoglyceride, which corresponded to 4 to 5% 2-monoglyceride in the distilled product. This figure is, of course, a minimum since the crystallization was not necessarily a quantitative separation.

As a result, it is believed that all commercial monoglyceride products contain 2-monoglycerides. The amount is probably 5 to 8% of the total monoglyceride content.

Summary

1. Commercial monoglycerides and mono-diglycerides contain 2-isomers. The amount is in the range of 5 to 8% of the total monoglyceride content.

2. 2-Mono-olein and 2-monostearin have been isolated from the reaction products of glycerin with oleic and with stearic acids.

3. At least for cake baking utility, 2-monoglycerides appear to be equivalent to 1-monoglycerides.

4. An analysis for total monoglyceride content is proposed. It incorporates perchloric acid isomerization directly into the usual periodic acid analysis.

Acknowledgments

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Accelerated Stability Test for Vitamin A in Oils and Fats by Means of Surface-Enlarging at Room Temperature

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MANY ACCELERATED METHODS have been proposed and used for the study of relative stability to oxidation of fats, oils, and oil-soluble vitamins. Among these are the Active Oxygen Method (frequently referred to as the "Swift" method) (1, 2, 4, 7), the Schaal oven test (2, 6, 7, 12), and the Barcroft-Warburg manometric method (8, 10, 14). All of these methods have the weakness of being conducted at temperatures higher than those of ordinary storage, thus raising a question as to the mechanism of the reactions, which would be involved under these conditions, as compared with those in more nearly "normal" circumstances (15). For example, it has been found that N.D.G.A. has a diminished effectiveness at relatively high temperatures (3, 9, and author's own data). Similar observations have been made with respect to isobutylgallate (11). The use of metallic catalysts such as copper (12) for accelerating oxidation also raises a question as to the reactions involved.

Dubouloz (5) has accelerated oxidation by expanding the surfaces through the medium of spreading vitamin A oil on filter paper. These papers are then suspended in a closed container at a constant temperature between 90° and 100°C. Taufel (15) also developed a method employing surface-expansion, using chromatographic paper in diffuse daylight at room temperature; but the use of diffuse daylight as a catalyst presents a condition not often found in regular storage conditions of vitamin A substances.

The present paper proposes an accelerated test for the relative stability of vitamin A-bearing oils which attempts to stay as closely as possible to ordinary storage conditions and still offer the merits of an