Influence of the Position of Unsaturated Fatty Acid Esterified Glycerol on the Oxidation Rate of Triglyceride

SHUN WADA and **CHIAKI KOIZUMI**, Department of Food Science and Technology, Tokyo University of Fisheries, Konan, Minato-ku, Tokyo, 108 Japan

ABSTRACT

The influence of the position of unsaturated fatty acid esterified glycerol on the oxidation rate of triglyceride was investigated at 50 C. Randomized triglycerides used were prepared by random interesterification between saturated and unsaturated monoacid triglycerides using sodium methoxide as catalyst. The monoacid triglycerides used were tripalmitin, tristearin, triolein and trilinolein. The molecular species of the randomized triglycerides were analyzed by high performance liquid chromatography (HPLC) in combination with gas liquid chromatography (GLC) and enzymatic hydrolysis. From the results of oxygen absorption measurement by GLC, the randomized triglycerides were more stable towards oxidation than the triglyceride mixtures which were prepared by mixing the equivalent quantities of the same monoacid triglycerides as used in the random interesterification. This may be due to the decrease in the contents of most unstable unsaturated monoacid triglycerides by random interesterification with saturated monoacid triglycerides. Furthermore, from the results obtained with the detailed analysis of the randomized triglycerides at different stages of oxidation, it became clear that the triglycerides having unsaturated fatty acids linked at the 2-position of glycerol are more stable towards oxidation than those linked at the 1(or 3)-position. The carbon chain length of saturated fatty acids has essentially no influence on the oxidation rates of unsaturated fatty acids esterified in the same glycerol.

INTRODUCTION

Extensive data have been published concerning the oxidation of unsaturated fatty acids. However, limited information is available on the oxidation rate of triglycerides as related to their structure.

Raghuveer and Hammond (1) suggested that the triglyceride having an unsaturated fatty acid linked at the 2-position of glycerol was more stable towards oxidation than that linked at the 1(or 3)-position. From this, it might be expected that the stability of the triglyceride could be improved by shifting unsaturated fatty acids from the 1,3-positions to the 2-position of glycerol. However, Zalewski and Gaddis (2) demonstrated that the oxidation of lard which was rich in triglycerides having unsaturated fatty acids linked at the 1,3-positions became faster after random interesterification. It is not certain whether the observations obtained with lard deny the abovementioned suggestion of Raghuveer and Hammond, since the shift of unsaturated fatty acid from the 1,3-positions to the 2-position of glycerol in lard triglycerides with random interesterification was not confirmed experimentally.

In a previous paper (3), the authors have proposed an analytical method for the triglyceride molecular species using high performance liquid chromatography (HPLC). In the present study, the influence of the position of unsaturated fatty acid esterified glycerol on the oxidation rate of triglyceride was investigated using the proposed method (3).

EXPERIMENTAL PROCEDURE

Triglyceride

Tripalmitin, tristearin, triolein and trilinolein, 99% pure, were obtained from the Applied Science Laboratories, Inc. The mixed triglycerides used for oxidation experiments were prepared from these triglycerides by random interesterification.

Random Interesterification

The random interesterification of triglycerides was performed in a glass apparatus as shown in Figure 1.

Equivalent quantities of two kinds of triglycerides to be randomized and sodium methoxide catalyst were placed, respectively, in the vessel (a) and a small compartment (b) which was connected to the vessel through teflon stop cock. After evacuation with an aspirator, the vessel was immersed in an oil bath at 80 C and the random esterification was initiated by adding the catalyst to the triglyceride mixture in the vessel. During the reaction, the triglycerides and catalyst were stirred continuously with a magnetic stirrer. After desired reaction times, hot distilled water was introduced into the vessel through the small compartment to stop the reaction. The randomized triglycerides formed were extracted from the reaction mixture with chloroform and purified by silicic acid chromatography after Ando et al. (4).

The triglyceride composition of these randomized triglycerides was analyzed by HPLC and gas liquid chromatography (GLC). The enzymatic method was used for estimation of fatty acid esterified on the 2-position of glycerol.

HPLC

Randomized triglycerides were fractionated by HPLC to analyze triglyceride composition. The high performance liquid chromatograph used was a Shimadzu LC-3A equipped with a differential refractometer detector and stainless steel tubing (1 ft × 1/4 in. id) packed with μ-Bondapak C_{18}. Methanol/chloroform (7:3) was used as a solvent system. The column was kept at 30 C by means of a water jacket.

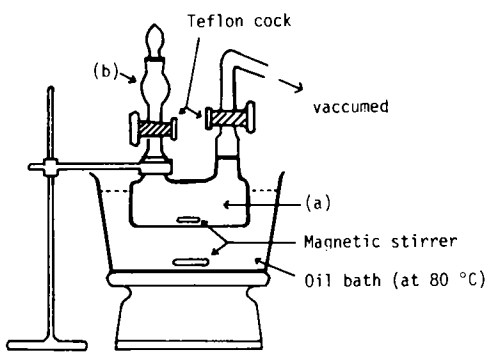

FIG. 1. Apparatus for triglyceride randomization. (a) Vessel; (b) compartment for catalyzer.

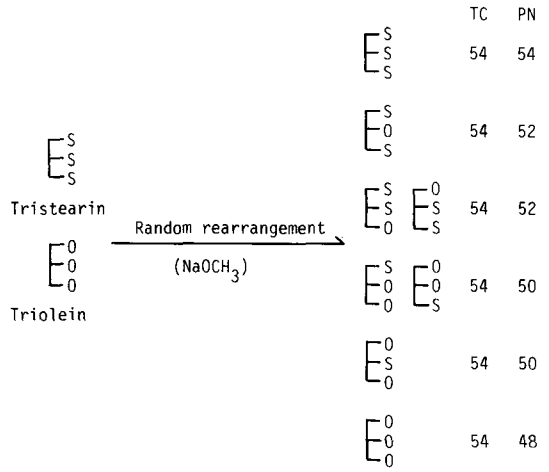

FIG. 2. Rearrangement of tristearin and triolein. TC: Total carbon number; PN: partition number.

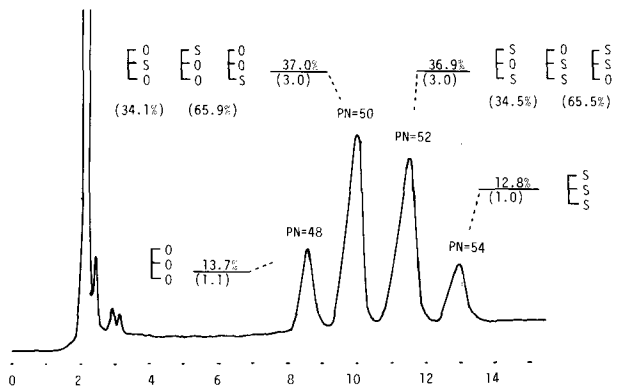

FIG. 3. HPLC chromatogram of randomized triglyceride (S-O). Column: μ-Bondapak C_{18}; eluent: methanol/chloroform (7:3); flow rate: 1.5 mL/min; detection: RI × 16. The figures on the chromatogram indicate PN and percentage (the ratio) of the triglyceride in each peak. The structures and percentages of the triglycerides are also shown above the peak on the chromatogram.

GLC

The gas chromatograph used for the triglyceride analysis was a Shimadzu GC-4BMPF with a flame ionization detector. The triglyceride composition, based on the total acyl carbon number (TC) of triglyceride, was determined by GLC under the following conditions: column, glass tubing (3 mm × 0.5 m) packed with 1% JXR on gas chrom Q (100–200 mesh); column temperature, 250–340 C programmed at 2 C/min. The fatty acid composition of the sample was also analyzed by a Shimadzu GC-6A gas chromatograph equipped with flame ionization detector and integrator of C-RIA. The conditions used were as follows: column, glass tubing (3 mm × 3 m) packed with 15% DEGS on chromsorb W (60–80 mesh); column temperature, 195 C. Methylation of fatty acid was done using BF_3/MeOH.

Enzymatic Hydrolysis

Enzymatic hydrolysis by pancreatic lipase (EC.3.1.1.3) obtained from Sigma Chemical Co. (type VI from porcine pancrease; 1,000,000 units) was conducted using a modification of Luddy's method. The procedure was described in detail in the previous paper (3).

Determination of Oxidation Rate of Triglyceride

A 40-mg portion of the sample triglyceride was put in a vial and the mouth was sealed tightly with a teflon/silicon septum by means of an aluminum ring. The vial was allowed to stand in an incubator at 50 C. At appropriate intervals, the oxygen in the head space of the vial was analyzed by GLC equipped with glass tubing (3 mm × 2 m) packed with molecular sieve 5A. A Shimadzu GC-3BT gas chromatograph equipped with a thermal conductivity detector was used. The oxidation rate of the triglyceride was calculated from the rate of decreasing amount of oxygen in the head space of the vial and expressed as oxygen absorption (mL) per gram of sample.

The decoloration of β-carotene when coupled with unsaturated triglyceride was measured by the method developed by Hammerschmidt (5).

RESULTS AND DISCUSSION

Preparation of Mixed Triglycerides by Random Interesterification

The random rearrangements of fatty acids in triglycerides have been explained theoretically (6). For example, when tristearin and triolein are randomized, six different types of triglycerides (excluding optical isomers) should be formed theoretically as shown in Figure 2. These six triglycerides formed are considered to be unresolved in GLC, because they have the same total carbon atoms of 54 in their acyl chains. However, it may be expected that these triglycerides are separated into four groups by HPLC, since the separation of triglycerides in reverse-phase HPLC depends on the partition number (PN), which is defined in the following equation: PN = TC − 2 × DB (number of double bond) and the PN of these six triglycerides are calculated to be 54, 52, 50 and 48.

The HPLC chromatograms of randomized triglycerides between tristearin and triolein at 80 C are shown in Figure 3. Four peaks were obtained as expected. The PN of these peaks were determined to be 54, 52, 50 and 48 from the linear relationship between PN and logarithm of retention times as mentioned in a previous paper (7). Tristearin and triolein, tripalmitin and trilinolein, and tristearin and trilinolein were randomized at 80 C until the reaction mixture changed to dark brown, indicating completion of the reaction. It usually required ca. 30 min. The HPLC chromatograms of the randomized triglycerides are shown in Figures 3–5. As shown in Figure 3, the randomized triglyceride (S-O) prepared from tristearin and triolein was composed of triolein (13.7%), 1,3-dioleo-2-stearin (12.6%; 37.0 × 0.341 = 12.6), 1(or 3)-monostearo-2,3(or 1,2)-diolein (24.4%; 37.0 × 0.659 = 24.4), 1,3-distearo-2-olein (12.7%; 36.9 × 0.345 = 12.7), 1(or 3)-monooleo-2,3(or 1,2)-stearin (24.2%; 36.9 × 0.655 = 24.2), and tristearin (12.8%). The components of randomized triglycerides (P-L) and (S-L) are also shown in Figures 4 and 5, respectively. The figure in parentheses in each chromatogram shows the ratio of triglycerides calculated from each peak areas in the HPLC chromatogram. These ratios of four types of triglycerides essentially coincided with the theoretical values (6). For instance, the ratio of triolein, dioleomonostearin, distearomonoolein, and tristearin was 1.1:3.0:3.0:1.0 as indicated in the chromatograms of Figure 3.

The peaks eluting just after the solvent peak were due to free fatty acids and other decomposed products of the triglycerides formed during the interesterification. These products were removed using silicic acid column chromatograhy.

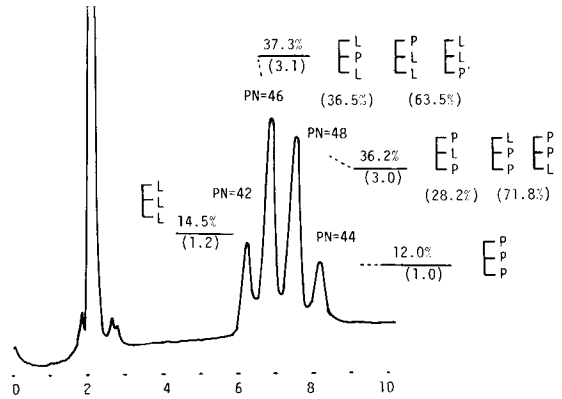

FIG. 4. HPLC chromatogram of randomized triglyceride (P-L). The designation of the figures is explained in Fig. 3.

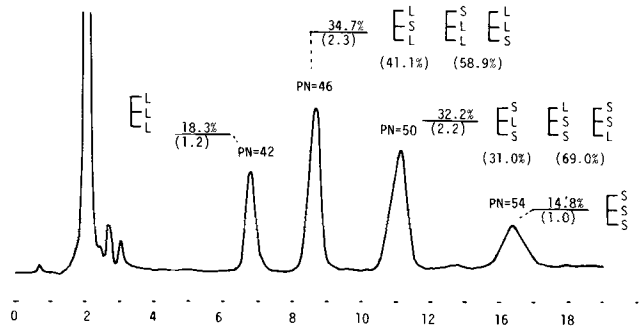

FIG. 5. HPLC chromatogram of randomized triglyceride (S-L). The designation of the figures is explained in Fig. 3.

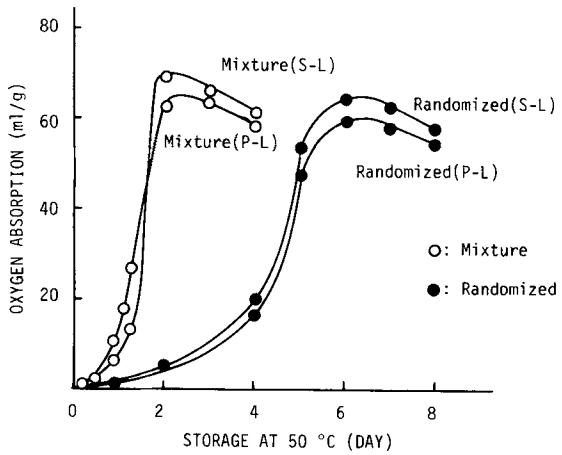

FIG. 6. Comparison of oxygen absorptions between triglyceride mixture and randomized triglyceride. (○) Triglyceride mixture; (●) randomized triglyceride.

Oxidation Rate of Triglyceride Prepared by Random Interesterification

Oxidation rates of the randomized triglycerides (S-L), (P-L), and (S-O) at 50 C were compared with those of the triglyceride mixtures which were prepared by mixing equivalent quantities of tristearin and triolein, tripalmitin and trilinolein, and tristearin and triolein, respectively. The rate of oxygen absorption of those samples are shown in Figures 6 and 7. The induction periods of the randomized triglycerides (S-L), (P-L), and (S-O) were longer than those of the mixtures of tristearin and trilinolein, tripalmitin and

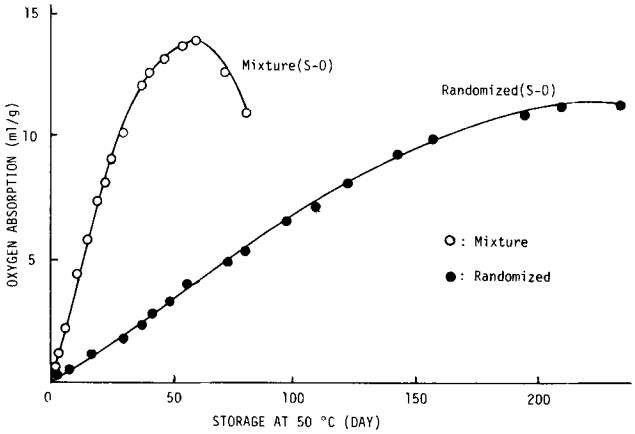

FIG. 7. Comparison of oxygen absorptions between triglyceride mixture (S-O) and randomized triglyceride (S-O). (○) Triglyceride mixture; (●) randomized triglyceride.

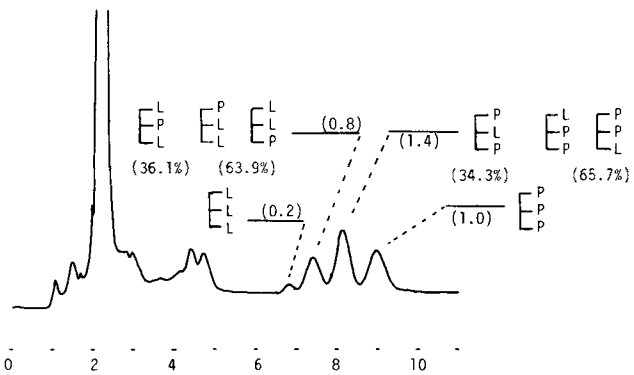

FIG. 8. HPLC chromatogram of randomized triglyceride (P-L) after 4 days oxidation at 50 C. The designation of the figure is explained in Fig. 3.

trilinolein, and tristearin and triolein, respectively. Thus, randomized triglycerides were more stable than the triglyceride mixtures, in spite of the fact that their fatty acid compositions are the same.

Influence of the Position of Unsaturated Fatty Acid Esterified Glycerol

To examine the influence of the position of unsaturated fatty acid esterified glycerol on the oxidation rate of triglyceride, the triglyceride compositions of samples at various stages of oxidation were analyzed by HPLC. For example, the HPLC chromatogram of randomized triglyceride (P-L) oxidized for 4 days is shown in Figure 8. The peaks ascribed as trilinolein almost disappeared, indicating that oxidative degradation of trilinolein and four types of diacid triglycerides (PLP), (LPP or PPL), (LPL), and (PLL or LLP) to tripalmitin were calculated. From these ratios, the percentages of triglycerides remaining were further calculated, provided that tripalmitin undergoes no oxidation throughout the period of oxidation experiment (Fig. 8). The percentages of (PLP) vs (LPP plus PPL) and (LPL) vs (PLL plus LLP) were 34.3%:65.7% and 36.1%:63.9%, respectively, from the lipase method. In this manner, the ratios and the percentages of various types of triglycerides remaining unoxidized were calculated and are shown in Tables I, II and III. From these results, it is clear that the oxidation rate of triglycerides depends on the degree of unsaturation.

TABLE I

Triglyceride (P-L) Remaining at the Two Different Stages of Oxidation

Day	0	4	5
O_2 abs (mL/g) triglyceride	0	31.0	47.2
PPP	100% (1.00)	100% (1.00)	100% (1.00)
PLP	100% (0.85)	56.5% (0.48)	11.8% (0.10)
LPP PPL	100% (2.15)	42.8% (0.92)	14.0% (0.30)
LPL	100% (1.13)	25.7% (0.29)	– (–)
PLL LLP	100% (1.97)	25.9% (0.51)	– (–)
LLL	100% (1.20)	16.7% (0.20)	– (–)

TABLE II

Triglyceride (S-L) Remaining at the Two Different Stages of Oxidation

Day	0	3	4
O_2 abs (mL/g) triglyceride	0	10.8	20.0
SSS	100% (1.00)	100% (1.00)	100% (1.00)
SLS	100% (0.68)	100% (0.68)	69.1% (0.47)
LSS SSL	100% (1.52)	99.3% (1.51)	67.8% (1.03)
LSL	100% (0.95)	72.6% (0.69)	40.0% (0.38)
SLL LLS	100% (1.35)	89.6% (1.21)	45.9% (0.40)
LLL	100% (1.20)	66.7% (0.80)	33.3% (0.40)

In the case of diacid triglycerides, the triglyceride (LPP or PPL) seemed to be oxidized more rapidly than the triglyceride (PLP) (Table I), in spite of the fact that their fatty acid compositions are the same. Similar results were obtained with the randomized triglycerides (S-L): the oxidation rate of the triglyceride (LSS or SSL) is higher than that of the triglyceride (SLS) (Table II). Furthermore, as shown in Table III, the oxidation rate of triglyceride (SSO or OSS) was higher than that of triglyceride (SOS). These results seem to indicate that the position of unsaturated fatty acids esterified in glycerol has an effect on the oxidation rate of triglyceride: the triglycerides having unsaturated fatty acids linked at the 2-position are more stable towards oxidation than those linked at the 1(or 3)-position.

Similar relationship between the oxidation rate and the position of unsaturated fatty acid in glycerol were found in the diacid triglycerides containing two molecules of unsaturated fatty acids (Tables I, II and III), though there were some exceptions in the advanced stages of the oxidation. These results supported the speculation of Raghuveer and Hammond (1) who concluded that the concentration of the unsaturated fatty acids on the 2-position of glycerol should stabilize a fat towards autoxidation.

TABLE III

Triglyceride (S-O) Remaining at the Two Different Stages of Oxidation

Day	0	74	111
O_2 abs (mL/g) triglyceride	0	4.9	7.0
SSS	100% (1.00)	100% (1.00)	100% (1.00)
SDS	100% (1.03)	98.1% (1.01)	74.8% (0.77)
SSO OSS	100% (1.95)	91.3% (1.78)	70.8% (1.38)
OSO	100% (1.02)	72.6% (0.74)	46.1% (0.47)
SOO OOS	100% (1.96)	79.6% (1.56)	43.9% (0.89)
OOO	100% (1.11)	55.9% (0.62)	28.8% (0.32)

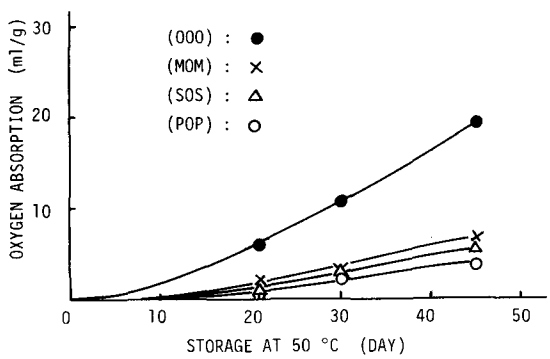

FIG. 9. Comparisons of oxygen absorptions of four types of triglycerides at 50 C.

Influence of the Carbon Chain Length of Saturated Fatty Acids

Wu et al. (8) demonstrated that the oxidation rate of unsaturated fatty acids were affected by the coexistence of saturated fatty acids. Therefore, the influence of the carbon chain length of the saturated fatty acid on the oxidation rate of triglyceride was examined using the triglycerides (MOM), (POP) and (SOS).

The rates of oxygen absorption of triglycerides (MOM), (POP) and (SOS) were determined during standing at 50 C and the results obtained are shown in Figure 9. Only slight differences in the rate of oxygen absorption were found among these triglycerides. These slight differences were judged as no effective disparity on the oxidation by repeated experiments. In the early stage of oxidation, the rate of oxidation of triglycerides were also determined by the method of Hammerschmidt. The results obtained are shown in Figure 10. There was essentially no difference in the rate of oxidation among these triglycerides.

From these results, it is concluded that even if myristic, palmitic and stearic acids esterified on the 1- and 3-positions of glycerol in triglycerides affect the oxidation rate of unsaturated fatty acid esterified on the 2-position,

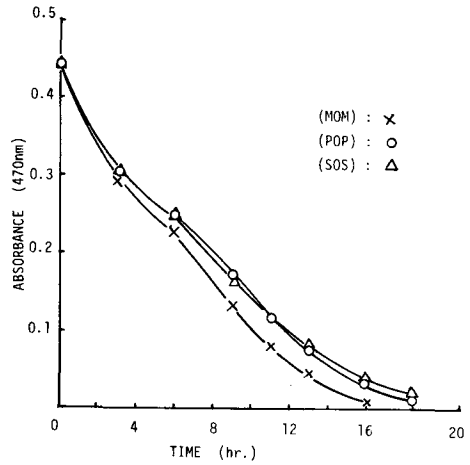

FIG. 10. Oxidation rates of triglyceride in the early stages of oxidation as measured by β-carotene decoloration.

the influences ascribed to the difference in carbon chain lengths are essentially the same.

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Hydrogen Bromide Titration for Soaps in Fat Products

Z.M. ZARINS, J.L. WHITE[1] and R.O. FEUGE[1], Southern Regional Research Center, US Department of Agriculture, New Orleans, LA 70179

ABSTRACT

Since none of the existing methods for determining soaps in fat products have been found to be entirely satisfactory, a method has been devised for the determination of alkali metal soaps by direct titration with Durbetaki reagent (hydrogen bromide dissolved in glacial acetic acid). When the titration was conducted at room temperature in acetic acid-benzene solution with crystal violet as indicator, soaps of potassium, sodium and lithium could be determined accurately in anhydrous oils, monoglycerides, and sucrose esters. The presence of alcohols, glycerol and sucrose did not interfere in the direct titration. However, oxidized oils, epoxides, and cyclopropenoid acids, which are known to consume hydrogen bromide, did interfere. Products containing the interfering substances could be analyzed by a modified procedure in which the alkali metal cations were extracted from a mixture of amyl acetate and *n*-butanol (1:3) into an aqueous solution of acetic acid, and titrated as the acetates.

INTRODUCTION

A number of methods have been proposed for the quantitative determination of soaps in fats and oils, the main emphasis being on soaps in refined oils. The oldest methods depend on ashing the sample (1-3). Others depend on the titration of the free fatty acids liberated when soapy oils are acidulated (4). A method enabling the spectrophotometric determination of calcium complexes of soaps has also been developed (5).

[1]Retired.

There are two official AOCS methods (6) for the determination of soap in oil: one measures the conductivity of soapy water obtained on washing the oil sample, the other is a titrimetric method that determines the alkalinity of the sample by an aqueous hydrochloric acid titration of the sample dissolved in acetone containing 2% water. The quantitative determination of soaps in fat products is difficult and so far no one method has been found universally acceptable.

In our experimental work with such fat derivatives as sucrose esters, we required a relatively simple method capable of measuring a wide range of soap concentrations. Haeberer and Maerker (7) demonstrated that the Durbetaki (8) reagent can be used to determine the purity of soaps.

The principle on which our method of titrating for soaps relies is based on a study of Kolthoff and Willman (9), who noted that electrolytes which are neutral in water may not be neutral when dissolved in glacial acetic acid. Differences in acidic and basic properties become quite pronounced. The alkali metal acetates, for example, are strongly basic in glacial acetic acid solution, and can be readily titrated. This basicity increases in the order Li < Na < K (9).

MATERIALS AND METHODS

Apparatus and Technique

The apparatus and technique employed in titrating for soap content are similar to those reported earlier (10). The