Figure 3 also shows two values of oleic hydrogenation rate coefficients which have been taken from the investigations of Swicklik and co-workers (4) (Table I) on the assumption that the first three results in Hydrogenation No. 3 are also due to an initial period. The corresponding experiments were carried out with 0.4% Ni, a hydrogen pressure of 65 lbs., vigorous agitation, and high hydrogen dispersion. Even though these conditions are quite different from ours, the results nevertheless show the same activation energy with a marked change in the frequency factor.

The final periods are independent of the catalyst only when oleic hydrogenations are examined, in which case they cannot be correlated with any common acid percentage. Hydrogenations of the linoleic components follow different patterns, depending on the state of the nickel. While, when hydrogenating with 0.25% fresh Ni, first-order kinetics can be observed down to linoleic percentages below 5%, the corresponding hydrogenations with 1% self-poisoned Ni seem to stop at about 10% linoleic acid. The experiments illustrated in Figure 4 were made in order to examine the stagnation of the linoleic hydrogenations. They were divided into five stages. (A) Sesame oil was hydrogenated with 1% self-poisoned Ni at 175°C. for 5 hrs. (B) What was left of the oil from stage (A) when samples had been drawn was doubled up with a fresh portion of sesame oil and hydrogenated for another 5 hrs. (C) A new portion of sesame oil was hydrogenated with 1% self-poisoned Ni at 175°C. for 6 hrs. After removal of the catalyst by filtration, what was left of this oil was divided into two equally large parts: (D) one part was hydrogenated in the same way as in stage (C) with a new portion of self-poisoned catalyst; (E) the other part was hydrogenated with 0.25% fresh Ni. On the basis of Figure 4 we draw the following conclusion. The decreasing hydrogenation rate coefficients cannot be caused by progressive catalyst poisoning since, after

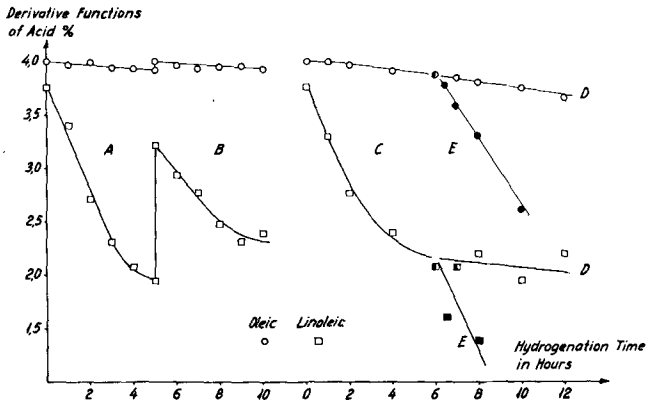

FIG. 4. Various hydrogenations to investigate the linoleic stagnation. (A) Hydrogenation with 1.0% self-poisoned Ni, 175°C.; (B) A continued after addition of fresh oil; (C) hydrogenation under the same conditions as A; (D) C continued after renewal of the 1.0% self-poisoned Ni; (E) C continued after exchange of the self-poisoned catalyst with 0.25% normal Ni.

an addition of fresh oil, the hydrogenation resumes what would have been its original rate, the reduced catalyst concentration taken into consideration. Once the final linoleic period has begun, the hydrogenation as a whole can best be characterized by assuming that the catalyst can no longer distinguish between linoleic and oleic components, and this applies whether fresh or self-poisoned nickel is used for further hydrogenations. We believe that these results are due to an isomerization of the linoleic acid.

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A Critical Study of the Oxidation Methods for the Determination of Glyceride Composition of Fats[1,2]

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THE OXIDATION OF FATS with permanganate in acetone was proposed by Hilditch and Lea (9,15) for the estimation of trisaturated glycerides (GS_3).[4] Kartha (21–23) carried out this oxidation in the presence of excess (3–6%) acetic acid and developed a method for the determination of the glyceride type of composition of natural fats by separating magnesium salts of azelao-glycerides. The results obtained for the glyceride type of composition of many natural fats by Kartha's method are different from those obtained by Hilditch's crystallization method (12), and the theories put forward by these two investigators (13,24) for the glyceride distribution in fats on the basis of these results also differ widely (10,11,14,24,28,36,41,42). Both the oxidation methods of Hilditch and Lea and Kartha are based on the assumptions that the unsaturated acids in the unsaturated glycerides (GS_2U, GSU_2, and GU_3) are cleaved and that the resultant azelao-glycerides are not hydrolyzed during the oxidation and the subsequent separation procedures.

[1] A portion of the Ph.D. (Technology) thesis submitted to the University of Bombay by G. Lakshminarayana.
[2] Presented at the 32nd fall meeting, American Oil Chemists' Society, Chicago, Ill., October 20–22, 1958.
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[4] Abbreviations used are as follows: G, glyceryl radical; S, saturated acid or radical containing 16 or more carbon atoms; U, unsaturated acid radical; O, oleic acid radical; L, linoleic acid radical; A, azelaic acid radical; and S°, oxygenated saturated acid radical (incomplete oxidation product of oleic acid).

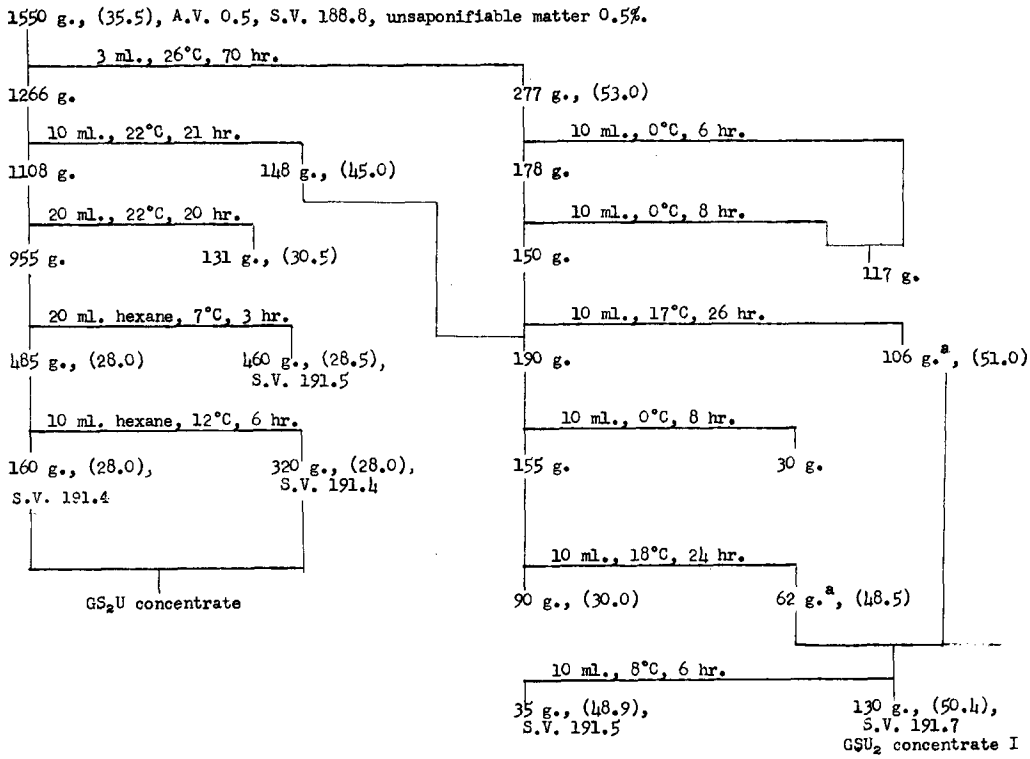

FIG. 1. Crystallization of GS_2U and GSU_2 concentrates from *G. indica* fat

Key for Figures 1 and 2: Volume of solvent (acetone unless otherwise stated) is expressed in milliliters per gram of glycerides. The figure in parentheses represents I.V. [a] Recrystallization under same conditions gave no precipitate showing absence of GS_3 (25).

The oxidation of oleic acid with aqueous permanganate results in the formation of cleavage products, α-diols, α-ketols, and other intermediate compounds (3,6,16,30,31,34,35,44). Armstrong and Hilditch (2) reported the presence of saturated esters in the permanganate oxidation products of alkyl esters of oleic acid in acetone and in acetic acid. These esters had a high saponification value and were cleaved on re-oxidation to pelargonic acid and half-ester of azelaic acid. The formation of an acetyl derivative of the hydroxy compound produced during the oxidation of oleic acid esters in acetic acid by hydrogen peroxide or air has also been reported (16,20,31). It is probable therefore that some of the intermediate oxidation products formed during the acetone-permanganate oxidation (alkaline) (23) and acetone-acetic acid-permanganate oxidation (acidic) (23) may be present as such, without being cleaved, in the oxidized fat even at the end of the oxidation. These products will contain oxygenated saturated fatty acids derived from the unsaturated acids. For future reference these products will be referred to as "incompletely oxidized glycerides" (*e.g.*, GS_2S^o).

Hilditch and Saletore (19) failed to isolate azelaoglycerides quantitatively because of hydrolysis during acetone-permanganate oxidation and purification. Although Kartha (21–23) showed that GS_2A do not hydrolyze in his oxidation by oxidizing a sample of GS_2U, he did not investigate the hydrolysis of GSA_2 in a similar manner.

A critical evaluation of the individual steps in the oxidation methods of Hilditch (15,18) and Kartha (23) was therefore undertaken in order to examine the limitations of these methods by investigating the completeness of oxidation and absence of azelao-glyceride hydrolysis. Earlier investigations (15,18,22,36) were carried out chiefly on natural fats of unknown composition. However in this investigation concentrates of GS_2U and GSU_2, prepared by intensive crystallization of fats, were used so that the results would be conclusive. During the course of this investigation a chromatographic procedure for the determination of GS_3 was standardized to overcome the limitations of Hilditch and Lea's method.

Experimental

Methods of Analysis. The methods of the A.O.C.S. (1) for acid (A.V.), saponification (S.V.), and iodine (I.V.) values were carried out on a semi-micro scale. The dihydroxystearic acid content was determined by the method of Sreenivasan *et al.* (38) except that chloroform was added before the reaction with periodic acid. Unsaponifiable matter was estimated by the pro-

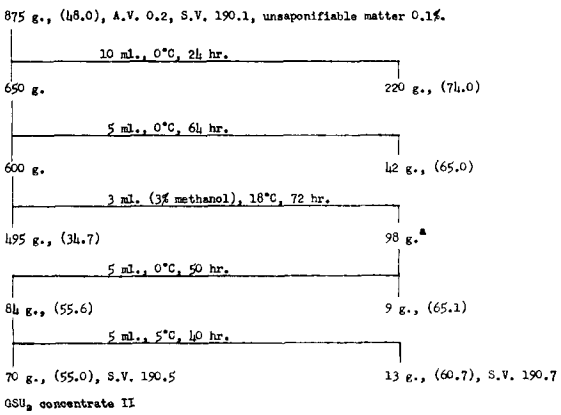

FIG. 2. Crystallization of GSU_2 concentrate from *G. morella* fat.

TABLE I
Composition of Glyceride Concentrates [a]

Glyceride concentrate	Source	Analytical constants		Fatty acids (wt. %)			Glycerides (wt. %)			
		I.V.	S.V.	S	O	L[b]	GS_3	GS_2O	GS_2L	GSO_2
GS_2U	*G. indica*	28.0	191.4	64.5	31.2	0.0	2.8	97.2	0.0	0.0
GSU_2 I	*G. indica*	50.4	191.7	40.4	54.5	0.8	0.0	25.4	2.4	72.2
GSU_2 II	*G. morella*	55.0	190.5	40.9	48.6	6.3	0.0	8.5	19.7	71.8

[a] The concentrates contained no free acids or unsaponifiable matter and were protected against oxidation with propyl gallate.
[b] No other polyunsaturated acid was found.

TABLE II
Oxidation of Glyceride Concentrates

Experiment No.[a]	Method of oxidation [b]	Glyceride concentrate (conc.)	Weight of conc.	Acetone	$KMnO_4$	Oxidized conc.	
						Yield	I.V.
			g.	*ml./g. conc.*	*g./g. conc.*	%	
1	Hilditch (15)	GS_2U	46.00	10	7	96.3	0.2
2	Hilditch	GS_2U	52.17	10	7	95.9	0.2
3	Hilditch [c]	GS_2U	22.13	20	6	78.3 [e]	0.2
4	Hilditch	GS_2U	48.31	20	7	68.3 [f]	...
5	Hilditch	GSU_2 II	20.61	20	9	86.6	1.0
6	Kartha [d] (23)	GS_2U	52.46	40	10	103.0	0.5
7	Kartha	GS_2U	10.16	40	10	88.3 [e]	0.3
8	Kartha	GSU_2 I	14.35	40	16	64.5 [e]	0.6
9	Kartha	GSU_2 II	12.00	40	16	67.9 [e]	0.3

[a] Same identification is used in the remaining tables.
[b] Duration of the oxidation: 33 hr. in Experiment 4 and 10–12 hr. in the remainder.
[c] $KMnO_4$ was mixed with anhydrous $MgSO_4$ (50 g.) (15).
[d] Acetic acid concentration varied from 3.0% to 3.6%.
[e] Insoluble azelao-glycerides obtained after Kartha's magnesium salt separation.
[f] Regenerated glycerides after $KHCO_3$ washing.

TABLE III
Estimation of GS_3 by Carbonate Washing Procedure

Experiment No.	Glyceride conc.	Oxidized conc.[a]	Crude GS_3			A.V. of last carbonate extracts (×)	GS_3[b] in conc.	
			Weight	I.V.	A.V.		Assuming (×)	Assuming GS_2A
		g.	*g.*				%	%
1	GS_2U	4.285	0.4051	1.7	42.9	.....	....	3.1
2	GS_2U	10.860	1.3230	0.7	18.1	90.2	9.0	8.4
3	GS_2U	5.585	0.5621	1.8	18.7	107.6	6.0	5.3
4	GS_2U	19.380	1.4890	1.0	19.1	114.8	4.2	3.7
5	GSU_2 II	4.714	0.4260	7.0	64.4	140.7	2.3	Negative
6	GS_2U	9.118	1.8680	1.1	37.3	.....	....	9.0

[a] Hilditch's oxidation in Experiments 1–5 and Kartha's oxidation in Experiment 6.
[b] Actual GS_3 content in concentrates: 2.8% in GS_2U and nil in GSU_2 II (Table I).

cedure of the Society of Public Analysts (5). Polyunsaturated acids were determined by the method of Brice *et al.* (4).

MATERIALS

Glyceride-Type Concentrates. Systematic fractional crystallization of refined *Garcinia indica* (43) and *Garcinia morella* (7) fats was carried out separately to obtain GS_2U and GSU_2 concentrates (Figures 1 and 2). These fats were chosen because the fatty acids are predominantly C_{18}, and the amounts of GS_3, GU_3, and linoleo-glycerides are small. The composition of each concentrate was calculated from its I.V., S.V., and linoleic acid content (Table I). It was assumed (22,25) that the GS_2U concentrate consisted of GS_3 and GS_2O and the GSU_2 concentrates GS_2O, GS_2L, and GSO_2 only.

GS_2A. The insoluble magnesium salts obtained from the GS_2U concentrate by Kartha's method (23) were washed with alcoholic ether. The regenerated GS_2A were further purified (9) by successive crystallizations from 10 volumes of ether (0°C.), acetone (0°C.), 90% ethanol (20°C.), and hexane (20°C.) (found—A.V. 70.4, S.V. 287.4; theoretical—A.V. 71.4, S.V. 285.5).

OXIDATION OF CONCENTRATES

The concentrates of GS_2U and GSU_2 were oxidized by the methods of Hilditch and Lea (15) and Kartha (23) (Table II).

ESTIMATION OF GS_3 BY CARBONATE WASHING PROCEDURE

An ether solution (300 ml.) of the pure GS_2A (1.563 g.) was washed with 10% aqueous K_2CO_3 solution (4 × 200 ml.) and water alternately. The GS_2A were recovered quantitatively from the ether and aqueous extracts with no appreciable change in characteristics (ether extract—1.238 g., A.V. 70.4, S.V. 286.8; aqueous extracts—0.31 g., A.V. 71.1), thereby showing the absence of hydrolysis.

The oxidized concentrates were subjected to the carbonate washing procedure (15) to determine the GS_3 contents (Table III). The unoxidized glycerides present in the crude GS_3 were calculated as GS_2O. The acidic substances were assumed to be GS_2A in one case, and in the other case the A.V. of the material extracted by the last two carbonate and water washes was used.

DETERMINATION OF GS_3 BY CHROMATOGRAPHIC PROCEDURE

Standardization of Chromatographic Procedure. A preliminary investigation of a chromatographic procedure for the determination of GS_3 was reported earlier (33). Merck's alkaline alumina (No. 1097) was heated at 300°C. for 1 hr. Hexane was found to be unsuitable as a solvent because of hydrolysis of triglycerides in the column. However columns packed wet with hexane and washed with ether (6 ml./g. of alumina) were found to be satisfactory. Chloroform

TABLE IV

Determination of GS_3 by Chromatographic Procedure

Experiment No.[a]	Glyceride conc.	Oxidized conc.[b]	Eluant vol.[d]	Eluted glycerides[c]		GS_3[e] in conc.	S.V.	
				Weight	I.V.		Eluted glycerides	GS_2S^o+ GSS_2^o
		mg.	l.	mg.		%		
1a	GS_2U	504	0.5	25.5	1.9	4.6	....	....
1b	GS_2U	2591	0.7	138.6	1.7	4.8	....	....
2	GS_2U	5218	1.0	300.8	2.2	5.1	201	214
3	GS_2U	4543	1.0	299.3	2.9	4.6	200	214
4	GS_2U	4749	1.6	374.8	1.6	5.1	201	214
5	GSU_2 II	4353	1.4	251.8	3.9	4.3	256	266
6a	GS_2U	6036	2.2	860.4	1.6	13.8	227	239
6b	GS_2U	4461	0.7	687.9	1.6	15.0	230	241
6c	GS_2U	4721	2.1	775.9	1.5	16.0	223	232
7	GS_2U	3366	0.8	329.5	1.8	8.1	228	250
8	GSU_2 I	584	0.3	59.3	7.0	5.0	....	....
9	GSU_2 II	687	0.3	99.1	2.8	8.8	230	234

[a] Letters after numbers represent different runs of the same sample.
[b] Hilditch's oxidation in Experiments 1–5 and Kartha's oxidation in Experiments 6–9.
[c] A.V. = 0.0.
[d] Ether in Experiments 1a and 6a and chloroform in the remainder. Column dimensions: 1.85 × 12 cm. in Experiments 1a, 8 and 9; 3.1 × 26 cm. in the remainder.
[e] Actual GS_3 content in concentrates: 2.8% in GS_2U and nil in GSU_2 concentrates (Table I).

was found to be an excellent solvent for packing as well as elution (32). Two columns (1.85 × 12 cm., 30 g. of alumina, and 3.1 × 26 cm., 180 g. of alumina) were standardized, using mixtures of GS_2A and hydrogenated *G. indica* fat (I.V. 0.0, A.V. 0.0). The optimum volume of solvent for the quantitative elution of neutral triglycerides was found to be 300 ml. of chloroform or 500 ml. of ether for the 12-cm. column and 2.2 liters of either solvent for the 26-cm. column. The recovery of neutral triglycerides was 97 to 99% (32).

Determination of GS_3 in Concentrates. The oxidized concentrates were chromatographed to determine the GS_3 contents (Table IV). Optimum volume of the eluant or less than the optimum amount was used to be sure that the eluted neutral triglycerides were free of other compounds. The GS_3 content of the concentrates was obtained by deducting the percentage of unoxidized glycerides (GS_2O) from the percentage of eluted glycerides. Higher values for GS_3 content than the actual indicate the presence of incompletely oxidized glycerides (GS_2S^o, GSS_2^o). The S.V. of the latter was calculated (Table IV) from the percentages of true GS_3 and unoxidized GS_2O, and the S.V. of these (theoretical) and the eluted glycerides (experimental).

STRUCTURE OF INCOMPLETELY OXIDIZED FATTY ACID

Determination and Analysis of Saturated Acids. The oxidized concentrates were hydrolyzed and subjected to Bertram separation twice according to the modifications of Kartha (3,26,37) to determine the S content (Table V). Even though the GS_3 contents of the concentrates as determined by the oxidation-chromatographic procedure were higher than the theoretical contents (Table IV), the S contents were not higher than the theoretical (Table V). To demonstrate that saturated acids and dihydroxystearic acid are not lost as soluble magnesium soaps, prepared mixtures of these acids with azelaic acid were subjected to the Bertram separation twice. The stearic and dihydroxystearic acids, free of azelaic acid, were recovered quantitatively (32). However the dihydroxystearic acid content of the saturated acids from the oxidized concentrates was negligible (Hilditch's oxidation, 0.3%; Kartha's oxidation, 0.1% on the basis of concentrates).

Analysis of the Eluted Glycerides. The percentage yields of acids from the eluted glycerides (Table IV) were lower than the expected yields for a mixture of GS_3 and GS_2O (found, 81–93%; expected, *ca.* 96%). For example, the percentage yield of acids from the eluted glycerides in Experiment 6 was 80.7% before Bertram separation and 69.8% after Bertram separation. The S.V. of the same acids was 226.5 before Bertram separation and 198.0 after Bertram separation (expected, 200.1). The α-glycol contents of the eluted glycerides and of the derived fatty acids were very low (Hilditch's oxidation: fatty acids, 0.2%; Kartha's oxidation: eluted glycerides, 0.3%, fatty acids, 0.3%, on the basis of concentrates).

HYDROLYSIS OF AZELAO-GLYCERIDES

The pure GS_2A (1.050 g.) were subjected to the magnesium salt separation procedure of Kartha and

TABLE V

Determination of Saturated Acids by Bertram Separation

Experiment No.[a]	Glyceride conc.	S from IAG[b]					S from SAG[b]		Total S in conc.[f]
		IAG	IBA[b]			S in conc.	IBA yield	S in conc.	
			Yield	I.V.	S.V.				
		mg.	mg.			%	mg.	%	%
2	GS_2U	2123[c]	1392	0.2	200.2	62.7	....	....	62.7
3	GS_2U	3064	2447	0.3	202.1	62.4	Nil	Nil	62.4
5	GSU_2 II	3871[c]	1738	0.2	204.0	38.8	....	....	38.8
7	GS_2U	3144	2300	0.5	199.5	64.3	Nil	Nil	64.3
8	GSU_2 I	1948	1186	1.3	203.6	38.7	251.6[d, e]	1.7	40.4
9	GSU_2 II	2715	1516	0.7	201.9	37.6	378.3[d]	3.2	40.8

[a] Hilditch's oxidation in Experiments 2, 3, and 5, and Kartha's oxidation in Experiments 7–9.
[b] IAG, insoluble azelao-glycerides; SAG, soluble azelao-glycerides; IBA, insoluble Bertram acids.
[c] Total oxidized concentrate (Table II) before magnesium salt separation.
[d] From the total concentrate oxidized (Table II).
[e] S.V. 215.2.
[f] Actual S content in concentrates: 64.5% in GS_2U, 40.4% in GSU_2 I, and 40.9% in GSU_2 II (Table I).

TABLE VI

Insoluble Azelao-Glycerides[a]: Yields, Analytical Values and Saturated Acid Contents

Experiment No.[b]	Glyceride conc.	Yield, %		A.V.		S.V.		S on conc. basis, %	
		F[c]	T[c]	F	T	F	T	F	T
2	GS_2U	80.3	89.7	107.3	68.9	276.0	282.0	62.1	64.5
3	GS_2U	78.3	89.7	118.5	68.7	275.2	281.9	62.4	64.5
6	GS_2U	91.3	89.9	62.7	67.3	277.7	280.1	....	....
7	GS_2U	88.3	89.7	73.3	68.5	280.0	281.7	64.3	64.5
8	GSU_2 I	64.5	77.2	134.2	131.2 106.2[d]	337.0	365.2 333.1[d]	38.7	38.7
9	GSU_2 II	67.8	74.0	141.0	129.4 118.5[d]	369.7	361.1 348.1[d]	37.6	37.7

[a] I.V. = 0.2 in Experiment 2 and 0.7 in Experiment 6. Others are given in Table II.
[b] Hilditch's oxidation in Experiments 2 and 3 and Kartha's oxidation in Experiments 6–9.
[c] F, found; T, theoretical.
[d] Calculated from the composition of the insoluble azelao-glycerides as determined by Kartha's method (Table VII).

were recovered quantitatively (1.048 g.) from the insoluble magnesium salts without hydrolysis (A.V. 70.2, S.V. 287.0).

The oxidized concentrates were subjected to Kartha's magnesium salt separation. The percentage yield, percentage S content (on the basis of the concentrate), A.V. and S.V. of the insoluble azelao-glycerides were determined and compared with the theoretical values (Table VI). The theoretical percentages of the yield and the S content in the case of GS_2U concentrate were calculated on the assumption that the GS_2U (after deducting the unoxidized GS_2O) were oxidized to GS_2A alone. The theoretical A.V. and S.V. were calculated from the percentages of the expected constituents of the insoluble azelao-glycerides, *viz.*, GS_3, GS_2A (theoretical), and unoxidized GS_2O (experimental) and the theoretical A.V. and S.V. of the same (Table I). In the case of GSU_2 concentrates, the theoretical percentage yield was obtained by adding the percentage unoxidized GS_2O (calculated from the I.V. of the saturated acids isolated), percentage GS_2A derived from the remainder of the GS_2U, and the percentage GSA_2 derived from the remainder of the GSU_2 after deducting the percentage of GSU_2 present in the soluble azelao-glycerides as GSA_2-magnesium salt (Tables I and VII). The theoretical A.V. and S.V. of the insoluble azelao-glycerides were calculated from the theoretical percentages of GS_2A and GSA_2 expected, the percentage unoxidized GS_2O, and the A.V. and S.V. of the same. The expected A.V. and S.V. of the insoluble azelao-glycerides were also calculated (Table VI) from the composition obtained by Kartha's method (Table VII).

COMPOSITION OF GSU_2 CONCENTRATED BY KARTHA'S METHOD

The glyceride-type composition of the GSU_2 concentrates was determined by the method of Kartha (23) (Table VII).

Discussion

PERMANGANATE OXIDATION OF MIXED SATURATED-UNSATURATED GLYCERIDES

Incomplete Oxidation of Unsaturated Acid. The GS_3 content of the GS_2U and GSU_2 concentrates as determined by the acetone-permanganate oxidation and chromatographic procedure is distinctly higher (2–4%, Table IV) than that present in the concentrate (Table I). This increase is larger when the oxidation is carried out in the presence of acetic acid (5–13%, Table IV). These results demonstrate that the oleic acid in a part of the unsaturated glycerides is not cleaved but gives an incomplete oxidation product having saturated acid characteristics. While this paper was in preparation, Eshelman and Hammond (8) also reported the formation of such products on oxidation of methyl oleate by Kartha's method.

Structure of the Incompletely Oxidized Fatty Acid. Epoxy (40) or dihydroxystearic (30) acids or the acetyl derivative of the latter are not the main products of incomplete oxidation, as is shown by the low α-glycol values of the saturated acids, the eluted glycerides, and the fatty acids of these glycerides. Furthermore the S contents of the GS_2U and GSU_2 concentrates as determined by Kartha's oxidation and Bertram separation procedure (Table V, Experiments 7–9) are the same as those determined independently of oxidation (Table I). If epoxy- (39) or dihydroxystearic acids are present, then the S content of the concentrates would be higher.

The above evidence therefore suggests that the incompletely oxidized acids are fissioned during saponification to lower acids which are lost during the Bertram separation. 9,10-Ketohydroxystearic acids are obtained by oxidation of oleic acid under controlled conditions (6,30,35,44). These α-ketols are split on saponification with alcoholic potassium hydroxide to give pelargonic and azelaic acids (17) and thus have a high S.V. The S.V. of the eluted glycerides and those calculated for glycerides containing incompletely oxidized acid (GS_2S^o, GSS_2^o) are higher (Table IV) than those of GS_2O (191.3) or GS_3 (191.9). The S.V. of the acids (226.5) of the eluted glycerides is higher than the value expected (200.1) for the mixed fatty acids of GS_2O and GS_3 (Experiment 6, Table IV). The presence of lower acids (*e.g.*, pelargonic and azelaic) in the recovered acids is thus indicated. The S.V. of the same acids is reduced to 198.0 by Bertram separation, thereby suggesting that these lower acids are lost during the Bertram separation. Furthermore the yield of the insoluble Bertram acids agrees fairly well with the yield calculated on the assumption that the 9,10-ketoacetoxystearic acid is lost during saponification and Bertram separation (found, 69.8%; calculated, 67.2%, on the basis of eluted glycerides).

The consumption of periodic acid by the eluted glycerides, isolated from the oxidized concentrates (Kartha's oxidation), was negligible, showing the absence of α-ketol (30). This suggests that the eluted glycerides contained acetylated α-ketols which do not seem to be cleaved readily by either periodic acid or permanganate. This could be the cause for the higher GS_3 contents obtained by Kartha's oxidation than by Hilditch's oxidation.

HYDROLYSIS OF GS_2A

Hilditch's Oxidation. The results in Table VI (Experiments 2 and 3) show definite evidence for hy-

drolysis of GS_2A to saturated acids in Hilditch's oxidation. The addition of magnesium sulfate (15) does not prevent the hydrolysis. These results do not support the opinion of earlier investigators (15,23) that the saturated fatty acid-glyceryl ester linkage in GS_2A and GSA_2 is stable during the oxidation.

Kartha's Oxidation. Results given in Table VI (Experiments 6 and 7) show that hydrolysis of GS_2A occurs to a very slight extent even in Kartha's oxidation. It has been shown that GS_2S^o increase the GS_3 content and therefore should also increase the yield of the insoluble azelao-glycerides. As the yields of the insoluble azelao-glycerides obtained from the GS_2U concentrate are almost the same as those calculated on the basis of complete oxidation, the percentage of hydrolysis is very small.

HYDROLYSIS OF GSA_2

Kartha's Oxidation. No attempt was made to study the hydrolysis of GSA_2 during Hilditch's oxidation as these are more susceptible to hydrolysis than GS_2A (19) which were shown to hydrolyze considerably.

Appreciable hydrolysis of the azelao-glycerides, obtained from GSU_2 concentrates by Kartha's oxidation, was found to take place during the oxidation and/or subsequent working procedure. The experimentally determined yields of insoluble azelao-glycerides (Table VI, Experiments 8 and 9) are lower than the theoretical. The acid values of the same are higher than the theoretical, calculated either from the expected composition or from the composition obtained by Kartha's method. As the GS_2A hydrolyze to a negligible extent, the difference in values suggests that the GSA_2, derived from GSU_2 in the concentrate, hydrolyze to an appreciable extent. Monoglycerides are the expected hydrolysis products of GSA_2; however the 1-monoglyceride content of the insoluble azelao-glycerides was found to be low (0.5% on the basis of concentrate). This proves that the monoglycerides are further hydrolyzed to free saturated acids. The percentages of GSU_2 hydrolyzed in GSU_2 I and GSU_2 II, calculated on the assumption that the loss in yield of the insoluble azelao-glycerides is entirely due to the loss of azelaic acid groups and glycerol from the GSA_2 molecules, were 37.1 and 18.2, respectively, on the basis of total GSU_2 present in the concentrates.

DETERMINATION OF SATURATED ACIDS

The values obtained in this investigation for the S contents of the GS_2U and GSU_2 concentrates by Hilditch's oxidation (15,18) are slightly lower (Table V, Experiments 2, 3, and 5) than the actual because of the chain degradation of free higher saturated acids (32). However the S content of the concentrates is accurately determined by Kartha's oxidation and Bertram separation (23,29) (Table V, Experiments 7–9).

ESTIMATION OF GS_3

Limitations of Hilditch and Lea's Method. There is definite evidence to show that GS_2A hydrolyze during Hilditch's oxidation. The high acid values of the material extracted by the last carbonate and water washes and also the inconsistent values for the percentage GS_3 calculated on the two assumptions, (Table III, Experiments 1–5) suggest definite hydrolysis of GS_2A during the oxidation and/or carbonate washing. But the GS_2A do not hydrolyze during the carbonate washing procedure, as proved by subjecting GS_2A to this procedure. The hydrolysis of GS_2A must have therefore taken place during the oxidation itself contrary to the opinion of Hilditch (10). However the error in the estimation of GS_3 is not as large as expected from the extent of hydrolysis of GS_2A to diglycerides because most of the latter are further hydrolyzed to saturated acids which are washed out by the carbonate solution. Further the error is more when GS_2U are more than GSU_2 in the concentrate because the monoglycerides derived from GSA_2 are hydrolyzed more readily than the diglycerides. The replacement of Hilditch's oxidation by Kartha's oxidation is not helpful because of the formation of incompletely oxidized glycerides (GS_2S^o, GSS_2^o) to a greater extent in the latter than in the former (Table III, Experiment 6).

Oxidation-Adsorption Method. The chromatographic separation procedure developed for the purpose of the present study gave a slightly higher value (by about 2%) than the actual value for the GS_3 content of the GS_2U concentrate oxidized by Hilditch's method (Table IV, Experiments 1–4). This error, due to the presence of incompletely oxidized glycerides, is not minimized by carrying out the oxidation for a longer period (Table IV, Experiment 4) but probably could be reduced by isolating and analyzing the insoluble Bertram acids of the eluted glycerides. The advantages of the method are that a) a small quantity of fat is sufficient, b) acidic compounds and hydrolysis products are strongly adsorbed, and c) emulsions encountered in the carbonate washing procedure are avoided.

KARTHA'S OXIDATION METHOD FOR GLYCERIDE-TYPE COMPOSITION

The accuracy of Kartha's oxidation method for the determination of glyceride type of composition of fats depends on the accurate determination of the insoluble azelao-glycerides and the saturated acids contained in these and in the soluble azelao-glycerides. From these data the proportions of GS_2U and GSU_2 are calculated after deduction of the amounts of GS_3 and unoxidized GS_2U. The GU_3 content is determined by difference.

Determination of GS_2U in the Absence of GSU_2 and GU_3. As there is no appreciable hydrolysis of GS_2A and as the S content is accurately determined by Kartha's method, the GS_2U content of a concentrate

TABLE VII

Composition of GSU_2 Concentrates by Kartha's Method [a]

Experiment No.	GSU_2 conc.	S, %	GS_2U, %				GSU_2, %				GU_3, %
			Unoxidized	As GS_2A in IAG	Oxidized	Total	As GSA_2 in IAG	From IAG	From SAG	Total	
8	I	40.4	1.78	37.10	41.60	43.4	25.64	32.65	5.81	38.4	18.2
9	II	40.8	0.97	31.02	34.77	35.7	35.86	45.64	9.99	55.6	8.7

[a] Actual composition of the concentrates: 27.8% GS_2U and 72.2% GSU_2 in GSU_2 I; 28.2% GS_2U and 71.8% GSU_2 in GSU_2 II (Table I).

containing 97.2% GS_2U and 2.8% GS_3 was accurately estimated (Table VI, Experiment 7: calculated value for the GS_2U content from the S content after deducting the amount of saturated acids in 2.8% GS_3 = 96.9%). This is in confirmation of Kartha's experimental observation with a mixture of GS_2U (99%) and GS_3 (23).

Determination of GS_2U in the Presence of GSU_2. According to Kartha, the insoluble azelao-glycerides obtained from an oxidized fat are composed of GS_3 (if any), unoxidized GS_2O, the whole of GS_2A, and a part of GSA_2 only. After correcting for the percentages of GS_3 [determined independently by crystallization (25)] and unoxidized GS_2O, calculated on total glyceride basis, and the percentage of saturated acids contained in these, calculated on the basis of insoluble azelao-glycerides, he obtained the percentage yield of (GS_2A + GSA_2) mixture together with its percentage of S content. From these data he calculated the GS_2A content on total glyceride basis using the following equation (22):

$$\%\ GS_2A = \%\ \text{yield of}\ (GS_2A + GSA_2) \times [\%\ S\ \text{of}\ (GS_2A + GSA_2) - \%\ S\ \text{of}\ GSA_2] / (\%\ S\ \text{of}\ GS_2A - \%\ S\ \text{of}\ GSA_2).$$

The GS_2A content can be determined accurately by this equation only if the assumptions that the oxidation of unsaturated glycerides to azelao-glycerides is complete and that the latter do not hydrolyze are valid. To test these assumptions and to show that GS_2U can be determined accurately in the presence of GSU_2 and GU_3, Kartha (22) added a definite amount of GS_2U to sesame oil and determined the total GS_2U content of the mixture. However this will not reveal any error since the composition of the mixture as well as the oil was determined by the same oxidation method.

Effect of Hydrolysis. The saturated acids, liberated mainly from GSA_2, are precipitated together with GS_2A and a part of unhydrolyzed GSA_2 as insoluble magnesium salts. Because of this, the percentage yield of the insoluble azelao-glycerides will be lower and its percentage of S content will be higher than the values calculated on the assumption of absence of hydrolysis. Furthermore the increase in the percentage of S content will be larger than the decrease in the percentage yield of the insoluble azelao-glycerides since the former is based on the latter, which in turn is on the total glyceride basis. Therefore the calculation of the composition of the insoluble azelao-glyceride mixture will result in a higher GS_2A content and a lower GSA_2 content. The decrease in the GSU_2 content of fat will be approximately twice the increase in the GS_2U content. As the GU_3 content is determined by difference, this will also be higher than the actual content. The increase is approximately equal to that in the GS_2U content.

Effect of Incomplete Oxidation. The incompletely oxidized glycerides (GS_2S^o, GSS_2^o, GSS^oA, etc.) containing acetylated α-ketols will be found in the precipitate of Kartha's magnesium salt separation. The effect of incomplete oxidation will therefore be the converse of the effect of hydrolysis of GSU_2.

Over-all Effect. The effect of incomplete oxidation can be equal to, more than, or less than the effect of hydrolysis, depending on the amount of incompletely oxidized glycerides formed and their nature (GS_2S^o, GSS_2^o, GSS^oA, etc.) and the amount of GSU_2 hydrolyzed. If these two effects are equal, Kartha's method will give the actual composition. If the effect of incomplete oxidation is greater than the effect of hydrolysis, then the GSU_2 content will be higher and the GS_2U and GU_3 contents will be lower than the actual. The converse will also be true. For the GSU_2 concentrates used in this investigation, the latter was the case since the amount of hydrolyzed GSU_2 (Table VI: GSU_2 I, 26.7%; GSU_2 II, 13.0% on the basis of concentrate) was larger than the amount of incompletely oxidized glycerides (Table IV: GSU_2 I, 5.0%; GSU_2 II, 8.8%). Thus Kartha's method gave higher values for GS_2U and GU_3 contents and lower values for GSU_2 content than the actual (Table VII).

Explanation of the Differences Between the Compositions Obtained by Hilditch's Crystallization and Kartha's Oxidation Methods. According to Kartha (22,24,27), for fats in which the GU_3 content is high, the value for GSU_2 content obtained by the crystallization procedure is higher than the actual because of intersolubility effects. Kartha's experiment (27) to demonstrate the effect of mutual solubility in the crystallization procedure involved azelao-glyceride analysis of the precipitate and filtrate fractions obtained from a single crystallization of peanut oil. He compared the results thus obtained with those calculated on the assumption that the precipitate and filtrate fractions contained only (GS_2U + GSU_2) and (GSU_2 + GU_3), respectively. However the azelao-glyceride analysis is now shown to be inaccurate, and thus Kartha's criticism becomes unsound.

The present study gives a suitable explanation for the differences (10, 11, 24, 28, 41, 42) between the results obtained for the glyceride type of composition of a number of natural fats by Kartha's oxidation method (23) or by the "glyceride type of distribution" rule (24) and those obtained by the crystallization method of Hilditch (12). For fats in which the GS_2U or GU_3 contents are high and GSU_2 contents low, the glyceride type of compositions recorded by both the methods are almost the same. This is mainly due to the facts that the GS_2A do not hydrolyze appreciably, the hydrolysis of GA_3 does not interfere with the quantitative determination of glyceride composition, and the effect of hydrolysis of a part of the GSU_2 is compensated by the effect of the incompletely oxidized glycerides. Where the GSU_2 content as determined by the crystallization method is high, the greatest divergence is noted because of the considerable hydrolysis of GSU_2 in Kartha's method. As the experimental basis of the restricted random distribution theory of Kartha (24), *viz.*, the oxidation method, is erroneous, the validity of the theory must be re-examined.

Summary

A critical evaluation of the individual steps in Hilditch's acetone-permanganate and Kartha's acetone-acetic acid-permanganate oxidation methods for the determination of the glyceride type of composition of fats was carried out in order to examine the limitations of these methods. Concentrates of mono-unsaturated-disaturated (GS_2U) and monosaturated-diunsaturated (GSU_2) glycerides, and pure monoazelao-disaturated glycerides (GS_2A) were used to obtain definite conclusions.

An alumina column chromatographic procedure was standardized for the quantitative separation of

neutral triglycerides from the acidic and hydrolysis products of oxidation. By this procedure both oxidation methods gave values higher than the actual for the trisaturated glyceride content of the concentrates. This increase was shown to be due to the probable formation of α-ketols from the unsaturated acids in Hilditch's oxidation and the corresponding acetyl derivatives in Kartha's oxidation. These incompletely oxidized glycerides were formed to a greater extent in Kartha's oxidation than in Hilditch's oxidation.

The saturated acid content was determined accurately by Kartha's oxidation and Bertram separation procedure whereas Hilditch's oxidation gave slightly lower values.

The GS_2A were unaffected by the carbonate washing procedure of Hilditch and Lea. However the GS_2U were found to hydrolyze considerably in Hilditch's oxidation. The GSU_2 were hydrolyzed appreciably in Kartha's oxidation procedure whereas the GS_2U were very slightly affected. As a result of this, Kartha's method, when applied to the GSU_2 concentrates, gave an increase in GS_2U and GU_3 contents and a decrease in GSU_2 content.

The effects of incomplete oxidation and hydrolysis on the determination of glyceride composition were demonstrated. This investigation explains the differences in the results obtained by Hilditch's crystallization method and Kartha's oxidation method. As the experimental basis of Kartha's restricted random distribution theory is unsound, this theory must be reexamined.

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Catalyzed Esterification of Oleic Acid[1]

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THE PURPOSE of this study was to determine quantitatively the effects of type and concentration of catalyst and temperature on the rate of esterification of oleic acid with ethylene glycol. The catalysts used were salts of divalent metals. In addition, a study was made of the rate of the catalyzed esterification of oleic acid by a variety of mono- and polyhydric alcohols. Some comments are made on the mechanism of esterification.

There have been many studies of catalysis of esterification, and no attempt will be made here to review them. Most of them are qualitative; some measure the time to reach a given low acid number. Flory (1, 2) showed that self-catalyzed esterification follows, for the most part, third-order kinetics; esterification catalyzed with *p*-toluenesulfonic acid follows second-order kinetics. This was also found by Othmer and Rao (3) in the esterification of oleic acid with butanol, using sulfuric acid catalyst. Rubin (4) calculated rate constants for the esterification of fatty acid with polyhydric epoxy resins, both self-catalyzed and catalyzed with acids or salts. He found no difference in rate between litharge and lead naphthenate; *p*-toluene sulfonic acid was about 30% faster. Calcium naphthenate was about 50% slower and its rate only a little greater than that of the self-catalyzed reaction. Feuge, Kraemer, and Bailey (5) compared the effectiveness of a variety of catalysts for the esterification of fatty acids with glycerol. They found zinc or tin chlorides the most effective and practical.

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