104. *The Component Glycerides of Partially Hydrogenated Fats. Part I. The Alterations in Glyceride Structure produced during Progressive Hydrogenation of Olive and Cottonseed Oils.*

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THE degree of similarity in physical and other properties between hydrogenated fats and natural fats (in substitution for which, or for mixtures thereof, the hydrogenated materials are at present widely used) depends (i) on the extent to which the component fatty acids in both classes are alike and (ii) on the manner in which these acids are combined to form the mixture of glycerides in either case. In addition to defining the general structure of hydrogenated fats at various stages of saturation, study of their component glycerides has provided opportunities for tracing the course of catalytic hydrogenation within the triglyceride molecule more closely than has been possible in former investigations, in which observations were confined to the changes in the component fatty acids as a whole, without reference to their mode of combination in the glycerides.

The choice of material for this investigation was determined by the suitability of the component fatty acids of a given oil for examination by the methods to be described. Thus, olive oil, which is rarely if ever hydrogenated on a technical scale, was chosen because of its comparatively simple structure and because it contains

a large amount (at least 60%) of tri-unsaturated glycerides (a considerable proportion of which is triolein). Later, when the results obtained from hydrogenated olive oil indicated that it would be instructive to commence from a fat which originally contained substantial proportions of a saturated acid (other, of course, than stearic), cottonseed oil (which contains over 20% of saturated acids, almost the whole of which is palmitic acid) offered a suitable starting point.

The glyceride structures of the partially hydrogenated fats which were prepared from these two sources were studied by means of the method proposed by one of us and Lea (J., 1927, 3106), which provides a means for the quantitative estimation of the fully-saturated glycerides of a fat by oxidising a weighed quantity in acetone solution with potassium permanganate. The unsaturated glycerides are attacked at the ethylenic linkings and converted into acidic derivatives, but saturated acid groups are unaltered. Consequently, since any triglyceride molecule which contains one or more unsaturated acid radicals is transformed into an acidic product of oxidation, the alkali salts of which are water-soluble, it is practicable to isolate the unattacked, neutral, fully-saturated triglyceride components of the fat. By using, in conjunction with this procedure, the ester-fractionation method for the analysis of the mixtures of fatty acids present (i) in the fully-saturated triglycerides and (ii) in the fats as a whole, the general constitution of the series of partially-hydrogenated products from each oil has been determined.

It has been shown (Collin and Hilditch, *Biochem. J.*, 1929, **23**, 1273) that in vegetable seed-fats the occurrence of fully-saturated glycerides is negligible until the molar amount of saturated acids in the combined acids of the whole fat approaches 60% and that, when higher proportions of saturated acids than this are present, the amount of fully-saturated glycerides is such that, in the remaining mixed saturated-unsaturated glycerides, the molar proportion of saturated acids is again in the neighbourhood of 60%. In other words, if the relations between saturated and unsaturated acids may be taken as indicative of those between any pair of component fatty acids, the latter are distributed amongst the glycerol molecules in more or less even fashion, so that, if the total proportions of the component acids concerned permit, the tendency is to form mixtures of mixed glycerides in which (if R and r respectively represent the more and the less abundant acyl residues) there are about 4 glyceride molecules R_2r to one of Rr_2.

The structure of butters (Hilditch and Sleightholme, *Biochem. J.*, 1931, **25**, 507), tallows (Banks and Hilditch, *ibid.*, p. 1168), pig body-

fats and other animal fats, as well as of vegetable mesocarp fats such as palm oil (Hilditch and Jones, *J. Soc. Chem. Ind.*, 1930, **49**, 363T), is quite different. In these fats the molar proportion of fully-saturated glycerides increases in a regular manner following corresponding increase in the proportion of saturated to unsaturated acids as a whole; the relation between fully-saturated glycerides and total molar proportion of saturated to unsaturated acids in this group is somewhat, but not exactly, similar to that for triglycerides prepared by heating glycerol with a mixture of saturated and unsaturated acids, or to that wherein fully-saturated glyceride content varies as the cube of the total molar percentage of saturated acids in the fat (Bhattacharya and Hilditch, *Proc. Roy. Soc.*, 1930, *A*, **129**, 468).

Of the vegetable oils now studied, olive oil is a mesocarp fat and contains about 14% (mols.) of saturated acids in its mixed fatty acids; it has a very small content (2%) of fully-saturated glycerides, but definitely more than would be expected from a seed-fat of the same total saturated : unsaturated acid ratio. Cottonseed oil, on the other hand, is a seed-fat with a total molar proportion of 27% saturated acids for the fat as a whole, but it contains less than 1% of fully-saturated glycerides (Hilditch and Lea, *loc. cit.*). In both oils all the remaining saturated acids are linked with unsaturated acids in the form of mixed glycerides, the composition of the latter probably approximating to that given by the " even-distribution " rule described above.

The amount of fully-saturated glycerides in a fat being determined, it is possible to give the limits between which must lie the amounts of either of the following classes of glycerides : mono-unsaturated-disaturated, di-unsaturated-monosaturated, and tri-unsaturated. Accordingly, the olive oil used in these experiments must have contained between 63 and 80%, and the cottonseed oil between 21 and 60%, of tri-unsaturated glycerides; if, as is exceedingly probable, there is considerable tendency towards " even-distribution," the actual value will lie in each case much nearer to the lower than to the upper limit suggested. The above statements summarise our present knowledge of the general glyceride structure of each of the materials on which this study is based.

Details of the experimental results are collected in Tables I and II. These tables show (i) the iodine value of each fat examined; (ii) the component fatty acids present in the whole fat (in cases marked with a dagger, these figures are calculated from the observed fall in iodine value in conjunction with the analysis of the mixed fatty acids of the original oil; in all other cases they represent the results of individual analyses); (iii) the percentage of fully-

TABLE I.

Hydrogenated olive oil.

Iodine value	83·6*	61·4	50·2	37·6	31·7	20·1	11·9
Composition of fatty acids in whole fat:							
Myristic	1·1	1·1†	1·1†	1·4	1·1†	—	1·1†
Palmitic	9·7	9·7	9·7	11·1	9·7	10·1	9·7
Stearic	1·0	18·3	31·4	44·4	53·1	68·1	76·0
Arachidic	0·9	0·9	0·9	0·8	0·9	0·4	0·9
Oleic and *iso*oleic	79·8	70·0	56·9	42·3	35·2	21·4	12·3
Linoleic	7·5	—	—	—	—	—	—
% fully-saturated glycerides	2	3·8	11·1	24·3	30·1	45·2	67·2
Composition of fatty acids in fully-saturated glycerides:							
Myristic	—	—	—	3·2	—	1·4	
Palmitic	100·0	34·4	37·6	31·9	34·3	26·9	
Stearic	—	65·6	62·4	64·9	65·7	70·3	
Arachidic	—	—	—	—	—	0·8	

Hydrogenated cottonseed oil.

Iodine value	103·4*	63·1	49·6	42·8	29·8	20·1	13·2	5·1
Composition of fatty acids in whole fat:								
Myristic	3·3	1·8	3·3†	3·3†	3·3†	3·3†	3·3†	3·3†
Palmitic	19·9	18·0	19·9	19·9	19·9	19·9	19·9	19·9
Stearic	1·3	9·5	19·3	27·2	42·5	53·8	61·9	71·2
Arachidic	0·6	0·8	0·6	0·6	0·6	0·6	0·6	0·6
Oleic and *iso*oleic	29·6	68·6	56·9	49·0	33·7	22·4	14·3	5·0
Linoleic	45·3	1·3	—	—	—	—	—	—
% fully-saturated glycerides	<1	1·5	7·3	15·7	33·7	43·9	64·4	87·4
Composition of fatty acids in fully-saturated glycerides:								
Myristic	—	—	0·5	—	—	—	—	—
Palmitic	100·0	36·8	35·9	34·5	31·3	29·4	28·7	27·1
Stearic	—	63·2	57·3	65·5	65·8	68·6	71·3	72·9
Arachidic (?)	—	—	6·3	—	2·9	2·0	—	—

* Original oil before hydrogenation.

† Calculated from iodine value of fat and analysis of original oil.

saturated glycerides; and (iv) the component fatty acids present in the latter.* Table I shows the percentages by weight as determined, whilst Table II gives the corresponding data in the form of molar percentages: it is essential to discuss variations in glyceride composition on a molar or equivalent basis in view of the varying molecular size of the component acyl radicals.

* The small proportions of myristic acid calculated to be present from the ester-fractionation analyses of the original oils were usually found to be much reduced, or even absent, after hydrogenation, and little or no myristic acid was observed in any of the fully-saturated glycerides. It is therefore possible that when minor proportions of myristic acid have been recorded in certain natural fats, at least part of this figure really represents palmitic acid contaminated by traces of esters of low molecular weight which are not derived from glycerides.

TABLE II.

Hydrogenated olive oil.

Iodine value	83·6*	61·4	50·2	37·6	31·7	20·1	11·9
Composition of fatty acids in whole fat :							
Myristic	1·4	1·3†	1·3†	1·7	1·3†	—	1·4†
Palmitic	10·6	10·6	10·6	12·0	10·6	11·1	10·6
Stearic	1·0	18·0	30·9	43·7	52·4	67·3	75·0
Arachidic	0·8	0·8	0·8	0·7	0·8	0·3	0·8
Oleic and *iso*oleic	78·7	69·3	56·4	41·9	34·9	21·4	12·2
Linoleic	7·5	—	—	—	—	—	—
% fully-saturated glycerides	2·0	4·0	11·2	25·5	30·8	46·5	67·5
Composition of fatty acids in fully-saturated glycerides : ‡							
Palmitic	100·0	36·8	40·0	37·6	36·8	30·8	
Stearic	—	63·2	60·0	62·4	63·2	69·2	

Hydrogenated cottonseed oil.

Iodine value	103·4*	63·1	49·6	42·8	29·8	20·1	13·2	5·1
Composition of fatty acids in whole fat :								
Myristic	4·0	2·2	4·0†	4·0†	4·0†	4·0†	4·0†	4·0†
Palmitic	21·4	19·4	21·4	21·4	21·4	21·4	21·4	21·4
Stearic	1·2	9·3	18·7	26·3	41·2	52·2	60·1	69·3
Arachidic	0·5	0·7	0·5	0·5	0·5	0·5	0·5	0·5
Oleic and *iso*oleic	28·7	67·1	55·4	47·8	32·9	21·9	14·0	4·8
Linoleic	44·2	1·3	—	—	—	—	—	—
% fully-saturated glycerides	<1	1·5	7·1	15·9	33·5	44·0	65·3	87·7
Composition of fatty acids in fully-saturated glycerides :‡								
Palmitic	100·0	39·2	39·1	36·9	33·7	31·7	30·9	29·2
Stearic	—	60·8	60·9	63·1	66·3	68·3	69·1	70·8

* Original oil before hydrogenation.

† Calculated from iodine value of fat and analysis of original oil.

‡ Myristic acid, in the few cases in which this acid was indicated by the fractionation results, has been included with the palmitic acid figure, and similarly, the small, occasionally recorded amounts of arachidic acid have been included with the stearic acid figures.

The limits within which the molar percentages of the three classes of glycerides containing unsaturated radicals may lie (in conformity with the observed molar content of fully-saturated components) are shown for each hydrogenated fat in Table III. This table also correlates the rate of increase in the fully-saturated glyceride contents with the increasing proportions of saturated acids in the fat as a whole during the progress of hydrogenation.

Owing to the selective action whereby linoleic groups are very largely converted into oleic or *iso*oleic groups before the latter are further hydrogenated (compare Hilditch and Moore, *J. Soc. Chem. Ind.*, 1923, **42**, 15T), little increase in the total amount of saturated acids, and consequently no appreciable addition to the fully-saturated glycerides, takes place until linoleo-glycerides have almost

TABLE III.

Iod. val.	Satd. acids, %.	F.S.G., %.	Mono-unsatd.-disatd. glycerides, %.	Di-unsatd.-monosatd. glycerides, %.	Tri-unsatd. glycerides, %.
			Hydrogenated olive oil.		
83·6	13·8	2	17·7—0	0—35·4	80·3—62·6
61·4	30·7	4	40·1—0	0—80·1	56·0—15·9
50·2	43·6	11·2	48·6—8·4	0—80·4	40·2—0
37·6	58·1	25·5	48·9—23·3	0—51·2	25·6—0
31·7	65·1	30·8	51·5—33·7	0—35·5	17·7—0
20·1	78·7	46·5	48·3—43·1	0—10·4	5·2—0
11·9	87·8	67·5	30·5—28·4	0—4·1	2·0—0
0	100·0	100	—	—	—
			Hydrogenated cottonseed oil.		
103·4	27·1	<1	39·1—0	0—78·3	59·9—20·7
63·1	31·6	1·5	45·1—0	0—90·3	53·4—8·2
49·6	44·6	7·1	56·3—19·6	0—73·3	36·6—0
42·8	52·2	15·9	54·5—24·8	0—59·3	29·6—0
29·8	67·1	33·5	50·4—33·3	0—33·2	16·1—0
20·1	78·1	44·0	51·2—46·3	0—9·7	4·8—0
13·2	86·0	65·3	31·0—27·4	0—7·3	3·7—0
5·1	95·2	87·7	10·8—10·2	0—2·1	1·5—0
0	100·0	100	—	—	—

disappeared. The proportion of linoleic acid in olive oil is not large, but in cottonseed oil it forms about 45% of the total acids, and reduction in iodine value from 103·4 to 63·1 leaves a fat the component acids of which (from the direct analysis, Table II) still contain 1·3% (mols.) of linoleic acid, the saturated acids having increased by only 4·5%.

After the linoleo-glycerides have disappeared, steady increase in the total saturated acids commences, but development of fully-saturated glycerides is relatively slow until towards the final stages of hydrogenation. The data in the last column of Table III indicate, indeed, that (whatever may be the actual content of tri-unsaturated glycerides) trioleins * at first disappear much more rapidly than fully-saturated components are produced, so that, in the earlier stages, the predominating action is conversion of trioleins into oleostearins. This observation discloses a previously unnoticed, but somewhat important, feature of the hydrogenation process, namely, that a molecule of triolein, adsorbed by nickel, is desorbed as soon as a single oleic group has undergone hydrogenation, so that direct transformation of molecules of triolein into tristearin at one and the same contact with the catalyst does not occur. A single unsaturated centre only is involved in each effective contact between catalyst and a triolein molecule.

There is good reason to suppose, as already mentioned, that the

* *I.e.*, the mixture of oleic and *iso*oleic triglycerides present after hydrogenation of the tri-unsaturated (oleic-linoleic) glycerides in the original oils.

mixed glycerides in both oils will conform to a large extent to the "evenly distributed" type and, in this case, the amounts of trioleins present at any stage will be much nearer to the lower than to the higher possible limits. It is therefore probably safe to assume (in the absence of an exact experimental means of determination of fully-unsaturated glycerides) that by the time the iodine value of either fat has been reduced to below about 40—45, trioleins will have completely disappeared. When triolein is absent, it becomes possible to give, within approximate limits, definite figures (instead of the limiting ranges of Table III) for the mono- and di-oleo-types of glycerides, and the values in question are given in Table IV.

TABLE IV.

Iod. val.	Satd. acids, %.	Glycerides. Fully-satd., %.	Mono-unsatd.-disatd., %.	Di-unsatd.-monosatd., %.
		Hydrogenated olive oil.		
37·6	58·1	25·5	23·3	51·2
31·7	65·1	30·8	33·7	35·5
20·1	78·7	46·5	43·1	10·4
11·9	87·8	67·5	28·4	4·1
		Hydrogenated cottonseed oil.		
42·8	52·2	15·9	24·8	59·3
29·8	67·1	33·5	33·3	33·2
20·1	78·1	44·0	46·3	9·7
13·2	86·0	65·3	27·4	7·3

If, therefore, we are correct in the belief that trioleins will have ceased to be present at the state of saturation mentioned, the next phase of the hydrogenation is characterised by increase in the production of both fully-saturated and mono-unsaturated-disaturated glycerides at approximately the same rate, and concurrent disappearance of di-unsaturated-monosaturated derivatives at approximately twice this rate: that is, di-oleo-glycerides are attacked in preference to mono-oleo-compounds.

Independent confirmation of this phenomenon has been afforded by examination of the composition of the fully-saturated glycerides insoluble in acetone at 0° present in the hydrogenated olive oils of iodine values 31·7, 20·1, and 11·9. Tristearin and the palmitodistearins are very sparingly soluble in this medium and advantage was taken of this (see p. 817) to remove a considerable portion of the fully-saturated glycerides present in the more saturated fats by this means during the determination of fully-saturated glyceride content. After recrystallisation from acetone at 0°, the approximate amounts of tristearin (equivalent, 296·7) and palmitodistearins (equivalent, 287·3) present were calculated from the observed

saponification equivalent of the mixture of glycerides separated.* The resulting data give minimum values for tristearin, since the small amount soluble in ice-cold acetone will have been left out of account; moreover the difference in equivalents (9·3) is too small to permit of any great accuracy. Nevertheless the results (Table V) show that tristearin is substantially absent until the hydrogenation has proceeded far towards completion and that, in the final stages, the increase in fully-saturated components is almost wholly accounted for by increase in tristearin.

TABLE V.

Hydrogenated Olive Oil.

		F.S.G. insoluble in acetone.		
Iod. val.	Fully-satd. glycerides, % (wt.).	% (wt.) of total F.S.G.	Sap. equiv.	Calculated % (wt.) of tristearin in whole fat.
31·7	30·1	83·8	288·4	3·3
20·1	45·2	88·5	290·3	13
11·9	67·2	92·7	294·8	33

So far, then, we have been considering the sequence of actions involved in the conversion of a molecule of triolein into tristearin, and the experimental results discussed above lead us to conclude: (i) that one oleic group only undergoes hydrogenation at each effective contact with the catalyst, the semi-hydrogenated glyceride then leaving the catalyst and requiring fresh adsorption thereat before further addition of hydrogen takes place; and (ii) that the hydrogenation of the different classes of unsaturated glycerides is definitely of a selective nature, trioleins being more readily attacked than dioleo-glycerides, and the latter more so than the mono-oleo-compounds.

The experimental data, however, indicate equally clearly that a still more delicate kind of selective hydrogenation operates throughout the process, namely, that mixed glycerides containing palmitic groups are preferentially attacked to those containing only stearic residues. Inspection of Table II reveals that, in the case of hydrogenated olive oil, the proportion of palmitic acid combined in the fully-saturated glycerides remains approximately constant (40—37 mols.%) until the total saturated acid content of the fat reaches 65%, but thereafter declines sharply; also, at the stage mentioned (65% total saturated acids) the amount of combined

* The figures obtained by this procedure suggest that similar examination of a completely hydrogenated fat will yield values for its tristearin content from which, in many cases, it may be possible to estimate within certain limits the amount of tri-unsaturated C_{18} glycerides in the original oil. This possibility is at present being studied, so far with favourable results.

palmitic acid in the fully-saturated components accounts for the whole of that present in the original fat. With cottonseed oil (which contains much more palmitic acid) the line of demarcation is not quite so sharply defined, but nevertheless the proportion of palmitic acid in the fully-saturated components only declines from 39% to 34% whilst the proportion of saturated acids in the whole fat is raised by hydrogenation to 67%, and, when the saturated acids amount to 78% of the whole, the palmitic acid combined in the fully-saturated part corresponds with the whole of that present in the original oil.

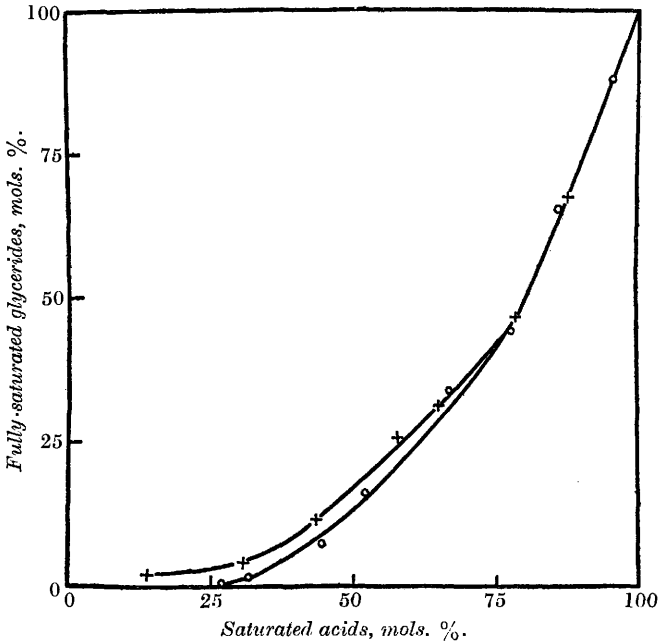
Determination of the mean equivalents of the portions of fully-saturated glycerides of hydrogenated olive oils obtained by recrystallisation from acetone (compare above, p. 812) gave confirmatory proof of the previous figures, a definite increase beyond that of palmitodistearin (287·3) only being observed with specimens hydrogenated to beyond the stage (65% total saturated acids) at which all the palmitic acid present was in the form of fully-saturated glycerides (Table VI). The corresponding data for cottonseed oil were not so definite : this is probably due (compare melting points) to dipalmitostearins which (in consequence of the high palmitic acid content of cottonseed oil) may have been present in sufficient quantity to prevent their complete separation by the acetone-crystallisation procedure.

TABLE VI.

Hydrogenated oil.	Satd. acids in whole fat, % (mols.).	Fully-satd. glycerides in whole fat, % (mols.).	F.S.G. recrystallised from acetone.		
			% (wt.) of total F.S.G.	Sap. equiv.	M. p.
Olive	30·7	4	68	286·5	
,,	43·6	11·2	71	287·7	
,,	58·1	25·5	64	288·6	61·5°
,,	65·1	30·8	84	288·4	
,,	78·7	46·5	89	290·3	63
,,	87·8	67·5	93	294·8	63
Cottonseed	52·2	15·9	73	285·9	60
,,	67·1	33·5	73	286·5	61
,,	78·1	44·0	93	286·5	60
,,	86·0	65·3	87	286·9	62
,,	95·2	87·7	89	287·3	62

Study of the glyceride structure of hydrogenated fats has therefore emphasised the numerous phases of hydrogenation which proceed, to a large extent, selectively and independently of each other. It has indicated not only the complexity of the glyceride hydrogenation process, but also its main stages, namely, saturation of only one ethenoid linking at each contact with the catalyst and preferential hydrogenation of triglycerides in the following order : trioleins, di-oleo-compounds, and mono-oleo-compounds. Further,

and lastly, although oleostearins must be produced in abundance by this sequence of changes, these are not converted into tristearin in any quantity until all palmito-oleins originally present have been saturated. Consequently, the comparatively slight difference in the triglyceride molecule introduced by the substitution of a palmitic for a stearic radical exerts a remarkable influence on the susceptibility to hydrogenation of an oleic group within the same molecule. This phenomenon is not, perhaps, so extraordinary as it may appear at first sight when it is considered in conjunction with the work of



× *Hydrogenated olive oil.* ○ *Hydrogenated cottonseed oil.*

Lebedev and his colleagues (J., 1925, **127**, 417; 1930, 321), who have pointed out the profound effect of substituent methyl or other groups in unsaturated compounds upon the susceptibility to hydrogenation of an ethylenic linking.

We would point out that the relationships, in hydrogenated olive and cottonseed oils, between the molar contents of fully-saturated glycerides and the molar percentages of saturated acids in the total acids of the fat (which are shown graphically in the figure) are in conformity with the general conclusions reached in the foregoing discussion. In the earlier stages, trioleins and di-oleo-glycerides are predominantly hydrogenated in stages, and little increase in fully-

saturated components occurs; subsequently, mono-oleo-glycerides which also contain palmitic groups are being preferentially hydrogenated, and a more rapid development of fully-saturated compounds (almost entirely palmitostearins) sets in; finally, oleostearins which have accumulated by reduction of (original) triolein and during saturation of palmito-oleo-glycerides are transformed into tristearin, and the amount of fully-saturated glycerides increases rapidly.

EXPERIMENTAL.

The oils employed, which consisted of an Italian olive oil of first quality and an alkali-refined cottonseed oil, possessed the following characteristics :

	Olive.	Cottonseed.
Acid value	2·2	0·4
Saponification equivalent	294·2	288·5
Iodine value	83·6	103·4
Unsaponifiable	0·8	0·8
(Iod. val. of unsaponifiable	194·5	120)

Conditions of Hydrogenation.—The oil (400 g.) was placed with reduced nickel–kieselguhr catalyst (10 g., containing about 1 g. of free metallic nickel) in an iron hydrogenation vessel of about 800 c.c. capacity fitted with a gas-tight cover carrying inlet and outlet gas-connections, a thermometer pocket, and a vertical stirrer to which was attached a horizontal blade. The contents of the vessel were heated with vigorous stirring in an atmosphere of hydrogen to 180° and the hydrogenation was conducted at this temperature in all cases. The hydrogen was passed through a meter prior to admission to the hydrogenation vessel and was led, after issuing therefrom, through a second meter; the process could thus be stopped when the meter-readings indicated that the absorption of hydrogen corresponded with the desired reduction in the iodine value of the fat. The general arrangements of the apparatus used have been described elsewhere (E. F. Armstrong and Hilditch, *Proc. Roy. Soc.*, 1919, *A*, **96**, 138). The actual time occupied in the hydrogenation of a 400 g. batch varied from about 25—30 minutes in the case of the olive oil product of iodine value 61·4 to about 180 minutes for the cottonseed oil product of iodine value 5·1.

With the more saturated products a single batch of 400 g. was sufficient, but in the case of the softer fats (iodine value from about 40 upwards) it was necessary to unite the fat from two or even three similar hydrogenations in order eventually to obtain suitable quantities of fully-saturated glycerides for the purpose of this study.

Determination of Component Fatty Acids.—The method employed has been described in full detail by our co-workers in several recent papers (compare, especially, *Analyst*, 1929, **54**, 80; *J. Soc. Chem.*

Ind., 1930, **49**, 364T; *Biochem. J.*, 1930, **24**, 270, 1102; 1931, **25**, 1169). The mixed fatty acids (about 200 g.) obtained by hydrolysis of a fat are converted into their lead salts in alcoholic solution (10 c.c. of 95% alcohol per gram of mixed acids) and the lead salts which are insoluble at room temperature are, after recrystallisation from alcohol, reconverted into fatty acids (" solid " acids) separately from those which are contained in the alcoholic liquors and which yield the mainly unsaturated (or " liquid ") acids. The " solid " acids obtained by this procedure from a natural fat should not possess an iodine value higher than 10, in which case the unsaturated acid represented by this figure may safely be taken as oleic acid; in hydrogenated fats " *iso*oleic " acids are present in varying amounts and also appear with the saturated acids in the " solid " group on account of the sparing solubilities of their lead salts in alcohol.

Each group of acids—" solid " and " liquid "—is separately converted into the corresponding neutral methyl esters and the latter are carefully distilled from a Willstätter bulb (heated in an oil-bath) under a pressure of 1 mm. or less. The operation is so conducted (primary fractions being refractionated when necessary) that a sufficient number of ester-fractions are obtained to ensure that, to within narrow limits, no single fraction contains more than two saturated and two unsaturated (oleic and linoleic) esters. If these conditions are satisfied and the whole sequence of operations has been carried out quantitatively, it is possible to estimate the composition of each ester-fraction in each group from its saponification equivalent and iodine value, and thence to arrive at the composition of the original mixture of acids. Since the complete fractionation data for a single analysis of this kind are extremely lengthy, and since typical records of similar analyses have been given in the papers cited, it has not been thought necessary to include detailed figures for the present series of analyses, the final results of which have been given in Tables I and II.

When determining the component fatty acids of the fully-saturated glycerides, it was of course possible to proceed directly with the esterification of the mixed acids, no separation by means of the lead salt process being necessary.

Determination of Fully-saturated Glyceride Content.—The general procedure adopted in the oxidation of a fat (1 part), dissolved in acetone (10 parts), by means of finely-powdered potassium permanganate (4 parts) was described by Hilditch and Lea (*loc. cit.*). It has been found convenient to vary the precise method used for the separation of the neutral and acidic portions of the oxidised fat according to the predominant characteristics of the fat under examination. Thus, whilst a fat which contains relatively large

amounts of di-unsaturated-monosaturated or of tri-unsaturated glycerides yields acidic oxidation products which are comparatively easily removable in the form of aqueous solutions of their potassium or ammonium salts, one in which mono-unsaturated-disaturated glycerides are present in considerable proportion gives rise to acidic glycerides, the alkali salts of which are particularly effective emulsifying agents. In the latter case, advantage may be taken of the fact that such acidic (mono-azelao-) glycerides readily yield sodium salts which may be crystallised from ether.

The less saturated fats did not always undergo complete oxidation in one operation. In such cases (indicated by an asterisk in Tables VII and VIII) the oxidation process was repeated on the crude neutral product isolated after the initial oxidation. (The weights of products recorded in Tables VII and VIII are corrected to correspond with the original weight of material employed in the initial oxidation.)

It was found desirable to carry out the purification of the saturated glycerides resulting respectively from hydrogenated olive oils and hydrogenated cottonseed oils by somewhat different means :

(i) *Hydrogenated olive oils.* Owing to the preponderance of palmitodistearins and tristearin in the fully-saturated portion of many of these fats, and to the very sparing solubility of these compounds in ice-cold ether, it was possible to effect a preliminary separation of part of the fully-saturated glycerides in a pure condition by cooling the ethereal solution of the whole of the products of oxidation to 0° and removing deposited solids by filtration. These were then further purified by crystallisation two or three times from acetone at 0°; their content of free acidic products was then quite negligible (A, Table VII).

The material removed by solution in acetone was recovered and added to the ethereal filtrates (made up to 2500 c.c. with ether), which were then treated with warm 30% aqueous sodium carbonate solution, followed by two rapid washings (without undue shaking) with warm distilled water; the withdrawn alkaline and aqueous solutions were extracted with ether and the regenerated acidic material was recovered from the aqueous phase (C). The ethereal solutions (which frequently contained substantial amounts of sodium salts of monoazelao-glycerides) were cooled in a freezing-mixture for one hour and filtered, the separated sodium salts being reconverted into acidic products (D); the ethereal filtrates (to which was added the ether used to extract the aqueous sodium carbonate washings previously mentioned) were finally washed with dilute aqueous potassium carbonate and then with water. The fully-saturated glycerides (B) recovered from the ether were somewhat contaminated

with acidic products, for which a correction was applied on the assumption that the acid value of the latter was approximately the same as that of the acidic material (E) regenerated from the aqueous potassium carbonate washings which had finally been given to the united ethereal extracts.

The experimental data for hydrogenated olive oils, on which the calculation of fully-saturated glyceride content is based, are collected in Table VII.

TABLE VII.

Oxidation of hydrogenated olive oils.

Hydrogenated oil							
Iodine value	83·6†	61·4	50·2	37·6	31·7	20·1	11·9
Weight oxidised (g.)	204·7*	204·5*	202·3*	68·2	98·9	103·8	104·9
Fully-saturated glycerides							
A. By crystallisation from ether and acetone (g.) ...	—	5·25	15·78	10·96	24·88	41·49	65·38
Acid value ...	—	0·3	0·4	1·4	0·3	0·2	0·2
B. Ether-soluble (g.)	4·61	2·78	7·77	6·50	5·88	6·17	6·22
Acid value ...	20·4	21·3	27·8	25·9	32·9	24·3	26·6
Acidic products							
C. From aqueous sodium carbonate (g.)	—	170·3	118·8	31·7	38·4	12·0	4·9
Acid value ...	—	247	235	225	200	272	237
D. From sodium salts ex ether (g.) ...	—	1·9	8·7	9·3	14·6	29·1	12·4
Saponification equivalent	—	180·1	192·3	199·8	191·5	193·1	191·3
E. From aqueous potassium carbonate (g.).....	—	3·5	14·8	3·1	1·5	2·4	14·1
Acid value ...	—	242·5	201	210	188·5	188	148
Total weight of pure F.S.G. (corrected)	4·2	7·79	22·48	16·59	29·73	46·87	70·48

† Original oil.

Previous experience with natural fats has shown (Hilditch and Saletore, *J. Soc. Chem. Ind.*, 1931, **50**, 468T) that only mono-azelao-glycerides yield sodium salts which are soluble in ether and that, unless corresponding mono-unsaturated-disaturated glycerides are present in considerable quantities, the amount of mono-azelao-compounds obtained as sodium salts from ether is inconsiderable. From Table VII it will be seen that this group (D) of acidic products amounts to an important proportion of the total acidic products of oxidation only when the iodine value of the fat has been reduced to 30 or below, and this is, in fact, further proof that, until this region is reached, di-unsaturated-monosaturated glycerides are present in quantity. It is also significant that the maximum yield of this

group (at iodine value 20·1) coincides with the maximum concentration of mono-oleo-glycerides as deduced in Table III on the assumption of general tendency towards "even distribution." The saponification equivalents of the acidic products D (which were not further purified by crystallisation) are of the order of that of azelaopalmitostearin (191·5).

TABLE VIII.

Oxidation of hydrogenated cottonseed oils.

Hydrogenated oil							
Iodine value	63·1	49·6	42·8	29·8	20·1	13·2	5·1
Weight oxidised (g.)	202·0*	200·6*	199·4*	201·5	104·6	101·5	99·5
Fully-saturated glycerides							
Weight of crude product (g.)	3·63†	18·55	35·92	81·76	58·00	68·38	88·24
Purification of F.S.G.							
Weight of crude F.S.G. used (g.)...	—	18·23	35·34	80·65	57·45	67·40	87·60
A. Fully-saturated glycerides, isolated as above...	—	13·05	26·64	53·82	44·21	57·10	63·42
Acid value ...	—	1·1	0·3	0·3	0·4	0·3	0·4
B. Fully-saturated glycerides, from ether extraction of washings ...	—	1·81	5·38	14·93	1·94	8·14	23·15
Acid value ...	—	19·3	24·8	15·6	28·3	7·8	0·9
C. Acidic products corresponding with B	—	3·37	3·32	11·90	11·30	2·16	1·03
Acid value ...	—	145	145·5	137	103	102	168
Weight of pure F.S.G. (corrected)	*ca.* 3·2	14·67	31·54	67·85	46·00	65·40	86·95

† Acid value 18·6.

(ii) *Hydrogenated cottonseed oils.* Probably as a result of the presence of relatively larger quantities of palmito-glycerides than in olive oil, it was found possible to omit the separation of sodium salts from ether in this series, and to remove most of the acidic products formed during oxidation by washing the ethereal solutions of the total reaction products repeatedly with dilute aqueous potassium carbonate and then with water. The crude fully-saturated material thus obtained was then further purified by boiling with dilute aqueous potassium carbonate, and then with water until all alkali salts were removed; the following compounds were then isolated :

A. Practically neutral fully-saturated glycerides (usually about 75% of the whole).

B. Fully-saturated glycerides still somewhat contaminated with acidic products, by extraction of the aqueous liquors with ether.

C. Acidic glycerides recovered from the extracted aqueous liquors, the mean equivalent (by direct titration) of which was assumed also to be that of the acidic impurities left in B.

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