CXLI. REGULARITIES IN THE GLYCERIDE STRUCTURE OF VEGETABLE SEED-FATS.

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There is now in existence a large amount of evidence that simple triglycerides, containing a single fatty acid, are only formed in any quantity in a natural fat if no other method of combining the fatty acids at the disposal of the plant or animal is possible.

Qualitative work on the fractional crystallisation of fats by Klimont [1902, 1903, 1904, 1905, 1912], Bömer [1907, 1909, 1913, 1920] and Amberger [1913, 1918, 1923, 1924], in the cases of cacao butter, beef and mutton tallows, butter, lard and other fats, and particularly Bömer's fractional crystallisations of coconut and palm kernel fats [Bömer and Schneider, 1924], proving the absence of trilaurin from both of them, although their lauric acid content is nearly 50 % of the total mixed acids, all show the wide extent of this generalisation.

Another body of evidence is provided by the work of Suzuki and Yokoyama [1927, 1928], and of Eibner [1927, 1928], on linseed, soya-bean, cod-liver and whale oils. By fractional crystallisation of the bromine-addition products of the glycerides present in these oils they have isolated, from linseed oil, dilinoleolinolenin, oleodilinolenin, and two varieties of linoleodilinolenin; from soya-bean oil, oleodilinolenin, linoleodilinolenin, dilinoleolinolenin, oleolinoleolinolenin and dipalmito-olein; and they have shown that similar mixtures occur in cod-liver and whale oils. The most interesting feature, however, is the almost complete absence of simple triglycerides, which brings these oils into harmony with the more saturated fats examined by the former group of workers.

During the past few years considerable attention has been given in our laboratory to the more saturated fats and it has been found possible quantitatively to remove any unsaturated glycerides by means of oxidation of the fat with anhydrous potassium permanganate in acetone solution, a procedure which converts all but the fully saturated triglycerides present into acidic products, and thus leads to a quantitative determination of the amount of completely saturated triglycerides in the original fat. Furthermore, when the compositions of the mixed fatty acids in the fully saturated glycerides and in the original fat have been ascertained, it becomes possible to obtain a measure of the average number of molecules of saturated fatty acid which are associated with one molecule of unsaturated acid in the mixed saturated-unsaturated

glycerides (plus tri-unsaturated glycerides) of the original fat; and, again, from this may be deduced limiting ranges for the proportions of mono-unsaturated-disaturated, di-unsaturated-monosaturated, and tri-unsaturated glycerides present in the original fat.

By taking advantage in this way of the amenability of unsaturated fatty acid residues to chemical change (oxidation), we are accumulating a good deal of evidence as to general relationships in the distribution of fatty acids in combination with glycerol, from the point of view of the manner in which saturated and unsaturated acids are associated. There is little, if any, reason to suppose that these relationships will differ fundamentally from those which govern the partition of individual saturated or unsaturated acids amongst the glycerol residues of a natural fat; on the contrary, the general similarity of all the higher fatty acids, saturated or unsaturated, in so far as matters of esterification or hydrolysis are concerned, justifies to some extent[1] the inference that relationships between saturated and unsaturated acids will largely hold for all the individual members of either group. At present, however, so far as semi-quantitative experimental investigation goes, we are confined almost exclusively by the chemical properties of the fats to observation of the two types of glyceride (i) fully saturated, and (ii) mixed saturated-unsaturated or wholly unsaturated.

The results which have been obtained by this method have verified, and have also brought out a difference in degree of, the tendency towards even distribution of fatty acids throughout a fat, which was indicated by the earlier work referred to at the commencement of this paper. We find that there is a

[1] So far as can be judged at present, it seems almost certain that the distribution of fatty acids in mixed saturated glycerides is governed by the same rules which operate in the saturated-unsaturated acid partition in mixed saturated-unsaturated glycerides; so that in seed-fats there is notably even distribution (cf. the examination of the fully saturated glycerides of coconut or palm kernel fat [Collin and Hilditch, 1928], in which trilaurin is not present, although lauric acid forms 50–60 % of the fatty acids), whilst in animal fats there is a more heterogeneous kind of assemblage in the fully saturated as in the whole of the glycerides (thus mutton tallow, the fatty acids of the fully saturated portion of which contain 50 % palmitic and 44 % stearic acid, contains 3 % of tristearin and possibly some tripalmitin [Collin, Hilditch and Lea, 1929], a result confirmed by Bömer [1907]; and tripalmitin or tristearin has been similarly reported by Bömer and by Amberger in certain other animal fats).

Concurrently with these general features, however, there is evidence that specific fatty acids may associate predominantly together in different ways in different fats. In some cases the composition of the saturated fatty acids (i) associated together, and (ii) associated with unsaturated acids in mixed saturated-unsaturated glycerides is much the same (*e.g.* dika fat in this paper, p. 1281), whilst in others it is essentially different. Thus in mutton tallow and cacao butter palmitic acid tends to concentrate in the fully saturated glycerides (as also in the case of *Myristica malabarica* fat, this paper, p. 1285), whilst in other cases (cf. nutmeg butter and laurel oil, this paper, pp. 1283–1287) it has been found mainly associated with the unsaturated acids.

This specific character of the association of different fatty acids in varying types of fat, which may be compared with the known specificity of fatty acid composition in the fats of certain botanical orders of plants [Hilditch, 1928], is apparently not in conflict with the operation of the general laws which govern the assemblage of the mixed fatty acids into triglycerides according to the biological origin of the fat, animal or vegetable.

marked difference between animal and vegetable fats, particularly vegetable seed (kernel) fats, in that the tendency referred to is much stronger in vegetable than in animal fats.

This is most clearly to be appreciated from a consideration of the relative number of molecules of saturated fatty acids present per molecule of unsaturated fatty acid in the whole original fat and in the portion thereof which does not consist of fully saturated triglycerides. To avoid repetition of a necessarily lengthy phrase, we shall refer to the number of molecules of saturated fatty acids present per molecule of unsaturated acid (more especially when in association in the form of mixed saturated-unsaturated triglycerides) as the "association ratio." The numerical value of the "association ratio" appears, from such data as are yet available, to be quite distinctly characteristic of the biological origin of the particular fat.

Up to the present, we have obtained information from less than twenty fats of widely varying type, but more detailed work is in progress on non-seed fats of vegetable origin (such as palm oil) and on a considerable number of animal fats of different kinds. We have, however, already completed a more thorough examination of a range of solid vegetable seed-fats, the results of which form the subject of this communication; it is in this group that the "association ratio" reaches the highest values observed so far, each molecule of oleic acid being linked, circumstances permitting, with an average of 1·3–1·6 molecules of saturated acid. In other words, the mixed saturated-unsaturated glycerides consist largely of mono-oleo-disaturated glycerides with some di-oleo-monosaturated glycerides and the even distribution of the fatty acids amongst the glycerol residues appears to reach a maximum in this class of fats.

The figures on which we base these deductions are summarised in Table I, wherein are also included, for the sake of illustration, such data as are yet available for vegetable non-seed fats and for various kinds of animal fats. Column I gives the fat examined, column II the percentages by weight of saturated and unsaturated fatty acids in the original fat, and column III the percentage of fully saturated triglycerides in the original fat, whilst the "association ratios" of the (molecular) proportions of saturated to unsaturated acids in the whole fat and in the mixed saturated-unsaturated (plus tri-unsaturated) glycerides are given respectively in columns IV and V.

In connection with Table I, we would point out that the association ratios for the mixed glycerides of kusum, cottonseed and ground-nut oils and of lard and rabbit fat have not been worked out in so much detail as in the other cases, owing to the small proportions of fully-saturated material present; whilst all the figures for beef tallow must be regarded as of a preliminary nature. Any uncertainties thus present in the values in column V for these six fats (which are consequently placed within brackets) are, however, sufficiently small not to interfere with their presentation in contrast to the remaining seed-fats.

Table I.

Fat examined	Acids in whole fat: Sat. %	Acids in whole fat: Unsat. %	Fully saturated triglycerides %	"Association ratios": In whole fat	"Association ratios": In sat.-unsat. glycerides	Reference
I	II		III	IV	V	VI
Vegetable seed-fats:						
Coconut	91·7	8·3	84	15·5	1·3–1·4	Collin and Hilditch [1928]
Dika	89·4	10·6	79	11·0	1·3	See this paper, p. 1280
Myristica officinalis	88·6	11·8	71	9·2	1·6	See this paper, p. 1281
Palm kernel	80·8	19·2	63	5·8	1·3–1·4	Collin and Hilditch [1928]
Illipé tallow	62·0	38·0	4·5	1·7	1·55	Hilditch and Priestman (forthcoming publication)
Cacao butter	58·8	41·2	2·5	1·5	1·4	Lea [1929]
Myristica malabarica	54·9	45·1	18	1·5	1·0	See this paper, p. 1283
Kusum oil	35·5	64·5	1–2	0·6	(0·6)	Dhingra, Hilditch and Vickery [1929]
Cottonseed oil	23·4	76·6	1 (?)	0·33	(0·33)	Hilditch and Lea [1927]
Ground-nut oil	15·1	84·9	1 (?)	0·18	(0·18)	Christian and Hilditch (forthcoming publication)
Vegetable non-seed fats:						
Palm oils	46·9	53·1	9	0·97	0·8	Hilditch and Jones (not yet published)
	48·6	51·4	10	1·04	0·8	
	41·6	58·4	7	0·78	0·65	
Laurel oil	44·7	55·3	26	1·09	0·37	See this paper, p. 1285
Animal fats:						
Butter fats	61·3	38·7	31	2·06	1·05	Hilditch and Jones [1929]
	59·9	40·1	29	1·95	1·04	
Mutton tallow	59·7	40·3	26	1·6	0·9	Collin, Hilditch and Lea [1929]
Beef tallow	51·0	49·0	14	1·1	(0·8)	Christian and Hilditch (not yet published)
Lard	44·1	55·9	8	0·85	(0·7)	Hilditch and Sleightholme (not yet published)
Rabbit fat	31·9	68·1	7	0·5	(0·4)	Vickery (private communication)

The main distinction which we wish to draw between animal and vegetable (seed) fats is sufficiently clear when cacao butter and Illipé tallow are compared with the two butter fats and mutton tallow. The unsaturated acid content of all five fats is almost equal, but, whereas the animal fats contain saturated glycerides varying between 26–31 %, the corresponding figures for the two vegetable fats are 2·5–4·5 %. It also appears that a similar distinction may be drawn between vegetable fats which are derived from seeds or kernels and those originating from other parts of the plant. Palm oil, a fruit pulp fat, and laurel oil (probably from a similar source but possibly mixed with a little seed-fat) show distinct differences in their glyceride construction from the other vegetable fats of seeds or kernels.

The clearest idea of the differences in the distribution of the fatty acids (saturated and unsaturated) is given by the association ratios in column V, which distinguish sharply between the animal fats and non-seed vegetable fats and the ten different varieties of seed-fats which have now been studied.

The data for the animal fats (the three last members of which have as yet only received preliminary investigation) show that in these there is markedly

less evenness of distribution of the fatty acids than in either class of vegetable fats; and such results as we have obtained up to the present indicate that uneven partition of the fatty acids with glycerol may be equally characteristic of vegetable fats originating elsewhere than in the seed itself. Laurel oil, with which (although not a seed-fat) we deal in this paper, appears to stand out quite exceptionally in this respect.

Turning now to the group of ten vegetable seed-fats, the "association ratio" of the saturated to unsaturated acids in the whole fats is equal to or greater than 1·5 : 1 in seven instances (three of which represent additional fats selected for study of their glyceride structure from the standpoint of the present communication); and in all but one of these the corresponding association ratio in the non-fully saturated glycerides present lies between 1·3 and 1·6 : 1, although the association ratio for the acids of the whole fat ranges from 1·5 to 15·5 : 1.

The remaining three fats contain preponderating amounts of oleic acid, and it is impossible at this juncture to discriminate between the amount of this acid present as triolein and that associated with saturated acids in mixed glycerides. The important point to observe in these liquid fats is the almost entire absence of fully saturated glycerides in spite of the presence of appreciable quantities of saturated acids (15–35 % by weight). The phenomenon of "even distribution" is evidenced here, as it were, from the opposite side: so long as there is sufficient excess of oleic acid present (in kusum oil the molecular ratio of oleic to saturated acids is about 1·8 : 1), all saturated acids are absorbed into mixed saturated-unsaturated glycerides.

It is not unreasonable to infer from this that, in those fats where the total molecular ratio of saturated : unsaturated acids exceeds the latter figure (say 2 : 1), triolein is similarly almost completely absent, so that from palm kernel fat upwards (in the table) the substantial absence of triolein may be presumed. The association ratio in the mixed glycerides is nevertheless of the same order as in the cases of Illipé tallow and cacao butter, thus again pointing to the general evenness of distribution of the fatty acids throughout kernel fats.

The principle of even distribution is seen at its maximum in cacao butter and Illipé tallow, where the proportions of saturated and unsaturated acids in the whole fats only slightly exceed the favoured mixed-glyceride "association ratio" of 1·3–1·6 : 1 and wherein, in point of fact, practically the whole of the fats are made up of mono-oleo-disaturated and di-oleo-monosaturated glycerides. Full details of the investigation of Illipé tallow will be published shortly elsewhere, but it may be said here that both this fat and cacao butter are further characterised by the presence of oleic, palmitic and stearic acids in proportions not far removed from the same molecular order, and that study of their oxidation products by special methods (of which a description has been given by Lea [1929] when dealing with cacao butter) has shown definitely that very large proportions of oleopalmitostearins, with subsidiary amounts of oleodistearins and oleodipalmitins, are present.

The fat from the seeds of *Myristica malabarica* is the only one of its type so far examined which does not fall within the scope of the generalisation suggested above. It is closely related, from the botanical standpoint, to nutmeg butter (the association ratio of which is in keeping with the rest) and the sample examined is undoubtedly a genuine kernel fat (prepared by extraction in this laboratory from nuts of authentic origin). The molecular ratio of saturated to unsaturated acids in the whole fat is much the same as in cacao butter, but the proportion of fully saturated glycerides is much higher (18 %) and the association ratio for the remainder of the fat is only 1 : 1. For the moment this must be counted as an exceptional case; it may be observed that this particular oil was accompanied by unusually large amounts of resinous and other non-fatty matter and that the mottled appearance of the kernels is some indication of unusual heterogeneity in the contents of the endosperm cells.

A few words may be added with reference to the specimen of laurel oil, the examination of which (although it is not a seed-fat) has been included in this paper owing to the interesting structure which has been disclosed. The mixed fatty acids (35 % lauric, 10 % palmitic, 36 % oleic, 19 % linoleic—molecular ratio of saturated : unsaturated, 1·1 : 1) were in proportions which would permit the association of all the saturated acid with the unsaturated acids in mixed glycerides, yet oxidation showed the presence of 26 % of fully saturated triglycerides. From the composition of the total fatty acids it follows, of course, that if saturated glycerides are present at all they must consist mainly of trilaurin; it is not the presence of trilaurin but rather the high proportion of fully saturated glycerides which is surprising, with its concurrent implication that the unsaturated acids are likewise linked with each other in an unusual degree. The low "association ratio" of 0·37 : 1 for the non-fully saturated glycerides connotes, in fact, the presence of between 15 and 48 % of triunsaturated glycerides, according to the respective amounts of di-oleo-monosaturated and mono-oleo-disaturated glycerides which may also occur in the fat.

Whether this predisposition to extreme heterogeneity is peculiar to the Lauraceae or is a frequent occurrence in vegetable non-seed fats cannot be known until this class of fats has received much more than the scanty attention so far given to them; but it may be pointed out as a matter of general interest that laurel oil is the only example we have met of a fat whose structure at all resembles the former erroneous conception of natural fats as a mixture of relatively simple triglycerides.

We would draw attention, in conclusion, to the two fats, laurel oil and nutmeg butter, wherein (from our estimations of the fully saturated glyceride contents and the composition of the saturated acids present in these fragments of the whole fats) we find evidence of the presence respectively of nearly 25 % of trilaurin and about 50 % of trimyristin. It is interesting to note that from these fats, which by chance contain large amounts of simple triglycerides,

Bömer and Ebach [1928] were able to isolate, by the fractional crystallisation process, quantities of these components of much the same order, namely, 30 % of trilaurin from laurel oil and 40 % of trimyristin from nutmeg butter; on the contrary, in spite of prolonged and laborious quantitative crystallisations, Bömer and the other workers failed to isolate more than insignificant quantities of any simple triglyceride from fats such as coconut, palm kernel, butter, tallow, etc.—all cases in which our own methods and the generalisations to which they have led us indicate that substantially only mixed glycerides are present. Bömer's procedure and our own, although fundamentally different in character, thus lead satisfactorily to exactly the same general conclusions.

Experimental.

The experimental methods employed in studying the four additional fats for which data are now given in this paper—dika fat, nutmeg butter, *Myristica malabarica* fat and laurel oil—were the same as those which have been given at length in several communications on glyceride structure from these laboratories (for example, in that [Collin and Hilditch, 1928] dealing with the component glycerides of coconut and palm kernel fats). It is thus unnecessary to recapitulate these in detail and the experimental figures given below are confined for the most part to a brief summary of the final results obtained.

So far as the determination of the composition of mixed fatty acids is concerned, we give only the final summary of the proportions estimated to be present in the "solid" and "liquid" acids (as obtained after separation by the lead salt-alcohol process) from the observed results of fractional distillation of the neutral methyl esters of each group. Fuller details of these analyses will be given elsewhere later for certain of these fats, the composition of which is of some general technical interest.

As regards the permanganate-acetone oxidations carried out in order to estimate the proportion of fully saturated glycerides present, the final weight of the latter is given after making the necessary corrections for any small amounts of acidic products of oxidation left in the purified neutral compounds. The only points of difference in the procedure now used from that adopted for coconut and palm kernel fats [Collin and Hilditch, 1928] were:

(i) the use of aqueous sodium carbonate for the separation of the oxidised fat into neutral and acidic portions was superseded by that of ammonia, which was found to have less tendency to produce emulsification;

(ii) in most of the present instances, the amount of fully saturated glycerides available was too small to permit of final purification by boiling with aqueous alkali carbonate solution, and in consequence traces of mono-azelaic glycerides were probably present in the neutral compounds when analysed for fatty acid composition. Correspondingly, traces of dimethyl azelate would be present in some of the methyl esters fractionated, leading to slightly lower saponification equivalents for the first fractions; thus the lowest fraction of

these esters from the fully saturated glycerides of laurel oil had a mean equivalent of 208·5, whereas the analysis of the original mixed fatty acids showed that no esters lower than methyl laurate (equivalent 214·0) were present. In such instances the fractions in question (in all cases small) were taken as being substantially methyl laurate.

1. Dika fat.

Dika fat is the kernel-fat from various species of *Irvingia*. The sample investigated was prepared by extracting with ether the kernels of a small consignment of the fruit of *I. Barteri*, which the Director of Forests, Ibadan, Nigeria, had been good enough to collect for us. Dika fat has previously been examined by Lewkowitsch [1905], who considered it to be a mixture of laurin and myristin with a very little olein.

The characteristics of the specimen we studied, and Lewkowitsch's corresponding data, are as follows:

	Present sample	Lewkowitsch
Saponification equivalent ...	233·9	224–232
Iodine value	9·07 %	4·3–5·2 %
Unsaponifiable matter... ...	1·05 %	—
M.P.	41–42°	38·9–41°
Mixed fatty acids (free from unsap.)		
Mean equivalent	220·3	—
M.P....	37·5–38°	—

Less than 70 g. of extracted fat was available for the complete investigation, and hence the fractionation and oxidation processes were perforce carried out on a much smaller scale than usual; the relative simplicity of the fatty acid mixture present and the high proportion of fully saturated glycerides, on the other hand, made it unnecessary to work with very large quantities of material in this particular case.

Composition of the total fatty acids of dika fat.

In view of the small quantity available and the very small proportion of unsaturated acids, the usual lead salt separation was omitted. The methyl esters of the mixed acids were resolved into six fractions by distillation under a high vacuum.

Lauric acid (anilide, M.P. 73·5°) was identified in the lowest fraction; the melting-point of the acid itself could not be raised above 36·5° by repeated crystallisation, although admixture with authentic lauric acid (M.P. 43°) raised the melting-point to 37–38°. We favour the view that traces of some lower acid rendered purification of the lauric acid unusually difficult, rather than the very unlikely alternative that an isomeric form of lauric acid is present in *Irvingia* fat.

The unsaturated acid present in small quantity was not definitely identified by conversion into a dihydroxystearic acid, but there is no reason to consider that it is other than the normal oleic acid.

The estimated composition of the total fatty acids was lauric (38·8 %), myristic (50·6 %) and oleic (10·6 %).

Oxidation of dika fat.

The fat (40 g.) was dissolved in acetone (400 cc.) and oxidised with finely-powdered potassium permanganate (80 g.), and finally yielded 31·55 g. (corrected weight) of neutral products (fully saturated glycerides), or 78·8 % of the whole fat.

Since 100 parts of original fat (sap. equiv. 233·9 and unsap. matter 1·05 %) gave 79 parts of fully saturated glycerides (sap. equiv. 228·9 and unsap. matter 0·7 %), it follows that the "association-ratio" in the non-fully saturated glycerides of the fat is 1·34 mols. saturated : 1 mol. oleic acid.

Composition of fatty acids in the fully saturated glycerides.

Methylation and fractionation of these fatty acids led to their composition being established as lauric (42·8 %) and myristic (57·2 %) acids. Lauric acid prepared from the two lowest ester fractions again possessed a low melting point, viz. 38·5° after three crystallisations (mixed with pure lauric acid, M.P. 38–41°); myristic acid was obtained from the fifth fraction (M.P. 52·5°, and, mixed with authentic myristic acid, 53°).

Distribution of the fatty acids in dika fat (estimated from the fractionation analyses).

	Total fat 100 g.	Fully sat. glycerides 79 g.	Mixed sat.-unsat. glycerides (by difference) 21 g.
Unsaponifiable matter	1·1	0·6	0·5
Glyceryl residue	5·4	4·3	1·1
Lauric acid	36·2	31·7	4·5
Myristic acid	47·4	42·4	5·0
Oleic acid	9·9	—	9·9

The data in the final column correspond with an "association ratio" of 1·3 : 1.

Percentage compositions of the saturated fatty acids present.

	(i) In the whole fat	(ii) In fully sat. glycerides	(iii) In the mixed glycerides
Lauric acid	43·3	42·8	47·5
Myristic acid	56·7	57·2	52·5

2. NUTMEG BUTTER (*Myristica officinalis*) FAT.

The fat examined was a specimen of guaranteed genuine origin supplied by Messrs Evans Sons, Lescher and Webb, Ltd. It contained 17·7 % of unsaponifiable matter and possessed an iodine value of 61·0 % (mainly due to essential oil), whilst the mean equivalent of the crude fatty acids present was 246·3.

Power and Salway [1907, 1908] gave the composition of nutmeg fat as essential oil (mainly *d*-pinene and *d*-camphene, 12·5 %), trimyristin (73·0 %),

triolein (3·0 %), trilinolein (0·5 %), resin (2·0 %) and unsaponifiable matter (8·5 %), whilst Bömer and Ebach [1928] isolated from it 40 % of trimyristin, M.P. 56·2°.

For our purposes we removed as much volatile oil as possible from the original pasty, brown fat by distillation with steam; the residue (about 70 % of the crude fat) was boiled with excess of aqueous sodium carbonate, well washed with boiling water and recovered, when it was much harder, and paler in colour, than the original fat, but still possessed an iodine value of 46 and saponification equivalent 295. These values were found to be mainly due to unsaponifiable matter which passed into solution in alcohol during the subsequent lead salt separation, and which was largely removed, prior to fractionation of the liquid acids as methyl esters, by converting the liquid acids into sodium soaps and extracting the aqueous solution of the latter repeatedly with ether.

Composition of the mixed fatty acids of nutmeg butter.

The following figures were thus finally obtained:

	"Solid" acids 71·0 %	"Liquid" acids 29·0 %	Total	Fatty acids % composition
Lauric acid	1·0	0·2	1·2	1·5
Myristic acid	60·1	1·5	61·6	76·6
Palmitic acid	8·2	—	8·2	10·1
Oleic acid	1·7	6·7	8·4	10·5
Linoleic acid	—	1·1	1·1	1·3
Unsaponifiable	—	19·5	19·5	—

From the main fraction of esters obtained on refractionation of the second primary fraction, myristic acid (M.P. 52·5–53·5°) was obtained after one crystallisation (mixed with authentic myristic acid, M.P. 52–53°), whilst the main fractions of acids from esters of the "liquid" acids yielded by oxidation with cold alkaline permanganate the characteristic dihydroxystearic acid corresponding with oleic acid, M.P. 128–129°.

Oxidation of nutmeg butter.

The refined fat (100 g.) was dissolved in acetone (1000 cc.) and treated in the usual manner with powdered potassium permanganate (400 g.); a large excess of permanganate was used in the hope that the non-fatty matter still present would be converted by oxidation into alkali-soluble products. This, as a matter of fact, took place to a large extent, since the final product contained only 1 % of non-fatty matter, as compared with 18·7 % in the material oxidised.

100 g. of the refined fat (containing 81·3 g. glycerides of mean saponification equivalent 248·1) gave 58·6 g. (corrected weight) of neutral products (fully saturated glycerides containing 1 % of non-fatty matter). Calculated on the glycerides originally present, the proportion of fully saturated components is therefore 71 %; the "association ratio" in the non-fully saturated glycerides, calculated from these figures, is 1·60 mol. saturated : 1 mol. oleic acid.

Composition of fatty acids in the fully saturated glycerides.

By fractionation of the methyl esters (35·6 g.) of the acids from the purified fully saturated material, it was estimated that there were present lauric (2·3 %), myristic (90·9 %) and palmitic (6·8 %) acids.

Distribution of the fatty acids in nutmeg butter (estimated from the fractionation analyses).

	Total fat 100 g.	Fully sat. glycerides 58·6 g.	Mixed sat.-unsat. glycerides (by difference) 41·4 g.
Unsaponifiable matter	18·7	0·6	18·1
Glyceryl residue ...	4·2	3·1	1·1
Lauric acid	1·2	1·2	—
Myristic acid... ...	59·0	50·0	9·0
Palmitic acid ...	7·8	3·7	4·1
Oleic acid	8·1	—	8·1
Linoleic acid... ...	1·0	—	1·0

The data in the final column correspond with an "association ratio" of 1·7 : 1. (The mean value 1·65 : 1 for the nutmeg butter "association ratio" corresponds with a mixture of 87 mols. mono-oleo-disaturated and 13 mols. di-oleo-monosaturated glycerides, if no triolein is present.)

Percentage composition of the saturated fatty acids present.

	(i) In the whole fat	(ii) In fully sat. glycerides	(iii) In the mixed glycerides
Lauric acid	1·7	2·2	—
Myristic acid ...	86·8	91·1	68·7
Palmitic acid ...	11·5	6·7	31·3

Owing to the very small proportion of mixed glycerides present in the fat, the values in the last column (which depend on small differences between relatively large numbers) are probably less reliable than the remainder.

3. *Myristica malabarica* fat.

The kernels of *M. malabarica* contain a fat in which oleic acid occurs in much higher proportion than in that from the related *M. officinalis*, the respective "association ratios" for the total fatty acids being 1·5 mol. and 9·2 mols. of saturated acids per mol. of unsaturated acids. The fat examined was prepared by extraction with carbon tetrachloride from seeds supplied by the District Forest Officer, Malabar District, Madras Presidency; it was a very dark-coloured, almost liquid fat and contained large amounts of resinous and non-fatty matter, which were extracted, as far as possible, by washing the fat (in ethereal solution) with aqueous ammonia and subsequently boiling it with excess of concentrated sodium carbonate solution. The fat, after refining in this manner, was semi-solid at the ordinary temperature, but still dark in colour; its apparent saponification equivalent (unsaponifiable matter still present) was 280, and its iodine value 77 %.

Composition of the mixed fatty acids of Myristica malabarica *fat.*

The following figures were obtained by analyses conducted on similar lines to those used for the nutmeg butter acids:

	"Solid" acids 36·2 %	"Liquid" acids 63·8 %	Total	Fatty acids % composition
Myristic acid ...	18·2	4·1	22·3	39·2
Palmitic acid ...	6·0	1·6	7·6	13·3
Other saturated acids	1·3	—	1·3	2·4
Oleic acid	2·7	22·4	25·1	44·1
Linoleic acid ...	—	0·6	0·6	1·0
Non-fatty matter ...	8·0	35·1	43·1	—

Palmitic acid (M.P. 60°) was identified in the penultimate fraction of distilled methyl esters of the "solid" acids, whilst from the residue from this distillation there was obtained in small amount the dihydroxystearic acid (M.P. 128°) corresponding with oleic acid and a mixture of saturated acids which, after three recrystallisations, melted alone at 63°, but, when mixed with pure palmitic acid, at 54–55°; for the purposes of calculation, the higher acid present was taken as stearic acid. Oleic acid was recognised by oxidising the acids from several of the "liquid" ester fractions with cold alkaline permanganate, when dihydroxystearic acid, M.P. 130°, was readily obtained.

The residue from the fractional distillation of the "liquid" esters contained most of the non-fatty matter originally present and was, of course, very large, in spite of carrying the distillation as far as possible by intensive heating; it was submitted to oxidation both by the cold alkaline permanganate process and by anhydrous permanganate in boiling acetone solution, and no evidence of the presence of more than traces of retained methyl oleate was obtained in either case. Consequently it was reckoned entirely as non-fatty matter in the calculation.

Oxidation of Myristica malabarica *fat.*

The oxidation by means of potassium permanganate in acetone solution was not completed in one operation and it was therefore necessary to submit the primary neutral products to a repetition of the process. Finally, from the refined fat (264·35 g.) there was obtained 28·3 g. (corrected weight) of fully saturated glycerides, or 10·7 % (without correction for unsaponifiable matter).

Thus, 100 parts of original material (containing 58·1 parts of glycerides, mean equivalent 255·0) yielded 10·7 parts of fully saturated material (containing 3 % non-fatty matter, mean equivalent of fatty compounds present 243·3); the "association ratio" in the non-fully saturated glycerides is thus 0·99 mol. saturated : 1 mol. unsaturated acids.

Composition of fatty acids in the fully saturated glycerides.

This was determined, by fractionation analysis of the methyl esters (15 g.), to be myristic (43·9 %), palmitic (54·4 %) and stearic (1·7 %) acids.

The main component of the earlier fractions of esters was methyl myristate (myristic acid, M.P. 52°, unchanged when mixed with authentic myristic acid,

after two crystallisations), whilst the residue consisted mainly of methyl palmitate with a small proportion of the methyl ester of a higher acid (calculated as stearic acid).

Distribution of the fatty acids in Myristica malabarica *fat (estimated from the fractionation analyses).*

	Total fat 100 g.	Fully sat. glycerides 10·7 g.	Mixed sat.-unsat. glycerides (by difference) 89·3 g.
Unsaponifiable matter	41·9	0·3	41·6
Glyceryl residue ...	2·7	0·5	2·2
Myristic acid... ...	21·7	4·3	17·4
Palmitic acid ...	7·4	5·4	2·0
Stearic acid	1·3	0·2	1·1
Oleic acid	24·4	—	24·4
Linoleic acid... ...	0·6	—	0·6

The data in the final column again correspond with an "association ratio" of 0·99 : 1.

Percentage composition of the saturated fatty acids present.

	(i) In the whole fat	(ii) In fully sat. glycerides	(iii) In the mixed glycerides
Myristic acid... ...	71·4	43·4	84·9
Palmitic acid ...	24·3	54·6	9·7
Stearic acid	4·3	2·0	5·4

4. Laurel fat.

The specimen investigated was supplied by Messrs Evans Sons, Lescher and Webb, Ltd. and had the following characteristics:

Saponification equivalent	269·8
Acid value	9·0
Iodine value	86·4 %
Unsaponifiable matter	6·2 %
Mean equivalent of mixed fatty acids (freed from unsaponifiable matter)	249·5

In the earlier literature [cf. Matthes and Sander, 1908] this fat was considered to be composed almost entirely of trilaurin and its high iodine value to be due to the presence of essential oil; whilst the latter doubtless contributes to the observed iodine value, we now show that over half of the total fatty acids are made up of oleic and linoleic acids [cf. also Lewkowitsch-Warburton, 1922].

Bömer and Ebach [1928] isolated 30 % of trilaurin, M.P. 45·6°, from laurel oil by fractional crystallisation of the fat from ether and acetone.

The oil which we examined was semi-solid and was dark green in colour; it was refined, prior to investigation, by boiling with aqueous sodium carbonate and subsequent thorough washing with boiling water.

Composition of the mixed fatty acids of laurel fat.

The following figures were obtained:

	"Solid" acids 50·7 %	"Liquid" acids 49·3 %	Total	Fatty acids % composition
Lauric acid	23·5	8·2	31·7	35·0
Palmitic acid ...	8·8	—	8·8	9·7
Oleic acid	12·9	20·1	33·0	36·6
Linoleic acid	2·5	14·4	16·9	18·7
Unsaponifiable matter	3·0	6·6	9·6	—

The fractionation of the "solid" methyl esters was abnormal in that between the second fraction (28·3 g., B.P. 87°/1 mm., sap. equiv. 214·9) and the fourth (17·9 g., B.P. 125–127°/1 mm., sap. equiv. 274·3) there was only a very small intermediate fraction (7·0 g., B.P. 87–125°/1 mm., sap. equiv. 253·8) covering a very wide range of boiling-point. Special attention was paid to the fatty acids present in this ester-fraction: palmitic acid (M.P. 62·5°) was readily obtained in quantity, but the more soluble portions present on further crystallisation eventually gave palmitic acid (M.P. 61–62°), except for one crop from the original mother liquors, which yielded a small amount of acid, M.P. 53–55°. This, however, on admixture with myristic acid, melted indefinitely at 44–46·5°. We conclude, therefore, that myristic acid is either absent, or present only in very small quantities; and we have based the analytical calculations on a mixture of lauric and palmitic acids.

Lauric acid (M.P. 43–44°) was isolated readily from the lower ester-fractions, and palmitic acid (M.P. 62–62·5°) from several of the higher fractions of the "solid" esters.

The main fractions of the liquid esters were hydrolysed and oxidised with ice-cold aqueous alkaline permanganate solution, when a tetrahydroxystearic acid soluble in hot water (M.P. 149–150°), a tetrahydroxystearic acid insoluble in hot water or ethyl acetate (M.P. 169–171°) and a dihydroxystearic acid soluble in hot ethyl acetate (M.P. 128–129°) were obtained in each case examined. These are the characteristic products of alkaline oxidation, under the conditions described, of ordinary oleic and linoleic acids.

Oxidation of laurel fat.

The refined fat (100 g.) was oxidised as in the cases previously described and the primary neutral products submitted to re-oxidation; there were finally produced 26·4 g. (corrected weight) of fully saturated glycerides. Allowing for unsaponifiable matter present in the material oxidised, this leads to an "association ratio" in the non-fully saturated glycerides of 0·37 mol. saturated per mol. unsaturated acids.

Composition of fatty acids in the fully saturated glycerides.

This was determined to be: lauric (94·0 %) and palmitic (6·0 %) acids.

Distribution of the fatty acids in laurel fat (estimated from the fractionation analyses).

	Total fat 100 g.	Fully sat. glycerides 26·4 g.	Mixed sat.-unsat. glycerides (by difference) 73·6 g.
Unsaponifiable matter	9·2	0·3	8·9
Glyceryl residue ...	4·5	1·6	2·9
Lauric acid	30·2	23·0	7·2
Palmitic acid ...	8·4	1·5	6·9
Oleic acid	31·6	—	31·6
Linoleic acid	16·1	—	16·1

The "association ratio" for the non-fully saturated glycerides from these data is the same as that given above, 0·37 : 1.

It should be observed that this figure is consonant with the presence of 81 mols. of di-unsaturated-monosaturated and 19 mols. of tri-unsaturated glycerides, or with that of 40 mols. of mono-unsaturated-disaturated and 60 mols. of tri-unsaturated glycerides in the non-fully saturated portion. This implies that in the whole fat there must be (by weight) at least 15 % of tri-unsaturated glycerides and there may be as much as 48 %; whilst the amounts of mono-unsaturated-disaturated and di-unsaturated-monosaturated glycerides must correspondingly lie between nil and, respectively, 26 % and 59 %.

Percentage composition of the saturated fatty acids present.

	(i) In the whole fat	(ii) In fully sat. glycerides	(iii) In the mixed glycerides
Lauric acid	78·2	94·0	50·7
Palmitic acid	21·8	6·0	49·3

SUMMARY.

1. Sufficient natural fats have now been examined with reference to their glyceride structure to justify the general statement that animal fats are built up on more heterogeneous lines than vegetable seed-fats, whilst it is beginning to appear that vegetable fats from parts of the plant other than the seed itself resemble the animal fats rather than kernel fats so far as the distribution of fatty acids amongst the glycerides is concerned.

2. Previous study of four seed-fats (coconut and palm kernel fats, cacao butter and Illipé tallow), in which the total molecular ratio of saturated to unsaturated acids is equal to or greater than 1·5 to 1, has disclosed the presence therein of fully saturated glycerides in amounts varying from 2·5 % to 84 % as the association ratio referred to increases from 1·5–15·5 to 1, and, correspondingly, the occurrence of saturated and unsaturated acids in mixed glycerides in proportions denoted by molecular association ratios varying from 1·3–1·55 to 1—an almost constant ratio in comparison with the widely differing characteristics of the fats in other respects.

In the present paper three further fats of similar total association ratio for the saturated and unsaturated acids have been investigated, and two of these

have been found to conform closely with the foregoing rules, whilst the third (*Myristica malabarica* fat) was found to be somewhat exceptional.

3. Three other seed-fats (kusum, cottonseed and ground-nut oils) which contain preponderating amounts of unsaturated acids, and have already been studied from this point of view, are all characterised by the almost complete absence of any fully saturated glycerides, although the proportion of saturated fatty acids in the whole fat ranges from 15 to 35 % in the oils in question.

4. All these data illustrate the pronounced tendency to even distribution of the fatty acids throughout the glycerides of seed-fats as a class. If the molecular proportion of unsaturated to saturated acids in the whole fat exceeds about 1·6 : 1, the saturated acids are almost wholly present in the form of mixed glycerides; if the aforesaid molecular proportions are reversed, increasing amounts of fully saturated glycerides are found to be present, but in every case but one so far examined the mixed saturated-unsaturated glycerides present contain saturated and unsaturated acids in molecular association ratios lying within the comparatively narrow limits of 1·3–1·6 to 1 (usually 1·3–1·4 to 1); finally, when fully saturated glycerides are present in quantity, no simple triglyceride has been detected (even when one acid forms 30–50 % of the whole of the saturated acids, *e.g.* lauric acid in coconut and palm kernel fats or palmitic and stearic acid in cacao butter or Illipé tallow) unless the composition of the saturated fatty acids is so simple that one acid is present in overwhelming excess (*e.g.* myristic acid in nutmeg butter, which contains a considerable proportion of trimyristin).

5. Laurel fat, although not a true seed-fat, has been included in this communication because of its interesting glyceride structure, which is strongly heterogeneous: although the molecular proportions of saturated and unsaturated acids in the whole fat are approximately equal, the fat contains 26 % of fully saturated glycerides (largely trilaurin) and the molecular association ratio for the acids of the mixed glycerides is 0·37 to 1, connoting the presence of tri-unsaturated glycerides in quantity.

6. Consideration of all these vegetable fats by the experimental methods described leads to the conclusion that simple saturated triglycerides exist in only two of them, namely, nutmeg butter and laurel fat; and it is of great interest to note that exhaustive fractional crystallisation of a number of these solid fats in the hands of Klimont, Bömer and their co-workers has given negative results in the isolation of simple saturated triglycerides, except in the two cases mentioned. In these instances, however, Bömer and Ebach [1928] isolated trimyristin and trilaurin respectively in amounts which are of the same quantitative order as the proportions disclosed by our present study.

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