Lectures of the 1960 Short Course on Edible Fats

conducted by

The American Oil Chemists' Society

at the

University of Illinois, Urbana, July 25-27

Under the sponsorship of the Education Committee, J. C. Cowan, chairman, with R. T. Milner as local chairman and L. R. Dugan Jr. as program chairman

Part II

The Glyceride Structure of Fats and Oils

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IF ONE WERE TO DEAL OUT, into piles of three, a well-shuffled deck of cards (from which the joker was excluded) and were to do so again and again, one would eventually lay down 64 different sequential combinations of the various suits. Some of these would be simply the reverse of others. For instance, the sequence clubs—diamonds—hearts is simply the reverse of hearts—diamonds—clubs. If these and other similar pairs are considered equals, there still remain 40 different and distinct combinations of three cards each, derivable from the four different suits.

The triglycerides of a natural fat composed of four different fatty acids esterified with glycerol likewise can exist in 64 different theoretical combinations, of which 40 are chemically distinguishable. A natural fat comprised of only four fatty acids would however be rare indeed. Butter fat is comprised of at least 28 different varieties capable of existing in nearly 15,000 quite distinct combinations, each of which is a specific triglyceride.

It would, of course, be a stupendous task to separate and identify each of these combinations even in a relatively simple fat and to determine its percentage of the whole fat. If all the components were dispersed at random, it would naturally be quite a simple matter to calculate the percentage of each variety of triglyceride from the component acid analysis of the whole fat by applying the simple rules of probability. But it is not so simple because usually distribution in natural fats is not completely at random.

After the discovery by Chevreul in 1823 that fats are glyceryl esters of fatty acids, it was more or less tacitly assumed, according to Hilditch (1), that they are mixtures of simple triglycerides, such as tristearine, triolein, etc. This belief was challenged near the middle of the century by Berthelot, who pointed out the possibility that they could contain molecules of the mixed type comprising two or three different fatty acids.

Hilditch (1) and his co-workers have studied the glyceride structure of fats by a variety of techniques, the best of which is fractional crystallization at moderate and low temperatures, followed by component acid and other analysis of the fractions. They found considerable evidence of nonrandom attachment of specific varieties of acyl groups at the 2-positions of the molecule, findings later confirmed and elaborated by Mattson and coworkers (2,3), and by some others cited by Mattson *et al.*

These discoveries have disposed of any likelihood that, except in rare instances, the fatty acyl groups in fats are distributed completely at random. It is therefore not ordinarily possible to calculate the proportions of the specific triglycerides in fats from the component acid analysis of the whole fat by application of the rules of probability.

The percentages of the glyceride types S_3, S_2U, SU_2, and U_3 in a wide variety of fats were estimated by Hilditch and his co-workers. In many cases the percentages of more specifically defined triglycerides, such as disaturated oleins, saturated dioleins, and dioleolinoleins, were also estimated.

THE WORK of Hilditch and his co-workers leads to the observation that there is a tendency in the natural fats toward heterogeneity in the distribution of the acyl groups among the various molecules. In other words, there is a tendency to produce as little as possible of the simple triglycerides and a corresponding tendency to produce mixed triglycerides. This came to be known as the principle of "even distribution" and is defined most recently by Hilditch in the third edition of his well-known volume.

The next major advances in the study of glyceride structure were disclosed by A. R. S. Kartha (4) in 1949. One of Kartha's contributions was a new method for determination of the glyceride types S_3, S_2U, SU_2, and U_3 by a less difficult and perhaps more consistently accurate procedure than those used by Hilditch and his colleagues.

This method has been replaced by still better procedures and need not be discussed in detail. It includes a process for modifying the unsaturated groups in fat molecules by oxidation, and partial separation of the reaction products by solubility differences after conversion of the acidic portions to the magnesium salts.

The results of analysis by this procedure agree very well, in most cases, with those obtained by another procedure devised by Kartha, by means of which the proportions of the glyceride types are calculated from the S and S_3 content. This method of calculation is based on the following assumptions: a) that in living tissue the acyl groups in the fat are constantly interchanging with each other, b) that they are all equally reactive, c) that acyl groups in the 1-, 2-, and 3-positions interchange with equal readiness, and d) that no more S_3 can be produced than will remain in the living tissue in a state of fluidity. Under these circumstances distribution of the acyl groups at any instant will be at random, providing that the quantity of S_3 so produced can exist in the living tissue in a fluid state. In case the fat contains so much S that the amount of S_3 which would be produced by strictly random distribution exceeds the amount which can remain fluid, the excess S must combine with U to form mixed triglycerides. The following example illustrates these variations.

In a natural fat in which the fatty acyl components consist of 50% of S and 50% of U, the percentages of the triglyceride types produced by random distribution would be:

S_3	S_2U	SU_2	U_3
12.5	37.5	37.5	12.5

If 12.5% of S_3 can exist as a fluid in the living tissue, the fat *in vivo* will have this composition (according to Kartha) at any instant. Upon the death of the tissue or maturation of the seed, the interchange of groups will stop, but the proportions of the glyceride types will not change.

If however no S_3 whatsoever can exist in the living tissue in the fluid state, no S_3 at all will be produced, according to the theory. Then the S which otherwise would have produced S_3 must combine with U to form S_2U or SU_2. Calculated by Kartha's method, from the percentage of S which is 50 and the percentage of S_3 which is 0, the percentages of the glyceride types will then be:

S_3	S_2U	SU_2	U_3
0	58.4	33.3	8.3

If any quantity at all of S_3 can be present in the tissue as a fluid, the proportions of the remaining types will vary accordingly. This type of distribution has been called "restricted random distribution."

The results obtained by Kartha's methods agree in almost all cases with the values for the glyceride types obtained through other procedures by Vander Wal (5), Youngs (6), and Hammond (8), which will be discussed later.

It was however soon pointed out that Kartha's dynamic equilibrium concept is not entirely in agreement with the facts. Shown in Figure 1 is a molecule of glycerol, the carbon atoms of which are numbered 1, 2, and 3. Esterification of this molecule with two molecules of saturated and one molecule of unsaturated fatty acid can occur in three ways, as shown in Figure 1. Molecule A is however not chemically distinguishable from Molecule B so actually only two chemical species are produced.

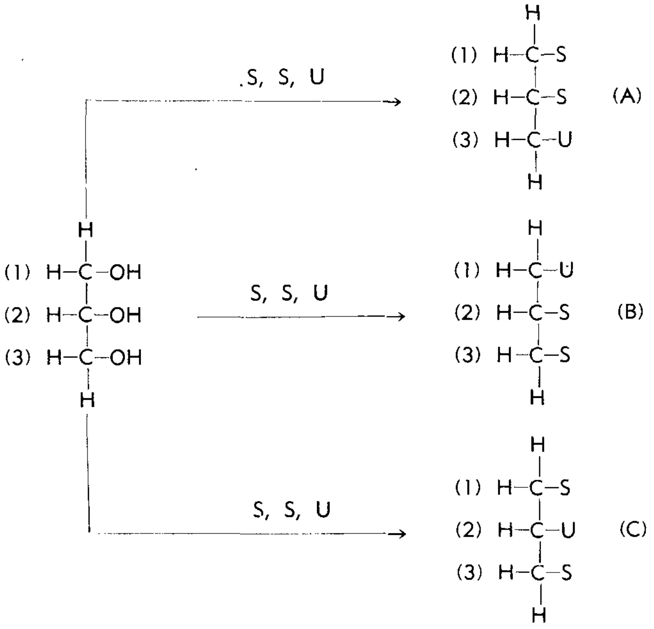

FIG. 1. Specific triglycerides obtainable by esterification of two saturated and one unsaturated acids with glycerol.

Note however that if A, B, and C are produced with equal facility, as when reaction occurs at random, there is twice as much opportunity for formation of the combined unsymmetrical forms of S_2U, which we will designate as SSU, as for formation of SUS. In the same way two molecules of SUU, the unsymmetrical form of SU_2, will be produced for each one of USU. A state of dynamic equilibrium resulting in random distribution of S and U in triglycerides will, then, result in two parts of the unsymmetrical

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TABLE I*
Triglyceride Types and Isomeric Forms in Some Hypothetical and Natural Fats

Fat		Composition: Types (% wt.)				Composition: Isomers (% wt.)			
		GS_3	GS_2U	GSU_2	GU_3	SUS	SSU	USU	UUS
Hypothetical, No. 1 a = 50; b = 50 Distribution, random	Calc., present method	12.5	37.5	37.5	12.5	12.5	25.0	12.5	25.0
	Calc., random distribution	12.5	37.5	37.5	12.5	12.5	25.0	12.5	25.0
Hypothetical, No. 2 a = 50; b = 0 Positional distribution random	Calc., present method	0	56.25	37.5	6.25	56.25	0	0	37.5
	Calc., restricted random dist'n	0	58.4	33.3	8.3	19.5	39.0	11.1	22.2
Kokum butter (2) a = 59.3; b = 3.7	Calc., present method	2.8	73.9	21.6	1.6	73.1	0.8	0	21.6
	Found, (58.9% S in sample) (4)	1.5	76.1	20.8	1.6	......	......	......	......
	Calc., restricted random dist'n (58.9% S in sample)	2 [a]	75.4 [a]	20.6 [a]	2 [a]	25.1	50.3	6.9	13.7
Pig fat (2) a = 36.0; b = 70.7	Calc., present method	2.5	22.4	55.7	19.4	1.0	21.4	46.9	8.8
	Found, (37.8% S in sample) (5)	2.9 [a]	25.3 [a]	53.3 [a]	18.5 [a]	......	......	......	......
Peanut oil (2) a = 20.9; b = 1.4	Calc., present method	0.1	9.9	42.5	47.5	9.3	0.6	0.7	41.8
	Found, (19.5% S in sample) (3)	0 [a]	9 [a]	42 [a]	49 [a]	......	......	......	......
	Calc., random dist'n, (20% S in sample)	0.8	9.6	38.4	51.2	3.2	6.4	12.8	25.6
Beef fat (2) a = 53.5; b = 29.2	Calc., present method	12.6	43.7	35.3	8.4	30.6	13.1	3.4	31.9
	Found, (59.4% S in sample) (5)	18.2 [a]	46.6 [a]	30.3 [a]	4.9 [a]	......	......	......	......
Cocoa butter (2) a = 59.9; b = 9.8	Calc., present method	7.1	67.5	23.3	2.1	65.0	2.5	0.2	23.1
	Found, (59.8% S in sample) (6)	2 [a]	77 [a]	21 [a]	......	......	......	......	......
Pig fat, safflower oil diet (2) a = 22.3; b = 44.0	Calc., present method	0.6	9.6	45.8	44.0	0.7	8.9	34.5	11.3
	Calc., random distribution	1.1	11.6	40.4	46.9	3.9	7.8	13.5	27.0
Rat fat, fat-free diet (2) a = 14.8; b = 17.2	Calc., present method	0.3	5.7	32.3	61.7	1.6	4.1	12.8	19.5
	Calc., random distribution	0.3	5.6	32.2	61.8	1.9	3.7	10.7	21.5
Soybean oil (2) a = 12.8; b = 0.0	Calc., present method	0	3.7	31.0	65.3	3.7	0	0	31.0
	Calc., random distribution	0.2	4.3	29.2	66.3	1.4	2.9	9.7	19.5

* Reprinted from Reference 5: [a] % Mol. In these fats the differences between % mol and % wt. are negligible.

forms of mixed triglycerides, SSU and UUS, for each part of the symmetrical forms, SUS and USU. Restriction of the proportion of S_3 formed in the reaction would make no difference in this ratio. But Hilditch long ago demonstrated that, in some fats at least, the unsymmetrical and symmetrical isomers of S_2U and SU_2 do not exist in the ratio of 2 to 1, and Lutton, Mattson, *et al.* (2,3) have supported and expanded these observations.

It is obvious therefore that even though Kartha's method of calculation of the glyceride types does give values usually agreeing with those of others, the theory of a dynamic equilibrium of equally reactive acyl groups among equally reactive positions of the glycerol component is not tenable. This defect in Kartha's theory shows up strongly when an attempt is made to calculate the glyceride type of composition of lard. The latter is sharply distinguished from all other fats on record by the fact that an extraordinarily large part of the 2-positions is occupied by saturated acyl groups. This circumstance makes it impossible to calculate the correct values by means of Kartha's formulas.

The basis for the next forward stride in the elucidation of glyceride structure was laid in 1956 by Mattson and Beck (2), who found that long-chain acyl groups in the 1- and 3-positions of triglyceride molecules can be selectively removed by digestion of the fat with pancreatic lipase. Analysis of the fragments enabled them to conclude, in agreement with the earlier observations of Hilditch, that, in some fats at least, the acyl groups are not distributed at random but that some of them are to be found in greater- or lesser-than-random proportions in the 2-positions.

In 1958 Mattson and Lutton (3) published the results of a series of quantitative analyses of various fats by which they determined the proportions of S in the whole fats and the proportions of S in the 2-monoglycerides that remained after the groups in the 1- and 3-positions were removed by means of pancreatic lipase hydrolysis.

USING THESE DATA, Vander Wal (5) published a procedure for calculating the proportions, not only of the S_3, S_2U, SU_2, and U_3 but also those of the isomeric forms of S_2U, which are SSU and SUS, and those of SU_2, which are UUS and USU.

The method is based on the following two postulates: whatever proportions of S and U occupy the collective 1-positions, the collective 2-positions, and the collective 3-positions in the triglycerides of a natural fat, they are dispersed therein at random; whatever proportions of S and U occupy the collective 1-positions, equal proportions occupy the collective 3-positions. A. S. Richardson (5) was quite likely the first to conceive of the pattern of distribution represented by these two postulates.

How well the values calculated by this method agree with some experimental and known values is shown in Table I. In the table the letter a, in the first column, represents the percentage of S in the whole fat while b represents the percentage of S in the acyl groups in the 2-position only. The mathematical procedure can be found in Reference 5.

It can be seen that there is good agreement between the experimental and calculated values where the samples are comparable except in the case of cocoa butter. Even in this instance the divergences are so small that they could well be due to experimental error. Calculated and experimental values agree well for lard, which has a large preponderance of S in the 2-position, and for kokum butter, in which

U greatly preponderates in the same position. Note that one is an animal fat and the other a vegetable fat. Application of the method to the hypothetical fat of known constitution (No. 1) shows that the mathematical process is a valid one. As will be described later, other investigators are in agreement with the results.

It seems reasonable to conclude that for fats such as these, in which C_{16}–C_{18} component acids predominate and in which shorter or longer components are present in relatively small proportions, the process is likely to give a good representation of the actual structure.

Shortly after the foregoing procedure was described in 1959, A. S. Richardson, in private correspondence, described a much simpler method of calculation giving the same results, which presumably he will describe.

Quite recently Youngs (6) has announced the discovery and development of another process for determining the proportions of the glyceride types and isomers, based on separation by means of chromatography of the products of partial oxidation of the unsaturated molecules. Analyses by Youngs confirm the accuracy of Vander Wal's results.

Dutton (7) has described recently the application of the counter-current distribution technique to the analysis of cocoa butter. His article, when published, will contain references to other work by means of the same procedure.

Hammond (8) has employed the temperature gradient technique in research on glyceride structure. His results also agree with those of Vander Wal.

It should be obvious by now that rather than being a helter-skelter, unpredictable, unordered mixture of triglyceride components, the natural fats are highly organized and quite predictable.

THE END is not yet. Another great problem is the explanation of how both plants and animals can produce such complicated but precisely organized substances as fats. This is partly the biochemist's and the enzymologist's job, but the mathematical relationships discussed in this paper must be satisfied by any system of synthesis they evolve. Indeed these individuals may very profitably be guided thereby.

From the point of view of the chemist in industrial fats and oils a knowledge of the structure of fats is as important as a road map to the traveller. Research heretofore has been done without real knowledge of the structure of the substances involved and has therefore been carried on in a state of semiblindness. Now we shall be able to see where we are, what we have to work with, and what we can hope to achieve. The results should be better products, more economically produced, with advantages to all concerned.

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Interesterification of Edible Fats

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DURING THE LAST TEN YEARS, rearranged lard has become a very important shortening ingredient. It is prepared from natural lard by redistributing the fatty acid radicals among the triglyceride molecules.

That esters can exchange acid groups has been known for almost one hundred years. In 1865, Friedel and Crafts, of Friedel-Crafts reactions fame, heated a mixture of amyl acetate and ethyl benzoate at 300°C. in a sealed tube. When they analyzed the contents of the tube, they found ethyl acetate and amyl benzoate as well as the original amyl acetate and ethyl benzoate (1). This classical experiment was the first demonstration of interesterification, the exchange of acyl and alkyl groups between esters:

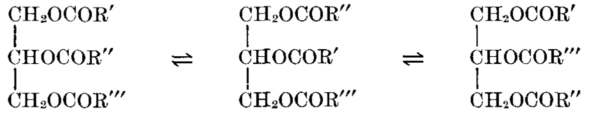
$$RCOOR' + R''COOR''' \rightleftharpoons RCOOR''' + R''COOR'$$

In this discussion the term interesterification will refer only to an exchange of acid or alcohol groups by esters. This is sometimes also called ester interchange or transesterification. Although we are primarily concerned with the interesterification of edible fats, it will be necessary to refer to a number of related ester reactions from time to time. 1. *Esterification* is the formation of an ester and water from an alcohol and acid. 2. *Hydrolysis* is the splitting of an ester by water. Free alcohol and acid are formed. 3. *Saponification* is the splitting of an ester by alkali. Soap and alcohol are formed. 4. *Alcoholysis* is a displacement of the alcohol radical of an ester by another alcohol. 5. *Acidolysis* is a displacement of the acid radical of an ester by another acid.

Interesterification of Triglycerides

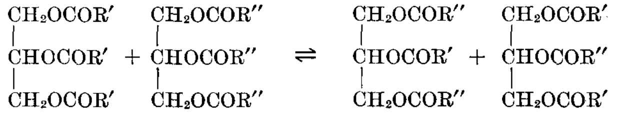
Fats have three ester linkages per molecule. Therefore the interchange possibilities are many. Not only can the fatty acid groups exchange positions within the molecule, but each fatty acid can also trade places with any one of three fatty acids in another molecule:

Interesterification within Molecules



$$\begin{array}{l} CH_2OCOR' \\ | \\ CHOCOR'' \\ | \\ CH_2OCOR''' \end{array} \rightleftharpoons \begin{array}{l} CH_2OCOR'' \\ | \\ CHOCOR' \\ | \\ CH_2OCOR''' \end{array} \rightleftharpoons \begin{array}{l} CH_2OCOR' \\ | \\ CHOCOR''' \\ | \\ CH_2OCOR'' \end{array}$$

Interesterification between Molecules



$$\begin{array}{l} CH_2OCOR' \\ | \\ CHOCOR' \\ | \\ CH_2OCOR' \end{array} + \begin{array}{l} CH_2OCOR'' \\ | \\ CHOCOR'' \\ | \\ CH_2OCOR'' \end{array} \rightleftharpoons \begin{array}{l} CH_2OCOR'' \\ | \\ CHOCOR' \\ | \\ CH_2OCOR'' \end{array} + \begin{array}{l} CH_2OCOR' \\ | \\ CHOCOR'' \\ | \\ CH_2OCOR' \end{array}$$