The Determination of Glyceride Structure[1]

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Abstract

Methods for the determination of glyceride structure are discussed. These fall, with some overlapping, into two categories: methods applicable to fats generally and methods applicable to the natural fats but not to fats generally.

Introduction

THE QUANTITATIVE STUDY of the component triglycerides in fats developed slowly over many years after Chevreul, in 1823, established the fact that fats are the glyceryl esters of long-chain fatty acids. For more than a century the separation of fats by physical means yielded mainly qualitative information, although some quantitative values were reported.

In 1927 Hilditch began to study glyceride composition by chemical procedures, and by combination of these with fractional crystallization. The latter technique became increasingly effective, and Hilditch and his coworkers employed it in a large part of their vast studies of the composition of the natural fats.

In 1951 two small related volumes were published by Kartha, the first containing an account of some rather startling observations and speculations on the structure of fats taken from his doctoral thesis, presented in 1949. Fortunately, they were widely distributed in this country by our colleagues at the U.S.D.A.'s Eastern Regional Laboratory. The concepts described by Kartha were novel and they were backed up by rather impressive analytical evidence. Further publicity in the JAOCS helped to awaken a great new interest in the subject of glyceride structure, both here and abroad.

Kartha's concepts, and his supporting evidence, must be recognized as brilliant contributions even though some of them have been the subjects of criticism and controversy. His work was provocative and provided the initial impetus for many of the advances made in recent years.

Methods and Discussion

The processes for the determination of glyceride structure fall, with some overlapping, into two categories. These are, methods applicable to fats generally, and methods applicable to the natural fats, but not to fats generally. These will be discussed in that order.

The following symbols and conventions will be used in the text: S = saturated acyloxy groups or acids. U = unsaturated acyloxy groups or acids.

Triglycerides will be designated by their acyloxy groups. Thus, S_2U represents a triglyceride type containing two saturated and one unsaturated acyloxy group, without regard to their orientation. SUS and SSU represent the two isomeric forms of S_2U and indicate how the groups are oriented.

When it is indicated that triglycerides are composed of or contain specific acids or groups of acids, it is to be understood that these are present in ester form.

Common fatty acids are sometimes designated by their carbon numbers with superscript marks to indicate the number of double bonds, if any. Thus, C_{18}'' stands for linoleic acid which has eighteen carbons and two double bonds.

Methods Applicable to Fats Generally

Several methods can be applied to the analysis of any mixture of triglycerides, including the natural fats.

Fractional crystallization. This procedure, mentioned earlier, has been described thoroughly by Hilditch in his well-known volume (1) and will not be discussed in detail here. Acetone is the preferred solvent and the initial separation is at the lowest temperature employed, which may be as low as −70C. Further separations are made at increasing temperatures and the intermediate fractions are recrystallized until little or no change occurs.

It is assumed that each final fraction contains no more than two contiguous members of the four possible categories, or glyceride types, S_3,S_2U,SU_2, and U_3; or of glycerides containing respectively 0,1,2 or 3 units of a single characterizing component, such as elaidic or linoleic acid.

The percentages of the component acids in each fraction are then determined and the glyceride compositions calculated, often with supplementary information such as may be gained by oxidation or hydrogenation followed by other analyses.

This process, obviously, is complex and time-consuming, but it has one outstanding advantage in that the separated components are not wholly destroyed. By today's techniques for component acid analysis and for determination of the positions occupied by component groups, much data are readily available that were unobtainable earlier without great expenditure of time and effort.

Kartha's oxidation-fractionation method. Kartha (15,5) produced two methods for determination of glyceride structure, one of which can be applied only to the natural fats. The other, which follows, is similar in principle to a procedure employed earlier by Hilditch, Lea, and others. Although Kartha applied it only to the natural fats, there is no obvious reason why it cannot be applied generally.

By Kartha's method the unsaturated components in the fat are oxidized, by means of potassium permanganate in acetone and acetic acid, to free-acid-containing residues (supposedly azelaides).[2] The result with natural fats is conversion of the usual mixture of S_3,S_2U,SU_2, and U_3 into the corresponding mixture of S_3,S_2A,SA_2 and A_3. The proportions of S_2A and SA_2 are determined through the differing solubilities of their magnesium salts, and from these the percentages of the parent compounds S_2U and SU_2 are available. The percentage of S_3 is determined by separate fractional crystallization and that of U_3 is found by difference.

Although the accuracy of this procedure has been attacked, it is true that in the hands of Luddy et al. (2,17) it has given results in moderately good accord with more recent analyses (3,4).

The analysis is difficult and tedious, and the identities of the components are, in part, destroyed. It

[1] Presented in part at the AOCS meeting in New Orleans, La., 1962.

[2] The symbol A here represents component azelaic acid, present in the glyceride as the half ester.

served very well, however, to support Kartha's theories, and his other process to be described later. It is, of course, applicable to fractions, mixtures, and synthetics as well as to natural fats.

Countercurrent distribution. In countercurrent distribution the substance to be fractionated is subjected to repeated partition between two immiscible liquid phases, in multistage apparatus such as was first described by Craig in 1944. The theory and practice have been described recently by Scholfield (6).

Using a 200 tube automatic apparatus Dutton, Scholfield and collaborators (7,8,9,10,11) have studied the glyceride structure of linseed oil, soybean oil, safflower oil, and cocoabutter. They used, almost exclusively, as the solvent system, a pentane-hexane-furfural-nitroethane mixture, which separates into two immiscible phases, and made from 800–1100 transfers (partitions) to achieve their separations.

Weight distribution, iodine value distribution, and fatty acid composition were determined after removal of the solvent from the separated fractions. In the recent study on cocoabutter using tracer substances, radioactivity distribution was also determined.

When plotted against the transfer numbers the distribution data gave a series of interrelated curves, the peaks of which indicate differences in molecular composition. From the data, and often with supplementary information obtained otherwise, estimates of the proportions of component triglycerides were made. In the recent report on cocoabutter, values are given for ten specific triglycerides such as tripalmitin, dipalmito-monoolein, dilinoleo-monoolein, etc.

The values obtained by countercurrent distribution are, in general, in broad agreement with those produced by other means. One instance of surprisingly close agreement, in corn oil, will be discussed later.

Countercurrent distribution has been used mainly on the natural fats, but obviously other mixtures can be resolved. The equipment is very costly and for this reason the process is not widely used.

Thermal gradient fractionation. In 1956 Baker and Williams (12) described an ingenious apparatus which automatically subjects a solute, or a mixture of solutes, to repeated solution and recrystallization.

The apparatus consists of a vertical column, packed with small glass beads, encased in a jacket and so heated that a linear thermal gradient exists from the higher temperatures at the top to the lower ones at the bottom.

The column is filled with the less effective of two selected solvents and the sample is placed at the top. As liquid is drawn off at the lower (colder) end of the column it is replaced at the top with a mixture of the two solvents increasingly and progressively richer in the better solvent. As the sample components dissolve at the top in the increasingly more effective solvent they pass down the column and at some point crystallize. They remain stationary until dissolved again by the improved solvent which follows, and are again precipitated at some point further down and therefore at a lower temperature. Finally, they pass from the column as a saturated solution at the lowest temperature, and are collected as fractions.

In the journey through the column the more soluble components move ahead of the others and are eluted first, provided they have a positive coefficient of solubility and do not form eutectic mixtures.

Hammond and coworkers (13,14) have applied this apparatus and procedure to the determination of glyceride structure. After some initial work with synthetic mixtures they studied the composition of cocoabutter, using acetone as the initial solvent and Skellysolve B as the eluting solvent. Eluted fractions were stripped of solvent and their weight and fatty acid composition determined, the latter by gas chromatography. They found that the greatest degree of separation was by the number of unsaturated fatty acids in the molecule, but that there was some separation by chain length and number of double bonds as well. They calculated values for individual glycerides and for glyceride types, the latter agreeing fairly well with values found by Kartha using procedures to be discussed later.

Thermal gradient analysis has the advantage of producing intact fractions for further analysis. It should be effective with modified fats, fractions, mixtures, and synthetics, as well as with natural fats. As with countercurrent distribution the technique is not standardized, supplementary information of various kinds frequently being needed before final calculations can be made. The apparatus is, however, much less elaborate than that used in countercurrent distribution. It is subject to precise control.

It is quite possible that this method can be used effectively in routine analysis under standardized conditions, especially where, as is often true in industrial applications, approximations are satisfactory. It is also possible that it can be developed further into a useful research tool.

Oxidation-fractionation—Method of Youngs. An excellent method for the glyceride structure analysis of all varieties of fats was published by Youngs in 1961 (3). It is today perhaps the best available method for the determination of the glyceride types and isomers in fats, generally.

The first step is periodate-permanganate oxidation of the sample, whereby the esterified, unsaturated, monocarboxylic acids are converted to saturated dicarboxylic half esters, without disturbing the configuration of the molecules. The resulting mixture is separated, quantitatively, into two fractions on a liquid-liquid partition column using 90-10 ethanol-water as the stationary phase and Skellysolve B as the mobile phase. As shown in Table I, fraction 1 consists of molecules holding zero or one free carboxyl group, while fraction 2 consists of molecules holding two or three free carboxyl groups.

The percentages of mono- and dicarboxylic acids in each fraction are determined by means of standard gas chromatography. The percentages of these components occupying the 1- and 3- positions in each fraction are found by pancreatic lipase analysis, and from these data the percentages of the parent glyceride forms SSS, SUS, SSU, USU, UUS, and UUU are found by calculation.

Analysis of known mixtures by this procedure gave good results. The maximum variation in triplicate

TABLE I
Sources and Composition of Fractions of Oxidized Fats

Fraction 1	Fraction 2
S S S → S S S	U –COOH U → –COOH S S
S S U → –COOH S S	U –COOH S → S U –COOH
S S S → S U –COOH	U –COOH U → –COOH U –COOH

determinations of lard was two units percent. Analysis of several natural fats gave results in good agreement with results by another method [3] (4).

A distinct advantage of this process is that it can be carried out on a small scale. The oxidation step requires only 250 mg of sample, 200 mg are sufficient for the separation, and 10–20 mg of each fraction is adequate for the pancreatic lipase analysis.

The identity of the unsaturated acids is, of course, destroyed but the percentages and the positions occupied by the saturated acids are known.

In case no more than the proportions of the glyceride types S_3, S_2U, SU_2, and U_3 are required, they can be calculated from the component acid analysis and the percentages of the fractions alone, the pancreatic lipase data being unnecessary for this purpose.

Thin-layer chromatography. Privett and Blank (27) have recently disclosed procedures for structural analysis of triglycerides which include separation by TLC, and in a forthcoming publication will describe further developments. The latest process consists, in brief, of ozonization, reduction of the ozonides to "aldehyde cores," separation of both ozonides and aldehyde cores by TLC, quantitative analysis by densitometry, and component analysis of the fractions by GLPC.

The process is in a state of development and progress is encouraging. It can be accomplished with only 2–5 mg of sample. At present it does not include means for determining isomeric forms.

Kaufmann et al. (28,29) have employed thin-layer chromatography for the qualitative separation of triglycerides in some natural fats.

Methods Applicable to the Natural Fats, but not to Fats Generally

The Rule for Glyceride Type Distribution—Kartha. In the 1951 publication of subject matter in his doctoral thesis Kartha (15) described a mathematical procedure for calculating the glyceride types, which he has designated the "rule for glyceride type distribution in natural fats." The rule was, according to Kartha, an expression of a quantitative relationship, resulting from experimental relationships alone. The experiments referred to are analyses by the method described above. The rule is stated thus in the 1951 publication:

> "The actual proportions of the different glyceride types are those which follow if we exchange one-third of the saturated acids in the factor GS_3 chance $-$ GS_3 actuals, with the same amount of unsaturated acids in the GSU_2 and GU_3 chance values, according to probability, the substitution of U by S in the GSU_2 leading to the formation of GS_2U alone, and the substitution of U by S in the GU_3 leading to the formation of GSU_2 alone."

Elsewhere in the volume is theory, an important part of which is that the acyloxy components of depot fats *in vivo* are in an equilibrium state of inter- and intra-molecular exchange. In natural fats all possible molecules are formed, according to theory, in random proportions provided they are fluid *in vivo*. In fats in which the random proportion of S_3 cannot all exist as a fluid the excess S, and preferentially that of highest molecular weight, will combine with U to form the more fluid molecules comprising S_2U and SU_2, in proportions governed by chance. The resultant non-random distribution is known as "restricted random" distribution. In a later publication (16) the rule is stated in other terms thus:

> "The glyceride-type composition of any natural fat is that obtained by interchange according to chance of one-third of the saturated acids represented by the difference between the GS_3 content required by chance and that actually present, with the unsaturated acids in the fat, without allowing the formation of any further GS_3."

In the same publication the following formulas are given for application of the rule to the calculation of glyceride structure:

$$GS_2U \text{ actual} = GS_2U \text{ chance} + (GS_3 \text{ chance} - GS_3 \text{ actual}) + 3a$$
$$GSU_2 \text{ actual} = GSU_2 \text{ chance} - 3a + 3b$$
$$GU_3 \text{ actual} = GU_3 \text{ chance} - 3b$$
$$GS_3 \text{ actual found by experiment}$$

where (a) is the amount of S substituting for U in GSU_2 and (b) is the amount of S substituting for U in GU_3.

The only experimental data required for calculation of the glyceride types are the percentages of S and S_3 in the fat. The results by Kartha agree well with those he derived by his analytical method which has given results agreeing, at least moderately well, with results by more recent procedures (3,4). When the procedure is applied to lard, however, the deviation is very large.

Hammond and Jones (18) have published a set of formulas, with reference to Kartha's, which will be mentioned later.

Methods based on the 1,3 random, 2 random pattern. If glycerol is fully esterified at the 2- positions with a mixture of fatty acids, and if the 1- and 3- positions, assumed to be identical, are then esterified at random with another mixture of fatty acids, the result is an instance of what is here designated as the 1,3 random, 2 random pattern. The 1- and 3- positions will be occupied by identical kinds and percentages of fatty acyloxy groups, distributed at random. The 2- positions will be occupied by another combination of acyloxy groups, also distributed at random. Total-random distribution is a special case of 1,3 random, 2 random distribution in which the same combination of acyloxy groups is distributed in the 2- positions as in the 1,3-positions.

As early as 1957, as shown in correspondence with the author, Richardson (19) had considered the possibility that this pattern of distribution may occur among the natural vegetable fats, and had devised means of calculating glyceride structure, based thereon. The only experimental data needed were the percentages of S and S_3 in the whole fat. From these the proportions of the remaining glyceride types and the remaining five glyceride forms SUS, SSU, USU, UUS, and UUU were obtainable. Using the same basic pattern he later calculated glyceride structure from pancreatic lipase analysis results. None of these procedures, nor the results thereof, were published. Methods and results dependent on the existence of the 1,3 random, 2 random pattern have been published by others.

It has not yet been proved that the pattern is valid for any natural fat.

Pancreatic lipase method—VanderWal. The acyloxy groups in the 1- and 3- positions in fats can be preferentially removed by the pancreatic lipase hydrolysis procedure of Mattson and Beck (20), de-

[3] Included are values for the isomers, found by a procedure based on 1,3 random 2, random distribution.

scribed in detail by Ast and VanderWal (21). It is possible to find by this means the percentage of each specific variety of acyloxy group among all the acyloxy groups occupying the 1- and 3- positions jointly, and the 2- positions separately, in any fat, natural or otherwise.

If this process is applied to a natural fat assumed to exist in the 1,3 random, 2 random pattern, the proportions of SSS, SUS, SSU, USU, UUS, and UUU can be readily calculated by several procedures.

A rather laborious method for doing this was published by VanderWal (4) in 1960 and applied to data published by Mattson and Lutton (22) on various fats.

The procedure, in brief, is to calculate, from pancreatic lipase data, the percentages in the sample of the four 1,3 random combinations represented as S— S,U — U,S — U, and U — S. The remaining S and U, found by pancreatic lipase analysis to occupy the 2- positions, is then distributed at random among these nuclei. The results are the percentages of the six molecular forms.

The results in Table II show how values by this procedure compare with those found by other processes.

The work of Youngs (3), Dutton et al. (11), and Jones and Hammond (14) provides further evidence of the validity of this procedure for calculation of glyceride types and Youngs found good agreement between values for the isomers, as well.

The process is, however, long and involved and the same results, and more, can be obtained by another procedure, based on the 1,3 random, 2 random pattern, which is described below.

Direct calculation from positional composition data. It is possible to calculate directly from positional composition data the percentage of any specific triglyceride, or group of triglycerides, in any fat in which the component acids are distributed in the 1,3 random, 2 random pattern. Coleman (24) and Coleman and Fulton (23) have published such a procedure, essentially the same as that to be discussed hereafter.

The percentage of each component acid among all those in the 2- positions and in the combined 1- and 3- positions can be found by pancreatic lipase analysis. If the 1- and 3- positions hold identical kinds and percentages of component acids, the percentage in each of these is the same as for the two in combination. With the percentage of each acid among all acids in the 1-, 2-, and 3- positions known, the proportion of any specific triglyceride can be calculated on the basis of probability, as will be demonstrated. The percentages of SSS, SUS, SSU, USU, UUS, and UUU can be calculated by the same process and the results will be the same as those calculated by the method of VanderWal (4), or by any other method based on 1,3 random, 2 random distribution.

TABLE III

Total and Positional Analyses of Corn Oil (% wt)

Component Acid	C 1,2,3	C 1,3	C 2
C_{16}	11.8	18.0	−0.6 (0)
C_{16}'	T	T	
C_{18}	1.9	3.1	−0.6 (0)
C_{18}'	29.1	28.7	30.0
C_{18}''	56.4	49.3	70.6
C_{18}'''	0.8	0.9	0.5
S	13.7	21.1	0.0
U	86.3	78.9	100.0

Formulas for the calculations are as follows: (The numerals following the letters A, B, and C indicate the positions occupied by the component acids represented.)

1. For triglycerides comprised of component acid A only
   % AAA = (% A 1) (% A 2) (% A 3) /10,000
2. For triglycerides comprised of two component acids, A and B
   % AAB = (% A 1) (% A 2) (% B 3) (2) /10,000
   % ABA = (% A 1) (% B 2) (% A 3) (2) /10,000
3. For triglycerides comprised of three component acids, A, B, and C
   % ABC = (% A 1) (% B 2) (% C 3) (2) /10,000
   % ACB = (% A 1) (% C 2) (% B 3) (2) /10,000
   % CAB = (% C 1) (% A 2) (% B 3) (2) /10,000

The application of these formulas will be demonstrated by calculation of some specific triglycerides in corn oil. Corn oil was employed for this demonstration because analysis by countercurrent distribution has provided a basis for some comparison with the present procedure.

In Table III are shown the results of pancreatic lipase analysis of a sample of corn oil. The headings C 1,2,3; C 1,3; and C 2 indicate the glycerol carbons to which the corresponding acyloxy groups are attached. Thus, under the heading C 1,2,3 is the analysis of the whole fat, under C 1,3 the analysis of the groups in the 1- and 3- positions only, and under C 2 the analysis (by calculation) of acyloxy groups attached at the 2-positions only. The values in the column headed C 2 are calculated from the others in accord with the following formula on the assumption of 1,3 random, 2 random distribution:

$$C\ 2 = (3)\ (C\ 1,2,3) - (2)\ (C\ 1,3)$$

It should be kept in mind that in 1,3 random, 2 random distribution the percentage of a specific group in the 1- position is the same as that in the 3- position, and both are numerically equal to the percentage in the combined 1- and 3- positions.

The percentages of some specific triglycerides calculated by this procedure are shown in Table IV with the equations whereby they are derived. Also in the table are the corresponding molecular percentages

TABLE II

Triglyceride Types and Isomeric Forms in Some Natural Fats

Fat:[b]		Composition: Types (% wt)				Composition: Isomers (% wt)			
		S_3	S_2U	SU_2	U_3	SUS	SSU	USU	UUS
Kokum butter (22)	Calc., present method	2.8	73.9	21.6	1.6	73.1	0.8	0	21.6
A = 59.3;	Found, (58.9% S in sample) (26)	1.5	76.1	20.8	1.6	.....	.....	.....	.....
B = 3.7	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 (22)	Calc., present method	2.5	22.4	55.7	19.4	1.0	21.4	46.9	8.8
A = 36.0;	Found, (37.8% S in sample) (17)								
B = 70.7		2.9[a]	25.3[a]	53.3[a]	18.5[a]	.....	.....	.....	.....
Peanut oil (22)	Calc., present method	0.1	9.9	42.5	47.5	9.3	0.6	0.7	41.8
A = 20.9;	Found, (19.5% S in sample)								
B = 1.4	(15) (16)	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

[a] % Mol. In these fats the differences between % mol and % wt are negligible.
[b] A = % S (total), B = % S among groups in 2-position.

TABLE IV

Wt % of Various Triglycerides in Corn Oil and How Derived [a]

Triglyceride	% Wt	Derivation
PPP	0	(18.0) (0) (18.0)/10,000
PPP	0.2	Random dist'n. (Calc.)
OLL	20.0	(28.7) (70.6) (49.3) (2) /10,000
LOL	7.3	(49.3) (30.0) (49.3) /10,000
L_2O	27.3	Sum OLL + LOL
L_2O	24.4(% mol)	Countercurrent dist'n, different sample
PLL	12.5	(18.0) (70.6) (49.3) (2) /10,000
LPL	0.0	(49.3) (0) (49.3) /10,000
L_2P	12.5	Sum PLL + LPL
L_2P	13.1(% mol)	Countercurrent dist'n, different sample
LLL	17.2	(49.3) (70.6) (49.3) /10,000
LLL	17.9	Random distribution. (Calc.)
LLL	22.0(% mol)	Countercurrent dist'n, different sample
LLL	22.4(% mol)	Random dist'n, sample above. (Calc.)

[a] P = palmitic, O = oleic, L = linoleic acids.

found by countercurrent distribution of a somewhat different sample by Scholfield and coworkers (25), together with some other values.

It will be noted that there is fairly good agreement, considering that the samples are different. In corn oil the differences between molecular and weight percent are small. The percentages of the six forms SSS, SUS, SSU, USU, UUS, and UUU can be calculated, in like manner, from the accumulated percentages of S and U.

Calculation from S and S_3 Content. It was stated earlier that Richardson had calculated the glyceride types and isomers in natural fats from the S and S_3 content alone, based on the assumption that the fats exist in the 1,3 random, 2 random pattern.

The mathematics are independent of the mechanism by which the fat arrives in the 1,3 random, 2 random pattern *in vivo* and Richardson did not fix on any particular mechanism although he mentioned some. One form in which the calculation can be carried out will be demonstrated here.

The proportions of the six molecular forms in a fat in which the S and U are distributed at random can be calculated by substitution in the following formulas:

$$\% \text{ SSS} = (S)\ (S)\ (S)\ /10{,}000$$
$$\% \text{ SUS} = (S)\ (U)\ (S)\ /10{,}000$$
$$\% \text{ SSU} = (S)\ (S)\ (U)\ (2)\ /10{,}000$$
$$\% \text{ USU} = (U)\ (S)\ (U)\ /10{,}000$$
$$\% \text{ UUS} = (U)\ (U)\ (S)\ (2)\ /10{,}000$$
$$\% \text{ UUU} = (U)\ (U)\ (U)\ /10{,}000$$

where S and U are the percentages of saturated and unsaturated groups in the whole fat.

The 1-, 2-, and 3- positions each hold the same proportions of S and U, distributed at random, and the pattern is a special case of 1,3 random, 2 random distribution.

It is possible for a fat of exactly the same composition as that just considered to be comprised in such a way that the 2- positions hold either less or more, and the 1,3- positions correspondingly more or less, than the random proportions of saturated components and still be in 1,3 random, 2 random distribution.

TABLE V

Individual Type-Members in Various Fats by Kartha's Approximation and by the Pancreatic Lipase Method

Fat	Type	Individual	% (Kartha)	% (Pancreatic lipase)
Corn Oil	SU_2	PL_2	12.3	12.5
	U_3	L_3	17.4	17.2
	U_3	L_2O	26.9	27.3
Cocoabutter	SU_2	PO_2	7.1	6.9
	S_2U	P_2O	12.2	13.9
Lard	SU_2	PO_2	19.5	23.6
	S_3	P_3	0.9	0.3
	S_2U	P_2O	8.3	4.8

P = palmitic, L = linoleic, and O = oleic component acids.

Under these circumstances the S displaced at the 2- position is replaced by an equal amount of U drawn equally from the 1- and 3- positions, and this U in turn is replaced in the 1- and 3- positions by the S excluded from the 2- position. The reverse is true if S is in excess.

If the percentage of S in the groups occupying the 2- positions is thus decreased by the value X, the molecular composition can be represented as follows:

$$\% \text{ SSS} = (S + X/2)\ (S - X)\ (S + X/2) \quad /10{,}000 \text{ I}$$
$$\% \text{ SUS} = (S + X/2)\ (U + X)\ (S + X/2) \quad /10{,}000 \text{ II}$$
$$\% \text{ SSU} = (S + X/2)\ (S - X)\ (U - X/2)\ (2) /10{,}000 \text{ III}$$
$$\% \text{ USU} = (U - X/2)\ (S - X)\ (U - X/2) \quad /10{,}000 \text{ IV}$$
$$\% \text{ UUS} = (U - X/2)\ (U + X)\ (S + X/2)\ (2) /10{,}000 \text{ V}$$
$$\% \text{ UUU} = (U - X/2)\ (U + X)\ (U - X/2) \quad /10{,}000 \text{ VI}$$

The value of X can be found by substitution of experimental values for S and S_3 in equation I, and these are the only data needed for calculation of the remaining five molecular forms.

If the value of S in the 2- position has increased by the percentage X the same equations apply, with a change in the sign before X.

Equation I is cubic and it is expedient to find the value of X by graphic means rather than by calculation. Its sign must be found experimentally as by some such means as pancreatic lipase analysis. The direction of the variation from random proportions is, however, well known for most of the common fats. It is only with new fats or fats produced *in vivo* under unusual circumstances that the sign of X need be determined experimentally.

Method of Hammond and Jones (18). These authors have developed equations based on equilibrium considerations for the determination of the glyceride types from S and S_3 content which vary somewhat from those of Kartha. They state that the equations of VanderWal can be developed on the same basis.

Approximation of the percentages of the individual members of the glyceride types from the component acid and glyceride type compositions. In 1951 Kartha (15) discussed briefly the approximation of the individual members of the glyceride types from the percentage of the type actually present and the proportions of the individual component acids in the whole fat.

Representing the percentages of saturated and unsaturated components generally as S and U, those of the various specific saturated components as S^a,S^b,S^c, etc., and the specific unsaturated components as U^a, U^b, U^c, etc., Kartha's formulas may be written as follows:

$$S^a{}_3 = (S_3 \text{ found})\ (S^a/S)^3$$
$$S^a{}_2S^b = (S_3 \text{ found})\ (S^a/S)^2\ (S^b/S)\ (3)$$
$$S^aS^bS^c = (S_3 \text{ found})\ [(S^a)(S^b)(S^c)/S^3]\ (6)$$
$$S^a{}_2U^a = (S_2U \text{ found})\ (S^a/S)^2(U^a/U)$$
$$S^aS^bU^a = (S_2U \text{ found})\ (S^a/S)\ (S^b/S)\ (U^a/U)\ (2)$$

The SU_2 and U_3 components are calculated by the same formulas, substituting S for U, and *vice versa*, as appropriate. The isomeric forms may be substituted for the mixed types.

In Table V are some values for individual type components calculated by these formulas compared with the corresponding values derived from pancreatic lipase results. The values for the type "found," necessary in the formula, are also calculated from pancreatic lipase data.

The values are sometimes good, and sometimes very rough, approximations. Perhaps the formulas are usable, under restricted circumstances, for special purposes.

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The Determination of Polymers in Fats and Oils

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Abstract

Fats and fatty acids are polymerized by oxidative or thermal processes. Structures have been deduced by using a number of chemical and physical techniques. General methods applicable to the analysis of polymerized oils include determinations of acetone number, iodine value (I.V.), molecular weight, dielectric constant, viscosity, and refractive index. Monomers, dimers, and trimers are separated generally by molecular distillation. In addition, urea fractionation and a number of chromatographic techniques are useful for the detection of monomers, dimers, and polymers.

Introduction

THE ANALYSIS of polymers present in fats and oils is of practical importance to both the chemist and the nutritionist. Knowledge of the chemistry and structure of polymeric products has led to the introduction of new industrial raw materials. On the other hand, nutritionists are interested in the nutritive value of substances formed in heated and polymerized fats.

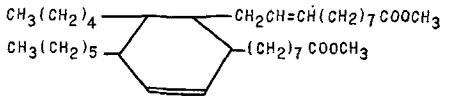
The purpose of this paper is 1), to survey the various structures found in heated and polymerized fats, noting procedures used for isolation and analysis; and 2), to describe techniques and methods useful for determining polymers in fats and oils.

Polymers are formed in fats and oils by processes which may be described as either thermal polymerization or oxidative polymerization. Thermal polymerizations proceed in the absence of oxygen. They involve primarily Diels-Alder addition of the double bond system at the 1, 4- position of a conjugated diene structure to form hydroaromatic cyclic compounds. On the other hand, oxidative polymerizations occur by free-radical mechanisms. Hydroperoxides, formed initially at low temperatures, decompose on heating to form principally dimeric products whose monomeric units are linked through carbon. Newman (1) and Perkins (2) have reviewed the chemical and nutritional changes that occur in heated fats. Sonntag (3) has reviewed reactions occurring during thermal polymerization of fatty acids. Privett (4) discussed autoxidation and oxidative polymerization of drying oils.

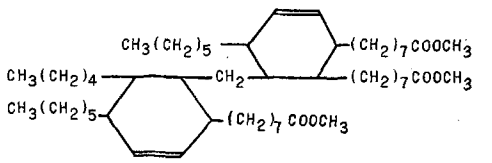
Experimental

Thermal Polymerization

Dimers and polymers. Bradley and coworkers (5, 6,7) observed changes in viscosity, molecular weight, iodine number, refractive index, density, and saponification number of methly or ethyl esters derived from thermally polymerized oils. On the basis of physical and chemical data obtained, the authors concluded that Diels-Alder addition reactions produce monocyclic and bicyclic dimers from linoleate and linolenate respectively, and further Diels-Alder addition produces trimeric cyclic structures. Paschke and coworkers (8,9) found that linoleate dimerizes largely by thermal conjugation followed by Diels-Alder addition of conjugated isomer with nonconjugated linoleate Dimers and trimers produced from heat polymerization of methyl linoleate are represented by the following structures:



DIMER



TRIMER

Chin (10), and Clingman and coworkers (11) presented formal proofs of the presence of six membered rings in the dimers isolated from thermally polymerized methyl eleostearate. Chin dehydrogenated the dimers with selenium and obtained derivatives of benzene and naphthalene. Clingman and coworkers, by substitutive bromination-dehydrobromination and oxidation obtained a 9% overall molar yield of methyl prehnitate (1,2,3,4-benzene-tetracarboxylate) from eleostearate dimers, indicating that the eleostearate dimer was a tetrasubstituted cyclohexene derivative.

The methyl eleostearate dimer that Chin isolated