Interesterification. I. Change of Glyceride Composition During the Course of Interesterification

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Abstract

Changes in composition produced in monounsaturated triglycerides during the course of interesterification, before full randomization is achieved, have been followed by using thin-layer chromatography. Results indicate that intraesterification, in which interchanges take place between positions on the same molecule, occurs at a faster rate than the general randomization which results from interesterification.

The reaction is found to proceed according to the expression log a α t, where 'a' is the proportion of fatty acid components not changed in position by the reaction. This expression is of general application to triglyceride interesterifications and provides a basic equation in terms of which the effects of temperature, catalysts, and other experimental conditions can be studied.

Introduction

Analyses of fully interesterified triglyceride mixtures indicate that the compositions do not differ significantly from those calculated for fully random fatty acid distributions (1–4). Only one case of significant differences has been reported, i.e., in the work on butterfat by Kuksis et al. (5). Butterfat is not typical, in that a much wider spread of fatty acid chain-lengths is involved, compared with most commercial fats. Even so, the differences were not great.

But although the end-product can be assumed to be a random mixture, the route by which this randomization has been achieved has not been explored so little is known regarding the kinetics of the reaction. Changes in physical properties provide an arbitrary measure, but they give no information on the compositional changes involved, and the results can only be applied to the particular substrate for which they were obtained.

Following changes in composition is difficult unless substrates are used which are as simple as possible. In the present work, substrates consisting solely of symmetrical monounsaturated triglycerides (SUS) have been chosen as these are the simplest compositions which will give indications both of the full randomization which results from interesterification and of the restricted randomization which results from intraesterification, i.e., the interchange of fatty acids on the same molecule.

The changes in composition have been followed during interesterification by using the technique of thin-layer chromatography on silver nitrate-impregnated silica gel (6), which separates triglycerides into fractions according to the amount of unsaturation in the molecule and also separates, to some extent, the symmetrical monounsaturated isomers (SUS) from the unsymmetrical isomers (USS).

Experimental Section

Substrates

The pure triglycerides, 2-oleodipalmitin (POP), 2-oleodistearin (SOS), and palmito-oleostearin (POS), were synthesized by standard methods. All gave single spots when examined by thin-layer chromatography.

A mid-fraction was prepared from cocoa butter by dissolving cocoa butter, 100 g, in hexane (1 liter) and cooling the solution to 0C. The residue, 78 g, was recrystallized from acetone (500 ml) at 0C and yielded 33 g of the desired cocoa butter fraction (CBF), which gave a single spot, corresponding to an SUS glyceride, on a thin-layer plate.

Interesterification

Interesterifications were carried out, over a range of temperatures, by using sodium and sodium alkoxides as catalysts. The glyceride, 1 g, was contained in a test tube supported in an oil bath on a magnetic stirrerhotplate. The glyceride was stirred vigorously, under nitrogen at 110C for 2 hr, to dry. The temperature was then adjusted and, when steady at the required value, the catalyst was added. Samples were taken immediately before the addition of the catalyst and at timed intervals afterwards. Samples of approximately 50 mg each were removed with a dropping pipette, and each sample was dropped immediately into a weighed sample tube containing 1% acetic acid (1 ml) in light petroleum.

These were then blown down under nitrogen until the smell of acetic acid could no longer be detected. Each sample was made up to a 1% solution in chloroform for spotting on a thin-layer plate.

Thin-Layer Chromatography

Silica Gel G, 60 g, was slurried with water (30 ml) containing silver nitrate (7 g). The slurry was spread onto plates, 20 cm × 20 cm, by using a Desaga spreader. The plates were dried in air for 15 min, then in an oven at 110C for 1 hr.

After the samples were spotted, the plates were developed with a solvent mixture containing cyclohexane 60%, di-isopropyl ether 40%, which was allowed to run to 15 cm. This treatment separates the interesterified mixture into five spots: trisaturated (S_3), symmetrical monounsaturated (SUS), unsymmetrical monounsaturated (USS), diunsaturated (SU_2), and triunsaturated (U_3). In only a few cases was the separation of SUS and USS sufficient to give separate estimation of these components.

After removal of the solvent the plates were sprayed with 50% phosphoric acid and charred by heating on a hotplate at 300C for half an hour. Intensities of spots were measured by using a Chromoscan Densitometer. All the samples from one reaction run were spotted onto one thin-layer plate which was calibrated by comparing the mixture obtained after one hour of reaction time with the composition calculated for a fully random distribution. Plates were run in duplicate, and compositions not correlating between plates were rejected.

Results

Table I indicates how R_1, the ratio of diunsaturated glycerides (SU_2) to monounsaturated glycerides

TABLE I

Chonge in $R_1 = \frac{SU_2}{S_2U}$ During Interesterification

Substrate	1 SOS		2 POP		3 POS		4 CBF		5 CBF	
Temperature	110C		100C		120C		65C		80C	
Catalyst[a]	Sodium		Sodium ethoxide		Sodium		Sodium methoxide		Sodium methoxide	
	t (min.)	R_1	t (min.)	R_1	t (min.)	R_1	t (min.)	R_1	t (min.)	R_1
	4	0.02	2	0.03	5	0.01	1	0.03	0.3	0
	5	0.16	5	0.14	7	0.03	2	0.09	1	0.15
	6	0.35	10	0.21	10	0.18	3	0.23	2	0.34
	8	0.45	20	0.29	15	0.40	5	0.38	3	0.45
	15	0.50	30	0.37	20	0.47	9	0.46	5	0.49
	60	0.50	60	0.50	30	0.49	60	0.50	20	0.50
					45	0.50			60	0.50
					60	0.50				

[a] Added weights of catalysts 0.1–0.4%.

(S_2U), changes with time. Since intraesterification will have the effect of altering the proportion of isomers within a fraction, without altering the total proportion of the fraction, R_1 will not be affected by intraesterification.

Table II lists the values of R_2, the ratio of symmetrical monounsaturated glycerides (SUS) to unsymmetical monounsaturated glycerides (USS), for those cases where separation of the isomers was achieved. As R_2 is a ratio of isomers within a fraction, it will reflect the extent of intraesterification.

Calculation

The process of interesterification can be considered as the removal of fatty acids at random from the glyceride molecules, the shuffling of these acids, and replacement of them on the glyceride molecules at random. At the stage of partial interesterification, not all of the fatty acids will have been mobilized in this way. There will be a residue of partial glycerides which retain the fatty acids in their original positions but with vacant positions distributed at random. Given the proportion of fatty acids mobilized, the distribution of vacant positions can be calculated on a simple basis. The triglyceride composition at this stage can then be calculated by refilling the vacant spaces according to the composition of the mobilized fatty acid pool.

If the proportion of unchanged fatty acids A = a and the proportion of mobilized fatty acids B = b, where a + b = 1, then, at any stage during interesterification, the following glyceride types will be present in the following proportions:

Unchanged glyceride	AAA	a^3
Partially changed glycerides	AAB	a^2b
	ABA	a^2b
	BAA	a^2b
	ABB	ab^2
	BAB	ab^2
	BBA	ab^2
Fully changed glycerides	BBB	b^3

By starting from a pure triglyceride SUS and assuming there is no distinct intraesterification, these glyceride types become:

Unchanged glyceride	SUS
Partially changed glycerides	SUB
	SBS
	BUS
	SBB
	BUB
	BBS
Fully changed glycerides	BBB

The proportion of each triglyceride component can be derived by replacing the B acids with the appropriate acid from the mobilized acid pool. In this case the composition of the mobilized acid pool will be S 66.7%, U 33.3%. Thus, at the partial interesterification stage, the component SUS will be derived as follows:

Unchanged triglyceride — a^3
SUB with B replaced by S — $2 \times a^2b \times 0.667$
SBS with B replaced by U — $a^2b \times 0.33$
SBB with B replaced by S and U — $2 \times ab^2 \times 0.33 \times 0.667$
BUB with B replaced by 2S — $ab^2 \times 0.667^2$
BBB with B replaced by 2S and U — $b^3 \times 0.667^2 \times 0.333$
So the total proportion of SUS will be

$$a^3 + 1.67a^2b + 0.88ab^2 + 0.15b^3$$

By substituting b = a − 1 and multiplying by 100, the equation for percentage of SUS in terms of 'a' is

$$SUS = 7.3a^3 + 33.3a^2 + 44.4a + 14.8$$

The percentage of the other components can be calculated in the same way except that slight variation arises for the unsymmetrical glycerides SUU and USS. Thus the B's in BUB can be replaced by S and U in two ways, giving SUU and UUS. Similarly BBB can give rise to USS and SSU, and SUU and UUS. A factors of two must therefore be introduced when these contributors are worked out.

The precentages of each component is then given by the following equations:

$$SUS = 14.8 + 44.4a + 33.3a^2 + 7.4a^3$$
$$SSS = 29.6 - 22.2a^2 - 7.4a^3$$
$$USS = 29.6 - 44.4a + 14.8a^3$$
$$SUU = 14.8 + 22.2a - 22.2a^2 - 14.8a^3$$
$$USU = 7.4 - 22.2a + 22.2a^2 - 14.8a^3$$
$$UUU = 3.7 - 11.1a^2 + 7.4a^3$$

From this $R_1 = \frac{SUU + USU}{SUS + USS} = \frac{2 - 2a^3}{4 + 3a^2 + 2a^3}$ (1)

which is the expression plotted in Fig. 1.

Also $R_2 = \frac{SUS}{USS} = \frac{4 + 12a + 9a^2 + 2a^3}{8 - 12a + 4a^3}$ (2)

By using Fig. 1 to derive values for 'a' corresponding to the experimental values of R_1, the values listed in Table III are obtained. Plotting log a against t shows a straight-line relationship in all cases (Fig. 2). The calculation has been applied to one of the Norris and Mattil results (Ref. 2 Table IV), which gives the variation in trisaturated glyceride content, during interesterification of tripalmitin, P_3, and triolein, O_3.

TABLE II

Change in $R_2 = \frac{SUS}{USS}$ During Interesterification

Experiment No.	Time (min.)	R_2
2	20	1.5
2	30	1.0
5	2	1.1
5	5	0.5

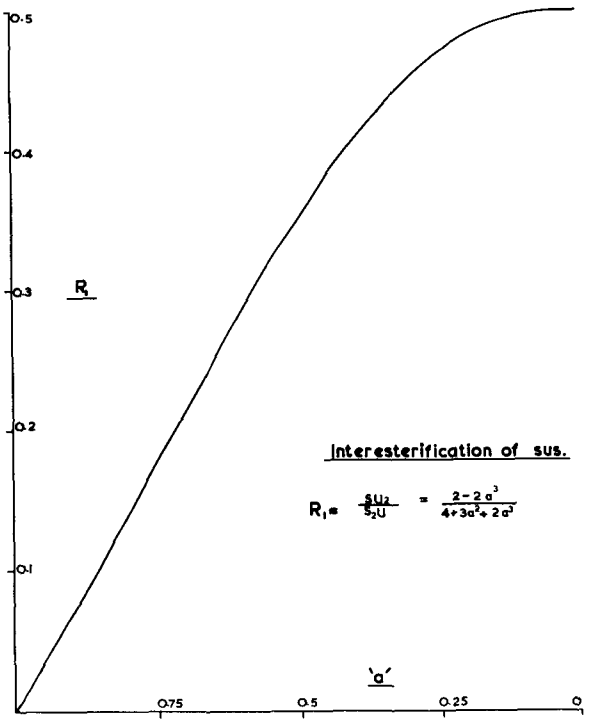

FIG. 1.

For the glyceride mixture $P_3 = 32\%$, $O_3 = 68\%$, results are:

AAA	PPP	$0.32\ a^3$
	OOO	$0.68\ a^3$
AAB	PPB	$0.32\ a^2b$
	OOB	$0.68\ a^2b$
ABA	PBP	$0.32\ a^2b$
	OBO	$0.68\ a^2b$
BAA	BPP	$0.32\ a^2b$
	BOO	$0.68\ a^2b$
ABB	PBB	$0.32\ b^2a$
	OBB	$0.68\ b^2a$
BAB	BPB	$0.32\ b^2a$
	BOB	$0.68\ b^2a$
BBA	BBP	$0.32\ b^2a$
	BBO	$0.68\ b^2a$
BBB		b^3

Contributors to PPP are then:

Unchanged PPPP	$0.32a^3$
PPB with B replaced by P	$2 \times 0.32\ a^2b \times 0.32$
PBP with B replaced by P	$0.32a^2b \times 0.32$
PBB with B replaced by P	$2 \times 0.32b^2a \times 0.32^2$
BPB with B replaced by P	$0.32b^2a \times 0.32^2$
BBB with B replaced by P	$b^3 \times 0.32^3$

which gives a total percentage of PPP

$$= 96.2a - 155.8a^2 + 88.3a + 3.3$$

which is the expression plotted in Fig. 3.

By using this expression to derive values of 'a' corresponding to the experimental values of P_3, the values listed in Table IV are obtained. The plot of log a vs. t is shown in Fig. 4. Considering the limitations of the method of analysis available, the experimental points show good agreement with a straight-line relationship.

If it is assumed that an intraesterification effect is occurring, the calculation must be modified. To have any observable effect, intraesterification must occur at a faster rate than interesterification since the latter will include interchanges equivalent to those resulting from the former. The effects of intraesterification therefore can be calculated if it is assumed that any glyceride molecule, which has been affected by the reaction at all, has been intraesterified. Thus all the residual partial glycerides must be considered to be derived from an intraesterified mixture, but the unchanged triglycerides will retain their original configuration.

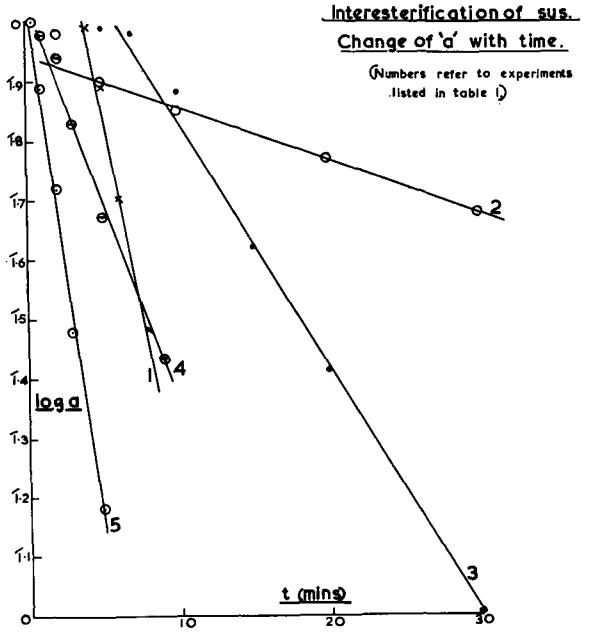

FIG. 2.

Then, by starting from SUS, the partially changed glycerides are derived from the intraesterification mixture, i.e., 66.7% USS 33.3% SUS. Results follow:

AAA	SUS	a^3
AAB	USB	$0.667a^2b$
	SUB	$0.33a^2b$
ABA	UBS	$0.667a^2b$
	SBS	$0.333a^2b$
BAA	BSS	$0.667a^2b$
	BUS	$0.333a^2b$
ABB	UBB	$0.667b^2a$
	SBB	$0.333b^2a$
BAB	BSB	$0.667b^2a$
	BUB	$0.333b^2a$
BBA	BBS	$0.667b^2a$
	BBS	$0.333b^2a$
BBB		b^3

By replacing the B acids as before, the following equations are obtained for percentages of each component in terms of a

$$SUS = 14.8 + 11.1a^2 + 75.0a^3$$
$$SSS = 29.6 - 22.2a^2 - 7.4a^3$$
$$USS = 29.6 + 22.2a^2 - 51.8a^3$$
$$SUU = 14.8 - 14.8a^3$$
$$USU = 7.4 - 7.4a^3$$
$$UUU = 3.7 - 11.1a^2 + 7.4a^3$$

from which

$$R_1 = \frac{2 - 2a^3}{4 + 3a^2 + 2a^3}$$

which is identical to equation (1).

TABLE III

Change in Proportion of Unchanged Fatty Acids (a) During Interesterification

1		2		3		4		5	
t	*a*	*t*	*a*	*t*	*a*	*t*	*a*	*t*	*a*
4	0.99	2	0.95	5	0.98	1	0.95	0.3	1.0
5	0.77	5	0.80	7	0.95	2	0.87	1	0.78
6	0.50	10	0.70	10	0.75	3	0.68	2	0.52
8	0.30	20	0.59	15	0.41	5	0.47	3	0.20
15	0	30	0.47	20	0.29	9	0.27	5	0.15
60	0	60	0	30	0.10	60	0	20	0
				45	0			60	0
				60	0				

TABLE IV

Interesterification of Tripalmitin and Triolein Based on results of Norris and Mattil [a]

t (min)[a]	P_3%[a]	a
0	28	0.92
30	13	0.18
60	8	0.06
85	6	0.03

[a] Ref. 2.

But for R_2 the equation is

$$R_2 = \frac{4 + 3a^2 + 20a^3}{8 + 6a^2 - 14a^3} \qquad (3)$$

Using values of 'a' from Table III, values of R_2 have been derived by a) assuming no distinct intraesterification is occurring (i.e. by using Equation 2) and b) assuming distinct intraesterification is occurring (i.e., by using Equation 3). These values are compared with the experimental values of R_2 in Table V. It is apparent that distinct intraesterification is taking place.

Discussion

It must be stressed that the concept of mobilized fatty acids in the calculation is not to be interpreted as a chemical mechanism. It means no more than that, at a given stage in the randomization, certain fatty acid moieties are found displaced from their original positions with no assumptions about how the displacements occur. It does not mean, for instance, that the fatty acids are displaced by a mechanism of hydrolysis and re-esterification or that fatty acids and partial glycerides are reaction intermediates in any sense.

Certain factors might be expected to affect the randomization which, because of the substrate used or the method of analysis, are not revealed by the present study. Such factors include a) differences in reactivity of different fatty acids, b) differences in reactivity between primary and secondary ester groups, or c) a combination of these resulting in certain fatty acids which favor certain ester positions.

Differences in fatty acids can be accommodated within the framework of the calculation by introducing a suitable factor. Thus the mobilized fatty acid pool will be enriched with respect to the more mobile fatty acids, and the vacant positions on the partially changed glycerides will be correspondingly altered. However, for this effect to be apparent, a wider range of fatty acid types is required than has been used in the systems which are described. These systems contain essentially only oleic, palmitic, and stearic acids. The method of analysis would not reveal differences between stearic and palmitic acids as it does not distinguish between corresponding palmitins and stearins. So the systems contain virtually one saturated acid and one unsaturated acid.

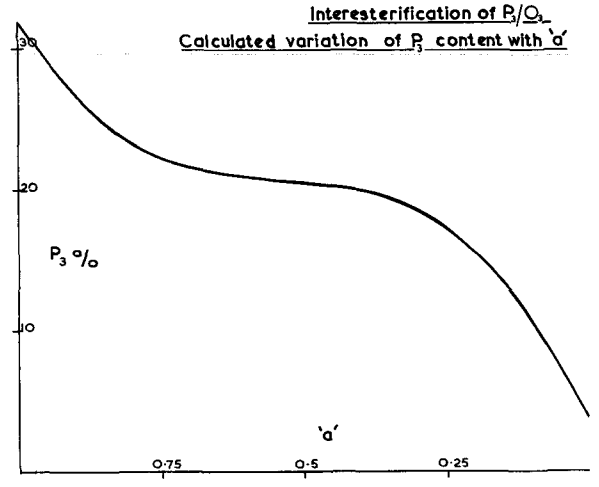

FIG. 3.

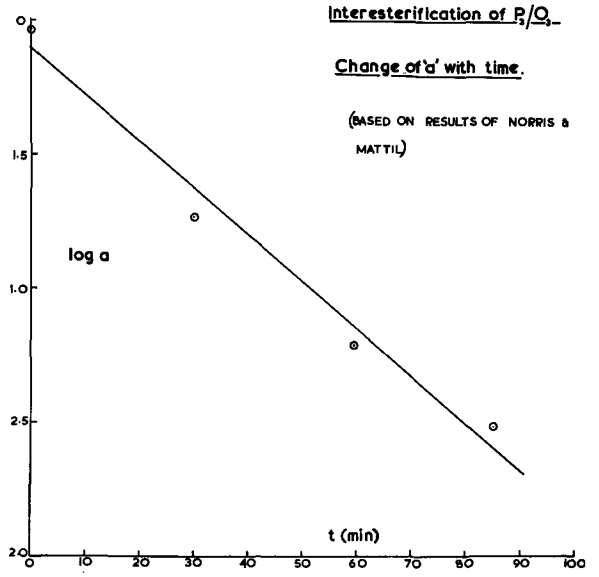

FIG. 4.

If the extreme case is considered, where the unsaturated acid is much more mobile than the saturated acid, then in the early stages of the reaction only unsaturated acids are mobilized. Also the vacated positions on the partially changed glycerides will be those originally occupied by unsaturated acids. Thus the over-all effect will be that unsaturated acid is replaced by unsaturated acid. So if only one unsaturated acid is present, no interesterification will be observed. If there is more than one unsaturated acid or a wider spread of chain length of saturated acids, then any differences of fatty acid mobility should become apparent, provided a suitable system of analysis is used. Differences because of chain length could be conveniently studied by using gas chromatography of triglycerides, for instance.

Differences in ester group positions would modify the relative distributions of the partially changed glycerides. Thus, by supposing acids at the primary position are less mobile than those at the secondary position, then the proportion of AAB and BAA type glycerides would be decreased in favor of the ABA type glycerides. Similarly the proportion of BAB type glycerides would be decreased in favor of the ABB and BBA type glycerides. This is analogous to the equilibration of 1:2– and 1:3– diglycerides and 1– and 2–monoglycerides.

These partial glycerides show wide divergences from the purely random distribution which are not apparent in the present results. This can be taken

TABLE V

Values of $R_2 = \frac{SUS}{USS}$

i	ii	iii
1.5	1.3	8.4
1.0	0.9	4.3
1.1	1.0	5.6
0.5	0.5	1.0

(i) Experimental.
(ii) Calculated by assuming distinct intraesterification.
(iii) Calculated by assuming no distinct intraesterification.

to mean that, when all the hydroxyl positions are esterified, differences between primary and secondary positions disappear. However it may be that an interaction of fatty acid effect and ester position effect does occur so that, with a wider range of fatty acids, the positional differences would become more marked. For instance, one can consider the acyl migration of partial glycerides to be a special case of interesterification in which the hydroxyl group represents the ester of an acid with a preference for secondary positions.

Such variations from the random would alter the equations relating 'a' to the percentage of each component, but they would not affect the relation log a α t. Thus bearing in mind these modifications, the calculation outlined above can be applied in principle to any mixture of glycerides of known composition. This includes natural fats which differ from the mixtures considered only in the complexity of their composition. So the expression log a α t will have general application and provides a basic equation in terms of which effects of temperature, catalysts, and other reaction conditions can be studied.

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