number of other laboratories for test under their operating conditions.

Since the official AOCS soybean analysis methods developed by this committee are now used satisfactorily in marketing the entire soybean crop for processing, this committee has completed its purpose. Any further modifications and study of the method may well be undertaken by the recently organized Seed and Meal Analysis Committee as one of its functions.

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Interesterification Reactions of Triglycerides[1]

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IT was suggested in a recent publication from this laboratory (10) that interesterification might possibly be one of the mechanisms involved in the digestion and metabolism of fats. Before an investigation of such a possibility it has been deemed essential that we investigate the mechanism of the interesterification reaction *in vitro*. This work has been incorporated into a general program of study of the glyceride structure of natural fats and their modification by chemical and physical means. In the present paper we shall attempt to show the probable glyceride structure produced in fats by interesterification.

Interesterification refers to the free exchange and redistribution of component fatty acids among the various triglycerides. Such an interchange has been known to occur under certain conditions of chemical and physical treatment. The process was first described in 1924 in a British patent by Van Loon (12), and since that time several patents have covered catalysts and conditions favoring the rearrangements (6, 7, 13).

It was deduced in our preliminary discussions that the end product of interesterification would probably be constituted according to the principles of random distribution. During the progress of this work a reference book by A. E. Bailey appeared, in which this same deduction was made without supporting experimental evidence being presented (1). It would be anticipated from chemical kinetics that when glycerol is esterified with a mixture of fatty acids of equal reactivity, the acids would distribute themselves in a random fashion among the glycerol molecules. Such an hypothesis is difficult to establish experimentally. Hilditch and his co-workers presented the first experimental evidence in support of random distribution when they showed that the structures of synthetic mixed glycerides corresponded to those of natural animal fats (3), and that in the latter the curve showing the relationship of trisaturated glycerides (Y) to mol percent of saturated acids (X) approximated that represented by the equation:

$$Y = K\ (X)^3 \qquad (2)$$

This principle has been further elucidated by Dean (5) and more recently Longenecker and his co-workers (9), who evolved the following set of equations (in which we shall use as an example a fat containing only palmitic, stearic, and oleic acids):

Percent tripalmitin =

$$\frac{P}{100} \times \frac{P}{100} \times \frac{P}{100} \times 100$$

Percent stearodipalmitin =

$$\frac{P}{100} \times \frac{P}{100} \times \frac{S}{100} \times 3(100)$$

Percent oleostearopalmitin =

$$\frac{P}{100} \times \frac{S}{100} \times \frac{O}{100} \times 6(100)$$

P, S, and O represent the molar percentages of the respective fatty acids in the mixture. The equations may be applied to any combinations of acids in mixtures of all degrees of complexity.

The only readily available method for establishing in part the random distribution of a fat is an analysis for trisaturated glycerides. This method has been applied in our investigation of the end products of interesterification. Thus, if a mixture of known saturated acid content is interesterified, its anticipated trisaturated glyceride composition may be calculated according to the above equations. If the experimentally determined amounts of trisaturated glycerides are in agreement over a range of values, one has rather good circumstantial evidence in favor of random distribution. In order to have a more quantitative measurement we have used not only mixtures of natural and hydrogenated fats, but also mixtures of known proportions of a synthetic saturated triglyceride (tripalmitin) with a synthetic unsaturated triglyceride (triolein).

Experimental

1. *Preparation of Synthetic Triglycerides.*

Methyl palmitate was prepared in a high degree of purity by a fractional distillation of the methyl esters of a commercial palmitic acid[2] through a Podbielniak Hyper-Cal column at a reflux ratio of 2.5:1 (11). This ester after conversion to the free acid exhibited a melting point of 62.5-63.0° C.

Tripalmitin was prepared by heating glycerol with 7% excess palmitic acid in the presence of 0.3% $SnCl_2 \cdot 2H_2O$ catalyst at 200° C. and 20 mm. pressure for 3 hours. The amount of water which was produced indicated that the reaction was more than 99%

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[1] Presented before the fall meeting of the American Oil Chemists' Society, Nov. 7-9, 1945, in Chicago.

[2] Neo-Fat 1-56.

complete. The excess fatty acids were removed by co-distillation at 250° C. and 2 mm. pressure with a stream of water vapor. No effort was made to remove the stannous chloride since this would entail significant losses of tripalmitin as a result of the necessary refining, and its presence should have no effect upon the final equilibria obtained in the interesterifications.

Methyl oleate was prepared by fractionally distilling the methyl esters of Olive Elaine[3] in a Podbielniak column at a 2.5:1 reflux ratio. The combined 18-carbon fractions were analyzed spectrometrically and iodometrically and found to contain about 5% polyunsaturated acids, 95% oleic acid, and no saturated acids. Triolein was synthesized from this methyl oleate by the method described above. The final product was refined by dissolving in petroleum ether and washing the solution free of organic acids with aqueous K_2CO_3. (This method obviously could not be applied to tripalmitin due to the comparative insolubility of the latter.) The ether solution was dried, filtered, and the solvent removed by distillation. Final traces of solvent were eliminated by heating the triolein to 150° C. under 2 mm. pressure with vigorous agitation.

2. *Crystallization Method for Determining Trisaturated Glycerides.*

The commonly accepted method for the determination of trisaturated glycerides is the tedious acetone-permanganate oxidation (8). This procedure is much too time-consuming for routine analysis such as required by the present work. Consequently an empirical crystallization method was devised to meet this need.

After some preliminary work it was finally decided to dissolve the glycerides in 10 ml. of hot acetone per gram of fat, and then hold the solution overnight in a constant temperature room at 74° F. The precipitate which formed was filtered, dried, and weighed. This method varies somewhat from that reported by Coffey and Spannuth (4), the modifications being necessitated by the relative insolubility of the tripalmitin. The parallelism between weights of precipitate and the respective trisaturated glyceride contents of fats is illustrated in Table I, where data are shown for various mixtures of lard with a hydrogenated lard (I.V. = 6.5). It may be seen that the percent recovery of saturated glycerides is quite consistent over a wide range. Obviously, the method is empirical and lacks precision, but it does yield a good approximation of the quantity of saturated triglycerides present in a mixture. A constant crystallization temperature was found to be important, so all succeeding analyses were made in a room maintained at 74° F. From the table it may be seen that when the amount of saturates is low, a 5:1 solvent ratio is desirable, as used by Coffey and Spannuth (4).

TABLE I.

Measurement of Trisaturated Glycerides by Crystallization From Acetone at 74° F., Using Known Mixtures of Lard and Hydrogenated Lard (Iodine Number 6.5).

Solvent ratio	Amount of hydrogenated lard added	Amount of precipitate due to hydrogenated lard	Recovery of hydrogenated lard	Iodine number of precipitate
(ml./gm.)	*(%)*	*(%)*	*(%)*	
10:1	0	0	....	15.0
10:1	2	1.2	60	11.7
10:1	5	3.8	76	14.2
10:1	10	7.4	74	15.8
10:1	20	17.4	86	16.6
10:1	30	27.2	90	19.4
10:1	40	35.4	88	18.4
10:1	50	43.2	86	16.8
5:1	2	1.7	87	17.6
5:1	5	4.3	86	19.3

3. *Interesterification of Mixtures of Lard and Hydrogenated Lard.*

In general, all the interesterifications have been conducted simply by heating mixtures of two fats or triglycerides in the presence of a catalyst at 225° C. under a stream of inert gas such as carbon dioxide or nitrogen. In all the cases which shall be reported here the catalyst used was Van Loon's wet stannous hydroxide precipitated on diatomaceous earth (1, 13). A single attempt to use dry NaOH as a catalyst failed to produce any detectable reaction. It may be well to point out that in using the wet stannous hydroxide catalyst (sufficient to represent about 0.4% Sn), the temperature of the reaction mixture must be raised slowly until all the moisture is removed, after which it is elevated rapidly. About 20-30 minutes usually were required to bring the temperature to 225° F This time is included in all cases in the first fraction.

TABLE II.

Interesterification of a 50:50 Mixture of Lard and Hydrogenated Lard Using Stannous Hydroxide Catalyst at 225° C.

Reaction time	Melting point	Softening point	Precipitate from 10:1 acetone solution	Iodine number of precipitate
(minutes)	*(°F.)*	*(°F.)*	*(%)*	
0	135	132	46	6.5
120	123	120	32	14.5
240	123	120	29	11.8

In Table II data are shown for the reaction between equal parts of lard and hydrogenated lard (I.V. 6.5). The lard used had the following composition:

Iodine Number	67.3
Polyunsaturated acids (spectrometrically)	13.4%
Oleic acid (calculated from above)	49.6%
Saturated acids (by difference)	37.0%

From these data and the iodine number of the hydrogenated lard, the total saturated acids of the mixture are calculated to be 66%. Thus, the amount of trisaturated glycerides anticipated by random distribution would be $(.66)^3$ or 28.7%. It may be seen that the percent of precipitate from acetone approaches that figure in the last fraction. The melting points and softening points appear to have reached a minimum after two hours, indicating that equilibrium probably had been attained by that time.

In Table III are data for a mixture containing 85% of the above lard and 15% of a hydrogenated

TABLE III.

Interesterification of 15 Parts of Hydrogenated Lard With 85 Parts of Lard Using Stannous Hydroxide Catalyst at 225° C.

Reaction time	Melting point	Softening point	Precipitate from 10:1 acetone solution
(minutes)	*(°F.)*	*(°F.)*	*(%)*
0	124	112	14
90	105	99	7
130	107	102	....
150	106	103	6
210	105	102	6
270	107	104	6

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[3] Emery's Special Red Oil.

lard (I.V. 18.1). In this case the calculated saturated acid content was 45%, and the trisaturated glycerides 9.0%. Again the percent of precipitate from acetone agrees fairly well with the calculated figure.

4. *Interesterification of Mixtures of Tripalmitin and Triolein.*

The data representing the reaction between 30 parts of tripalmitin and 70 parts of triolein are shown in Table IV. Again both the melting point and the softening point fell rapidly to an equilibrium position. From the data and the short reaction time it appears that the reaction was stopped at, or just barely before, equilibrium.

TABLE IV.

Interesterification of 30 Parts Tripalmitin and 70 Parts Triolein Using Stannous Hydroxide Catalyst at 225° C.

Reaction time	Melting point	Softening point	Precipitate from 10:1 acetone solution	Iodine number of precipitate
(minutes)	*(°F.)*	*(°F.)*	*(%)*	
0	136	133	28	5.0
30	129	123	15	11.0
60	122	92	8	11.7
85	119	91	6[1]	11.8

[1] Mol percent palmitic acid in mixture = 32. Calculated mol percent of tripalmitin by random distribution = 3.3.

TABLE V.

Interesterification of 50 Parts Tripalmitin and 50 Parts Triolein Using Stannous Hydroxide Catalyst at 225° C.

Reaction time	Melting point	Softening point	Precipitate from 10:1 acetone solution	Iodine number of precipitate
(minutes)	*(°F.)*	*(°F.)*	*(%)*	
0	....	....	....	....
25	133	125	28	10.7
55	124	113	13	14.8
85	124	119	15	13.7
115	123	111	11[1]	12.6

[1] Mol percent of palmitic acid in mixture = 52.3. Calculated mol percent of tripalmitin by random distribution = 14.3.

A mixture of equal parts of tripalmitin and triolein was interesterified, and the data was presented in Table V. Here the reaction time was longer and apparently equilibrium was attained.

Discussion

In every case above there were sufficiently great changes observed in the melting characteristics and acetone solubilities of the mixed fats or glycerides to prove conclusively that redistribution of the fatty acid radicals had occurred. The actual agreement between the amounts of trisaturated glycerides found in the end-products of interesterification and the amounts calculated according to the principle of random distribution is illustrated in Table VI.

TABLE VI.

Agreement of Composition of End-Products of Interesterification With the Theory of Random Distribution.

Sample	Mol percent saturated acids	Calculated trisaturated glycerides	Experimentally determined trisaturated glycerides
	(%)	*(%)*	*(%)*
Lard Mixture 1	66.0	28.7	29
Lard Mixture 2	45.0	9.0	6
Glyceride Mixture 1	32.0	3.3	6
Glyceride Mixture 2	52.3	14.3	11-15

Considering the limitations of the crystallization method used, it may be observed that the agreement has been quite satisfactory. The greatest discrepancies appear in the less-saturated mixtures where experimental errors are magnified. On the whole, our results appear to provide excellent circumstantial evidence for the hypothesis that fatty acids distribute themselves at random over the available glycerol molecules during the reaction conditions employed.

Summary

A study of the interesterification reaction has been undertaken using synthetic triglyceride systems (triolein in varying proportions with tripalmitin) and also combinations of lard and hydrogenated lard. An empirical crystallization method has been used in determining the amount of trisaturated glycerides present in a mixed fat. The results indicate that the end-products of interesterification approach the composition expected from the principle of random distribution.

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