method for the determination of dienoic conjugated acids, alpha-eleostearic acid, beta-eleostearic acid, linoleic acid, oleic acid, and total saturated fatty acids.

Comparisons of the results obtained with similar values, calculated from the known composition of the mixtures, prove that the proposed method gives reasonable results. Standard deviations between determined and calculated results vary from 0.36 for diene conjugated acids to 1.40 for oleic acid.

The method has been applied to the analysis of foreign and domestic tung oils.

REFERENCES

1. American Oil Chemists' Society, Official and Tentative Methods, 2nd Ed., edited by V. C. Mehlenbacher, Chicago, 1946, rev. to May 1951.
2. Bradley, T. F., and Richardson, David, Ind. Eng. Chem., *34*, 237-242 (1942).
3. Brice, B. A., and Swain, M. L., J. Opt. Soc. Am., *35*, 532-544 (1945).
4. Brice, B. A., Swain, M. L., Herb, S. F., Nichols, P. L. Jr., and Riemenschneider, R. W., J. Am. Oil Chem. Soc., *29*, 279-287 (1952).
5. Hilditch, T. P., Morton, R. A., and Riley, J. P., Analyst, *70*, 68-74 (1945).
6. Hilditch, T. P., and Riley, J. P., J. Soc. Chem. Ind., *65*, 74-81 (1946).
7. Mitchell, J. H. Jr., Kraybill, H. R., and Zscheile, F. P., Ind. Eng. Chem., Anal. Ed., *15*, 1-7 (1943).
8. O'Connor, R. T., Heinzelman, D. C., McKinney, R. S., and Pack, F. C., J. Am. Oil Chem. Soc., *24*, 212-216 (1947).
9. O'Connor, R. T., Stansbury, M. F., Damaré, H. G., and Stark, S. M. Jr., J. Am. Oil Chem. Soc., *29*, 461-466 (1952).
10. Pack, F. C., Planck, R. W., and Dollear, F. G., J. Am. Oil Chem. Soc., *29*, 227-228 (1952).
11. Youden, W. J., "Statistical Methods for Chemists," John Wiley and Sons Inc., New York, N. Y., 1951.

[Received October 29, 1952]

On the Glyceride Composition of Animal Fats

O. T. QUIMBY, R. L. WILLE, and E. S. LUTTON, Chemical Division, Procter and Gamble Company, Cincinnati, Ohio

WHILE the glyceride composition of natural fats has received considerable attention, there are few cases where the composition is well-established, and general principles are not broadly or closely applicable. For many vegetable fats, especially seed fats, Hilditch and associates (1) have established an approximately "even distribution" of fatty acids in the glycerides, but there are many departures from perfect correspondence to the rule. For example, in a detailed study of corn oil Doerschuk and Daubert (2) observed a "partial random distribution."

There is less information on glycerides of animal fats than on those of vegetable origin. Evidence has been presented by Hilditch (3) that the glycerides of lard are largely 2-palmityl glycerides (and therefore non-random), but Norris and Mattil (4), in a study which included lard and tallow, concluded that "the results further substantiate the hypothesis that animal fats, in contradistinction to seed fats, are essentially randomly distributed." Kartha (5) has noted, on the basis of his own and others' results, that the trisaturated in animal fats is measurably less and the disaturated is measurably more than the "chance values."

The present authors propose to show that certain animal fats are quite non-random in their glyceride structure just as vegetable seed fats have been shown to be. The study involves a reexamination of lard, beef tallow, and mutton tallow by fractional crystallization and examination of fractions and products of their complete hydrogenation by familiar thermal techniques as well as by x-ray diffraction.

Experimental

A 50-lb. sample of edible lard (unhydrogenated) was obtained from E. Kahn's Sons (Cincinnati, O.). Approximately 10 lbs. of beef tallow were obtained from beef suet by dry rendering followed by a Super-filtrol bleach. Ten lbs. of mutton tallow were obtained from Swift and Company, Chicago, Ill. The original fats were analyzed and their methyl esters fractionated in a Podbielniak still. Data appear in Tables I and II.

TABLE I

Distillation Analysis of Methyl Esters

	Lard	Beef Tallow	Mutton tallow
% C_{14}	2.6	5	6.6
% C_{16}	27.9	30	24.4
% C_{18}	69.5	65	69.0

After analysis the fats were subjected to fractional solvent crystallization. In the case of the lard and mutton tallow a preliminary rough fractionation was followed by more careful detailed fractionation to obtain nearly representative S_3, S_2U, and SU_2 (and U_3) fractions. Two fractionations were performed for lard: one as a pilot run, therefore more detailed; the

TABLE II

Composition of Major Fractions

	Original	S_3	S_2U		SU_2	"U_3"
			Composite	Pure		
	From lard (large scale fractionation)					
I.V.	64.4	3.1[b]	35.8	33.4	62.2	
% Oleic	45.6[a]		25.4	24.5	53.3	
% Linoleic	10.6		6.3	5.2	6.9	
% Linolenic	1.2		0.25	0.15	0.31	
% Arachidonic	0.3		0.18	0.11	0.20	
% Conjugated	0.2		0.13	0.11	0.16	
% Saturated	37.8		63.3	65.5	34.7	
	From beef tallow					
I.V.	37.4	2.5	30.0		59.8	
% Oleic	35.8		29.2		55.2	
% Linoleic	1.8		0.86		3.4	
% Linolenic	0.22		0.10		0.71	
% Arachidonic	0.09		0.00		0.11	
% Conjugated	0.54		0.38		0.94	
% Saturated	57.1		64.6		35.3	
	From mutton tallow					
I.V.	42.6	8.5	33.5		59.3	79.2
% Oleic	40.8		32.5		50.8	62.0
% Linoleic	1.6		0.63		1.7	2.9
% Linolenic	1.1		0.51		1.8	2.6
% Arachidonic	0.34		0.03		0.48	1.2
% Conjugated	1.5		0.93		2.3	3.7
% Saturated	50.2		61.0		38.6	23.2

[a] 95.6% basis for total fatty acids.
[b] From original fractionation.

other to prepare substantial amounts of material for further experimentation. (See Figures 1-3).

Original Fractionation

2 kg. Lard
8 l. Acetone

60°F — A, 344 g., cmp 58.8°C, IV 36
40°F — B, 320 g., cmp 36.2°, IV 42
20°F — C, 520 g., cmp 18.3°, IV 61
6°F — D, 307 g., cmp 10.8°, IV 74
d, 509 g., IV 92

Isolation of S_3

276 g. A
1380 cc Acetone

90°F — A', 41 g., cmp 64.5°, IV 3.1
a'

Isolation of S_2U Fractions

High Melting

From a', (a'-I)-164 g., cmp 40.9, IV 31
From B, (B-1)-104 g., cmp 40.3, IV 27

Low Melting

From a', B, and C, (a'BC-I)-177 g., cmp approx. 32°, IV approx. 30

Isolation of SU_2 Fraction

High Melting

From a', B, and C, (a'BC-II)-317 g., cmp 15.8°, IV 57
From D, (D-1)-157 g., cmp 12.5, IV 66

Low Melting

From a', B, C, and D, (a'BCD-I)-225 g., cmp approx. 0°, IV 58-70.

FIG. 1. Detailed fractionation of lard (adjusted to give 100% yield).

The beef tallow was fractionated very much according to classical methods of fractional crystallization as indicated in Fig. 4 for the early stages of the operation. The procedure was carried on to sort out the various desired fractions, S_3, etc., until 60 crystallizations in all had been performed, representing a maximum of 22 stages of fractionation. In the course of this separation samples were tested against the curve of Figure 5 for I. V. *vs.* refractive index, the curve having been established with data from a few fractions which covered the whole range.

The final isolated fractions representing S_3, S_2U, SU_2, and U_3 were analyzed. The final glyceride compositions that appear in Table III were calculated from the fractions with the assumptions: 1) that only two "neighboring" types of glycerides, *e.g.*, S_3 and S_2U, appeared in one fraction, and 2) that the unsaturated acids were representative of the unsaturated

17.5 kg. Lard
51 l. Acetone

50°F — A, 5710 g., cmp 55.3°C, IV 33.5
0°C — B 8290 g., cmp 24.5°, IV 60.7
3500 g., IV 91.0

Isolation of "Pure S_2U"

From 2650 g. A, 610 g. "Pure S_2U," cmp 41.5, IV 33.4

Isolation of Technical S_2U

From A, 2900 g. Technical S_2U, cmp approx. 40°, IV approx. 36

Isolation of Technical SU_2

From B, 4090 g. Technical SU_2, cmp 18°, IV 62.2.

FIG. 2. Large scale fractionation of lard (adjusted to give 100% yield).

Original Fractionation

1. kg. Mutton Tallow, IV 42.6

70°F — A, 169 g., cmp 58.2°C, IV 14.5
50°F — B, 162 g., cmp 51.2°, IV 24.3
30°F — C, 211 g., cmp 32°, IV 36.0
−5°F — D, 148 g., cmp 26.0°, IV 42.7
d, 310 g., cmp 11.0°, IV 75

Isolation of S_3

From A and B, (AB-I)-189 g., cmp approx. 59°, IV approx. 8.5

Isolation of S_2U

From C and D, (CD-I)-300 g., IV 33.5

Isolation SU_2

From d and D, (dD-I)-235 g., IV approx. 59.3

Isolation U_3

From d, (d-I)-81 g., IV 79.2.

FIG. 3. Fractionation of mutton tallow (adjusted to give 100% yield).

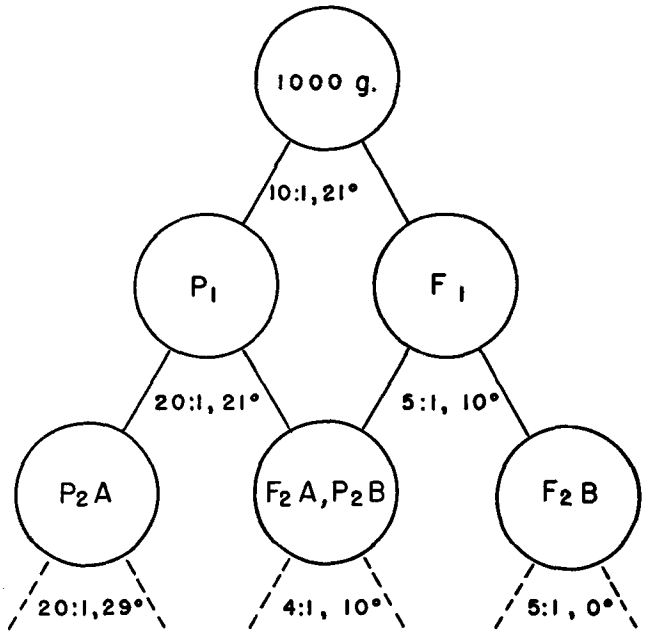

FIG. 4. Tallow recrystallization.

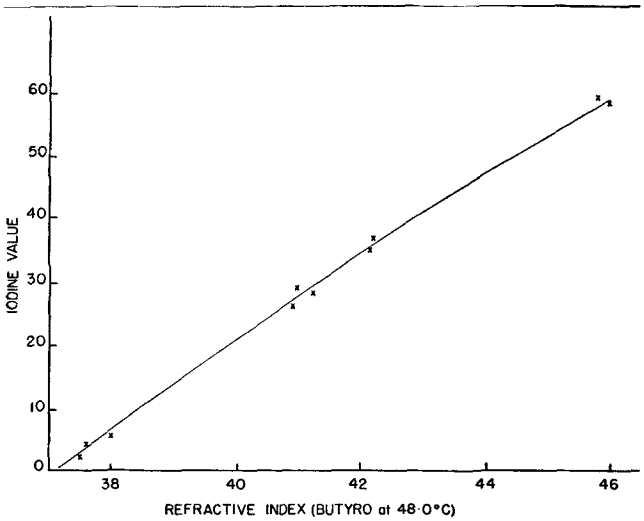

FIG. 5. Relationship of iodine value to refractive index for beef tallow glycerides.

TABLE III

Glyceride Composition of Lard, Beef Tallow, and Mutton Tallow

	Lard		Beef tallow		Mutton tallow	
	QWL	Lit. (6)	QWL	Lit. (6)	QWL	Lit. (7) (English)
S_3	2.4	1.9	17.9	14.7	14.8	26
S_2U	28.0	25.7	40.9	45.9	42.6	30-52
SU_2	40.1	54.4	41.2	37.1	37.9	0-44
U_3	29.5	18.0	<1%	2.2	4.7	

acids of the whole sample. Detailed analyses and calculations are not shown but are available from the authors. (In the lard case, the glyceride composition was obtained from the small detailed fractions. Similar results were obtained with the larger fractionation.)

From each fat, the fractions were appropriately grouped as S_3, S_2U, SU_2 (and U_3) and analyzed as shown in Table II. These samples were then hydrogenated to approximately 0 I. V. and subjected to thermal and diffraction study. The melting point and diffraction techniques have been described elsewhere (8). Cooling curves were obtained by following the temperature on a thermometer inserted in a 75-g. sample of fat, melted and cooled without stirring in air at 80°F.(See Table IV and Figures 6-8.)

TABLE IV

Properties of Completely Hydrogenated Fractions

	From original	From S_3	From S_2U From lard: Technical	From S_2U From lard: "Pure"	From SU_2	From U_3
I.V.	0.1		0.0	0.1	0.0	
α m.p. °C.	50.8		50.2	50.6(51.8[a])	51.1	
C.M.P. °C.	63.6		66.8	66.6(67.9[a])	64.5	
From beef tallow						
S.V.	197.8	198.4		197.3	196.3	
I.V.	0.5	2.5		0.4	0.7	
α m.p. °C.	49.6	49.2		50.2	50.4	
C.M.P. °C.	65.6	61.7		66.1	66.0	
From mutton tallow						
S.V.	197.8	199.3		198.5	198.5	197.2
I.V.	0.5	0.2		0.2	0.7	2.6
α m.p. °C.	49.6	48.3		49.7	49.0	47.5
C.M.P. °C.	63.7	61.7		64.2	63.4	63.2
Pure glycerides (8)						
			PSS	SPS		
α m.p. °C.			50.6	51.8		
C.M.P. °C.			65.2	68.5		

[a] On crystallization from solvent with slight fractionation.

The disaturated portions of Table II were examined by x-ray diffraction for comparison with data on the known disaturated compounds. (See Table V.)

TABLE V

Diffraction Data on S_2U Portions

Source	Melted and chilled: Pattern	Melted and chilled: M.P.°C.	Solvent crystallized: Pattern	Solvent crystallized: M.P.°C.
Lard	.....	21.1	β'-3(L.S.67 Å)	41.4
Beef tallow	α-2(L.S.[a] 47.6 Å)	20.6	β-3(L.S.64.4 Å)	35.6
Mutton tallow	α-2(L.S.48 Å)	21	60% β-2(L.S.42 Å) 40% β-3(L.S.65 Å)	33-41
OPS(9)	α-2(L.S.40.3 Å)	25.3	β'-3(L.S.66.9 Å)	40
POS(9)	α-2(L.S.47.8 Å)	18.2	β-3(L.S.63.1 Å)	38

[a] L.S.—long spacing.

Norris and Mattil (4) had proposed a random interesterification technique to study the glyceride arrangement in a group of fats including lard and beef

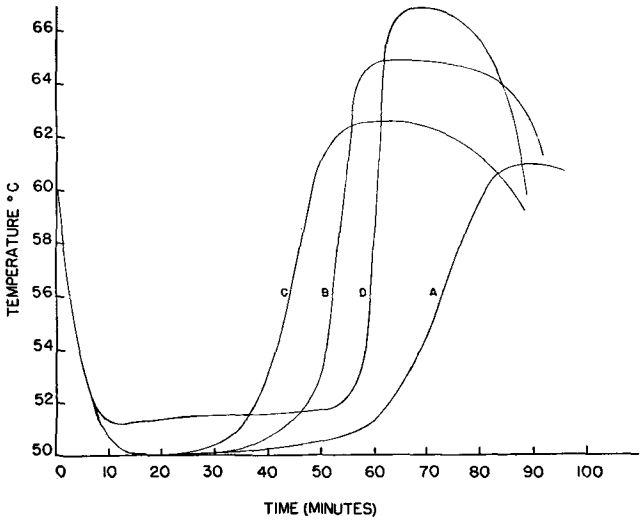

FIG. 6. Cooling curves of completely hydrogenated lard fractions.

A—Lard
B—Lard S_2U
C—Lard SU_2
D—Purified lard S_2U

TABLE VI

Calculated (Random) vs. Experimental Values for S_3 and S_2U

Fat	% S_3 Calc.	% S_3 Exp.	% S_2U Calc.	% S_2U Exp.
Lard	6.2	2.4	28.3	28.0
Beef tallow	21.3	17.9	43.1	40.9
Mutton tallow	14.5	14.8	39.3	42.6

tallow. They compared S_3 contents before and after randomization. With a somewhat different approach random rearrangement was used here for studying completely hydrogenated fractions of fats. The main basis for comparison was the cooling curve technique. Randomization was accomplished by treatment with 0.1% sodium methylate, added in xylene suspension, for ½ hour at 70°C. and subsequent neutralization with dilute acetic acid to kill the catalyst.

Some have assumed, since the S_3 content of animal depot fats is near the value calculated from probability, that animal fat acids are randomly arranged in the glycerides. This is not the view of Hilditch who has said (10) ". . . it was noticed that the proportions of fully saturated glycerides in those animal fats which had been examined and which contained over 35% of saturated acids in their total fatty acids were not very different from that calculated on the basis of 'random' or 'indiscriminate' rather than 'even' distribution. . . . The circumstance that some approach to 'indiscriminate' distribution of the saturated acids was apparent in the glycerides of animal depot (and) milk fats with more than about 35% of saturated acid in their total fatty acids did not however commend itself as an adequate reason for assuming that, in these fats, so fundamentally different a mode of assemblage of triglycerides takes place from that which occurs in wide ranges of other natural fats, both vegetable and animal. Moreover it soon became clear that this apparent similarity to results based on 'probability' consideration only held for the saturated acids . . . [Hilditch] and his colleagues therefore put forward the hypothesis that, in the animal depot fats under discussion, the final mixture of component glycerides is the consequence of a bio-hydrogenation process which has operated

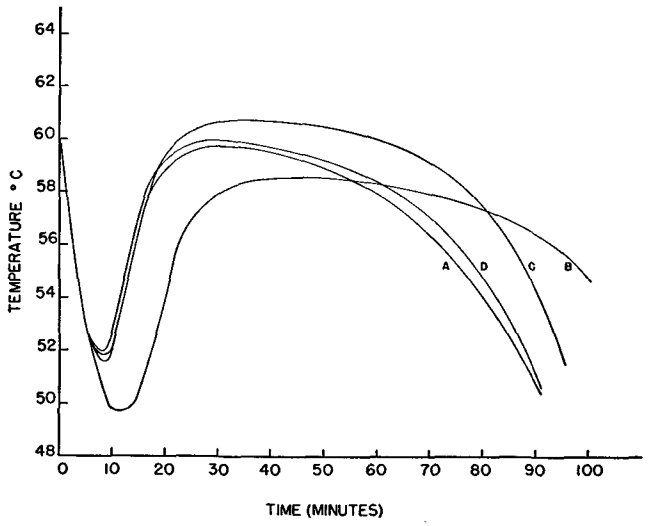

FIG. 7. Cooling curves of completely hydrogenated mutton tallow fractions.

A—Mutton tallow
B—Mutton tallow S_3
C—Mutton tallow S_2U
D—Mutton tallow SU_2

after the precursor fatty acids (mainly palmitic and oleic) have been assembled . . . according to . . . the rule of . . . 'evenly distributed' glyceride production."

The evidence of this study is very much in line with the views of Hilditch with regard to non-randomness of distribution in animal fats whether or not this is associated with a bio-hydrogenation process.

The comparison of cooling curves on randomized and original, completely hydrogenated fats is strong evidence that none of the three fats of study was originally randomly arranged. By the randomization they are brought close together in physical behavior as one would expect from the fatty acid composition, but the original cooling curves suggest two types of composition different from each other and from the random state. One is the lard type, the other is the tallow type associated with largely 2-palmityl and with 1-palmityl glycerides respectively as other evidence suggests. In random distribution a 2-palmityl configuration is half as probable as a 1-palmityl configuration. Therefore the randomized hydrogenated fats should resemble hydrogenated tallow more than hydrogenated lard as, indeed, the cooling curves show. Moreover the randomized hydrogenated fats and hydrogenated original tallow crystallize in bulk substantially without voids whereas the hydrogenated lard crystallizes with a remarkable swelling characteristic of strongly beta-tending fats, *e.g.*, tristearin and 2-palmityldistearin.

That a similar pattern of arrangement, with respect to palmityl position, exists for S_3, S_2U, and SU_2 glycerides of a given fat is strongly suggested by the similarity in cooling curves of the completely hydrogenated fractions of a given fat. The lard fractions show the long "alpha halt," characteristic of 2-palmityldistearin (11) while the tallow fractions show quick rise from the minimum due presumably to the 1-palmityldistearin content.

Further evidence of the 2-palmityl content of lard is given by the diffraction patterns of the hydrogenated fractions which, in the beta phase, show the close approach of the outer short spacing lines at approximately 3.8 Å which is characteristic of 2-palmityldistearin. In contrast, two lines in this region, at about 3.85 and 3.6 Å, appear distinctly for hydrogenated fractions from tallow. Moreover the disaturated fraction of lard, before hydrogenation, shows a diffraction pattern of a beta prime-3 type closely similar to the pattern of 2-palmityloleylstearin treated in similar fashion and quite different from patterns of the 2-oleyl or 2-stearyl isomers, fatty acid analysis calling for an approximate composition of ⅓ palmitic, ⅓ oleic, and ⅓ stearic. These conclusions are in line with those of Hilditch (3).

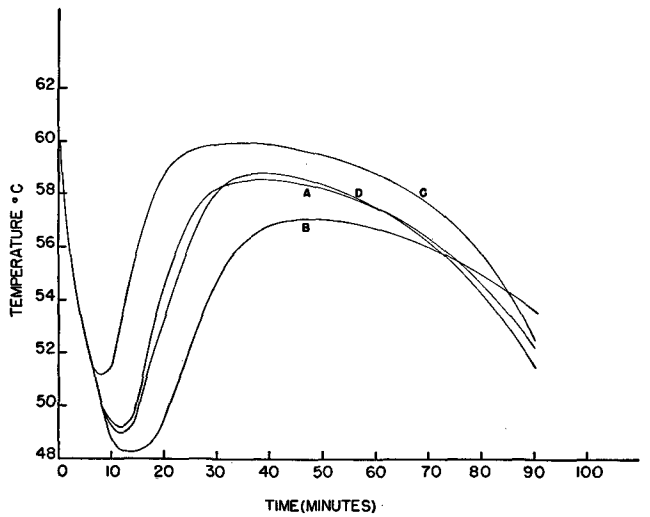

FIG. 8. Cooling curves of completely hydrogenated beef tallow fractions.

A—Beef tallow
B—Beef tallow S_3
C—Beef tallow S_2U
D—Beef tallow SU_2

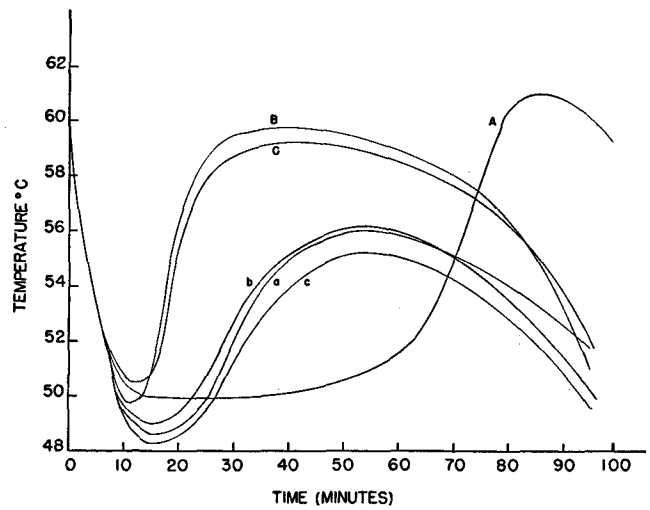

FIG. 9. Cooling curves of completely hydrogenated fats. Effect of rearrangement.

A—Lard
B—Beef tallow
C—Mutton tallow
a—Randomized lard
b—Randomized beef tallow
c—Randomized mutton tallow

Similar evidence for the 1-palmityl arrangement of beef tallow comes from the close similarity in thermal data and in diffraction patterns of the hydrogenated fractions to 1-palmityl distearin. Moreover the disaturated fraction gives a pattern corresponding only to 2-oleylpalmitylstearin, among the 3 possible isomers.

The mixture of β-3 and β-2 forms for mutton tallow disaturated is also highly suggestive of 2-oleyl

arrangement since only such disaturated components give β-3 in the pure state (9) and since β-2 has been observed for nearly pure 2-oleyl samples (12); in addition β-2 has been observed for 50-50 binary mixes of disaturated glycerides (12) only when one component was a 2-oleyl glyceride but the other a 1-oleyl compound.

While agreeing with the general conclusion of Kartha (5) (and Hilditch) that animal fats are not randomly arranged, present results as shown in Table VI do not agree entirely with his conclusions that in animal fats S_3 is less and S_2U is more than the corresponding "chance value."

The nature of the evidence presented is not such as to establish within extremely close limits the glyceride composition of the various fats, but it does indicate:

a) that animal fats are non-random in their fatty acid distribution.

b) that tallows differ sharply from lard in fatty acid distribution; the latter definitely is composed largely of 2-palmityl glycerides, the former probably of 1-palmityl glycerides.

c) for a given animal fat there is considerable constancy of palmityl position throughout its S_3, S_2U, and SU_2 glycerides.

Summary

In agreement with comments of Hilditch but in contrast with certain other statements in the literature it is concluded that lard, beef tallow, and mutton tallow are non-random in distribution of fatty acid chains among their glycerides. From thermal and x-ray diffraction techniques applied to S_3, S_2U, SU_2, and U_3 fractions of these fats and to the various products of complete hydrogenation it is concluded that lard is composed largely of 2-palmityl glycerides as proposed by Hilditch while the tallows are composed largely of 1-palmityl glycerides.

Acknowledgment

The authors express their appreciation to members of this laboratory who have given advice and assistance, and especially to Fred R. Hugenberg, who performed most of the experimental work.

REFERENCES

1. Hilditch, T. P., "The Chemical Constitution of Natural Fats," 2nd Ed., John Wiley and Sons Inc., N. Y. (1947). Chapter VI, pp. 231-287.
2. Doerschuk, A. P., and Daubert, B. F., J. Am. Oil Chem. Soc., *24*, 274 (1947).
3. Hilditch, T. P., *loc. cit.*, p. 315.
4. Norris, F. A., and Mattil, K. F., J. Am. Oil Chem. Soc., *24*, 274 (1947).
5. Kartha, A. R. S., *ibid.*, *29*, 109 (1952).
6. Riemenschneider, R. W., Luddy, F. E., Swain, M. L., and Ault, W. C., *ibid.* *23*, 276 (1946).
7. Collin, G., Hilditch, T. P., and Lea, C. H., J. Soc. Chem. Ind., *48*, 46 (1929).
8. Lutton, E. S., Jackson, F. L., and Quimby O. T., J. Am. Chem. Soc., *70*, 2441 (1948).
9. Lutton, E. S., *ibid.*, *73*, 5595 (1951).
10. Hilditch, T. P., *loc. cit.*, p. 300, 301, 302, 315.
11. Malkin, T., and Meara, M. L., J. Chem. Soc., 103 (1939).
12. Lutton, E. S., unreported.

[Received October 29, 1952]

Some Factors Influencing Foam Stability

C. R. SPORCK, Division of Research and Development, Lever Brothers Company, Edgewater, N. J.

IT is evident that the foaming power of liquids does not depend on the surface tension alone. A liquid hydrocarbon of surface tension about 25 dynes/cm at 25°C. foams no more readily than water of surface tension about 72 dynes/cm at 25°C. Gibbs (1) points out that for persistence a liquid film must have a means of withstanding shock, a sort of elasticity. Stated succinctly, for stability a film must have an elastic property so that if the film is increased in area by an external agency, a force will arise in the film tending to oppose this increase of area and if the film is decreased in area, a force will arise to resist the change. Only if this compensatory reaction can be elicited from the film, can the shocks to which by chance it is subjected be prevented from destroying the film. This elasticity can be provided for liquid films between two gas masses only if the film contains at least two components (water is conventionally considered the first component).

Now there are two cases to be considered, A and B:

A. The second of the two components may be a component of the surface alone, being insoluble in the bulk.

B. This case may arise in two ways:

1. The second component may be a component of the surface and the bulk phases, but the bulk phase may be not large enough to constitute a reservoir. Therefore it cannot fix the chemical potential in the surface of component two as the surface phase changes its extent.
2. The bulk phase may be large enough to act as a reservoir, but the time required for adjustment of the chemical potential of component two in the surface after a disturbance may be long compared to the duration of the disturbance.

The physical picture implicit in the above is clear. In general, in two component aqueous solutions the surface tension decreases as the Gibbs surface excess increases and increases as the surface excess decreases. If the surface-active component is confined to the surface alone, (Case A) a contraction or extension of total surface area causes an increase or decrease of surface concentration which causes a decrease or increase in surface tension. Hence we have a force which always resists a change in surface area whether this change is an increase or decrease. With the surface-active component confined to the surface and bulk liquid phases (Case B1) i.e., a compound of low vapor pressure, the situation is similar. If the adjustment to equilibrium is slow after change in surface area as in Part 2 of Case B above, the elasticity is even greater than the equilibrium value.

To illustrate the importance of this last point we may most effectively use a three-component system.