tions although, as yet, only crude results have been obtained.

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## Summary

Details of apparatus construction and refinements are presented for a micro capillary rise method of determining absolute values of surface tension. Experimental values of surface tension for benzene, n-pentadecane, and methyl laurate are recorded and compared with previous literature values. Parachors are determined. The method requires a minute amount of sample and is simple, quick, precise, and accurate.

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# The Trans Fatty Acids of Margarines and Shortenings[1, 2]

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A PRESENT TREND in the American diet is the rapid increase in use of shortenings and margarines. For example, in 1953 the consumption of margarine in the United States exceeded that of butter for the first time; lard consumption has declined along with the increase in use of shortenings.

Margarines and shortenings can be, and sometimes are, formulated by blending natural fats, but currently it is almost the universal practice to use hydrogenated fats for this purpose. This use of what one might properly call a "synthetic fat" as a major item in our diet makes the problem of the composition of hydrogenated fats important, and more particularly that of the nature of the unsaturated acids in these fats. The unsaturated acids in natural fats are almost always of the so-called *cis* configuration, the principal exception being vaccenic acid, *trans*-11-octadecenoic acid, which has been found in small amounts in several animal fats (4). Recent work in this laboratory (5) has shown that summer butterfat contains up to 9.5% *trans* acids. In contrast to the natural fats, hydrogenated fats are known to contain large quantities of *trans* acids, not presently known to occur naturally. Another important difference in composition between the hydrogenated fats and the oils from which they have been made is their content of essential fatty acids. Hydrogenation destroys a major part of these acids, thus impairing their nutritive value in this respect.

The almost hopelessly complex nature of the mixed fatty acids in hydrogenated fats can best be pointed out by citing a few of the numerous investigations of the chemistry of hydrogenation of fatty acids and their esters and glycerides, which involves more than simple addition of hydrogen to double bonds. Various types of isomerization take place in this process so that in partially hydrogenated products there is always an increase in the number and types of fatty acids present in the product. Thus Moore (16) showed that, in the course of hydrogenating ethyl oleate, the resulting iso-oleic acids consisted of a mixture of 8-, 9-, and 10-octadecenoic acids. Likewise Hilditch and Vidyarthi (8), Benedict and Daubert (3), and others (15, 17) found that partial hydrogenation of oleic acid and its esters resulted in formation of numerous positional and geometric isomers of oleic acid. The trans 6-, 7-, 8-, 9-, 10-, and 11-octadecenoic acids, described by Huber (9) melt much higher than the corresponding *cis* isomers, and at least several of them are responsible for a considerable part of the desired high melting point of commercial hydrogenated fats.

The partial hydrogenation of a dienoic acid, such as linoleic acid, or of its esters, results in a still more complicated mixture of products including numerous octadecenoic acids, (16, 8, 20) both *cis* and *trans,* as well as various isomers, both positional and geometric, of linoleic acid itself. Most of these isolinoleic acids have not been identified reliably. Hydrogenation of the 12,13-double bond to give oleic acid is always accompanied by less reaction of the 9,10-double bond to give 12-octadecenoic acid (24). (Cf. also 1, 6, 14, 20, 22.)

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[2] Presented at the June, 1955, meeting of the Institute of Food Technologists, Columbus, Ohio.

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The use of soybean oil in making edible hydrogenated fats introduces a still further complication in that this oil contains important amounts of linolenic acid, the products of partial hydrogenation of which must be added to those resulting from oleic and linoleic acids. Thus van der Veen (24) demonstrated the formation of two iso-linoleic acids on hydrogenating methyl linolenate. Lemon (12) noted that some of these isomers had double bonds separated by more than one methylene group; these are only slowly conjugated by alkali and hence are not estimated by the standard ultraviolet spectrophotometric procedure. These findings were substantiated by Bailey and Fisher (2), who hydrogenated cottonseed, soybean, and linseed oils under selective conditions and found 9,15-octadecadienoic acid among the products and were further confirmed with soybean oil by Mattil (14), and by Daubert and Filer (6). (Cf. [7].) With linseed oil Lemon and Cross (13) concluded that hydrogenation is accompanied by a *cis* to *trans* change in some of the double bonds and that the resulting iso-linoleic acid has at least one *trans* double bond, a finding that was confirmed by Rebello and Daubert (18) by infrared examination. On partial hydrogenation of methyl linolenate Ueno (23) found that the 15,16-position hydrogenates first, then the 9,10-position, while the 12,13-position is the least susceptible one.

The preceding literature has been cited in order to point out the complex nature of the component fatty acids in partially hydrogenated fats made from cottonseed, peanut, and soybean oils, and others occasionally used in food technology to prepare margarines and shortenings. These oils contain large amounts of oleic and linoleic acids, even as much as 50% of the latter while soybean oil contains up to 10% of linolenic acid. Further, the preceding citations have been concerned primarily with fundamental investigations of hydrogenation of acids, simple esters, and glycerides. At the time this work was completed, almost no work had been reported on the actual composition of commercial margarines and shortenings. Consequently objectives of the present investigation were to study the nature of the fatty acids in several typical margarines and shortenings as manufactured in the United States, to determine the *trans* acid contents of these products, and to study the distribution of *trans* acids in the several carbon series. These results were designed to throw light on the nature of these most important food products.

## Experimental

*Materials.* Five specimens of shortenings and six of margarines were purchased on the open market. The shortenings were analyzed as received; the margarines were melted, filtered to remove salt and curd, and dried under reduced pressure. The origins of the several specimens and the sample designations are described in Table I.

*Analytical Methods.* Iodine values, saponification numbers, saponification equivalents, and ultraviolet analyses before and after alkali-isomerization were determined according to the Official and Tentative Methods of The American Oil Chemists' Society. The *trans* acids were determined both as triglycerides and as methyl esters by the infrared-spectrophotometric method described by Shreve *et al.* (19) and by Swern and co-workers (21). It was necessary to develop new infrared reference values for several pure acids and esters in order to standardize the procedure with the Beckman IR spectrophotometer used in the present investigation. Infrared absorption was measured at 10.36 $\mu$ in optically pure carbon disulphide. The total concentration of the solution was adjusted where possible to give solute optical densities corresponding to k-values of 0.300 to 0.600. The extinction, k, was calculated from the following relationship:

$$k = \frac{d}{cL}$$

where d is the optical density, c is the concentration in grams per liter, and L is the cell thickness in centimeters. The k-values found for the standard compounds used in this investigation are recorded in Table II.

TABLE I
Description of Shortenings and Margarines

| Sample | Brand | Company | Origin of Fat |
|---|---|---|---|
| | | Shortenings | |
| $S_1$ | Crisco | Procter and Gamble | Vegetable oils |
| $S_2$ | Spry | Lever Brothers Co. | Vegetable oils |
| $S_3$ | Swift'ning | Swift and Company | Meat fats and vegetable fats[a] |
| $S_4$ | Durkee's | Durkee Famous Foods | Vegetable fats |
| $S_5$ | Food Club | Topco Assoc. Inc. | Vegetable fats |
| | | Margarines | |
| $M_1$ | All Sweet | Swift and Company | Cottonseed and soybean oils |
| $M_2$ | Parkay[b] | Kraft Foods Co. | Soybean and cottonseed oils |
| $M_3$ | Good Luck | Lever Brothers Co. | Soybean and cottonseed oils |
| $M_4$ | Dixie | The Capital City Products Co. | Cottonseed and soybean oils |
| $M_5$ | Nu-Maid | The Miami Margarine Co. | Cottonseed and soybean oils |
| $M_6$ | Parkay[b] | Kraft Foods Co. | Soybean and cottonseed oils |

[a] The unique properties of this shortening are due to the fact that it is a blended composition and only slightly, if any, hydrogenated.
[b] Two specimens purchased at different times.

TABLE II
Infrared Extinction Coefficient[a] of Reference Compounds

| Triglycerides | k | Methyl Esters | k | Acids | k |
|---|---|---|---|---|---|
| Tristearin | 0.082 | Laurate | 0.044 | Lauric | 0.132 |
| Triolein | 0.106 | Myristate | 0.044 | Myristic | 0.117 |
| Trielaidin | 0.563 | Palmitate | 0.029 | Palmitic | 0.135 |
| | | Stearate | 0.034 | Hexadecenoic | 0.178 |
| | | Oleate | 0.044[b] | Stearic | 0.136 |
| | | Elaidate | 0.523[b] | Oleic | 0.144[b] |
| | | | | Elaidic | 0.560 |

[a] All k-values at 10.36 $\mu$.
[b] The extinction coefficient values of oleic acid, methyl oleate, and methyl elaidate were determined in this laboratory by R. H. Backderf.

*Spectrophotometric Examination of Margarines and Shortenings.* The several specimens of margarines and shortenings were examined by ultraviolet and infrared spectrophotometry; the results are given in Table III.

With one exception the margarines and shortenings in Table III have high iodine values (72.5–84.7) similar in fact to olive oil, and contain high percentages of *trans* acids (22.6–41.7%). The high melting points and plastic properties of these products are primarily due to their content of high melting (44–53°) *trans* monounsaturated acids. The exception noted is for the blended shortening, $S_3$, which is formulated with a minimum of hydrogenation and hence is of negligible *trans* acid content. The analytical values for the several classes of fatty acids in Table III are determined by empirical spectrophotometric proce-

TABLE III

Spectrophotometric Analysis of Shortenings and Margarines

| Sample | Sap. No. | Iod. Val. | Fatty Acids % | | | | | Conjugated Diene % [b] | k Value 10.36 μ | Trans Glyceride % [c] |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Saturated | Oleic | Linoleic [a] | Linolenic [a] | Arachidonic [a] | | | |
| $S_1$ | 191.2 | 76.79 | 23.7 | 63.9 | 11.8 | 0.4 | 0.0 | 0.2 | 0.204 | 22.7 |
| $S_2$ | 191.4 | 75.40 | 16.6 | 79.3 | 3.9 | 0.0 | 0.0 | 0.2 | 0.258 | 33.4 |
| $S_3$ | 196.1 | 59.40 | 44.9 | 42.5 | 11.3 | 0.6 | 0.5 | 0.2 | 0.097 | 00.4 |
| $S_4$ | 192.9 | 73.67 | 20.0 | 74.6 | 4.4 | 0.2 | 0.0 | 0.8 | 0.272 | 36.6 |
| $S_5$ | 196.3 | 72.55 | 21.5 | 72.8 | 4.7 | 0.2 | 0.0 | 0.8 | 0.264 | 34.9 |
| $M_1$ | 193.1 | 75.53 | 17.6 | 76.9 | 4.9 | 0.2 | 0.0 | 0.4 | 0.268 | 35.5 |
| $M_2$ | 193.2 | 81.36 | 15.2 | 75.6 | 8.1 | 0.6 | 0.0 | 0.5 | 0.272 | 37.1 |
| $M_3$ | 192.3 | 78.67 | 20.5 | 68.0 | 10.6 | 0.5 | 0.0 | 0.4 | 0.260 | 34.8 |
| $M_4$ | 192.2 | 74.49 | 23.0 | 66.3 | 10.1 | 0.1 | 0.0 | 0.5 | 0.251 | 32.9 |
| $M_5$ | 191.1 | 84.77 | 15.5 | 71.3 | 10.8 | 0.9 | 0.0 | 1.5 | 0.293 | 41.7 |
| $M_6$ | 192.3 | 80.26 | 15.2 | 75.6 | 8.2 | 0.5 | 0.0 | 0.5 | 0.254 | 33.2 |

[a] Values for 2-, 3-, and 4-double acids are stated as linoleic, linolenic, and arachidonic acids, respectively, but the method is not specific for these acids.

[b] Conjugated triene and tetraene acids less than 0.1%.

[c] Estimated as trielaidin.

dures and, because of the inherent nature of hydrogenated fats, are of strictly limited accuracy. Linoleic acid values, 3.9–11.8%, avg. 7.3, are subject to the limitation of the presence of small amounts of non- or slowly-conjugatable diene. Linolenic acid values range from 0.0–0.9%. Both linoleic and linolenic acid values include all conjugatable diene and triene; in other words, iso-acids are known to be present.

*Fractional Distillation of Methyl Esters of Selected Specimens of a Margarine and a Shortening.* One specimen each of a margarine and a shortening was converted to methyl esters by the usual procedure, and the esters were fractionally distilled. Analytical data on the original esters and on the resulting fractions are described in Tables IV and V.

From Tables IV and V it is quite clear that *trans* components occur in all fractions except $S_2$–3. Therefore both $C_{16}$ and $C_{18}$ *trans* fatty acids occur in these specimens of margarine and shortening. *Trans* unsaturated fatty acid esters show gradual increase in succeeding $C_{18}$ fractions. Thus, with $M_6$, fractions 4–9 show gradual increases from 35.3% to a maximum of 43.4% in fraction 9. Ultraviolet analyses of the fractions 4–11 of $M_6$ are shown in Table VI.

TABLE IV

Analysis of Methyl Ester Fractions of $M_6$

| Frac. No. | Wt. g. | Iod. Val. | Mean Mol. Wt., acids | k 10.36 μ | Trans Esters % [a] |
|---|---|---|---|---|---|
| 1 | 6.15 | 5.13 | 234.2 | 0.048 | 3.5 |
| 2 | 123.40 | 10.99 | 257.6 | 0.058 | 5.4 |
| 3 | 73.50 | 73.85 | 274.5 | 0.168 | 30.4 |
| 4 | 151.00 | 95.37 | 279.5 | 0.208 | 35.1 |
| 5 | 146.40 | 98.07 | 281.8 | 0.219 | 37.4 |
| 6 | 112.90 | 97.15 | 281.9 | 0.221 | 37.8 |
| 7 | 112.90 | 93.65 | 281.5 | 0.234 | 41.0 |
| 8 | 87.40 | 88.04 | 282.8 | 0.240 | 42.2 |
| 9 | 15.60 | 85.19 | 278.8 | 0.245 | 43.4 |
| 10 | 72.80 | 75.30 | 282.1 | 0.233 | 40.9 |
| 11 | 6.35 | 68.85 | 281.2 | 0.205 | 35.3 |
| Column hold-up | 10.00 | ........ | ........ | 0.148 | ...... |
| Total | 918.40 | | | | |
| Pot residue | 17.40 | ........ | ........ | 0.130 | ...... |
| Original esters | 935.80 | 78.91 | 276.8 | 0.214 | 36.9 |

[a] Calculated as methyl elaidate.

TABLE V

Fractional Distillation of Methyl Esters of $S_2$ and Infrared Examination of Fractions

| Frac. No. | Wt. g. | Iod. Val. | Mean Mol. Wt., acids | k 10.36 μ | Trans Esters % [a] |
|---|---|---|---|---|---|
| 1 | 1.60 | 10.87 | 249.2 | 0.069 | 7.1 |
| 2 | 22.70 | 1.52 | 253.1 | 0.049 | 2.5 |
| 3 | 48.70 | ........ | 257.1 | 0.040 | 0.0 |
| 4 | 18.20 | 76.88 | 270.7 | 0.177 | 27.8 |
| 5 | 132.70 | 85.81 | 277.8 | 0.235 | 39.9 |
| 6 | 302.40 | 85.28 | 281.9 | 0.250 | 43.0 |
| 7 | 47.95 | 76.30 | 282.5 | 0.260 | 45.3 |
| 8 | 71.50 | 65.12 | 282.8 | 0.260 | 45.7 |
| Column hold-up | 10.75 | 46.81 | ........ | 0.201 | 33.8 |
| Pot residue | 28.20 | 61.37 | ........ | 0.194 | 31.7 |
| Original esters Total | 687.80 | 74.25 | 276.3 | 0.217 | 36.3 |

[a] Calculated as elaidate. In Fractions 1 and 2 the material is likely to be actually trans hexadecenoate.

TABLE VI

Ultraviolet Analysis of Ester Fractions of $M_6$ a

| Frac. | Stearic Acid % | Mono-ethenoic Acid % | Dienoic Acid | | Trienoic Acid | |
|---|---|---|---|---|---|---|
| | | | Conj. % | Non-conj. % | Conj. % | Non-conj.[b] % |
| 4 | 3.3 | 81.7 | 0.2 | 14.3 | 0.0 | 0.5 |
| 5 | 2.8 | 81.0 | 0.2 | 15.4 | 0.0 | 0.5 |
| 6 | 0.8 | 86.1 | 0.2 | 12.8 | 0.0 | 0.5 |
| 7 | 2.4 | 87.0 | 0.2 | 9.9 | 0.0 | 0.5 |
| 8 | 7.5 | 83.2 | 0.3 | 8.3 | 0.0 | 0.6 |
| 9 | 14.8 | 71.7 | 0.6 | 12.4 | 0.0 | 0.4 |
| 10 | 19.9 | 72.7 | 1.9 | 5.5 | 0.0 | 0.0 |
| 11 | 32.0 | 56.6 | 7.7 | 3.2 | 0.0 | 0.5 |
| Original esters | 19.0 | 70.9 | 0.5 | 9.1 | 0.0 | 0.5 |

[a] All fractions calculated as $C_{18}$ esters.

[b] No detectable amounts of conjugated trienoic acids.

As distillation proceeds (Table VI), the amounts of non-conjugated polyunsaturated esters tend to decrease while conjugated octadecadienoate rises. Its highest percentage is found in fraction 11, 7.7%. As would be expected, the percentage of methyl stearate increases gradually in the successive $C_{18}$ fractions, behaving thus like the *trans* unsaturated esters.

*Further Separation of the $C_{18}$ Esters of $M_1$ by Low Temperature Crystallization. The Isolation of a $C_{18}$ Trans Esters Concentrate.* A composite $C_{18}$ ester fraction, made by combining fractions 4–10 of $M_1$, was

TABLE VII

Ultraviolet Analysis of Crystallized Fractions

| Fract. | Saturated Ester % | Octa-decenoate % | Octadecadienoate | | Octadeca-trienoate Nonconj. % |
|---|---|---|---|---|---|
| | | | Conj. % | Nonconj. % | |
| $F_1$ | 0.0 | (41.3)[a] | 8.1 | 49.6 | 2.0 |
| $F_2$ | 0.0 | (56.8)[a] | 1.7 | 40.2 | 1.3 |
| $F_3$ | 7.9 | 83.0 | 1.6 | 7.4 | 0.0 |
| $C_2$ | 1.1 | 98.7 | 0.0 | 0.2 | 0.0 |
| Composition of combined $C_{18}$ fraction, subjected to Low Temperature Crystallization | | | | | |
| | 5.8 | 81.8 | 0.3 | 11.1 | 0.8 |

[a] These values by difference but in serious error because not all dienoate has been estimated.

TABLE VIII
Approximate Composition of Crystallization Fractions Calculated from Iodine Values

| Frac. | Wt. g. | °C. | I.V. | Stearate | Octadecenoate | Octadeca-dienoate | Trans %[a] |
|---|---|---|---|---|---|---|---|
| $F_1$ | 13.5 | —65[b] | 152.54 | 00.0 | 22.9 | 77.1[c] | 43.8 |
| $F_2$ | 12.0 | —65[b] | 132.40 | 00.0 | 46.1 | 53.9 | 41.6 |
| $F_3$ | 3.9 | —60[b] | 87.65 | 00.0 | 97.2 | 2.8 | 19.0 |
| $C_2$ | 40.9 | —60[d] | 84.79 | 00.9 | 99.1 | 00'0 | 29.5 |
| $F_4$ | 21.6 | 0 | 74.53 | 12.9 | 87.1 | 00'0 | 90.6 |
| $C_1$ | 7.8 | 0 | 11.35 | 86.7 | 13.3 | 00'0 | 12.3 |

[a] Trans % is calculated as Me elaidate in all fractions.
[b] Calculated here as binary mixtures of octadecenoate and octadecadienoate, on the assumption that no stearate is present in —60° to —65° filtrates.
[c] But u.v. analysis gives 49.6% non-conj. and 8.1% conj. dienoate and 2% trienoate.
[d] Calculated as a mixture of stearate and octadecenoate.

crystallized as shown in Chart 1. Analytical data on the resultant fractions are given in Tables VII and VIII.

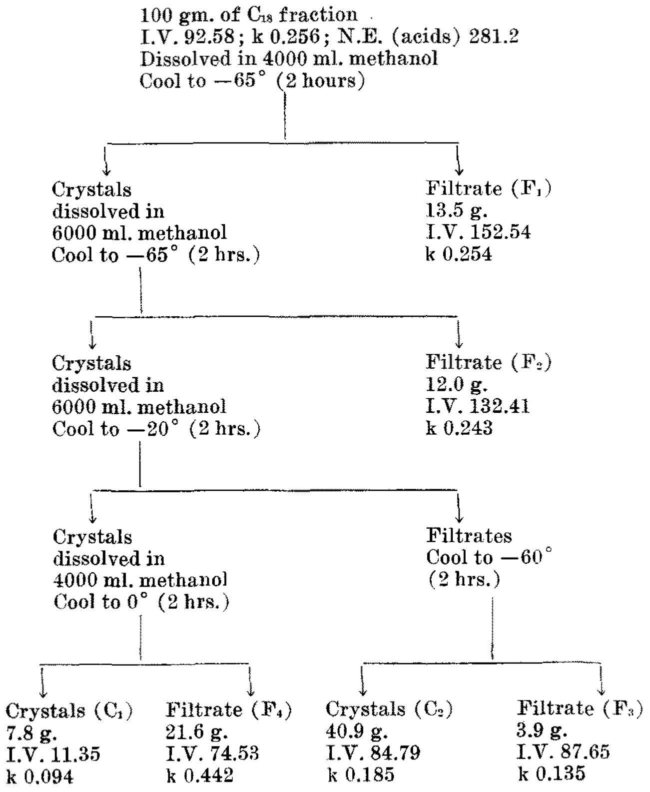
By calculation from the iodine value, $F_1$ (Table VIII) contains 22.9% of octadecenoate and 77.1% octadecadienoate. Ultraviolet analysis shows $F_1$ to contain 49.6 and 8.1%, respectively, of nonconjugated and conjugated dienoates, a total of 57.7%, and, in addition, 2% of triene ester. The difference, about 20%, may be due to octadecadienoates which will not rearrange to conjugated diene systems with alkali, as, for example, 9,15-octadecadienoate, and in part to other esters which require longer periods of alkali isomerization such as *trans,trans-*, *cis,trans-*, and *trans,cis-*9,12-, and 12,15-octadecadienoate. These latter types are not determined quantitatively under our experimental conditions. To estimate them it would be necessary to isomerize for 60 to 360 min., as suggested by Jackson *et al.* (10). This was not done. Solubility considerations (see discussion later) would likewise confirm the assumption that the diene acids found in $F_1$ are these several types of geometric isomers of octadecadienoates. It is to be further noted that $F_1$ contains 8% of conjugated diene, which in turn will tend to confuse the result by only partly showing up in the iodine value. Thus no presently available methods will accurately assess the composition of this mixture. The octadecenoate in $F_1$ is largely methyl oleate, but it may contain other soluble isomers of this ester, such as methyl 15-octadecenoate, etc. *Trans* octadecenoates are not likely to be present in appreciable amounts since these tend to be sparingly soluble at —60°. Most of the *trans* material in this fraction is likely diene in nature. Octadecadienoate values for $F_2$ in Tables VII and VIII are widely different, 41.9 and 53.9%, respectively, for the reasons mentioned above. Trans esters in this fraction, 51.6%, quite certainly include both monoene and diene esters. *Trans* components in fraction $F_3$ are likely to be principally octadecenoates with the double bond beyond the 9-position.

The —60° crystals, $C_2$, contain about 99% octadecenoate, about one-third of which is trans ester. The *cis* and *trans* octadecenoates in this fraction may include many of the predictable types, including oleate and elaidate. The highest concentration of *trans* material is found in fraction $F_4$. This fraction consists of stearate and trans-9-octadecenoate and possible *cis*-octadecenoates of lesser solubility than oleate. Ninety per cent of the octadecenoate in this fraction is *trans*. The major component of $C_1$ is methyl stearate contaminated with *trans,* and *cis*-octadecenoates. From the data of Tables VII and VIII it is quite obvious that the scheme used in Chart 1 produced a fraction, $F_4$, with the highest (90.6%) concentration of *trans* esters. The advantages of low temperature crystallization in separating the $C_{18}$ esters into six simplified fractions are borne out by the data in Tables VII and VIII, but each of the simplified fractions still contains three or usually many more components.

## Discussion

The $C_{18}$ fatty acids of most vegetable fats consist mainly of three solubility types, stearic, oleic, and linoleic (and possibly linolenic) acids, which can be separated readily by cooling solutions of these acids to —20° and to —60°. Stearic acid comes out at —20°, oleic acid at —60°, and linoleic (and linolenic) acid remains in solution at —60°. Esters behave similarly although they are somewhat more soluble. In hydrogenated fats two more types of acids exist, namely, positional and geometrical isomers of both octadecenoic acids and octadecadienoic acids. *Trans* octadecenoic acids are less soluble than the corresponding *cis* acids. Thus the solubility of elaidic acid is intermediate between that of stearic and oleic acids. The position of the double bond in the carbon

CHART 1
Crystallization of $C_{18}$ Ester Fraction of $M_6$ to Produce Trans Ester Concentrates



chain of octadecenoic acids has a definite effect on the melting points and solubility of both *cis-* and *trans-*acids. For example, recently Kolb and Brown (11) showed that as the double bond of a *cis* octadecenoic acid is moved away from the carboxyl group, the acid becomes somewhat more soluble. Therefore we may expect to find *cis* 12- and 15-octadecenoic acids, such as may conceivably be present in partially hydrogenated cottonseed and soybean oils, to be more soluble than oleic acid and to appear in the linoleic acid (filtrate) fractions.

A second double bond in any given fatty acid further increases its solubility; linoleic acid is about 30 times as soluble as oleic acid. Rebello and Daubert (18), as cited previously, applied more than 30 crystallizations to obtain an isolinoleic ester fraction (95.8% pure) consisting of at least three isomers, the 8,14-, 9,15-, and 10,14-octadecadienoic esters. Conjugated diethenoic esters are less soluble than non-conjugated. *Cis,trans-* and *trans,cis-*octadecadienoic acids would appear to be an intermediate solubility class between *cis,cis-* (the more soluble) and *trans,trans-* (the less soluble) esters although actual solubility data on pure compounds are not available.

One concluding statement should be made, appraising ultraviolet and infrared spectrophotometric methods as applied to partially hydrogenated fats and oils. Ultraviolet analysis, before alkali isomerization, will estimate the small amount of pre-conjugated acids in these fats. After alkali isomerization the method has decided limitations because of the presence of fatty acids which either do not isomerize or which isomerize slowly, or, at least, at rates different from those established in the official procedures for conjugated systems. Infrared examination for *trans* acids in hydrogenated fats is an extremely useful procedure, but it also is limited at the moment by the difficulty of establishing the base line and reference values for the numerous *cis* and *trans* components in these mixtures. Values are somewhat more accurate for simplified distillation and crystallization fractions.

A final comment is in order. The American people are eating nearly three billion pounds of margarines and shortenings annually. The average *trans* acid content of these products is about 30%. Thus we are consuming nearly a billion pounds of *trans* fatty acids, by far the largest part of which have been introduced into these food products by hydrogenation. Work is already under way in this laboratory which indicates that human body fat contains appreciable amounts of *trans* acids. These are doubtless of dietary origin. It is indeed fortunate that at present there is no evidence to indicate that these unique acids are in any sense deleterious. The principal disadvantage of hydrogenation is that it removes large amounts of the essential fatty acids which are present in the natural oils. We are currently investigating the problem of actually how much linoleic acid is to be found in these important food products.

## Conclusions

1. Six specimens of margarines and five of shortenings have been examined for *trans* fatty acids by infrared absorption at 10.36 $\mu$. With one exception *trans* components calculated as trielaidin were found to range from 22.7–41.7%; the single example is a specimen of shortening prepared by blending vegetable and animal fats and subjected to minor hydrogenation. The iodine values of these materials were close to the range found for olive oil; the high melting point of the products is due, principally, to the presence of large amounts of *trans* acids.
2. Methyl esters were prepared from one specimen each of margarine and shortening, and the several distillation fractions were studied by infrared and ultraviolet spectrophotometry. All fractions contained *trans* acids, but large amounts were found in the $C_{18}$ fractions. It is probable that small amounts of *trans*-hexadecenoic acid occur in these fats.
3. In order to produce concentrates of *trans* esters and further to study the nature of the *trans* unsaturated acids of the $C_{18}$ series, the combined $C_{18}$ esters of a specimen of margarine were fractionally crystallized at low temperature. Six simplified fractions were thus obtained, and their composition was studied from their iodine values and infrared and ultraviolet absorptions. One of these fractions contained 90% of its unsaturated acids as *trans* acids.
4. Evidence is presented that the unsaturated fatty acids of a typical margarine, and probably generally of margarines and shortenings, are actually an extremely complex mixture of many of the theoretically possible positional and geometric isomers of the octadecenoic and octadecadienoic acids. When the hydrogenated fat is made from an oil containing linolenic acid (soybean oil), the mixture is even more complex.
5. The spectrophotometric method of analysis of hydrogenated fats is evaluated and shown to be subject to definite limitations.

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