

Octadecadienoic Acids of Shortenings and Margarines

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OF THE NUMEROUS FATTY ACIDS found naturally in fats and oils, linoleic, linolenic, and arachidonic acids alone have been shown to be essential components of the animal diet (2-5, 9, 23, 24). They are also efficacious in the treatment of some human skin diseases (10). Linoleic and arachidonic acids are biologically effective by themselves, but linolenic acid has been observed to be active in promoting growth only in the presence of linoleic acid (9). Recent work has indicated that arachidonic acid is the most important of the three acids in animal nutrition (7) and that it can be synthesized by the animal organism from linoleic acid (1, 16, 20, 21, 26). For children there is no dietary requirement for arachidonic acid when linoleic acid is supplied (27). Furthermore, of the three acids, linoleic acid is reported to be most effective in alleviating pyridoxine deficiency (18). These observations and the fact that arachidonic acid occurs in significant amounts only in some animal fats and fish oils and is not found in vegetable fats and oils demonstrate clearly that linoleic acid is the principal dietary source of essential fatty acid.

In this country the consumption of hydrogenated fats in the form of margarines and shortenings has grown steadily in recent years until presently they constitute one of the important components of the American diet. In one of the important competitions, for example, the use of margarine is now about equal to that of butter. Margarines and shortenings are commonly made by the partial hydrogenation of soybean and cottonseed oils. It is well known that under the conditions of commercial hydrogenation the component linoleic and linolenic acids are largely destroyed. In a recent paper Mabrouk and Brown (14) have called attention to the various isomeric monoethenoic and diethenoic acids produced as a result of hydrogenation. Of the latter, the conjugated, the 9,12-*cis,trans*, and the isolated (positional) *cis,trans* isomers of linoleic acid have been reported to occur in hydrogenated fats (8, 12, 13, 19). Only the (9,11-) conjugated, the 9,12-*trans,trans* (linolelaidic), and the 9,12-*cis,trans* octadecadienoic acids have been biologically tested and found to be ineffective in relieving fat deficiency symptoms although the last mentioned has the "sparking effect" on linolenic acid activity (5, 17). Hydrogenated coconut oil and fats rich in elaidin have been observed to accentuate fat deficiency symptoms (7). Hydrogenated fats similar to margarines and shortenings have nutritive values comparable to a natural fat of the same firmness (15). The objectives of the present study are to assess the linoleic acid content of typical

margarines and shortenings and to shed some light on the nature of other octadecadienoic acids present in these fats. In connection with this study we have been successful in concentrating most of the polyunsaturated acids into one fraction.

Experimental

Materials. Three samples each of shortenings and margarines, purchased on the market in Columbus, O., were used in the present investigation. Dates of purchase and brands were as follows: Goodluck, Parkay, and Dixie margarines, April, 1955; Spry shortening, Dec., 1954; Crisco and Durkee shortenings, date not known, but approximately Summer, 1952, and stored until use at -20° .

Methods of Analysis. Most of the methods employed are those described in the "Official and Tentative Methods" of the American Oil Chemists' Society, and for the estimation of polyunsaturated acids the revised (1953) procedure was followed. Linoleic acid was specifically estimated by the tetrabromide method (25), and *trans* acids were estimated by the infrared spectrophotometric method (6, 14, 22). Extinction coefficients used as reference standards in calculations of *trans* acids are as follows: palmitic acid, 0.151; stearic acid, 0.140; oleic acid, 0.145; linoleic acid, 0.171; linolenic acid, 0.193; elaidic acid, 0.584.

Fractionation of Fatty Acids of Margarines and Shortenings by Low Temperature Crystallization. A principal objective was to get the polyunsaturated acids concentrated into one fraction as far as possible free from *trans* monoethenoic acids. Preliminary crystallization studies were conducted with methyl esters. Trials with such solvents as pentane, acetone, and methanol at temperatures between -20 and -70°C . showed that, although a final filtrate fraction containing all the polyethenoic esters could be obtained, yet it also contained monoethenoic esters as the major component. The presence of *trans* monoethenoic esters makes it impossible to evaluate the *trans* dienoic ester content. Consequently in the work described below the mixed acids were crystallized from dilute solutions under which conditions complications due to association, etc., are likely to be at a minimum. After several preliminary crystallizations of the acids of one of the shortenings from acetone at different temperatures the scheme described in Figure 1 was adopted.

The scheme in Figure 1 separated each specimen of mixed acids into four end-fractions. In calculating the composition of these fractions, the following points were taken into consideration:

Fraction 1. All unsaturation in this fraction was assumed to be due to octadecenoic acids and was calculated as such from the iodine value. The *trans* acids were calculated as

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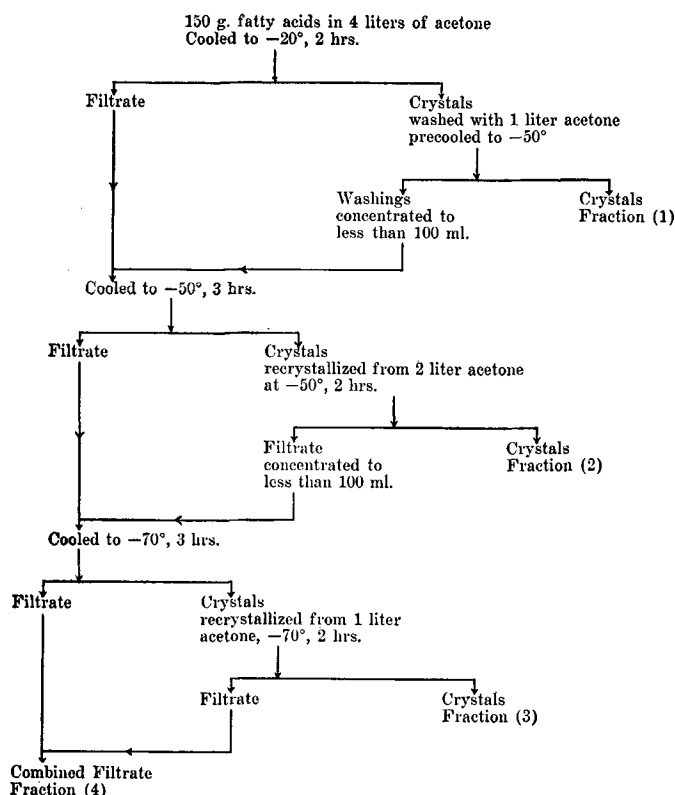


Fig. 1. Scheme for the fractionation of fatty acids from hydrogenated fats by low temperature crystallization.

elaic acid from the equation derived for a mixture of saturated and *trans* monoethenoic acids. It should be pointed out that palmitic acid is present to a considerable extent in this fraction (N. Eq.: SP₁, 272.6; DX₁, 270.7). As a near approximation this fraction may be considered as a 50:50 mixture of C₁₆ and C₁₈ acids and therefore, in correcting for the extinction of the saturated components, the mean extinction of palmitic and stearic acids was used.

Fraction 2. The polyunsaturated acids in this fraction were assumed to have been fully estimated by the spectrophotometric method, and the residual unsaturated acids were calculated from the iodine value as octadecenoic acids. Saturated acids were found by difference. *Trans* acids, as before, were calculated as elaidic acid. The neutralization equivalent (SP₂, 277.3; DX₂, 282.3) showed that the major component of this fraction is C₁₈ and therefore, in correcting for the absorption of the *cis* component, the value obtained for pure oleic acid was used.

Fraction 3. Composition of this fraction was calculated, assuming that a) no saturated acids are present, b) that for every 1% of conjugated dienoic acid present the experimental iodine value is 0.5 units lower than the theoretical (8), and c) that all the triene present has been detected by the u.v. spectrophotometric procedure. The *trans* acids, again, were estimated as elaidic acid. The neutralization equivalent (SP₃, 282.3; DX₃, 283) showed that this fraction is comprised entirely of C₁₈ acids and thus that the correction for the *cis* component as oleic acid is valid.

Fraction 4. The same assumptions as under Fraction 3 were made in calculating the contents of polyunsaturated acids and the total dienoic acids from the iodine value. The *trans* acids were assumed to be isomeric linoleic acids with one double bond *trans* (*cis,trans* or *trans,cis*) with the same absorption as elaidic acid (11, 21). Consequently the *k* value for elaidic acid was used for the pure *cis,trans* component and, in correcting for the *cis* acid, the value obtained for pure linoleic acid was used. From the weight of tetrabromides obtained from 1 g. of this fraction the content of linoleic acid was read off the standard curve (25).

Analysis and composition of the original fats, fatty acids, and different fractions are recorded in Table I.

Trend of Crystallization. The presence of geometric and positional isomers of unsaturated acids makes

the separation of the fatty acids of partially hydrogenated oils into simpler components a difficult one. In the scheme described in the preceding figure most of the saturated acids are found in Fraction 1, together with 14–30% of unsaturated acids. *Trans* acid contents in the several fractions are actually slightly higher than total monoethenoic acids, calculated from the iodine value, a discrepancy we are unable to explain. Only traces of polyethenoic acid (diene) are noted in this fraction.

Fraction 2 is the major fraction in all instances, constituting 40–50% of the total acids. This fraction is composed mainly of monoethenoic acids; the *trans* acid content varies from 30–65% of the fraction. In all cases maximum *trans* acid content is found in this fraction. The dienoic acid content is small, 1–1.5%. Most of the *trans* acids in these specimens of margarines and shortenings are therefore monoethenoic.

Fraction 3 represents an intermediate between the monoethenoic acids (Fraction 2) and polyethenoic acids (Fraction 4) both with respect to proportions and contents of *trans* acids. Again the major component is monoethenoic, comprising about 95% and above, about 20–25% of which is *trans*. With all samples the *trans* acid content of this fraction is significantly lower than that preceding or following it. Thus it is reasonable to assume that the residual *trans* monoethenoic acids which did not separate out in the previous fractions have crystallized out in this fraction, in which case the *trans* acids of the next (last) fraction are likely to be dienoic acids. The content of dienoic acids in Fraction 3 is of the order of 3–5%, and in most cases the experimental value for the total dienoic acids (conjugated and non-conjugated) is in fair agreement with that calculated from the iodine value after correcting for conjugated unsaturation. With one sample (SP₃) the values are unusually high as the fraction was not recrystallized but was simply washed once with cold acetone.

It must be pointed out that the maximum concentration of fatty acids in solution at the time this fraction comes down is about 1.5% and even at such dilutions significant amounts of diene crystallize out, thus demonstrating the difficulty attendant upon the attempt to isolate the isomers of linoleic acid into one fraction.

Fraction 4 is the most important fraction of the present study as it contains most of the polyunsaturated acids of the original fat. Only in this fraction could the content of triene (1–3%) be estimated with any degree of accuracy by the alkali isomerization method. Conjugated diene is present in significant proportion, 1–4%. This is also the fraction where the tetrabromide method (25) for the estimation of linoleic acid could be applied with reasonable accuracy. The content of linoleic acid ranged from 13–40% of the fraction. This amounts to 2–8% of the original mixed fatty acids. The recently reported observation that shortenings and margarines have essential fatty acid activity is obviously due to this presence of linoleic acid and therefore is not necessarily due to isomers of linoleic acid, as contended by Melnick and Deuel (15). With most samples the proportion of diene other than linoleic acid (difference between total diene from iodine value and linoleic acid as estimated by tetrabromide method) comes pretty close to and agrees well with the content of *trans* acids of this fraction, indicating that the other

TABLE I
Analysis of Shortenings, Margarines, and Fatty Acids and Crystallization Fractions of These Acids

Specimen	Yield %	I.V.	Sat'd acids %	Monoethenoid %	Conj. diene ^b %	Nonconjugated		Total diene from I.V. %	Linoleic acid by T.B. No. %	I.R. analysis		Diene other than linoleic acid %
						Diene %	Triene %			k _{10.36μ} (0.4 mm.) slit	Trans acid %	
SPRY												
Fat.....	—	75.5	—	—	0.3	6.6	< 0.2	—	—	0.235	—	—
Fatty acids ^a	—	78.6	24.5	64.0	0.3	6.8	< 0.2	11.6	3.2	0.276	28.8	—
SP ₁ (-20°C.).....	25.4	12.6	86.1	13.9	< .05	< .05	—	—	—	0.215	15.9	—
SP ₂ (-50°C.) ^c	50.5	87.1	5.1	93.2	0.2	1.5	< 0.05	—	—	0.323	40.6	—
SP ₃ (-70°C.) ^c	9.8	101.8	—	87.1	0.4	6.4	0.1	12.8	—	0.246	24.0	—
SP ₄ (Filtrate).....	14.1	150.2	—	34.2	1.6	37.1	1.2	65.6	22.4	0.337	42.9	42.2
CRISCO												
Fat.....	—	76.7	—	—	0.2	10.3	0.4	—	—	0.196	—	—
Fatty acids ^a	—	79.5	25.9	60.7	0.3	10.7	0.6	12.5	8.1	0.253	24.6	—
CR ₁ (-20°C.).....	27.5	15.0	83.5	16.5	< 0.1	0.1	—	—	—	0.224	18.0	—
CR ₂ (-50°C.).....	40.0	84.4	7.3	91.8	0.1	0.8	—	—	—	0.288	32.6	—
CR ₃ (-70°C.).....	11.7	92.6	—	96.8	0.2	3.0	< 0.1	3.0	—	0.225	18.2	—
CR ₄ (Filtrate).....	20.5	146.9	—	39.7	2.2	48.9	3.3	57.0	39.5	0.265	25.2	17.5
DURKEE'S												
Fat.....	—	74.0	—	—	0.8	4.4	0.1	—	—	0.260	—	—
Fatty acids ^a	—	77.9	24.0	67.1	0.8	4.6	0.1	9.1	2.0	0.336	43.5	—
DU ₁ (-20°C.).....	31.0	27.0	70.1	29.7	0.1	0.1	—	—	—	0.282	31.4	—
DU ₂ (-50°C.).....	41.1	85.6	5.5	83.7	0.4	0.4	< .05	—	—	0.401	58.3	—
DU ₃ (-70°C.).....	12.4	94.0	—	95.0	0.9	2.1	< .05	5.0	—	0.237	20.5	—
DU ₄ (Filtrate).....	15.6	131.4	—	55.0	3.8	24.0	.50	48.5	12.5	0.332	42.6 (40.1) ^d	36.0
GOOD LUCK												
Fat.....	—	78.7	—	—	0.3	9.7	0.4	—	—	0.247	—	—
Fatty acids ^a	—	82.0	23.1	62.4	0.4	9.7	0.5	13.6	4.8	0.319	39.6	—
GL ₁ (-20°C.).....	23.7	14.6	83.9	16.1	0.1	< 0.1	—	—	—	0.223	17.4	—
GL ₂ (-50°C.).....	44.2	82.5	7.4	91.8	0.1	0.4	—	—	—	0.387	55.1	—
GL ₃ (-70°C.).....	11.3	92.7	—	97.0	0.3	3.10	—	3.0	—	0.253	24.6	—
GL ₄ (Filtrate).....	20.8	151.8	—	33.7	1.2	43.9	2.2	64.1	28.1	0.350	40.7	36.0
PARKAY												
Fat.....	—	82.0	—	—	1.4	11.2	0.6	—	—	0.200	—	—
Fatty acids ^a	—	85.0	23.2	59.0	1.5	11.2	0.6	16.2	7.5	0.335	43.5	—
PK ₁ (-20°C.).....	26.6	21.7	76.5	22.7	0.2	0.6	—	—	—	0.264	27.0	—
PK ₂ (-50°C.).....	38.1	84.4	7.5	91.1	0.7	0.7	—	—	—	0.430	64.9	—
PK ₃ (-70°C.).....	13.0	92.6	—	96.1	1.6	2.6	< 0.1	3.8	—	0.219	17.1	—
PK ₄ (Filtrate).....	22.1	158.1	—	26.2	4.4	47.5	3.4	70.4	33.9	0.315	36.6	36.5
DIXIE												
Fat.....	—	77.6	—	—	1.1	9.6	< 0.1	—	—	0.250	—	—
Fatty acids ^a	—	80.3	25.1	60.3	1.2	9.5	< 0.1	14.3	4.8	0.310	37.6	—
DX ₁ (-20°C.).....	26.6	18.2	80.0	19.8	0.1	0.1	—	—	—	0.248	23.1	—
DX ₂ (-50°C.).....	37.3	81.7	10.3	88.6	0.5	0.6	—	—	—	0.388	55.4	—
DX ₃ (-70°C.).....	15.0	92.0	—	97.1	1.1	2.0	< .05	2.9	—	0.252	24.4	—
DX ₄ (Filtrate).....	21.2	148.0	—	35.1	3.4	40.9	0.6	64.3	22.6	0.342	43.8	41.7

^a The contents of saturated, monoethenoid, and linoleic acids are those obtained by back calculations from respective fractions. The total diene was calculated from the iodine value of this product after correcting for triene, the true proportion of which was calculated from that of the final filtrate fractions.
^b Conjugated triene is present in detectable amounts in the final filtrate fractions only. It is present to about 0.5% in DU₄, and in the rest it is less than 0.1%.
^c With SP₂ the crystals were washed with 1 liter of acetone, precooled to -75°, and with SP₃ the crystals were washed with 200 ml. of acetone precooled to -75°. ^d Calculated as elaidic acid.

dienes have at least one *trans* double bond and hence the only *cis,cis* diene present is linoleic acid.

Nature of the Octadecadienoic Acids. It has been reported that geometric isomers of linoleic acid are isomerized to the respective conjugated acids at different rates (11). A study of isomerization at times 25 min., 60 min., and 4.5 hrs., respectively, was therefore conducted on several of the fractions described in Table I. The final filtrate fractions (SP₄, DX₄) of these specimens were also isomerized for 150 min. From the k₂₃₂ values obtained after isomerization for 25 (k₁) and 60 (k₂) min., respectively, the proportions of *cis,cis* and *cis,trans* (*trans,cis*) linoleic acids were calculated from the following equations (11):

$$87.2x + 57.4y = 100k_1$$

$$88.7x + 70.4y = 100k_2$$

which when solved becomes:

$$x = 4.19k_1 - 2.99k_2$$

$$y = 4.54k_2 - 4.52k_1$$

where

$$x = \% \text{ 9,12-dienoic (cis,cis)}$$

$$y = \% \text{ 9,12-dienoic (cis,trans)}$$

$$k_1 = k_{232} \text{ m}\mu \text{ after 25 min. isomerization corrected for conjugated diene}$$

$$k_2 = k_{232} \text{ m}\mu \text{ after 60 min. isomerization corrected for conjugated diene}$$

It must be noted that, in deriving these relationships, the standard values used for pure *cis,cis* and *cis,trans* dienes are those reported for the methyl esters (11). The results are recorded in Table II.

The data in Table II reveal that the k₂₃₂ mμ values increase with an increasing time of isomerization.

TABLE II
Isomerization Study at Different Times on Selected Fractions^a
(6.6% KOH in Ethylene Glycol)

Fraction	k ₂₃₂ (Pre-conjugated)	k ₂₃₂ Isomerized			Conjugatable diene ^c	
		25	60	4.5	<i>cis,cis</i> %	<i>cis,trans</i> %
		minutes	minutes	hours		
SP ₁	< 0.05	< 0.1	< 0.1	< 0.1	—	—
SP ₂	0.2	1.5	1.8	2.0	—	—
SP ₃	0.5	6.9	8.6	8.8	2.5	7.5
SP ₄ ^b	2.0	37.0	41.0	44.7	28.7	18.0
DX ₁	0.1	0.2	0.2	0.2	—	—
DX ₂	0.6	1.2	1.2	1.3	—	—
DX ₃	1.4	3.3	3.8	4.2	0.8	2.2
DX ₄ ^b	4.0	41.4	46.4	48.9	28.6	22.4

^a Significant k₂₃₂ values (about 1.0) were obtained with SP₄ and DX₄, and these declined with increasing time of isomerization.
^b K₂₃₂ values after 150 minutes of isomerization: SP₄, 44.2; DX₄, 48.7.
^c K₂₃₂ values of columns 3 and 4 were corrected for pre-conjugated dienes, adjusted to those of methyl esters, and then substituted in the relationships derived earlier.

This rise is not significant with SP_2 and DX_2 , and thus the assumption made earlier in the calculations that all dienes present in these fractions are completely estimated by the ultraviolet absorption method is valid. With the fraction DX_3 the rise is not serious, and the total isomerizable diene (sum of *cis,cis* and *cis,trans*), about 2.9%, agrees admirably with the total diene calculated from iodine value about 2.9% (Table I), further supporting the assumption made under calculations that all dienes present are estimated by the ultraviolet absorption method. In interpreting the results with SP_3 , the fact that it was not recrystallized must be borne in mind. If the *trans,trans* 9,12-isomer was present, it must have appeared in either of these fractions (cryst -50 or $-70^\circ C.$) because of its higher melting point $28^\circ C.$ (11). The almost constant k_{232} value, even after 4-5 hrs. of isomerization with these fractions, shows that this isomer, even if present, is negligible. With SP_4 and DX_4 the increase in the values is considerable, revealing the presence of isomerizable geometric isomers of linoleic acid. With a synthetic mixture of linoleic and linoleic acid (*trans* 9, *trans* 12) acids Jackson *et al.* (11) noted that the values increased even after 150 min. of isomerization and became steady beyond 4.5 hrs. In our study we note that, with SP_4 and DX_4 , the values at the end of 150 min. and 4.5 hrs. are about the same. Therefore it is concluded that no *trans,trans* isomer is present and the only conjugatable isomer present is of the *cis,trans* or *trans,cis* type. The contents of *cis,cis* and *cis,trans* diene were calculated on this basis. It is noted that the values for linoleic acid by this calculation are higher than that by the tetrabromide method. From the empirical nature of the k_{232} $m\mu$ values for the *cis,trans* isomers of linoleic acid, as pointed out by Jackson *et al.* (11), this is about the best agreement that can be expected. However, for the content of linoleic acid, the tetrabromide method is the one to be relied upon as this is a direct estimation. A comparison of the proportions of total *trans* ($SP_4 - 42.9\%$, $DX_4 - 43.8$) and that of *cis,trans* ($SP_4 - 18.0$, $DX_4 - 22.4$) shows that considerable amounts (24.9, 21.4) of isolated *cis,trans* dienes are present. Placing reliance on the tetrabromide method for estimation of linoleic acid, it can be concluded that for all practical purposes these isomers, 9,12 *cis,trans* or *trans,cis* and isolated *cis,trans* are present in about equal amounts.

Summary

A scheme is described to separate the fatty acids of shortenings and margarines into four fractions, the final filtrate of which contains most of the polyunsaturated acids. The nature of the unsaturated acids in these fractions is discussed. It is observed that these fats contain 25-40% of *trans* monoethenoic acids and 2-8% of linoleic acid and considerable proportions of both 9,12-*cis,trans* or *trans,cis* and isolated *cis,trans* isomers of linoleic acid.

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Refining Cottonseed Oil at High Rates of Shear¹

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A LARGE PERCENTAGE of the cottonseed oil being produced does not readily refine and bleach to the light color demanded in present-day shortening and other edible oil products. Any one or several of a number of factors may be responsible for a given oil having an unsatisfactory color after processing. Adverse environmental conditions during the growing of the cottonseed may result in immature or

damaged seed being harvested. Prolonged storage or storage at a relatively high temperature or storage of seed having a high moisture content may be responsible. Finally the conditions under which an oil is extracted from the seed and stored may have an adverse effect on the ease with which color bodies can be subsequently removed. It is certain that cottonseed oils which are difficult to process to a light color by standard methods will continue to be produced.

It has been demonstrated that re-refining, the accepted method of treating off-color cottonseed oils,

¹ Presented at the 29th fall meeting of the American Oil Chemists' Society, Minneapolis, Minn., Oct. 11-13, 1954.

² One of the laboratories of the Southern Utilization Research Branch, Agricultural Research Service, U. S. Department of Agriculture.