Lipid Composition of Selected Margarines

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ABSTRACT

Results of analytical studies on the composition of 10 selected margarines representative of consumer-available hard and soft types are presented. Paired hard and soft products from the same manufacturer were chosen where possible. All of the margarines were compared on the basis of total fatty acid composition, polyunsaturated to saturated fatty acid ratios, total *trans* and the *trans* content of the monoene and diene fractions, location of the double bond in the monoene isomers, per cent conjugation, distribution of the fatty acids at the 2 position of the triglycerides, tocopherol content, and the ratios of α-tocopherol to polyunsaturated fatty acids. As expected the soft margarines contained more polyunsaturated fatty acids than their companion hard types, but all soft margarines did not contain more polyunsaturated fatty acids than all of the hard margarines. The one margarine containing safflower oil had the highest polyunsaturated to saturated ratio. Eight of the ten margarines contained more than 15% *trans* monoene and nine contained less than 5% *trans* diene. Positional isomers in the monoene fraction were Δ6 to Δ12 with the *cis* Δ9 isomer predominating. All of the margarines contained less than 1.9% conjugation. The percentage of *trans* monoene at the 2 position was greater for some margarines than that in the total fatty acid. This was attributed to the preferential placement of polyunsaturated fatty acids at the 2 position in the original vegetable oils. The forms of tocopherol found were characteristic of the original vegetable oils. Ratios of α-tocopherol to PUFA varied from 0.1 to 0.5 mg/g. Determination of the relationship of the amount of tocopherol content to either source or hardness is not possible on the basis of our data.

INTRODUCTION

In recent years there has been an increasing awareness of the influence of diet on human well-being, especially the effect of dietary fat on heart disease. Although the major emphasis has been on the fatty acid composition of food fats, other characteristics have been implicated. These include triglyceride structure, geometric and positional isomers (1-3) and tocopherol content (4). As a consequence of this awareness, there have been numerous proposals that limitations be put on both the kind and amount of dietary fat. One of the most recent of these proposals was the report of the Intersociety Commission for Heart Disease Resources (5). One of the results of these proposals is that it has become increasingly imperative that more information on the nature of food fats be available.

Margarines are widely used and frequently mentioned as components of recommended or therapeutic diets. Margarines are manufactured from polyunsaturated vegetable fats, which are usually either partially hydrogenated or mixed with hydrogenated fats to achieve the oxidative stability necessary for good flavor and to make a spreadable product. Partially hydrogenated fats contain *trans* unsaturation, positional isomers and conjugation not naturally found in vegetable fats. The physical properties of these altered fatty acids are different from those of naturally occurring fatty acids; there are limited data indicating that their nutritional effects are also different (6).

This paper reports data on the natural and altered fatty acid composition, triglyceride structure and tocopherols of 10 consumer-available margarines.

MATERIALS

Margarines were bought in local markets in July 1970. They were selected on the basis of availability, oil seed derivation and degree of hardness. The coding of the five hard cube, one whipped and four soft tub-type margarines, and the label information regarding origin and processing are given in Table I. Each margarine was heated to 50 C and the melted oil decanted from the salts and other solids. All samples were stored in isooctane under N_2 at -40 C.

EXPERIMENTAL PROCEDURES

Analytical and Preparative Gas Liquid Chromatography

Methyl esters were prepared by transesterification with BF_3/methanol (7). Methyl pentadecanoate was used as an internal standard. Fatty acids were identified by comparison with known standards.

An F&M Model 775 preparative gas chromatography was used for all fatty acid separations. A splitter, flame ionization detector and collection port were added to the basic unit to permit the use of small analytical sample sizes and to provide for the manual collection of selected fractions.

A glass column, 17 ft x 0.25 in. packed with 12% DEGS

TABLE I

Margarine Type and Label Information

Composition (from label)	Code for brands		
	Hard cube	Whipped cube	Soft tub
Partially hydrogenated soybean and cottonseed oils	A,B	C	BB[a]
Partially hydrogenated soybean oil, partially hydrogenated and liquid cottonseed oil			AA[a]
Liquid corn oil, partially hydrogenated soybean and cottonseed oil	D		
Liquid corn oil, partially hydrogenated corn oil	E		EE[a]
Partially hydrogenated soybean oil, liquid safflower oil			F
Coconut oil, liquid sunflower oil, hardened coconut oil, hardened palm oil, cream	G		

[a]AA has same brand name as A, BB as B, and EE as E.

on 100/110 mesh Anakrom ABS (Analabs), was used for both analytical and preparative chromatography. Operating conditions for isothermal analytical separations were: column temperature, 195 C; injection port temperature, 196 C; detector temperature, 300 C; carrier gas, helium at 60 ml/min. One sample (G, Table I) containing both "cream" and coconut oil required temperature programing to separate the short and medium chain fatty acids. For this sample the oven temperature was programed at 2.5 C/min from 80 to 180 C. For preparative separations the operating conditions were: column temperature, 184 C; injection port temperature, 190 C; detector temperature, 285 C; and helium flow rate, 60 ml/min. Fractions were collected by passing the effluent from the collection port through 18 in. lengths of 16 gauge Teflon tubing. The collected lipid was recovered from the tubing by rinsing with petroleum ether and separated from column bleed contaminants by passing the petroleum ether solution through a column of 60-80 mesh alumina (8). The purity of the collected fractions was evaluated by analytical gas liquid chromatography (GLC). The efficiency of the recovery was 50% for the monoenes and 30% for the dienes, as determined by known standards.

Determination of *trans* Unsaturation

Trans unsaturation in the total margarine lipid and in the monoene and diene fractions from preparative GLC was determined by IR, according to the method described by Allen (9). This method relates the *trans* content to the ratio absorbance at 10.3 μ to absorbance at 8.67 μ and is independent of concentration and cell length. A NaCl microcell with 0.5 mm path and a minimum usable volume of 20 μl was used. Standard curves were used to relate the per cent *trans* to the ratio A10.3/A8.67. Trielaidin was the reference for the total margarine lipid, methyl elaidate for the monene fraction and methyl linoelaidate for the diene fraction.

Determination of the Positional Isomers

The fatty acid methyl esters were separated by silver nitrate thin layer chromatography (TLC) into fractions differing in the number and geometrical configuration of the double bonds (10). Plates coated with SilicAR TLC 7G silicic acid (Mallinckrodt Chemical Works), to which was added 25% silver nitrate, were developed with petroleum ether-ethyl ether 95:5 v/v. Plates were air-dried, sprayed with 0.1% 2,7-dichlorofluorescein in ethanol and visualized with UV light. The zones containing *cis*-monoene, *trans*-monoene, *cis,cis*-diene and *cis,trans*-diene plus *trans,cis*-diene were scraped from the plates, eluted with anhydrous diethyl ether and freed of solvent in a stream of N_2. Purity of each fraction was determined by GLC.

The location of the double bonds was determined by ozonolysis, followed by GLC separation of the fragments. The ozone generator was a modification of that described by Bonner (11). A solution of ca. 35 μg methyl esters was prepared in 30 μl dichloromethane (12). The solution was cooled in a methanol-dry ice bath, and ozonized oxygen was bubbled through it for 10-35 sec. Methyl pentadecanoate was added after ozonolysis to serve as an internal standard. Reduction of the untreated ozonide to fragments identifiable by GLC took place in the injection port of the gas chromatograph (13) (Varian Aerograph Model 1520) after injection onto a 9 ft x 1/8 in. glass column packed with 12% DEGS on 100/110 Anakrom ABS. The injection port temperature was 210 C, and the column temperature was programed from 55 to 175 C at 4 C/min. Identities of the fragments were established by comparison with those derived by ozonizing known unsaturated fatty acid methyl esters of high purity. Identifications made on this basis were confirmed by GLC/mass spectrometry.

The per cent of conjugated double bonds was determined spectrophotometrically by using AOCS Official Method Cd 7-58 (14).

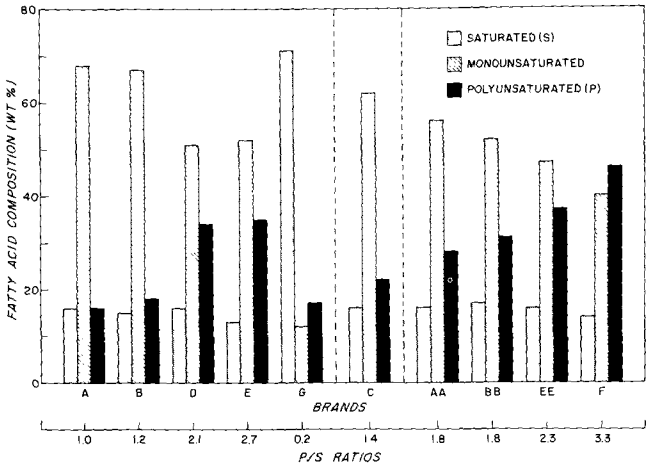

FIG. 1. Composition of polyunsaturated, monounsaturated, and saturated fatty acids of margarines and ratios of polyunsaturated and saturated fatty acids.

Glyceride Analysis

The glycerides were analyzed for their fatty acid composition at the 2 position by using a pancreatic lipase (EC 3.1.1.3, glycerol ester hydrolase) digestion by the method of Sampugna and Jensen (15). The melted samples (600 mg) were emulsified in a Sorval Mini Omni Mixer with 24 ml 5% Gum Arabic in 0.25 M tris (hydroxymethyl)-aminomethane (TRIS) buffer (pH 8.4), $CaCl_2$, and sodium taurocholate. One milligram of Worthington Pancreatic Lipase (Hog) was added in TRIS buffer. The samples were incubated in a Dubnoff Shaking Water Bath for 5 min at 37 C. After the action of the lipase was stopped with 20% H_2SO_4, samples were extracted, evaporated under nitrogen and transferred to vials.

Preparative plates of SilicAR TLC 7G were used to separate the lipolysis products. The solvent system was petroleum ether-ethyl ether-acetic acid 90:20:1 v/v/v. The plates were sprayed with dichlorofluorescein solution and viewed under UV light. The fatty acid and monoglyceride zones were scraped and eluted with ethyl ether, converted to methyl esters and their fatty acid composition determined by GLC. The mixture of methyl esters was further separated on silver nitrate TLC into fractions according to the number of double bonds. The fatty acid composition of these fractions was determined by GLC.

Tocopherol Determination

Tocopherols were determined by saponification of the fat, extraction, conversion to trimethylsilyl eters and quantitative GLC (16).

RESULTS AND DISCUSSION

Fatty Acid Composition

The fatty acid composition of the margarines (Table II) was quite variable, even for apparently similar products. Saturated acids, principally palmitate (16:0) and stearate (18:0), varied least. Only sample G, based on coconut oil, contained large amounts of saturated acids. These were mainly medium chain fatty acids of less than 14 carbons; the major acid was laurate (12:0). Oleate (18:1) varied most, from 12.3 to 67.6%. The linoleic acid (18:2) content of some of the hard cube types was higher than that of some of the soft tub types, showing that degree of hardness was not an adequate criterion of polyunsaturated fatty acids (PUFA) content. In fact two of the related corn oil margarines, E and EE, were difficult to distinguish on the basis of fatty acid composition, although one was described as hard and the other as soft.

TABLE II

Fatty Acid Composition (wt %) of Margarines

Type	Brand	Fatty acids										
		14:0 and lower	16:0	18:0	18:1	18:2	X_1[a]	X_2[a]	18:3	20:0	20:2	22:0
Hard cube	A	tr[b]	9.6	5.5	67.6	11.0	1.6	2.6	0.7	0.4	0.3	0.5
	B	0.1	8.7	5.2	67.2	10.6	1.9	3.9	0.9	0.3	0.3	0.6
	D	0.7[c]	9.6	4.9	50.4	31.0	0.8	0.9	0.7	0.4	0.2	0.3
	E	tr	8.4	4.3	51.5	31.7	—	3.1	0.6	0.3	tr	tr
	G	54.8[d]	9.5	6.2	12.3	16.3	—	—	0.2	0.2	tr	0.2
Whipped cube	C	tr	9.2	5.7	62.6	15.3	1.2	3.3	1.1	0.3	0.3	0.5
Soft tub	AA	0.1	9.2	5.6	55.8	24.2	0.8	1.6	1.3	0.3	0.3	0.5
	BB	0.1	9.9	5.8	52.0	25.8	0.8	2.2	1.8	0.4	0.5	0.5
	EE	tr	11.2	4.3	47.1	33.6	0.4	1.5	1.1	0.3	0.1	0.4
	F	0.1	8.2	5.0	40.2	43.3	0.6	0.6	1.4	0.3	0.2	tr

[a]Tentatively identified as isomers of 18:2 (see text).
[b]tr = trace (<0.1%).
[c]Includes: 0.5% 12:0 and 0.2% 14:0.
[d]Includes: 0.3% 6:0; 5.1% 8:0; 4.0% 10:0; 38.7% 12:0; and 6.7% 14:0.

During preparative GLC, 18:2 Δ9,12 *cis,cis* was consistently accompanied by two other peaks, shown in Table II as X_1 and X_2. When these two compounds were collected and ozonized together only Δ9 and Δ12 unsaturation was found, suggesting that these unknown compounds were Δ9,12 geometrical isomers. X_1 had the retention time of 18:2 Δ9,12 *trans,trans* and X_2 the retention time of 18:2 Δ9,12 *cis,trans* or 18:2 Δ9,12 *trans,cis*. However, since only traces of the all-*trans* isomer were found by silver nitrate TLC, identities of X_1 and X_2 are in doubt. These two compounds, the products of hydrogenation if their tentative identifications are correct, were both absent only in brand G.

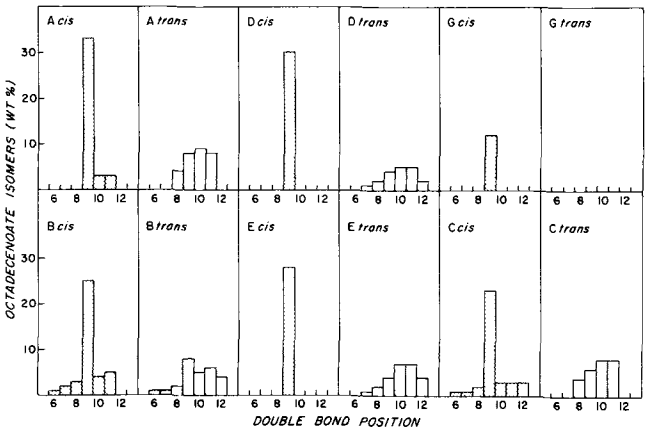

FIG. 2. Double bond position in *cis* and *trans* octadecenoate fractions from hard and whipped cube margarines.

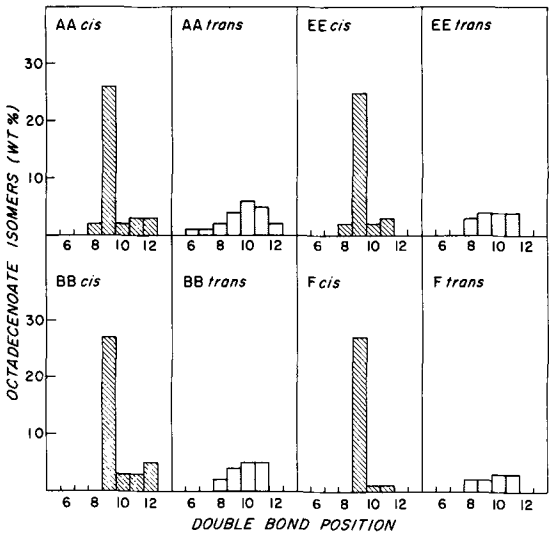

FIG. 3. Double bond position in *cis* and *trans* octadecenoate fractions from soft tube margarines.

The polyunsaturated to saturated (P/S) ratios (Fig. 1) are sometimes considered an important characteristic of food fats. The P/S ratios were calculated as the sum of the percentages of all acids with two or more double bonds divided by the sum of all saturated acids. The P/S ratios of soft AA and BB were higher than their companion hard margarines A and B, but the P/S ratio of EE was lower than the ratio for E. The soft safflower-containing product (F) had the highest P/S ratio. The fatty acid compositions of these selected margarines were similar to the compositions reported by Ostwald (17), Bernfield et al. (18) and Miljanich and Ostwald (19), although there was less saturated fatty acid in these margarines than in those analyzed early in the 1960's. The differences reflect technological changes in both analysis and margarine manufacture.

Altered Fatty Acid Composition

Although *trans* acids are natural components of beef, lamb and dairy products (20,21) because of the hydrogenation that takes place in the rumen, they do not occur naturally in vegetable fats. Sample G contained no *trans* unsaturation. The total *trans* fatty acid content of the remaining margarines (Table III) varied from 14 to 36%. Most of the *trans* unsaturation was found in the monoene fraction (>10%); the diene fraction contained up to 4.5% *trans*. These results suggest that the *trans* content of hard margarines need not greatly exceed those of the soft types. This may reflect improvements in processing, since Zalewski and Kummerow (22) found 41-47% *trans* in hard margarines and 18-20% *trans* in soft margarines. Jensen et al. (23), on the other hand, found 26% *trans* in a hard margarine. The per cent conjugation (Table III) in each of the margarines was small (0.4-1.9%) and was probably due to hydrogenated fat, although oxidation may also lead to conjugation. However little oxidation had occurred as indicated by peroxide value determinations (data not shown)

The monoenes contained positional isomers from Δ6 to Δ12 (Figs. 2 and 3). The double bond in the isomers of the *trans* monoene was more widely distributed over the carbon chain than in the isomers of the *cis* monoene fraction, which was predominantly Δ9 or oleic acid. The distribution of the positional isomers was similar to that reported by

TABLE III

trans and Conjugated Fatty Acid Composition (wt %) of Margarines

Composition	Brands									
	A	B	D	E	G	C	AA	BB	EE	G
Total *trans*	33.0	36.0	22.0	30.0	—	33.0	28.0	24.0	21.0	14.0
Monoene (18:1) *trans*	27.7	26.2	19.7	23.7	—	26.3	20.6	15.6	15.1	10.4
Diene (18:2) *trans*	2.4	4.3	1.2	4.5	—	3.6	2.3	4.2	2.9	2.3
Total conjugation	1.4	1.4	0.7	0.7	0.4	1.9	1.6	1.6	1.3	0.4

TABLE IV

Composition (wt %) of Fatty Acids at 2 Position of Margarine Triglycerides

Fatty acid	Brands									
	A	B	D	E	G	C	AA	BB	EE	F
16:0 or lower	0.7	0.9	2.5	1.5	63.6[a]	0.8	0.8	1.0	1.5	1.3
18:0	2.6	4.8	6.5	4.4	6.3	4.7	3.8	3.8	4.6	3.2
18:1 *cis*	43.2	45.9	28.5	13.5	12.8	30.7	36.9	41.2	22.2	27.2
18:1 *trans*	39.9	27.0	25.3	36.4	—	34.7	18.2	16.0	25.1	12.9
X_1	0.6	0.7	—	—	—	0.8	—	0.6	—	—
X_2	1.9	4.2	1.5	3.3	—	3.2	1.6	3.3	—	—
18:2	10.3	16.4	35.8	40.5	17.3	23.9	36.6	32.6	46.6	54.2
18:3	—	—	—	0.4	—	1.3	2.1	1.5	—	0.9
22:0	0.7	—	—	—	—	—	—	—	—	—

[a]Includes: 2.4% 8:0; 2.7% 10:0; 39.6% 12:0; 11.2% 14:0; and 7.7% 16:0.

Scholfield et al. (24).

The diene fraction contained only Δ9,12, whether it was *cis-cis, cis-trans* or *trans-cis*. Sample G contained neither *trans* fatty acids nor positional isomers; there were no unsaturated compounds left in this sample because of hydrogenation, indicating that the hardened coconut and palm oils were completely hydrogenated.

Glyceride Structure

The fatty acid composition at the 2 position is given in Table IV. In most of the samples there was a higher percentage diene at the 2 position than in the total sample. There was 40-83% monoene at the 2 position and of this 13 to 40% was *trans* monoene. For some of the margarines the percentage *trans* monoene at the 2 position was greater than in the total fatty acid. This results because of the preferential placement of PUFA at the 2 position in the original vegetable oils (25-27), and hydrogenation yields monoene at the 2 position. This is in contrast to those animal fats containing *trans* acids naturally, which preferentially have them at the 1 and 3 positions of triglycerides (20,21). Jensen et al. (23) found 28.3% *trans* at the 2 position of the margarine they analyzed.

Tocopherol Composition

Table V gives the tocopherol content and ratios of α-tocopherol to PUFA for the tested margarines. Most of the margarines contained more γ-tocopherol than α-tocopherol, a characteristic of either soybean or corn oil (16). The forms peculiar to sample G were derived from palm and coconut oil (16).

The need for dietary vitamin E (as α-tocopherol) has been related to the intake of PUFA; Harris and Embree (28) have estimated that the preferred dietary ratio should be 0.6 mg α-tocopherol per gram PUFA. If this is an acceptable criterion, all of the margarines were deficient in vitamin E. Sample G had the highest ratio, due to its low PUFA content. The α-tocopherol/PUFA ratios for the soft tub margarines were lower than those for the hard cube types. The soft tub margarines not only contained more PUFA, but some contained less α-tocopherol. This was true for two of the companion pairs, B/BB and E/EE. If this is true of other polyunsaturated food fats, high PUFA diets may be low in vitamin E.

In conclusion, these margarines varied widely in PUFA, the component of greatest current interest, in the amount of *trans* unsaturation, positional isomers and tocopherols, and in their triglyceride structures and α-tocopherol/PUFA ratios. The new food labeling regulations (29) may provide some information on PUFA (as cis-cis-methylene-interrupted PUFA), saturated fatty acids (12:0, 14:0, 16:0 and 18:0) and "other fatty acids." Other than this the only information available to the consumer is the source of the oil and the hardness of the margarines. How can these clues be related to the variations reported here? *Trans* unsaturation and amounts of positional isomers were in general lower in the soft margarines than in the hard types, both in total fatty acids and at the 2 position. Determination of the relation of tocopherol content to either source or hardness was not possible on the basis of these data. Both the highest and lowest values were found in the hard margarines. Hard margarines had higher ratios of α-tocopherol/PUFA; none was higher than 0.5 mg/g.

REFERENCES

1. Elson, C.E., L.R. Dugan, Jr., L.R. Bratzler and A.M. Pearson, Lipids 1:322 (1966).
2. Yamamoto, I., M. Suzano, A. Kadote, Y. Sato and M. Wada, Jap. J. Biochem. 66:685 (1969).

TABLE V

Tocopherol Composition and Ratios of α-Tocopherol to PUFA[a]

Brand	α-T[b]	mg/100 g γ-T[b]	δ-T[b]	mg α-T/gm PUFA ratio
A	3.4	14.4	3.5	0.2
B	6.8	18.3	3.8	0.4
D	6.9	22.7	3.5	0.2
E	15.0	53.6	2.0	0.4
G[c]	8.8	0.8	0.4	0.5
C	3.2	15.1	3.0	0.2
AA	4.1	10.3	2.7	0.1
BB	5.8	40.4	12.6	0.2
EE	6.6	21.1	1.9	0.2
F	11.7	29.0	8.1	0.2

[a]PUFA, polyunsaturated fatty acids.

[b]α-Tocopherol, γ-tocopherol and δ-tocopherol.

[c]Also contains β-tocopherol (.6 mg), α-tocotrienols (0.5 mg) and traces of β- and γ-tocotrienols.

3. Beare, J.L., JAOCS 47:487 (1970).
4. Nutr. Rev. 30:70 (1972).
5. Inter-Society Commission for Heart Disease Resources, "Primary Prevention of the Atherosclerotic Diseases," Circulation XLII, A 84, 1970.
6. Sgoutas, D., and F.A. Kummerow, Amer. J. Clin. Nutr. 23:111 (1970).
7. Metcalf, L.D., and A.A. Smith, Anal. Chem. 33:363 (1961).
8. Allen, R.R., Lipids 4:627 (1969).
9. Allen, R.R., JAOCS 46:522 (1969).
10. Morris, L.J., J. Lipid Res. 7:717 (1966).
11. Bonner, W.A., J. Chem. Educ. 30:452 (1953).
12. Kleiman, G.F., F.R. Spencer, F.R. Earle and I.A. Wolff, Lipids 4:135 (1969).
13. Davison, V.L., and H.I. Dutton, Anal. Chem. 38:1302 (1966).
14. "Official and Tentative Methods of The American Oil Chemists' Society," Vol. I, Second edition, AOCS, Champaign, Ill., 1964 (revised to 1972), Method Cd 7-58.
15. Sampugna, J., and R.G. Jensen, Lipids 3:519 (1968).
16. Slover, H.T., J. Lehmann and R.J. Valis, JAOCS 46:417 (1969).
17. Ostwald, R., J. Amer. Diet. Ass. 39:313 (1961).
18. Bernfield, P., F. Homburger and T.F. Kelley, Amer. J. Clin. Nutr. 11:554 (1962).
19. Miljanich, P., and R. Ostwald, J. Amer. Diet. Ass. 56:29 (1970).
20. Duncan, R.D., and G.A. Gartan, J. Sci. Food Agr. 18:99 (1967).
21. Woodrow, I.L., and J.M. DeMann, Biochim. Biophys. Acta 152:472 (1968).
22. Zalewski, S., and F.A. Kummerow, JAOCS 45:87 (1968).
23. Jensen, R.G., J. Sampugna, J.G. Quinn, D.L. Carpenter, T.A. Marks and J.A. Alford, Ibid. 42:1029 (1965).
24. Scholfield, C.R., V.L. Davison and H.J. Dutton, Ibid. 44:648 (1967).
25. Gunstone, F.D., and M. Ilyas Qureshi, Ibid. 42:961 (1965).
26. Jurriens, G., and A.C.J. Kroesen, Ibid. 42:9 (1965).
27. Brockerhoff, H., and M. Yurkowski, J. Lipid Res. 7:62 (1966).
28. Harris, P.L., and N.D. Embree, Amer. J. Clin. Nutr. 13:385 (1963).
29. Fed. Reg. 38:2136 (1973).

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