Composition and Structure of Some Consumer-available Edible Fats

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ABSTRACT

Samples of polyunsaturated margarines, table margarines, hard cube polyunsaturated vegetable oil, hard cube vegetable oil, animal fat, and blends of vegetable oil and animal fat were analyzed for fatty acid composition, percentage of *cis,cis*-methylene interrupted polyunsaturated fatty acids, percentage isolated *trans*-unsaturation, and percentage conjugated diene. Gas liquid chromatography was used to separate and measure the geometric isomers of the octadecaenoic and octadecadienoic acids. Selected samples were analyzed for the content of positional isomers in their *cis* monoene and *trans* monoene fractions, and for the percentage of fatty acid esterified in the 2-position of their triglycerides.

INTRODUCTION

There is a growing need for thorough and reliable information on the nutrient composition of all human foods (1). Sources of dietary fat have been changing continuously since the early part of this century. Consumption of vegetable oils has risen, and there has been a shift from lard to shortening and from butter to margarine and an increase in the use of solid and cooking oils (2). In countries where these changes have occurred, the fatty acid content of the average diet has changed accordingly.

A large percentage of dietary fats has been hydrogenated, either chemically by the use of catalysts in the case of processed vegetable oils, or biologically by rumen microorganisms in the case of milkfat and the depot fat of ruminants. During hydrogenation, triglyceride fatty acids are isomerized both in positional and geometric configuration of their double bonds. Recent improved methodology allows the determination of isomeric fatty acids produced as a result of hydrogenation. Studies on the biological utilization of these modified fatty acids have been reviewed (3-5).

While the composition and structure of milkfat are well documented (6,7), those of other consumer-available dietary fats are not often recorded. This paper reports compositional data for margarines, cooking oils, and fat.

EXPERIMENTAL PROCEDURES

Samples

Samples of 1 lb cubes of table margarine, polyunsaturated table margarine, hard cube vegetable oil, hard cube polyunsaturated vegetable oil, animal fat, and blends of vegetable oil and animal fat were purchased at regular intervals from supermarkets in Brisbane, Australia, from August, 1973, to July, 1974. In the Australian context, table margarine usually refers to margarine manufactured from vegetable oils and hydrogenated vegetable oils as the sole or major component. Polyunsaturated table margarine is made solely from vegetable oils and hydrogenated vegetable oils, and contains not less than 40% *cis,cis*-methylene interrupted polyunsaturated fatty acids and not more than 20% of saturated fatty acids. Animal fat refers to refined beef tallow or mutton tallow or mixtures of the two. Fat was extracted from the margarines by melting at 45 C, centrifuging, and filtering through filter paper to dry the samples. Oil and fat samples were melted and used without further treatment, and all samples were stored under nitrogen at -20 C until required for analysis.

Determination of *trans*-Unsaturation

Isolated *trans*-unsaturation was determined by IR spectroscopy using AOAC method 28.047 (8). Methyl elaidate (Applied Science Laboratories, Inc., State College, PA) was used as a standard.

Determination of Conjugated Double Bonds

The percentage of conjugated double bonds was determined spectrophotometrically using AOCS Official Method Cd 7-58 (9).

Enzymatic Determination of Polyunsaturated Fatty Acids

The content of *cis,cis*-methylene interrupted polyunsaturated fatty acids was determined using the interim methodology instructions of Sheppard et al. (10). Lipoxidase was obtained from Sigma Chemical Company, St. Louis, MO. Methyl linoleate (Applied Science Laboratories, Inc.) was used for standardization.

Pancreatic Lipase Deacylation of Triglycerides

The percentage of fatty acids in the *sn*-2-position of the triglycerides was determined using the semi-micro procedure of Luddy et al. (11) with modifications reported by Litchfield (12). After deacylation, the components were applied as a streak onto a 20 cm x 20 cm glass thin layer chromatography (TLC) plate, coated to a thickness of 0.3 mm with Kieselgel G (E. Merck, Darmstadt, Germany) containing 8% (W:W) boric acid. The plates were developed using the solvent system diethyl ether:hexane (60:40). Bands were visualized under UV light after being sprayed with a methanolic solution containing 0.025% 2',7'-dichlorofluorescein and 0.005% Rhodamine B. The band representing 2-monoglycerides was scraped from the plate into a chromatography column containing a 3 cm plug of Florisil and eluted with diethyl ether.

Argentation Thin Layer Chromatography

Fatty acid methyl esters were separated according to the number, and geometric configuration of the double bonds by TLC on 20 cm x 20 cm glass TLC plates coated to a thickness of 0.3 mm with Kieselgel G containing 10% (W:W) silver nitrate. The plates were developed using the solvent system hexane:diethyl ether (92:8).

Determination of Positional Isomers

The position of the double bonds in *cis* and *trans* monenes was determined by ozonolysis (13). The methyl esters dissolved in carbon disulphide were cooled to -65 C when ozonized oxygen was passed for up to 40 sec using a Supelco microozonizer. Ozonides were decomposed in situ with triphenyl phosphine. The resulting aldehydes and aldehyde esters were measured quantitatively by gas liquid chromatography (GLC). Glass columns, 2 m x 2 mm ID, packed with a mixture of 75% of 10% OV 17 on 100-120 mesh Gas-Chrom Q and 25% of 10% OV 225 on 100-120 mesh Gas-Chrom Q (14) (Applied Science Laboratories, Inc.), were temperature programmed from 40 C to 275 C at 4 C/min. Injector and detector temperatures were 225 C and 250 C, respectively. Carrier gas flow rate was 25 ml/min. Identification of fragments was made using an

homologous series of aldehydes (Supelco, Inc., Bellefonte, PA) and by ozonolysis of fatty acid methyl esters of known positional configuration. Response factors were applied to correct for lack of response in the flame ionization detector by carboxyl and carbonyl carbon atoms.

Gas Liquid Chromatography of Fatty Acid Methyl Esters

Methyl esters were prepared from triglycerides and 2-monoglycerides by the method of Christopherson and Glass (15). They were analyzed isothermally at 175 C on a 2 m x 2 mm ID glass column packed with 10% EGSS-X on 100-120 mesh Gas-Chrom P (Applied Science Laboratories, Inc.). Injector and detector temperatures were 225 C and 250 C, respectively. Carrier gas flow rate was 25 ml/min.

A GLC separation of geometric isomers of methyl octadecenoates and methyl octadecadienoates was obtained using a 3.65 m x 2 mm ID stainless steel column packed with 10% Apolar-10C on 100-120 mesh Gas-Chrom Q (Applied Science Laboratories, Inc.). The column was operated isothermally at 170 C. Injector and detector temperatures were 230 C, and 250 C, respectively. Carrier gas flow rate was 20 ml/min.

Standard fatty acid methyl esters obtained from Applied Science Laboratories, Inc., were used to obtain correction factors for nonlinearity of detector response. Methyl *cis, trans-(trans,cis)* octadecadienoate was prepared from linoleic acid by isomerization with nitrous acid (16) followed by esterification and separation by preparative argentation TLC. All GLC analyses were carried out using a Varian-Aerograph Model 1840 Gas Chromatograph. Quantitative results were obtained with the aid of a Varian-Aerograph Model 480 Digital Integrator.

Sterol Determination

The sterol composition of most of the samples used in this study was previously reported (17).

RESULTS AND DISCUSSION

Fatty Acid Composition

The composition of the 71 samples is given in Table I. In Australia the use of the term "polyunsaturated" when used in connection with fat products is restricted to those products in which the total acids present contain not less than 40% of *cis,cis*-methylene interrupted polyunsaturated fatty acids and not more than 20% of saturated fatty acids. At the time of this survey, there was no legal requirement for a manufacturer to comply with this definition. All 40 samples of polyunsaturated margarine contained < 20% of saturated fatty acids and > 40% 18:2 acids as measured by GLC. However, only half of the samples contained > 40% *cis,cis*-methylene interrupted polyunsaturated fatty acids. Table margarines contained approximately half the 18:2 content of polyunsaturated table margarines. This was compensated for by increased palmitic acid content.

Hard cube polyunsaturated vegetable oils, hard cube vegetable oils, animal fats, and blends of vegetable oil and animal fat are used for frying, deep frying, and roasting. The hard cube polyunsaturated vegetable oils had a composition similar to the polyunsaturated table margarines. On the basis of sterol composition (17) and fatty acid composition, the hard cube vegetable oils are considered to consist mainly of palm oil. Of the 12 samples labeled "blended vegetable and animal oil," 5 samples did not contain vegetable oil at all as judged by the absence of phytosterols in the GLC sterol profile (17). The remaining 7 samples contained little vegetable oil on the evidence of their cholesterol and phytosterol content. The fatty acid profile of the animal fat was typical of that from the depot fat of oxen and sheep.

TABLE I

Composition of Margarines, Hard Cube Vegetable Oils, and Animal Fats

Classification	Number of samples	Fatty acid composition (wt % acid)												*cis,cis*-methylene interrupted polyunsaturated fatty acids (wt %)			% Isolated *trans*-unsaturation			% Conjugated dienes		
		16:0			18:0			18:1			18:2			Others								
		Min.	Max.	Avg.	Min.	Max.	Avg.	Min.	Max.	Avg.	Min.	Max.	Avg.	Min.	Max.	Avg.	Min.	Max.	Avg.	Min.	Max.	Avg.
Polyunsaturated table margarine	40[a]	6.5	12.9	9.2	5.2	9.6	7.3	33.7	43.2	38.2	40.2	50.0	44.8	0.1	0.4	0.2[c]	35.5	46.0	39.8	12.9	22.1	17.1	0.26	0.80	0.47
Table margarine	7[b]	20.3	29.4	24.6	5.4	8.1	6.8	33.9	45.2	40.3	16.0	27.8	21.6	0.5	15.3	6.8[d]	13.8	23.3	18.9	10.8	19.0	14.9	0.27	0.47	0.35
Hard cube polyunsaturated vegetable oil	4[b]	8.6	9.4	9.1	9.1	11.0	9.7	37.3	39.1	38.2	41.9	44.3	42.7	0.2	0.3	0.3[c]	35.8	39.0	36.9	21.9	25.1	23.2	0.28	0.56	0.45
Hard cube vegetable oil	4[b]	41.0	42.6	42.1	4.7	5.3	5.0	39.9	42.3	40.9	9.5	11.5	10.5	1.7	1.8	1.8			ND[e]	0.0	4.4	2.1	0.15	0.23	0.18
Blended vegetable oil and animal fat	12[a]	23.2	27.6	24.1	15.1	21.7	19.4	32.9	40.7	36.7	1.9	7.7	3.6	13.7	20.1	16.1			ND	4.6	6.9	5.7	0.68	1.30	0.94
Animal fat	4[b]	21.9	23.2	22.6	18.8	20.0	19.5	38.9	40.0	39.5	2.0	2.8	2.4	15.7	16.3	16.0			ND	6.7	8.8	7.8	0.94	1.18	1.11

[a]Representing three brands from three different manufacturers.
[b]One single brand.
[c]Representing 14:0.
[d]Representing short chain fatty acids.
[e]Not determined.

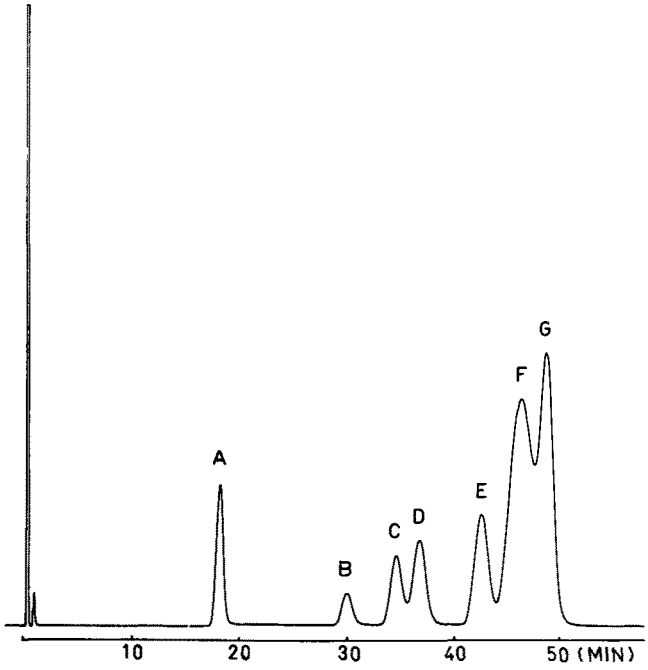

FIG. 1. Chromatogram illustrating the GLC separation of methyl esters of nitrous acid isomerized safflower oil on an Apolar-10C column. (A) Methyl palmitate, (B) methyl stearate, (C) methyl *trans*-octadecaenoates, (D) methyl *cis*-octadecaenoates, (E) methyl *trans,trans*-octadecadienoates, (F) methyl *cis,trans-(trans,cis)* octadecadienoates; (G) methyl *cis,cis*-octadecadienoates.

trans Fatty Acids

The polyunsaturated table margarines, table margarines, and hard cube polyunsaturated vegetable oils had isolated *trans*-unsaturation ranging from 10.8 to 25.1%. These values are somewhat lower than the range 14.0 to 36.0% recently reported by Carpenter and Slover (18) and lower than the range 14.5 to 49.1% reported by Kummerow (19,20). The *trans* fatty acid content, however, is higher than that found in milkfat (21). The role of *trans* fatty acids in biological systems is receiving increased attention. This subject has recently been reviewed (3-5).

Jolliffe (22) introduced the concept of P/S ratio (the ratio of polyunsaturated to saturated fatty acids) as a means of characterizing a dietary fat. Although the use of the P/S ratio has been criticized (23), it is still widely used, and indeed the polyunsaturated products sampled in this study were labeled as having a P/S ratio of greater than 2:1. This 2:1 ratio is significant, as it has been shown by Keys et al. (23) that in man in caloric equilibrium, changes in dietary fat produce responses in the serum cholesterol level that, on the average, are predictable from the percentages of total calories provided by saturated (S) and polyunsaturated (P) fatty acid glycerides in the diets. S and P have opposing actions, and, in general, Δ cholesterol (mg/100 ml) = 2.7 Δ S-1.3 Δ P, where Δ refers to the difference between the two diets (23).

Reports on the effect of *trans* fatty acids on serum cholesterol levels have been conflicting. However, a more recent study by Vergroesen (24) would indicate that dietary *trans* fatty acids when fed with dietary cholesterol will elevate serum cholesterol levels. If this is the case, the content of *trans* acids should be taken into account when calculating P/S ratio and other formulas for predicting serum cholesterol levels.

GLC and TLC were used to detect the presence of geometric octadecadienoate isomers. TLC, however, will only detect *cis,trans(trans,cis)* dienes; the *trans,trans* dienes migrate with the *cis* monoenes. Countercurrent distribution using argentation systems (25) and ion-exchange resins (26) have previously been used to separate diene geometric isomers. On an analytical scale, 200 ft capillary gas chromatographic columns have been required to separate these isomers (27,28). With the recent introduction of the GLC liquid phase Apolar-10C (29), separation of *cis* and *trans* isomers can be achieved with packed analytical GLC columns which previously could only be achieved with capillary columns.

Figure 1 is a chromatogram illustrating the separation of *cis* and *trans* isomeric monoenes and dienes on a 3.65 m packed column containing 10% Apolar-10C. The performance characteristics of the column are given in Table II. The broadened peak representing methyl 18:2, 9^c, 12^t plus 18:2, 9^t, 12^c is due to some separation of *cis,trans* and *trans,cis* isomers.

The Apolar-10C column was used to determine the content of 18:2 *cis,trans (trans,cis)* and 18:2 *trans,trans* fatty acids in the samples. TLC was also used to examine the content of 18:2 *cis,trans (trans,cis)* fatty acids. In all samples the content of both isomers did not exceed 0.5% for each. It was found that the content of nonconjugated dienes containing *trans* unsaturation plus the content of conjugated diene did not always make up the difference in value between 18:2 measured by GLC and the percentage of *cis,cis*-methylene interrupted fatty acids, measured by enzymatic methods.

The Apolar-10C column was also used to measure quantitatively the content of *cis* and *trans* monoene. Fats having isolated *trans* unsaturation (expressed as % methyl elaidate) of 12.9, 15.0, 17.8, 21.7, and 25.1% by the standard IR method (8) have values of 11.6, 12.0, 14.8, 17.0, and 23.1% by GLC. As the IR method measured only small amounts of *trans* diene, the discrepancy between the two methods may possibly be due to the fact that there is also some separation of positional isomers resulting in a widening of peaks; this affects the separation factor for *cis* and *trans* monoenes. Preparation of a longer and more efficient column may overcome the discrepancies.

Positional Isomers

The positional isomers present in the *cis* monoene and

TABLE II

Relative Retention Times and Separation Factors for the Apolar-10C Column

Number	Fatty acid methyl ester	Relative retention time	Separation factor
1	18:0	1.00	
2	18:1,9^t	1.15	$3/2^a = 1.06$
3	18:1,9^c	1.22	
4	18:2,9^t,12^t	1.40	$6/4 = 1.13$
5	18:2,9^c,12^t 18:2,9^t,12^c	1.50	$6/5 = 1.05$
6	18:2,9^c,12^c	1.58	

[a]Refers to the numbers in column 1.

TABLE III

Composition of *Cis* and *Trans* Monoene Isomers in Some Edible Fats

Sample[a]	Wt %	Position of Double Bond and Wt % of Isomer								
		6	7	8	9	10	11	12	13	14
		cis monoene								
A1	23.4	0.5	2.0	2.6	72.1	4.5	7.2	8.5	1.7	0.9
A2	27.6	0.7	1.8	2.5	83.3	3.2	4.4	2.5	1.1	0.5
A3	19.9	0.4	0.9	2.8	76.3	4.7	6.4	7.0	1.2	0.3
B	27.5	0.3	1.2	2.4	79.8	4.3	5.8	4.2	1.3	0.7
C	15.2	1.0	2.6	5.4	58.0	8.6	10.6	8.7	3.3	1.8
D	38.1	-	0.7	0.8	92.3	1.1	3.4	1.3	0.3	0.1
		trans monoene								
A1	19.3	1.0	3.0	9.8	16.6	23.6	21.4	12.1	8.5	4.0
A2	13.3	3.0	6.1	12.6	16.5	18.7	16.6	12.3	8.9	5.3
A3	15.7	-	1.4	8.7	28.1	22.3	18.9	14.0	5.2	1.4
B	16.4	1.2	3.2	10.7	22.3	22.2	18.5	11.9	6.7	3.3
C	23.9	1.7	4.5	12.1	17.9	21.1	17.6	12.1	8.4	4.6
D	4.2	0.4	1.6	7.6	29.8	20.6	20.5	12.8	5.2	1.5

[a](A) Represents samples of polyunsaturated table margarines, (B) sample of table margarine, (C) sample of hard cube polyunsaturated vegetable oil, and (D) sample of hard cube vegetable oil.

trans monoene fractions of six fats made solely from vegetable oil and purchased on the same day is given in Table III. The *cis* monoene fractions consisted predominately of the 18:2, 9^c isomer. The *trans* monoenes had a wider distribution of isomers. The distribution of positional isomers is similar to that reported by Jones et al. (25), Scholfield, et al. (30), and Carpenter and Slover (18).

TRIGLYCERIDE STRUCTURE

The percentage fatty acid esterified in the 2-position of samples of polyunsaturated table margarine, table margarine, and hard cube polyunsaturated vegetable oil is given in Table IV. There is a tendency for 18:2 to be preferentially esterified in the 2-position. Palmitic acid is preferentially esterified in the 1- or 3-positions.

The position a fatty acid occupies in the triglyceride molecule may be of importance in nutrition. It has been shown by Elson et al. (31) and Yamamoto et al. (32,33) that in rats essential fatty acids esterified in the 1- and 3-positions in triglycerides have less hypocholesterolaemic action than essential fatty acids esterified at the 2-position. Filer et al. (34) reported that palmitic acid is better absorbed for the 2-position than from the 1- and 3-positions of triglycerides.

TABLE IV

Proportion of Individual Fatty Acid Esterified in the 2-Position of Fats[a]

Sample[b]	Fatty acids			
	16:0	18:0	18:1	18:2
A1	9.3	12.2	34.9	53.3
A2	7.7	29.6	31.0	41.5
A3	6.9	30.5	35.6	39.3
B	12.3	17.5	37.4	50.7
C	10.9	24.9	34.2	39.9

[a]Calculated from M/3T x 100, where M = mol % of the acid in the monoglyceride and T = mol % of the same acid in the triglyceride before hydrolysis.

[b]Same sample designations as in Table III.

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