Fatty Acid Composition and *trans* Isomer Content of Hardened Olive Oil

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ABSTRACT

Cooking fats and margarines prepared from olive oil were analyzed gas chromatographically for fatty acid composition and *trans* isomer content. Argentation thin layer chromatography and mass spectrometry were used to assist in identifications of minor components. Olive oil margarines and cooking fats contained 70–76% monoenoic acids, of which 30–37% was in the *trans* configuration, and 5% dienoic acids. Twenty percent of the dienes were in the form of *trans-trans* and *cis-trans* octadecadienoic acid.

INTRODUCTION

The amount and nature of fat in human diet and its effect on the general health of the population are the subject of extensive research. In recent years, much concern was voiced among the scientists that geometrical isomers of fatty acids might not be safe, since biological organisms might not be able to utilize properly new "fats" produced via hydrogenation (1, 2). Thus, the analysis of *trans* fatty acids in hardened products has assumed renewed importance.

Sources of dietary fat have been changing in Greece in the last four decades. There has been a considerable shift from butter and animal fat to vegetable margarines and shortenings. Recommendations by the medical profession to avoid cholesterol gave this shift a significant impetus.

Hydrogenated olive oil is extensively used in the Greek diet, although data which might be useful to ascertain its nutritional characteristics is lacking. In the present work, results are presented which were obtained from the analysis of 3 samples of commercially hydrogenated olive oil and 18 samples of margarines and cooking fats prepared from hardened olive oil.

EXPERIMENTAL PROCEDURES

Samples

Ten samples of household margarine and eight samples of cooking fats were purchased in the retail market during a period of two years (June 1979 to July 1981). Three authentic samples of industrially hydrogenated olive oil were obtained from a local plant and a plant located in the area of Athens.

Fat was extracted from the margarines by melting at 40 C. The upper layer was dried with sodium sulfate and filtered through filter paper. Cooking fats and hydrogenated olive oil samples were melted and used without further treatment. All samples were stored under nitrogen at −17 C until required for analysis.

Preparation of Methyl Esters

Methyl esters were prepared by transesterification of fatty acids with BF_3-methanol (3), after saponification and separation of the unsaponifiables. The esters were separated according to the number of double bonds by thin layer chromatography on 20 × 20 cm plates, which were coated with Silica Gel G containing 10% silver nitrate (4). The plates were developed with hexane/diethyl ether (9:1). Fractions were visualized with dichlorofluorescein.

Gas Chromatography and Mass Spectrometry

Fatty acid compositions were determined with a Hewlett-Packard, Model 7620A, flame ionization gas chromatograph. The conditions employed were: column 20 ft × 1/8 in., stainless steel, packed with 15% OV-275 on 100–120 mesh Chromosorb P, AW-DMCS; column temperature 220 C; injection port 240 C; detector 250 C; carrier gas flow rate 25 mL/min.

Standard fatty acid methyl esters (16:0, 18:0, 18:1*t*, 18:1*c*, 18:2*cc*, Fluka puriss, standard for GC) were used to obtain relative retention times (RRT) and response factors. Methyl palmitelaidate and methyl linolelaidate were purchased from Supelco. Methyl *cis,trans*-octadecadienoate was prepared from pure linoleic acid by isomerication with nitrous acid, esterification with BF_3-methanol and purification with argentation thin layer chromatography (5, 6). All these esters were used to assist in identifications of minor peaks in the gas chromatograms.

Combined gas chromatography-mass spectrometry (GC-MS) analyses were performed on a Perkin-Elmer, Model 950, gas chromatograph linked to a Perkin-Elmer RMU-6L, 70 eV mass spectrometer. The chromatograph was fitted with the same column as for gas chromatographic analysis.

RESULTS AND DISCUSSION

A reproduction of a typical chromatogram of margarine methyl esters is given in Figure 1. Cooking fats give similar chromatograms, but the peaks corresponding to lower

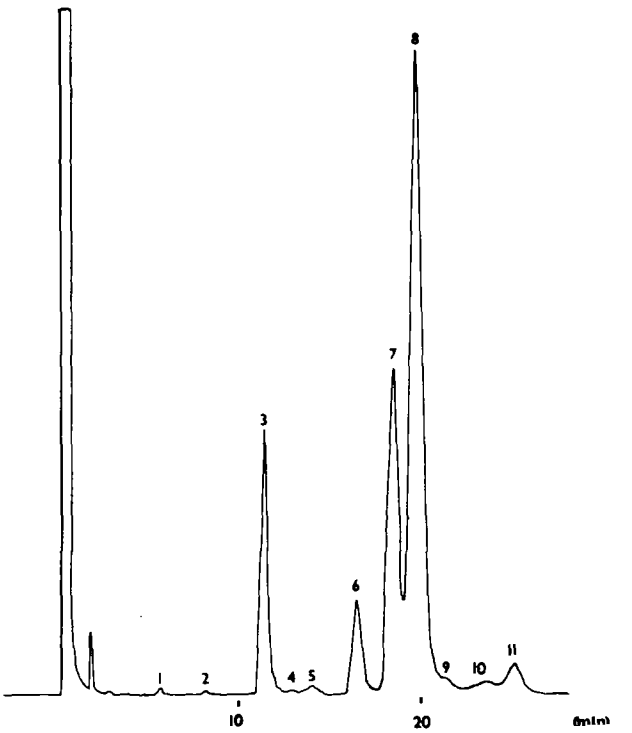

FIG. 1. Separation of margarine methyl esters. (1) 12:0. (2) 14:0. (3) 16:0. (4) *t*-16:1. (5) *c*-16:1. (6) 18:0. (7) *t*-18:1. (8) *c*-18:1. (9) *tt*-18:2. (10) *ct*-18:2 (or *tc*-18:2). (11) *cc*-18:2.

TABLE I

Fatty Acid Composition of Hydrogenated Olive Oil

Samples	Fatty acids (%)											
	12:0	14:0	16:0	16:1*t*	16:1*c*	18:0	18:1*t*	18:1*c*	18:2*tt*	18:2*ct*	18:2*cc*	P/S
1	0.1	0.1	12.1	0.2	0.6	7.4	27.1	49.1	0.2	0.9	2.4	0.18
2	tr	tr	11.7	0.2	0.5	7.5	28.9	46.0	0.2	0.7	4.2	0.27
3	0.1	0.2	12.9	0.3	0.6	10.3	26.6	45.5	0.3	0.5	2.7	0.13

TABLE II

Fatty Acid Composition of Margarines and Cooking Fats Prepared from Olive Oil

	Number of samples	Fatty acids (%)																	
		10:0			12:0			14:0			16:0			16:1*t*			16:1*c*			
		Min	Max	MV	Min	Max	MV	Min	Max	MV	Min	Max	MV	Min	Max	MV	Min	Max	MV	
Margarine	10	–	–	–	–	0.2	0.1	–	0.2	0.1	11.4	13.9	12.5	0.2	0.4	0.2	0.4	0.8	0.7	
Cooking fats	8	–	0.2	0.2	0.1	0.3	0.3	0.3	0.7	0.5	11.5	14.0	12.3	0.2	0.3	0.3	0.4	0.8	0.6	
	Number of samples	18:0			18:1*t*			18:1*c*			18:2*tt*			18:2*ct*			18:2*cc*			P/S
		Min	Max	MV	Min	Max	MV	Min	Max	MV	Min	Max	MV	Min	Max	MV	Min	Max	MV	
Margarine	10	5.8	9.5	7.1	19.4	28.3	23.2	45.5	56.5	49.9	tr	0.2	0.2	0.6	1.3	1.1	3.0	4.6	3.9	0.26
Cooking fats	8	6.5	11.3	9.0	23.3	26.9	25.7	43.5	50.5	45.9	tr	0.2	0.2	0.6	1.6	0.9	3.0	5.0	4.1	0.23

molecular weight fatty acids (10:0, 12:0, 14:0) are slightly higher. This is due to the small quantity of butter fat present in these products, which is added to enhance the flavor.

As demonstrated in the chromatogram, minor constituents give very small or poorly resolved peaks. Methyl esters were, therefore, preseparated by silver nitrate thin layer chromatography and the monoene and diene fractions were rechromatographed (4, 5).

The monoene fraction contained a very small amount of dienes. This is apparently due to the *trans,trans*-octadecanoic acid methyl esters which migrate with the monoenes in the argentation thin layer chromatography (4). These contaminants were disregarded in calculating fatty acid compositions of the monoene fractions.

Since the *tt*-18:2 isomers cannot be separated from the monoenes by the technique employed, the dienes were additionally isolated from the fatty acid mixture by preparative gas liquid chromatography of the methyl esters using a 20% DEGS column. This fraction was used in the combined GC-MS analysis and in checking the level of octadecadienoic acids other than linoleic.

Peak 4 (Fig. 1) was present in the chromatogram of the monoene fraction. Its RRT was the same with methyl palmitoelaidate. Peaks 9 and 10 correspond to components present in the diene fraction. These components gave mass spectra with fragmentations that are essentially similar to those reported for 18:2 acids (7, 8). The molecular ion was at m/e 294, with other ions at m/e 263 (loss of a methoxy radical), 262 (elimination of methanol) and 178 (loss of the ester moiety plus a rearranged hydrogen by cleavage of the C_5-C_6 bond). Peak 9 had the same RRT in the gas chromatograms with standard linoleilaidate, while the RRT of peak 10 was very close to that of *cis,trans*-octadecadienoic acid methyl ester obtained from isomerized linoleic acid. These findings are in agreement with those reported by other authors, who showed that *tt*-18:2 and *ct*-18:2 emerge in front of the linoleic acid when polar columns are used in the gas chromatographic analysis of methyl esters (4, 5, 9, 10). Thus, it is concluded that peaks 9 and 10 are due to mono- and di-*trans* dienes. Jackson reported (11) that some *cis-cis* or mono-*trans* 18:2 positional isomers have retention times similar to that of 18:2*tt*. It is, therefore, reasonable to assume that peak 9 is partly due to 18:2*tt* and partly to other 18:2 isomers.

The results of analysis carried out on 3 samples of hydrogenated olive oil and 18 samples of olive oil cooking fats and margarines are presented in Tables I and II. As indicated in the tables, hydrogenated olive oil and its products have a low percentage of dienoic acids (ca. 5%). The percentage of *cis-cis*-linoleic acid is smaller (4%) because 20% of the dienes is in the form of geometrical isomers.

Polyunsaturated/saturated ratio is very low, too (<0.3). This ratio becomes much lower if *trans* fatty acids are included in the calculations as saturated components, as suggested by Beare-Rogers and others (12, 13).

These results should be taken into account by those concerned with dietary fat and its effect on coronary heart disease, if overestimation of the effectiveness of olive oil is to be avoided. Natural olive oil has been reported to have beneficial effects on the hepatobiliary function and cholesterol level (14, 15). Hydrogenated products, however, differ from the oil from which they are derived and there is no evidence that they meet requirements based on present-day knowledge about dietary linoleic acid and isomeric fats. It is, therefore, reasonable to consider whether some pressure must be on the olive oil industry to direct technology to produce fats of higher nutritional quality. Blending of the oil with other vegetable oils before or after hydrogenation and modification of processing conditions may result in elimination of *trans*-unsaturation and an increase in the concentration of 9,12-*cis,cis*-octadecadienoic acid.

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