Content of *trans*-Fatty Acids in Edible Margarines

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ABSTRACT: Fatty acid composition, including *trans*-isomers, was determined for four types of imported margarines consumed by the Bulgarian population. The results were compared with data obtained from a Bulgarian edible margarine produced under German license. Fatty acid composition and *trans*-isomer content were determined by gas chromatography of fatty acid methyl esters on a packed and capillary column, respectively. The total contents of *trans*-isomers of oleic and linoleic acid were within the ranges of 1.9–8.0% and 0.4–1.4%, respectively. The Bulgarian margarine contained similar quantities of *trans*-isomers.

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KEY WORDS: Fatty acids, margarine, *trans*-isomers.

Hydrogenated oils are still the major materials used in the production of margarines (halvarines), although other technologies, such as interesterification, are being implemented. It is known that hydrogenation, applied by the producer, is the major contributor to the presence of geometric and positional unsaturated fatty acid (FA) isomers. They end up in the margarines depending on their content in the processed material (hydrogenated oil) and go from there into the tissue after human consumption.

Much evidence has been published during the last few years about adverse effects of *trans*-isomers on human health. According to some authors, *trans*-FA are associated with an increased risk of cardiovascular disease in that they behave like saturated FA in the human metabolism (1–3). Others have reported that *trans*-FA raise the level of cholesterol in low-density lipoproteins (LDL) and decrease it in high-density lipoproteins (HDL), i.e., they have an adverse effect on the serum lipoprotein profile (3,4). These potential cardiovascular risks are greater in population groups with lower essential fatty acid (EFA) intakes, where a greater prevalence of cardiovascular disease is recorded (1,2). The reason for the latter is that *trans*-FA inhibit the biosynthesis of the essential arachidonic acid (C20:4), which is important for tissue growth. Accordingly, the intake of *trans*-FA isomers, i.e., through margarine consumption by children, is not recommended (4).

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Information that relates the adverse effect of *trans*-FA in human diets is the basis for many studies that determine their content in margarines (halvarines). This information reports their level in margarine products and thus serves as a basis for recommendations of the level of *trans*-FA in the human diet. This paper, a continuation of a study performed 3 yr ago (5), presents the results of a study of FA composition and of *trans*-FA isomer content of several imported margarines (halvarines) available in the Bulgarian market place. The results are compared with those of a Bulgarian margarine, produced under German license and obtained during a previous study (5). Market samples were selected to mimic the consumption of *trans*-FA by the general population of Bulgaria.

EXPERIMENTAL PROCEDURES

Market samples of one margarine and three halvarine batches (six samples of each batch), produced in the Netherlands and Austria, were analyzed. All samples were of the same expiration date.

The samples were dehydrated and cleaned up, and the FA present were converted to methyl esters by reaction with boron trifluoride/methanol for 24 h at room temperature in sealed tubes. Esters were extracted twice with *n*-hexane, the extracts were combined, dehydrated with sodium sulfate, and neutralized with sodium carbonate, and were then ready to be injected in the gas chromatograph.

Aliquots of 1 μL were injected simultaneously into two gas chromatographs—one equipped with a packed column, and the other with a capillary column.

Total FA composition was determined on a Pye Unicam, series 304 gas chromatograph (Philips Scientific, Cambridge, United Kingdom), equipped with flame-ionization detector (FID) and a glass packed column of 3 m × 2 mm i.d. Packing material was 7.5% DEGS (LAC 728) (Carlo Erba, Milan, Italy) on Chromosorb W, AW-HMDS, 100–200 mesh (Sigma Chemical Co., St. Louis, MO). Working temperatures of the column, injector, and detector were 190, 250, and 263°C, respectively. Carrier gas was nitrogen at a flow rate of 20 mL/min. Flow rates of hydrogen and air were selected to attain maximum FID signal response. Recoveries were 98%, and relative standard deviations were 2.2 to 3.4%.

Trans-isomers of oleic ($C_{18:1}$) and linoleic ($C_{18:2}$) acids were determined on a Perkin-Elmer 8500 gas chromatograph (Beaconsfield, United Kingdom), equipped with an FID and

TABLE 1
Fatty Acid Composition of Margarines[a]

Fatty acid	VO/MW/N	VOF/A	VO/AF/N	VO/N	VO/B
8:0	0.5 ± 0.1	1.9 ± 0.2	1.1 ± 0.1	1.1 ± 0.5	—
10:0	0.5 ± 0.2	0.5 ± 0.2	0.6 ± 0.2	1.7 ± 0.9	1.1 ± 0.3
12:0	1.7 ± 0.6	0.3 ± 0.1	0.6 ± 0.2	6.5 ± 0.8	17.9 ± 1.0
14:0	1.1 ± 0.5	0.6 ± 0.4	2.8 ± 0.3	2.2 ± 0.3	6.5 ± 0.8
15:0	—	—	0.3 ± 0.1	—	—
16:0	21.2 ± 2.2	13.5 ± 0.5	11.7 ± 0.8	15.4 ± 0.5	12.3 ± 0.9
16:1	—	—	1.3 ± 0.3	—	0.4 ± 0.1
18:0	7.6 ± 2.3	6.9 ± 0.9	5.4 ± 0.7	8.0 ± 0.5	4.1 ± 0.1
18:1 *cis*	31.5 ± 3.5	49.2 ± 1.8	40.8 ± 3.0	18.8 ± 1.9	20.6 ± 1.7
18:1 *trans*	6.5 ± 2.8	8.0 ± 2.2	1.9 ± 0.9	3.0 ± 0.9	0.8 ± 0.2
18:2 *cis*	28.1 ± 4.1	13.0 ± 1.2	19.1 ± 2.7	37.0 ± 1.2	35.0 ± 1.6
18:2 *trans*	1.4 ± 0.5	0.4 ± 0.1	0.9 ± 0.3	1.0 ± 0.2	1.3 ± 0.4
20:0	—	—	1.6 ± 0.7	—	—
18:3	—	4.9 ± 0.7	10.9 ± 0.8	4.5 ± 0.8	—
Unidentified	—	0.9 ± 0.2	1.0 ± 0.3	0.9 ± 0.2	—

[a]Results expressed in percentage and standard deviation; "—" = not detected; MW = milk whey; VOF = vegetable oils and fats; AF = animal fats; VO = vegetable oils; N = the Netherlands; A = Austria; B = Bulgaria.

capillary column, Stabilwax from Restek Corporation (Bellefonte, PA), 60 m × 0.25 mm × 0.25 µm. Temperatures of injector and detector were 250 and 300°C, respectively. Initial column temperature was 68°C, raised to 175°C at a fixed heating rate (1°C/min). Hydrogen was used as the carrier gas at 8 psig, split ratio 1:50, detector hydrogen 100 kPa, and air 140 kPa.

Fatty acid methyl ester (FAME) peaks were identified by comparison with those of standard solutions of individual FAME at a concentration of 0.4 mg/mL. Areas were integrated, and results were calculated as µg FAME per µL injected.

A FAME mixture to simulate margarine FA pattern was analyzed on the Stabilwax capillary column. Correction factors (K) for FA, calculated according to ISO 5508:1990(E), were as follows: $K_{8:0} = 0.72$, $K_{10:0} = 0.93$, $K_{12:0} = 1.16$, $K_{14:0} = 1.26$, $K_{16:0} = 1.23$, $K_{18:0} = 1.65$, $K_{18:1cis} = 1.21$, $K_{18:1trans} = 0.78$, $K_{18:2cis} = 0.86$, $K_{18:2trans} = 0.71$, and $K_{18:3} = 1.30$. Resolutions (R) were calculated with formulas of the AOAC Official Method Cd14b-93, revised 1995, for mixtures of $C_{18:1cis}/C_{18:1trans}$ (*trans*-9-octadecenoic acid), and $C_{18:2cis}/C_{18:2trans}$ (*trans*-9-*trans*-12-octadecadienoic acid): $R_{C18:1cis/trans} = 0.74$ and $R_{C18:2cis/trans} = 1.6$. Amounts of *cis*- and *trans*-$C_{18:1}$ and $C_{18:2}$ isomers were determined by standard additions. Ratios of $C_{18:1}$ *cis/trans* and $C_{18:2}$ *cis/trans* were calculated, and results for $C_{18:1}$ and $C_{18:2}$ FAME, obtained on the packed column, were computed as $C_{18:1cis}$, $C_{18:1trans}$, $C_{18:2cis}$, $C_{18:2trans}$ content, respectively. Final results are all expressed as relative percentages.

RESULTS AND DISCUSSION

FA compositions of the margarines are presented in Table 1. The amount of *trans*-isomers in the samples is within the range 1.9 to 8% for oleic acid ($C_{18:1}$) and 0.4 to 1.4% for linoleic acid ($C_{18:2}$). This paper does not discuss positional isomers.

Table 2 presents the FA groups and ratios between them. The content of polyunsaturated fatty acids (PUFA) is within the range 18.3–42.5% (Table 2). Because EFA are included in this group, the PUFA content is of major importance for the biological and nutritional value of these products. The value for the Bulgarian margarine is 36.3% PUFA, which is in the upper region of the PUFA range. The ratio of *trans*-isomers to total unsaturated FA content varied from 0.04 to 0.12 (Table 2) and seems to be favorable compared with the 0.08 to 0.31 values for 11 types of imported margarines analyzed in a former study (5).

TABLE 2
Groups and Ratios Between the Types of Fatty Acids from the Composition of Margarines

Margarine[a]	Saturated (SFA) (%)	Monounsaturated (MFA) (%)	Polyunsaturated (PUFA) (%)	*Trans*-/unsaturated	Saturated/unsaturated	*Trans*-/*cis*-[b]
VO/MW/N	32.6	38.0	29.5	0.12	0.48	0.13
VOF/A	23.7	57.2	18.3	0.11	0.31	0.14
VO/MW/N	24.1	44.0	30.9	0.04	0.32	0.05
VO/N	34.9	21.8	42.5	0.06	0.54	0.07
VO/B	41.9	21.8	36.3	0.04	0.72	0.04

[a]See Table 1 for abbreviations.
[b]Ratios calculated on the basis of mean values.

The ratio of saturated/unsaturated FA shows the relation between the two major FA groups of the fat composition (Table 2). Its value varies from 0.29 to 0.54. The Bulgarian product has a larger ratio (0.78), which indicates a high proportion of saturated FA. This ratio was 0.17 to 1.54 for the group of 11 margarines analyzed previously (5). The prevalence of unsaturated over saturated FA (smaller ratio) is considered positive from the viewpoint of nutritional content (Table 2).

The ratio *trans*-/*cis*-FA expresses the degree of formation of the *cis*- or *trans*-forms of $C_{18:1}$ and $C_{18:2}$ FA in the analyzed margarines. It depends on the conditions used in hydrogenation of the oil used for margarine production. The ratio varies between 0.05 and 0.14 for the margarines studied (Table 2). The Bulgarian margarine has a ratio of 0.04, which corresponds to a smaller content of *trans*-isomers. This ratio for the margarines previously analyzed varied between 0.08 and 0.56 (5).

The analyzed margarines have low total *trans*-FA content. Data are lower than in the former study (5) and may be the result of technological changes in processing. Bulgarian margarine has a low *trans*-isomer content, but the amount of saturated FA in it is undesirably high (41.9%) (Table 2).

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