Fatty Acid Composition and Contents of *trans* Monounsaturated Fatty Acids in Frying Fats, and in Margarines and Shortenings Marketed in Denmark

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ABSTRACT: This study examined *trans* monounsaturated fatty acid contents in all margarines and shortenings marketed in Denmark, and in frying fats used by the fast-food restaurants Burger King and McDonald's. *Trans* $C_{18:1}$ content was 4.1 ± 3.8% (g per 100 g fatty acids) in hard margarines, significantly higher than the content in soft margarines of 0.4 ± 0.8%. Shortenings had an even higher content of *trans* $C_{18:1}$, 6.7 ± 2.3%, than the hard margarines. Margarines and shortenings with high contents of long-chain fatty acids had about 20% total *trans* monoenoic of which close to 50% were made up of *trans* long-chain fatty acids. Both fast-food frying fats contained large amounts of *trans* $C_{18:1}$, 21.9 ± 2.9% in Burger King and 16.6 ± 0.4% in McDonald's. In Denmark the per capita supply of *trans* $C_{18:1}$ from margarines and shortenings and frying fats has decreased steadily during recent years. The supply of *trans* $C_{18:1}$ from margarines and shortenings in the Danish diet is now 1.1 g per day.

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Margarine products, shortenings, and fats used for frying are major sources of *trans* fatty acids (TFA) in the Danish diet. *Trans* unsaturation, occurring during the industrial hydrogenation of *cis* unsaturated vegetable and marine oils, allows the fatty acids to pack together more closely than their corresponding *cis* isomers, resulting in a harder fat with more desirable physical properties, texture, and keeping quality (1). The most abundant *trans* isomers of $C_{18:1}$ (octadecenoic acid) from industrial hydrogenation are those with unsaturation at positions 9, 10, and 11 (2). TFA also occur naturally in ruminant fat and in meat and milk, as a result of intestinal bacterial hydrogenation of dietary unsaturated fatty acids (3). In ruminant fat the $C_{18:1}$ isomer with a *trans* bond in position 11 predominates.

TFA in high levels in the diet have repeatably been shown to affect serum lipids/lipoproteins unfavorably (4–11), with higher intake resulting in higher serum low-density lipoprotein (LDL) cholesterol (4,7,9,10) and lipoprotein(a) (5,6), and lower serum high-density lipoprotein (HDL) cholesterol concentrations (4,7,9–11). Several population studies have examined the relationship between TFA intake and risk of coronary heart disease (12–18). Except for one (14), all these studies have found a positive association, attributed to the intake of hydrogenated vegetable oil. In addition, high intake of TFA may have other health consequences. A study published recently demonstrated an association between adipose stores of TFA and risk of developing breast cancer in postmenopausal women (19). Consequently, recommendations to reduce the content of TFA in margarine products to less than 5% of fatty acids, the level found in dairy products, have been advanced by the Danish Nutrition Council (20), and follow-up data have demonstrated that the industry complied with these recommendations within a short time (21).

While fatty acid composition in margarine products has been extensively analyzed, the fatty acid composition of shortenings used by the baking industry and of frying fats used by the fast-food industry is less well known. As hydrogenated fats constitute a significant proportion of dietary fat intake, a detailed and current knowledge of the composition of these foods is necessary when issuing dietary guidelines for the public. This paper reports the fatty acid composition, including the proportion of TFA, in all Danish-produced margarine products for the retail market and all shortenings for the baking industry. The fatty acid composition of new and used frying fats used by two major international fast-food chains is also reported.

EXPERIMENTAL PROCEDURES

Sample description. All Danish margarine brands available on the retail market and all shortening brands intended for use in the baking industry in 1996 (in Denmark shortenings are sold only for industrial purposes) were analyzed. Of each brand, one package representing 500 g was taken at random directly from the production line from the three margarine- and shortening-producing factories in Denmark, namely Vejle Margarinefabrik (Vejle, Denmark), Dragsbæk Margarinefabrik (Thisted, Denmark), and Van den Bergh Foods (Sønderborg, Denmark), in November 1996. A total of 97 brands was drawn, consisting of 59 brands of margarines and 43 brands of shortenings. There was only minimal import of margarine and shortening, so the sampling covers the entire Danish market for the retail outlet and the baking industry.

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Samples of 12 new and 12 used frying fats from the fast-food restaurants McDonald's and Burger King were also examined. The used fats were sampled after they had been in use for 1 to 7 d (mean: 4 d for both fast-food restaurants). Samples of frying fat were taken six times during 1996 from the same McDonald's and Burger King restaurants. Each sample of frying fat was about 500 g.

Sample preparation. Margarines, shortenings, and new frying fats were considered homogenous and consequently not homogenized. Used frying fats were mixed by stirring and warming. All samples were filled in bags and frozen at −20°C until analysis.

Methods of analysis. The fatty acids were determined by boiling the fat in methanolic potassium hydroxide, methylation by boiling with methanol and boron trifluoride, and extraction of the fatty acid methyl esters with isooctane, followed by gas–liquid chromatography (GLC) (PE 8500; Perkin-Elmer Corp., Norwalk, CT) on a 50-m capillary column, CP Sil 88, 0.25 mm i.d., 0.2 µm df. (Chrompack International, Middelburg, the Netherlands) with flame-ionization detection (21). C_{17} was used as an internal standard for quantification of the fatty acids. Temperature program was: start temperature 120°C, ramp rate 2°C/min, oven temperature 220°C, detector temperature 350°C, programmed temperature vaporizer injection system from 40 to 300°C. As reported by others (22) a complete separation of all *trans* and *cis* $C_{18:1}$ isomers is not possible, however, with this temperature program and column good separation was obtained, especially for *trans* $C_{18:1}$ amounts below 10% of fatty acids (23). The overlap between some of the isomers will underestimate the *trans* content by about 20% as reported earlier (24).

Analytical quality assurance. The results were ascertained by making the so-called R-charts over the deviation on the double determinations of the fatty acids palmitic ($C_{16:0}$), stearic ($C_{18:0}$), oleic (*cis* $C_{18:1n\text{-}9}$), and linoleic (*cis* $C_{18:2n\text{-}6}$). Reference materials were an in-house material of margarine, certified BCR reference material CRM 162 Soya-maize oil (Community Bureau of Reference, Brussels, Belgium) and Nu-Chek-Prep Standards GLC 68 and 17A[1] (Nu-Chek-Prep, Inc., Elysian, MN). For margarines, shortenings, and frying fats, a relative standard deviation of about 4% was found for all four fatty acids with about 200 double determinations. The results were well controlled, both regarding precision and variation, during the entire investigation.

Argentation thin-layer chromatography. The long-chain TFA from hydrogenated fish oils cannot be sufficiently separated to be quantified by GLC on a 50-m polar capillary column. To quantify these TFA, the fatty acid methyl esters were separated by thin-layer chromatography by application in a spot on Kiselgel plates (Merck, Darmstadt, Germany), impregnated with a solution of 5% silver nitrate, and eluted with petroleum ether/diethyl ether. The spots were visualized by spraying with dichlorfluorescein, scraped off, and eluted with diethyl ether (25). The spots with the saturated fatty acids and TFA were combined. The fatty acids were then determined as described previously, again using C_{17} from the spot with saturated fatty acids as internal standard for the determination of TFA.

Statistics and data presentation. One-way analysis of variance (ANOVA) was used, followed by the Tukey post-test if $P < 0.05$. Results of contents of the various fatty acids are given as mean ± SD in g per 100 g total fatty acids.

RESULTS

Table 1 shows the contents (mean ± SD; range) of individual fatty acids in margarines and shortenings. For practical reasons the margarines are divided into three categories based on their

TABLE 1
Fatty Acid Composition (g/100 g fatty acids, mean ± SD; range) of Danish Margarines and Shortenings

	Margarines			Shortenings
	<20% Linoleic acid (*n* = 32)	20–40% Linoleic acid (*n* = 19)	>40% Linoleic acid (*n* = 8)	<20% Linoleic acid (*n* = 38)
Fatty acid				
$C_{8:0}$	0.4 ± 1.1; 0–1.7	0.2 ± 0.4; 0–1.0	—	0.1 ± 0.3; 0–1.3
$C_{10:0}$	0.1 ± 0.3; 0–1.2	0.1 ± 0.3; 0–0.8	—	0.1 ± 0.3; 0–0.9
$C_{12:0}$	2.5 ± 3.0; 0–9.2	2.9 ± 3.2; 0–9.5	3.1 ± 2.5; 0–6.4	1.1 ± 2.5; 0–4.8
$C_{14:0}$	2.0 ± 1.9; 0–6.9	1.7 ± 1.6; 0–4.6	1.2 ± 1.0; 0–2.6	1.8 ± 1.5; 0–4.8
$C_{16:0}$	22.3 ± 7.9; 10.0–37.1	16.0 ± 5.4; 5.1–26.1	14.4 ± 4.1; 8.8–23.4	25.6 ± 6.3; 12.0–34.4
Trans $C_{16:1}$	0.4 ± 1.0; 0–3.7	0.3 ± 0.7; 0–2.9	—	0.6 ± 1.3; 0–3.9
Cis $C_{16:1}$	0.1 ± 0.2; 0–0.9	0.1 ± 0.3; 0–1.1	—	0.2 ± 0.4; 0–2.0
$C_{18:0}$	7.0 ± 2.5; 3.1–12.4	7.2 ± 5.3; 2.3–23.1	5.6 ± 1.5; 3.9–8.3	6.3 ± 1.2; 3.5–9.0
Trans $C_{18:1}$	4.1 ± 3.8; 0–14.2	3.1 ± 3.3; 0–5.8	0.4 ± 0.8; 0–1.9	6.7 ± 2.3; 3.0–13.6
Cis $C_{18:1}$	39.2 ± 9.0; 15.3–48.5	33.7 ± 12.2; 17.1–58.4	21.9 ± 3.1; 17.8–26.6	37.2 ± 6.8; 23.0–54.0
$C_{18:2}$	12.7 ± 3.9; 3.8–19.0	27.7 ± 7.7; 20.1–37.6	51.7 ± 5.4; 44.3–61.2	12.1 ± 4.2; 5.7–17.5
$C_{18:3}$	4.4 ± 2.1; 0.7–8.0	3.9 ± 2.6; 0.7–9.1	1.4 ± 1,1; 0–2.7	3.5 ± 1.3; 1.5–7.9
$C_{20:0}$	0.9 ± 1.1; 0–4.7	0.6 ± 0.9; 0–3.3	—	0.7 ± 0.9; 0–3.0
$C_{20:1}$	1.1 ± 0.6; 0–3.2	0.7 ± 0.6; 0–1.9	—	0.9 ± 0.7; 0–3.0
$C_{22:0}$	0.5 ± 1.2; 0–5.2	0.5 ± 1.0; 0–3.5	0.4 ± 0.5; 0–1.1	0.5 ± 0.9; 0–2.8
$C_{22:1}$	—[a]	0.1 ± 0.3; 0–1.0	—	—
Other[b]	2.8 ± 6.0; 0–19.6	1.2 ± 3.0; 0–10.9	—	2.9 ± 5.9; 0–19.6

[a]Not detected, or trace amounts (<0.1%).
[b]Include mainly isomers of long-chain fatty acids.

content of linoleic acid (LA): (i) margarines with less than 20% (g per 100 g fatty acids) LA are hard margarines used mainly for frying; (ii) margarines with 20–40% LA are semisoft margarines used mainly as spreads on bread; and (iii) margarines with more than 40% LA are soft margarines also used as spreads. Shortenings for the baking industry are hard fats with less than 20% LA.

Within categories, comparatively large compositional variations between brands were observed (Table 1). Mean *trans* $C_{18:1}$ contents were significantly higher in hard than in soft margarines [ANOVA: $P < 0.05$; hard vs. semisoft: not significant (NS), hard vs. soft: $P < 0.05$, semihard vs. soft: NS], but generally low in all three categories. Of the hard margarines 34% (11 brands) were "*trans*-free" (containing less than 1% *trans* $C_{18:1}$), while 42 and 75% (8 and 6 brands) of the semisoft and soft margarines, respectively, could be considered "*trans*-free." Only small amounts of *trans* $C_{16:1}$ were present. A significantly lower *trans* $C_{18:1}$ was demonstrated in the hard margarines than in shortenings ($P < 0.005$). None of the shortening brands was "*trans*-free."

The sums of the saturated lauric, myristic, and palmitic fatty acids, suspected to have a higher atherogenic potential than stearic acid (26), were 26.3 ± 8.6, 20.9 ± 6 .5, and 18.7 ± 7.1% in hard, semisoft, and soft margarines, respectively (ANOVA: $P < 0.05$; hard vs. semisoft: NS, hard vs. soft: $P < 0.05$, semihard vs. soft: NS). Lauric, myristic, and palmitic acids were 28.7 ± 6.1% in shortenings, not significantly different from contents in hard margarines. In hard, semisoft, and soft margarines and in shortenings the total monounsaturated fatty acids—chiefly oleic acid—were 40.3 ± 8.8, 34.5 ± 12.6, 21.9 ± 3.1, and 38.3 ± 6.7%, respectively, and total polyunsaturated fatty acids—chiefly LA—were 16.9 ± 5.9, 31.7 ± 6.2, 53.1 ± 4.7, and 15.5 ± 4.9%, respectively.

Within categories (between brands) no significant correlations were found between contents of *trans* $C_{18:1}$ and total or individual saturated fatty acids.

Nine margarines and seven shortenings had a high content (more than 5%) of long-chain fatty acids. These brands had an average of about 20% total *trans* monoenoic fatty acids, of which close to 50% were made up of *trans* long-chain fatty acids ($C_{20:1}$, $C_{22:1}$) (Table 2). n-3 Polyunsaturated fatty acids were not present in significant amounts (less than 0.2%) after hardening of the fish oils.

TABLE 2
***Trans* Monoenoic Fatty Acids (g/100 g fatty acids; mean ± SD; range) in Danish Margarines and Shortenings Containing More than 5% Long-Chain Fatty Acids**

Trans $C_{16:1}$	2.7 ± 1.2; 0–3.6
Trans $C_{18:1}$	7.1 ± 1.7; 4.2–10.6
Trans $C_{20:1}$	4.2 ± 1.9; 0–6.6
Trans $C_{22:1}$	5.3 ± 2.5; 0–8.4
Σ Trans monoenoic	20.0 ± 6.0; 12.8–25.9

Fatty acid composition (mean ± SD; range) in frying fats from Burger King and McDonald's is shown in Table 3. The frying fat from Burger King was characterized by a substantially higher content of total saturated fatty acids (25.1 ± 0.5 vs. 8.8 ± 0.4%; $P < 0.0001$; new frying fat) and LA (14.6 ± 0.9 vs. 2.0 ± 0.3; $P < 0001$; new frying fat), and a lower content of oleic acid (21.9 ± 2.9 vs. 62.5 ± 1.1; $P < 0.0001$; new frying fat) compared to the frying fat from McDonald's. Both frying fats contained large amounts of *trans* $C_{18:1}$, 21.9 ± 2.9% in Burger King and 16.6 ± 0.4% in McDonald's ($P < 0.01$; new frying fat). Only small amounts of *trans* $C_{18:2}$ were present.

The fatty acid composition of the frying fat from Burger King did not change much after use, although *cis* $C_{18:2}$ decreased somewhat ($P < 0.005$) and *trans* $C_{18:1}$ increased slightly ($P = 0.2$). Larger changes were seen in the frying fat from McDonald's after use, with increases in saturated fatty acids (to 17.7 ± 7.1%; $P < 0.01$) and *trans* $C_{18:1}$, while decreases were demonstrated for *cis* $C_{18:1}$ ($P < 0.002$).

Mean fat content was close to 100% in the new frying fats, and after use the fat content was reduced slightly, by about 2%.

DISCUSSION

Recently, TFA data for margarines, shortenings, and some frying fats have been made available for 14 European countries in

TABLE 3
Fatty Acid Composition (g/100 g fatty acids, mean ± SD; range) in Frying Fat from Burger King and McDonald's

	Burger King		McDonald's	
	New	Used	New	Used
Fatty acid				
$C_{16:0}$	12.7 ± 0.6; 12.4–13.4	14.2 ± 2.4; 12.4–18.8	4.1 ± 2.0; 4.8–5.1	10.2 ± 5.1; 7.4–19.7
$C_{18:0}$	12.5 ± 0.6; 11.8–13.6	13.5 ± 1.5; 11.7–15.3	3.8 ± 0.3; 3.2–4.1	7.5 ± 2.1; 4.9–10.1
Trans $C_{18:1}$	21.9 ± 2.9; 18.5–25.5	24.8 ± 3.7; 20.7–30.1	16.6 ± 0.4; 16.1–17.0	21.1 ± 3.4; 16.8–24.7
Cis $C_{18:1}$	29.4 ± 2.5; 26.0–32.0	28.8 ± 3.6; 24.0–32.0	62.5 ± 1.1; 61.1–64.0	52.5 ± 8.4; 41.0–61.3
Trans $C_{18:2}$	1.2 ± 0.5; 0.7–1.9	0.7 ± 0.5; 0–1.5	1.3 ± 0.5; 0.8–1.7	0.3 ± 0.5; 0–1.0
Cis $C_{18:2}$	14.6 ± 0.9; 13.5–15.7	10.6 ± 2.0; 8.2–12.9	2.0 ± 0.3; 1.4–2.4	1.4 ± 0.4; 1.0–2.0
$C_{18:3}$	0.7 ± 0.4; 0–1.0	0.3 ± 0.4; 0–1.1	—	—
$C_{20:0}$	—[a]	—	—	—
$C_{20:1}$	—	—	1.1 ± 0.1; 1.1–1.2	0.8 ± 0.4; 0–1.1
$C_{22:1}$	—	—	0.2 ± 0.3 ;0–0.7	—
Other[b]	8.3 ± 1.0; 7.2–10.2	7.9 ± 1.9; 6.2–10.3	8.3 ± 0.7; 7.4–9.2	5.9 ± 1.2; 4.0–7.6

[a]Not detected, or trace amounts (<0.1%).
[b]Mainly positional isomers around the unsaturated fatty acids.

connection with the TRANSFAIR study (27). In the TRANSFAIR study a large variation of the TFA content in various fat products was found. The proportion of TFA in soft margarines ranged between 0.1 and 16.5% of total fatty acids; the hard household margarines and the industrial fats for cooking and baking had a slightly higher proportion of TFA, and the highest amounts were found in fats for deep frying. The results from our study were similar: the lowest contents of TFA were found in the soft margarines and higher contents in the hard margarines and shortenings, and an even higher content was found in the industrially hardened fats used for frying.

The reductions during the recent years in the average contents of *trans* $C_{18:1}$ in margarines, and the generally unaltered and higher *trans* contents in shortenings compared to margarines for household use are similar to observations described for other European countries (28–30). A comparison of all margarine products available on the Danish market in 1992 and 1995 showed, especially for the semisoft margarines, marked reductions in *trans* $C_{18:1}$ contents during these 3 yr, largely replaced by polyunsaturated fatty acids, mainly LA (21). In 1995 the *trans* $C_{18:1}$ content in shortenings was 6.8 ± 3.1%, similar to contents in the present study for 1996, and in Danish margarines no further reductions in the *trans* content occurred from 1995 to 1996.

The saturated fatty acids, especially the short-chain fatty acids palmitic acid, lauric acid, and myristic acid elevate blood cholesterol (26), albeit probably not having the same atherogenic potential as TFA (18). Consequently, from a health point of view less would be gained if "*trans*-free" margarines were hardened with saturated fatty acids instead of TFA. However, no correlation could be shown between contents of *trans* $C_{18:1}$ and saturated fatty acids in margarines and shortenings.

In the Nordic countries marine oils are often used as ingredients in margarines, primarily of the hard category, and in shortenings. So far most of the research into the effects of TFA on blood lipids and risk of coronary heart disease has concentrated on C_{18} carbon acids, whereas the effects of longer chainlengths are largely unknown. Recent Norwegian studies have shown that high intakes of TFA from partially hydrogenated marine oils increase total- and LDL-cholesterol and decreases HDL-cholesterol (10), but seem to have more favorable effects on fibrinolysis compared to the same amount of TFA from partially hydrogenated soybean oil (31). The hydrogenation of both vegetable oils and marine oils results in a number of *trans* isomers, of which *trans* monoenoic dominate (1). In our study 16 brands were found to have more than 5% long-chain fatty acids. In these margarines and shortenings, marine *trans* monoenoic acids, $C_{20:1}$ and $C_{22:1}$, constituted more than half of total *trans* monoenoic acids.

New frying fat from the fast food restaurants Burger King and McDonald's had a high content of *trans* $C_{18:1}$ compared to margarines and shortenings, which increased somewhat after being used for a mean of 4 d, indicating that TFA are formed during frying. The changes in fatty acid composition with use were most pronounced in the frying fat from McDonald's. The explanation for the difference in stability of the two frying fats is difficult to give, but could depend on their contents of antioxidants.

In 1992 the National Food Agency of Denmark conducted a study on the fat content of french fries and the fatty acid distribution of the fats used for frying from almost 100 fast-food restaurants, including five Burger King and McDonald's restaurants (32). During frying the fat content increased by 11 g/100 g french fries (from 5.2 ± 1.5 to 16.2 ± 4.5 g/100 g). With an average content of 20% *trans* $C_{18:1}$ in the frying fat, eating 100 g of french fries will supply more than 3 g of TFA. In 1992 the frying fat used by these fast food restaurants had a *trans* $C_{18:1}$ content of 32.5 ± 8.9%. Thus, during the 4 yr from 1992 to 1996, a substantial decrease occurred in the *trans* content of the frying fat. This decrease happened at the expense of an increase in *cis* $C_{18:1}$ and *cis* $C_{18:2}$.

In Denmark, average daily intakes of TFA based on available data from 1991 were estimated to be 5 g per person (20). Half of the daily TFA intake, 2.5 g, was calculated to come from foods incorporating industrially hardened fats, and the other half from fats of ruminant origin. Total per capita supply of industrial fats has decreased substantially, from 16.8 to 9.4 kg per person per year during the last 10 years (Danish Statistical Bureau). A significant decline in the intake of TFA since 1991 has most likely occurred because of a decreased intake of mainly hard fats and reformulations of the semisoft margarines with lower contents of *trans* $C_{18:1}$. Based on Danish sales figures for margarines and shortenings in 1996 (kindly supplied by the Association of Danish Margarine Manufacturers, which include factories supplying close to 100% of the Danish market), total sale for the Danish market corresponds to a daily per capita supply of 27 g, of which 9 g come from shortenings, and 14, 3, and 1 g from hard, semisoft, and soft margarines, respectively. Based on these figures, total daily *trans* $C_{18:1}$ supply for each Dane is 1.1 g from shortenings and margarines, and the daily supply of saturated, monunsaturated, and polyunsaturated fatty acids from these fats is 7.5, 8.3, and 4.7 g, respectively.

The intake of TFA from industrially hydrogenated fats has been reduced during recent years because manufacturers have reduced TFA content in many of their products. Compared with the health consequences of the high total fat content in the Danish diet and the high proportion of dairy saturated fatty acids, the intake of TFA may seem trivial and unlikely to cause harm. However, the intake of TFA has in one large prospective epidemiological study been shown to be associated with a higher atherogenic potential than the intake of saturated fatty acids and total fat (18). Further, it has been shown that TFA can be substantially reduced in hardened vegetable oils without discernible changes in their cost to the consumer or in their technical and culinary properties. Therefore, it seems prudent to limit their content as much as is practicable in foods.

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