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ORIGINAL CONTRIBUTION

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Determination of *trans*-octadecenoic acids in german margarines, shortenings, cooking and dietary fats by Ag-TLC/GC

Bestimmung von *trans*-Octadecensäuren in deutschen Margarinesorten, Back-, Brat- und Diätfetten mittels Ag-TLC/GC

Summary According to numerous, even recent studies, *trans* fatty acids (TFA) are related to coronary heart disease. Thus, for the evaluation of the daily TFA-intake precise data on the content of TFA in currently available edible fats are of great interest. The present study gives a comprehensive overview on total *trans*-octadecenoic acid contents in 93 brands of German margarines, shortenings, cooking and dietary fats purchased in 1994, that were obtained by a two-dimensional chromatography method (Ag-TLC/GC). Conventional margarines were found to contain 0.17 - 25.90 wt% (n = 46) and shortenings/cooking fats 0.04–32.51 wt% (n = 16) TFA related to total fatty acids. Mean values were 9.32 resp. 9.79 %, whereas dietary and reformatory fats exhibited a mean of 0.65 % (n = 31) TFA. Among conventional margarines the highest TFA contents were detected in products (n = 11) derived exclusively from sunflower oil (mean value = 20.71 %). Seasonal changes in single brands were relatively small.

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Zusammenfassung Zahlreichen, auch neueren Studien zufolge werden *trans*-Fettsäuren (TFA) mit koronaren Herzerkrankungen in Zusammenhang gebracht. Für eine Berechnung der täglichen TFA-Aufnahme sind deshalb präzise Angaben über den aktuellen Gehalt von TFA in handelsüblichen Speisefetten von großem Interesse. Die vorliegende Arbeit gibt einen umfassenden Überblick der Gesamtgehalte an *trans*-Octadecensäuren in 93 Marken von im Jahre 1994 gekauften deutschen Margarinen, Back-, Brat- und Diätfetten, die mit Hilfe einer zweidimensionalen chromatographischen Methode (Ag-TLC/GC) ermittelt wurden. Danach enthielten konventionelle Margarinen 0,17–25,90 Gew% (n = 46) und Back-/Bratfette 0,04–32,51 Gew% (n = 16) TFA bezogen auf die gesamten Fettsäuren. Die Mittelwerte betrugen 9,32 % bzw. 9,79 %, während Diät- und Reformfette im Mittel 0,65 % (n = 31) TFA aufwiesen. Unter den konventionellen Margarinen zeigten die ausschließlich aus Sonnenblumenöl hergestellten Produkte (n = 11) die höchsten TFA-Gehalte (Mittelwert = 20,71 %). Jahreszeitliche Schwankungen innerhalb einzelner Marken waren relativ gering.

Key words *trans* fatty acids – edible fats – gas chromatography – argentation thin-layer chromatography

Schlüsselwörter *trans*-Fettsäuren – Speisefette – Gaschromatographie – Silberionen-Dünnschichtchromatographie

Abbreviations *FA* = fatty acid *FAME* = fatty acid methyl ester *GC* = gas chromatography · *TFA* = *trans* fatty acid · *Ag-TLC* = argentation thin-layer chromatography *C18:1* = octadecenoic acids

Introduction

Since *trans* fatty acids are suspected to influence serum lipid levels (among others 14, 31, 32) and therefore seem to be a risk factor for coronary heart diseases (2, 29), knowledge about the content of TFA in edible fats is of current interest. The greatest contribution to the TFA intake with food results from partially hydrogenated vegetable fats and oils (26), which were found to contain up to over 30 wt% *trans*-octadecenoic acids (27), related to total FA.

Thus, the present study aims at the exact determination of *trans*-octadecenoic acids, which comprise more than 80 % of all *trans* isomers in food, in a market-comprising range of conventional margarines, shortenings and cooking fats available today. For reasons of comparison, the C18:1 TFA content in a variety of dietary and reformatory fats was analyzed as well. The latest extensive study concerning TFA contents in a large number (n = 110) of partially hydrogenated edible fats on the German market has been published in 1978 (10). On the one hand, the analysis of TFA has been improved substantially up to now, on the other hand, changes due to technological development should be revealed by an overview of the current fat market.

Materials and methods

Fat samples and preparation of FAME

Altogether, 108 commercial products purchased during August (n = 93) and December (n = 15) 1994 in the northern part of Germany were melted at 70 °C in an oven. After separation of water the fat layer was passed through a filter, dissolved in n-heptane and then dried over Na_2SO_4. FAME were obtained by transesterification with sodium methylate using a modified method (20) similar to that of Christopherson and Glass (3).

Analysis of TFA

Trans-octadecenoic acids were analyzed gas chromatographically at an isothermal oven temperature of 175 °C using a 100-m capillary column (Chrompack CP-Sil 88). To avoid an overlapping of *trans*- and cis-C18:1 peaks a prefractionation into saturates, *trans*-monoenes and *cis*-monoenes by Ag-TLC has been applied, allowing an exact determination of the total C18:1 TFA content. Quantitation was done by relating *trans* Δ11 to the total C18:1 group (100-m column), which had been determined by means of total FA analysis on a 25-m capillary column (Chrompack CP-Wax 58). Thus, with the known content of *trans* Δ11 all other isomers of *trans*-C18:1 could be computed. Calibration of the total FA profile had been achieved with a standard mixture containing the methyl esters of the main fatty acids in a similar ratio (20). Analytical conditions have been specified elsewhere (15).

Results and discussion

Whereas *trans*-octadecenoic acids in milk fats result from biohydrogenation in the rumen, the isomeric pattern of C18:1 TFA in other edible fats is caused by technological hydrogenation aiming at the improvement of consistency, which partly leads to relatively high TFA contents. The upper part of Fig. 1 shows a partial gas chromatogram of FAME derived from a typical margarine. The corresponding analytic can be considered as most progressive regarding the resolution of isomeric TFA today (15). The identification of C18:1 isomers was achieved by commercial FAME standards and Ag-TLC technique as published in our earlier paper (15). In a recent study a comparable identification was found (30).

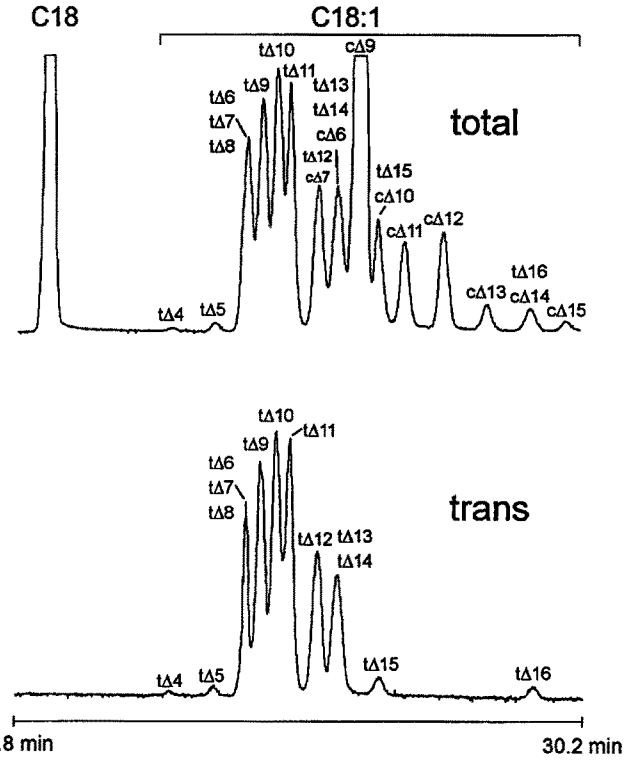

Fig. 1 Partial gas chromatograms of FAME derived from margarine (upper part) and from a TFA fraction of the same margarine obtained by Ag-TLC (lower part).

Numerous studies about the content of TFA in partially hydrogenated fats have been published in the past (5), but most of these were only performed with a relative small, nonrepresentative number of samples (1, 4, 8, 9, 13, 16-19, 21-23, 25, 28) and/or concerned products from other countries (6, 7, 12, 24). The latest extensive study on German fats (n = 110) was published in 1978 (10). Part of the applied methods appear to be more or less imprecise, e.g., certain isomers of C18:1 TFA have been neglected because of a lack in GC resolution (8, 10, 12, 17-19, 22), problems with the transesterification into FAME were observed (11) or relative inexact infrared spectroscopy methods have been used (9, 13). Even a quite new study (18) is based on a GC method, which

does not consider the isomers *trans* Δ12-16 and probably Δ4-5.

Since an exact determination of the total content of *trans*-octadecenoic acids by GC affords a consideration of the *cis*-portions (Δ6, Δ7, Δ10, Δ14) lying under some *trans*-peaks, the TFA analysis was performed with *trans*-FAME fractions (lower part of Fig. 1) gained by Ag-TLC (15), which avoids a sometimes occurring overlap of *cis* Δ9 with *trans* Δ15 as well. The combination of Ag-TLC with GC has widely been applied with TFA analysis in partially hydrogenated fats (1, 7, 16, 21, 23, 25, 28) and can be considered as a reference method. In Table 1 the evaluated total *trans* C18:1 contents in 46 margarines and 16 shortenings/cooking fats are listed. Though the mean values of 9.32 % resp. 9.79 % are close to one another, the maximal TFA content of 32.51 % found in a cooking fat exeeds the highest value of margarines by far. This is due to the desired property of such a fat, which should be as inert as possible to heating. Thus, the C18:2 and C18:3 fatty acids are reduced by hydrogenation resulting in an increase of C18:1 TFA.

Table 1 Total *trans*-octadecenoic acids in German margarines, shortenings/cooking fats and dietary/reformatory* fats (wt% of total fatty acids; mean, min, max = mean, minimal, maximal value; sd = standard deviation)

	n	mean	min	max	sd
Margarines	46	9.32	0.17	25.90	7.35
Shortenings, cooking fats	16	9.79	0.04	32.51	8.51
Dietary fats, reformatory fats	31	0.65	0.03	2.94	0.65
Sunflower-margarines	11	20.71	12.93	25.90	3.40

* terms restricted to margarine of special composition in Germany

Furthermore, the great variance of TFA contents is remarkable. The frequency distribution of total *trans*-octadecenoic acids in margarines, shortenings/cooking fats and dietary/reformatory fats is shown in Fig. 2. Though all fats of the first two categories were declared to contain partially hydrogenated vegetable oils, there are brands, which exhibit very low TFA contents of down to 0.17 resp. 0.04 %. Similar values are mostly to be found only for dietary or reformatory fats (Table 1) that must not be hydrogenated at all. Here, the low amounts of 0.03–2.94 % could be attributable to heat treatment during the production process, as consistency of those fats is mainly adjusted by transesterification. However, it should be emphasized that most dietary/reformatory fats contain less than 1 % of TFA (Fig. 2). Regarding the number of samples in the different categories, which represent an almost complete spectrum of the market in

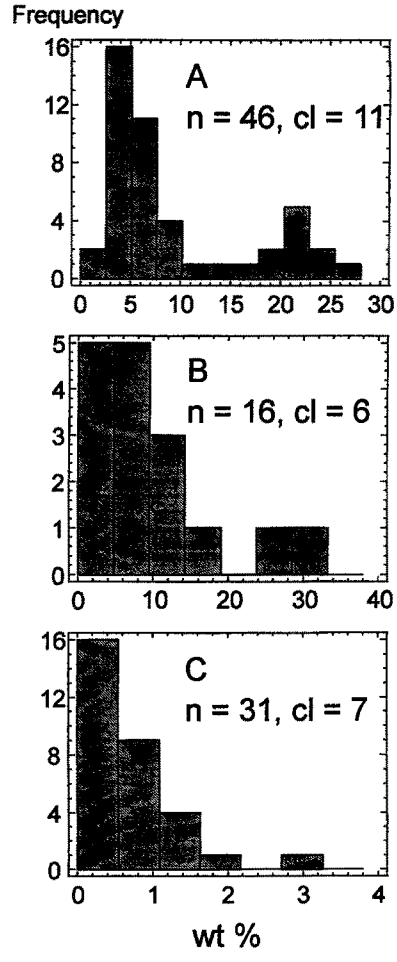

Fig. 2 Frequency distribution for total contents of *trans*-octadecenoic acids in edible fats: A = margarines, B = shortenings/cooking fats, C = dietary/reformatory fats, n = number of samples, cl = number of classes.

Germany, it can be assumed that changes in nutritional consciousness have already led to an increase of dietary and reformatory fats.

On the other hand, there are various margarines containing 15–28 % TFA (Fig. 2), since, depending on the raw material and the process parameters, the degree of isomerization into TFA varies markedly. These high contents of *trans*-octadecenoic acids are found in margarines derived from pure sunflower oil (Table 1), which exhibits very high amounts of unsaturated fatty acids, especially linoleic acid (C18:2). Therefore, the required hydrogenation for getting a good consistency leads to relatively high TFA contents. The group of sunflower margarines is clearly to been seen in the frequency distribution (Fig. 2). For other margarines derived from several vegetable oils with different FA composition acceptable product parameters can be achieved without producing as much TFA.

Seasonal changes in the composition of single brands of margarines and shortenings/ cooking fats are shown in Table 2. The TFA content in two batches of the same brand purchased in 8/94 resp. 12/94 decreased in the mean. Whereas changes in shortenings/cooking fats of

Table 2 Seasonal changes in TFA content of partially hydrogenated vegetable fats (wt% of total fatty acids; Δ_{abs} = absolute difference; mean = mean value)

	Margarine			Shortening/cooking fat		
Sample	8/94	12/94	Δ_{abs}	8/94	12/94	Δ_{abs}
1	8.79	6.99	-1.80	11.25	6.13	-5.12
2	3.69	3.15	-0.54	12.78	11.35	-1.44
3	5.34	2.87	-2.47	32.51	34.62	+2.11
4	12.93	11.69	-1.23	8.82	9.77	+0.95
5	5.33	0.95	-4.38	1.87	1.76	-0.11
6	25.90	23.71	-2.19	0.50	1.60	+1.11
7	6.75	5.21	-1.54			
8	4.21	2.91	-1.29			
9	7.39	5.52	-1.87			
Mean	8.93	7.00	-1.93	11.29	10.87	-0.42

0.42 % are negligible, there was a greater decrease of 1.93 % for margarines, but these changes were mainly caused by a few products. The smallest relative deviation was found for sunflower margarines (high in TFA), where hardly any compositional alteration by blending of different raw materials such as with other products is possible. Altogether, the changes were relatively small. Thus, the data listed in Table 1 may vary slightly but can be regarded as representative for the current production.

Though edible fats without any TFA are not to be found, the values of most dietary and reformatory fats can be considered as harmless. Whereas, compared to former studies about German edible fats (10), the range of these dietary and reformatory fats has increased, there are still a lot of edible fats with high TFA contents. Thus, with regard to several recent studies about physiological aspects of TFA intake (among others 2, 14, 29, 31, 32), there remains the old demand for technological changes in edible fat production. In connection with nutritional statistics, the presented data allow a new estimation of the daily per capita intake of TFA by edible fats derived from vegetable oils to be made, which will update former evaluations (11, 26).

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