Z Ernährungswiss 34: 27-29 (1995)

SHORT COMMUNICATION

D. Precht

Variation of trans fatty acids in milk fats

Variation von *trans*-Fettsäuren in Milchfetten

Zusammenfassung *Trans*-Fettsäuren werden in Zusammenhang mit einem erhöhten Atherosklerose-risiko diskutiert. Die Entwicklung einer schnellen und exakten Meßmethode zur Bestimmung von *trans*-Fettsäuren in Milchfett ist deshalb von großem Interesse. Anhand der gaschromatographischen Analyse der *trans*-Octadecensäuren sowie der Triglyceride von 100 unterschiedlichen Milchfetten wurde mit statistischen Methoden eine aus verschiedenen Triglyceriden bestehende Formel zur schnellen Feststellung der *trans*-Gehalte entwickelt (Standardabw. = 0,293 %, r = 0,9977). Mit Hilfe schnell durchführbarer Triglyceridanalysen wurden anschließend von einem großen Milcheinzugsgebiet die saisonalen Veränderungen der *trans*-Gehalte im Milchfett ermittelt. Für die 100 Milchfettproben sowie die Proben des Milcheinzugsgebietes konnten für die *trans*-Fettsäurengehalte Schwankungsbereiche von 1,91–6,34 Gew.% bzw. 1,97–4,37 Gew.% ermittelt werden. Die Mittelwerte betrugen hierbei 3,83 und 3,18 Gew.% und die Medianwerte 3,67 bzw. 3,30 Gew.%.

Received: 9 September 1994
Accepted: 3 December 1994

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Summary *Trans* fatty acids are discussed in connection with an increased risk of atherosclerosis. Therefore, the development of a rapid and exact measuring method for the determination of *trans* fatty acids in milk fat is of great interest. Using gas chromatographic analysis of the *trans*-octadecenoic fatty acids as well as of the triglycerides of 100 different milk fat samples a formula consisting of different triglycerides for the quick determination of *trans* contents was developed by means of statistical methods (standard deviation = 0.293 %, r = 0.9977). Subsequently, the seasonal variations of the *trans* contents in milk fat samples from a large milk collection area were determined using rapid triglyceride analyses. For the *trans* fatty acid contents of the 100 milk fat samples and the samples from the milk collection area scattering ranges of 1.91–6.34 wt% resp. 1.97–4.37 wt% were found; the mean contents were 3.83 and 3.18 wt%, and the median values 3.67 and 3.30 wt%, respectively.

Schlüsselwörter *trans*-Fettsäuren – Milchfett – Gaschromatographie – Fütterungseinflüsse

Key words *Trans* fatty acids – milk fat – gas liquid chromatography – feeding influences

Introduction

Whereas, according to some authors, the saturated fatty acids C12–C16 increase both the LDL- and the HDL-cholesterol level (12), clinical nutritional studies have shown that *trans* fatty acids increase the LDL-cholesterol level and lead to a decrease in the HDL-cholesterol level (4, 11). *Trans* fatty acids exert, therefore, a particularly unfavorable influence on the LDL/HDL ratio. Furthermore, an increase in the Lp(a)-lipoprotein level has been observed after the uptake of *trans* fatty acids (5). According to numerous authors both effects are associated with an increased risk of premature atherosclerosis (1, 3, 5).

In the past, it has been shown that a number of studies concerning the determination of *trans* fatty acid contents have led to rather varying results, if different methods,

e.g., the use of too short gas chromatographic columns or of infrared spectroscopy, were applied to identical samples (10). So far, exact determinations were mainly possible by performing time-consuming gas chromatographic methods in combination with thin-layer chromatographic extractions (6, 10).

The development of a time-saving and accurate analytical method for the quantitative determination of *trans* fatty acids in fats is, therefore, of great interest in food analysis. In the following, a method is described which enables the measurement of isomeric C18:1-fatty acids on the basis of a triglyceride analysis within a few minutes. Based on this method the seasonal variations of *trans*-octadecenoic acid contents in milk fat samples obtained from a larger milk collection area were analyzed. Similar measurements have not yet been performed in Germany and, as far as other European countries are concerned, also were restricted to a few milk fat samples only (10).

The studies carried out refer, in the following, exclusively to octadecenoic acids, which comprise more than 80 % of all *trans* isomers in the food.

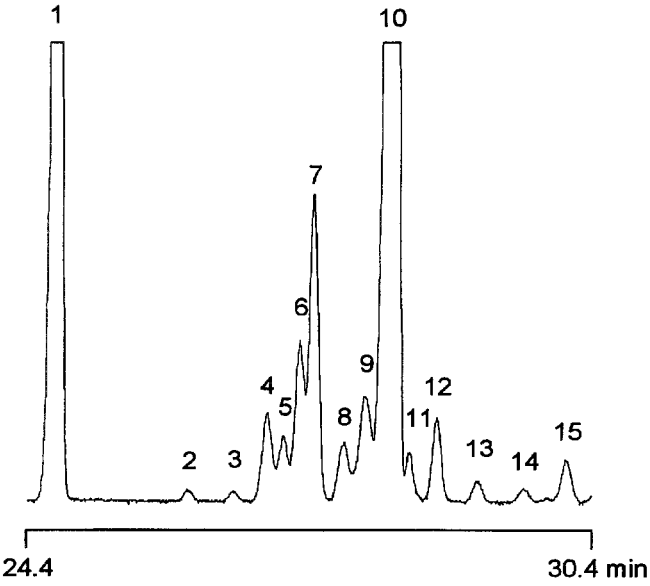

Fig. 1 Partial chromatogram of C18 and C18:1 *trans/cis* fatty acid methyl esters analyzed on a 100-m CP-Sil 88 capillary column with: 1, C18; 2, *trans*-Δ5; 3, *trans*-Δ7; 4, *trans*-Δ6/8; 5, *trans*-Δ9; 6, *trans*-Δ10; 7, *trans*-Δ11; 8, *trans*-Δ12; 9, *trans*-Δ13/Δ14; 10, *cis*-Δ9; 11, *trans*-Δ15; 12, *cis*-Δ11; 13, *cis*-Δ12; 14, *cis*-Δ13; 15, *trans*-Δ16.

Materials and methods

All milk fats analyzed were obtained by melting butter at 50° C followed by filtration of the fat layer. *Trans* fatty acid contents were determined gas chromatographically after preparation of the methyl esters according to a modified method (7) similar to that of Christopherson and Glass (2). The column used was a 100-m fused-silica-capillary column coated with 100 % cyanopropyl polysiloxane (CP-Sil 88, 0.25 mm i.d.) and was operated isothermally at 175° C. Calibration was done by means of total fatty acid spectrum analysis using a 25 m CP-Wax 58-capillary column, i.e., the trans-octadecenoic acids were calibrated in relation to stearic acid; the individual fatty acids of the total FA spectrum were calibrated using a test mixture consisting of the methyl esters of the main fatty acids, the ratio of which had been adjusted to milk fat. Low contents of *trans* fatty acids (Δ15-C18:1) in the oleic acid peak and small amounts of *cis* isomers in the *trans* fatty acid peaks were taken into account by fractionation of the fatty acid methyl esters by TLC on silica-gel plates impregnated with $AgNO_3$.

Gas chromatographic triglyceride analysis (separation of triglycerides by carbon number) has been described elsewhere (8).

Results and discussion

Isomeric octadecenoic fatty acids in milk fat occur as a result of biohydrogenation in the rumen of cows. Figure 1 shows the octadecenoic acids of milk fat, based on typical summer feeding conditions of the cows. The tentative identification of the peaks is done according to specifications in the literature (10, 13), comparison with FAME standards, and own argentation thin-layer chromatography results. Regarding the resolution the 100 m Sil 88-column used can today be considered as the most progressive in *trans* fatty acid separation; its resolution is clearly better compared with the phases used frequently up to now as, e.g., SP1000, SP2100 or SP2340. A similar resolution compared with the Sil 88-column is achieved by a SP2560-column of same length. It is obvious that in milk fat the most frequently occurring *trans* fatty acid is vaccenic acid, where the double bond is located in position 11 from the carboxyl group. The elaidic acid (*trans* Δ9) appears in relatively small amounts, in contrast to previous studies.

Triglyceride analysis with separation of triglycerides restricted to different carbon numbers has proved to be a rapid method for the determination of *trans*-octadecenoic acid contents. As has been shown earlier this technique allows a detailed characterization of milk fats by means of individual triglycerides or combinations of triglycerides (9).

Based upon *trans*-octadecenoic acid contents and triglyceride analysis of 100 different milk fats, covering a wide scope of feeding conditions, a triglyceride formula was developed by means of a multiple regression analysis. With the 15 triglycerides C26, C28 to C54 more than 32 000 different triglyceride combinations were tested by a computer with respect to their suitability for

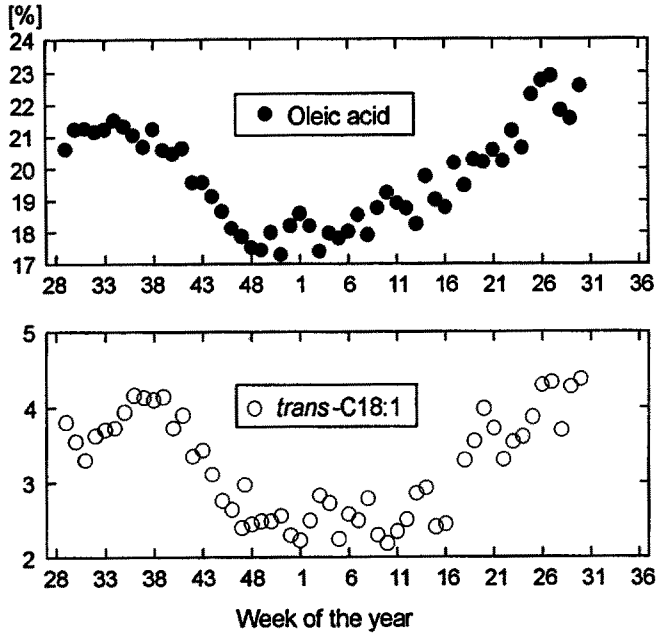

Fig. 2 Weekly variation in the weight percentages of oleic acid and of the sum of all *trans*-octadecenoic acids of a large milk collection area.

the theoretical computation of the *trans*-octadecenoic contents. Of all triglyceride formulae the following one has proved to be the most accurate (sum of Δ5, Δ6 to Δ16):

trans-C18:1 = 3.7190 · C26 + 2.4439 · C28 - 8.1505 · C30 + 3.5187 · C32 - 0.4819 · C34 - 0.3737 · C36 + 0.3514 · C40 + 1.3285 · C42 - 1.3429 · C44 - 0.2689 · C46 + 0.9557 · C48 - 0.7356 · C52 + 1.0775 · C54.

The formula produces a normal probability plot of the residuals. The standard error of estimation (square root of the residual mean square) was 0.293 %, the mean absolute error (average of the absolute values of the residuals) was 0.212 % and the correlation coefficient 0.9977. The formula was checked with a further set of 14 very different milk fats covering a wide scope of feeding conditions from which the triglycerides and the sum of all *trans*-octadecenoic acids were analyzed. All theoretical calculated *trans*-values agreed to a high extent with the experimental data (standard deviation = 0.12 %).

The triglyceride formula allows to determine the *trans* C18:1-fatty acid content within 10–20 min by inserting the corresponding triglyceride contents from a triglyceride analysis of an unknown milk fat sample.

The underlying 100 milk fat samples exhibited a scattering range between 1.91 and 6.34 wt%, a mean content of 3.83 wt% and a median value of 3.67 wt% for the *trans* C18:1 isomers.

Furthermore, butter samples from a West German milk collection area, obtained from bulk milks of always the same herds, were analyzed weekly. Figure 2 shows the weekly measuring results for the oleic acid and the *trans*-octadecenoic acid contents. It is obvious that the *trans* and oleic acid contents take a similar course. As can be seen in the middle of the figure, markedly lower *trans* and oleic acid contents are found during the winter. This effect is connected with the high amount of polyunsaturated C18-fatty acids of the pasture feeding, the linoleic and the linolenic acid, which contribute up to 75 % to this fodder. By the biohydrogenation in the rumen the polyunsaturated fatty acids are transferred into stearic and oleic acid but also in *trans*-octadecenoic proportions. The *trans* fatty acid contents throughout the year gave a scattering range of 1.97–4.37 wt% and a mean content of 3.18 wt% (median value: 3.30 wt%).

The studies performed are particularly important for estimating the uptake of *trans*-octadecenoic acids by dairy fats. Since all milk products, such as butter, cheese, whipping cream etc. are subject to great seasonal variations of the *trans* contents, which are attributable to feeding or lactation conditions, the measurement of only a few samples can lead to wrong conclusions as regards the mean *trans* content.

Acknowledgements

We would like to thank Mrs. B. Fischer and B. Krumbeck for their assistance in performing analytical experiments.

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