Content of individual *cis/trans* isomers of 16:1, 18:1 and 18:2 fatty acids in the reference milk fat CRM164

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1. Introduction

Since the beginning of the last decade, the consumption of *trans* fatty acids (TFA) has increasingly been discussed with regard to adverse nutritional or health aspects. In particular, the unfavourable influence on the cholesterol level in different lipoprotein classes has been related to an increasing risk for atherosclerosis and cardiovascular disease (see reviews (1-7)). TFA are found in ruminant fats due to rumenic bio-hydrogenation. Further, substantial amounts of TFA may arise from partial hydrogenation of vegetable oils during production of margarines or cooking fats. However, irrespective of the amount epidemiological studies suggested a different effect of TFA from ruminant fats and from partially hydrogenated vegetable oils (8). This might be attributable to the different isomeric distribution of the respective TFA. Thus, the negative physiological effects might be related to certain individual isomers of *trans*-16:1, *trans*-18:1 or *trans*-18:2 rather than to the total amount of ingested TFA. Accordingly, positive effects have been associated with the isomer t11-18:1 predominating TFA in dairy fats which can act as a metabolic precursor of the bioactive conjugated linoleic acid (CLA) isomer c9t11-18:2 (9).

Regarding the complex physiological potential, the assessment of TFA requires the exact qualitative and in particular the quantitative analysis of individual isomers. For calibration of the analysis of TFA isomers by gas chromatography (GC) a reference standard is a useful aid. Fatty acid analysis in milk fat can be calibrated using the certified reference material CRM164 (10). This material is an anhydrous milk fat that has been certified for the contents of the main fatty acids. Further, indicative (not certified) values are given for several minor fatty acids. However, only total contents are given for 18:1 and 18:2 *cis/trans* isomers, respectively. The aim of the present study was to establish the complete isomeric pattern of *cis*- and *trans*-16:1, 18:1 and 18:2 fatty acid isomers and, thus, to have a generally available reference milk fat with known contents of isomeric and total TFA. Of course in practice the isomeric quantification requires more effort than only a single GC analysis. Up to now, there is no such material on the market. Recently, CRM164 can not be obtained from the Institute for Reference Materials and Measurements (IRMM, Geel/Belgium) anymore, but the presented data can still be of use for laboratories having this reference material in stock.

2. Materials and methods

2.1 Samples and preparation of fatty acid methyl esters (FAME)

The reference material CRM164 was obtained from the Institute for Reference Materials and Measurements (IRMM, Geel/Belgium). CRM164 is an anhydrous milk fat with certified fatty acid composition.

FAME were obtained from the fat sample by *trans*-esterification with sodium methoxide. For this purpose a mixture of 1200 ml *n*-heptane, 300 ml of a 10% solution of fat in *n*-heptane and 30 ml of a 2 N solution of sodium methoxide in methanol was shaken vigorously for 3 min (Vortex-Mixer) and centrifuged (2 min at 2000 min^{-1}). The supernatant or a suitable dilution was used for direct injection into the GC. For argentation thin-layer chromatography the procedure was changed to obtain a 20% solution of FAME.

2.2 Argentation thin-layer chromatography (Ag-TLC)

Ag-TLC was performed similarly to the principles described by *Morris* (11) but introducing some modifications. TLC glass plates coated with 0.25 mm Silica Gel 60 (20 x 20 cm, Merck, Darmstadt/Germany) were dipped into a 20% aqueous solution (w/v) of silver nitrate for 20 min and air-dried in the dark. Directly before chromatography, the plates were activated at 120°C for 30 min. After cooling to ambient temperature, a 20% solution (v/v) of FAME in *n*-heptane was applied to the plate in a narrow band with a concentration of 0.4 mg FAME cm^{-1}, leaving a space of 1.5 cm at both edges. For the separation of *trans*-monoenes and *cis*-monoenes (16:1, 18:1) the plates were developed with a solvent mixture of *n*-heptane/diethyl ether (90:10). The dienes (18:2) were separated by developing with *n*-heptane/diethyl ether (70:30). Subsequently, the dried plates were lightly sprayed with 0.2% 2´,7´-dichlorofluorescein in *iso*-propanol to visualize the individual bands (from top: saturates, *trans*-monoenes, *cis*-monoenes, dienes; identification by methyl esters of stearic acid, elaidic acid, oleic acid and linoleic acid in preliminary tests). The fractions were marked under UV light, scraped off separately and eluted with diethyl ether several times. Combined elutes of each fraction were evaporated by a nitrogen flow and dissolved and diluted adequately in *n*-heptane for GC analysis. In order to obtain correct results, cross-contamination of TLC-fractions was checked after GC analysis on a 100-m column by mutually comparing the fractions and the total FAME.

2.3 Gas chromatography

In order to imitate the resolution underlying the certification process of CRM164 (10) total FAME and TLC-fractions were analysed on a CP9001 gas chromatograph (Chrompack, Middelburg/The Netherlands) equipped with a split injection port (1:50), FID and a 25-m fused silica capillary column (i.d.: 0.25 mm) coated with 0.20 µm of CP-Wax 58CB (Chrompack-Varian, Darmstadt/Germany). Hydrogen was used as the carrier gas with a column head pressure of 69 kPa (1.5 ml/min). Injector and detector temperatures were both 265°C. Oven program: 50°C, 1 min isothermal, then with 5°C/min to 225°C, 15 min isothermal, then with 5°C/min to 260°C. Results obtained with this short 25-m column were used for a qualitative comparison only.

For the quantification of isomeric contents high resolution analyses of total FAME and TLC-fractions were performed on a CP9002 gas chromatograph (Chrompack) equipped with a split injection port (1:25), FID and a 100-m fused silica capillary column (i.d.: 0.25 mm) coated with 0.20 µm of CP-Sil88 (Chrompack). Hydrogen column head pressure was 160 kPa (145°C) or 220 kPa (125°C). The temperatures of injector and detector were both 255°C. Analyses of TLC-fractions were performed isothermally at 145°C (18:2) or at 125°C (16:1, 18:1). In order to obtain a corresponding resolution, total FAME were analysed at both temperatures. In addition, total FAME were analysed at 172°C (160 kPa) for 18:1 analysis.

The concentration of GC samples was adapted individually to obtain an optimum resolution for the respective range of evaluated FAME. All samples were injected manually using the 'hot injection'-technique.

2.4 Identification and calibration of FAME isomers

The identification of *cis* and *trans* isomers of 16:1 (12-14) and of 18:1 (15) is based on our previous work. Also the identification of *cis/trans*-18:2 isomers was done according to our former investigations (16) which were based on the use of FAME standards and particularly on studies by *Ratnayake* and *Pelletier* (17) as well as *Ulberth* and *Henninger* (18).

Quantitative results were obtained from analyses on the 100-m column only. After the analysis of total FAME, non-overlapped peaks were quantified directly. This was achieved by calibration via neighbouring peaks having similar response factors. Thus, 16:1 acids were calibrated by the known content of 16:0. For the calibration of 18:1 and 18:2 acids the known 18:0 content was used. Before, the 16:0 and 18:0 content had been calculated from the certified values of 16:0 total and 18:0 total by subtraction of 16:0 iso and 18:0 iso, respectively.

Only those peaks, that overlapped in total FAME analysis, were quantified from analyses of the TLC-fractions. Calibration within the TLC-fractions was done by isomers which could be quantified in the total FAME analysis before. Thus, *cis*-16:1 isomers were quantified via c9-16:1 and *trans*-16:1 isomers via t9-16:1. Quantification of *trans*-18:1 isomers in the TLC-fraction was achieved via t6-11. Overlapped isomers of *cis*-18:1 were quantified by calculating the differences between total and *trans* FAME or from the *cis*-fraction by c11. 18:2 isomers that could not be obtained in total FAME analysis were quantified from the TLC-fraction via c9c12-18:2. For further details on quantification see "Results and discussion".

The integration of chromatograms was performed using a Hewlett Packard 3365 II ChemStation. Results are given in g per 100 g of total fatty acids (%).

3. Results and discussion

In the past decade the determination of fatty acid profiles mostly was based on the gas chromatographic analysis of FAME using capillary columns with stationary phases of the nitroterephtalic acid-modified or unmodified polyethylenglycol type. Such columns have partly been used for the certification exercise of the anhydrous milk fat reference material CRM164 (10) as well. These columns showed overlaps especially of unsaturated fatty acid isomers to a considerable extent. Even today, there is no GC column capable of resolving all fatty acid isomers present in milk fat within a single analysis. In Fig. 1 the overlaps occurring with a 25-m CP Wax58 column (nitroterephtalic acid-modified polyethylenglycol type) are demonstrated for a milk fat. Though this is another typical milk fat, a corresponding resolution results with CRM164 as well. The upper chromatogram shows the total FAME in the region comprising most of the 18:1 and 18:2 isomers. The other chromatograms were obtained from TLC-fractions using identical GC settings.

On the one hand, it can be seen that there are complex overlaps between *cis*- and *trans*-18:1 isomers. That's why the reference material CRM164 was certified only for the total 18:1 content. The vertical dotted line indicates the separation presumably applied between the peak groups of 18:1 and 18:2 in the certification process. Also 18:2 isomers could only

be certified as total 18:2 because neither c9c12-18:2 nor c9t11-18:2 (18:2 conj.) showed a sufficient precision singly. The latter isomer is not contained in this part of the chromatogram (Fig. 1) but is also subject to variable overlaps with other fatty acids.

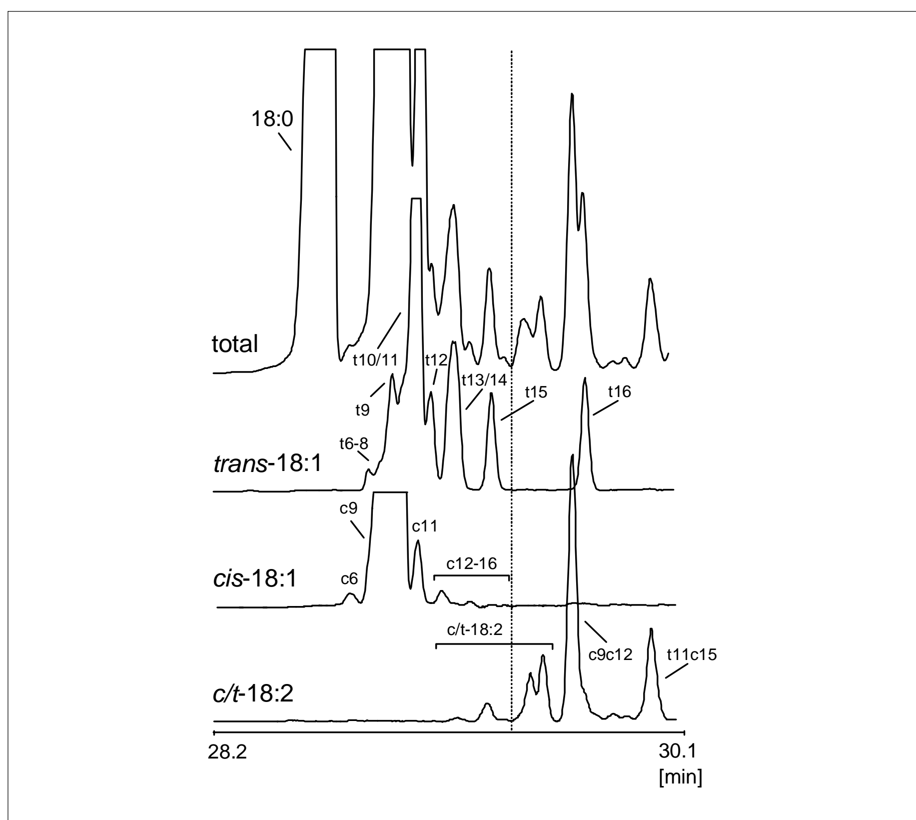

Fig. 1: Partial gas chromatograms of a milk and its TLC-fractions in the 18:1 and 18:2 region (25-m CP-Wax58 column)

On the other hand, there is an overlap between c9c12-18:2 and t16-18:1. Under the special conditions used here the two peaks could partly be resolved. Using standard conditions these peaks often co-elute completely. That's probably the reason why c9c12-18:2 could not be certified in CRM164. Further, some minor 18:2 isomers located on the left side of the dotted line fall inside the 18:1 group.

The aim of determining the individual isomers of 18:1 and 18:2 in the reference milk fat CRM164 does not only require the TLC-fractionation into *trans*-monoenes, *cis*-monoenes and dienes but an increased resolution between the isomers as well. Thus, in order to split up the contents of total 16:1, 18:1 and 18:2, the corresponding TLC-fractions of CRM164 were analysed using a 100-m CP-Sil88 column. To achieve the highest possible resolution, analyses were performed at isothermal temperatures of only 145°C or 125°C. All analyses were performed in duplicate using fractions from two independent TLC-separations. This high-resolution low-temperature GC has already been used in our previous studies on *trans* isomers (19).

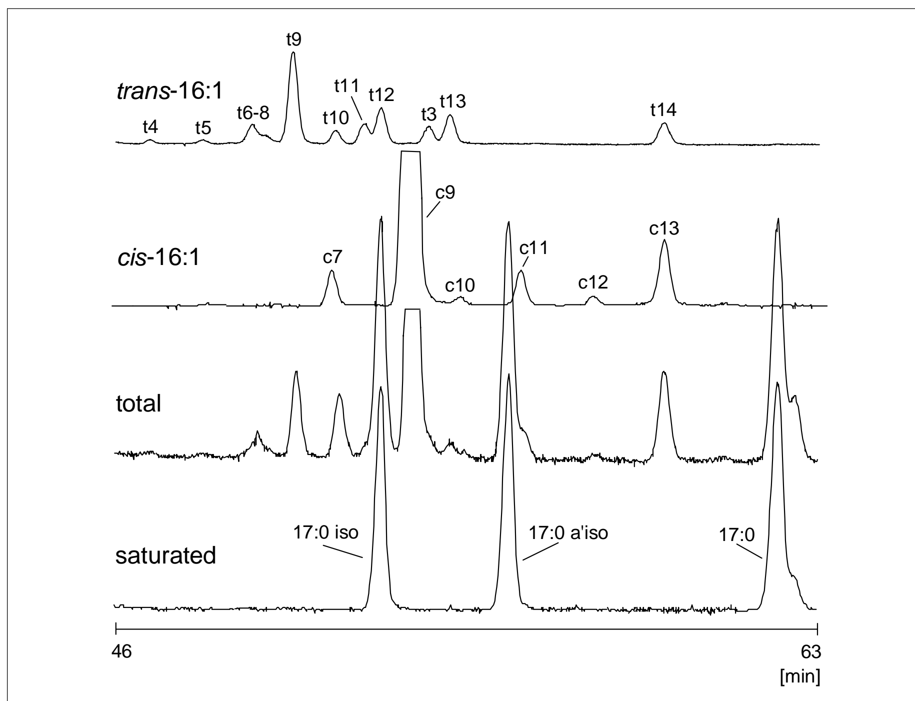

Fig. 2: Partial gas chromatograms of CRM164 and its TLC-fractions in the 16:1 region (100-m CP-Sil88 column at 125°C)

The analyses performed to quantify the isomers of 16:1 are shown in Fig. 2. The isomer c9 could be integrated without overlaps in the chromatogram of total FAME as there are no co-eluting peaks in the *trans*-monoenoic or the saturated acid fraction. The isomer t3 could be separated from c9 as a shoulder. By the known content of 16:0 (not to be seen in that part of the chromatogram shown in Fig. 2) the isomer c9 could be quantified. However, the certified 16:0 content was corrected by subtraction of 16:0 iso before. Finally, the other *cis*-16:1 isomers were quantified by c9 using the *cis*-fraction chromatogram. For the quantification of *trans*-16:1 isomers the peaks t4 to t9 as well as t13 could be calibrated by 16:0 directly in the chromatogram of total FAME. The isomers t10 to t14 and t3 were quantified in the *trans*-fraction chromatogram using the isomer t9. Further, the isomers 17:0, 17:0 iso and 17:0 a'iso were determined in the saturated fraction using both 16:0 and 18:0. Finally, it was confirmed that the sum of the contents of all *cis*- and *trans*-16:1 isomers and the three 17:0 isomers was identical to the total content of that whole group calculated from the total FAME chromatogram. The results of the individual 16:1 isomers are given in Tab. 1. Since the contents neither of these isomers nor of their sum had been certified in CRM164, the contents are not adjusted to a certain content now.

Tab. 1: Contents of *cis* and *trans* isomers of 16:1, 18:1 and 18:2 in the reference milk fat CRM164, means of duplicate determinations [%]

	Isomer											Total	
16:1 *cis*				**c7**	**c9**	**c10**	**c11**	**c12**	**c13**				
				0.06	1.29	0.01	0.05	0.02	0.11			1.54	
trans	**t3**	**t4**	**t5**	**t6-8**	**t9**	**t10**	**t11**	**t12**	**t13**	**t14**			
	0.04	0.01	0.01	0.05	0.15	0.02	0.04	0.09	0.06	0.05			0.52
18:1 *cis*			**c6**	**c9**		**c11**	**c12**	**c13**	**c14**	**c15**	**c16**		
			0.06	19.64		0.48	0.20	0.07	0.03	0.15	0.07	20.70	
trans	**t4**	**t5**	**t6-8**	**t9**	**t10**	**t11**	**t12**	**t13**	**t14**	**t15**	**t16**		
	0.01	0.01	0.20	0.26	0.43	1.66	0.33	0.29	0.50	0.31	0.41		4.41
18:2 *cis*									**c9c12**	**c9c15**			
									1.35	0.10		1.45	
trans	**tt-NMID**	**t9t12**	**c/t**	**c9t13/ t8c12**	**t8c13**	**c9t12**	**t9c12**	**t11c15**					
	0.16	0.01	0.10	0.16	0.11	0.05	0.01	0.21					0.81
Total *cis*												23.69	
trans													5.74

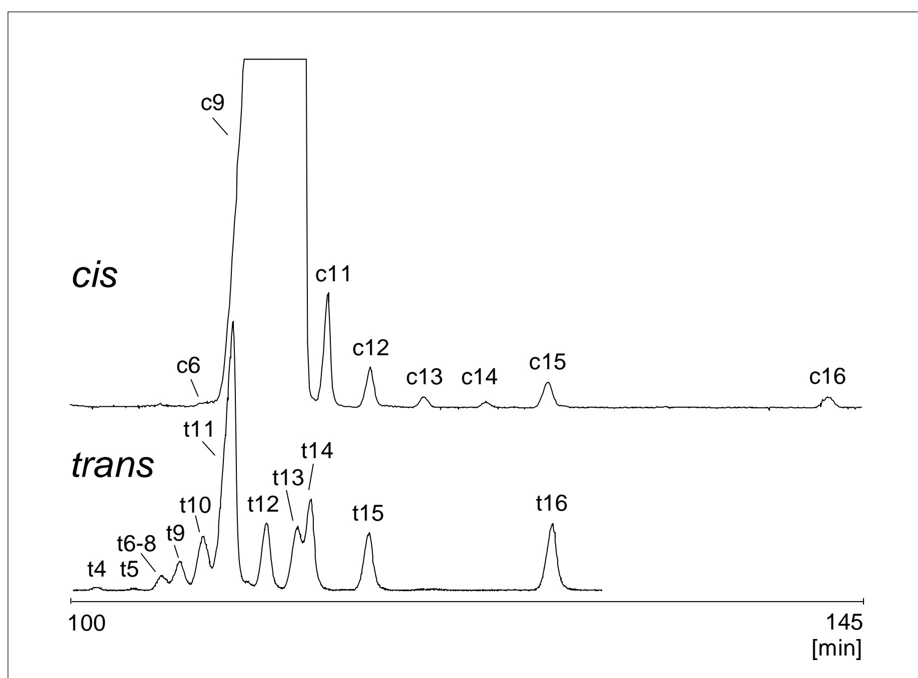

Fig. 3: Partial gas chromatograms of TLC-fractions from CRM164 in the 18:1 region (100-m CP-Sil88 column at 125°C)

The isomeric distribution of 18:1 isomers is shown in Fig. 3. The GC analysis at 125°C allowed to partly resolve the *trans* isomers t10 from t11 and t13 from t14. However, at 125°C the isomer t11 would partly co-elute with c9 in the chromatogram of total FAME. So, total FAME were analysed at 172°C allowing to quantify the *trans* isomers t4 to t11 by the certified and corrected content of 18:0 directly without overlaps (Fig. 4). The isomers t12 to t16 were quantified from the *trans*-monoenoic acid fraction by the group content of t6 to t11. Direct quantification of the *cis*-18:1 isomers c11, c12, c13 and c15 was possible from the total FAME chromatogram (Fig. 4) using 18:0 for calibration. The isomers c6, c9 and c14 were also calculated from the total FAME chromatogram (Fig. 4) by subtraction of the overlapping *trans* isomers t13-14, t15 and t16, respectively, which had been quantified before as mentioned above. Only the c16 content was determined from the *cis*-monoenoic fraction by the isomer c11.

As can be seen from Fig. 1, the certified content of the total 18:1 presumably contains some minor 18:2 isomers (tt-NMID) with a total content of 0.12%. Quantification of these 18:2 isomers is described below. If the certified 18:1 total content of 24.82% is corrected for these overlaps, it should be 24.70% actually. On the other hand, the content of t16-18:1 is not included in the certified value and has to be added to obtain a correct figure for total 18:1. This results in a true value of 25.11%. Thus, the contents of all individual *cis*- and *trans*-18:1 isomers newly determined in the present study were adjusted to come to a total content of 25.11%. Tab. 1 contains the finally resulting contents of individual 18:1 isomers.

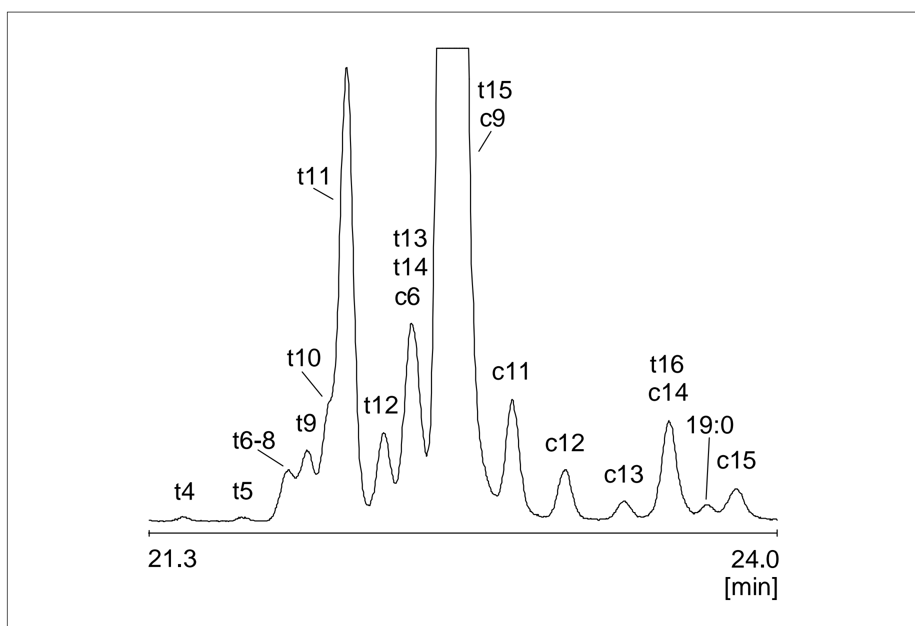

Fig. 4: Partial gas chromatogram of total FAME from CRM164 in the 18:1 region (100-m CP-Sil88 column at 172°C)

The analysis of 18:2 isomers comprised different c/t-isomers (cc, ct, tc, tt) except for conjugated isomers (CLA) which in contrast to other TFA are regarded as beneficial for health. CLA show a different chromatographic behaviour and do not interfere with the other 18:2 isomers. Fig. 5 illustrates the isomeric distribution of 18:2 isomers other than CLA in

CRM164 with a tentative identification of the isomers t8c12 and t8c13. Most isomers shown here could be quantified directly from the chromatogram of total FAME at 145°C by the certified content of 18:0 without overlaps. The isomer t9t12 had to be determined differently because of an overlap with an unidentified 18:2 isomer (c/t) and was quantified directly from the total FAME chromatogram at 172°C using t5-18:1 (16). Further, the various small isomers indicated as t,t-NMID (*trans,trans* non methylene interrupted dienes) had to be quantified from the TLC fraction analysed at 145°C by the content of c9c12-18:2 which had been determined from the total FAME analysis before. These are t,t-18:2 isomers with more than one methylene group between the double bonds that have not been identified in greater detail. From comparison of chromatograms it could be deduced that t,t-NMID located on the left side of the double arrow (a) co-elute with the group of 18:1 isomers marked in Fig. 1, while t,t-NMID on the right side (b) fall inside the 18:2 group on the 25-m column.

Due to various possible overlaps it was impossible to understand which isomers actually had been included in the certified 18:2 total content of CRM164. At least, it can be assumed that considerably more isomers are included in the analysis now than at that time. During the certification exercise of CRM164 a certified value could be established neither for c9c12-18:2 nor for c9t11-18:2 (CLA), but the sum of both was certified as 18:2 total content. However, the present study did not comprise CLA. Due to these different reasons, the certified value could not be used as a reference. Thus, the obtained contents of individual 18:2 isomers are not adjusted to the certified 18:2 total content originally given for CRM164. The new 18:2 data shown in Tab. 1 should be regarded as a correction instead.

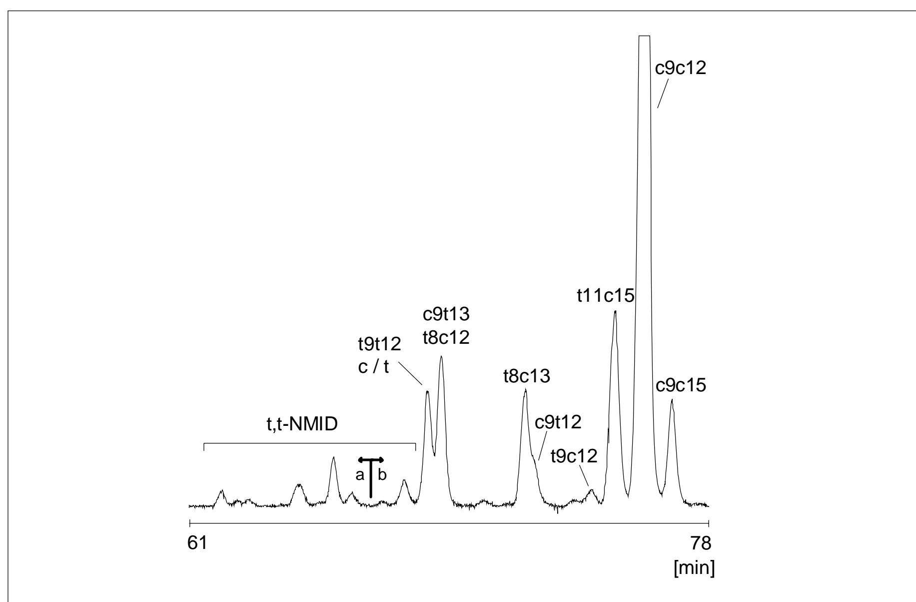

Fig. 5: Partial gas chromatogram of the dienoic acid TLC fraction from CRM164 in the 18:2 region (100-m CP-Sil88 column at 145°C; c/t: unidentified C18:2 isomer)

The overall content of 16:1, 18:1 and 18:2 *cis* isomers determined in the present study amounts to 23.69%, while the content of *trans* isomers in CRM164 is 5.74% in total (Tab. 1). Isomers of CLA are not included in these figures. With a 18:1 TFA content of

4.41% and an indicative content of C54 triglycerides of 5.0% (10) CRM164 could be identified as a milk fat originating from the transition period between barn and pasture feeding and thus having a medium composition (20).

4. Conclusions

The isomeric distribution determined now allows to extend the use of CRM164. In addition to the calibration of total fatty acid analysis CRM164 can as well be used to calibrate the analysis of total or isomeric TFA contents in dairy fats or to check the performance of such methods. Moreover, these data can be used to estimate overlaps on shorter GC columns and, thus, to optimise the total fatty acid calibration as well. Although the new isomeric data have not been obtained in a collaborative trial, these can at least be regarded as indicative particularly as the calibration was based on certified data.

Acknowledgement

The authors thank Mrs. Birte Fischer and Mrs. Bärbel Krumbeck for their assistance in performing the analytical work.

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6. Summary

Molkentin, J., Precht, D.: **Content of individual *cis/trans* isomers of 16:1, 18:1 and 18:2 fatty acids in the reference milk fat CRM164.** Kieler Milchwirtschaftliche Forschungsberichte **56** (1) 53-63 (2004)

24* Trans *fatty acids (milk fat, calibration, gas chromatography, reference material)

Trans fatty acids (TFA) are generally discussed in the context of numerous adverse health aspects, whereby individual isomers possibly cause varying effects. For instance, positive effects are associated with the isomer t11-18:1 predominating TFA in dairy fats. This isomer can act as a metabolic precursor of the bioactive conjugated linoleic acid (CLA) isomer c9t11-18:2. Thus, the determination of TFA contents and in particular of their isomeric distribution in edible fats is of importance. Due to overlapping peaks during gas chromatography (GC) of fatty acid methyl esters, TFA can only be quantified correctly with the Ag-TLC/GC method. Thus, for calibration and quality assurance of TFA analysis in milk fat, a reference material with known composition like CRM 164 (anhydrous milk fat, IRMM, Geel/Belgium) is useful. As there exist neither certified nor indicative values for TFA contents of this reference material, the isomeric composition was determined in this study. For this purpose, CRM 164 and – to avoid of overlaps – its fractions obtained by argentation thin-layer chromatography (Ag-TLC) containing saturated, *cis*-monoenoic, *trans*-monoenoic or dienoic fatty acids were analyzed by GC. The GC analyses were performed on a 100-m column (CP-Sil88) at different isothermal temperatures, and allowed to quantify the individual *cis* and *trans* isomers of 16:1, 18:1 and 18:2 excluding CLA. The isomeric data were calibrated using the certified contents of main fatty acids and fitted into the total fatty acid composition of CRM 164. As a result, there now exists a commercial reference material with known isomeric distribution and total content of TFA (5.74%), which can be used for calibration of TFA analysis. Moreover, these data are of importance with regard to the estimation of overlaps on shorter GC columns and, thus, for calibration of the total fatty acid composition as well.

Zusammenfassung

Molkentin, J., Precht, D.: **Gehalt einzelner *cis/trans*-Isomere von 16:1-, 18:1- und 18:2-Fettsäuren in dem Referenz-Milchfett CRM164.** Kieler Milchwirtschaftliche Forschungsberichte **56** (1) 53-63 (2004)

24* Trans*-Fettsäuren (Milchfett, Kalibrierung, Gaschromatographie, Referenzmaterial)

Allgemein werden *trans*-Fettsäuren (TFA) im Zusammenhang mit zahlreichen ungünstigen gesundheitlichen Aspekten diskutiert, wobei möglicherweise einzelnen Isomeren unterschiedliche Wirkungen zukommen. Positive Wirkungen werden z. B. dem in Milchfett vorherrschenden TFA-Isomer t11-18:1 zugeschrieben. Dieses Isomer kann als metabolische Vorstufe des bioaktiven Isomers c9t11-18:2 der konjugierten Linolsäure (CLA) fungieren. Eine Bestimmung von TFA-Gehalten und insbesondere ihrer Isomerenverteilung in Speisefetten ist daher von Bedeutung. Aufgrund überlappender Peaks in der Gaschromatographie (GC) von Fettsäure-Methylestern können TFA nur mit der Ag-TLC/GC-Methode korrekt quantifiziert werden. Deshalb ist ein Referenzmaterial mit bekannter Zusammensetzung wie CRM 164 (wasserfreies Milchfett, IRMM, Geel/Belgien) für die Kalibrierung und Qualitätssicherung der Analyse von TFA in Milchfett von Nutzen. Da weder zertifizierte noch hinweisende Werte für die TFA-Gehalte dieses Referenzmaterials

existieren, wurde die Isomeren-Zusammensetzung in diesen Untersuchungen bestimmt. Dazu wurden CRM 164 und – zur Vermeidung von Überlappungen – dessen mit Hilfe von Silberionen-Dünnschichtchromatographie (Ag-TLC) gewonnene Fraktionen, die jeweils gesättigte, *cis*-Monoen-, *trans*-Monoen- und Dien-Fettsäuren enthielten, mittels GC analysiert. Die GC-Analysen wurden auf einer 100 m-Säule (CP-Sil88) bei verschiedenen isothermen Temperaturen durchgeführt und ermöglichten die Quantifizierung einzelner *cis*- und *trans*-Isomere von 16:1, 18:1 und 18:2 mit Ausnahme der CLA. Die Isomerendaten wurden mit Hilfe der zertifizierten Gehalte von Hauptfettsäuren kalibriert und in die Gesamt-Fettsäurenzusammensetzung von CRM 164 eingepasst. Somit liegt nun ein kommerzielles Referenzmaterial mit bekannter Isomerenverteilung und bekanntem Gesamtgehalt an TFA (5,74%) vor, das zur Kalibrierung von TFA-Analysen verwendet werden kann. Darüber hinaus sind diese Daten hinsichtlich der Abschätzung von Überlappungen auf kürzeren GC-Säulen und somit auch der Kalibrierung der Gesamt-Fettsäurenzusammensetzung von Nutzen.

Résumé

Molkentin, J., Precht, D.: **Teneurs d'isomères *cis/trans* individuels en acides gras 16:1-, 18:1- et 18:2 dans la matière grasse de référence, notamment celle du lait CRM164.** Kieler Milchwirtschaftliche Forschungsberichte **56** (1) 53-63 (2004)

***24 Acides gras* trans** (matière grasse de lait, calibrage, chromatographie gazeuse (GC), substance de référence)

Généralement les acides gras *trans* (AGT) sont discutés sous leur aspect potentiellement nocif à la santé. Ceci n'empêche que des effets variés sont attribués aux isomères individuels. Par exemple, des effets positifs à l'isomère AGT t11-18:1 prévalant dans la matière grasse de lait. Cet isomère peut fonctionner comme stade préliminaire métabolique de l'isomère bioactif c9t11-18:2 de l'acide linoléique conjugué (conjugated linoleic acids: CLA). C'est la raison pour laquelle une détermination des teneurs des AGT et avant tout leur répartition isomérique dans des graisses alimentaires sont importantes. A cause des pics dans la chromatographie gazeuse (GC) de méthyle esters d'acides gras, les AGT ne peuvent être quantifiés correctement qu'avec la méthode Ag-TLC/GC. C'est pourquoi une substance de référence à composition connue comme CRM 164 (graisse de lait anhydre, IRMM, Geel/Belgique) est utile pour le calibrage et l'assurance de la qualité de l'analyse des AGT dans la graisse de lait. Comme il n'existe ni de valeurs certifiées ni indicatrices pour les teneurs des AGT pour cette substance de référence la composition isomérique a été déterminée dans le cadre de ces examens. Pour ce faire des CRM 164 et – pour éviter des recoupements – dont les fractions obtenues par chromatographie sur couche mince à ions argentés (Ag-TLC) contenaient les acides gras saturés *cis*-monoen-, *trans*-monoen- et dien, ont été analysés par chromatographie gazeuse (GC). Les analyses par GC ont été réalisées sur une colonne 100 m (CP-Sil88) sous différentes températures isothermiques et ont permis une quantification d'isomères individuels *cis* et *trans* de 16:1, 18:1 et de 18:2 à l'exception des CLA. Les données isomériques ont été calibrées à l'aide de teneurs certifiées des acides gras principaux et adaptées à la composition globale des acides gras CRM 164. Ainsi il n'existe qu'une substance de référence commerciale, à répartition isomérique connue et avec une teneur connue en ACF (5,74%) pouvant être utilisée pour calibrer des analyses de AGT. En plus, ces données sont utiles pour estimer des recoupements sur des colonnes GC plus courtes et par conséquent aussi pour calibrer la composition globale en acides gras.