

- (6) FEIER, U., GOETSCH, P.-H.: *Milchwissenschaft* **45** 440–441 (1990)
- (7) FINK, R., KESSLER, H.G.: *Milchwissenschaft* **43** 275–280 (1988)
- (8) FEIER, H., KLOSTERMEYER, H.: *Milchwissenschaft* **38** 475–477 (1983)
- (9) JIMENEZ-PEREZ, S., CORZO, N., MORALES, F.J., DELGADO, T., OLANO, A.: *J. Food Prot.* **55** 304–306 (1992)
- (10) KEENEY, M., BASSETTE, R.: *J. Dairy Sci.* **42** 945–960 (1959)
- (11) KIESNER, V.C., JIMENEZ, S., KLOBES, H., MOLTZEN, B., EINHOF, K.: *Kieler Milchw. Forschungsber.* **46** 15–31 (1994)
- (12) MARTINEZ-CASTRO, I., OLANO, A.: *Rev. Esp. Lech.* **110** 213–217 (1978)
- (13) MARTINEZ-CASTRO, I., OLANO, A.: *Milchwissenschaft* **35** 5–8 (1980)
- (14) MOTTAR, J., NAUDTS, M.: *Lait* **588** 476–488 (1979)
- (15) MOTTAR, J., WAES, G., MOERMANS, R., NAUDTS, M.: *Milchwissenschaft* **34** 257–262 (1979)
- (16) NANGPAL, A.: Diss., Univ. Kiel (1988)
- (17) OLANO, A., CALVO, M.M., REGLERO, G.: *Chromatographia* **21** 538–540 (1986)
- (18) OLANO, A., CALVO, M.M., RAMOS, M.: *Milchwissenschaft* **44** 80–82 (1989)
- (19) PARK, Y.H., HONG, Y.H.: *J. Korean Soc. Food Nutr.* **19** 330–334 (1990)
- (20) PEREZ, S.J., CORZO, N., MORALES, F.J., DELGADO, T., OLANO, A.: *J. Food Prot.* **55** 304–305 (1992)
- (21) RADECKER, I.C., KUHLMANN, B., WEISS, G., KLOSTERMEYER, H., SCHLIMME, E.: *Kieler Milchw. Forschungsber.* **44** 129–141 (1992)
- (22) RENNER, E., DORGUTH, H.: *Dtsch. Milchwirtsch.* **14** 505–508 (1980)

5. Summary

AKALIN, A.S., GÖNÇ, S.: **Lactulose and 5-HMF contents in market milks.** *Milchwissenschaft* **52** (7) 377–380 (1997).

34 Market milk (lactulose, HMF)

The lactulose and HMF contents in market milks were analyzed by gas chromatographic and colorimetric methods, respectively. UHT milk samples, directly or indirectly heat-treated, were also examined for lactulose and HMF during a period of 120 d storage in 4-week intervals at room temperature or 30°C. The gas chromatographic method was evaluated for precision and accuracy using phenyl-β-glucoside as internal standard. The mean recovery was found to be 99.1%. The mean lactulose content of the market milks determined was 1.2–1.9 mg/100 ml in pasteurized milks and 7.6–45.4 mg/100 ml in UHT milks and were found to be within the limits considered acceptable. The HMF contents of UHT milks varied between 5.2 and 15.1 μmol/l. The lactulose content has been shown to be useful in distinguishing pasteurized, directly heated UHT and indirectly heated UHT milks. The production dates of UHT milks were found to be important in definition of the lactulose level because of the increase of lactulose during the storage at 30°C.

AKALIN, A.S., GÖNÇ, S.: **Laktulose und 5-HMF-Gehalt in Konsummilch.** *Milchwissenschaft* **52** (7) 377–380 (1997).

34 Konsummilch (Laktulose, HMF)

Die Laktulose- und HMF-Gehalte von Konsummilch wurden gaschromatographisch und kolorimetrisch untersucht. Direkt oder indirekt erhitzte UHT-Milchproben wurden ebenfalls während einer 120tägigen Lagerung bei Zimmertemperatur oder 30°C in 4wöchigem Abstand auf Laktulose und HMF-Gehalt analysiert. Als interner Standard wurde das gaschromatographische Verfahren auf Präzision und Richtigkeit der Werte mit Phenyl-β-Glukosid geprüft. Der durchschnittliche Rückgewinnungswert lag bei 99.1%. Die durchschnittlichen Laktulosewerte von pasteurisierter Milch lagen bei 1,2–1,9 mg/100 ml, von UHT-Milch bei 7,6–45,4 mg/100 ml und erreichten damit akzeptable Werte. Der HMF-Gehalt in UHT-Milch schwankte zwischen 5.2 und 15.1 μmol/l. Es zeigte sich, daß der Laktulosegehalt zur Differenzierung von pasteurisierter sowie direkt und indirekt erhitzter UHT-Milch geeignet ist. Während der Lagerung bei 30°C erhöht sich der Laktulosegehalt; daher ist bei der Definition des Laktulosegehaltes in UHT-Milch das Herstellungsdatum wichtig.

Occurrence of *trans*-C16:1 acids in bovine milkfats and partially hydrogenated edible fats

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1. Introduction

In connection with the consumption of *trans* fatty acids, in particular *trans*-octadecenoic acids (*trans*-C18:1), a large number of risks to health is discussed (1, 2). In recent years, the risk of CHD (coronary heart disease) was partly also found to be correlated with the content of *trans*-hexadecenoic acids (*trans*-C16:1) in plasma (3) or adipose tissue (4) of human subjects, whereas another study did not find a correlation between *trans*-C16:1 in adipose tissue and CHD risk factors (5). The re-

port of the "Expert Panel on *Trans* Fatty Acids and Coronary Heart Disease" (6) even concludes that the association between total intake of *trans* fatty acids and CHD is more likely attributable to *trans*-C16:1 than to *trans*-C18:1. An association with *trans*-C16:1 would mean that animal fats and not partially hydrogenated vegetable fats cause the negative physiological effects, as according to previous studies only animal fats are supposed to contain substantial amounts of *trans*-C16:1 (6).

Concerning gas chromatography of FAME on packed columns, already THOMAS and WINTER (4) as well as AITCHISON *et al.* (7) described overlaps of *trans*-C16:1 with saturated C17 acids. These overlaps were detected by comparison with hydrogenated fat samples and argentation thin-layer chromatography (Ag-TLC), respectively. AITCHISON *et al.* found that the real contents of *trans*-C16:1 in different fats could be 2.9 to 7.6 times lower compared with data from direct GC. So, possibly too high *trans*-C16:1 contents were measured in probably most studies based on direct GC. According to recent examinations such overlaps even occur with capillary columns usually applied at present. Thus, the present study was performed to determine reliable data on *trans*-C16:1 contents in bovine milkfats and partially hydrogenated edible fats.

2. Materials and methods

2.1 Samples

Milkfats were obtained from butter by melting and filtering the fat layer through a folded filter MN 615ff ¼ (Macherey-Nagel, Düren, Germany) at 50 °C in an oven. The study comprised 27 butter samples originating from various areas throughout Germany covering most different feeding and lactation conditions which are characterized by the content of C54 triglycerides (all triglycerides with a total acyl carbon number of 54) (8). Thus, samples were selected to regularly cover the whole variation range of C54 contents. Moreover, 62 brands of partially hydrogenated edible fats were purchased in August 1994 in the northern part of Germany. Commercial products were melted and, after separation of the water, passed through a filter at 70 °C in an oven. The isolated fats were dissolved in *n*-heptane and then dried over Na₂SO₄. Spot checks for free fatty acids exhibited no increased hydrolysis of triglycerides by the temperature applied.

2.2 Preparation of fatty acid methyl esters

Triglycerides were *trans*-esterified to fatty acid methyl esters (FAME) by a modified method (9) similar to that of SCHULTE and WEBER (10) using sodium methylate.

2.3 Argentation thin-layer chromatography (Ag-TLC) of fatty acid methyl esters

The separation and isolation of *trans* monoenoic fatty acids by argentation thin-layer chromatography has already been described elsewhere (11).

2.4 Gas chromatography of the total fatty acid composition

The total fatty acid profile was recorded by analysis of the methyl esters (FAME) on a CP 9001 gas chromatograph (Chrompack, Middelburg, Netherlands) equipped with split/splitless injection port, flame ionization detector and a 25-m fused silica capillary column (ID = 0.25 mm) coated with 0.20 µm of CP-Wax 58CB (Chrompack). Hydrogen carrier gas flow was 0.9 ml/min (38 kPa) at a split ratio of 1:50. Injector and detector temperatures were both 265 °C. The oven temperature was 45°C for 1 min initially, then programmed at 5°C/min to

225 °C, held at this temperature for 15 min and finally raised by 5°C/min to 260 °C to completely elute even long-chain fatty acids. Samples were injected manually (1 µl) using the 'hot-injection-technique'.

2.5 Gas chromatography of *trans* fatty acids

Trans monoenoic fatty acids were analyzed as FAME on a CP 9001 gas chromatograph (Chrompack, Middelburg, Netherlands) equipped with split/splitless injection port and flame ionization detector using a 100-m fused-silica capillary column (ID = 0.25 mm) coated with 0.2 µm of CP-Sil 88 (Chrompack). The carrier gas flow was 0.6 ml H₂/min (160 kPa) at a split ratio of 1:100. The oven was operated isothermally at 175 °C. In slight variation the *isomeric distributions* of *trans*-hexadecenoic acids were determined at 125 °C oven temperature and 220 kPa H₂. Injector and detector temperatures were both 255 °C. Samples (FAME in *n*-heptane) were injected manually using the 'hot-injection-technique'.

2.6 Identification of *trans*-hexadecenoic acids and other *trans* isomers

Besides by Ag-TLC/GC the differentiation of *trans*-hexadecenoic acids from other fatty acids was done by GC/MS of the *trans* fraction as well using a 100-m column as described above. For GC/MS analysis a Carlo Erba HRGC 5300 gas chromatograph linked to a Carlo Erba QMD 1000 quadrupole mass analyser was used. The mass spectrometer was operated under electron impact ionization (EI⁺, 70 eV) and individual peaks in the chromatogram were identified using the single ion mode. In the same way isomeric peaks of *trans*-C17:1 and *trans*-C20:1 were identified.

After identification of *trans* Δ9-C16:1 by GC using a FAME standard obtained from Sigma (St. Louis, MO 63178 USA), the remaining isomers were identified tentatively in analogy to the order of *trans*-octadecenoic acids (11).

2.7 Calibration and quantitation of fatty acids

Quantitation of the sum of *trans*-hexadecenoic acid isomers was performed in the gas chromatogram of the Ag-TLC *trans* fraction by relating it to the content of the *trans*-C18:1 group Δ6-Δ11. Before, the content of this group has been determined and calibrated in each sample by GC analysis of the total fatty acid profile using an oven temperature program as described above. For calibrating the individual fatty acids of this total FA chromatogram, a standard consisting of the methyl esters of the main fatty acids was applied. The integration of chromatograms was performed using a Hewlett Packard 3365 II Chemstation. Results are given in g per 100 g total fatty acids.

3. Results

For an exact gas chromatographic determination of *trans*-C16:1 fatty acids in different fats the isolation of the *trans*-monoene fraction by Ag-TLC prior to gas chromatography is indispensable. In Fig. 1 the result of fractionating FAME derived from a bovine milkfat into saturates and *trans*-monoenes is illustrated. It is obvious that *trans*-C16:1 does not only comprise one single peak. Moreover, even on the 100-m capillary column

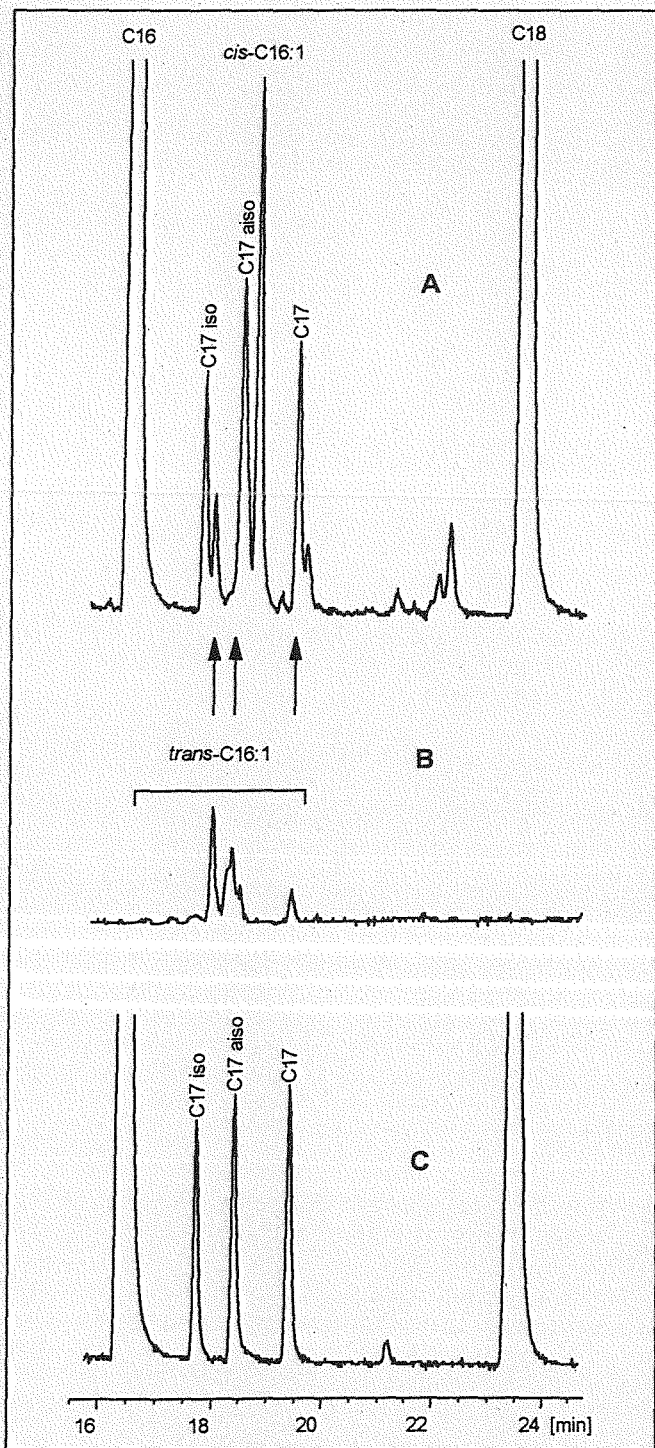


Fig. 1: Partial gas chromatograms of total FAME (A) as well *trans* (B) and saturated (C) FAME isolated by Ag-TLC showing the overlaps of peaks on a 100 m column for bovine milkfat.

applied in this study (100 % cyanopropyl polysiloxane) *trans*-C16:1 isomers coelute with saturated C17:0 acids to a great extent. Additionally, on 50-m columns the left *trans*-C16:1 isomeric peak (Fig. 1) overlaps with the peak of C17iso. According to published chromatograms, previous studies using 50-m columns (100 % cyanopropyl polysiloxane) without Ag-TLC quantified these two last-mentioned overlapped peaks on the assumption that the result represents the total *trans*-C16:1 content (e.g. 12, 13). As the proportion of this single

peak of positional *trans*-C16:1 isomers towards the combined peak as well as to the total *trans*-C16:1 content varies in wide ranges, the extent of the error varies as well considering different fats.

In the present study 27 German bovine milkfats covering most different feeding and lactation conditions as well as 62 brands of German margarines, cooking fats and shortenings were analyzed by the combination of Ag-TLC and GC to obtain exact data on the content of *trans*-hexadecenoic acids in edible fats. The results are given in Table 1 and exhibit that both bovine milkfat as well as partially hydrogenated margarines and cooking fats/shortenings made from vegetable oils contain relatively low amounts of *trans*-C16:1 of 0.13 %, 0.04 % and 0.01 %, on average. Especially in milkfat the contents are distinctly lower compared to previous findings of e.g. 0.60 % (14) obtained without Ag-TLC. Thus, there are no significant differences between milkfat and partially hydrogenated vegetable fats concerning *trans*-C16:1 total contents. In dietetic fats (n = 10) analyzed for reasons of comparison *trans*-C16:1 contents of 0.01%, on average, were found. The mean *trans*-C16:1 content in German milkfats given in Table 1 is in good agreement with recent data on French (15) milkfats (0.11%) as well as with older values on British (16) milkfats of 0.17% and on Danish (17) milkfats of 0.18% all obtained by Ag-TLC/GC.

In contrast, *trans*-C16:1 contents in margarines, cooking fats and shortenings containing partially hydrogenated fish oil are higher by far. The mean content in 6 samples was 1.89% with a maximum of 3.03% (Table 1). Fig. 2 shows a typical chromatogram of the different positional isomers of *trans*-C16:1 from such a fat. In Table 2 the mean isomeric distribution from these 6 samples is given with $\Delta 9$ being the main isomer. So, these results exhibit that the main source of *trans*-C16:1 fatty acids is partially hydrogenated fish oil contained in some margarines, cooking fats and shortenings.

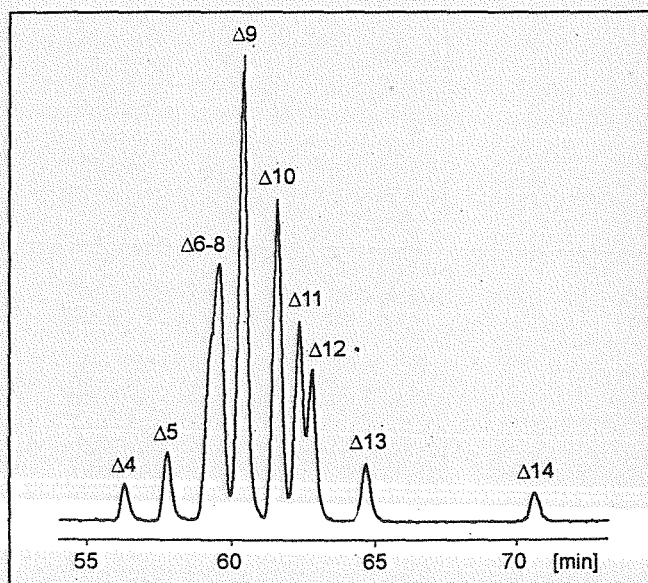


Fig. 2: Isomeric distribution of *trans*-hexadecenoic FAME (tentative identification) in a cooking fat containing partially hydrogenated fish oil obtained by gas chromatography at reduced temperature (125 °C).

Table 1: Contents of *trans*-hexadecenoic (t C16:1) and *trans*-eicosaenoic (t C20:1) acids in edible fats from different origin (g/100 g total fatty acids)

Fat Origin	Milkfat Cow	Margarine Vegetable oil	Cook ¹ Vegetable oil	Margarine/Cook Fish oil	Margarine/Cook Vegetable oil/fish oil	Dietetic fat Vegetable oil	
No. of samples	27	44	12	6	62	10	
t C16:1	Mean value	0.13	0.04	0.01	1.89	0.21	0.01
	Minimal value	0.05	0.00	0.00	1.33	0.00	0.00
	Maximal value	0.25	0.24	0.08	3.03	3.03	0.02
	SD ²	0.05	0.06	0.03	0.63	0.58	0.01
t C20:1	Mean	0.00	0.16	0.07	4.49	0.56	0.13

¹Cook: cooking fat/shortening; ²SD: standard deviation.

Table 2: Isomeric distribution of *trans*-hexadecenoic acids (tentative identification) in fats containing partially hydrogenated fish oil (g/100 g total fatty acids)

	$\Delta 4$	$\Delta 5$	$\Delta 6-8$	$\Delta 9$	$\Delta 10$	$\Delta 11$	$\Delta 12$	$\Delta 13$	$\Delta 14$	Total
Mean value	0.06	0.07	0.28	0.46	0.37	0.26	0.23	0.09	0.07	1.89
Minimum	0.03	0.04	0.02	0.10	0.23	0.21	0.19	0.04	0.05	1.33
Maximum	0.11	0.10	0.59	0.96	0.62	0.32	0.31	0.14	0.12	3.03
SD	0.02	0.02	0.23	0.29	0.13	0.04	0.04	0.03	0.02	0.63

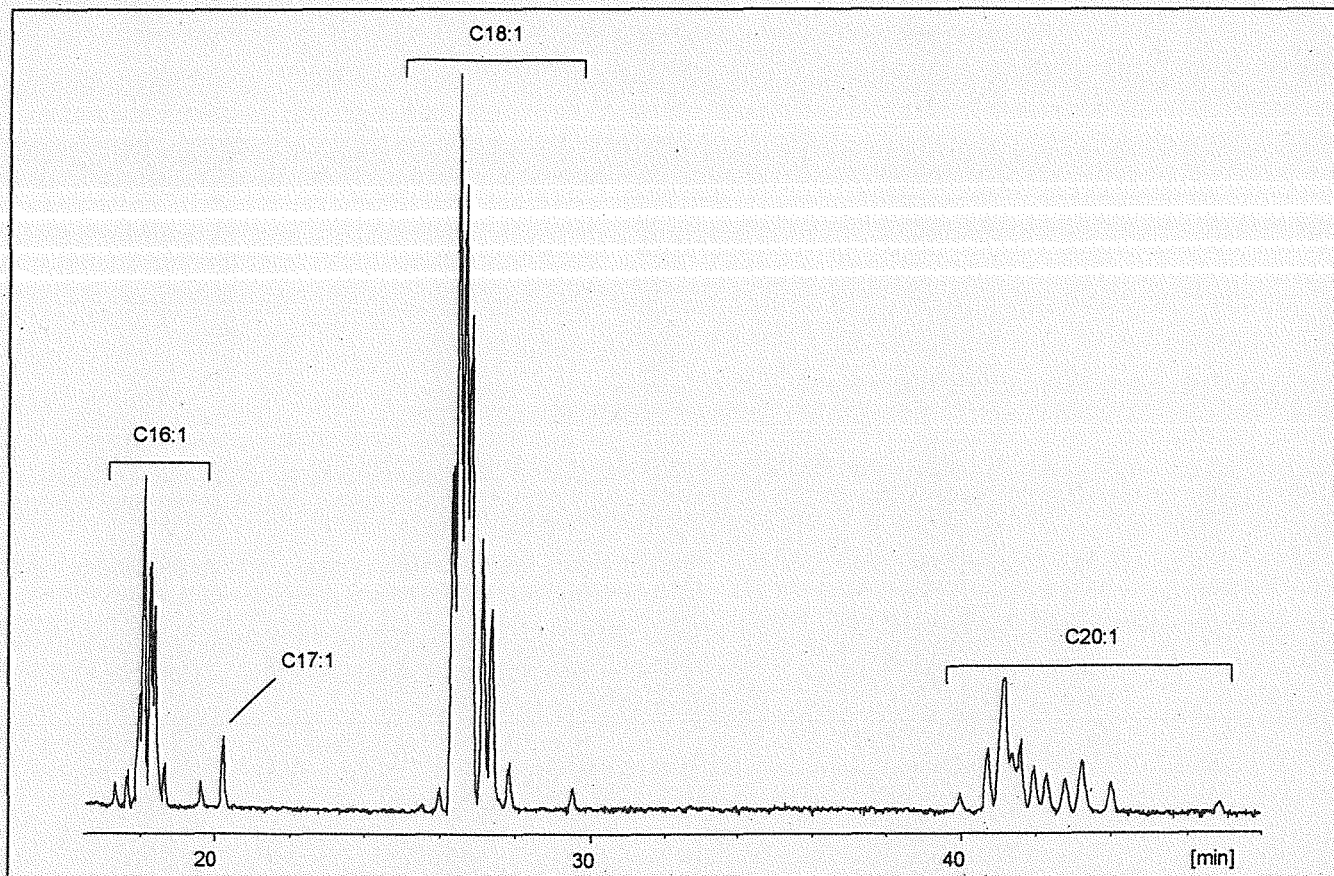


Fig. 3: Gas chromatogram of *trans* FAME (Ag-TLC fraction, identification by GC/MS) derived from a cooking fat containing partially hydrogenated fish oil.

Already THOMAS and WINTER (4) therefore attributed high *trans*-C16:1 levels in human adipose tissue to the consumption of higher proportions of hydrogenated marine oil products. However, it was proposed that despite the association of high *trans*-C16:1 levels in adipose tissue with the incidence of CHD, *trans*-C16:1 might ac-

tually not be the cause for CHD. Instead of *trans*-C16:1 THOMAS and WINTER suspected C20 and C22 di- and trienoic acid *cis* and *trans* isomers which are present in hydrogenated marine oils in high amounts as well.

It can be seen from Table 1 that in the present study products with hydrogenated fish oil (n = 6) also con-

tained high amounts of *trans*-eicosanoic acids (*trans*-C20:1) of 4.49 %, on average. These *trans*-C20:1 acids are found at most in traces of 0.01% in milkfats (18) and only in low amounts of 0.00 - 0.24% in hydrogenated vegetable fats. However, the physiological effects of *trans*-C20:1 have not been investigated so far. Fig. 3 shows a gas chromatogram of *trans* acids from a cooking fat containing partially hydrogenated fish oil. Besides *trans* isomers of C16:1, C18:1 and C20:1 also a small amount of *trans*-C17:1 can be seen.

Regarding the risk to health possibly related to *trans*-C16:1, hydrogenated vegetable fats as well as bovine milkfats can be considered of minor importance. A comparison of *trans*-C18:1 contents in these two types of fat has already been published elsewhere (19). However, a threat of products containing hydrogenated fish oil attributable to the high contents of *trans*-C16:1 and *trans*-C20:1, in addition to an average *trans*-C18:1 content of 6.61 % analyzed in these samples, can not be excluded. Therefore, the consumption of such products should be avoided. This can easily be followed as their market share is still decreasing. Further, one of the few products still available in 1997 exhibited a decreased *trans*-C16:1 content of 1.27 % instead of 3.03 % and a *trans*-C18:1 content of 4.68% instead of 6.54% in comparison to 1994. However, the content of *trans*-C20:1 has increased from 3.01% to 3.48% within the same time.

4. Conclusions

Concerning the mean content of *trans*-C16:1, there is no significant difference between bovine milkfats and partially hydrogenated vegetable fats both exhibiting rather low *trans*-C16:1 contents. So, not animal fats in general, but hydrogenated fish oils contain high amounts of *trans*-C16:1 and additionally *trans*-C20:1 acids as well as possibly other long-chain *trans* acids as well. Therefore, theories concerning the association of *trans*-C16:1 contents in adipose tissues or plasma and incidence of CHD in humans have to be thought over as regards the contribution of different edible fats. Further, uncertainties may arise if data on human lipids were obtained without Ag-TLC.

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5. References

- (1) ZOCK, P.L.: Dietary fatty acids and risk factors for coronary heart disease. Thesis, Wageningen (1995)
- (2) PRECHT, D., MÖLKENTIN, J.: *Nahrung - Food* **39** 343-374 (1995)
- (3) SIGUEL, E.N., LERMAN, R. H.: *Am. J. Cardiol.* **71** 916-920 (1993)
- (4) THOMAS, L.H., WINTER, J.H.: *Hum. Nutr. Food Sci. Nutr.* **41F** 153-165 (1987)
- (5) HUDGINS, L.C., HIRSCH, J., EMKEN, E.A.: *Am. J. Clin. Nutr.* **53** 474-482 (1991)
- (6) Report of the Expert Panel on *Trans* Fatty Acids and Coronary Heart Disease. (Ed. P.M. Kris-Etherton), *Am. J. Clin. Nutr.* **62** 655S-707S (1995)
- (7) AITCHISON, J.M., DUNKLEY, W.L., CANOLTY, N.L., SMITH, L.M.: *Am. J. Clin. Nutr.* **30** 2006-2015 (1977)

- (8) PRECHT, D., MÖLKENTIN, J.: *Intern. Dairy J.* **6** 791-809 (1996)
- (9) PRECHT, D.: *Fat Sci. Technol.* **92** 153-161 (1990)
- (10) SCHULTE, E., WEBER, K.: *Fat Sci. Technol.* **91** 181-183 (1989)
- (11) MÖLKENTIN, J., PRECHT, D.: *Nahrung - Food* **40** 297-304 (1996)
- (12) KOLETZKO, B.: *Nahrung* **35** 229-283 (1991)
- (13) BEATRIZ, M.B.P.P., FERREIRA, M.A.: *J. High Resol. Chromatogr.* **19** 180-182 (1996)
- (14) PFALZGRAF, A., TIMM, M., STEINHART, H.: *Z. Ernährungswiss.* **33** 24-43 (1993)
- (15) WOLFF, R.L.: *J. Am. Oil Chem. Soc.* **71** 277-283 (1994)
- (16) HAY, J.D., MORRISON, W.R.: *Biochim. Biophys. Acta* **202** 237-243 (1970)
- (17) LUND, P., JENSEN, F.: *Milchwissenschaft* **38** 193-196 (1983)
- (18) STROCCHI, A., HOLMAN, R.T.: *Riv. Ital. Sostanze Grasse* **48** 617-622 (1971)
- (19) PRECHT, D., MÖLKENTIN, J.: *Kieler Milchwirtsch. Forsch.ber.* **49** 17-34 (1997)

6. Summary

MÖLKENTIN, J., PRECHT, D.: **Occurrence of *trans*-C16:1 acids in bovine milkfats and partially hydrogenated edible fats.** *Milchwissenschaft* **52** (7) 380-385 (1997).

44 Fats (*trans*-fatty acids)

Applying a combination of argentation thin-layer chromatography (Ag-TLC) and gas chromatography, contents of *trans*-hexadecenoic acids (*trans*-C16:1) have been determined in 27 German bovine milkfats and 62 German partially hydrogenated edible fats. It could be demonstrated that due to an overlap with C17 fatty acids frequently too high *trans*-C16:1 contents have been measured particularly for milkfats if no pre-separation by Ag-TLC was applied. According to the present studies, milkfats contain 0.13 % and partially hydrogenated margarines and cooking fats/shortenings of vegetable origin 0.04 % resp. 0.01 % *trans*-C16:1, on average. In contrast, products containing partially hydrogenated fish oil ($n = 6$) exhibited relatively high *trans*-C16:1 contents of 1.89 %, on average. Moreover, besides the widespread *trans*-octadecenoic acids (*trans*-C18:1) these products additionally contain high amounts of *trans*-eicosanoic acids (*trans*-C20:1) of 4.49 %, on average. The presented results could be of use for future considerations concerning theories of an association of *trans*-C16:1 contents in human lipids with the incidence of coronary heart diseases.

MÖLKENTIN, J., PRECHT, D.: **Vorkommen von *trans*-C16:1 Säuren in bovinen Milchfetten und partiell hydrierten Speisefetten.** *Milchwissenschaft* **52** (7) 380-385 (1997).

44 Fette (*trans*-Fettsäuren)

Mit Hilfe der Kombination von Silberionen-Dünnschichtchromatographie (Ag-TLC) und Gaschromatographie wurden in 27 deutschen bovinen Milchfetten und 62 deutschen teilhydrierten Speisefetten die Gehalte an *trans*-Hexadecensäuren (*trans*-C16:1) bestimmt. Es konnte gezeigt werden, daß ohne Ag-TLC insbesondere für Milchfett infolge einer Überlappung mit C17-Fettsäuren häufig zu hohe *trans*-C16:1-Gehalte gemessen wurden. Nach den vorliegenden Studien kommen in Milchfetten durchschnittlich 0,13%

und in teilhydrierten Margarinen und Brat-/Backfetten auf pflanzlicher Basis 0,04 % bzw. 0,01 % *trans*-C16:1 vor. Dagegen wiesen Produkte, die teilhydriertes Fischöl enthielten ($n = 6$), relativ hohe *trans*-C16:1-Gehalte von durchschnittlich 1,89 % auf. Des Weiteren enthielten diese Produkte neben den in vielen Fetten vorkommenden *trans*-

Octadecensäuren (*trans*-C18:1) zusätzlich hohe Mengen an *trans*-Eicosaensäuren (*trans*-C20:1) von durchschnittlich 4,49%. Die vorgestellten Ergebnisse könnten für zukünftige Überlegungen zu Theorien über eine Assoziation von *trans*-C16:1-Gehalten in menschlichen Lipiden mit dem Auftreten koronarer Herzerkrankungen von Nutzen sein.

The compositional, textural and maturation characteristics of reduced-fat Cheddar made from milk containing added Dairy-Lo™

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1. Introduction

The association between the type and level of dietary fat and the risk of arteriosclerosis and related health problems, has led to a greater consumer awareness of dietary fat *per se* (1–3) and a dramatic increase in the supply of, and demand for, low fat foods, including cheese (4). Compared to other low fat foods the consumption of low/reduced fat cheese as a percentage of overall cheese production/consumption is still low (e.g. 8% in the UK) but is growing at a faster rate (though from a smaller base volume) than the mainstream full fat cheese market (5). The low consumption has been attributed to poor consumer perceptions of the products, based on taste and texture (5–9). Textural defects include increased firmness, rubberiness elasticity, hardness, dryness and graininess. The negative flavour attributes associated with reduced-fat Cheddar include low intensities of typical Cheddar cheese aroma and flavour (10–12) and flavour defects such as bitterness, astringency, meaty-brothiness and unclear flavours (13–15).

Attempts to address these problems have focused on improvement via modification of manufacturing process, addition of hydrocolloids and/or starters and/or starter culture adjuncts (11–14, 16–21). Mimetics have found most application in natural cheeses, and of these the microparticulated milk protein-based products Simplese® 100 (18, 19, 22) and Dairy-Lo™ have been mostly investigated (20).

LUCEY and GORRY (19) reported that reduced-fat Cheddar made from milk containing 2% Simplese® 100 had higher moisture levels, lower hardness and yield stresses than controls, made using the same make-procedure, at 4 months. The addition of Simplese® 100 resulted in slightly lower levels of water soluble- and 5% phosphotungstic acid-soluble-N (as % of total N) at 4 months. KUCOKONER and HAQUE (20) reported the addition of Dairy-Lo™ resulted in an increase in the moisture content and a decrease in hardness of low-fat Cheddar cheese.

This communication reports on the use of Dairy-Lo™ in reduced-fat Cheddar cheese.

2. Materials and methods

2.1 Addition of micro-particulated whey protein (Dairy-Lo™) to the cheese milk

A commercial whey protein-based fat substitute, Dairy-Lo™, provided by Pfizer Food Science (Nutrition

Supplies, Innishannon, Co. Cork, Ireland), was specified with the following composition: dry matter, 96%; protein, 35%; fat, 5%; lactose, 52.5%. Dairy-Lo™ was added to a final level of 1% (w/w) to the cheese milk. The Dairy-Lo™ was added to a portion (25% total weight) of the cheesemilk and thoroughly mixed using a Silver-son Mixer (Model EX, Silver-son Machines Ltd., Water-side, Chesham, UK); the Dairy-Lo™-fortified milk was then heated to 77°C and cooled to 31°C. Following pasteurisation, under normal conditions (72°C x 15 s) and cooling, the other portion of cheesemilk (75% total weight) was blended with the Dairy-Lo™-fortified fraction to give reduced-fat milk containing 1% (w/w) Dairy-Lo™.

2.2 Rennet coagulation properties

Milk samples were withdrawn from the cheese vat prior to starter culture addition and tested within 1 h. The rennet coagulation properties were recorded continuously as a function of time from rennet addition in a controlled-strain rheometer (Bohlin VOR, Bohlin Reologi, Lund, Sweden) using low amplitude oscillation, as described by GUINEE *et al.* (23). Immediately prior to testing, the pH was adjusted to 6.55 using 1 N HCl at 31°C and tempered at 31°C for 15 min. Double strength fermentation-produced chymosin (Chy-max®, Pfizer Dairy Products Division, Pfizer Inc., Milwaukee, WI, USA), diluted 1:100 was added at a rate of 7.0 ml per l. The renneted sample was placed in the rheometer cell, where the temperature was maintained at 31°C; liquid paraffin was layered onto the surface of the milk to prevent evaporation during coagulation and measurements were taken over 3600 s.

2.3 Manufacture of reduced-fat Cheddar cheese

Pasteurized, reduced-fat milks (454 kg) were cooled to 31°C and inoculated with equal quantities of defined strain starter cultures *Lactococcus lactis* subsp. *cremoris* strains 303 and 227 (Hansens, Little Island, Cork) at a rate of 14 g/kg. The strains were grown separately in reconstituted skim milk powder, 10% (w/w). After a 30 min ripening period at 31°C, chymosin (Double Strength Chy-max - 50,000 MCU/ml; Pfizer Inc., Milwaukee, WI, USA) was added at a level of 0.07 ml/kg. The curd was cut, allowed to heal for 10 min, cooked at a rate of 0.2 K/min to 38°C, pitched at pH 6.15, cheddared, milled at