

Precision of Milk Fat Quantitation in Mixed Fats by Analysis of Butyric Acid

J. Molquentin* / D. Precht

Institut für Chemie und Physik, Bundesanstalt für Milchwissenschaft, PO Box 6069, 24121 Kiel, Germany

Key Words

Gas chromatography
Butyric acid
Milk fat content
Mixed fats

Summary

Quantitation of the milk fat content in mixed fats is of interest in food analysis. It can be achieved by calculation from the butyric acid (C4) content in the mixed fat and a mean C4 content for pure milk fats. Alternatively, instead of applying a C4 mean value, the C4 content in the actual milk fat can be used if this reference sample is available. A recently improved analytical method based on gas chromatography of butyric acid methyl ester using an internal standard has been applied to determine the milk fat content in mixtures with beef tallow, lard, soybean oil, olive oil, sunflower oil, palm kernel oil and palm oil ranging from 1 to 90 % milk fat content. Within the whole range of mixtures, mean absolute deviations between the determined and the actual milk fat content were below 0.5 % and the maximum absolute deviation was not more than 1.0 % when using the reference sample. The overall mean absolute deviation was 0.04 % for milk fat contents of 1 to 10 % and 0.28 % for milk fat contents of 10 to 90 %.

Introduction

Control of the declared composition of mixed edible fats as well as of fats from processed foods such as chocolate, ice-cream, cakes and pastries, which besides milk fat can contain other non-milk fats, requires the quantitation of milk fat. A suitable method particularly for the determination of small amounts of foreign fat in milk fat is the analysis of triglycerides in conjunction with triglyceride formulae [1], which is applied in the purity control of butter submitted for intervention in the EU [2]. Using this method to determine mixtures of

beef tallow in milk fat between 10 and 90 % resulted in average absolute deviations of ca. 2 and 3 % for two different formulae [3]. The present study was in particular aimed at the quantitation of medium and small amounts of milk fat in mixtures containing a variety of foreign fats. Such a method is of current interest for European market and customs regulations, but for this purpose a higher precision is desirable.

Since butyric acid (C4) only occurs in milk fat from cows and other mammals, but not in animal adipose or vegetable fats, gas chromatographic analysis of C4 in the mixed fat and the pure milk fat enables the content of milk fat to be calculated [4, 5]. This method is well-known and has for instance already been applied to quantitate small amounts of milk fat in cocoa butter or chocolate fat [6, 7]. If a sample of the pure milk fat is not available, a typical mean C4 content may be used instead [5]. Such a representative C4 content has recently been determined for European milk fats using the most practical unit of $\text{g}\cdot 100\text{ g}^{-1}$ fat [8]. In the present study these two variants of milk fat quantitation, (with and without availability of a reference sample), were compared with respect to the absolute deviation between actual and determined milk fat content within a wide range of proportions. To this end C4 contents were analysed, with slight modifications, by a recently optimized GC method involving analysis of fatty acid methyl esters with an internal standard [9] that enables rapid quantitation by only evaluating two fatty acids in each chromatogram.

Experimental

Fat Samples

The base milk fat (reference sample) was obtained from a butter resulting from pasture feeding (summer) by melting and passing the fat layer through a folded filter MN 615ff ¼ (Macherey-Nagel, Düren, Germany) at 50 °C in an oven. Beef tallow and lard were obtained in a similar way at 80 °C. Pure vegetable fats were purchased at retail stores.

Mixtures were prepared with 1, 2, 3, 4, 5, 8, 10, 15, 20, 30, 40, 50, 60, 70, 80 and 90 % w/w milk fat.

Chemicals

The purity of all chemicals used was of analytical grade or higher. Sodium methylate, methanol, sodium hydrogen sulphate-monohydrate and *n*-heptane were obtained from Merck (Darmstadt, Germany). The calibration substances butyric and valeric acid methyl esters, were obtained from Fluka (Deisenhofen, Germany).

Preparation of Samples for Gas Chromatography

100 ± 20 mg of fat were weighed to the nearest 0.1 mg and dissolved in 5 mL methyl valerate standard (*n*-heptane, containing 0.4 mg mL⁻¹ methyl valerate). 1 mL of this solution was mixed with 20 µL sodium methylate solution (2 M in methanol) in a sample vial, shaken vigorously for 3 min (Vortex mixer) and centrifuged for 1 min (2000 rpm). After addition of 100 mg sodium hydrogen sulphate-monohydrate the vial was recapped, mixed again for 2 min and centrifuged for 1 min. The clear supernatant was used for GC analysis.

Gas Chromatography

Analyses of fatty acid methyl esters (FAME) were performed on a CP 9002 gas chromatograph (Chrompack, Middelburg, The Netherlands) equipped with split injection port, flame ionization detector and a 25 m fused silica capillary column (I.D. 0.25 mm) coated with 0.20 µm of CP-Wax 58 CB (Chrompack). Hydrogen carrier gas flow was 0.9 mL min⁻¹ (38 kPa) at a split ratio of 1:50. Injector and detector temperatures both were 265 °C. The oven temperature initially was set at 45 °C for 1 min, then programmed at 5 °C min⁻¹ to 75 °C, then at 10 °C min⁻¹ to 225 °C, and after 7 min isothermal was finally raised by 10 °C min⁻¹ to 260 °C to completely elute any long-chain FAMEs. Samples were injected manually (1 µL) using the 'hot-injection-technique' [6].

Calibration and Quantitation

Except for some modifications, calibration and quantitation were done by a method applied in several analytical standards [10–12] using methyl valerate as an internal standard. Only the peak areas of methyl butyrate and methyl valerate were integrated with a Hewlett Packard 3365 II ChemStation. In the following, the ratio $Q = C4/C5$ was considered. Analysing 5 different calibration solutions (*n*-heptane) which contained different concentrations of methyl butyrate (0.15, 0.30, 0.60, 0.90 and 1.20 mg mL⁻¹) but always the same concentration of methyl valerate (0.4 mg mL⁻¹) allowed a calibration line (Q against $C4$ content) and an average response factor for Q to be calculated. After preparing a solution of milk fat FAME while adding methyl valerate, the evaluation of Q from the resulting analytical data and consideration of the weighed fat quantity allowed the determination of the content of butyric acid methyl ester and of butyric acid in an unknown sample (g·100 g⁻¹ fat). All calculations were done by a macro programmed

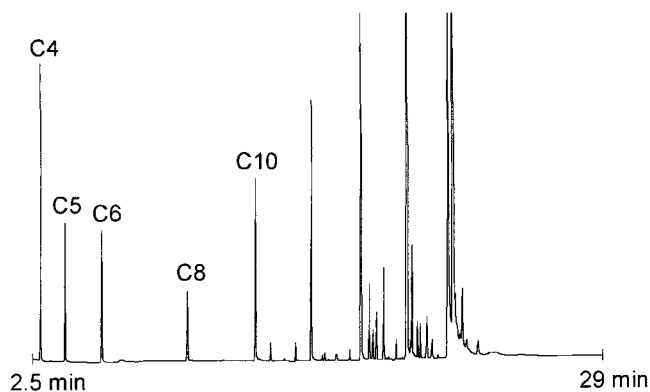


Figure 1

Gas chromatogram of fatty acid methyl esters derived from milk fat with valeric acid methyl ester as an internal standard (25 m CP-Wax 58 CB, resolution reduced in the later part).

in Excel 5.0™ that only required the input of the integrated areas and the quantity of fat weighed out. Milk fat percentages (x) were calculated from the analysed $C4$ contents in the pure milk fat (a) and in the mixed fat (b) using the equation $x = b/a \times 100$. In additional calculations (a) was replaced by an average $C4$ content previously determined for milk fats [8].

Results and Discussion

The present study was performed to determine the precision of milk fat quantitation in mixtures by analysis of butyric acid using an improved analytical method [9]. To that end a base milk fat was blended with different proportions of 7 vegetable and animal fats. Subsequently, the butyric acid ($C4$) contents were quantitated in g·100 g⁻¹ fat by GC analysis of the butyric acid methyl ester using an internal standard. Thus, quantitation using the optimized method [9] was checked over a wide range of milk fat proportions and of non-milk fats for the first time. Figure 1 shows a chromatogram of the base milk fat.

The calculation of milk fat contents in each mixture was based on its $C4$ content relative to the actual $C4$ content of the pure base milk fat. Milk fat contents were also calculated using a mean $C4$ content of 3.42 g·100 g⁻¹ fat for pure milk fats instead of analysing the reference sample. This mean value has recently been determined for European milk fats in a representative way considering most different fat compositions [8]. To minimize variations due to the analytical instrument when using the reference sample for calculation, the $C4$ content of the base milk fat was reanalysed every second day. The corresponding value was used to calculate the milk fat content in the subsequent samples.

Table I shows the results of milk fat quantitation in mixtures with beef tallow, lard, soybean oil, olive oil, sunflower oil, palm kernel oil and palm oil using the composition of the reference milk fat in the calculation. Despite some variations in the precision, no systematic dif-

Table I. Contents of milk fat in mixed fat samples calculated using a reference sample of the base milk fat [%] (MV: mean value; r: repeatability, $r = 2.83 \cdot$ standard deviation).

Actual content	Content calculated from mixture of milk fat with							MV	r
	Beef tallow	Lard	Soybean oil	Olive oil	Sunflower oil	Palm kernel oil	Palm oil		
1	0.99	1.01	0.96	0.98	1.00	1.03	0.95	0.99	0.08
2	1.98	2.02	2.11	2.02	2.03	1.98	1.96	2.01	0.14
3	2.94	3.06	2.98	2.99	3.03	3.03	2.92	2.99	0.14
4	3.96	4.01	3.99	4.06	4.08	4.09	4.01	4.03	0.14
5	4.98	4.96	4.98	5.04	5.00	5.07	4.99	5.00	0.11
8	8.06	8.02	8.10	8.06	8.05	7.99	8.02	8.04	0.10
10	10.01	10.13	9.90	10.05	9.99	10.08	9.99	10.02	0.21
15	14.99	15.12	14.93	15.09	14.96	15.12	15.12	15.05	0.24
20	20.14	20.49	20.49	20.07	20.21	20.14	20.09	20.23	0.51
30	30.19	30.87	30.35	30.26	30.23	30.48	30.14	30.36	0.71
40	40.06	40.69	40.23	39.97	40.15	40.22	40.52	40.26	0.72
50	50.01	50.89	50.43	50.19	50.22	50.11	50.68	50.36	0.91
60	60.20	60.92	60.48	60.13	60.31	60.55	59.99	60.37	0.88
70	69.67	70.04	70.31	69.85	70.90	70.21	70.49	70.21	1.16
80	79.83	80.36	80.03	80.05	80.89	80.41	79.99	80.22	1.02
90	89.27	90.41	89.48	90.00	89.57	90.32	89.07	89.73	1.47

Table II. Contents of milk fat in mixed fat samples determined assuming a mean butyric acid content for the base milk fat [%] (MV: mean value; r: repeatability, $r = 2.83 \cdot$ standard deviation).

Actual content	Content calculated from mixture of milk fat with							MV	r
	Beef tallow	Lard	Soybean oil	Olive oil	Sunflower oil	Palm kernel oil	Palm oil		
1	1.02	1.02	1.02	0.99	1.05	1.08	0.99	1.02	0.09
2	2.05	2.05	2.25	2.05	2.13	2.08	2.05	2.09	0.21
3	3.04	3.10	3.19	3.04	3.19	3.19	3.04	3.11	0.21
4	4.09	4.06	4.27	4.12	4.30	4.30	4.18	4.19	0.29
5	5.15	5.03	5.32	5.12	5.26	5.32	5.20	5.20	0.31
8	8.19	8.13	8.65	8.19	8.48	8.39	8.36	8.34	0.53
10	10.18	10.26	10.58	10.20	10.53	10.58	10.41	10.39	0.50
15	15.23	15.32	15.96	15.32	15.76	15.88	15.58	15.58	0.84
20	20.47	20.44	21.93	20.38	21.49	21.35	20.70	20.97	1.75
30	30.67	30.79	32.49	30.73	32.13	32.31	31.05	31.45	2.31
40	40.70	40.58	43.07	40.58	42.69	42.63	41.75	41.71	3.11
50	50.82	50.76	54.74	50.91	53.39	54.39	52.22	52.46	4.88
60	61.17	60.76	64.74	60.99	64.12	64.18	61.81	62.54	4.90
70	70.79	69.85	75.26	70.85	75.38	74.42	72.63	72.74	6.52
80	81.11	80.15	85.67	81.20	85.99	85.23	82.43	83.11	6.95
90	90.70	90.18	95.79	91.29	97.22	95.73	91.78	93.24	8.19

ferences were found between the different kinds of fat. The mean absolute deviations between the determined and the actual milk fat contents are plotted in Figure 2. Starting from small milk fat contents the mean deviation increased at first, while above 30 % milk fat it remained nearly constant not exceeding 0.5 % (black spots). A similar plot showed the maximal absolute deviation found in these studies with values below 1 % (black triangles). The overall mean absolute deviation was 0.04 % for milk fat contents of 1 to 10 % and 0.28 % for milk fat contents of 10 to 90 %. In addition the graph shows the course of the mean relative deviation that, as expected, decreased with increasing milk fat content (white spots).

Using the mean C4 value [8] instead of the C4 content of the actual milk fat sample for calculation resulted in the data in Table II. The deviations from the actual milk fat content are higher than in Table I. This is partly attributable to the fact that variations due to the analytical instrument could not be reduced by reanalysis of the real milk fat sample as described above, but the greater deviations are caused by an average C4 content of the base milk fat of $3.56 \text{ g} \cdot 100 \text{ g}^{-1}$ fat rather than the assumed value of $3.42 \text{ g} \cdot 100 \text{ g}^{-1}$ fat. The maximal deviation attributable to the natural variation of C4 contents of $3.07\text{--}3.75 \text{ g} \cdot 100 \text{ g}^{-1}$ fat [8] amounts to ca. $\pm 0.34 \text{ g} \cdot 100 \text{ g}^{-1}$ fat. The theoretically resulting maximum deviation in the milk fat quantitation (99 % confidence level) is illus-

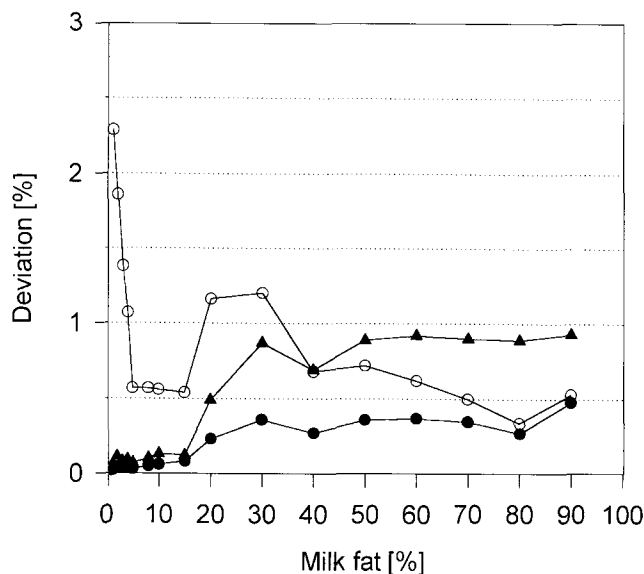


Figure 2

Deviations between actual and determined milk fat contents in mixed fats using a reference sample of the base milk fat: \blacktriangle = maximal absolute deviation; \bullet = mean absolute deviation ($n = 7$); \circ = mean relative deviation ($n = 7$).

trated in Figure 3 (black dots). This line is equivalent to a maximal relative deviation of ca. 9%. In practice there is an additional instrumental and experimental error. In the present study an almost linear increase of the mean absolute experimental deviation from 0.02% up to ca. 3% was found with increasing milk fat content (Figure 3, black triangles). Due to the variations from the analytical instrument the maximal experimental deviation rose to ca. 7% (black squares). However, the overall mean absolute deviation in the range of 1 to 10% milk fat was only 0.19% and for milk fat contents of 10 to 90% this mean deviation amounted to 1.92%.

As the precision of milk fat quantitation without a reference sample depends on the actual C4 content of the base milk fat, the precision figures presented here could be worse if a milk fat with a more extreme C4 content were used. However, without knowledge of the actual C4 content the exact range of error cannot be calculated. Only the maximal relative error of ca. 9% shown in Figure 3 can be considered which may in some cases still be exceeded due to analytical errors. A reduction of the analytical variations, which occur despite checks made weekly on the calibration curve can, however, be achieved by daily determination of a correction factor using a reference material as e.g. CRM 164 [13].

It should be emphasized that the two sources of error described above do not occur in the rather precise milk fat quantitation applying as reference a sample of the base milk fat used in the blends.

Quantitation of small contents of milk fat in cocoa butter or chocolate fat using C4 ($\text{g}\cdot 100\text{ g}^{-1}$ fatty acids) without a milk fat reference sample have been performed earlier [6, 7]. The resulting mean absolute devia-

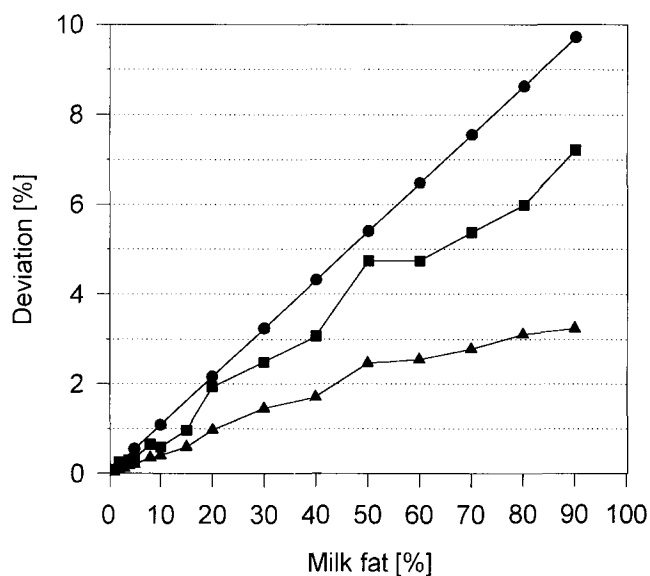


Figure 3

Deviations between actual and determined milk fat contents in mixed fats assuming a mean butyric acid (C4) content for the base milk fat: \bullet = maximal absolute theoretical deviation due to C4 variation; \blacksquare = maximal absolute experimental deviation; \blacktriangle = mean absolute experimental deviation ($n = 7$).

tions of 0.38% (4–8% milk fat) and 0.64% (4–12% milk fat), respectively, were slightly higher than those calculated from the present study.

For mixed fats with a high milk fat content, i.e. milk fats with admixtures of up to 15% foreign fat, the analysis of triglycerides in conjunction with triglyceride formulae gives quite good results considering that no reference sample was used. Admixtures of different foreign fats (3–15%) or combinations of foreign fats (4–7%) could be quantitated with mean absolute deviations of 0.7 to 0.8% [1]. Thus, in the range of milk fat contents $\geq 85\%$ the triglyceride method is superior to the C4 method without the use of a reference sample.

Studies on the quantitation of milk fat in mixed fats based on the fatty acid analysis of both the mixed fat and the pure components (component samples) have been performed by Muuse and Martens earlier [14]. A ring trial with 16 participating laboratories led to repeatabilities of $r = 4.3$ to 5.2% with mixtures containing 41–63% milk fat when exclusively using butyric acid for the calculation of the milk fat content. In the present study, determinations within this concentration range showed repeatabilities of $r = 0.7$ to 0.9% (Table I). Using several fatty acids simultaneously for the milk fat quantitation, Muuse and Martens [14] found repeatabilities of $r = 2.0$ to 5.4% . However, this requires the analysis of three fats, i.e. besides the mixed fat a sample of the milk fat and of the non-milk fat have to be available. As can be seen from Table II, repeatabilities of $r = 3.1$ – 4.9% (range 40–60% milk fat) can be achieved without any reference sample (only using a mean C4 value) with the method used in the present study.

Besides minor statistical effects due to the different study design that possibly might impair a direct comparison of repeatabilities (r), the higher precision of the procedure presented here, using a reference sample, may be due to the analytical method. The participants in the above-mentioned ring trial [14] determined the total fatty acid composition ($\text{g}\cdot 100\text{ g}^{-1}$ fatty acids) which involves greater analytical variations, in particular concerning butyric acid. The results of the present study are based on the exclusive analysis of butyric acid ($\text{g}\cdot 100\text{ g}^{-1}$ fat) using an internal standard. Carried out in this way the butyric acid determination exhibits an excellent repeatability of $r = 0.04\%$ (determined on a pure milk fat with $n = 10$; relative standard deviation of repeated measurements $\text{RSD} = 0.44\%$) [9] that has a direct effect on the milk fat quantitation. As the separation and calculation of medium- and long chain fatty acids is not required, the increase in the GC temperature program enables some time to be saved (total duration ca. 30 min, cp. Figure 1).

Conclusions

The analysis of butyric acid (C_4 , in $\text{g}\cdot 100\text{ g}^{-1}$ fat) in a mixed fat and its underlying milk fat using a recently improved method allows the quantitation of milk fat with mean absolute deviations below 0.5% and maximum errors below 1.0% at any milk fat content within the entire range of 1 to 90%. The use of a mean value instead of the actual C_4 content of the milk fat involved as a rule leads to higher deviations caused by the natural variation of the C_4 content in milk fats. Performing analyses of the mixed fat and the respective reference milk fat avoids this problem and enables precise determinations of milk fat in mixed fats to be made. The achievable pre-

cision appears to be higher than in former investigations.

Acknowledgements

The authors thank Mrs. B. Krumbeck and Mrs. B. Fischer for their assistance in performing analytical experiments.

References

- [1] D. Precht, *Z. Lebensm. Unters. Forsch.* **194**, 107 (1992).
- [2] Commission Regulation (EC) No 454/95, annex III, Official Journal L 46, 1 (1995).
- [3] D. Precht, *Fat Sci. Technol.* **93**, 538 (1991).
- [4] J. Molkentin, D. Precht, *Schriftenreihe des Bundesministeriums für Ernährung, Landwirtschaft und Forsten, Reihe A: Angewandte Wissenschaft, Landwirtschaftsverlag GmbH Münster, Heft 469*, 47 (1998).
- [5] H. Hadorn, K. Zürcher, *Rev. Int. Choc.* **27**, 82 (1972).
- [6] D. Precht, *Fat Sci. Technol.* **92**, 153 (1990).
- [7] D. Precht, *Fat Sci. Technol.* **92**, 275 (1990).
- [8] J. Molkentin, D. Precht, *Milchwissenschaft* **52**, 82 (1997).
- [9] J. Molkentin, D. Precht, *Z. Lebensm. Unters. Forsch. A* **206**, 213 (1998).
- [10] M. Arens, Chr. Gertz, *Fat Sci. Technol.* **92**, 61 (1990).
- [11] W. D. Pocklington, A. Hautfenne, *Pure & Appl. Chem.* **58**, 1419 (1986).
- [12] Amtliche Sammlung von Untersuchungsverfahren nach § 35 LMBG, Methode L 17.00-12, Beuth Verlag, Berlin, 1985.
- [13] *Community Bureau of Reference*, Reference Material CRM 164 (anhydrous milk fat; certified butyric acid content $3.49 \pm 0.06\text{ g}/100\text{ g}$ fat).
- [14] B. Muuse, R. Martens, *Bulletin of the IDF* **285**, 65 (1993).

Received: May 25, 1998
 Revised manuscript
 received: Aug 3, 1998
 Accepted: Aug 14, 1998