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Densitometric determination of the fat content of milk and milk products

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Abstract

An automatable method for determining the fat content of milk and milk products was developed using a precise densitometer based on the principle of the resonant frequency U-tube oscillation. The sample is first decomposed with hydrochloric acid and the fat extracted with hexane. The density of the fat/hexane solution obtained varies with the fat content of the sample. Milk, cream and cheese samples were analysed. The mean values from the densitometric determination of the fat content did not differ from the mean values obtained by the corresponding gravimetric reference methods.

Keywords: Milk; Milk products; Dairy products; Fat content; Densitometry

1. Introduction

The fat content of a milk product is an important indication of quality, both economically and physiologically. In the dairy industry, it is mainly determined by using "quick methods". Spectrometric measuring methods are often used (IDF, 2000). However, these methods have to be calibrated, which is costly. Many laboratories, therefore, are using a method developed by the Swiss chemist and dairy-owner Niklaus Gerber, patented in 1891 under the name "Acid-Butyrometrie" (Gerber, 1891). This method is still used because it is simple, fast, low-cost and suitable for a relatively high sample throughput. However, butyrometry has several disadvantages. The determination cannot be automated and involves a certain risk in handling highly concentrated sulphuric acid, especially while reading the butyrometer. Handling the butyrometer requires practical skills, which has a negative effect on the robustness of the method. A great disadvantage is the varying definition of fat compared with the reference methods. In the latter, fat is always defined by extraction with a non-polar solvent (IDF, 1986; IDF, 1987; IDF, 1996). In the butyrometric method, this extraction step is missing. Several attempts have been made to harmonize the fixed butyrometer scale with the values from reference analysis (Hänni, 1960; Trossat, 2000). Even with the costly gravimetric reference methods, attempts were made to automate or simplify the procedure (Evers et al., 2000; Manganiello, Ríos, Valcárcel, Ligero, & Tena, 2000; Schulte, 2001).

Applying the FOSS-LET instrument (Foss Electric, Hillerød, Denmark) an attempt was made as early as 1973 to determine the fat content of milk products by measuring the density of a fat solution (Montag, 1973). The fat was extracted with tetrachloroethene and the content was determined using a potentiometrically defined hydrometer level adjustment (Pfeiffer, Wellhäuser, & Gehra, 1973). Depending on the fat content, samples in a range of 20 and 45 g per determination were weighed and extracted with 120 mL tetrachloroethene.

Recently, a possibility for an alternative method for the determination of the fat content emerged from the development of new densitometers based on the principle of the resonant frequency U-tube oscillation. Such densitometers allow a very precise density analysis of relatively small volumes. Some instrument manufacturers offer such instruments with samplers already. This paper shows the development of a densitometric fat determination applied to various milk products.

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2. Material and methods

2.1. Samples, digestion and densitometry

The samples were commercial milk products obtained from Swiss market (milk, cream, cheese). They were homogenised as described in the reference standards (see Section 2.2). To account for different fat contents, either 5.0 g milk or 2.0 g cream or cheese were weighed, to the nearest 0.001 g, in 30 mL centrifugation tubes made of teflon (FEP Oak Ridge-Typ, Semadeni AG, Nr. 2661, Ostermundigen, Switzerland) and decomposed with 5 mL (milk, cream) or 10 mL (cheese) concentrated hydrochloric acid (HCl, 25%) for 40 min in a water bath at 90 to 95°C. After cooling the digested samples to room temperature using a water bath, the lipids were extracted with 3.0 mL n-hexane (Merck, Darmstadt, Germany) on a shaker (Heidolph UNIMAX 2010, VWR International GmbH, Dietikon, Switzerland) at 400 rpm for 30 min.The measurement uncertainty was reduced by weighing the added hexane volume. Addition of one glass bead (12 mm diameter) improved extraction of the lipids through turbulence. Both phases were then separated for 10 min in a centrifuge (Sigma-4K10, VWR International GmbH, Dietikon, Switzerland) at 5300*g*.

Determination of the density of the upper solvent phase was carried out at 25 °C using a densitometer (Paar DMA 5000, Anton Paar GmbH, Graz, Austria). Calibration was carried out with solutions of commercial dehydrated molten butter fat in n-hexane. The molten butter was dehydrated using a hydrophobic folded filter (Nr. $597^{1/2}$ hy, Schleicher & Schuell, Bottmingen, Switzerland).

2.2. Reference methods and butyrometric methods

Determinations using the gravimetric reference methods were carried out in accordance with the standards of the International Dairy Federation IDF 5B (IDF, 1986), IDF 16C (IDF, 1987) and IDF 1D (IDF, 1996). For the butyrometric determinations the standards of the International Organization for Standardization ISO 3433 (ISO, 1975) and ISO 2446 (ISO, 1976) were applied and for the butyrometric determination of cream fat content the Swiss Food Manual method (SLMB, 2002).

3. Results and discussion

3.1. Calibration function

For the determination of the relationship between density and mass of dehydrated molten butter fat in n-hexane, the density values of twenty different weighings of dehydrated molten butter fat in 3 mL n-hexane were measured. The obtained values are shown in Fig. 1.

From the data in Fig. 1 an empirical function (1) was then calculated by using the least-squares method

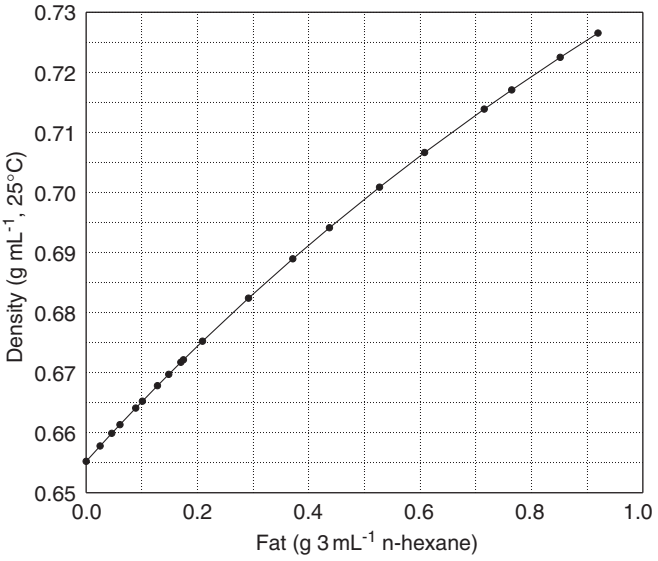

Fig. 1. Calibration curve developed for dehydrated molten butter in n-hexane using the densitometric fat determination.

(TableCurve 2D, 1998):

$$\rho_s = \frac{a + cm_f}{1 + bm_f}, \tag{1}$$

where ρ_s is the density of the fat solution in g mL^{-1} and m_f is the mass of fat in g.

The coefficients a, b and c of the empirically calculated function (1) can be identified by the theoretical considerations expressed in Eqs. 2–8. The mass and volume balance of the fat solution are

$$m_s = m_f + m_h, \tag{2}$$

$$V_s = V_f k + V_h, \tag{3}$$

where m_s is the mass of the fat solution in g, m_f the mass of fat in g, m_h the mass of n-hexane in g, V_s the volume of the fat solution in mL, V_f the volume of fat in mL, V_h the volume of n-hexane in mL and k the correction factor (due to volume contraction) for the fat volume with constant volume of n-hexane.

From the balances in Eqs. (2) and (3) the density of the fat solution can be calculated:

$$\rho_s = \frac{m_s}{V_s} = \frac{m_f + m_h}{V_f k + V_h}. \tag{4}$$

By using the density of the n-hexane (ρ_h) and the density of the pure fat (ρ_f) in the liquid state, Eq. (4) can be extended as follows:

$$\rho_s = \frac{m_f + V_h \rho_h}{\frac{m_f}{\rho_f} k + V_h}, \tag{5}$$

where ρ_h is the density of n-hexane in g mL^{-1} and ρ_f the density of the pure fat in gmL^{-1}.

By transposing the Eq. (5) and dividing both numerator and denominater by V_h, the following function, similar to the empirically calculated function (1), is obtained:

$$\rho_s = \frac{\rho_h + \frac{m_f}{V_h}}{1 + \frac{k}{\rho_f V_h} m_f} \quad (6)$$

Let us define coefficients a, b and c of the empirically calculated function (1) as follows:

$$a = \rho_h \quad b = \frac{k}{\rho_f V_h} \quad c = \frac{1}{V_h}.$$

For practical application, only coefficient b has to be calculated from the calibration data. Determination of the density of the pure fat (ρ_f) is therefore not required and the calibration function can be described as follows:

$$\rho_s = \frac{\rho_h + \frac{1}{V_h} m_f}{1 + b m_f}. \quad (7)$$

The following parameters were used in the calculation of the calibration function (8): ρ_h, 0.65516 g mL^{-1} (n-hexane measured at 25 °C); V_h 3 mL (selected volume of n-hexane); and b, 0.35587 g^{-1} (calculated using least-squares-fit of function (7) and data from Fig. 1).

$$\rho_{s;25\,^\circ C} = \frac{0.65516 + \frac{1}{3} m_f}{1 + 0.35587 m_f}. \quad (8)$$

3.2. Comparison of repeatability limits between reference, butyrometric and densitometric methods of fat determination

The repeatability limit r of different fat determination methods was calculated from n duplicate determinations with formula (9) in accordance with ISO 5725-2 (ISO, 1994); the repeatabilities and numbers of duplicate determinations are presented in Table 1.

$$r = 2.83\sqrt{\frac{\sum_{i=1}^{n} d_i^2}{2n}}, \quad (9)$$

where r is the repeatability in g kg^{-1}, d_i is the difference of the ith- duplicate determination in g kg^{-1} and n the number of duplicate determinations.

Table 1 shows that in the case of cheese the repeatability limit of the three fat determination methods was similar. The measurement uncertainty due to inhomogeneous fat distribution in cheese was obviously of secondary importance. In the case of cream, the same repeatability limit was reached with the densitometric method as with the butyrometric method. The gravimetric reference method gave considerably more precise values. The repeatability limit of densitometric fat determination in milk samples was closer to the reference method value and was about half of that for the butyrometric method.

Table 1
Repeatability limit r (g kg^{-1}) of three fat determination methods in various dairy matrices with number of repeated measurements n as indicated

	Gravimetric (Reference)			Butyrometric			Densitometric	
Matrix	r	n	Method	r	n	Method	r	n
Cheese	3.1	34	IDF 5B	3.4	13844[a]	ISO 3433	3.3	32
Cream	1.0	100	IDF 16C	3.2	230	SLMB 3/04	3.0	25
Milk	0.30	100	IDF 1C	0.84	250	ISO 2446	0.46	34

[a]Duplicates of routine measurements from ALP laboratory between 1990 and 1999.

Table 2
Comparison of the mean values (g kg^{-1}) of fat content in various dairy products tested by the reference methods and the densitometric fat determination method

	Reference method ($n = 2$)	Densitometric method ($n = 5$)	
Matrix	Mean value	Mean value	s[a]
Skimmed milk	0.84	0.85	0.04
Raw milk	39.8	40.1	0.2
Coffee cream	152	152	0.2
Half-cream	254	253	0.4
Couronne (soft cheese)	277	276	0.7
Tilsit cheese	284	284	0.3
Emmental cheese	316	317	0.7
Appenzell cheese	328	326	1.6
Gruyère cheese	345	344	0.3
Sbrinz cheese	348	347	0.4
Full cream	369	370	0.2

[a]Standard deviation (g kg^{-1}) of the five replicate densitometric determinations.

Table 3
Parameters of the regression between fat contents obtained by the reference method and the densitometric method

Parameter	Value	Unit
Number of data pairs	11	—
Slope b	0.998	—
95%-confidence interval of b	[0.994, 1.003]	—
Standard error of b	0.002	—
Intercept a	0.245	$g\,kg^{-1}$
95%-confidence interval of a	[−0.929, 1.418]	$g\,kg^{-1}$
Standard error of a	0.519	$g\,kg^{-1}$
Standard error of the regression	0.757	$g\,kg^{-1}$

3.3. Comparison of the mean values of fat content obtained by the reference and the densitometric method

For assessing the accuracy of each matrix given in Table 2, the mean values from a duplicate determination of the fat content of the reference method were compared with the mean values from a five-fold determination using the densitometric method.

The densitometric method of fat determination was compared with the corresponding reference methods using a classical regression analysis of these values (Systat for Windows, 2000). The parameters of the regression are presented in Table 3.

The slope b and the intercept a are not significantly different ($\alpha = 0.05$) from 1 and 0, respectively. The mean values from the densitometric fat determination did not differ from the mean values of the corresponding gravimetric reference methods for any of the products analysed.

4. Conclusions

Densities of butter fat solutions in n-hexane can be measured using a high-precision density measurement equipement. With these density values an empirical function can be calculated and its coefficients are identifiable by theoretical considerations. The resulting calibration function is applicable to the determination of fat contents of different milk products. The obtained results proved to be in good agreement with the reference values and results of butyrometric measurements. Cost and time needed for the densitometric determination are comparable to those for butyrometry and considerably less than those for the gravimetric method. A further advantage is the possibility for automatisation of the proposed method.

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References

Evers, J. M., Wightman, L. M., Crawford, A., Contarini, G., Coors, U., Farrington, D. S., et al. (2000). A precise method to measure the total fat content of spreadable fats. *International Dairy Journal*, *10*(12), 815–827.

Gerber, N. (1891). Neuer Butyrometer. Patent CH2621, (1891-01-31), Bern: Eidgenössisches Institut für Geistiges Eigentum.

Hänni, H. (1960). Die Rolle des Amylalkohols bei der Fettbestimmung nach Gerber. *Mitteilungen aus dem Gebiete der Lebensmitteluntersuchung und Hygiene*, *51*(6), 514–524.

IDF. (1986). *International Standard 5B. Cheese & processed cheese products—Determination of Fat content—Gravimetric method (reference method)*. Brussels, Belgium: International Dairy Federation.

IDF. (1987). *International Standard 16C. Cream—Determination of Fat content—Gravimetric method (Röse Gottlieb reference method)*. Brussels, Belgium: International Dairy Federation.

IDF. (1996). *International Standard 1D. Milk—Determination of Fat content—Gravimetric method (Röse Gottlieb reference method)*. Brussels, Belgium: International Dairy Federation.

IDF. (2000). *International Standard 141C. Whole milk—Determination of milkfat, protein and lactose content—Guidance on the operation of mid-infrared instruments*. Brussels, Belgium: International Dairy Federation.

ISO. (1975). *International Standard 3433. Cheese—Determination of fat content – (Butyrometric routine method)*. Geneva, Switzerland: International Standards Organisation.

ISO. (1976). *International Standard 2446. Milk—Determination of fat content (Butyrometric routine method)*. Geneva, Switzerland: International Standards Organisation.

ISO. (1994). *International Standard 5725-2. Accuracy (trueness and precision) of measurement methods and results—Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method*. Geneva, Switzerland: International Standards Organisation.

Manganiello, L., Ríos, A., Valcárcel, M., Ligero, A., & Tena, T. (2000). Automatic determination of fat in milk by use of a flow injection system with a piezoelectric detector. *Analytica Chimica Acta*, *406*(2), 309–315.

Montag, A. (1973). Zur densitometrischen Fettbestimmung in milcheiweissreichen Erzeugnissen mit dem FOSS-LET-Gerät. *Deutsche Lebensmittel-Rundschau*, *69*(12), 470–472.

Pfeiffer, G., Wellhäuser, R., & Gehra, H. (1973). "Foss-Let", ein Fett-Analyzer für die Standardisierung der Produktion und die Fertigproduktkontrolle. *Die Fleischwirtschaft*, *7*, 985–991.

Schulte, E. (2001). Micro method for rapid gravimetric determination of fat content in food after acid treatment. *Deutsche Lebensmittel-Rundschau*, *97*(3), 85–89.

SLMB. (2002). *Schweizerische Lebensmittelbuchmethode 3/04. Rahm—Fett nach Gerber—Roeder–Liebefeld (Butyrometrische Routinemethode)*. Bern, Switzerland: Eidg. Drucksachen- und Materialzentrale.

Systat for Windows (2000). Version 10.0 (CD and manual). Chicago, IL, USA: SPSS Inc.

TableCurve 2D (1998). Version 4.07 for Windows (CD and manual). Chicago, IL, USA: SPSS Inc.

Trossat, P. (2000). Détermination de la matière grasse. Evolution de la méthode Gerber. *Revue Laitière Française*, *599*, 28–30.