INTERNATIONAL STANDARD

ISO 17678

IDF 202

First edition
2010-02-15

Milk and milk products — Determination of milk fat purity by gas chromatographic analysis of triglycerides (Reference method)

Lait et produits laitiers — Détermination de la pureté des matières grasses laitières par analyse chromatographique en phase gazeuse des triglycérides (Méthode de référence)

Reference numbers
ISO 17678:2010(E)
IDF 202:2010(E)

ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

International Dairy Federation
Diamant Building • Boulevard Auguste Reyers 80 • B-1030 Brussels
Tel. + 32 2 733 98 88
Fax + 32 2 733 04 13
E-mail info@fil-idf.org
Web www.fil-idf.org

Published in Switzerland

Contents

Page

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 17678 | IDF 202 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*, and the International Dairy Federation (IDF). It is being published jointly by ISO and IDF.

Foreword

IDF (the International Dairy Federation) is a non-profit organization representing the dairy sector worldwide. IDF membership comprises National Committees in every member country as well as regional dairy associations having signed a formal agreement on cooperation with IDF. All members of IDF have the right to be represented on the IDF Standing Committees carrying out the technical work. IDF collaborates with ISO in the development of standard methods of analysis and sampling for milk and milk products.

The main task of Standing Committees is to prepare International Standards. Draft International Standards adopted by the Standing Committees are circulated to the National Committees for endorsement prior to publication as an International Standard. Publication as an International Standard requires approval by at least 50 % of the IDF National Committees casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. IDF shall not be held responsible for identifying any or all such patent rights.

ISO 17678 | IDF 202 was prepared by the International Dairy Federation (IDF) and Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*. It is being published jointly by IDF and ISO.

All work was carried out by the Joint ISO-IDF Project Group on *Foreign fats* of the Standing Committee on *Analytical methods for composition* under the aegis of its project leader, Dr J. Molkentin (DE).

Milk and milk products — Determination of milk fat purity by gas chromatographic analysis of triglycerides (Reference method)

1 Scope

This International Standard specifies a reference method for the determination of milk fat purity using gas chromatographic analysis of triglycerides. Both vegetable fats and animal fats such as beef tallow and lard can be detected. By using defined triglyceride equations, the integrity of milk fat is determined.

Basically, the method applies to bulk milk, or products made thereof, irrespective of feeding, breed or lactation conditions. In particular, the method is applicable to fat extracted from milk products purporting to contain pure milk fat with unchanged composition, such as butter, cream, milk, and milk powder.

However, under the circumstances listed hereafter, a false positive result can be obtained. Hence, the method is not applicable to milk fat:

a) obtained from bovine milk other than cow's milk;

b) obtained from single cows;

c) obtained from cows which received an exceptionally high feeding of pure vegetable oils such as rapeseed oil;

d) obtained from colostrum;

e) subjected to technological treatment such as removal of cholesterol or fractionation;

f) obtained from skim milk or buttermilk;

g) extracted by using the Gerber, Weibull–Berntrop or Schmid–Bondzynski–Ratzlaff methods, or that has been isolated using detergents (e.g. the Bureau of Dairy Industries method).

With the extraction methods specified in g), substantial quantities of partial glycerides or phospholipids can pass into the fat phase. Consequently, the scope of this International Standard excludes certain products and particularly cheese, whose ripening process can also affect the fat composition to such a degree that a false positive result is obtained.

NOTE 1 In nature, butyric (*n*-butanoic) acid (C4) occurs exclusively in milk fat and enables quantitative estimations of low to moderate amounts of milk fat in vegetable and animal fats to be made. However, due to the large variation of C4, whose approximate content ranges from 3,1 % mass fraction to 3,8 % mass fraction, it is difficult to provide qualitative and quantitative information for foreign fat to pure milk fat ratios of up to 20 % mass fraction (see Reference [11]).

NOTE 2 In practice, quantitative results cannot be derived from the sterol content of vegetable fats, because they depend on production and processing conditions. Furthermore, the qualitative determination of foreign fat using sterols is ambiguous.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 1211|IDF 1, *Milk — Determination of fat content — Gravimetric method* (*Reference method*)

ISO 2450|IDF 16, *Cream — Determination of fat content — Gravimetric method* (*Reference method*)

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

ISO 7328|IDF 116, *Milk-based edible ices and ice mixes — Determination of fat content — Gravimetric method* (*Reference method*)

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
milk fat purity
absence of vegetable and animal fats determined by the procedure specified in this International Standard

NOTE The purity is determined using S-values, which are calculated from the content of triglycerides. Triglyceride mass fractions are expressed as percentages.

4 Principle

Fat extracted from milk or milk products is analysed by gas chromatography (GC) using a packed or a short capillary column to determine triglycerides (TGs), separated by total carbon numbers. By inserting the mass fraction, expressed as a percentage, of fat molecules of different sizes (C24 to C54, using even C numbers only) into suitable TG equations, S-values are calculated. If the S-values exceed the limits established with pure milk fat, the presence of foreign fat is detected.

NOTE 1 The suitability and equivalence of both packed and capillary columns have been demonstrated previously (see References [8] to [10]).

NOTE 2 An S-value is the sum of weighted TG mass fractions.

5 Reagents

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade.

5.1 Water complying with the requirements of ISO 3696, grade 2.

5.2 Carrier gas, nitrogen or, alternatively, helium or hydrogen, all with a purity of at least 99,995 % volume fraction.

5.3 Fat standards, purity at least 99 % mass fraction, for standardizing the milk fat standard described in 8.3.3.

5.3.1 Triglyceride standards, saturated; suitable products are available commercially.

5.3.2 Cholesterol standard.

5.4 Methanol (CH_3OH), with a water content of not more than 0,05 % mass fraction.

5.5 ***n*-Hexane** [$CH_3(CH_2)_4CH_3$].

5.6 ***n*-Heptane** [$CH_3(CH_2)_5CH_3$].

5.7 **Other gases**, hydrogen, purity at least 99,995 % volume fraction, free from organic impurities (C_nH_m < 1 µl/l); synthetic air, free from organic impurities (C_nH_m < 1 µl/l).

5.8 **Anhydrous sodium sulfate** (Na_2SO_4).

6 Apparatus

Usual laboratory equipment and, in particular, the following.

6.1 **High-temperature gas chromatograph**, suitable for use at temperatures of at least 400 °C and equipped with a flame ionization detector (FID). For capillary GC, an on-column or a programmed temperature vaporization injector is indispensable while a split injector is unsuitable.

Septa used in the injector shall withstand high temperatures and exhibit a very low degree of “bleeding”. Always use graphite seals to connect the column as well as injector and/or detector inserts (where applicable).

6.2 **Chromatography column**.

6.2.1 **Packed column**, glass, of internal diameter 2 mm and length 500 mm, packed with a stationary phase of 3 % OV-1 on 125 µm to 150 µm (100 mesh to 120 mesh) Gas ChromQ [1].

The preparation, silanization, packing and conditioning of the packed column is described in Annex A.

Alternatively, a capillary column (6.2.2) may be used.

6.2.2 **Capillary column**, short, e.g. of length 5 m, with a non-polar stationary phase that can withstand temperatures up to 400 °C or more [2].

Condition the column by performing 20 analyses of a milk fat solution (8.2) within no more than 2 days by using the settings given in 8.3.4.2. After that, ensure that the response factors (8.3.3) are close to 1 and not higher than 1,250 0.

Because of the variable overlap between C24 and cholesterol, a higher response factor may be accepted for C24.

Columns with different dimensions and a different non-polar, highly temperature-resistant phase may be used as long as their performance is consistent with this International Standard. However, the column length is restricted by the indispensable limitation in resolution as shown in Figure 1. See also 8.3.4.2.

6.3 **Extrelut column** [1], capacity 1 ml to 3 ml, filled with silica gel, for the extraction of milk fat in accordance with 8.1.4 only.

6.4 **Graphite seals**, capable of withstanding temperatures of at least 400 °C; for the connection of the GC column as well as for the injector and/or detector inserts.

6.5 **Water bath**, capable of being maintained at 50 °C ± 2 °C.

6.6 **Oven**, capable of operating at 50 °C ± 2 °C and 100 °C ± 2 °C.

---

1) Example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO or by IDF of this product.

2) CP-Ultimetal SimDist (5 m, 0,53 mm, 0,17 µm) is an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO or by IDF of this product.

6.7 Micropipette.

6.8 Graduated pipette, capacity 5 ml, ISO 835[2] class A.

6.9 Round-bottomed flask, capacity 50 ml.

6.10 Erlenmeyer flask, nominal capacity 250 ml.

6.11 Funnel.

6.12 Fine-pored filter paper.

6.13 Rotary evaporator.

6.14 Ampoules, nominal capacity 1 ml, fitted with a polytetrafluoroethylene-lined aluminium crimp cap or screw cap.

6.15 Injection syringe, with syringe plunger not reaching into the tip of the needle (packed column GC).

NOTE With these syringes, better repeatability of the results is obtained.

6.16 Analytical balance, capable of weighing to the nearest 1 mg, with a readability of 0,1 mg.

7 Sampling

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 707 | IDF 50[1].

A representative sample should have been sent to the laboratory. It should not have been damaged or changed during transport or storage.

8 Procedure

8.1 Preparation of test samples

8.1.1 General

For the preparation of test samples, use one of the milk fat isolation or extraction methods specified in 8.1.2 to 8.1.4.

8.1.2 Isolation from butter or butteroil

Melt 50 g to 100 g of test sample in the water bath (6.5) or the oven (6.6) at 50 °C.

Add 0,5 g to 1,0 g of sodium sulfate (5.8) to a folded filter paper (6.12). Preheat a 250 ml Erlenmeyer flask (6.10) and a funnel (6.11) with the filter paper inserted, containing the sodium sulfate, in the oven (6.6) at 50 °C.

When a limited amount of test sample is available, use a smaller test sample and adapt the procedure accordingly.

However, note that the handling of a smaller test portion involves a higher risk of obtaining a non-representative sample.

While keeping the preheated flask, funnel, and inserted filter device in the oven, filter the fat layer of the molten sample without transferring any serum.

NOTE 1 Butter can be obtained from cream by churning and thorough washing of the resulting butter grain.

NOTE 2 The milk fat obtained using the procedure in this subclause is almost free of phospholipids.

8.1.3 Extraction according to the Röse–Gottlieb gravimetric method

Extract the fat fraction from the test sample by using the gravimetric method specified in one of: ISO 1211|IDF 1, ISO 2450|IDF 16 or ISO 7328|IDF 116.

8.1.4 Extraction from milk using silica gel columns

Temper the milk to 20 °C. Using a micropipette (6.7), add 0,7 ml of the sample thus prepared into a 1 ml to 3 ml Extrelut column (6.3). Allow the sample to distribute uniformly on the silica gel for approximately 5 min.

To denature the protein–lipid complexes, using the graduated pipette (6.8), add 1,5 ml of methanol (5.4) to the Extrelut column. Subsequently, extract the fat fraction from the test sample with 20 ml of *n*-hexane (5.5). Add the *n*-hexane slowly in small amounts. Collect the solvent draining off in a 50 ml round-bottomed flask (6.9), previously dried to a constant, known mass weighed to the nearest 1 mg and record the mass to 0,1 mg.

Allow the column to drain until empty after the extraction. Distil off the solvents from the eluate on a rotary evaporator (6.13) with its water bath maintained at between 40 °C and 50 °C.

After distilling off the solvents, dry and subsequently weigh the round-bottomed flask and its contents to the nearest 1 mg, recording the mass to 0,1 mg. Determine the fat mass yield by subtracting the mass of the dried empty round-bottomed flask from the mass obtained.

Depending on the fat content of the milk and the required concentration of the sample solution, check whether it is necessary to combine the yield of two or more extractions to obtain an adequate amount of fat.

8.2 Preparation of fat sample solution

For gas chromatography with a packed column, prepare a 5 % volume fraction solution of the fat obtained in 8.1.2, 8.1.3 or 8.1.4 in *n*-hexane (5.5) or *n*-heptane (5.6). Depending on the column dimensions, use a concentration of 1 % [0,53 mm internal diameter (ID), wide-bore] or lower for on-column injection with a capillary column.

When using the fat sample prepared in 8.1.4, calculate the amount of solvent (5.5 or 5.6) to be added to the test sample in the flask based on the mass of fat obtained.

Completely dissolve the fat in the solvent used. Transfer approximately 0,5 ml to 1 ml of the fat sample solution obtained into an ampoule (6.14).

8.3 Chromatographic triglyceride determination

8.3.1 Baseline drift

To minimize baseline rising, condition the column as specified in 6.2.2 (capillary column) or in Clause A.4 (packed column).

NOTE Because of the high column temperature, the analysis of TGs is particularly susceptible to a rise of the baseline in the high carbon-number range.

8.3.2 Injection technique

8.3.2.1 Packed column

To avoid discrimination effects and to improve the quantification of the high-boiling TG components, apply the hot-needle technique.

Fill the needle with air by drawing up the fat solution into the body of the syringe. Insert the needle into the injector. Heat the needle prior to injection for about 3 s. Then, rapidly inject the syringe contents.

8.3.2.2 Capillary column

When using cool on-column injection (8.3.4.2), insert the needle of the syringe and inject immediately. Choose a suitable subsequent dwell time of the needle in the injector so as to avoid broad tailing of the solvent peak.

NOTE The optimum dwell time is typically about 3 s.

8.3.3 Calibration

8.3.3.1 General

For the calibration of test samples, perform two to three analyses of standardized milk fat at the beginning of every working day. Use the last analysis of the standardized milk fat to determine the response factors, f_i (mass fraction divided by area fraction), of the TGs and of cholesterol and apply these to the subsequent test samples (see 10.1):

$$f_i = \frac{w_i \sum A_i}{\sum w_i \; A_i} \tag{1}$$

where

w_i is the mass fraction, expressed as a percentage, of each TG or cholesterol in the standardized milk fat;

A_i is the numerical value of the peak area of each TG or cholesterol in the standardized milk fat.

Express the response factors to four decimal places.

Proceed in accordance with either 8.3.3.2 or 8.3.3.3 to obtain a standardized milk fat with a known TG composition.

8.3.3.2 Commercial milk fat standard

Use a standardized milk fat with a certified TG composition[3)] to determine the response factor of each constituent of the test sample.

8.3.3.3 Laboratory milk fat standard

Prepare about 1 g of a mixture of the fat standards (5.3) — containing at least the saturated TGs, C24, C30, C36, C42, C48 and C54, as well as cholesterol; plus, preferably, C50 and C52 — by weighing to the nearest 1 mg and recording the mass to 0,1 mg to obtain a relative TG composition similar to milk fat.

Analyse repeatedly a solution of the fat standards mixture in *n*-hexane (5.5) or *n*-heptane (5.6) in accordance with 8.3.4. In the same sequence, analyse repeatedly milk fat of typical composition.

Determine the TG response factors from the fat standards mixture. Calculate the intermediate response factors of TGs not present in the mixture by mathematical interpolation. Apply the response factors obtained to the milk fat in order to obtain a standardized composition.

The standardized milk fat thus obtained has a stock life of several years, if stored under nitrogen at a maximum temperature of −18 °C.

---

3) CRM 519 (anhydrous milk fat) is an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO or by IDF of this product.