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**Milk — Determination of fat content —  
Gravimetric method (Reference method)**

*Lait — Détermination de la teneur en matière grasse — Méthode  
gravimétrique (Méthode de référence)*

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## 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 1211|IDF 1 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.

This third edition of ISO 1211|IDF 1 cancels and replaces the second edition (ISO 1211:1999), which has been technically revised.

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## 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 1211|IDF 1 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 *Fat in milk* of the Standing Committee on *Analytical methods for composition* under the aegis of its project leader, Mrs. S. Orlandini (IT).

This edition of ISO 1211|IDF 1 cancels and replaces IDF 1D:1996, which has been technically revised.

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# Milk — Determination of fat content — Gravimetric method (Reference method)

**WARNING** — Persons using this International Standard should be familiar with normal laboratory practice. This International Standard does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions.

## 1 Scope

This International Standard specifies the reference method for the determination of the fat content of milk of good physicochemical quality.

The method is applicable to raw cow milk, raw sheep milk, raw goat milk, reduced fat milk, skimmed milk, chemically preserved milk, and processed liquid milk.

It is not applicable when greater accuracy is required for skimmed milk, e.g. to establish the operating efficiency of cream separators.

NOTE ISO 7208<sup>[7]</sup> specifies a special method for skimmed milk products.

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## 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 3889 | IDF 219, *Milk and milk products — Specification of Mojonnier-type fat extraction flasks*

## 3 Terms and definitions

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

### 3.1

#### fat content of milk

mass fraction of substances determined by the procedure specified in this International Standard

NOTE The fat content is expressed as a percentage mass fraction.

## 4 Principle

An ammoniacal ethanolic solution of a test sample is extracted with diethyl ether and light petroleum. The solvents are removed by distillation or evaporation. The mass of the substances extracted is determined.

NOTE This is usually known as the Röse-Gottlieb principle.

## 5 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified, and distilled or demineralized water or water of equivalent purity.

The reagents shall leave no appreciable residue when the determination is carried out by the method specified (see 9.3.2).

**5.1 Ammonia solution**, containing a mass fraction of  $\text{NH}_3$  of approximately 25 % [ $\rho_{20}(\text{NH}_3) = 910 \text{ g/l}$ ].

If an ammonia solution of this concentration is not available, a more concentrated solution of known concentration may be used (see 9.5.1).

**5.2 Ethanol** ( $\text{C}_2\text{H}_5\text{OH}$ ), or ethanol denatured by methanol, containing a volume fraction of ethanol of at least 94 % (see A.4).

**5.3 Congo red solution.**

Dissolve 1 g of Congo red ( $\text{C}_{32}\text{H}_{22}\text{N}_6\text{Na}_2\text{O}_6\text{S}_2$ ) in water in a 100 ml one-mark volumetric flask (6.14). Make up to the mark with water.

NOTE The use of this solution, which allows the interface between the solvent and aqueous layers to be seen more clearly, is optional (see 9.5.2). Other aqueous colour solutions can be used provided that they do not affect the result of the determination.

**WARNING — Congo red is carcinogenic.**

**5.4 Diethyl ether** ( $\text{C}_2\text{H}_5\text{OC}_2\text{H}_5$ ), free from peroxides (see A.3), and complying with the requirements for the blank test (see 9.3.2 and A.2).

**WARNING — The use of diethyl ether can lead to hazardous situations. Observe current safety precautions for handling, use, and disposal.**

**5.5 Light petroleum**, with any boiling range between 30 °C and 60 °C or, as equivalent, **pentane** ( $\text{CH}_3[\text{CH}_2]_3\text{CH}_3$ ) with a boiling point of 36 °C and complying with the requirements for the blank test (see 9.3.2, A.1 and A.2).

**5.6 Mixed solvent.**

Shortly before use, mix equal volumes of diethyl ether (5.4) and light petroleum (5.5).

## 6 Apparatus

**WARNING — Since the determination involves the use of volatile flammable solvents, all electrical apparatus employed shall comply with legislation relating to the hazards in using such solvents.**

Usual laboratory equipment and, in particular, the following.

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

**6.2 Centrifuge**, capable of holding the fat-extraction flasks or tubes (6.6) and capable of spinning at a rotational frequency of  $500 \text{ min}^{-1}$  to  $600 \text{ min}^{-1}$  to produce a radial acceleration of  $80g$  to  $90g$  at the outer end of the flasks or tubes.

The use of the centrifuge is optional, but recommended (see 9.5.5).



**6.3 Distillation or evaporation apparatus**, suitable for distilling the solvents and ethanol from the boiling or conical flasks, or evaporating from dishes (see 9.5.12) at a temperature not exceeding 100 °C.

**6.4 Drying oven**, electrically heated, with ventilation port(s) fully open, capable of operating at a temperature of 102 °C ± 2 °C throughout its working space.

The oven shall be fitted with a suitable thermometer.

**6.5 Water bath**, capable of maintaining a temperature between 35 °C and 40 °C.

**6.6 Mojonnier type fat-extraction flasks**, as specified in ISO 3889 | IDF 219.

NOTE It is also possible to use fat-extraction tubes, with siphon or wash-bottle fittings, but the procedure is then different (see Annex B).

The fat-extraction flasks shall be provided with good quality cork bungs or stoppers of other material [e.g. silicone rubber or polytetrafluoroethylene (PTFE)] unaffected by the reagents used. Cork bungs shall be extracted with the diethyl ether (5.4), kept in water at a temperature of 60 °C or more for at least 15 min, and shall then be allowed to cool in the water so that they are saturated when used.

**6.7 Rack**, suitable for holding the fat-extraction flasks (or tubes) (6.6).

**6.8 Wash bottle**, suitable for use with the mixed solvent (5.6).

A plastics wash bottle shall not be used.

**6.9 Fat-collecting vessels**, such as boiling flasks (flat-bottomed), of capacities 125 ml to 250 ml, conical flasks, of capacity 250 ml, or metal dishes.

If metal dishes are used, they shall be of stainless steel, flat-bottomed with a diameter of 80 mm to 100 mm and a height of approximately 50 mm.

**6.10 Boiling aids**, fat-free, of non-porous porcelain, silicon carbide or glass. Their use is optional.

**6.11 Measuring cylinders**, capacities 5 ml and 25 ml, ISO 4788<sup>[4]</sup> class A, or any other apparatus suitable for the product concerned.

**6.12 Pipettes**, graduated, capacity 10 ml, ISO 835<sup>[2]</sup> class A.

**6.13 Tongs**, made of metal, suitable for holding flasks, beakers or dishes.

**6.14 One-mark volumetric flask**, capacity 100 ml, ISO 1042<sup>[3]</sup> class A.

## 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<sup>[1]</sup>.

It is important the laboratory receive a truly representative sample which has not been damaged or changed during transport or storage.

Store laboratory samples at a temperature of between 2 °C and 6 °C from the time of sampling to the time of commencing the procedure.

## 8 Preparation of test sample

Using the water bath (6.5), warm the test sample to a temperature of  $38\text{ °C} \pm 2\text{ °C}$ . Gently mix the test sample thoroughly without causing frothing or churning. Then cool the test sample quickly to  $20\text{ °C} \pm 2\text{ °C}$ .

If a homogeneous test sample can be obtained without pre-warming (e.g. for samples of skimmed milk), bring the test sample to a temperature of  $20\text{ °C} \pm 2\text{ °C}$  and gently mix thoroughly by repeatedly inverting the sample bottle.

A reliable value for the fat content cannot be expected:

- a) if the milk is churned;
- b) when a distinct smell of free fatty acids is perceptible;

NOTE Goat milk naturally contains a low level of free fatty acids, which are not completely extracted in this method.

- c) if, during or after preparation of the test sample, white particles are visible on the walls of the sample bottle or fat droplets float on the surface of the sample.

## 9 Procedure

### 9.1 General

If it is required to check whether the repeatability limit (11.2) is met, carry out two single determinations in accordance with 9.2 to 9.5.

NOTE An alternative procedure using fat-extraction tubes with siphon or wash-bottle fittings (see Note to 6.6) is given in Annex B.

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### 9.2 Test portion

Mix the prepared test sample (Clause 8) by gently inverting the bottle three or four times. Immediately weigh, to the nearest 1 mg, 10 g to 11 g of the test sample, directly or by difference, in a fat-extraction flask (6.6).

Transfer the test portion as completely as possible into the lower (small) bulb of the fat-extraction flask.

### 9.3 Blank tests

#### 9.3.1 Blank test for method

Carry out a blank test simultaneously with the determination using the same procedure and same reagents, but replacing the test portion in 9.2 by 10 ml of water (see A.1).

When a batch of test samples is analysed, the number of drying cycles may differ between different samples. If one blank sample is used for the entire batch, ensure that the blank value, used in the calculation of the fat content of any individual sample, was obtained under the same conditions as the individual test sample.

If the value obtained in the blank test regularly exceeds 1,0 mg, check the reagents if this has not been recently done (9.3.2). Corrections of more than 2,5 mg should be mentioned in the test report.

#### 9.3.2 Blank test for reagents

To test the quality of the reagents, carry out a blank test as specified in 9.3.1. Additionally use an empty fat-collecting vessel, prepared as specified in 9.4, for mass control purposes. The reagents shall leave no residue greater than 1,0 mg (see Clause A.2).

If the residue of the complete reagent blank test is greater than 1,0 mg, determine the residue of the solvents separately by distilling 100 ml of the diethyl ether (5.4) and light petroleum (5.5), respectively. Use an empty fat-collecting vessel, prepared for control purposes as in the preceding paragraph, to obtain the real mass of the residue which shall not exceed 1,0 mg.

Replace unsatisfactory reagents or solvents, or redistil solvents.

#### 9.4 Preparation of fat-collecting vessel

Dry a fat-collecting vessel (6.9) with a few boiling aids (6.10) in the oven (6.4) maintained at  $102\text{ °C} \pm 2\text{ °C}$  for 1 h.

NOTE 1 Boiling aids are optional to promote gentle boiling during the subsequent removal of solvents, especially when using glass fat-collecting vessels.

Protected from dust, allow the fat-collecting vessel to cool to the temperature of the weighing room. Cool a glass fat-collecting vessel for at least 1 h and a metal dish for at least 30 min. To avoid insufficient cooling or unduly long cooling times, do not cool the fat-collecting vessel in a desiccator.

Use tongs (6.13) to place the fat-collecting vessel on the balance. Weigh the fat-collecting vessel to the nearest 1,0 mg.

NOTE 2 The use of tongs effectively avoids temperature variations.

#### 9.5 Determination

9.5.1 Start the determination within 1 h of weighing the sample.

Add 2 ml of ammonia solution (5.1), or an equivalent volume of a more concentrated ammonia solution (see 5.1), to the test portion in the fat-extraction flask (9.2). Mix thoroughly with the test portion in the small bulb of the fat-extraction flask. <https://standards.iteh.ai/catalog/standards/sist/e91b7bcc-6f2d-4f80-a313-29797a9e4722/iso-1211-2010>

9.5.2 Add 10 ml of ethanol (5.2). Mix gently but thoroughly by allowing the contents of the fat-extraction flask to flow backwards and forwards between the small and large bulb. Avoid bringing the liquid too close to the neck of the flask. If desired, add 2 drops of the Congo red solution (5.3).

9.5.3 Add 25 ml of diethyl ether (5.4). Close the fat-extraction flask with a cork bung saturated with water or with a stopper of other material wetted with water (6.6). For 1 min, shake the flask vigorously, but not excessively, to avoid the formation of persistent emulsions.

While shaking, keep the fat-extraction flask in a horizontal position with the small bulb extending upwards, periodically allowing the liquid to run from the large bulb into the small bulb. Carefully remove the bung or stopper and rinse it and the inside of the neck of the fat-extraction flask with a little mixed solvent (5.6). Use the wash bottle (6.8) so that the rinsings run into the flask.

9.5.4 Add 25 ml of the light petroleum (5.5). Close the fat-extraction flask with the bung or stopper. Mix again for 30 s as specified in 9.5.3.

9.5.5 Centrifuge the closed fat-extraction flask for between 1 min and 5 min at a radial acceleration of 80g to 90g. If a centrifuge (6.2) is not available, allow the closed flask to stand in a rack (6.7) for at least 30 min until the supernatant layer is clear and distinctly separated from the aqueous layer.

9.5.6 Carefully remove the cork or stopper and rinse it and the inside of the neck of the fat-extraction flask with a little mixed solvent (5.6). Use the wash bottle (6.8) so that the rinsings run into the flask. If the interface is below the bottom of the stem of the flask, raise it slightly above this level by gently adding water down the side of the flask (see Figure 1) to facilitate the decantation of solvent.

NOTE In Figures 1 and 2, one of the three types of fat-extraction flask specified in ISO 3889|IDF 219 has been chosen, but this does not imply any preference over the other types.