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

*Crème — 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 2450|IDF 16 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.

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This fourth edition of ISO 2450|IDF 16 cancels and replaces the third edition (ISO 2450:1999), of which it constitutes a minor revision.

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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 at 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.

Draft International Standards adopted by the Action Teams and Standing Committees are circulated to the National Committees for voting. 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 2450|IDF 16 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 Action Team *Fat* of the Standing Committee on *Main components in milk* under the aegis of its project leader, Mr G.J. Beutick (NL).

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

**WARNING** — The use of this International Standard may involve hazardous materials, operations and equipment. This International Standard does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this International Standard to establish safety and health practices and determine the applicability of regulatory limitations prior to use.

## 1 Scope

This International Standard specifies the reference method for the determination of the fat content of raw, processed and sour cream in which no appreciable separation or breakdown of fat, due to lipolysis, has occurred.

The method is not applicable to sour creams with starch or other thickening agents.

**NOTE** If the method is not applicable and when separation or breakdown of fat occurs, a method utilizing the Weibull-Berntrop principle is suitable (see ISO 8262-3|IDF 124-3<sup>[3]</sup>).

## 2 Normative reference

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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 835, *Laboratory glassware — Graduated pipettes*

ISO 1042, *Laboratory glassware — One-mark volumetric flasks*

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

ISO 4788, *Laboratory glassware — Graduated measuring cylinders*

## 3 Terms and definitions

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

### 3.1

#### fat content of cream

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 portion 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 only 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 9.2.2).

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

NOTE If ammonia solution of this concentration is not available, a more concentrated solution of known concentration may be used (see 9.4.2).

**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 Clause A.5.)

**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 9.4.3). Other aqueous indicator solutions can be used provided that they do not affect the result of the determination.

**5.4 Diethyl ether** ( $\text{C}_2\text{H}_5\text{OC}_2\text{H}_5$ ), free from peroxides (see Clause A.3), containing no more than 2 mg/kg of antioxidants, and complying with the requirements for the blank test (see 9.2.2, Clauses A.1 and A.4).

**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 9.2.2, Clauses A.1 and A.4).

The use of pentane is recommended because of its higher purity and consistent quality.

**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 9.4.6).

**6.3 Distillation or evaporation apparatus**, for distilling the solvents and ethanol from the boiling or conical flasks, or evaporating from beakers and dishes (see 9.4.13) at a temperature not exceeding  $100 \text{ }^\circ\text{C}$ .

**6.4 Drying oven**, electrically heated, with ventilation port(s) fully open, capable of being maintained at a temperature of  $102 \text{ }^\circ\text{C} \pm 2 \text{ }^\circ\text{C}$  throughout its working space.

The oven shall be fitted with a suitable thermometer.

**6.5 Water bath**, capable of being maintained at a temperature between  $35 \text{ }^\circ\text{C}$  and  $50 \text{ }^\circ\text{C}$ .

**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 then the procedure is different. The alternative procedure is given in 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  $60 \text{ }^\circ\text{C}$  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**, 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. [ISO 2450:2008  
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**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 or silicon carbide (optional when metal dishes are used).

**6.11 Measuring cylinders**, of capacities 5 ml and 25 ml, complying with the requirements of ISO 4788, class A, or any other apparatus suitable for the product concerned.

**6.12 Pipettes**, graduated, of capacity 10 ml, complying with the requirements of ISO 835, class A.

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

**6.14 Volumetric flask**, one-mark, of capacity 100 ml, complying with the requirements of ISO 1042, class A.

## 7 Sampling

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

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>.

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

## 8 Preparation of test sample

Warm the test sample to a temperature of between 35 °C and 40 °C by means of the water bath (6.5), if necessary. Thoroughly, but gently, mix the test sample by repeatedly inverting the sample bottle, or, if the cream is very thick, by stirring with a spatula, without causing frothing or churning, and cool quickly to approximately 20 °C.

Sour cream should not be warmed but should be thoroughly stirred.

Churned cream should not be cooled as it has to be weighed at a temperature of between 30 °C and 40 °C (see 9.1).

NOTE A reliable value for the fat content cannot be expected:

- a) when a distinct smell of free fatty acids is perceptible;
- b) 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.

In these cases, a method utilizing the Weibull-Berntrop principle is suitable (see ISO 8262-3 | IDF 124-3<sup>[3]</sup>).

## 9 Procedure

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NOTE 1 If it is required to check whether the repeatability limit (11.2) is met, carry out two single determinations in accordance with 9.1 to 9.4.

NOTE 2 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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### 9.1 Test portion

Mix the test sample (Clause 8) by gently stirring or rotating and inverting the container several times. Immediately weigh to the nearest 1 mg, directly or by difference, a test portion yielding 0,3 g to 0,6 g of extracted fat (depending on the fat content of the cream) 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.

### 9.2 Blank tests

#### 9.2.1 Blank test for method

Carry out a blank test simultaneously with the determination using the same procedure and the same reagents, but replacing the dispersed test portion in 9.4.1 by 10 ml of water (see Clause A.2).

When one blank sample is used for a batch of test samples of which the individual samples may not have exactly the same conditions, ensure that the procedure for obtaining the value of the blank used in the calculation of the result corresponds exactly to that of 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 (9.2.2). Corrections of more than 2,5 mg should be mentioned in the test report.

#### 9.2.2 Blank test for reagents

To test the quality of the reagents, carry out a blank test as specified in 9.2.1. Additionally, use an empty fat-collecting vessel, prepared as specified in 9.3, for mass control purposes. The reagents shall leave no residue greater than 1,0 mg (see Clause A.1).

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 residue, which shall not exceed 1,0 mg.

Very occasionally, the solvents may contain volatile matter which is strongly retained in fat. If there are indications of the presence of such substances, carry out blank tests on all the reagents and for each solvent using a fat-collecting vessel with about 1 g of anhydrous butterfat. If necessary, redistil solvents in the presence of 1 g of anhydrous butterfat per 100 ml of solvent. Use the solvents only shortly after the redistillation.

Replace unsatisfactory reagents and solvents, or redistil solvents.

### 9.3 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 °C for 1 h.

NOTE 1 Boiling aids are desirable to promote gentle boiling during the subsequent removal of solvents, especially when using glass fat-collecting vessels; their use is optional with metal dishes.

Protect the fat-collecting vessel from dust and allow it to cool to the temperature of the weighing room (glass fat-collecting vessel for at least 1 h; metal dish for at least 30 min).

To avoid insufficient cooling or unduly long cooling times, the fat-collecting vessel should not be placed in a desiccator.

Use tongs 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, in particular, inducing temperature variations.

### 9.4 Determination

9.4.1 Carry out the determination without delay.

Add an amount of water at about 50 °C to the test portion in the fat-extraction flask (9.1) to obtain a total volume of 10 ml to 11 ml. Use the water to wash the test portion into the small bulb of the fat-extraction flask. Mix thoroughly with the test portion in the small bulb. Cool in running water to room temperature.

9.4.2 Add 2 ml of ammonia solution (5.1) to the test portion (9.4.1), or an equivalent volume of a more concentrated ammonia solution (see Note to 5.1). Mix thoroughly with the test portion in the small bulb of the fat-extraction flask.

9.4.3 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 near to the neck of the flask. If desired, add 2 drops of the Congo red solution (5.3).

9.4.4 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). Shake the flask vigorously, but not excessively, for 1 min, 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. If necessary, cool the flask in running water to about room temperature. Carefully remove the bung or stopper and rinse it and the neck of the flask with a little mixed solvent (5.6). Use the wash bottle (6.8) so that the rinsings run into the flask.

9.4.5 Add 25 ml of the light petroleum (5.5). Close the fat-extraction flask with the rewetted (by dipping into water) bung or stopper. Shake the flask gently again for 30 s as specified in 9.4.3. Proceed with shaking as specified in 9.4.4.