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**Whey cheese — Determination of fat  
content — Gravimetric method (Reference  
method)**

*Fromage de sérum — 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 3.

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.

International Standard ISO 1854 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, Subcommittee SC 5, *Milk and milk products*, in collaboration with the International Dairy Federation (IDF) and AOAC International, and will also be published by these organizations.

This third edition cancels and replaces the second edition (ISO 1854:1987), which has been technically revised.

Annexes A and B of this International Standard are for information only.

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# Whey cheese — Determination of fat content — Gravimetric method (Reference method)

**WARNING:** The use of this International Standard may involve hazardous materials, operations and equipment. This standard does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this 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 fat content of whey cheese.

**NOTE** If the whey cheese does not dissolve completely in the ammonia or if it contains free fatty acids in significant quantities (only in exceptional cases and then noticeable by a distinct smell), the result of the determination will be too low. With such products recourse should be made to a method utilizing the Weibull-Berntrop principle (see ISO 8262-3).

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## 2 Normative reference

The following normative document contains provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, this publications do not apply. However, parties to agreement based on this International Standard are encouraged to investigate the possibility of applying the most recent edition of the normative document indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

ISO 3889, *Milk and milk products — Determination of fat content — Mojonnier-type fat extraction flasks.*

## 3 Term and definition

For the purposes of this International Standard, the following term and definition apply.

### 3.1

#### fat content of whey cheese

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

**NOTE** The fat content is expressed as a mass fraction, in percent [formerly given as % (*m/m*)].

## 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 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 9.4.3).

**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.5.)

**5.3 Congo red solution**

Dissolve 1 g of Congo red in water in a 100 ml one-mark volumetric flask (6.14). Dilute 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 9.4.4). Other aqueous colour solutions may 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 A.3), containing no more than 2 mg/kg of antioxidants, and complying with the requirements for the blank test (see 9.2.2, A.1 and A.4).

NOTE The use of diethyl ether could lead to hazardous situations. Due to expected changes in safety regulations studies are ongoing to replace diethyl ether by another reagent provided that it does not affect the end result of the determination.

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

NOTE The use of pentane is recommended because of its higher purity and constant 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 80 g to 90 g at the outer end of the flasks or tubes.

NOTE The use of the centrifuge is optional but recommended (see 9.4.7).

**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 9.4.14) at a temperature not exceeding 100 °C.

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

The oven shall be fitted with a suitable thermometer.

**6.5 Water bath**, capable of boiling water.

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

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 bark corks or stoppers of other material [e.g. silicone rubber or polytetrafluoroethylene (PTFE)] unaffected by the reagents used. Bark corks 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**, 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 or silicon carbide (optional when metal dishes are used).

**6.11 Measuring cylinders**, of capacities 5 ml and 25 ml.

**6.12 Pipettes**, graduated, of capacity 10 ml. [ISO 1854:1999](https://standards.iteh.ai/catalog/standards/sist/0631baf6-7d88-4564-8cf9-154b6a877000/iso-1854-1999)

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

**6.14 Appropriate grinding device**.

**6.15 Volumetric flasks**, one-mark, of capacity 100 ml.

## 7 Sampling

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 707.

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

## 8 Preparation of test sample

Prepare the test sample using an appropriate device (6.14). Quickly mix the ground or grated mass and, if possible, grind it a second time. Again mix thoroughly. Clean the device after preparing each test sample.

If the test sample cannot be ground or grated, mix it thoroughly by intensive kneading, for example with a pestle in a mortar. The danger of moisture loss during grinding or grating of the sample should be avoided as far as practically possible.

Keep the prepared test sample in an airtight sample container until the time of analysis, which should be carried out on the same day. If delay is unavoidable, take every precaution to ensure proper storage of the test sample. When refrigerated, ensure that any condensation of moisture on the inside surface of the container is thoroughly and uniformly reincorporated into the test sample.

## 9 Procedure

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 in 6.6) is given in annex B.

### 9.1 Test portion

Mix the test sample (clause 8) by gently stirring or rotating and inverting the sample container several times. Immediately weigh to the nearest 1 mg, about 3 g of the prepared 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 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 same reagents, but replacing the dissolved test portion in 9.4.1 by 10 ml of water (see A.2).

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 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 and light petroleum, respectively. Use an empty fat-collecting vessel, prepared for control purposes as described above, 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 or solvents, or redistil the 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) set 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).



NOTE 2 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 3 Tongs should preferably be used to avoid, in particular, temperature variations.

## 9.4 Determination

### 9.4.1 Carry out the determination without delay.

Add 10 ml of pre-heated water at a temperature of  $65\text{ }^{\circ}\text{C} \pm 5\text{ }^{\circ}\text{C}$  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 completely into the small bulb of the fat-extraction flask. Mix thoroughly with the test portion in the small bulb.

9.4.2 Heat the contents of the fat-extraction flask in the boiling water bath (6.5). Shake gently occasionally until the test portion is completely dispersed. Leave the flask for 20 min in the boiling water bath. Then cool the flask in running water to room temperature.

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

9.4.4 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). If necessary, cool the flask in running water to room temperature.

9.4.5 Add 25 ml of diethyl ether (5.4). Close the fat-extraction flask with a cork 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 cork 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.

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

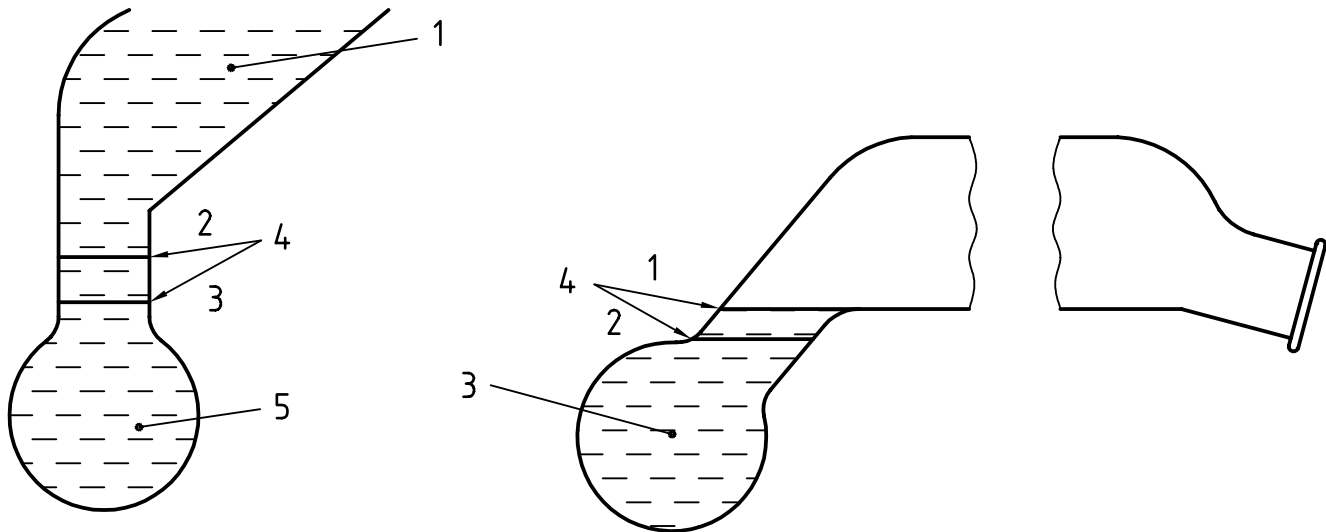
9.4.7 Centrifuge the closed fat-extraction flask for between 1 min and 5 min at a radial acceleration of 80 *g* to 90 *g*. If a centrifuge is not available, allow the closed flask to stand in the rack (6.7) for at least 30 min until the supernatant layer is clear and distinctly separated from the aqueous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

9.4.8 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 decanting of solvent.

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

9.4.9 Hold the fat-extraction flask by the small bulb and carefully decant as much as possible of the supernatant layer into the prepared fat-collecting vessel (see 9.3) containing a few boiling aids (6.10) in the case of a boiling or conical flask (optional with metal dishes). Avoid decanting any of the aqueous layer (see Figure 2).

9.4.10 Rinse the outside of the neck of the fat-extraction flask with a little mixed solvent (5.6). Collect the rinsings in the fat-collecting vessel. Take care that the mixed solvent does not spread over the outside of the fat-extraction flask. If desired, remove the solvent or a part of it from the fat-collecting vessel by distillation or evaporation as described in 9.4.14.



**Key**

- 1 Solvent
- 2 At second and third extraction
- 3 At first extraction
- 4 Interface
- 5 Aqueous layer

**Key**

- 1 At second and third extraction
- 2 At first extraction
- 3 Aqueous layer
- 4 Interface

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**Figure 1 — Before decanting**

**Figure 2 — After decanting**

**9.4.11** Add 5 ml of ethanol (5.2) to the contents of the fat-extraction flask. Use the ethanol to rinse the inside of the neck of the flask and mix as described in 9.4.4.

**9.4.12** Carry out a second extraction by repeating the operations described in 9.4.5 to 9.4.9 inclusive. Instead of 25 ml, use only 15 ml of diethyl ether (5.4) and 15 ml of light petroleum (5.5). Using the diethyl ether, rinse the inside of the neck of the fat-extraction flask too.

If necessary, raise the interface slightly to the middle of the stem of the flask by gently adding water down the side of the flask (see Figure 1) to enable the final decanting of solvent to be as complete as possible (see Figure 2).

**9.4.13** Carry out a third extraction without addition of ethanol by again repeating the operations described in 9.4.5 to 9.4.9 inclusive. Again, use only 15 ml of diethyl ether (5.4) and 15 ml of light petroleum (5.5). Using the diethyl ether, rinse the inside of the neck of the fat-extraction flask again.

If necessary, raise the interface slightly to the middle of the stem of the flask by gently adding water down the side of the flask (see Figure 1) to enable the final decanting of solvent to be as complete as possible (see Figure 2).

**NOTE** The third extraction may be omitted for products with a fat content of less than 3 %.

**9.4.14** Remove the solvents (including the ethanol) as completely as possible from the fat-collecting vessel, by distillation if using a boiling or conical flask, or by evaporation if using a beaker or dish (6.3). Rinse the inside of the neck of the boiling or conical flask with a little mixed solvent (5.6) before commencing the distillation.

**9.4.15** Heat the fat-collecting vessel, with the boiling or conical flask placed on its side to allow solvent vapour to escape, for 1 h in the drying oven (6.4) set at 102 °C. Remove the fat-collecting vessel from the oven and immediately verify whether or not the fat is clear. If the fat is not clear, fatty extraneous matter is presumed to be present and the whole procedure shall be repeated. If the fat is clear, protect the fat-collecting vessel from dust and allow the fat-collecting vessel to cool (preferably not in a desiccator) to the temperature of the weighing room (a glass fat-collecting vessel for at least 1 h, a metal dish for at least 30 min).

Do not wipe the fat-collecting vessel immediately before weighing. Use tongs to place the fat-collecting vessel on the balance. Weigh the fat-collecting vessel to the nearest 1,0 mg.

**9.4.16** Heat the fat-collecting vessel, with the boiling or conical flask placed on its side to allow solvent vapour to escape, for a further 30 min in the drying oven (6.4) set at 102 °C. Cool and reweigh as described in 9.4.15. If necessary, repeat the heating and weighing procedures until the mass of the fat-collecting vessel decreases by 1,0 mg or less, or increases between two successive weighings. Record the minimum mass as the mass of the fat-collecting vessel and extracted matter.

## 10 Calculation and expression of results

### 10.1 Calculation

Calculate the fat content of the sample using the following equation:

$$w_f = \frac{(m_1 - m_2) - (m_3 - m_4)}{m_0} \times 100 \%$$

where

$w_f$  is the mass fraction of fat in the sample, in percent;

$m_0$  is the mass of the test portion (9.1), in grams;

$m_1$  is the mass of the fat-collecting vessel and extracted matter, determined in 9.4.16, in grams;

$m_2$  is the mass of the prepared fat-collecting vessel (9.3), in grams;

$m_3$  is the mass of the fat-collecting vessel used in the blank test (9.2) and any extracted matter determined in 9.4.16, in grams;

$m_4$  is the mass of the fat-collecting vessel (9.3) used in the blank test (9.2), in grams.

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### 10.2 Expression of results

Round the result to two decimal places.

## 11 Precision

### 11.1 Interlaboratory test

Details of an interlaboratory test in accordance with ISO 5725<sup>1)</sup> on the precision of the method have been published (see reference [6]).

The values for repeatability and reproducibility limits are expressed for the 95 % probability level and may not be applicable to concentration ranges and matrices other than those given.

### 11.2 Repeatability

The absolute difference between two independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, will in not more than 5 % of cases be greater than a mass fraction of 0,2 %.

<sup>1)</sup> ISO 5725:1986 (now withdrawn) was used to obtain the precision data.