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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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## Contents

Page

Foreword.....	iv
Foreword.....	v
1 Scope .....	1
2 Normative reference .....	1
3 Terms and definitions .....	1
4 Principle.....	2
5 Reagents.....	2
6 Apparatus .....	2
7 Sampling.....	3
8 Preparation of test sample.....	4
9 Procedure .....	4
9.1 Test portion .....	4
9.2 Blank tests.....	4
9.3 Preparation of fat-collecting vessel.....	5
9.4 Determination.....	5
10 Calculation and expression of results.....	7
10.1 Calculation.....	7
10.2 Expression of results .....	8
11 Precision.....	8
11.1 Interlaboratory test .....	8
11.2 Repeatability.....	8
11.3 Reproducibility.....	8
12 Test report .....	8
Annex A (informative) Notes on procedures .....	9
Annex B (informative) Alternative procedure using fat-extraction tubes with siphon or wash-bottle fittings .....	11
Bibliography .....	14

## 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 1854|IDF 59 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 fourth edition of ISO 1854|IDF 59 cancels and replaces the third edition (ISO 1854:1999), 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 1854|IDF 59 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).

This edition of ISO 1854|IDF 59 cancels and replaces IDF 59:1986, of which it constitutes a minor revision.

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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 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 fat content of whey cheese.

The method is not applicable to products which do not dissolve completely in ammonia solution or which contain free fatty acids in significant quantities.

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

## 2 Normative reference

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 whey cheese**

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 9.4.3).

**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 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 9.4.4). 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 9.4.7).

**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  $100 \text{ }^{\circ}\text{C}$ .

**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 maintaining water at boiling point.

**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) 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.

**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 Appropriate grinding device.**

**6.15 Volumetric flasks**, 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>.

## **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 to 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 3,000 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 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 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) 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 (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, in particular, inducing 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  $65\text{ °C} \pm 5\text{ °C}$  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 to 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 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.

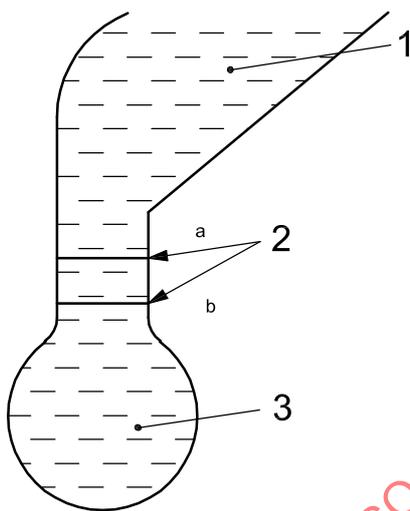
9.4.6 Add 25 ml of the light petroleum (5.5). Close the fat-extraction flask with the rewetted (by dipping into water) bung or stopper. Shake the flask gently again for 30 s as specified in 9.4.4. Proceed with shaking as specified in 9.4.5.

9.4.7 Centrifuge the closed fat-extraction flask for between 1 min and 5 min at a radial acceleration of 80g to 90g. If a centrifuge (6.2) 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 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. 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.

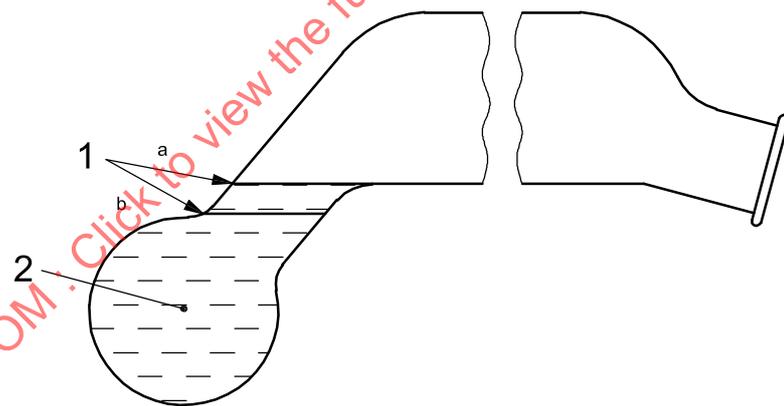
NOTE In Figures 1 and 2, one of the three types of fat-extraction flasks as specified in ISO 3889|IDF 219 has been chosen, but this does not imply any preference over other types.

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).



**Key**  
 1 solvent  
 2 interface  
 3 aqueous layer  
 a At second and third extraction.  
 b At first extraction.

Figure 1 — Before decanting



**Key**  
 1 interface  
 2 aqueous layer  
 a At second and third extraction.  
 b At first extraction.

Figure 2 — After decanting

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 specified in 9.4.14.

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 specified in 9.4.4.

**9.4.12** Carry out a second extraction by repeating the operations specified 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, also rinse the inner wall of the neck of the fat-extraction flask.

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 specified 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 % mass fraction.

**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) maintained 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) maintained at 102 °C. Cool and reweigh as specified 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,  $w_f$ , expressed as a percentage mass fraction, of the sample using Equation (1):

$$w_f = \frac{(m_1 - m_2) - (m_3 - m_4)}{m_0} \times 100 \quad (1)$$

where

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

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

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

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

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

## 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 5725:1986<sup>1)</sup> (see Reference [2]) on the precision of the method appear in Reference [4].

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 fat of 0,2 %.

### 11.3 Reproducibility

The absolute difference between two independent single test results, obtained using the same method on identical test material in different laboratories with different operators using different equipment, will in not more than 5 % of cases be greater than a mass fraction of fat of 0,3 %.

## 12 Test report

The test report shall include at least the following information:

- a) all information necessary for the complete identification of the sample;
- b) the sampling method used, if known;
- c) the test method used, together with reference to this International Standard;
- d) all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents which may have influenced the test result(s);
- e) the corrections made, if a value of more than 2,5 mg is obtained in the blank test for the method;
- f) the test result(s) obtained or, if the repeatability has been checked, the final quoted result obtained.

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1) ISO 5725:1986 (now superseded) was used to obtain the precision data.

## Annex A (informative)

### Notes on procedures

#### A.1 Blank test to check the reagents (see 9.2.2)

In this blank test, a fat-collecting vessel for mass control purposes has to be used so that changes in the atmospheric condition of the balance room or temperature effects of the fat-collecting vessel do not falsely suggest the presence or absence of non-volatile matter in the extract of the reagents. This fat-collecting vessel may be used as a counterweight vessel in the case of a two-pan balance. Otherwise, deviations of the apparent mass ( $m_3 - m_4$  in 10.1) of the fat-collecting vessel for control purposes should be considered when checking the mass of the fat-collecting vessel used for the blank test. Hence, the change in apparent mass of the fat-collecting vessel, corrected for the apparent change in mass of the fat-collecting vessel for control purposes, shall show no increase in mass greater than 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 redistillation.

#### A.2 Blank test carried out simultaneously with the determination (see 9.2.1)

The value obtained in the blank test, carried out simultaneously with the determination, enables the apparent mass of substances extracted from a test portion ( $m_1 - m_2$ ) to be corrected for the presence of any non-volatile matter derived from the reagents and also for any change of atmospheric conditions in the balance room and some temperature difference between the fat-collecting vessel and the balance room at the two weighings (9.4.16 and 9.3).

Under favourable conditions (low value in the blank test on reagents, constant temperature of the balance room, sufficient cooling time for fat-collecting vessel), the value will usually be less than 0,5 mg and can then be neglected in the calculation in the case of routine determinations. Slightly higher values (positive and negative) up to 2,5 mg are also often encountered. After correction for these values, the results will still be accurate. Corrections of more than 2,5 mg should be mentioned in the test report (Clause 12).

If the value obtained in this blank test regularly exceeds 1,0 mg, the reagents should be checked, if no recent check has been made. Any impure reagent or reagents traced should be replaced or purified (see 9.2.2 and Clause A.1).

#### A.3 Test for peroxides

To test for peroxides, add 1 ml of a freshly prepared 100 g/l potassium iodide solution to 10 ml of diethyl ether (5.4) in a small glass-stoppered cylinder which has been previously rinsed with the ether. Shake the cylinder and allow to stand for 1 min. No yellow colour should be observed in the diethyl ether layer.

Other suitable methods of testing for peroxides may be used.

To ensure that the diethyl ether is free, and is maintained free, from peroxides, treat the diethyl ether, at least 3 days before it is to be used, as follows.

Cut zinc foil into strips that will reach at least halfway up the bottle containing the diethyl ether, using approximately 8 000 mm<sup>2</sup> of foil per litre of diethyl ether.

Before use, completely immerse the strips of foil for 1 min in a solution containing 10 g of copper(II) sulfate pentahydrate (CuSO<sub>4</sub>·5H<sub>2</sub>O) and 2 ml/l of concentrated (98 % mass fraction) sulfuric acid. Wash the strips

gently but thoroughly with water, place the wet copper-plated strips in the bottle containing the diethyl ether, and leave the strips in the bottle.

Other methods may be used provided that they do not affect the result of the determination.

#### **A.4 Diethyl ether containing antioxidants**

Diethyl ether containing about 1 mg of antioxidants per kilogram is available in some countries, especially for fat determinations. This content does not exclude its use for reference purposes.

In other countries, diethyl ether with higher antioxidant contents, e.g. up to 7 mg/kg, is available. Such ether should only be used for routine determinations with an obligatory blank test carried out simultaneously with the determination(s) to correct for systematic errors due to the antioxidant residue. For reference purposes, such diethyl ether shall always be distilled before use.

#### **A.5 Ethanol**

Ethanol denatured otherwise than by the addition of methanol may be used, provided that the denaturant does not affect the result of the determination.

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## Annex B (informative)

### Alternative procedure using fat-extraction tubes with siphon or wash-bottle fittings

#### B.1 General

If fat-extraction tubes with siphon or wash-bottle fittings are to be used, use the procedure specified in this annex. The tubes shall be provided with good quality cork bungs or stoppers as specified for the flasks in 6.6 (see Figure B.1 as an example).

#### B.2 Procedure

##### B.2.1 Preparation of test sample

See Clause 8.

##### B.2.2 Test portion

Proceed as specified in 9.1 but using the fat-extraction tubes (see Note to 6.6 and Figure B.1).

The test portion shall be delivered as completely as possible to the bottom of the fat-extraction tube.

##### B.2.3 Blank test

See 9.2 and Clause A.2.

##### B.2.4 Preparation of fat-collecting vessel

See 9.3.

##### B.2.5 Determination

**B.2.5.1** Carry out the determination without delay.

Add about 10 ml of pre-heated water at a temperature of about 65 °C to the test portion in the fat-extraction tube (B.2.2) to obtain a total volume of 10 ml to 11 ml. Use the water to wash the test portion on to the bottom of the tube. Mix thoroughly.

**B.2.5.2** Heat the contents of the tube in the boiling water bath (6.5). Shake gently occasionally until the cheese is completely dispersed. Leave the tube for 20 min in the boiling water bath.

**B.2.5.3** Add 2 ml of ammonia solution (5.1) or an equivalent volume of a more concentrated ammonia solution (see Note to 5.1) to the fat-extraction tube. Mix thoroughly with the pretreated test portion at the bottom of the tube.

**B.2.5.4** Add 10 ml of ethanol (5.2). Mix gently but thoroughly at the bottom of the fat-extraction tube. If desired, add 2 drops of the Congo red solution (5.3).