SRI LANKA STANDARD 735: PART 1/SECTION 5 : 2011
ISO 1211: 2010

METHODS OF TEST FOR
MILK AND MILK PRODUCTS
PART 1 – DETERMINATION OF FAT CONTENT
Section 5 : Milk -Gravimetric method (Reference method)
(Third Revision)

SRI LANKA STANDARDS INSTITUTION

Sri Lanka Standard

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SLS 735: Part 1/Section 5 : 2011
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Sri Lanka Standards are subject to periodical revision in order to accommodate the progress made by industry. Suggestions for improvement will be recorded and brought to the notice of the Committees to which the revisions are entrusted.

This standard does not purport to include all the necessary provisions of a contract.

Sri Lanka Standard
METHODS OF TEST FOR MILK AND MILK PRODUCTS
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Section 5 : Milk -Gravimetric method (Reference method)
(Third Revision)

NATIONAL FOREWORD

This Sri Lanka standard was approved by the Sectoral Committee on Agricultural and Food Products and was authorized for adoption and publication as a Sri Lanka Standard by the Council of the Sri Lanka Standards Institution on 2011-10-27.

This Sri Lanka Standard was first published in 1986 and subsequently revised in 2006 and in 2009.
This standard prescribes the general methods for determining whether the material conforms to the requirements of the relevant individual standards and thus form a necessary adjunct to series of Sri Lanka Standard Specification for individual milk and milk products. However, keeping in view the experience gained during the years and various International Standards brought out by the International Organization for Standardization (ISO) on the subject of testing milk and milk products, it was decided to adopt this test method as a national standard.

In order to accommodate the large number of test methods within the scope of one standard, this standard is published in several parts.

Part 1 of the standard consists of several sections.
Section 5 of the standard is identical with ISO 1211 : 2010, Milk-Determination of fat content-Gravimetric method (Reference method), published by the International Organization for Standardization (ISO) and also IDF 1 : 2010, published by the International Dairy Federation (IDF).

Terminology and Conventions:

The text of the International Standard has been accepted as suitable for publication, without deviation, as a Sri Lanka Standard. However, certain terminology and conventions are not identical with those used in Sri Lanka Standards. Attention is therefore drawn to the following:

a) Wherever the words “International Standard” appear referring to this standard should be interpreted as “Sri Lanka Standard”.

b) The comma has been used throughout as a decimal marker. In Sri Lanka Standards it is the current practice to use the full point on the base line as the decimal marker.

c) Wherever page numbers are quoted, they are ISO page numbers.

Cross References

International Standard	Corresponding Sri Lanka standard
ISO 3889, Milk and milk products-Specification of Mojonnier-type fat extraction flasks	No corresponding Sri Lanka standard

INTERNATIONAL STANDARD

ISO 1211

IDF 1

Third edition
2010-06-01

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

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

Reference numbers
ISO 1211:2010(E)
IDF 1:2010(E)

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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.

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.

INTERNATIONAL STANDARD

ISO 1211:2010(E)
IDF 1:2010(E)

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[7] specifies a special method for skimmed milk products.

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 NH_3 of approximately 25 % [ρ_{20} (NH_3) = 910 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 (C_2H_5OH), 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 ($C_{32}H_{22}N_6Na_2O_6S_2$) 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 ($C_2H_5OC_2H_5$), 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** ($CH_3[CH_2]_3CH_3$) 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 min^{-1} to 600 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[4] class A, or any other apparatus suitable for the product concerned.

6.12 Pipettes, graduated, capacity 10 ml, ISO 835[2] 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[3] 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[1].

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 °C ± 2 °C. Gently mix the test sample thoroughly without causing frothing or churning. Then cool the test sample quickly to 20 °C ± 2 °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 °C ± 2 °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.

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 °C ± 2 °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.

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.

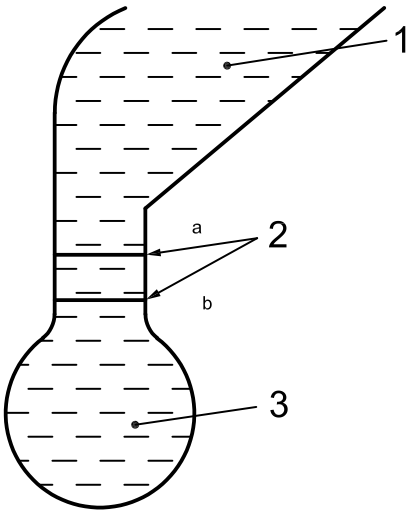
9.5.4 Add 25 ml of the light petroleum (5.5). Close the fat-extraction flask with the bung or stopper. Mix again for 30 s as specified in 9.5.3.

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.

9.5.7 Hold the fat-extraction flask by the small bulb and carefully decant as much as possible of the supernatant layer (solvent Figure 1) into the prepared fat-collecting vessel (see 9.4). Avoid decantation of any of the aqueous layer (see Figure 2).

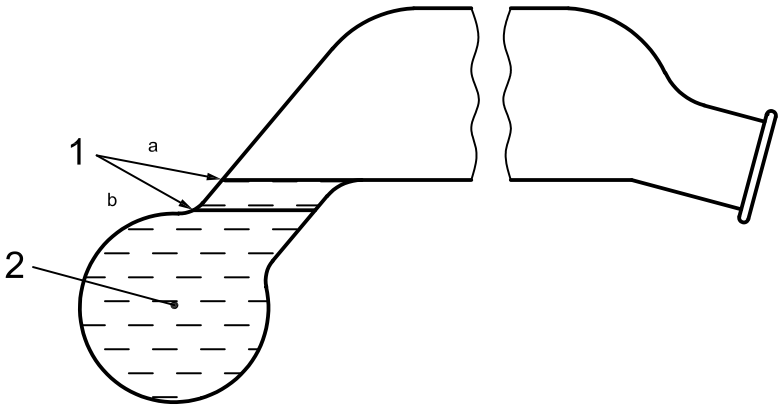


Key

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

9.5.8 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 9.5.12.

9.5.9 Add 5 ml of ethanol (5.2) to the contents of the fat-extraction flask. Mix as specified in 9.5.2. If Congo red solution (5.3) has been previously added, add no further solution.

9.5.10 Carry out a second extraction by repeating the operations specified in 9.5.3 to 9.5.7 inclusive. Instead of 25 ml, use only 15 ml of diethyl ether (5.4) and 15 ml of light petroleum (5.5). 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 decantation of solvent to be as complete as possible (see Figure 2).

9.5.11 Carry out a third extraction without addition of ethanol by again repeating the operations specified in 9.5.3 to 9.5.7 inclusive. Again, use only 15 ml of diethyl ether (5.4) and 15 ml of light petroleum (5.5). 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 decantation of solvent to be as complete as possible (see Figure 2).

The third extraction may be omitted for milk with a fat content of less than 0,5 % mass fraction.

9.5.12 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 neck of the boiling or conical flask with a little mixed solvent (5.6) before commencing the distillation.

9.5.13 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 ± 2 °C.

Remove the fat-collecting vessel from the oven while immediately verifying whether the fat is clear. If the fat is not clear, fat-extraneous matter is presumed to be present and the whole procedure shall be repeated. If clear, protect the fat-collecting vessel from dust and allow it 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.

Do not wipe the fat-collecting vessel immediately before weighing. Use tongs (6.13) to place the fat-collecting vessel on the analytical balance (6.1). Weigh the fat-collecting vessel to the nearest 1,0 mg.

9.5.14 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 ± 2 °C. Cool and reweigh as specified in 9.5.13. If necessary, repeat the heating and weighing procedures until the mass of the fat-collecting vessel decreases by 2,0 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 test sample, w_f, expressed as a percentage mass fraction, by 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 (9.2);

m_1 is the mass, in grams, of the fat-collecting vessel and extracted matter, determined in 9.5.14;

m_2 is the mass, in grams, of the prepared fat-collecting vessel (9.4);

m_3 is the mass, in grams, of the fat-collecting vessel used in the blank test (9.3.1) and any extracted matter determined in 9.5.14;

m_4 is the mass, in grams, of the fat-collecting vessel (9.4) used in the blank test (9.3.1).

10.2 Expression of results

Round the result to two decimal places.

11 Precision

11.1 Interlaboratory test

The values for repeatability and reproducibility derived from this interlaboratory test were determined in accordance with ISO 5725-1[5] and ISO 5725-2[6].

Details of interlaboratory tests on the precision of the method are given in Annex C and Annex D, respectively (see also Reference [8]). The values obtained may not be applicable to concentration ranges and matrices other than those given.

11.2 Repeatability

The absolute difference between two individual single test results, obtained with 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) 0,031 % mass fraction for skimmed cow milk;

b) 0,036 % mass fraction for reduced fat cow milk;

c) 0,043 % mass fraction for whole cow milk;

d) 0,030 % mass fraction for goat milk;

e) 0,069 % mass fraction for sheep milk.

11.3 Reproducibility

The absolute difference between two individual single test results, obtained with 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) 0,043 % mass fraction for skimmed cow milk;

b) 0,042 % mass fraction for reduced fat cow milk;

c) 0,056 % mass fraction for whole cow milk;

d) 0,052 % mass fraction for goat milk;

e) 0,096 % mass fraction for sheep milk.

12 Test report

The test report shall contain 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 (ISO 1211 | IDF 1:2010);

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; and if the repeatability has been checked, the final quoted result obtained.

Annex A
(informative)

Notes on procedures

A.1 Blank test carried out simultaneously with the determination (see 9.3.1)

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 temperature difference between the fat-collecting vessel and the balance room at the two weighings (9.5.14 and 9.4). When one blank sample is used for a batch of test samples, ensure that the blank vessel accompanies the test sample vessels until the last vessel has reached constant mass.

To calculate the fat content, use the mass of the blank vessel that corresponds to the drying cycle of the test samples [i.e. for a test vessel that reached constant mass at drying cycle n, use the blank mass at drying cycle n; for drying cycle $(n + 1)$ use the blank mass of drying cycle $(n + 1)$ etc. (see 9.3.1)].

Under favourable conditions, such as a low value in the blank test on reagents, constant temperature of the balance room, sufficient cooling time for fat-collecting vessel, the value is usually less than 1,0 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 are still 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 9.3.2 and Clause A.2).

A.2 Blank test to check the reagents (see 9.3.2)

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.

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. The 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, solvents can 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 solvents only shortly after redistillation.

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 previously rinsed with the same solvent. Shake the cylinder and allow to stand for 1 min. No yellow colour should be observed in either layer.

Other suitable methods of testing for peroxides may also be used.

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

Cut zinc foil into strips reaching at least half-way up the bottle containing the diethyl ether. As such that needs approximately 8 000 mm^2 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 ($CuSO_4 \cdot 5H_2O$) and 2 ml of concentrated sulfuric acid (98 % mass fraction) per litre. 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 Ethanol

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

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 employed, 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 9.2, 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 9.3 and A.1.

B.2.4 Preparation of fat-collecting vessel

See 9.4.

B.2.5 Determination

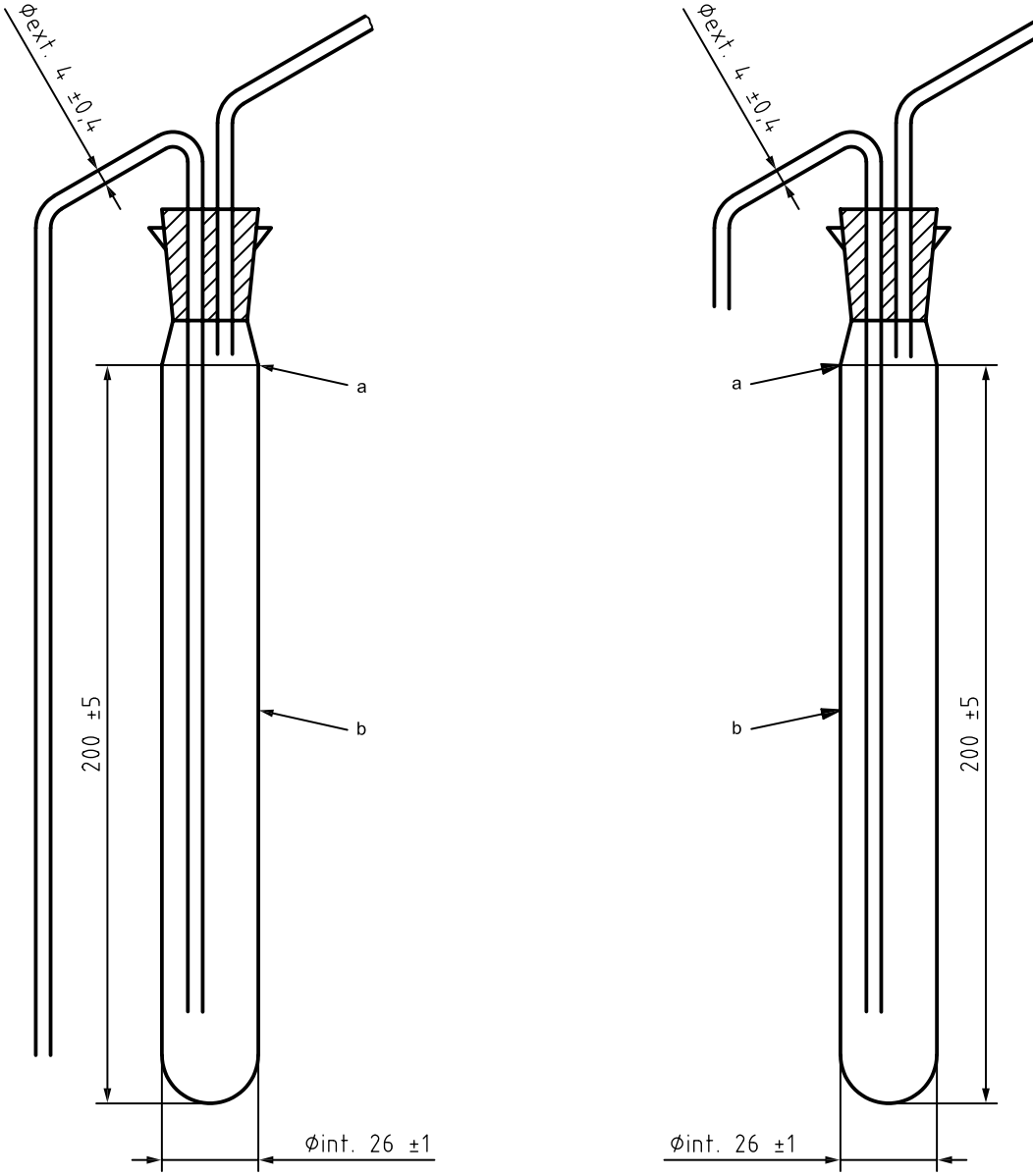
B.2.5.1 Carry out the determination without delay.

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 tube (B.2.2) Mix thoroughly with the pretreated test portion at the bottom of the fat-extraction tube.

B.2.5.2 Add 10 ml of ethanol (5.2). Mix gently but thoroughly with the mixture at the bottom of the fat-extraction tube. If desired, add 2 drops of the Congo red solution (5.3).

B.2.5.3 Add 25 ml of diethyl ether (5.4). Close the fat-extraction tube with a cork bung saturated with water or with a stopper of other material wetted with water (6.6). Shake the tube vigorously, but not excessively, with repeated inversions, for 1 min, to avoid the formation of persistent emulsions. Carefully remove the bung or stopper and rinse it and the neck of the tube with a little mixed solvent (5.6). Use the wash bottle (6.8) so that the rinsings run into the tube.

Dimensions in millimetre



a) With siphon fitting **b) With wash-bottle fitting**

[a] Capacity to this level with fittings removed 105 ml ± 5 ml.

[b] Wall thickness 1,5 mm ± 0,5 mm.

Figure B.1 — Examples of fat-extraction tubes

B.2.5.4 Add 25 ml of light petroleum (5.5). Close the fat-extraction tube with the rewetted (by dipping in water) bung or stopper. Shake the tube gently for 30 s, as described in B.2.5.3.

B.2.5.5 Centrifuge the closed fat-extraction tube for 1 min to 5 min at a radial acceleration of 80*g* to 90*g*. If a centrifuge (6.2) is not available, allow the closed tube 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. If necessary, cool the tube in running water to room temperature.

B.2.5.6 Carefully remove the cork or stopper and rinse it and the neck of the fat-extraction tube with a little mixed solvent (5.6). Use the wash bottle (6.8) so that the rinsings run into the tube.

B.2.5.7 Insert a siphon fitting or a wash-bottle fitting into the fat-extraction tube. Push down the long inner limb of the fitting until the inlet is approximately 4 mm above the interface between the layers. The inner limb of the fitting shall be parallel to the axis of the fat-extraction tube.

Carefully transfer the supernatant layer out of the fat-extraction tube into the fat-collecting vessel (see 9.4) containing a few boiling aids (6.10) in the case of boiling or conical flasks (optional). Avoid the transfer of any of the aqueous layer. Rinse the outlet of the fitting with a little mixed solvent collecting the rinsings in the fat-collecting vessel.

NOTE The supernatant layer can be transferred out of the fat-extraction tube by using, for example, a rubber bulb attached to the short stem to apply pressure.

B.2.5.8 Loosen the fitting from the neck of the fat-extraction tube. Slightly raise the fitting and rinse the lower part of its long inner limb with a little mixed solvent (5.6). Lower and re-insert the fitting and transfer the rinsings to the fat-collecting vessel.

Rinse the outlet of the fitting with a little mixed solvent again, collecting the rinsings in the fat-collecting vessel. If desired, remove the solvent or a part of it from the fat-collecting vessel by distillation or evaporation as specified in 9.5.12.

B.2.5.9 Again loosen the fitting from the neck. Slightly raise the fitting and add 5 ml of ethanol to the content of the fat-extraction tube. Using the ethanol, rinse the long inner limb of the fitting. Mix as described in B.2.5.2.

B.2.5.10 Carry out a second extraction by repeating the operations described in B.2.5.3 to B.2.5.8. 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 long inner limb of the fitting during the removal of the fitting from the fat-extraction tube after the previous extraction.

B.2.5.11 Carry out a third extraction without the addition of ethanol by again repeating the operations described in B.2.5.3 to B.2.5.8. Again, use only 15 ml of diethyl ether and 15 ml of light petroleum. Using the diethyl ether, rinse the long inner limb of the fitting as described in B.2.5.10.

The third extraction may be omitted for milk with a fat content of less than 0,5 % mass fraction.

B.2.5.12 Proceed as specified in 9.5.12 to 9.5.14.

Annex C
(informative)

Interlaboratory trial on raw milk

C.1 General

An international collaborative test involving 19 laboratories from 13 countries was carried out in 2005-12 (see Reference [8]). The test was carried out on 12 pairs of blind duplicate samples comprising:

a) three pairs of skimmed milk samples with a fat content, $w_f < 0{,}5$ g/100 g;

b) three pairs of reduced fat milk samples with a fat content in the range 0,5 g/100 g $\leqslant w_f \leqslant$ 2 g/100 g;

c) six pairs of raw milk samples with a fat content in the range 3 g/100 g $\leqslant w_f \leqslant$ 6 g/100 g.

The test was organized by the Associazione Italiana Allevatori, Laboratorio Standard Latte, Maccarese, Italy.

The results obtained were subject to statistical analyses in accordance with ISO 5725-1[5] and ISO 5725-2[6] to give the precision data shown in Tables C.1, C.2 and C.3, respectively.

C.2 Test results

Table C.1 — Results for skimmed milk

Parameters	Sample			Grand Mean[a]
	3	12	1	
No. of participating laboratories after eliminating outliers	11	10	11	—
Mean value, g/100 g	0,222	0,336	0,487	0,348
Repeatability standard deviation, s_r, g/100 g	0,011	0,010	0,012	0,011
Repeatability limit r (2,8s_r), g/100 g	0,030	0,028	0,034	0,031
Coefficient of variation of repeatability, $C_{V,r}$, %	13,7	8,3	7,0	8,9
Reproducibility standard deviation, s_R, g/100 g	0,018	0,010	0,017	0,016
Reproducibility limit R (2,8s_R), g/100 g	0,051	0,028	0,047	0,043
Coefficient of variation of reproducibility, $C_{V,R}$, %	23,0	8,5	9,6	12,5

[a] The mean values were calculated using only sample data with outliers removed. All other statistical means were calculated from the square root of the average of the squared deviations.

Table C.2 — Results for reduced fat milk

Parameters	Sample			Grand Mean[a]
	7	6	2	
No. of participating laboratories after eliminating outliers	11	11	11	—
Mean value, g/100 g	0,561	1,368	2,039	1,323
Repeatability standard deviation, s_r, g/100 g	0,011	0,011	0,016	0,013
Repeatability limit r (2,8·s_r), g/100 g	0,031	0,032	0,044	0,036
Coefficient of variation of repeatability, $C_{V,\,r}$, %	5,5	2,4	2,2	2,7
Reproducibility standard deviation, s_R, g/100 g	0,016	0,013	0,016	0,015
Reproducibility limit R (2,8·s_R), g/100 g	0,044	0,036	0,045	0,042
Coefficient of variation of reproducibility, $C_{V,\,R}$, %	7,8	2,6	2,2	3,2

[a] The mean values were calculated using only sample data with outliers removed. All other statistical means were calculated from the square root of the average of the squared deviations.

Table C.3 — Results for whole milk

Parameters	Sample						Grand Mean[a]
	9	5	10	4	11	8	
No. of participating laboratories after eliminating outliers	10	11	10	11	9	11	—
Mean value, g/100 g	3,032	3,287	4,052	4,305	5,503	5,825	4,334
Repeatability standard deviation, s_r, g/100 g	0,010	0,017	0,011	0,022	0,014	0,013	0,015
Repeatability limit r (2,8·s_r), g/100 g	0,028	0,047	0,031	0,063	0,040	0,038	0,043
Coefficient of variation of repeatability, $C_{V,\,r}$, %	0,9	1,4	0,8	1,5	0,7	0,7	1,0
Reproducibility standard deviation, s_R, g/100 g	0,014	0,021	0,013	0,025	0,015	0,025	0,020
Reproducibility limit R (2,8·s_R), g/100 g	0,040	0,059	0,037	0,071	0,043	0,069	0,056
Coefficient of variation of reproducibility, $C_{V,\,R}$, %	1,3	1,8	0,9	1,7	0,8	1,2	1,3

[a] The mean values were calculated using only sample data with outliers removed. All other statistical means were calculated from the square root of the average of the squared deviations.

Annex D
(informative)

Interlaboratory trial on raw sheep milk and raw goat milk

D.1 General

An international collaborative test (see Reference [8]) involving 16 laboratories from nine countries was carried out in 2006-11.

The test included six pairs of blind duplicate samples for each type of milk. Those of sheep milk samples had a fat content of 4,5 g per 100 g to 8,5 g per 100 g, those of goat milk samples a fat content of 1,5 g per 100 g to 5,0 g per 100 g.

The studies were organized by the Associazione Italiana Allevatori, Laboratorio Standard Latte, Maccarese, Italy. The results obtained were subject to statistical analyses in accordance with ISO 5725-1[5] and ISO 5725-2[6] to give the precision data shown in Tables D1 and D.2, respectively.

D.2 Test results

Table D.1 — Results for sheep milk

Parameters	Sample						Grand Mean[a]
	9	5	10	4	11	8	
No. of participating laboratories after eliminating outliers	14	12	13	14	12	14	—
Mean value, g/100 g	6,492	4,497	5,554	8,334	7,312	7,877	6,678
Repeatability standard deviation, s_r, g/100 g	0,032	0,022	0,013	0,032	0,012	0,028	0,025
Repeatability limit r (2,8s_r), g/100 g	0,090	0,062	0,038	0,090	0,033	0,078	0,069
Coefficient of variation of repeatability, $C_{V,\,r}$, %	1,4	1,4	0,7	1,1	0,4	1,0	1,0
Reproducibility standard deviation, s_R, g/100 g	0,044	0,022	0,033	0,042	0,025	0,033	0,034
Reproducibility limit R (2,8s_R), g/100 g	0,123	0,062	0,091	0,119	0,069	0,092	0,096
Coefficient of variation of reproducibility, $C_{V,\,R}$, %	1,9	1,4	1,6	1,4	0,9	1,2	1,4

[a] The mean values were calculated using only sample data with outliers removed. All other statistical means were calculated from the square root of the average of the squared deviations.

Table D.2 — Results for goat milk

Parameters	Sample						Grand Mean[a]
	1	2	3	4	5	6	
No. of participating laboratories after eliminating outliers	12	14	12	14	14	13	—
Mean value, g/100 g	3,017	1,542	4,870	2,200	4,405	3,673	3,285
Repeatability standard deviation, s_r, g/100 g	0,008	0,012	0,011	0,008	0,012	0,010	0,011
Repeatability limit r (2,8·s_r), g/100 g	0,023	0,035	0,031	0,023	0,035	0,029	0,030
Coefficient of variation of repeatability, $C_{V,\,r}$, %	0,7	2,3	0,6	1,1	0,8	0,8	0,9
Reproducibility standard deviation, s_R, g/100 g	0,017	0,018	0,020	0,019	0,023	0,015	0,019
Reproducibility limit R (2,8·s_R), g/100 g	0,048	0,051	0,055	0,053	0,063	0,042	0,052
Coefficient of variation of reproducibility, $C_{V,\,R}$, %	1,6	3,3	1,1	2,4	1,4	1,1	1,6

[a] The mean values were calculated using only sample data with outliers removed. All other statistical means were calculated from the square root of the average of the squared deviations.

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