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Milk, dried milk products and cream — Determination of fat content — Gravimetric method

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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.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document 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.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

IDF (the International Dairy Federation) is a non-profit private sector organization representing the interests of various stakeholders in dairying at the global level. IDF members are organized in National Committees, which are national associations composed of representatives of dairy-related national interest groups including dairy farmers, dairy processing industry, dairy suppliers, academics and governments/food control authorities.

ISO and IDF collaborate closely on all matters of standardization relating to methods of analysis and sampling for milk and milk products. Since 2001, ISO and IDF jointly publish their International Standards using the logos and reference numbers of both organizations.

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This document was prepared by the IDF *Standing Committee on Analytical Methods for Composition* and ISO Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*. It is being published jointly by ISO and IDF.

The work was carried out by the ISO/IDF Action Team on C32 of the *Standing Committee on Analytical Methods for Composition* under the aegis of its project leader, Mr P. Trossat (FR).

Milk, dried milk products and cream — Determination of fat content — Gravimetric method

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

1 Scope

This document specifies the method for the determination of fat content.

The method is applicable to:

a) raw milk (cow, sheep, goat), reduced fat milk, skimmed milk, chemically preserved milk and processed liquid milk;

b) dried milk products (e.g. whole, partially skimmed, skimmed milk powder; dairy permeate powder; whey powder; blend skimmed milk powder and vegetable fat; milk based infant formula powder);

c) raw, processed and sour cream.

For the following products, the precision figures are given in Annex H. These precision figures are derived from interlaboratory studies not conforming to the requirements from ISO 5725-2 in terms of number of samples (< 6) and number of participating laboratories (< 8).

d) evaporated milk and sweetened condensed milk (e.g. liquid sweetened and unsweetened concentrated milk);

e) whey cheese as defined in CODEX CXS 284-1999;

f) liquid whey and buttermilk;

g) milk-based edible ices and ice mixes;

h) liquid concentrated infant foods.

The method does not apply in the following cases:

— For b), when the powder contains hard lumps which do not dissolve in ammonia solution. This is noticeable by a distinct smell and the result of the determination will be too low. In such cases, a method using the Weibull-Berntrop principle is suitable, e.g. ISO 8262-3|IDF 124-3.

— For c), The method is not applicable to sour creams with starch or other thickening agents. When separation or breakdown of fat occurs, a method using the Weibull-Berntrop principle is suitable, e.g. ISO 8262-3|IDF 124-3.

— For e), to products which do not dissolve completely in ammonia solution, as the result of the determination will be too low. With such products, a method using the Weibull-Berntrop principle is suitable, e.g. ISO 8262-3|IDF 124-3.

— For g), to milk-based edible ices and ice mixes in which the level of emulsifier, stabilizer or thickening agent or of egg yolk or of fruits, or of combinations of these constituents, makes the Röse-Gottlieb method unsuitable. With such products, a method using the Weibull-Berntrop principle is suitable, e.g. ISO 8262-2|IDF 124-2.

— For h), to products which do not dissolve completely in ammonia due to the presence of starch or dextrin at mass fractions of more than 5 % (in dry matter), or to the presence of hard lumps. For such products, a method using the Weibull-Berntrop principle is suitable, e.g. ISO 8262-1|IDF 124-1.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1
fat content
mass fraction of substances

Note 1 to entry: Fat content is determined by the procedure specified in this document.

Note 2 to entry: The fat content is expressed as a percentage by mass.

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 principle is usually described 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 Annex A).

5.1 Ammonia solution, containing a mass fraction of NH_3 of approximately 25 %.

If ammonia solution of this concentration is not available, a more concentrated solution of known concentration may be used (see 9.4.2.1).

5.2 Ethanol (C_2H_5OH), or ethanol denatured by methanol, containing a volume fraction of ethanol of at least 94 %, see Clause A.5.

5.3 Indicator solution (optional). The use of indicator solutions allows the interface between the solvent and aqueous layers to be seen more clearly (see 9.4.2.3). Other aqueous indicator solutions can be used provided that they do not affect the result of the determination.

5.3.1 Bromocresol purple solution, mass concentration 1 g/100 ml. Dissolve 1 g of bromocresol purple in 10 ml of ethanol and dilute to 100 ml with water.

5.3.2 Patent blue V. Dissolve 1 g of patent blue V (sodium salt) in water and dilute to 100 ml with water.

5.4 Diethyl ether ($C_2H_5OC_2H_5$), free from peroxides (see Clause A.3), containing no more than 7 mg/kg of antioxidants, and conforming to the requirements for the blank test (see 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 ($CH_3(CH_2)_3CH_3$) with a boiling point of 36 °C and conforming to the requirements for the blank test (see Clauses A.1 and A.4).

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

NOTE Petroleum ether and petroleum benzine with an appropriate boiling range are some suitable commercial names of this reagent.

5.6 Mixed solvent.

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

6 Apparatus

The usual laboratory equipment and, in particular, the following shall be used.

Use graduated pipettes in accordance with ISO 835, one-mark volumetric flasks in accordance with ISO 1042 and graduated measuring cylinders in accordance with ISO 4788.

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 to produce a radial acceleration of around 80*g* to 90*g* at the outer end of the flasks or tubes.

The use of the centrifuge is optional but recommended (see 9.4.2.6).

6.3 Distillation or evaporation apparatus, for distilling the solvents and ethanol from the boiling or conical flasks, or evaporating from beakers and dishes (see 9.4.2.12) 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 °C ± 2 °C throughout its working space. The oven shall be fitted with a suitable thermometer.

6.5 Water bath, capable of being maintained at a temperature of 37,5 °C ± 2,5 °C, 50 °C ± 5 °C, 65 °C ± 5 °C and at boiling point.

6.6 Fat-extraction flasks:

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

6.6.2 Extraction tubes-type fat extraction flask. It is also possible to use fat extraction tubes with siphon or wash-bottle fittings (see the model given in Annex B).

6.6.3 Stoppers. The flasks or tubes shall be provided with stoppers of different material [bark cork, silicone rubber, polytetrafluoroethylene (PTFE) or glass] unaffected by the reagents used. Bark corks shall be washed with diethyl ether (5.4), kept in water at 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 plastic wash bottle shall not be used.

6.9 Fat-collecting vessels, e.g.:

— boiling flasks, flat-bottomed, of capacity 125 ml to 250 ml;

— conical flasks, of capacity 250 ml;

— 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 Measuring cylinders or delivering systems, compatible with the use of solvents of capacities 5 ml and 25 ml.

6.11 Pipettes or delivering systems, of capacity 10 ml.

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

6.13 Blender, fitted with a bowl with a capacity of 1 l with its lid or any other device suitable for preparing the test sample.

6.14 Boiling aids (optional), fat free in non-porous porcelain, silicon carbide or glass.

7 Sampling

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

Sampling is not part of the method specified in this document. A recommended sampling method is given in ISO 707|IDF 50.

From the time of sampling to the time of commencing the procedure, store laboratory liquid samples at a temperature of 4 °C ± 2 °C and dried products at room temperature. For evaporated milk, sweetened condensed milk and milk-based infant foods, store samples in sealed cans or bottles unopened at a temperature below 20 °C, until the time of starting the procedure.

8 Preparation of test sample

8.1 Milk

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 approximately 20 °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.

8.2 Dried milk products

Thoroughly mix the test sample by repeatedly rotating and inverting the sample container. If necessary, transfer all the test sample to an airtight container of approximately twice the volume of the test sample to allow this operation to be carried out.

8.3 Evaporated milk

Shake and invert the sample container. Open the sample container and pour the sample slowly into a second sample container, provided with an airtight lid. Mix by repeated transfer, taking care to incorporate in the sample any fat or other constituent adhering to the wall and ends of the first container. Finally, transfer the product as completely as possible to the second container.

If necessary in the case of samples in sealed cans, condition the unopened container in the water bath (6.5) maintained at a temperature of 50 °C ± 5 °C. Remove and shake the can vigorously every 15 min. After 2 h, remove the can and allow it to cool to room temperature.

Remove the lid entirely and thoroughly mix the sample by stirring with a spoon or spatula. If fat separates, do not test the sample.

8.4 Sweetened condensed milk

Open the sample container and mix thoroughly with a spoon or spatula. Use an up-and-down rotary movement in such a way that the top layers and the content of the lower corners of the container are moved and mixed. Take care to incorporate in the sample any milk adhering to the wall and ends of the container. Transfer the sample as completely as possible to a second sample container, provided with an airtight lid. Close the second container.

If necessary, in the case of samples in sealed cans, condition the unopened can in the water bath (6.5) at a temperature of 38 °C ± 2 °C. Open the can, scrape out all milk adhering to the interior of the can, transfer to a dish large enough to permit stirring thoroughly and mix until the whole mass is homogeneous.

In the case of a sample in a collapsible tube, open the tube and transfer the contents to a jar. Then cut the tube and scrape out all material adhering to the interior and add to the contents of the jar.

8.5 Whey cheese

Prepare the test sample using an appropriate device. 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, e.g. with a pestle in a mortar. The risk 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.

8.6 Cream

Warm the test sample to a temperature of 38 °C ± 2 °C by means of the water bath (6.5), if necessary. Thoroughly, but gently, mix the test sample by repeatedly inverting the sample bottle, or, if the cream is very thick, by stirring with a spatula, without causing frothing or churning, and cool quickly to approximately 20 °C.

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

NOTE A reliable value for the fat content cannot be expected when a distinct smell of free fatty acids is perceptible, and/or if during or after preparation of the test sample white particles are visible on the walls of the sample bottle or fat droplets float on the surface of the sample. In such cases, a method utilizing the Weibull-Berntrop principle is suitable, such as ISO 8262-3|IDF 124-3.

8.7 Skimmed milk, whey and buttermilk

Warm the test sample to a temperature of 38 °C ± 2 °C by means of the water bath (6.5), if necessary. Gently mix the test sample thoroughly by repeatedly inverting the sample bottle without causing frothing or churning. Cool the test sample quickly to approximately 20 °C.

NOTE A reliable value for the fat content cannot be expected if the milk is churned, when a distinct smell of free fatty acids is perceptible and/or 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.

8.8 Milk-based infant foods

8.8.1 Liquid products

Shake and invert the sample container. Open the container to pour the product slowly into a second sample container provided with an airtight lid. Mix by repeated transfer of the product, taking care to incorporate in the sample any fat or other constituent adhering to the wall and ends of the first container. Transfer the test sample as completely as possible to the second sample container. Close this container.

If necessary, condition the unopened sample container in the water bath (6.5) at a temperature of 50 °C ± 5 °C. Remove and shake the container vigorously every 15 min. After 2 h, remove the container, dry the outside with a tissue and allow to cool to room temperature. Remove the lid or cap entirely and thoroughly mix the contents by stirring with a spoon or spatula. If fat separates, do not test the sample. Transfer the test sample as completely as possible to a second sample container provided with an airtight lid. Close this container.

8.8.2 Viscous or pasty products

Open the sample container and thoroughly mix the contents with a spoon or spatula. If possible, use an up-and-down rotary movement in such a way that the top layers and the contents of the lower corners of the container are moved and mixed. Take care to incorporate in the test sample any fat or other constituents adhering to the wall and ends of the container. Transfer the test sample as completely as possible to a second sample container provided with an airtight lid. Close this container.

If necessary, condition the unopened sample container in the water bath (6.5) maintained 38 °C ± 2 °C. Remove the container, dry the outside with a tissue and open it. Scrape out all test sample adhering to the interior of the container. Transfer the test sample to a dish large enough to permit thorough stirring and mix until the whole mass is homogeneous. Transfer the test sample as completely as possible to a second sample container provided with an airtight lid. Close this container.

8.9 Milk-based edible ices and ice mixes

8.9.1 Edible ices, ice mixes and concentrated ice mixes

8.9.1.1 Do not allow the temperature to exceed 12 °C at any time during the preparation of the (ice) pieces. Remove any coating of non-ice character from the test sample.

8.9.1.2 If possible, separate the layers of multilayer products, in which the layers possibly have different fat contents, while the product is still frozen. Prepare individual test samples from each layer as specified in 8.9.1.3.

8.9.1.3 Cut the test sample into pieces. Select several pieces at random to give a total mass of approximately 100 g, if possible. Place the pieces in a blender jar. Cover the jar with its lid and allow the pieces to soften at room temperature. Mix plain test samples for 2 min, and test samples containing particulate matter (e.g. nuts, hard candy chips) for not more than 7 min, to obtain a homogeneous mixture.

If fat separates or churning occurs, discard the mixture and repeat the preparation process using a shorter mixing time. Immediately transfer the mixed test sample to a suitable airtight container and proceed with the determination within 1 h.

8.9.2 Dried ice mixes

Mix thoroughly by rotating and inverting the sample container. If necessary, transfer the test sample to a suitable airtight container of adequate capacity to allow mixing. If the test sample still contains lumps or pieces of ingredients, homogenize it in an appropriate blender (6.13).

9 Procedure

9.1 Test portion

Mix the test sample by gently stirring or rotating and inverting the container several times.

For skimmed milk, whey and buttermilk, two test portions are extracted in two fat-extraction flasks (6.6). The extracts of the two flasks are poured into one prepared fat-collecting vessel (see 9.3).

Immediately weigh, to the nearest 1 mg, directly or by difference, in a fat-extraction flask (6.6), one of the test portions as shown in Table 1.

Transfer the test portion in the fat extraction flask as completely as possible into the lower (small) bulb for the Mojonnier.

Table 1 — Mass of the test portion for the different matrices

Matrix	Test portion
Milk, skimmed milk, whey and buttermilk	10 g to 11 g
Dried milk products	a) about 1,0 g of dried high-fat milk, of dried whole milk or of dried butter serum; b) about 1,5 g of dried partially skimmed milk; c) about 1,5 g of dried skimmed milk; d) about 1,5 g of dried whey; e) about 1,5 g of dried buttermilk.
Evaporated milk	4 g to 5 g
Sweetened condensed milk	2,0 g to 2,5 g
Whey cheese	3,0 g ± 0,2 g
Cream	0,3 g to 0,6 g of extracted fat
Milk-based infant foods	1,5 g to 10,0 g corresponding to 1,0 g to 1,5 g of dry matter
Edible ices, ice mixes	4 g to 5g
Concentrated ice mixes	2,0 g to 2,5 g
Dried ice mixes	0,9 g to 1,1 g

9.2 Blank tests

Carry out a blank test simultaneously with the determination using the same procedure and the same reagents, but replacing the test portion in 9.1 by 10 ml of water (see Clause A.2).

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 done recently (see Clause A.1). Corrections for values of more than 2,5 mg in the blank test shall be reported in the test report (see Clause A.2).

9.3 Preparation of fat-collecting vessel

Dry a fat-collecting vessel (6.9) with a few boiling aids (6.14) in the oven (6.4) maintained at 102 ± 2 °C for at least 1 h.

Protect the fat-collecting vessel from dust and allow it to cool 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).

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

Use tongs (6.12) to place the fat-collecting vessel on the balance. Weigh the fat-collecting vessel to the nearest 1,0 mg.

NOTE In particular, the use of tongs effectively avoids temperature variations.

9.4 Determination

9.4.1 Preparation steps

9.4.1.1 Milk, skimmed milk, whey and buttermilk

Carry out the determination without delay.

9.4.1.2 Dried milk products

Carry out the determination without delay. Add about 10 ml of preheated water at a temperature of 65 °C ± 5 °C to the test portion in the fat-extraction flask (see Table 1) 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 flask (into the small bulb for Mojonnier type flask). Mix thoroughly with the test portion (in the small bulb for Mojonnier type flask) until the test portion is completely dispersed.

9.4.1.3 Whey cheese

Carry out the determination without delay. Add about 10 ml of preheated water at a temperature of 65 °C ± 5 °C to the test portion in the fat-extraction flask (see Table 1) 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 flask (into the small bulb for Mojonnier type flask). Mix thoroughly with the test portion (in the small bulb for Mojonnier type flask) until the test portion is completely dispersed.

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.

9.4.1.4 Evaporated milk

Carry out the determination without delay. Add water at a temperature of about 50 °C to the test portion in the fat-extraction flask (see Table 1) 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 flask (into the small bulb for Mojonnier type flask). Shake gently with slight warming at about 50 °C in the water bath (6.5) until the test portion is completely dispersed. Cool in running water to room temperature.

9.4.1.5 Cream

Carry out the determination without delay. Add an amount of water at about 50 °C to the test portion in the fat-extraction flask (see Table 1) 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 flask (into the small bulb for Mojonnier type flask). Mix thoroughly with the test portion (in the small bulb for Mojonnier type flask). Cool in running water to room temperature.

9.4.1.6 Milk-based edible ices and ice mixes

Carry out the determination without delay. Add 6 ml, 8 ml or 10 ml of preheated water at a temperature of 65 °C ± 5 °C to the test portion in the fat-extraction flask (see Table 1) as appropriate 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 flask (into the small bulb for Mojonnier type flask). Mix thoroughly with the test portion (in the small bulb for Mojonnier type flask). Cool, except for the test portion of dried ice mixes, in running water to room temperature.

9.4.1.7 Milk-based infant foods

Carry out the determination without delay. If necessary, add preheated water at a temperature of 65 °C ± 5 °C to the test portion in the fat-extraction flask (see Table 1) 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 flask (into the small bulb for Mojonnier type flask). Shake gently with slight warming in a water bath (6.5) maintained at 50 °C ± 5 °C until the test portion is completely dispersed.

9.4.2 Determination

9.4.2.1 Add 2 ml of ammonia solution (5.1) to the test portion (9.4.1), or an equivalent amount of a more concentrated ammonia solution (see 5.1). Mix thoroughly with the test portion in the flask (in the small bulb for Mojonnier type flask).

9.4.2.2 Only when testing dried milk, milk-based infant foods and milk-based edible ices and ice mixes, close the flask and heat to 65 °C ± 5 °C in the water bath (6.5) for 15 min to 20 min with occasional shaking. Cool in running water to room temperature.

9.4.2.3 Add 10 ml of ethanol (5.2). Mix gently but thoroughly by allowing the contents of the flask to flow backwards and forwards and for Mojonnier type flask, between the two bulbs while not bringing the liquid too near to the neck. If desired, add two drops of the indicator solution (5.3). For whey cheese, if necessary, cool the flask in running water to room temperature.

9.4.2.4 Add 25 ml of diethyl ether (5.4). Close the fat-extraction flask with a stopper (6.6.3).

Shake the flask vigorously for 1 min, but not so vigorously as to cause formation of a persistent emulsion. For Mojonnier type flask only, while shaking, keep the flask in a horizontal position with the small bulb extending upwards, periodically allowing the liquid in the large bulb to run 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.

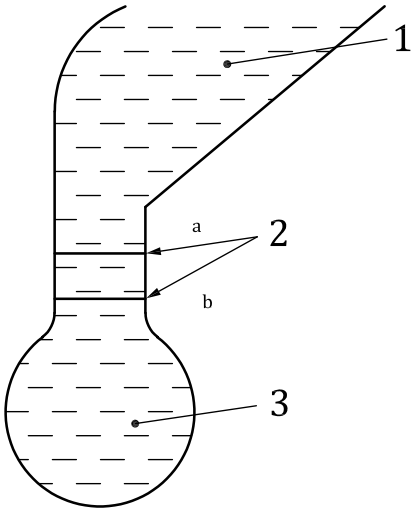
9.4.2.5 Add 25 ml of light petroleum (5.5). Close the fat-extraction flask with the stopper. Mix gently again for 30 s as specified in 9.4.2.4.

9.4.2.6 Centrifuge the closed fat-extraction flask for between 1 min and 5 min at a radial acceleration of around 80*g* to 90*g*. 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.

9.4.2.7 Carefully remove 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. For Mojonnier only, 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.

For Mojonnier type fat extraction flasks, 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 (6.14) (optional with metal dishes). Avoid decanting any of the aqueous layer (see Figure 2).

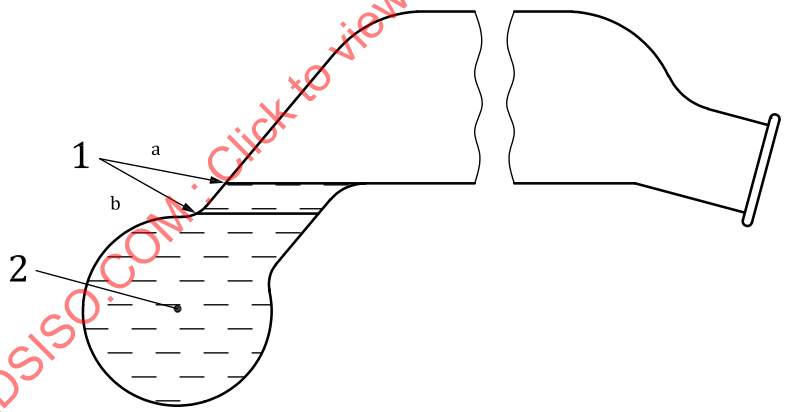
NOTE In Figures 1 and 2, one of the three types of fat-extraction flasks (6.6.1) specified in ISO 3889|IDF 219 has been chosen, but this does not imply any preference over other types.



Key

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

Figure 1 — Before decanting



Key

1 solvent
2 aqueous layer
a At second and third extraction.
b At first extraction.

Figure 2 — After decanting

For extraction tubes type fat extraction flask, insert a siphon or a wash-bottle fitting into the tube (see Annex B). 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 extraction tube.

Carefully transfer the supernatant layer out of the tube into the fat-collecting vessel (see 9.3) containing a few boiling aids (6.14) in the case of flasks (optional with metal dishes). Avoid 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.

Loosen the fitting from the neck of the 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.

9.4.2.8 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 9.4.2.12.

9.4.2.9 Add 5 ml of ethanol (5.2) to the contents of the fat-extraction flask. Using the ethanol, rinse the inside of the neck of the flask and mix as specified in 9.4.2.3.

9.4.2.10 Carry out a second extraction by repeating the operations specified in 9.4.2.4 to 9.4.2.8 inclusive. Instead of 25 ml, use only 15 ml of diethyl ether (5.4) and 15 ml of light petroleum (5.5). Using diethyl ether, also rinse the inner wall of the neck of the fat-extraction flask.

For Mojonnier type 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).

9.4.2.11 Carry out a third extraction without addition of ethanol by again repeating the operations specified in 9.4.2.4 to 9.4.2.8 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.

For Mojonnier, 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).

The third extraction may be omitted for products with a fat content of less than given in Table 2.

Table 2 — Fat content below which a third extraction may be omitted

Matrix	Mass fraction
Dried milk products, milk-based infant foods	5 %
Milk	0,5 %
Whey cheese	3 %
Milk-based edible ices and ice mixes	0,5 %
Evaporated milk and sweetened condensed milk	1 %

9.4.2.12 Remove the solvents (including 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.

9.4.2.13 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 and immediately verify whether 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 (6.12) to place the fat-collecting vessel on the balance. Weigh the fat-collecting vessel to the nearest 1,0 mg.

9.4.2.14 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 9.4.2.13. If necessary, repeat the heating and weighing procedures until the mass of the fat-collecting vessel decreases by 2,0 mg or less for milk and by 1,0 mg or less for the other products, 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 by mass of the sample using Formula (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 (see 9.1);

m_1 is the mass, in grams, of the fat-collecting vessel and extracted matter, determined in 9.4.2.14;

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

m_3 is the mass, in grams, of the fat-collecting vessel used in the blank test (see 9.2) and any extracted matter determined in 9.4.2.14;

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

10.2 Expression of results

Round the result to the decimal places specified in Table 3.

Table 3 — Number of decimal places per matrix

Matrix	Decimal places
Milk	2
Dried milk products	2
Milk-based infant foods	2
Whey cheese	2
Milk-based edible ices and ice mixes	2
Cream	2
Evaporated milk and sweetened condensed milk	2
Skimmed milk, whey and buttermilk	3

11 Precision

11.1 Interlaboratory test

The values for repeatability and reproducibility limits are expressed for the 95 % probability level and are not necessarily applicable to concentration ranges and matrices other than those given. The raw results of the interlaboratory studies are presented in Annex C (raw milk), Annex D (raw sheep milk and raw goat milk), Annex E (dried milk products), Annex F (cream) and Annex G (skimmed milk).

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 not in more than 5 % of cases be greater than:

a) a mass fraction of 0,031 g/100 g for skimmed milk;

b) a mass fraction of 0,010 g/100 g for skimmed milk (according to operating conditions in 9.1);

c) a mass fraction of 0,036 g/100 g for reduced fat milk;

d) a mass fraction of 0,043 g/100 g for whole milk;

e) a mass fraction of 0,030 g/100 g for goat milk;

f) a mass fraction of 0,069 g/100 g for sheep milk;

g) 0,67 % of the average of the fat content for cream;

   For example, the absolute difference between two independent results for cream with a 20 % mass fraction of fat should not be greater than 0,13 g of fat per 100 g of sample, and that difference for cream with an 80 % mass fraction of fat should not be greater than 0,54 g of fat per 100 g of sample.

h) a mass fraction of 0,252 g/100 g for dried milk products with a fat content from 0,3 % to 1 %;

i) a mass fraction of 0,346 g/100 g for dried milk products with a fat content more than 1 %.

NOTE For dried products with a fat content below 0,3 %, no precision figures can be given considering the closeness of the fat content to the limit of detection of the method.

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 not in more than 5 % of cases be greater than:

a) a mass fraction of 0,043 g/100 g for skimmed milk;

b) a mass fraction of 0,017 g/100 g for skimmed milk (according to operating conditions in 9.1);

c) a mass fraction of 0,042 g/100 g for reduced fat milk;

d) a mass fraction of 0,056 g/100 g for whole milk;

e) a mass fraction of 0,052 g/100 g for goat milk;

f) a mass fraction of 0,096 g/100 g for sheep milk;

g) 1,13 % of the average of the fat content of the cream;

   For example, the absolute difference between two independent test results for cream with a 20 % mass fraction of fat should not be greater than 0,23 g of fat per 100 g of sample, and that difference

for cream with an 80 % mass fraction of fat should not be greater than 0,92 g of fat per 100 g of sample.

h) a mass fraction of 0,313 g/100 g for dried milk products with a fat content from 0,3 % to 1 %;

i) a mass fraction of 0,449 g/100 g for dried milk products with a fat content of more than 1 %.

NOTE For dried products with a fat content below 0,3 %, no precision figures can be given considering the closeness of the fat content to the limit of detection of the method.

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 a reference to this document, i.e. ISO 23318|IDF 249;

d) all operating details not specified in this document, or regarded as optional, together with details of any incidents which can 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.

Annex A
(informative)

Additional procedures

A.1 Blank test to check the reagents

To test the quality of the reagents, carry out a blank test as specified in 9.2. In this blank test, a fat-collecting vessel (6.9) for mass control purposes shall be used in order that changes in the atmospheric condition of the balance room or temperature effects on the fat-collecting vessel will not falsely suggest the presence or absence of non-volatile matter in the extract of the reagents. The control 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 control vessel shall be considered when checking the mass of the fat-collecting vessel used for the blank test. Hence, the change in apparent mass of the blank test 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.

The reagents shall leave no residue greater than 0,5 mg. 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. Replace unsatisfactory reagents or solvents, or redistil solvents.

Very occasionally, the 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 the solvents only shortly after redistillation.

A.2 Blank test carried out simultaneously with the determination (see 9.2)

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. However, corrections are also needed for any change of atmospheric conditions in the balance room and some temperature difference between the fat-collecting vessel (6.9) and the balance room at the two weighings (9.4.2.14 and 9.3).

Under favourable conditions (low value in the blank test on reagents, stable temperature of the balance room, sufficient cooling time for fat vessel), the value will usually be less than 1,0 mg and may 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. When corrections for a value of more than 2,5 mg are applied, this shall be mentioned in the test report (see 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 shall be replaced or purified (see Clause A.1).

A.3 Test for peroxides

To test for peroxides, add 1 ml of a freshly prepared 100 g/l of 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 either 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 ether at least three days before it is to be used, as follows.

Cut zinc foil into strips that will reach at least half-way up the bottle containing the diethyl ether, using approximately 80 cm^2 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 (98 % mass fraction) sulfuric acid 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 Diethyl ether containing antioxidants

Diethyl ether containing up to 7 mg/kg of antioxidants can be used for such fat determination method if a blank test is carried out as described in 9.2.

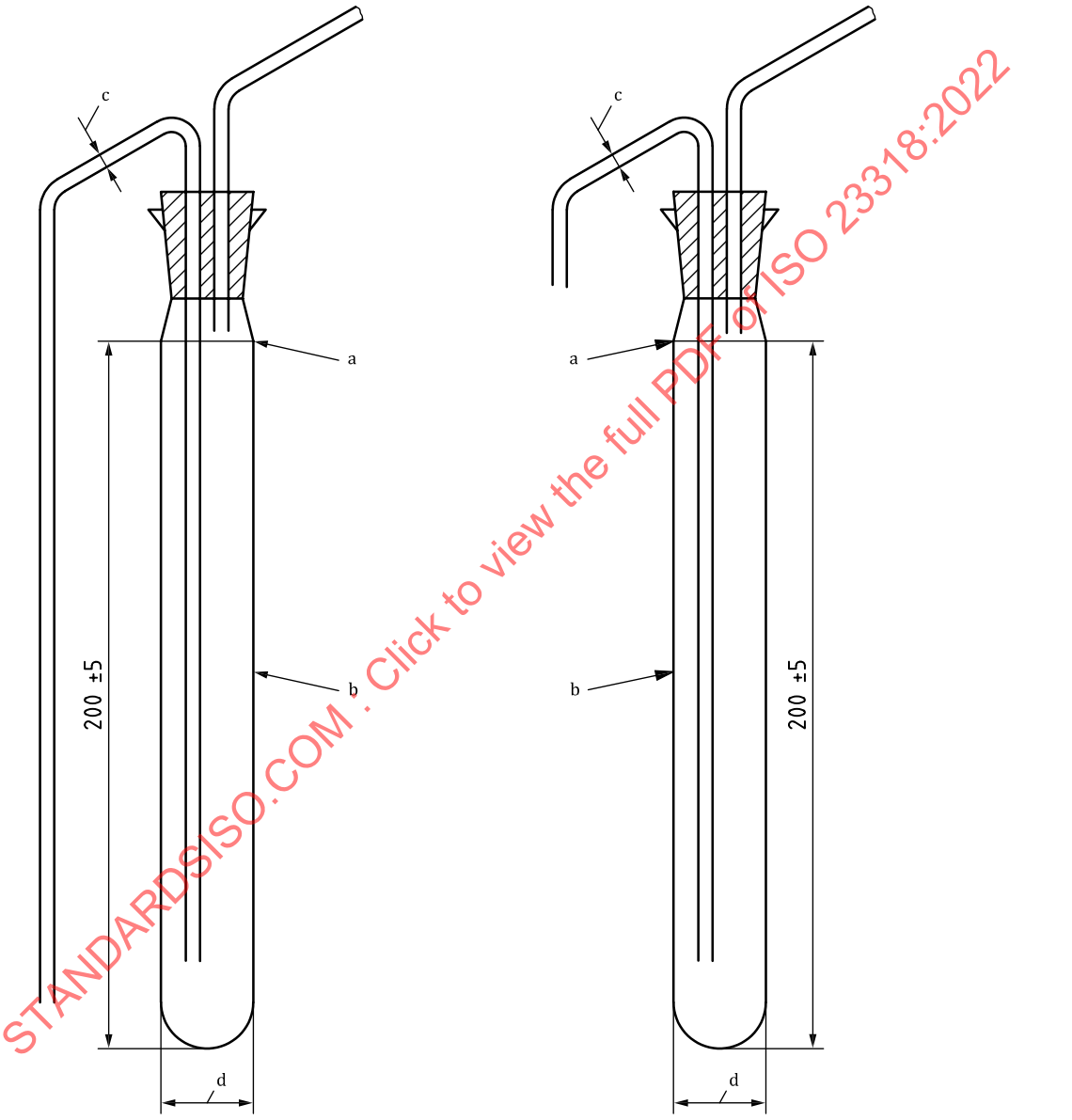
A.5 Ethanol

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

Annex B
(informative)

Fat-extraction tube model with siphon or wash-bottle fittings

Dimensions in millimetres



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

b Wall thickness 1,5 mm ± 0,5 mm.

c Φ ext. 4 ± 0,4.

d Φ int. 26 ± 1.

Figure B.1 — Examples of fat-extraction tubes

Annex C
(informative)

Interlaboratory study on raw milk

An international interlaboratory study involving 19 laboratories from 13 countries was carried out in 2005–12[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 < w_f < 2 g/100 g;

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

The test was organized by the Associazione Italiana Allevatori, Laboratorio Standard Latte, Maccarese, Italy. The results obtained were subjected to statistical analyses in accordance with ISO 5725-1 and ISO 5725-2 to give the precision data shown in Tables C.1, C.2 and C.3.

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 (2,8 × s_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 (2,8 × s_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 study on raw sheep milk and raw goat milk

An international interlaboratory study involving 16 laboratories from 9 countries was carried out in 2006–11 (see ISO 5725-1).

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 had 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 subjected to statistical analyses in accordance with ISO 5725-1 and ISO 5725-2 to give the precision data shown in Tables D.1 and D.2.

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 (2,8 × s_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 (2,8 × s_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.