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Cheese and processed cheese products, caseins and caseinates — Determination of fat content — Gravimetric method

Fromages et fromages fondus, caséines et caséinates — Détermination de la teneur en matière grasse — Méthode gravimétrique

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Forewords

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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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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), in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 302, *Milk and milk products - Methods of sampling and analysis,* in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement). It is being published jointly by ISO and IDF.

This first edition cancels and replaces ISO 1735 | IDF 5:2004 and ISO 5543 | IDF 127:2004, which have been merged and technically revised.

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*, in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 302, *Milk and milk products - Methods of sampling and analysis*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement). It is being published jointly by ISO and IDF.

The work was carried out by the IDF/ISO Action Team (C34) of the *Standing Committee on Analytical Methods for Composition* under the aegis of its project leader, Mr Philippe Trossat (FR).

Cheese and processed cheese products, caseins and caseinates — Determination of fat content — Gravimetric method

WARNING — The use of this document can 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 to determine the applicability of regulatory limitations prior to use.

1 Scope

This document specifies a method for the determination of the fat content of all types of cheese and processed cheese products containing lactose of below 5 % (mass fraction) of non-fat solids, and all types of caseins and caseinates.

The method is not applicable to fresh cheese types containing, for example, fruits, syrup or muesli. For such products, the Schmid-Bondzynski-Ratzlaff (SBR) principle is not applicable due to high concentrations of sugars. For these products, the method using the Weibull-Berntrop principle (see ISO 8262-3 | IDF 124-3[4]) is appropriate.

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 565, *Test sieves — Metal wire cloth, perforated metal plate and electroformed sheet — Nominal sizes of openings*

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.

ISO and IEC maintain terminological 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 determined by the procedure specified in this document

Note 1 to entry: The fat content is expressed as a percentage by mass.

4 Principle

A test portion is digested with hydrochloric acid, then ethanol is added. The acid-ethanolic solution is subsequently extracted with diethyl ether and light petroleum. The solvents are removed by

distillation or evaporation. The mass of the substances extracted, which are soluble in light petroleum, is determined.

NOTE This is usually known as the Schmid-Bondzynski-Ratzlaff 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 Annex A).

5.1 Concentrated hydrochloric acid, containing a mass fraction of HCl of approximately 36 % (ρ_{20} = 1,18 g/ml).

5.2 Dilute hydrochloric acid, containing a mass fraction of approximately 25 % (ρ_{20} = 1,125 g/ml).

Dilute 675 ml of concentrated hydrochloric acid (5.1) to 1 000 ml with water and mix, or use dilute hydrochloric acid if commercially available.

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

Ethanol denatured otherwise than by methanol may be used provided that the denaturant does not affect the result of the determination (see A.5).

5.4 Diethyl ether ($C_2H_5OC_2H_5$), free from peroxides (see A.3) and containing none or not more than 7 mg/kg of antioxidants (see A.4).

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.

5.6 Mixed solvent, prepared shortly before use by mixing equal volumes of diethyl ether (5.4) and light petroleum (5.5).

6 Apparatus

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 6.7) and capable of producing a radial acceleration of around 80*g* to 90*g* at the outer end of the flasks or tubes.

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

6.3 Distillation or evaporation apparatus to enable the solvents and ethanol to be distilled from the fat-collecting flasks or to be evaporated from beakers and dishes 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 the working space. Alternatively, a **vacuum drying oven**, capable of being maintained at 72,5 °C ± 2,5 °C. A pressure less than 600 mbar (50 mmHg) may be used. The drying oven shall be fitted with a suitable thermometer.

6.5 Boiling water bath or **hot plate.**

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

6.7 Extraction tubes-type fat-extraction flasks.

It is also possible to use fat-extraction tubes with siphon or wash-bottle fittings. For an example, see the model in Figure B.1.

6.8 Stoppers.

The flasks or tubes (6.6 and 6.7) shall be provided with stoppers of different material, such as bark cork, silicone rubber, polytetrafluoroethylene (PTFE) or glass, unaffected by the reagents used. Bark corks shall be washed with the diethyl ether (5.4) and kept in water at 60 °C or more for at least 15 min. They shall then be allowed to cool in the water so that they are saturated when used.

6.9 Rack, to hold the fat-extraction flasks or tubes.

6.10 Wash bottle, suitable for use with the mixed solvent (5.6). A plastic wash bottle shall not be used.

6.11 Fat-collecting vessels.

For example:

— 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 preferably be of stainless steel, be flat-bottomed, and have a diameter of 80 mm to 100 mm and a height of approximately 50 mm. Do not use aluminium dishes.

6.12 Boiling aids, fat-free, of non-porous porcelain or silicon carbide, or glass beads. The use of glass beads is optional in the case of metal dishes.

6.13 Measuring cylinders or dispensers, of capacities 5 ml and 25 ml.

6.14 Pipette or dispenser, graduated, to deliver 10 ml.

6.15 Tongs, made of metal, capable of holding flasks, beakers or dishes.

6.16 Sheets of cellulose film, unlacquered, soluble in hydrochloric acid, of thickness 0,03 mm to 0,05 mm, of dimensions 50 mm × 75 mm approximately. The sheets shall be inert under the test conditions.

6.17 Grinding or grating device, for grinding or grating the laboratory sample if necessary. This device should be such that no undue heat will be developed and no loss of moisture occurs. A hammer mill shall not be used.

6.18 Test sieve, of woven wire cloth, diameter 200 mm, nominal size of opening 500 µm, with receiver, conforming to the requirements of ISO 565.

6.19 Container with lid, airtight, of capacity such that the test sample can be mixed by shaking.

6.20 Beaker or flask, of capacity of 100 ml.

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[1].

From the time of sampling to the time of commencing the procedure, the test samples shall be kept at a temperature of between 2 °C and 6 °C for cheese and at room temperature for casein and caseinates.

8 Preparation of test sample

8.1 Cheese

Prior to analysis, remove the rind, the smear or the mouldy surface layer of the cheese in such a way as to obtain a test sample representative of the cheese.

Grind or grate the test sample by using an appropriate grinding or grating device (6.17). Mix the ground mass quickly and, if necessary for semi-hard and hard cheeses, grind it a second time and again mix thoroughly.

For hard and semi-hard cheeses, previously to grind or grate, preferably cut into cubes of about 15 mm × 15 mm. Mix the cubes by shaking in a container and grind or grate the prepared sample as specified before.

Clean the device after preparing each sample.

If the test sample cannot be ground or grated, mix it thoroughly by intensive kneading, for example with a pestle in a mortar. Care should be taken to avoid moisture loss.

Store the test sample in an airtight container until commencing the analysis, which shall be carried out as soon as possible after grinding.

If, however, a delay is unavoidable, take all precautions to ensure proper preservation of the test sample. When refrigerated, bring the test sample to room temperature. Thoroughly mix the sample to obviate the well-documented transfer of moisture within the cheese that occurs during cooling and warming. Ensure that any condensation of moisture on the inside surface of the container is thoroughly and uniformly re-incorporated into the test sample. Do not examine ground cheese showing unwanted mould growth or signs of deterioration.

All sample preparation should be carried out in a manner which minimizes moisture loss. Such moisture loss will have the effect of increasing the apparent fat content.

8.2 Caseins and caseinates

Thoroughly mix the laboratory sample, if necessary, after transferring all of it to an airtight container of suitable capacity, by repeatedly shaking and inverting the container.

Transfer 50 g of the laboratory sample to the test sieve (6.18).

If it does not pass completely through the sieve, use the grinding device to achieve this condition. Immediately transfer all the sieved sample to the container (6.19) and mix thoroughly in the closed container. During these operations, take precautions to avoid any change in the water content of the product.

If the 50 g portion directly passes through the sieve, or nearly completely passes, use this test sample for the determination.

After the test sample has been prepared, proceed with the determination (see 9.4) as soon as possible.

9 Procedure

9.1 Test portion

Mix the test sample by gently stirring. Immediately weigh, to the nearest 1 mg, directly or by difference, 1 g to 3 g of test sample for cheese and 2 g to 3 g for caseins and caseinates into a fat-extraction flask (6.6 or 6.7), a 100 ml beaker or flask (6.20).

For cheeses having a mass fraction of fat of more than 30 %, adapt the mass of the test portion so as to obtain a mass of extracted fat of between 750 mg and 1 000 mg.

The test portion may also be weighed on a sheet of cellulose film (6.16), which is subsequently folded and introduced into the chosen vessel. For the Mojonnier type flask, deliver the test portion as completely as possible into the lower (small) bulb of the fat-extraction flask.

9.2 Blank test

Carry out a blank test simultaneously with the determination, using the same procedure and same reagents but omitting the test portion.

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

9.3 Preparation of a fat-collecting vessel

Dry a fat-collecting vessel (6.11) with a few boiling aids (6.12) in the drying oven (6.4) for at least 1 h.

NOTE Boiling aids are desirable to promote gentle boiling during the subsequent removal of solvent, especially in the case of glass vessels; their use is optional in the case of metal dishes.

Allow the fat-collecting vessel to cool (protected from dust) to the temperature of the weighing room (glass vessel for at least 1 h, metal dish for at least 30 min).

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

Use tongs to place the fat-collecting vessel on the balance to avoid, in particular, temperature variations. Weigh the fat-collecting vessel to the nearest 1 mg.

9.4 Determination

9.4.1 Depending on the shape of the extraction apparatus and the size of the test portion, add 8 ml to 10 ml for cheese and 7,5 ml to 10 ml (6.14) for caseins and caseinates of dilute hydrochloric acid (5.2). Add the hydrochloric acid so as to wash the test portion and for Mojonnier type flask into the small bulb of the fat-extraction flask (6.6 or 6.7) or onto the bottom of the beaker or flask (6.20), and mix.

9.4.2 Heat by gently moving the fat-extraction flask or vessel (to avoid charring) in a boiling water bath or on a hot plate (6.5), until all particles are completely dissolved.

9.4.3 Allow the fat-extraction flask or vessel to stand for 20 min to 30 min in the boiling water bath (6.5) or keep it gently boiling on the hot plate (6.5) for 10 min. Cool under running water.

9.4.4 If the digestion has been carried out in the fat-extraction flask apparatus, add 10 ml (6.14) of ethanol (5.3). Mix gently but thoroughly by allowing the contents of the flask to flow backwards and forwards and, for a Mojonnier type flask, between the two bulbs while not bringing the liquid too near to the neck.

Alternatively, if the digestion has been carried out in a vessel other than the fat-extraction flask (6.6 or 6.7), pour the contents of the vessel into a fat-extraction flask (6.6 or 6.7). Rinse the vessel successively with 10 ml of ethanol (5.3). Mix gently as described above. Then rinse the vessel with 25 ml of diethyl ether (5.4) and pour the vessel contents into the fat-extraction flask, while rinsing the tip or rim with some additional diethyl ether or mixed solvent (5.6). Close the fat-extraction flask with a stopper (6.8) and shake as described in 9.4.5. Finally rinse the vessel again with 25 ml of light petroleum (5.5) and pour that solvent into the fat-extraction flask, while also rinsing the tip or rim with some additional light petroleum. Close the fat-extraction flask again and shake its contents as described in 9.4.6. Then continue with the centrifugation procedure as in 9.4.7.

9.4.5 Add 25 ml of diethyl ether (5.4). Close the fat-extraction flask with a stopper (6.8).

Shake the flask vigorously for 1 min, but not so vigorously as to cause formation of a persistent emulsion. For a 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 under running water.

Carefully remove stopper and rinse it and the neck of the flask with a little mixed solvent (5.6). Use the wash bottle (6.10) so that the rinsings run into the fat-extraction flask.

9.4.6 Add 25 ml of the light petroleum (5.5). Close the fat-extraction flask with the stopper (6.8).

Gently shake the flask as described in 9.4.5, but for 30 s only.

9.4.7 Centrifuge the closed fat-extraction flask at an acceleration of around 80*g* to 90*g* for 1 min to 5 min. If a centrifuge is not available, allow the closed flask to stand in the rack (6.9) for at least 30 min until the supernatant layer is clear and distinctly separated from the aqueous layer. If necessary, cool the fat-extraction flask under running water.

9.4.8 Carefully remove the 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 (6.10) so that the rinsings run into the flask.

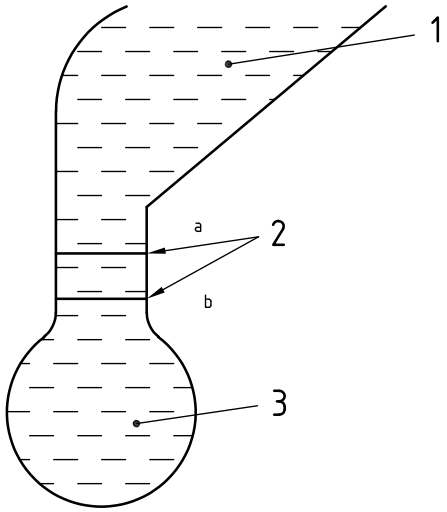
If the interface is below the bottom of the stem of the flask, raise it slightly above this level by gently adding water down the side of the flask (see Figure 1) to facilitate the decanting of solvent.

9.4.9 For Mojonnier-type fat-extraction flasks, use the following procedure:

— 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 (6.12) (optional with metal dishes).

— Avoid decanting any of the aqueous layer (see Figure 2).

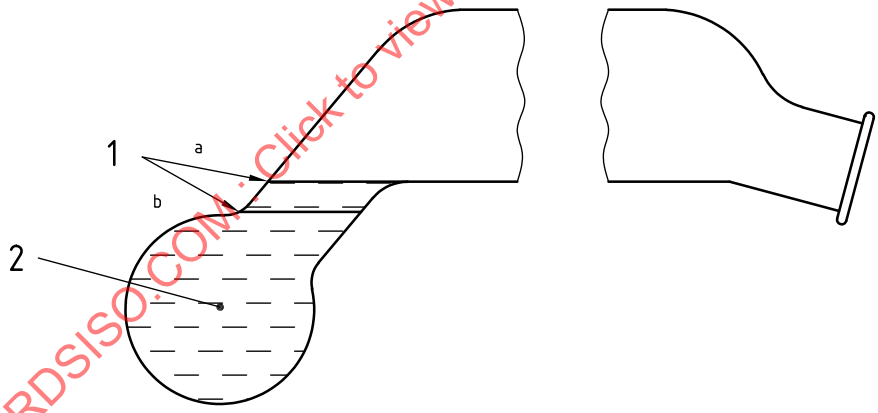
NOTE In Figures 1 and 2, one of the three types of fat-extraction flasks (6.6) specified in ISO 3889 |IDF 219 has been chosen, but this does not imply any preference over other types.



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

9.4.10 For extraction tubes-type fat-extraction flasks, use the following procedure:

— 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 (6.12) 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.

9.4.11 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 extraction flask. If desired, remove the solvent or a part of it from the fat-collecting vessel by distillation or evaporation as described in 9.4.14.

9.4.12 Carry out a second extraction by repeating the operations described in 9.4.5 to 9.4.11 inclusive, using only 15 ml of diethyl ether (5.4) and 15 ml of light petroleum (5.5).

9.4.13 Carry out a third extraction by repeating the operations described in 9.4.5 to 9.4.11 inclusive. Again, use only 15 ml of diethyl ether (5.4) and 15 ml of light petroleum (5.5). Use the ether to rinse as described in 9.4.12.

The third extraction may be omitted for products with a mass fraction of fat of less than 3 %.

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

9.4.15 Heat the fat-collecting vessel in the drying oven (6.4) for 1 h. Remove the fat-collecting vessel from the oven and immediately verify whether or not 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 the fat is clear, protect the fat-collecting vessel from dust and allow the vessel to cool to the temperature of the weighing room (glass vessel for at least 1 h, metal dish for at least 30 min).

NOTE Depending on the fat-collecting vessel used, placing it on its side in the drying oven can allow the solvent vapour to escape more readily.

Do not wipe the fat-collecting vessel immediately before weighing. Use tongs to place the fat-collecting vessel on the balance to avoid, in particular, temperature variations. Weigh to the nearest 1 mg.

9.4.16 Heat the fat-collecting vessel in the drying oven (6.4) for 30 min. Reweigh and record as described in 9.4.15. Repeat the heating and weighing procedure until the mass of the fat-collecting vessel decreases by 1,0 mg or less, or increases between two successive weighings. Record the minimum mass as the mass of the fat-collecting vessel and extracted matter.

10 Calculation and expression of results

The fat content, w_f, expressed as a percentage by mass, is given in 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 9.4.15;

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 9.4.15;

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

Report the result to the nearest 0,01 % (mass fraction).

11 Precision

11.1 Interlaboratory test

Details of an interlaboratory test on the precision of the method are given in Annex C (cheeses) and Annex D (caseins and caseinates). The values derived from this test may not be applicable to concentration ranges and matrices other than those given.

Laboratories that make use of this method should note that for certain types of cheese higher values for *r* and *R* might be found in practice.

For caseins and caseinates, the values for repeatability and reproducibility are expressed at the 95 % probability level and derived from the results of an interlaboratory trial carried out in accordance with ISO 5725-2[3].

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 0,30 % for cheeses.

The absolute difference between two independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, will in not more than 5 % of cases be greater than 0,10 % for caseins and caseinates.

11.3 Reproducibility

The absolute differences between two 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 0,40 % for cheeses.

The absolute difference between two single test results, obtained using the same method on identical test material in different laboratories with different operators using different equipment, will in not more than 5 % of cases be greater than 0,20 % for caseins and caseinates.

11.4 Test report

The test report shall specify:

a) all information necessary for the complete identification of the sample;

b) the sampling method used, if known;

c) the test method used, with reference to this document, i.e. ISO 23319 | IDF 250;

d) all operating details not specified in this document, or regarded as optional, together with details of any incidents that could have influenced the test results;

e) the test results obtained or, if the repeatability has been checked, the final quoted result obtained;

f) the date of the test;

g) the blank value [($m_3 - m_4$), see 9.2] shall be reported if it exceeds 2,5 mg.

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 (6.11) 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_3) in Clause 10] 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 1,0 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 that 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. But corrections are also needed for any change of atmospheric conditions in the balance room and for some temperature differences between the fat-collecting vessel (6.11) and the balance room at the two weighings (9.4.16 and 9.3).

Under favourable conditions (low value in the blank test on reagents, 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, it shall be mentioned in the test report (see 11.4).

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 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 that 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 per kilogram can be used for such fat determination method if a blank test is carried out as described in 9.2.

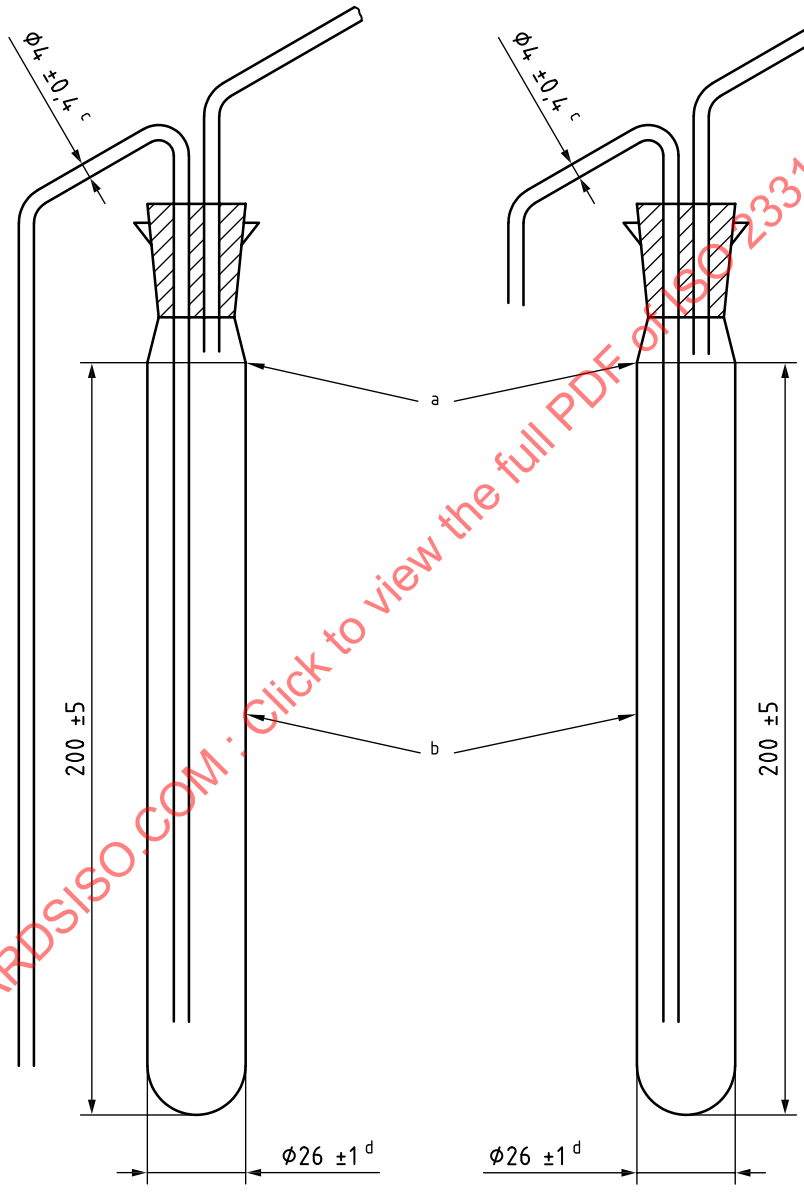
A.5 Ethanol

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 External diameter.

d Internal diameter.

Figure B.1 — Examples of fat extraction