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

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

Reference number
ISO 1854 : 1987 (E)

Foreword

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Draft International Standards adopted by the technical committees are circulated to the member bodies for approval before their acceptance as International Standards by the ISO Council. They are approved in accordance with ISO procedures requiring at least 75 % approval by the member bodies voting.

International Standard ISO 1854 was prepared by Technical Committee ISO/TC 34, *Agricultural food products,* in collaboration with the International Dairy Federation (IDF) and the Association of Official Analytical Chemists (AOAC) and will also be published by these organizations.

This second edition cancels and replaces the first edition (ISO 1854 : 1972), of which it constitutes a technical revision (see introduction).

Users should note that all International Standards undergo revision from time to time and that any reference made herein to any other International Standard implies its latest edition, unless otherwise stated.

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

0 Introduction

This second edition of ISO 1854 has been prepared within the framework of producing a series of methods, which are harmonized to the greatest possible extent, for the gravimetric determination of the fat content of milk, milk products and milk-based foods.

The method is based on the revised Röse-Gottlieb method for milk specified in ISO 1211[1)], to which a number of modifications to improve the precision of the method have been made. A more complete explanation of these modifications to the basic Röse-Gottlieb (RG) procedure is given in the introduction to ISO 1211.

Whey cheese contains a high percentage of lactose; it cannot, therefore, be analysed according to the method of Schmid-Bondzynski-Ratzlaff[1], which is generally used for most types of cheese. However, as whey cheese usually dissolves easily in ammonia, the fat content can be determined according to the RG method.

Although fresh cheeses such as cottage cheese and quarg also have high lactose contents, the RG method is less suitable, probably owing to extreme inhomogeneity of the product and the impracticability of attaining homogeneity. The method is also less suitable for whey protein cheese. In such cases, a method based on the Weibull-Berntrop principle, as given in ISO 8262-3[2)], is to be preferred.

1 Scope and field of application

This International Standard specifies the reference method for the determination of the fat content of whey cheese.

An alternative procedure to that using fat-extraction flasks is given in the annex, which forms an integral part of this International Standard.

NOTE — If the whey cheese does not dissolve completely in the ammonia or if it contains free fatty acids in significant quantities (this occurs only in exceptional cases, and is noticeable by a distinct smell), the result of the determination will be too low. With such products recourse has to be made to a method based on the Weibull-Berntrop principle as given in ISO 8262-3[2)].

2 References

ISO 707, *Milk and milk products — Methods of sampling.*

ISO 2920, *Whey cheese — Determination of dry matter content (Reference method).*

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

3 Definition

For the purposes of this International Standard, the following definition applies.

fat content of whey cheese : All the substances determined by the method specified in this International Standard.

It is expressed as a percentage by mass.

4 Principle

Extraction of an ammoniacal ethanolic solution of a test portion with diethyl ether and light petroleum, removal of the solvents by distillation or evaporation, and determination of the mass of the substances extracted which are soluble in light petroleum. (This is usually known as the Röse-Gottlieb principle.)

5 Reagents

All reagents shall be of recognized analytical grade and shall leave no appreciable residue when the determination is carried out by the method specified. The water used shall be distilled water or water of at least equivalent purity.

To test the quality of the reagents, carry out a blank test as specified in 8.3. Use an empty fat-collecting vessel (6.9), prepared as specified in 8.4, for mass control purposes. The reagents shall leave no residue greater than 0,5 mg (see 10.1).

If the residue of the complete reagent blank test is greater than 0,5 mg, determine the residue of the solvents separately by

1) ISO 1211, *Milk — Determination of fat content — Gravimetric method (Reference method).*

2) ISO 8262-3, *Milk products and milk-based foods — Determination of fat content by the Weibull-Berntrop gravimetric method (Reference method) — Part 3 : Special cases.* (At present at the stage of draft.)

distilling 100 ml of the diethyl ether and light petroleum respectively. Use an empty control vessel to obtain the real mass of residue of each solvent, which shall not exceed 0,5 mg.

Replace unsatisfactory reagents or solvents, or redistil solvents.

5.1 Ammonia solution, containing approximately 25 % (m/m) of NH_3, $\varrho_{20} \approx 910$ g/l.

NOTE — If ammonia solution of this concentration is not available, a more concentrated solution of known concentration may be used (see 8.5.3).

5.2 Ethanol, or **ethanol denatured by methanol,** at least 94 % (V/V).

(See 10.5.)

5.3 Congo-red solution.

Dissolve 1 g of Congo-red in water and dilute to 100 ml.

NOTE — The use of this solution, which allows the interface between the solvent and aqueous layers to be seen more clearly, is optional (see 8.5.4). Other aqueous colour solutions may be used provided that they do not affect the result of the determination.

5.4 Diethyl ether, free from peroxides (see 10.3) and containing no or not more than 2 mg/kg of antioxidants and complying with the requirements for the blank test (see clause 5, and also 10.1 and 10.4).

5.5 Light petroleum, having any boiling range between 30 and 60 °C.

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

6 Apparatus

WARNING — Since the determination involves the use of volatile flammable solvents, electrical apparatus employed may be required to comply with legislation relating to the hazards in using such solvents.

Usual laboratory equipment, and in particular

6.1 Analytical balance.

6.2 Centrifuge, in which the fat-extraction flasks or tubes (6.6) can be spun at a rotational frequency of 500 to 600 min^{-1} to produce an acceleration of 80g to 90g at the outer end of the flasks or tubes.

NOTE — The use of the centrifuge is optional but recommended (see 8.5.7).

6.3 Distillation or **evaporation apparatus,** to enable the solvents and ethanol to be distilled from fat-collecting flasks or to be evaporated from beakers and dishes (see 8.5.10 and 8.5.14) at a temperature not exceeding 100 °C.

6.4 Drying oven, electrically heated, with ventilation port(s) fully open, capable of being maintained at 102 ± 2 °C throughout the working space. The oven shall be fitted with a suitable thermometer.

6.5 Boiling water bath.

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

NOTE — It is also possible to use **fat-extraction tubes** (or **flasks**) with **siphon** or **wash-bottle fittings,** but the procedure is then different. This procedure is specified in the annex. The long inner limb of the fitting may have a hooked end if desired.

The flasks (or tubes, see the note) shall be provided with good quality bark corks or stoppers of other material [for example silicone rubber or PTFE[1]] which are unaffected by the reagents used. Bark corks shall be washed with the 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, to hold the fat-extraction flasks (or tubes) (see 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, for example boiling flasks (flat-bottomed) of capacity 125 to 250 ml, conical beakers of capacity 250 ml, or metal dishes. If metal dishes are used, they shall be made of stainless steel, shall be flat-bottomed, preferably with a spout, and shall have a diameter of 80 to 100 mm and a height of approximately 50 mm.

6.10 Boiling aids, fat-free, of non-porous porcelain or silicon carbide (optional in the case of metal dishes).

6.11 Measuring cylinders, of capacities 5 and 25 ml.

6.12 Pipettes, graduated, of capacity 10 ml.

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

6.14 Appropriate grinding or grating device, easy to clean, for preparing the sample.

1) Polytetrafluoroethylene.

7 Sampling

See ISO 707.

8 Procedure

NOTE — The alternative procedure using fat-extraction tubes with siphon or wash-bottle fittings (see the note to 6.6) is described in the annex.

8.1 Preparation of the test sample

Prepare the sample using an appropriate device (6.14). Quickly mix the ground or grated mass and, if possible, grind it a second time and again mix thoroughly. Clean the device after preparing each sample. If the sample cannot be ground or grated, mix it thoroughly by intensive kneading, for example with a pestle in a mortar.

Keep the prepared sample in an airtight 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 sample. When refrigerated, ensure that any condensation of moisture on the inside surface of the container is thoroughly and uniformly re-incorporated into the test sample.

8.2 Test portion

Mix the test sample (8.1) by gently stirring or rotating and inverting the container and immediately weigh, to the nearest 1 mg, directly or by difference, into a fat-extraction flask (6.6) about 3 g of the prepared sample.

The test portion shall be delivered as completely as possible into the lower (small) bulb of the extraction flask.

8.3 Blank test

Carry out a blank test simultaneously with the determination, using the same procedure and same reagents, but replacing the diluted test portion in 8.5.1 by 10 ml of water (see 10.2).

8.4 Preparation of fat-collecting vessel

Dry a vessel (6.9) with a few boiling aids (6.10) in the oven (6.4) for 1 h (see note 1).

Allow the 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 0,5 h) (see note 2).

Using tongs (6.13) (to avoid, in particular, temperature variations), place the vessel on the balance (6.1) and weigh to the nearest 0,1 mg.

NOTES

1 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.

2 The vessel should not be placed in a desiccator, to avoid insufficient cooling or unduly long cooling times.

8.5 Determination

8.5.1 Add 10 ml of warm water, so as to wash the test portion into the small bulb of the flask, and mix thoroughly in the small bulb.

8.5.2 Heat the flask in the boiling water bath (6.5), shaking gently occasionally until the test portion is completely dispersed. Leave the flask for 20 min in the water bath.

8.5.3 Add 2 ml of the ammonia solution (5.1), or an equivalent volume of a more concentrated ammonia solution (see the note to 5.1), mix thoroughly with the dispersed test portion in the small bulb of the flask and cool, for example in running water. After the addition of the ammonia, continue the determination without delay.

8.5.4 Add 10 ml of the ethanol (5.2) and mix gently but thoroughly by allowing the contents of the flask to flow backward and forward between the two bulbs; avoid bringing the liquid too near to the neck of the flask. If desired, add 2 drops of the Congo-red solution (5.3).

8.5.5 Add 25 ml of the diethyl ether (5.4), close the flask with a cork (see 6.6) saturated with water or with a stopper wetted with water, and shake the flask vigorously, but not excessively (in order to avoid the formation of persistent emulsions), for 1 min with the flask in a horizontal position and 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, then carefully remove the cork or stopper and rinse it and the neck of the flask with a little of the mixed solvent (5.6), using the wash bottle (6.8), so that the rinsings run into the flask or the prepared fat-collecting vessel (see 8.4).

8.5.6 Add 25 ml of the light petroleum (5.5), close the flask with the rewetted cork or rewetted stopper (by dipping in water), and shake the flask gently for 30 s as described in 8.5.5.

8.5.7 Centrifuge the closed flask for 1 to 5 min at a rotational frequency of 500 to 600 min^{-1} (see 6.2). If a centrifuge is not available, allow the closed flask to stand in the rack (6.7) for at least 30 min until the supernatant layer is clear and distinctly separated from the aqueous layer. If necessary, cool the flask in running water.

8.5.8 Carefully remove the cork or stopper and rinse it and the inside of the neck of the flask with a little of the mixed solvent so that the rinsings run into the flask or the fat-collecting vessel.

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 flasks as specified in ISO 3889 is shown, but this does not imply any preference over the other types.

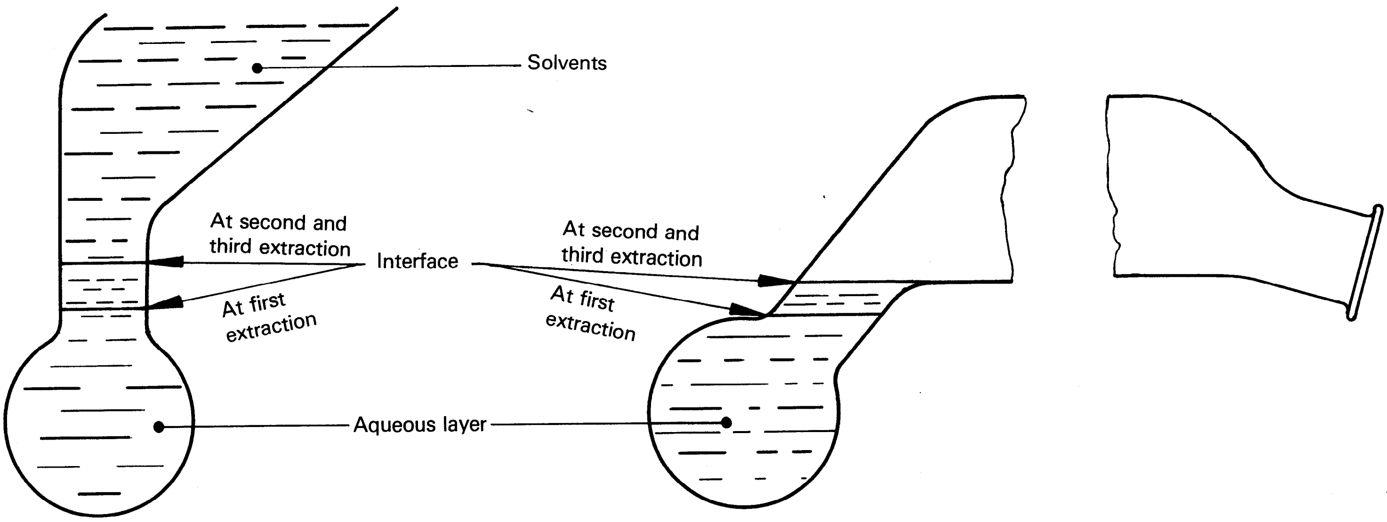

Figure 1 — Before decantation
(8.5.8, 8.5.12, 8.5.13)

Figure 2 — After decantation
(8.5.9, 8.5.12, 8.5.13)

8.5.9 Holding the extraction flask by the small bulb, carefully decant as much as possible of the supernatant layer into the prepared fat-collecting vessel (see 8.4) containing a few boiling aids (6.10) in the case of flasks (optional with metal dishes), avoiding decantation of any of the aqueous layer (see figure 2).

8.5.10 Rinse the outside of the neck of the extraction flask with a little of the mixed solvent, collecting the rinsings in the fat-collecting vessel and taking care that the mixed solvent does not spread over the outside of the extraction flask.

If desired, the solvent or part of the solvent may be removed from the vessel by distillation or evaporation as described in 8.5.14.

8.5.11 Add 5 ml of the ethanol (5.2) to the contents of the extraction flask, using the ethanol to rinse the inside of the neck of the flask, and mix as described in 8.5.4.

8.5.12 Carry out a second extraction by repeating the operations described in 8.5.5 to 8.5.10 inclusive, but using only 15 ml of the diethyl ether (5.4) and 15 ml of the light petroleum (5.5); use the ether to rinse the inside of the neck of the extraction flask.

If necessary, raise the interface to slightly above the middle of the stem of the flask (see figure 1) to enable the final decantation of solvent to be as complete as possible (see figure 2).

8.5.13 Carry out a third extraction without addition of ethanol by again repeating the operations described in 8.5.5 to 8.5.9 inclusive, but using only 15 ml of the diethyl ether (5.4) and 15 ml of the light petroleum (5.5); use the ether to rinse the inside of the neck of the extraction flask.

If necessary, raise the interface to slightly above the middle of the stem of the flask (see figure 1) to enable the final decantation of solvent to be as complete as possible (see figure 2).

NOTE — The third extraction should be omitted for products having fat contents of 3 % (*m*/*m*) or less.

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

8.5.15 Heat the fat-collecting vessel (flask placed on its side to allow solvent vapour to escape) for 1 h in the drying oven (6.4), controlled at 102 ± 2 °C. Remove the fat-collecting vessel from the oven, allow to cool (not in a desiccator, but protected from dust) to the temperature of the weighing room (glass vessel for at least 1 h, metal dish for at least 0,5 h) and weigh to the nearest 0,1 mg.

Do not wipe the vessel immediately before weighing. Place the vessel on the balance using tongs (to avoid, in particular, temperature variations).

8.5.16 Repeat the operations described in 8.5.15 until the mass of the fat-collecting vessel decreases by 0,5 mg or less, or increases, between two successive weighings. Record the minimum mass as the mass of the fat-collecting vessel and extracted matter.

8.5.17 Add 25 ml of the light petroleum to the fat-collecting vessel in order to verify whether or not the extracted matter is wholly soluble. Warm gently and swirl the solvent until all the fat is dissolved.

If the extracted matter is wholly soluble in the light petroleum, take the mass of fat as the difference between the final mass of the vessel containing the extracted matter (see 8.5.16) and its initial mass (see 8.4).

8.5.18 If the extracted matter is not wholly soluble in the light petroleum, or in case of doubt, extract the fat completely from the vessel by repeatedly washing with warm light petroleum.

NOTE — National legislation may mandatorily prescribe such an extraction, either in general or in particular cases.

Allow any trace of insoluble material to settle and carefully decant the light petroleum without removing any insoluble material. Repeat this operation three more times, using the light petroleum to rinse the inside of the neck of the vessel.

Finally, rinse the outside of the top of the vessel with mixed solvent so that the solvent does not spread over the outside of the vessel. Remove light petroleum vapour from the vessel by heating the vessel for 1 h in the drying oven (6.4), controlled at 102 ± 2 °C, allow to cool and weigh, as described in 8.5.15 and 8.5.16.

Take the mass of fat as the difference between the mass determined in 8.5.16 and this final mass.

9 Expression of results

9.1 Method of calculation and formula

9.1.1 The fat content, w_f, of the sample, expressed as a percentage by mass, is equal to

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

where

m_0 is the mass, in grams, of the test portion (8.2);

m_1 is the mass, in grams, of the fat-collecting vessel and extracted matter determined in 8.5.16;

m_2 is the mass, in grams, of the prepared fat-collecting vessel (see 8.4), or, in the case of undissolved material, of the fat-collecting vessel and insoluble residue determined in 8.5.18;

m_3 is the mass, in grams, of the fat-collecting vessel used in the blank test (8.3) and any extracted matter determined in 8.5.16;

m_4 is the mass, in grams, of the prepared fat-collecting vessel (see 8.4) used in the blank test (8.3), or, in the case of undissolved material, of the fat-collecting vessel and insoluble residue determined in 8.5.18.

Report the result to the nearest 0,01 % (m/m).

9.1.2 The fat content of the dry matter, expressed as a percentage by mass, is equal to

$$w_f \times \frac{100}{w_d}$$

where

w_f is the fat content of the sample calculated in 9.1.1;

w_d is the dry matter content of the sample, determined in accordance with ISO 2920.

9.2 Precision

9.2.1 Repeatability

The difference between two single results found on identical test material by one analyst within a short time interval should not exceed 0,2 g of fat per 100 g of product.

9.2.2 Reproducibility

The difference between two single and independent results found by two operators working in different laboratories on identical test material should not exceed 0,3 g[1] of fat per 100 g of product.

10 Notes on procedure

10.1 Blank test to check the reagents

In this blank test, a vessel for mass control purposes has to be used in order that changes in the atmospheric condition of the balance room or temperature effects of the fat-collecting vessel will not falsely suggest the presence or absence of non-volatile matter in the extract of the reagent. This 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 the formula given in 9.1.1) 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 fat-collecting vessel, corrected for the apparent change in mass of the control vessel, shall not exceed 0,5 mg.

Very occasionally, the solvents may contain volatile matter which is strongly retained in fat. If there are indications of the presence of such substances, carry out blank tests on all the reagents and for each solvent using a fat-collecting vessel with about 1 g of fresh anhydrous butterfat. If necessary, redistil solvents in the presence of 1 g of anhydrous butterfat per 100 ml of solvent. Solvents treated in this way should only be stored for short periods following distillation.

10.2 Blank test carried out simultaneously with the determination

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 in atmospheric conditions of the balance room and any temperature difference between the fat-collecting vessel and the balance room at the two weighings (8.5.16 and 8.4 or 8.5.18).

1) This value is tentative.

Under favourable conditions (low value in the blank test on reagents, equable temperature of the balance room, sufficient cooling time for the fat-collecting vessel), the value will usually be less than 0,5 mg and can then be neglected in the calculation in the case of routine determinations. Slightly higher values (positive and negative) up to 2,5 mg are also often encountered. After correction for these values, the results will still be accurate. When corrections for a value of more than 2,5 mg are applied, this fact should be mentioned in the test report (clause 11).

If the value obtained in this blank test regularly exceeds 0,5 mg, the reagents should be checked if this has not been recently done. Any impure reagent or reagents traced should be replaced or purified (see clause 5 and 10.1).

10.3 Test for peroxides in diethyl ether

To test for peroxides, add 1 ml of a freshly prepared 100 g/l potassium iodide solution to 10 ml of the diethyl ether 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 diethyl ether (without antioxidants) is free, and is maintained free, from peroxides, treat the ether as follows at least 3 days before it is to be used.

Cut zinc foil into strips that will reach at least half-way up the bottle containing the ether, using approximately 80 cm^2 of foil per litre of 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 % (m/m)] sulfuric acid per litre. Wash the strips gently but thoroughly with water, place the wet copper-plated strips in the bottle containing the ether, and leave the strips in the bottle.

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

10.4 Diethyl ether containing antioxidants

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

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

10.5 Ethanol

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

11 Test report

The test report shall show the method used and the result obtained. It shall also mention all operating conditions not specified in this International Standard, or regarded as optional, as well as any incidents likely to have influenced the results. The blank value ($m_3 - m_4$, see 9.1.1) shall be reported if it exceeds 2,5 mg.

The test report shall include all the information necessary for the complete identification of the sample.

Bibliography

[1] HOSTETTLER, H. IDF Report No. 51 (1957).

Annex

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

(see figure 3 for example)
(This annex forms an integral part of the standard.)

A.0 Introduction

If fat-extraction tubes with siphon or wash-bottle fittings are to be used (see the note to 6.6), use the procedure specified in this annex.

A.1 Procedure

A.1.1 Preparation of the test sample

See 8.1.

A.1.2 Test portion

Proceed as specified in 8.2 but using the fat-extraction tubes (see 6.6).

The test portion shall be delivered as completely as possible onto the bottom of the extraction tube.

A.1.3 Blank test

See 8.3 and 10.2.

A.1.4 Preparation of fat-collecting vessel

See 8.4.

A.1.5 Determination

A.1.5.1 Add 10 ml of warm water, so as to wash the test portion onto the bottom of the tube, and mix thoroughly.

A.1.5.2 Heat the tube in the boiling water bath (6.5), shaking gently occasionally until the test portion is completely dispersed. Leave the tube for 20 min in the water bath.

A.1.5.3 Add 2 ml of the ammonia solution (5.1), or an equivalent volume of a more concentrated ammonia solution (see the note to 5.1), mix thoroughly with the dispersed test portion at the bottom of the tube and cool, for example in running water. After the mixture has been cooled, continue the determination without delay.

A.1.5.4 Add 10 ml of the ethanol (5.2) and mix gently but thoroughly at the bottom of the tube. If desired, add 2 drops of the Congo-red solution (5.3).

A.1.5.5 Add 25 ml of the diethyl ether (5.4), close the tube with a cork (see 6.6) saturated with water or with a stopper wetted with water, and shake the tube vigorously, but not excessively (in order to avoid the formation of persistent emulsions), with repeated inversions for 1 min. If necessary, cool the tube in running water, then carefully remove the cork or stopper and rinse it and the neck of the tube with a little of the mixed solvent (5.6), using the wash bottle (6.8), so that the rinsings run into the tube.

A.1.5.6 Add 25 ml of the light petroleum (5.5), close the tube with the rewetted cork or rewetted stopper (by dipping in water), and shake the tube gently for 30 s as described in A.1.5.5.

A.1.5.7 Centrifuge the closed tube for 1 to 5 min at a rotational frequency of 500 to 600 min^{-1} (see 6.2). If a centrifuge is not available, allow the closed tube 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 tube in running water.

A.1.5.8 Carefully remove the cork or stopper and rinse it and the neck of the tube with a little of the mixed solvent so that the rinsings run into the tube.

A.1.5.9 Insert a siphon fitting or a wash-bottle fitting into the tube and 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 prepared fat-collecting vessel (see 8.4) containing a few boiling aids (6.10) in the case of flasks (optional with metal dishes), avoiding the transfer of any of the aqueous layer. Rinse the outlet of the fitting with a little of the mixed solvent, collecting the rinsings in the fat-collecting vessel.

A.1.5.10 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 of the mixed solvent. Lower and re-insert the fitting and transfer the rinsings to the fat-collecting vessel.

Rinse the outlet of the fitting with a little of the mixed solvent, collecting the rinsings in the fat-collecting vessel. If desired, the solvent or part of the solvent may be removed from the vessel by distillation or evaporation as described in 8.5.14.

A.1.5.11 Again loosen the fitting from the neck, slightly raise the fitting and add 5 ml of the ethanol to the contents of the tube, using the ethanol to rinse the long inner limb of the fitting; mix as described in A.1.5.4.