
**Caseins and caseinates — Determination
of fat content — Gravimetric method
(Reference method)**

*Caséines et caséinates — Détermination de la teneur en matière
grasse — Méthode gravimétrique (Méthode de référence)*

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 5543|IDF 127 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 AOAC International. It is being published jointly by ISO and IDF and separately by AOAC International.

This edition of ISO 5543|IDF 127 cancels and replaces ISO 5543:1986, of which it constitutes a minor revision. Only editorial changes have been made.

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Foreword

IDF (the International Dairy Federation) is a worldwide federation of the dairy sector with a National Committee in every member country. Every National Committee has the right to be represented on the IDF Standing Committees carrying out the technical work. IDF collaborates with ISO and AOAC International in the development of standard methods of analysis and sampling for milk and milk products.

Draft International Standards adopted by the Action Teams and Standing Committees are circulated to the National Committees for voting. Publication as an International Standard requires approval by at least 50 % of the National Committees casting a vote.

ISO 5543|IDF 127 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 AOAC International. It is being published jointly by ISO and IDF and separately by AOAC International.

All work was carried out by the Joint ISO/IDF/AOAC Group of Experts, *Fat determination* (E31), under the aegis of its project leader, Mr J. Eisses (NL).

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Introduction

This International Standard 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 food.

A method based on the principle of Schmid-Bondzynski-Ratzlaff (SBR), involving digestion with hydrochloric acid, has been chosen for the following reasons:

- a) many caseins do not readily dissolve in ammonia, either because they contain or consist of hard lumps, or because they are not soluble, or are only poorly soluble (e.g. rennet casein), and therefore cannot be examined according to the method based on the Röse-Gottlieb (RG) principle as used for milk and most milk products;
- b) all caseins and caseinates, due to their low lactose content [less than 5 % (mass fraction) of dry matter], can be examined according to the SBR principle; this has the advantage over the Weibull method in that the method can be carried out using the same apparatus as that specified for the RG method and, at the same time, is less time consuming;
- c) methods based on the SBR principle have already found wide application in many countries as official or standardized methods for the examination of all caseins and caseinates.

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Caseins and caseinates — Determination of fat content — Gravimetric method (Reference method)

1 Scope

This International Standard specifies the reference method for the determination of the fat content of all types of caseins and caseinates.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

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

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

ISO 5550, *Caseins and caseinates — Determination of water content (Reference method)*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1

fat content of caseins and caseinates

all the substances determined by the method specified in this International Standard

NOTE It is expressed as a mass fraction in percent.

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, then the solvents are removed by distillation or evaporation. The mass of the substances extracted, which are soluble in light petroleum, is determined. (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.

To test the quality of the reagents, carry out a blank test as specified in 8.3. Use an empty fat-collecting vessel, prepared as specified in 8.4, for mass control purposes (see 11.1). The reagents shall leave no residue greater than 0,5 mg.

If the residue of the complete reagent blank test is greater than 0,5 mg, determine the residue of the solvents separately by distilling 100 ml of the diethyl ether and light petroleum respectively. Use an empty control vessel to obtain the real mass of residue, which shall not exceed 0,5 mg.

Replace unsatisfactory reagents or solvents, or redistil solvents.

5.1 Dilute hydrochloric acid solution, ρ_{20} (HCl) \approx 1,125 g/ml. (See also Note in 8.5.1.)

Dilute 675 ml of hydrochloric acid [ρ_{20} (HCl) = 1,18 g/ml] to 1 000 ml with water.

5.2 Ethanol (C₂H₅OH) or **methanol** (CH₃OH), at least 94 % (volume fraction).

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

5.3 Diethyl ether (C₂H₅OC₂H₅), free from peroxides (see 11.3) and containing none or not more than 2 mg/kg of antioxidants, and complying with the requirements for the blank test (see the introductory paragraphs to this clause, and also 11.1 and 11.4).

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

5.5 Mixed solvent, prepared shortly before use by mixing equal volumes of the diethyl ether (5.3) and the light petroleum (5.4).

5.6 Congo red solution.

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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) and only useful with products giving colourless or only slightly coloured digests. Other aqueous colour solutions may be used provided that they do not affect the result of the determination.

6 Apparatus and materials

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, the following.

6.1 Analytical balance, capable of weighing to the nearest 1 mg, with a readability of 0,1 mg.

6.2 Centrifuge, in which the stoppered fat-extraction flasks or tubes (6.6) can be spun at a rotational frequency of 500 min⁻¹ to 600 min⁻¹ to produce a gravitational field of 80g and 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 the fat-collecting flasks or to be evaporated from beakers and dishes (see 8.5.10 and 8.5.12) at a temperature not exceeding 100 °C.

6.4 Drying oven, electrically heated, with ventilation port(s) fully open, capable of being maintained at $102\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ throughout the working space, and fitted with a suitable thermometer; or **vacuum drying oven**, capable of being maintained at $70\text{ }^{\circ}\text{C}$ to $75\text{ }^{\circ}\text{C}$, at a pressure less than 600 mbar (50 mmHg).

6.5 Boiling water bath or hotplate (see 8.5.2).

6.6 Mojonnier-type fat-extraction flasks, as specified in ISO 3889 (but see the Note in 8.5.2).

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

The flasks (or tubes, see Note) shall be provided with good quality bark corks or stoppers of another material [e.g. silicone rubber or polytetrafluoroethylene (PTFE)] unaffected by the reagents used. Bark corks shall be washed with the diethyl ether (5.3), kept in water at $60\text{ }^{\circ}\text{C}$ or more for at least 15 min, and shall then be allowed to cool in the water so that they are saturated when used.

6.7 Rack, to hold the fat-extraction flasks (or tubes) (see 6.6).

6.8 Wash bottle, suitable for use with the mixed solvent (5.5). A plastic wash bottle shall not be used.

6.9 Fat-collecting vessels, for example boiling flasks (flat-bottomed) of capacity 125 ml to 250 ml, conical beakers of capacity 250 ml or metal dishes.

If metal dishes are used, they shall preferably be of stainless steel, and shall be flat-bottomed, preferably with a spout, and shall have a diameter of 80 mm to 100 mm and a height of approximately 50 mm.

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

6.11 Measuring cylinders, of capacities 5 ml 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 Grinding device, for grinding 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.

6.15 Test sieve, of woven wire cloth, diameter 200 mm, nominal size of opening $500\text{ }\mu\text{m}$, with receiver, complying with the requirements of ISO 565.

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

7 Sampling

A representative sample should have been sent to the laboratory. It should not have been damaged or changed during transport or storage. The laboratory sample shall be stored in a securely closed airtight container.

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 707^[1].

8 Procedure

NOTE The alternative procedure using fat-extraction tubes with siphon or wash-bottle fittings (see Note in 6.6) is described in the Annex A.

8.1 Preparation of test sample

8.1.1 Thoroughly mix the laboratory sample (Clause 7), if necessary after transferring all of it to an airtight container of suitable capacity, by repeatedly shaking and inverting the container.

8.1.2 Transfer 50 g of the laboratory sample to the test sieve (6.15). 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 (6.16) and mix thoroughly in the closed container. During these operations, take precautions to avoid any change in the water content of the product.

8.1.3 After the test sample has been prepared, proceed with the determination (8.5) as soon as possible.

If the 50 g portion directly passes through the sieve, or nearly completely passes, use the prepared test sample (see 8.1.1) for the determination.

8.2 Test portion

Mix the test sample (8.1) by gently stirring, or by rotating and inverting the container. Immediately weigh, to the nearest 1 mg, directly or by difference, into a fat-extraction flask (6.6) or into a 100 ml beaker or flask, 2 g to 3 g of the test 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 omitting the test portion as in 8.5.1 (see 11.2).

8.4 Preparation of fat-collecting vessel

Dry a vessel (6.9) containing a few boiling aids (6.10) in the oven (6.4) for 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 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). The vessel should not be placed in a desiccator to avoid insufficient cooling or unduly long cooling times.

Using tongs (6.13) (to avoid, in particular, temperature variations), place the vessel on the balance and weigh to the nearest 1 mg, recording the mass to four decimal places.

8.5 Determination

8.5.1 Add 7,5 ml to 10 ml, depending on the shape of the extraction apparatus, of the dilute hydrochloric acid (5.1) so as to wash the test portion into the small bulb of the extraction flask or onto the bottom of the beaker or flask, and mix.

NOTE Some laboratories prefer to use 7,5 ml to 8,5 ml of 1,15 g/ml hydrochloric acid instead of 7,5 ml to 10 ml of dilute hydrochloric acid (5.1).

8.5.2 Heat by gently moving the vessel in a boiling water bath, or over a flame or on a hotplate, until all the particles are entirely dissolved.

NOTE Mojonnier-type flasks (6.6) with a spherical lower bulb (forms B and C in ISO 3889) are particularly suitable for direct heating over a flame or on a hotplate.

8.5.3 Allow the vessel to stand for 20 min to 60 min in the boiling water bath, shaking occasionally during the initial 15 min, or keep it gently boiling over the flame or on the hotplate for 10 min. Cool, for example under running water.

If, at a later stage of the procedure, difficulties are encountered due to a viscous aqueous phase, repeat the determination with a smaller test portion and a longer heating or boiling time.

8.5.4 If the digestion has been carried out in the extraction apparatus, add 10 ml of the ethanol (5.2) and mix gently but thoroughly by allowing the contents of the flask to flow backwards and forwards between the two bulbs; avoid bringing the liquid too near to the neck of the flask. If desired, add two drops of the Congo red solution (see 5.6).

If the digestion has been carried out in a vessel other than the extraction flask, pour the contents of the vessel into the extraction flask. Rinse successively with 10 ml of ethanol (5.2), 25 ml of diethyl ether (5.3) and 25 ml of light petroleum (5.4), each time pouring the solvent into the extraction flask. Mix after the addition of the ethanol as described above, and shake the extraction flask after the addition of diethyl ether and light petroleum, as described in 8.5.5 and 8.5.6 respectively.

8.5.5 Add 25 ml of the diethyl ether (5.3), 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 under running water, then carefully remove the cork or stopper and rinse it and the neck of the flask of the flask with a little of the mixed solvent (5.5), 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 (5.4), 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 min to 5 min at a rotational frequency of 500 min⁻¹ 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 under 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 decanting the solvent.

NOTE In Figures 1 and 2, one of the three types of flasks as specified in ISO 3889 has been chosen, but this does not imply any preference over the other types (see, however, the Note in 8.5.2).

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), taking care to avoid decanting 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 8.5.12.