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Cheese and processed cheese products — Determination of fat content — Gravimetric method (Reference method)

Fromage et fromage fondu — Détermination de la teneur en matière grasse — Méthode gravimétrique (Méthode de référence)

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Foreword

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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 International Standard may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 1735 | IDF 5 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 1735 | IDF 5 cancels and replaces ISO 1735:1987, which has been technically revised.

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 IDF National Committees casting a vote.

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All work was carried out by the Joint ISO/IDF/AOAC Action Team, *Fat*, of the Standing Committee, *Main components in milk*, under the aegis of its project leader, Mr G.J. Beutick (NL).

This edition of ISO 1735 | IDF 5 cancels and replaces IDF 5B:1986, which has been technically revised.

Cheese and processed cheese products — Determination of fat content — Gravimetric method (Reference method)

WARNING — The use of ISO 1735 | IDF 5 may involve hazardous materials, operations and equipment. This standard does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this standard to establish safety and health practices and to determine the applicability of regulatory limitations prior to use.

1 Scope

This International Standard specifies the reference method for the determination of the fat content of all types of cheese and processed cheese products having lactose contents of below 5 % (mass fraction) of non-fat solids.

2 Normative references

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

ISO 3889:1977, *Milk and milk products — Determination of fat content — Mojonnier-type fat extraction flasks*

3 Terms and definitions

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

3.1
fat content of cheese and processed cheese products
mass fraction of substances determined by the procedure specified in this International Standard

NOTE 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 extracted with diethyl ether and light petroleum and the solvents are removed by distillation or evaporation. The mass of the substances extracted 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. The reagents shall leave no appreciable residue when the determination is carried out by the method specified (see 5.1).

5.1 Purity of reagents

To test the quality of the reagents, carry out a blank test as specified in 9.2. Use an empty fat-collecting vessel, prepared as specified in 9.3, for mass control purposes.

The reagents shall leave no residue greater than 0,5 mg (see A.1 of Annex A).

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.

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 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 the redistillation.

5.2 Concentrated hydrochloric acid, ρ_{20} (HCl) = 1,18 g/ml.

5.3 Dilute hydrochloric acid, ρ_{20} (HCl) = 1,125 g/ml approximately.

Dilute 675 ml of concentrated hydrochloric acid (5.2) to 1 000 ml with water and mix.

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

5.5 Diethyl ether, free from peroxides (see A.4), complying with the requirements for the blank test.

5.6 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.

NOTE The use of pentane is recommended due to its higher purity and constant quality.

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

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, the following.

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

6.2 Centrifuge, capable of holding the fat-extraction flasks or tubes (6.6) and capable of spinning at a rotational frequency of 500 min^{-1} to 600 min^{-1} to produce a radial acceleration of 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**, capable of distilling the solvents and ethanol from the flasks or capable of evaporating from beakers and dishes (9.4.13) 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. The oven shall be fitted with a suitable thermometer.

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

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

NOTE It is also possible to use fat-extraction tubes, with siphon or wash-bottle fittings, but the procedure is slightly different then. The alternative procedure is specified in Annex B.

The flasks shall be provided with good quality bark corks or stoppers of other material unaffected by the reagents used. Extract bark corks with the diethyl ether (5.5), then place in water at 60 °C or more for at least 15 min. Then allow them to cool in the water so that they are saturated when used.

6.7 Rack, capable of holding the fat-extraction flasks (or tubes) (6.6).

6.8 Wash bottle, suitable for use with the mixed solvent (5.7). Do not use plastic wash bottles.

6.9 Fat-collecting vessels, for example: boiling flasks, flat-bottomed, of capacity 125 ml to 250 ml; conical flasks, of capacity 250 ml; or metal dishes made of stainless steel, flat-bottomed, of diameter 80 mm to 100 mm, of height approximately 50 mm. Do not use aluminium dishes.

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

6.11 Measuring cylinders, of capacities 5 ml and 25 ml.

6.12 Pipettes, graduated, to deliver 10 ml.

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

6.14 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.

6.15 Appropriate grinding or **grating device**, easy to clean, for preparing the test sample.

7 Sampling

It is important that the laboratory receive a test sample which is truly representative and has not been damaged or changed during transport or storage.

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

The test samples shall be kept at a temperature of between 0 °C and 20 °C from the time of sampling to the time of commencing the procedure.

8 Preparation of test sample

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 as it is usually consumed.

Grind or grate the test sample by using an appropriate grinding or grating device (6.15). Mix the ground mass quickly and, if necessary for semi-hard and hard cheeses, grind it a second time and again mix thoroughly. Preferably, cut hard and semi-hard cheeses into cubes of about 15 mm × 15 mm. Mix the cubes by shaking in a container. Grind or grate the test 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.

9 Procedure

NOTE An alternative procedure using fat-extraction tubes with siphon or wash-bottle fittings is specified in Annex B.

9.1 Test portion

Mix the test sample (Clause 8) by gently stirring. Immediately weigh, to the nearest 1 mg, directly or by difference, 1 g to 3 g of test sample into a fat-extraction flask (6.6), a 100 ml beaker or flask.

Weigh an amount of 3 g for cheeses having a mass fraction of fat of up to 30 %. 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 (6.14), which is subsequently folded and introduced into the chosen vessel. 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 (see A.2).

If the value obtained in the blank test regularly exceeds 1,0 mg, check the reagents if this has not been done recently (see 5.1). Corrections for values of more than 2,5 mg in the blank test shall be reported in the test report.

9.3 Preparation of fat-collecting vessel

Dry a fat-collecting vessel (6.9) with a few boiling aids (6.10) in the drying oven (6.4) set at 102 °C 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 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 dish 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 of dilute hydrochloric acid (5.3). Add the hydrochloric acid so as to wash the test portion into the small bulb of the fat-extraction flask (6.6) or onto the bottom of the beaker or flask, 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) or over a flame, until all the particles are entirely dissolved.

NOTE Some Mojonnier-type fat-extraction flasks cannot be heated over a flame.

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 over the flame or 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 of ethanol (5.4). Mix gently but thoroughly by allowing the contents of the flask to flow backwards and forwards between the two bulbs while not bringing the liquid too near the neck of the flask. Proceed as in 9.4.5.

Alternatively, if the digestion has been carried out in a vessel other than the fat-extraction flask (6.6), pour the contents of the vessel into a fat-extraction flask. Rinse the vessel successively with 10 ml of ethanol (5.4). Mix gently but thoroughly by allowing the contents of the flask to flow backwards and forwards between the two bulbs while not bringing the liquid too near the neck of the flask. Then rinse the vessel with 25 ml of diethyl ether (5.5) and pour the vessel contents into the fat-extraction flask, while rinsing the tip or rim with some additional diethyl ether. Close the fat-extraction flask with a cork saturated with water or with a stopper of other material wetted with water (see 6.6) and shake as described in 9.4.5. Finally rinse the vessel again with 25 ml of light petroleum (5.6) 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 (5.5). Close the fat-extraction flask with a cork saturated with water or with a stopper of other material wetted with water (see 6.6).

Shake the flask vigorously for 1 min, but not so vigorously as to cause formation of a persistent emulsion. 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 the cork or stopper and rinse it and the neck of the flask with a little mixed solvent (5.7). Use the wash bottle (6.8) so that the rinsings run into the fat-extraction flask.

9.4.6 Add 25 ml of the light petroleum (5.6). Close the fat-extraction flask with the rewetted cork or rewetted stopper (by dipping in water).

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 a rotational frequency of 500 min^{-1} to 600 min^{-1} for 1 min to 5 min. 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 fat-extraction flask under running water.

9.4.8 Carefully remove the cork or stopper and rinse it and the inside of the neck of the fat-extraction flask with a little mixed solvent (5.7). Use the wash bottle (6.8) so that the rinsings run into the flask.

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

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

9.4.9 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 (6.10) (optional with metal dishes). Avoid decanting any of the aqueous layer (see Figure 2).