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Skimmed milk, whey and buttermilk — Determination of fat content — Gravimetric method (Reference method)

Lait écrémé, sérum et babeurre — Détermination de la teneur en matière grasse — Méthode gravimétrique (Méthode de référence)

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Foreword

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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 7208 | IDF 22 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*, and the International Dairy Federation (IDF). It is being published jointly by ISO and IDF.

This third edition of ISO 7208 | IDF 22 cancels and replaces the second edition (ISO 7208:1999), of which it constitutes a minor revision.

Foreword

IDF (the International Dairy Federation) is a non-profit organization representing the dairy sector worldwide. IDF membership comprises National Committees in every member country as well as regional dairy associations having signed a formal agreement on cooperation with IDF. All members of IDF have the right to be represented at the IDF Standing Committees carrying out the technical work. IDF collaborates with ISO 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 IDF National Committees casting a vote.

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ISO 7208 | IDF 22 was prepared by the International Dairy Federation (IDF) and Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*. It is being published jointly by IDF and ISO.

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

This edition of ISO 7208 | IDF 22 cancels and replaces IDF 22:1987, of which it constitutes a minor revision.

Skimmed milk, whey and buttermilk — Determination of fat content — Gravimetric method (Reference method)

WARNING — The use of this International Standard may involve hazardous materials, operations and equipment. This International Standard does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this International Standard to establish safety and health practices and 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 liquid skimmed milk, whey and buttermilk. It is a particularly accurate gravimetric method especially for the purpose of establishing the operating efficiency of cream separators.

This International Standard also specifies the reference method for establishing correction tables for procedures with skimmed milk butyrometers.

NOTE When a high accuracy of determination is not required, the method specified in ISO 1211 | IDF 1[2] is suitable.

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 835, *Laboratory glassware — Graduated pipettes*

ISO 1042, *Laboratory glassware — One-mark volumetric flasks*

ISO 3889 | IDF 219, *Milk and milk products — Specification of Mojonnier-type fat extraction flasks*

ISO 4788, *Laboratory glassware — Graduated measuring cylinders*

3 Terms and definitions

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

3.1
fat content of skimmed milk, whey and buttermilk
mass fraction of substances determined by the procedure specified in this International Standard

NOTE The fat content is expressed as a percentage mass fraction.

4 Principle

An ammoniacal ethanolic solution of a test portion is extracted with diethyl ether and light petroleum. The solvents are removed by distillation or evaporation. The mass of the substances extracted is determined.

NOTE This is usually known as the Röse-Gottlieb principle.

5 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified, and only distilled or demineralized water or water of equivalent purity.

The reagents shall leave no appreciable residue when the determination is carried out by the method specified (see 9.2.2).

5.1 Ammonia solution, containing a mass fraction of NH_3 of approximately 25 % (ρ_{20} = 910 g/l).

NOTE If ammonia solution of this concentration is not available, a more concentrated solution of known concentration may be used (see 9.4.2).

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

5.3 Congo red solution

Dissolve 1 g of Congo red ($C_{32}H_{22}N_6Na_2O_6S_2$) in water in a 100 ml one-mark volumetric flask (6.14). Make up to the mark with water.

NOTE The use of this solution, which allows the interface between the solvent and aqueous layers to be seen more clearly, is optional (see 9.4.3). Other aqueous indicator solutions can be used provided that they do not affect the result of the determination.

5.4 Diethyl ether ($C_2H_5OC_2H_5$), free from peroxides (see Clause A.3), containing no more than 2 mg/kg of antioxidants, and complying with the requirements for the blank test (see 9.2.2, Clauses A.1 and A.4).

WARNING — The use of diethyl ether can lead to hazardous situations. Observe current safety precautions for handling, use, and disposal.

5.5 Light petroleum, with any boiling range between 30 °C and 60 °C or, as equivalent, **pentane** ($CH_3[CH_2]_3CH_3$) with a boiling point of 36 °C and complying with the requirements for the blank test (see 9.2.2, Clauses A.1 and A.4).

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

5.6 Mixed solvent.

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

6 Apparatus

WARNING — Since the determination involves the use of volatile flammable solvents, all electrical apparatus employed shall 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 80g to 90g at the outer end of the flasks or tubes.

The use of the centrifuge is optional but recommended (see 9.4.6).

6.3 Distillation or evaporation apparatus, for distilling the solvents and ethanol from the boiling or conical flasks, or evaporating from beakers and dishes (see 9.4.13) 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 its working space.

The oven shall be fitted with a suitable thermometer.

6.5 Water bath, capable of being maintained at a temperature of between 35 °C and 40 °C.

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

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

The fat-extraction flasks shall be provided with good quality cork bungs or stoppers of other material (e.g. silicone rubber or polytetrafluoroethylene) unaffected by the reagents used. Cork bungs shall be extracted with the diethyl ether (5.4), kept in water at a temperature of 60 °C or more for at least 15 min, and shall then be allowed to cool in the water so that they are saturated when used.

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

6.8 Wash bottle, suitable for use with the mixed solvent (5.6).

A plastics wash bottle shall not be used.

6.9 Fat-collecting vessels, such as boiling flasks (flat-bottomed), of capacities 125 ml to 250 ml, conical flasks, of capacity 250 ml, or metal dishes.

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

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

6.11 Measuring cylinders, of capacities 5 ml and 25 ml, complying with the requirements of ISO 4788, class A, or any other apparatus suitable for the product concerned.

6.12 Pipettes, graduated, of capacity 10 ml, complying with the requirements of ISO 835, class A.

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

6.14 Volumetric flask, one-mark, of capacity 100 ml, complying with the requirements of ISO 1042, class A.

7 Sampling

A representative sample should have been sent to the laboratory. It should not have 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 | IDF 50[1].

Store laboratory samples at a temperature between 2 °C and 6 °C from the time of sampling to the time of commencing the procedure.

8 Preparation of test sample

Warm the test sample to a temperature between 35 °C and 40 °C by means of the water bath (6.5), if necessary. Gently mix the test sample thoroughly by repeatedly inverting the sample bottle without causing frothing or churning. Cool the test sample quickly to approximately 20 °C.

NOTE A reliable value for the fat content cannot be expected:

a) if the milk is churned;

b) when a distinct smell of free fatty acids is perceptible;

c) if during or after preparation of the test sample, white particles are visible on the walls of the sample bottle or fat droplets float on the surface of the sample.

9 Procedure

NOTE 1 In the determination, two test portions (9.1) are extracted in two Mojonnier-type fat-extraction flasks (6.6). The extracts of the two flasks are poured into one prepared fat-collecting vessel (9.3).

NOTE 2 If it is required to check whether the repeatability limit (11.2) is met, carry out two single determinations in accordance with 9.1 to 9.4.

NOTE 3 An alternative procedure using fat-extraction tubes with siphon or wash-bottle fittings (see Note to 6.6) is given in Annex B.

9.1 Test portion

Mix the test sample (Clause 8) by gently inverting the bottle three or four times. Immediately weigh, to the nearest 1 mg, 10,000 g to 11,000 g of the test sample, directly or by difference, in each of two fat-extraction flasks (6.6).

Transfer the test portion as completely as possible into the lower (small) bulb of the fat-extraction flask.

9.2 Blank tests

9.2.1 Blank test for method

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

When one blank sample is used for a batch of test samples of which the individual samples may not have exactly the same conditions, ensure that the procedure for obtaining the value of the blank used in the calculation of the result corresponds exactly to that of 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 recently done (9.2.2). Corrections of more than 2,5 mg should be mentioned in the test report.

9.2.2 Blank test for reagents

To test the quality of the reagents, carry out a blank test as specified in 9.2.1. Additionally, use an empty fat-collecting vessel, prepared as specified in 9.3, for mass control purposes. The reagents shall leave no residue greater than 1,0 mg (see Clause A.1).

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 (5.4) and light petroleum (5.5), respectively. Use an empty fat-collecting vessel, prepared for control purposes as in the preceding paragraph, to obtain the real mass of residue which shall not exceed 1,0 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 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.

Replace unsatisfactory reagents and solvents, or redistil solvents.

9.3 Preparation of fat-collecting vessel

Dry a fat-collecting vessel (6.9) with a few boiling aids (6.10) in the oven (6.4) maintained at 102 °C for 1 h.

NOTE 1 Boiling aids are desirable to promote gentle boiling during the subsequent removal of solvents, especially when using glass fat-collecting vessels; their use is optional with metal dishes.

Protect the fat-collecting vessel from dust and allow it to cool to the temperature of the weighing room (glass fat-collecting 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 (6.13) to place the fat-collecting vessel on the balance. Weigh the fat-collecting vessel to the nearest 1,0 mg.

NOTE 2 The use of tongs effectively avoids, in particular, inducing temperature variations.

9.4 Determination

9.4.1 Carry out the determination without delay.

Carry out the operations specified in 9.4.2 to 9.4.13 on both pretreated test portions (9.1).

9.4.2 Add 2 ml of ammonia solution (5.1) to the two test portions in the fat-extraction flasks (9.1), or an equivalent volume of a more concentrated ammonia solution (see Note to 5.1). Mix thoroughly with the test portion in the small bulb of each of the two fat-extraction flasks.

9.4.3 Add 10 ml of ethanol (5.2). Mix gently but thoroughly by allowing the contents of the two fat-extraction flasks to flow backwards and forwards between the small and large bulb. Avoid bringing the liquid too near to the necks of the flasks. If desired, add 2 drops of the Congo red solution (5.3).

9.4.4 Add 25 ml of diethyl ether (5.4). Close both fat-extraction flasks with a cork bung saturated with water or with a stopper of other material wetted with water (6.6). Shake the flasks vigorously, but not excessively, for 1 min, to avoid the formation of persistent emulsions.

While shaking, keep both fat-extraction flasks in a horizontal position with their small bulbs extending upwards, periodically allowing the liquid to run from the large bulb into the small bulb. If necessary, cool the flasks in running water to about room temperature. Carefully remove the bungs or stoppers and rinse them and the necks of both flasks with a little mixed solvent (5.6). Use the wash bottle (6.8) so that the rinsings run into the flask concerned.

9.4.5 Add 25 ml of the light petroleum (5.5). Close both fat-extraction flasks with the rewetted (by dipping into water) bungs or stoppers. Shake the flasks gently again for 30 s as specified in 9.4.3. Proceed with shaking as specified in 9.4.4.

9.4.6 Centrifuge the two closed fat-extraction flasks for between 1 min and 5 min at a radial acceleration of $80g$ to $90g$. If a centrifuge (6.2) is not available, allow the closed flasks 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 both flasks in running water to room temperature.