INTERNATIONAL STANDARD

ISO 23318

IDF 249

First edition
2022-11

Milk, dried milk products and cream — Determination of fat content — Gravimetric method

Lait, produits laitiers secs et crème — Détermination de la teneur en matière grasse — Méthode gravimétrique

Reference numbers
ISO 23318:2022(E)
IDF 249:2022(E)

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11

Email: copyright@iso.org
Website: www.iso.org

International Dairy Federation
Silver Building • Bd Auguste Reyers 70/B
B-1030 Brussels
Phone: +32 2 325 67 40
Fax: +32 2 325 67 41
Email: info@fil-idf.org
Website: www.fil-idf.org

Published in Switzerland

Contents

Page

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.

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).

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

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). It is being published jointly by ISO and IDF.

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.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. IDF shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

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*. It is being published jointly by ISO and IDF.

The work was carried out by the ISO/IDF Action Team on C32 of the *Standing Committee on Analytical Methods for Composition* under the aegis of its project leader, Mr P. Trossat (FR).

Milk, dried milk products and cream — Determination of fat content — Gravimetric method

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

1 Scope

This document specifies the method for the determination of fat content.

The method is applicable to:

a) raw milk (cow, sheep, goat), reduced fat milk, skimmed milk, chemically preserved milk and processed liquid milk;

b) dried milk products (e.g. whole, partially skimmed, skimmed milk powder; dairy permeate powder; whey powder; blend skimmed milk powder and vegetable fat; milk based infant formula powder);

c) raw, processed and sour cream.

For the following products, the precision figures are given in Annex H. These precision figures are derived from interlaboratory studies not conforming to the requirements from ISO 5725-2 in terms of number of samples (< 6) and number of participating laboratories (< 8).

d) evaporated milk and sweetened condensed milk (e.g. liquid sweetened and unsweetened concentrated milk);

e) whey cheese as defined in CODEX CXS 284-1999;

f) liquid whey and buttermilk;

g) milk-based edible ices and ice mixes;

h) liquid concentrated infant foods.

The method does not apply in the following cases:

— For b), when the powder contains hard lumps which do not dissolve in ammonia solution. This is noticeable by a distinct smell and the result of the determination will be too low. In such cases, a method using the Weibull-Berntrop principle is suitable, e.g. ISO 8262-3|IDF 124-3.

— For c), The method is not applicable to sour creams with starch or other thickening agents. When separation or breakdown of fat occurs, a method using the Weibull-Berntrop principle is suitable, e.g. ISO 8262-3|IDF 124-3.

— For e), to products which do not dissolve completely in ammonia solution, as the result of the determination will be too low. With such products, a method using the Weibull-Berntrop principle is suitable, e.g. ISO 8262-3|IDF 124-3.

— For g), to milk-based edible ices and ice mixes in which the level of emulsifier, stabilizer or thickening agent or of egg yolk or of fruits, or of combinations of these constituents, makes the Röse-Gottlieb method unsuitable. With such products, a method using the Weibull-Berntrop principle is suitable, e.g. ISO 8262-2|IDF 124-2.

— For h), to products which do not dissolve completely in ammonia due to the presence of starch or dextrin at mass fractions of more than 5 % (in dry matter), or to the presence of hard lumps. For such products, a method using the Weibull-Berntrop principle is suitable, e.g. ISO 8262-1|IDF 124-1.

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 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.

ISO and IEC maintain terminology 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

Note 1 to entry: Fat content is determined by the procedure specified in this document.

Note 2 to entry: The fat content is expressed as a percentage by mass.

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 principle is usually described 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 Annex A).

5.1 Ammonia solution, containing a mass fraction of NH_3 of approximately 25 %.

If ammonia solution of this concentration is not available, a more concentrated solution of known concentration may be used (see 9.4.2.1).

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 Indicator solution (optional). The use of indicator solutions allows the interface between the solvent and aqueous layers to be seen more clearly (see 9.4.2.3). Other aqueous indicator solutions can be used provided that they do not affect the result of the determination.

5.3.1 Bromocresol purple solution, mass concentration 1 g/100 ml. Dissolve 1 g of bromocresol purple in 10 ml of ethanol and dilute to 100 ml with water.

5.3.2 Patent blue V. Dissolve 1 g of patent blue V (sodium salt) in water and dilute to 100 ml with water.

5.4 Diethyl ether ($C_2H_5OC_2H_5$), free from peroxides (see Clause A.3), containing no more than 7 mg/kg of antioxidants, and conforming to the requirements for the blank test (see 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 conforming to the requirements for the blank test (see Clauses A.1 and A.4).

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

NOTE Petroleum ether and petroleum benzine with an appropriate boiling range are some suitable commercial names of this reagent.

5.6 Mixed solvent.

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

6 Apparatus

The usual laboratory equipment and, in particular, the following shall be used.

Use graduated pipettes in accordance with ISO 835, one-mark volumetric flasks in accordance with ISO 1042 and graduated measuring cylinders in accordance with ISO 4788.

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 to produce a radial acceleration of around 80*g* to 90*g* at the outer end of the flasks or tubes.

The use of the centrifuge is optional but recommended (see 9.4.2.6).

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 9.4.2.12) at a temperature not exceeding 100 °C.

6.4 Drying oven, electrically heated, with ventilation port(s) fully open, capable of being maintained at 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 37,5 °C ± 2,5 °C, 50 °C ± 5 °C, 65 °C ± 5 °C and at boiling point.

6.6 Fat-extraction flasks:

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

6.6.2 Extraction tubes-type fat extraction flask. It is also possible to use fat extraction tubes with siphon or wash-bottle fittings (see the model given in Annex B).

6.6.3 Stoppers. The flasks or tubes shall be provided with stoppers of different material [bark cork, silicone rubber, polytetrafluoroethylene (PTFE) or glass] unaffected by the reagents used. Bark corks shall be washed with 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, for holding the fat-extraction flasks (or tubes) (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, e.g.:

— 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 be of stainless steel, flat-bottomed with a diameter of 80 mm to 100 mm and a height of approximately 50 mm.

6.10 Measuring cylinders or delivering systems, compatible with the use of solvents of capacities 5 ml and 25 ml.

6.11 Pipettes or delivering systems, of capacity 10 ml.

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

6.13 Blender, fitted with a bowl with a capacity of 1 l with its lid or any other device suitable for preparing the test sample.

6.14 Boiling aids (optional), fat free in non-porous porcelain, silicon carbide or glass.

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.

From the time of sampling to the time of commencing the procedure, store laboratory liquid samples at a temperature of 4 °C ± 2 °C and dried products at room temperature. For evaporated milk, sweetened condensed milk and milk-based infant foods, store samples in sealed cans or bottles unopened at a temperature below 20 °C, until the time of starting the procedure.

8 Preparation of test sample

8.1 Milk

Using the water bath (6.5), warm the test sample to a temperature of 38 °C ± 2 °C. Gently mix the test sample thoroughly without causing frothing or churning. Then cool the test sample quickly to approximately 20 °C.

If a homogeneous test sample can be obtained without pre-warming (e.g. for samples of skimmed milk), bring the test sample to a temperature of 20 °C ± 2 °C and gently mix thoroughly by repeatedly inverting the sample bottle.

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;

NOTE Goat milk naturally contains a low level of free fatty acids, which are not completely extracted in this method.

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.

8.2 Dried milk products

Thoroughly mix the test sample by repeatedly rotating and inverting the sample container. If necessary, transfer all the test sample to an airtight container of approximately twice the volume of the test sample to allow this operation to be carried out.

8.3 Evaporated milk

Shake and invert the sample container. Open the sample container and pour the sample slowly into a second sample container, provided with an airtight lid. Mix by repeated transfer, taking care to incorporate in the sample any fat or other constituent adhering to the wall and ends of the first container. Finally, transfer the product as completely as possible to the second container.

If necessary in the case of samples in sealed cans, condition the unopened container in the water bath (6.5) maintained at a temperature of 50 °C ± 5 °C. Remove and shake the can vigorously every 15 min. After 2 h, remove the can and allow it to cool to room temperature.

Remove the lid entirely and thoroughly mix the sample by stirring with a spoon or spatula. If fat separates, do not test the sample.

8.4 Sweetened condensed milk

Open the sample container and mix thoroughly with a spoon or spatula. Use an up-and-down rotary movement in such a way that the top layers and the content of the lower corners of the container are moved and mixed. Take care to incorporate in the sample any milk adhering to the wall and ends of the container. Transfer the sample as completely as possible to a second sample container, provided with an airtight lid. Close the second container.

If necessary, in the case of samples in sealed cans, condition the unopened can in the water bath (6.5) at a temperature of 38 °C ± 2 °C. Open the can, scrape out all milk adhering to the interior of the can, transfer to a dish large enough to permit stirring thoroughly and mix until the whole mass is homogeneous.

In the case of a sample in a collapsible tube, open the tube and transfer the contents to a jar. Then cut the tube and scrape out all material adhering to the interior and add to the contents of the jar.

8.5 Whey cheese

Prepare the test sample using an appropriate device. Quickly mix the ground or grated mass and, if possible, grind it a second time. Again mix thoroughly. Clean the device after preparing each test sample.

If the test sample cannot be ground or grated, mix it thoroughly by intensive kneading, e.g. with a pestle in a mortar. The risk of moisture loss during grinding or grating of the sample should be avoided as far as practically possible.

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

8.6 Cream

Warm the test sample to a temperature of 38 °C ± 2 °C by means of the water bath (6.5), if necessary. Thoroughly, but gently, mix the test sample by repeatedly inverting the sample bottle, or, if the cream is very thick, by stirring with a spatula, without causing frothing or churning, and cool quickly to approximately 20 °C.

Churned cream should not be cooled as it has to be weighed at a temperature of between 30 °C and 40 °C (see 9.1).

NOTE A reliable value for the fat content cannot be expected when a distinct smell of free fatty acids is perceptible, and/or 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. In such cases, a method utilizing the Weibull-Berntrop principle is suitable, such as ISO 8262-3|IDF 124-3.

8.7 Skimmed milk, whey and buttermilk

Warm the test sample to a temperature of 38 °C ± 2 °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 if the milk is churned, when a distinct smell of free fatty acids is perceptible and/or 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.

8.8 Milk-based infant foods

8.8.1 Liquid products

Shake and invert the sample container. Open the container to pour the product slowly into a second sample container provided with an airtight lid. Mix by repeated transfer of the product, taking care to incorporate in the sample any fat or other constituent adhering to the wall and ends of the first container. Transfer the test sample as completely as possible to the second sample container. Close this container.

If necessary, condition the unopened sample container in the water bath (6.5) at a temperature of 50 °C ± 5 °C. Remove and shake the container vigorously every 15 min. After 2 h, remove the container, dry the outside with a tissue and allow to cool to room temperature. Remove the lid or cap entirely and thoroughly mix the contents by stirring with a spoon or spatula. If fat separates, do not test the sample. Transfer the test sample as completely as possible to a second sample container provided with an airtight lid. Close this container.