INTERNATIONAL STANDARD

ISO 6732

IDF 103

Second edition
2010-06-01

Milk and milk products — Determination of iron content — Spectrometric method (Reference method)

Lait et produits laitiers — Détermination de la teneur en fer — Méthode spectrométrique (Méthode de référence)

Reference numbers
ISO 6732:2010(E)
IDF 103:2010(E)

ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

International Dairy Federation
Diamant Building • Boulevard Auguste Reyers 80 • B-1030 Brussels
Tel. + 32 2 733 98 88
Fax + 32 2 733 04 13
E-mail info@fil-idf.org
Web www.fil-idf.org

Published in Switzerland

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 6732 | IDF 103 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 second edition of ISO 6732 | IDF 103 cancels and replaces the first edition (ISO 6732:1985), 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 on 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.

The main task of Standing Committees is to prepare International Standards. Draft International Standards adopted by the Standing Committees are circulated to the National Committees for endorsement prior to publication as an International Standard. Publication as an International Standard requires approval by at least 50% of IDF National Committees casting a vote.

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.

ISO 6732|IDF 103 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 former Joint ISO-IDF Action Team on *Minor compounds*, now part of the Standing Committee on *Analytical methods for composition*.

This edition of ISO 6732|IDF 103 cancels and replaces IDF 103A:1986, of which it constitutes a minor revision.

Milk and milk products — Determination of iron content — Spectrometric method (Reference method)

1 Scope

This International Standard specifies a spectrometric reference method for the determination of the iron content of milk and milk products.

This method is applicable to

— milk, skimmed milk, whey and buttermilk;

— plain yogurt and skimmed yogurt;

— evaporated milk and sweetened condensed milk;

— dried whole and skimmed milk, dried whey and dried buttermilk;

— cream and butter;

— anhydrous butterfat, butteroil, butterfat and ghee;

— ice-cream;

— cheese of various ages, and processed cheese;

— caseins, caseinates and coprecipitates.

2 Terms and definitions

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

2.1
iron content in milk and milk products
mass fraction of substances determined by the procedure specified in this International Standard

NOTE The iron content is conventionally expressed in milligrams per kilogram of sample.

3 Principle

Organic material is digested with a mixture of nitric and sulfuric acids, preceded, in the case of cream and anhydrous butterfat, butteroil, butterfat and ghee, by removal of the fat. In the case of butter, serum is separated and digested.

Iron(II) ions, obtained by reduction of iron(III) ions, are complexed with bathophenanthroline. The iron(II) compound is extracted with isoamyl alcohol. The absorbance of the red solution thus obtained is measured spectrometrically at a wavelength of 533 nm.

4 Reagents and materials

IMPORTANT — Maintain reagents, glassware and equipment, as well as the laboratory environment as clean as possible in order to avoid contamination by rust. Each laboratory should check and identify its own sources of contamination.

Use only reagents of very pure analytical grade and which, with the exception of the iron standard solutions (4.14 and 4.15), are free from iron.

4.1 Water, complying with grade 2 as defined in ISO 3696[5].

4.2 Ethanol (C_2H_5OH), about 96 % volume fraction.

Distil, if necessary, in an iron-free distillation unit.

4.3 Diethyl ether ($C_2H_5OC_2H_5$).

Distil, if necessary, in an iron-free distillation unit.

4.4 Light petroleum, boiling range 40 °C to 60 °C.

Distil, if necessary, in an iron-free distillation unit.

4.5 Nitric acid (HNO_3), concentrated, ρ_{20} = 1,42 g/ml.

Distil in an iron-free distillation unit. Discard the first 50 ml of distillate. Do not store the nitric acid in a brown glass bottle.

4.6 Sulfuric acid[1)] (H_2SO_4), concentrated, ρ_{20} = 1,84 g/ml.

4.7 Potassium sulfate[1)], solution in sulfuric acid.

Dissolve 25 g of anhydrous potassium sulfate (K_2SO_4) in sulfuric acid (4.6) and make up to 100 ml with the same acid. Filter the solution, without suction, through an all-glass, iron-free, filter crucible, of porosity grade P 100 (pore diameter 40 µm to 100 µm).

If the potassium sulfate available is not iron-free, purify it as follows.

Dissolve 40 g of potassium sulfate in 500 ml of water (4.1) and add 3 ml of the hydroxylammonium chloride solution (4.10). Extract the solution with 10 ml of the bathophenanthroline solution (4.12). Remove the upper layer. Repeat these two operations until the upper layer remains colourless. Evaporate the water in a clean oven.

4.8 Hydrogen peroxide[1)] (H_2O_2), solution, ρ_{20} = 1,099 g/ml to 1,103 g/ml.

Store in a refrigerator.

---

1) Aristar, Suprapur and Ultrex reagents are examples of suitable products available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO or IDF of these products.

4.9 Sodium acetate[1)], saturated solution.

Dissolve 232,5 g of anhydrous sodium acetate (CH_3COONa) in 500 ml of water (4.1).

If the sodium acetate available is not iron-free, purify it as follows.

Dissolve 232,5 g of sodium acetate in 500 ml of water. Filter through a filter paper. Add 3 ml of the hydroxylammonium chloride solution (4.10). Extract the solution with 10 ml of the bathophenanthroline solution (4.12). Remove the upper layer. Repeat these two operations until the upper layer remains colourless.

4.10 Hydroxylammonium chloride, solution.

Dissolve 20 g of hydroxylammonium chloride ($HONH_3Cl$) in water (4.1) and make up to 100 ml. Filter through a filter paper. Extract the solution with 5 ml of the bathophenanthroline solution (4.12). Allow the layers to separate properly. Remove the upper layer. Repeat these two operations until the upper layer remains colourless.

NOTE Generally, five extractions are sufficient.

If the solution was prepared more than 24 h before use, it is advisable to repeat the extraction with the bathophenanthroline.

Instead of the hydroxylammonium chloride solution, a freshly prepared solution of ascorbic acid can be used as a reducing agent. The ascorbic acid solution can be made by dissolving 10 g of ascorbic acid in 100 ml of water. The solution should be extracted with the bathophenanthroline solution in exactly the same way as described for the hydroxylammonium chloride solution. It should be stored in a refrigerator. Instead of 3 ml of the hydroxylammonium chloride solution, 3 ml of this ascorbic acid solution can be used in 4.7, 4.9 and 8.2.1.4.

4.11 Isoamyl alcohol (3-methyl-1-butanol).

Distil, if necessary, in an iron-free distillation unit.

4.12 Bathophenanthroline, solution.

Dissolve 83,1 mg of bathophenanthroline [4,7-diphenyl-1,10-phenanthroline ($C_{24}H_{16}N_2$)] in 100 ml of the isoamyl alcohol (4.11).

4.13 Potassium permanganate, solution.

Dissolve 100 mg of potassium permanganate ($KMnO_4$) in 50 ml of water (4.1).

4.14 Iron, standard solution corresponding to 1 000 mg of iron per litre.

Dissolve 7,022 g of ammonium iron(II) sulfate hexahydrate [$(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$] in 250 ml of water (4.1). Add 8 ml of sulfuric acid (4.6) and cool to room temperature. Make up to 1 000 ml with water.

A volume of 1 ml of this standard solution contains 1 mg of iron.

NOTE Commercially available preparations which contain 1 000 mg of iron can be used instead of the ammonium iron(II) sulfate hexahydrate.

4.15 Iron, standard solution corresponding to 1 mg of iron per litre.

On the day of use, pipette (5.11) 1 ml of the standard iron solution (4.14) into 250 ml of water (4.1). Add 1 ml of sulfuric acid (4.6) and make up to 1 000 ml with water.

A volume of 1 ml of this standard solution contains 1 µg of iron.

5 Apparatus

IMPORTANT — Maintain glassware and equipment, as well as the laboratory environment as clean as possible in order to avoid contamination by rust. Each laboratory should check and identify its own sources of contamination.

Store clean glassware, including the glass beads (5.8), in 10 % mass fraction nitric acid solution. Rinse three times before use with distilled water and then three times with double-distilled water. If necessary, dry by successively rinsing with ethanol (4.2) and diethyl ether (4.3).

Usual laboratory equipment and in particular the following.

5.1 Analytical balance.

5.2 Centrifuge, capable of producing a radial acceleration of 2 500g, with tubes of capacity at least 150 ml.

5.3 Grinding device, appropriate to the nature of the sample.

5.4 Sieve, nominal size of openings 500 µm, ISO 565[1], made of iron-free material.

5.5 Water baths.

5.6 Micro-burners or **electric heaters**, which do not emit iron-containing particles.

5.7 Digestion flasks (Kjeldahl), capacity approximately 70 ml, with ground-glass stoppers, calibrated on the lower part of the neck at 50 ml.

5.8 Glass beads, preferably made of quartz, which do not release iron during the digestion procedure (see 8.2.1).

5.9 Measuring cylinders, capacities 5 ml, 10 ml and 25 ml, ISO 4788[6].

5.10 Graduated pipettes, capacities 1 ml, 2 ml and 5 ml, graduated in divisions of 0,1 ml, ISO 835[4].

5.11 One-mark pipettes, capacities 1 ml, 2 ml, 3 ml, 4 ml, 5 ml, 10 ml and 25 ml, ISO 648[2] class A.

5.12 Spectrometer, suitable for measuring absorbance at 533 nm, equipped with cells of optical pathlength 10 mm.

6 Sampling

IMPORTANT — Avoid contamination by iron. Store glass sampling jars in 10 % mass fraction nitric acid solution. Rinse them thoroughly and dry before use.

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 707|IDF 50[3].

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

7 Preparation of test sample

IMPORTANT — Avoid contamination by iron.

7.1 Milk, skimmed milk and whey

Bring the sample to (20 ± 2) °C and mix carefully. If, in the case of milk, the fat is not evenly dispersed, heat the sample slowly to 40 °C, mix gently by inversion only, and cool quickly to (20 ± 2) °C.

7.2 Buttermilk

If necessary, remove butter granules. Bring the sample to (20 ± 2) °C and mix carefully, immediately before weighing (see 8.1.1).

7.3 Plain yogurt and skimmed yogurt

Bring the sample to (20 ± 2) °C and mix carefully. If serum separates, stir vigorously, immediately before weighing (see 8.1.1).

7.4 Cream

Bring the sample to (20 ± 2) °C. Mix or stir thoroughly, but not so vigorously as to cause frothing or churning.

If the cream is very thick, or if the fat is not evenly dispersed, warm slowly to 40 °C to facilitate mixing.

Cool the sample quickly to (20 ± 2) °C. Stir the sample in the container thoroughly. Mix until the whole mass is homogeneous. Close the container.

Correct results cannot be expected if adequate mixing of the sample is not achieved or if the sample shows any evidence of churning or any other signs of abnormality.

7.5 Evaporated milk

Shake the container thoroughly, inverting it frequently. Open the container and pour the milk slowly into another container made of glass, provided with an airtight lid, taking care to incorporate in the sample any fat or other constituents adhering to the wall of the original container. Stir vigorously and close the container.

Heat the closed container in a water bath at 40 °C to 60 °C. Remove and shake the container vigorously every 15 min. After 2 h, remove the container and cool to (20 ± 2) °C. Remove the lid and mix thoroughly by stirring the sample with a spoon or spatula.

If the fat separates, correct results cannot be expected.

7.6 Sweetened condensed milk

Open the container and thoroughly mix the milk with a spoon or spatula, using an up-and-down rotary movement in such a way that the top and bottom layers 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 container made of glass, provided with an airtight lid, and close this container. Heat the closed container in a water bath at 30 °C to 40 °C. Cool to (20 ± 2) °C. Stir the sample in the container thoroughly. Mix until the whole mass is homogeneous. Close the container.

In the case of a collapsible tube, open it and transfer the contents to a glass container. Cut open the tube and transfer as completely as possible all material adhering to the interior of the container.